Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

tert-butyl 2-ethylperoxyhexanoate

EC Number: 221-110-7
CAS Number: 3006-82-4

CLH-O-0000007217-74-01/F

Adopted

1 December 2022

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON TERT-BUTYL 2-ETHYLPEROXYHEXANOATE

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: *tert*-butyl 2-ethylperoxyhexanoate
EC number: 221-110-7
CAS number: 3006-82-4
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2022	Germany		MemberState	1
Comment received				
If proposing to classify the substance as Skin Sens. 1B, H317 a labeling with GHS07 is required despite assigned GHS08. According to Article 26 (1) of Regulation (EC) No. 1272/2008 a non-award of the GHS07 with a simultaneous award of GHS08 is only possible if GHS08 must be labeled for respiratory sensitization. Therefore, in the CLH dossier "GHS07" has to be added in Table 6 subsection 3.1 in the - Row "Dossier submitters proposal" and column "Labelling/ Pictogram, Signal Word Code(s)" and - Row "Resulting Annex VI entry if agreed by RAC and COM" and column "Labelling/ Pictogram, signal word code(s)".				
Dossier Submitter's Response				
Thank you for your support. Agrees that the labelling with GHS07 is required.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2022	Germany	United Initiators GmbH on behalf of the Organic Peroxides Consortium	Company-Manufacturer	2
Comment received				
Please refer to the attached document.				

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to CLP Proposal TBPEH_2022-04-28.pdf
Dossier Submitter's Response
Thank you for your support concerning SKIN SENS 1B (H 317) and Repr. 1B for fertility.
RAC's response
Noted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2022	Estonia		MemberState	3
Comment received				
The MSCA could support the DS proposal on classification of TBPEH as Repr. 1B – H360FD (May damage fertility. May damage the unborn child.). With regards to adverse effects on sexual function and fertility, the key evidence for classification is from the EOGRTS (following OECD 443) study in rats (from 2020). Sexual function and fertility was adversely effected by TBPEH, causing reduced fertility indices (% of females pregnant) in 300 (P1 generation) and 1000 mg/kg bw/d (P0 and P1 generation) dose groups and changes in estrous cycle (prolonged estrous) at 1000 mg/kg bw/d (P0 and P1). Quantitative examinations of ovaries revealed decreased number of developing follicles and increased follicular atresia at 1000 mg/kg bw/d (P0); in 3/24 females one or both sided follicular cyst was detected at 1000 mg/kg bw/d (P0), and the slightly longer mean duration of pregnancy of dams at 1000 mg/kg bw/day was statistically significant (22.37 days versus 21.97 days) (P0). These effects occurred without overt general toxicity in parental animals. At 1000 mg/kg bw/d lower bodyweight in P1 (mainly in males) animals and changes in organ weights were described. Pyelectasia was observed at 300 mg/kg bw/d (P1) and 1000 mg/kg bw/d (P1 and F1A). In addition, lower T3 and T4 levels were measured at 1000 mg/kg bw/d in P0 and P1 animals, although without accompanying TSH or histopathological changes. In conclusion, the data provides clear evidence of adverse effects on sexual function and fertility which are considered not to be a secondary non-specific consequence of other toxic effects, and the criteria for classification as Reproductive toxicity Category 1B are fulfilled. Regarding adverse effects on development, two specific prenatal developmental toxicity studies (OECD 414, in rats and rabbits), and in addition, EOGRTS and reproduction/developmental screening study (OECD 421, in rats) provided results relevant for classification. In rats, developmental toxicity was mainly induced at 1000 mg/kg bw/d, although at 300 mg/kg bw/d statistically significantly lower mean pup bodyweight was observed in F2 generation in EOGRTS, and slightly but statistically significantly increased late embryonic deaths were observed at 400 mg/kg bw/d in the PNDT test. 1000 mg/kg bw/d of TBPEH caused slightly (without statistical significance) increased late embryonic deaths in the PNDT study. In EOGRTS, extra-uterine mortality increased and bodyweight of pups decreased in both, F1 and F2 generations at 1000 mg/kg bw/d. Also in the reproduction/developmental screening study, post-implantation loss and post-natal loss were increased and mean bodyweight of pups was decreased at 1000 mg/kg bw/d. Therefore, the high dose of TBPEH (1000 mg/kg bw/d) had a consistent effect on the viability of offspring in several rat studies. In addition, in EOGRTS at 1000 mg/kg bw/d effects were seen on F1 offspring's (Cohort 1B) development (surface righting reflex, pinna detachment, eye opening), and sexual maturation (Cohort 1A females) as observed by statistically significantly longer period of vaginal patency and longer period of appearance of the first cornified vaginal smear. In the PNDT study in rats, slightly but statistically significantly decreased fetus bodyweight (-6%) and increased incidence of				

fetuses with visceral abnormalities (4% vs 1% in control) and increased incidence of incomplete ossification of the skull bones and metacarpal/metatarsal was observed at 1000 mg/kg bw/d without maternal adverse effects.
In the rabbit prenatal developmental toxicity study, the high dose of 300 mg/kg bw/d caused statistically significantly increased early embryonic death and post-implantation loss, significantly lower fetal weight and reduced crown-rump length and increased incidence of delayed ossification of proximal and middle phalanges, although in rabbits this dose level caused significant general toxicity in dams, where 3 moribund animals were euthanized and reduced bodyweight and food consumption and bodyweight loss were evident. Also, the incidence of abortions increased with increasing dose levels (0, 1, 2 and 8 at control, 30, 100 and 300 mg/kg bw/d, respectively).
Concluding the developmental toxicity data on TBPEH, the fetal development was adversely effected in rats and rabbits by exposure to TBPEH and was not associated with other serious toxicity in parental animals in rats, thus, classification as Reproductive toxicity Category 1B is appropriate according to the CLP criteria.

Dossier Submitter's Response

Thank you for your support for Repr. 1B for fertility and development.

RAC's response

The view of the Estonian CA is noted.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2022	Germany		MemberState	4

Comment received

Adverse effects on sexual function and fertility
The classification of tert-butyl 2-ethylperoxyhexanoate (TBPEH) as reproductive toxicant for fertility is supported.
An extended one-generation reproduction toxicity study (EOGRTS) in rats (OECD TG 443) conducted with TBPEH is available (Cohort 1B animals were mated to produce a second (F2) generation). Oral exposure (gavage) of TBPEH (OECD TG 443) resulted in a reduced reproduction index (= percentage of pregnant females) for P0 animals at the high dose (1000 mg/kg bw/day; 67 % vs. 91 % in controls) and Cohort 1B animals at the mid (300 mg/kg bw/day; 80 % vs. 95 % in controls) and high dose (56 % versus 95 % in controls). Cohort 1A animals were not mated to produce a second generation. Obviously, the decrease in fertility of treated animals originates from an alteration of reproductive function of the females, because (P0) males exposed to TBPEH (1000 mg/kg bw/day), which did not fertilise their P0 females of the main treatment group, mated successfully with non-treated females.
Further relevant findings included a dose-dependent increase of irregular oestrus cycling in P0 females during the two last weeks of the ten-week pre-mating period (54 % at 1000 mg/kg bw/d (statistically significant), 63 % at 300 mg/kg bw/d, 79 % at 100 mg/kg bw/d with regular cycle vs. 83 % in controls). Furthermore, there was a dose-dependent decrease in the number of females with days in prooestrus (3 days in controls, 2.6 days at 100 mg/kg bw/d, 2.5 days at 300 mg/kg bw/d, and 1.5 days at 1000 mg/kg bw/d; statistically significant at the high dose). The number of females in prolonged oestrous cycle was also higher than in the control group at 1000 mg/kg bw/day (29 % vs. 0 %). In F1 females (Cohort 1A), the number of female animals in prolonged oestrous cycle was slightly higher than in the control group at 1000 mg/kg bw/day (15 % versus 0 %), relative to controls.
P0 females at 1000 mg/kg bw/day showed a (statistically significant) decreased number of developing follicles and an increased number of follicular atresia compared to controls (mean number of follicular atresia: 13.2 vs. 5.3 in controls upon quantitative evaluation

of ovaries). The same effects were seen in F0 females not achieving pregnancy (but not in female animals in Cohort 1A and Cohort 1B).
Effects occurred in the absence of severe maternal toxicity and were considered treatment-related. Mean body weight and body weight gain were comparable in the control and test item-treated females at 100, 300 and 1000 mg/kg bw/day during the pre-mating, gestation and lactation periods. Human data for TBPEH on reproductive toxicity were not available.
Other relevant studies performed with TBPEH, including a reproduction/developmental toxicity screening test (OECD TG 421) and 90-day oral toxicity study (OECD TG 408), did not result in adverse effects on female fertility and/or oestrus cycling. However, exposure during pre-mating is much shorter during the screening study (14 days) and lower doses were used during the 90-day oral toxicity study (≤ 450 mg/kg bw/d) compared to the EOGRTS (exposure for ten weeks pre-mating, ≤ 1000 mg/kg bw/d).
Altogether, the DE-CA supports classification of TBPEH as Repr. 1B for fertility.
Some quantitative data and findings in the lower dose groups that are relevant for an assessment of the reproductive toxic properties of TBPEH were reported in Annex I, but not in the dossier. To support the relevance of classification it is recommended to add these data in the dossier (e.g. dose-dependent increase of irregular oestrus cycling and dose-dependent decrease of days in prooestrus, etc.).

Adverse effects on development
In a reproduction/developmental toxicity screening test in rats (OECD TG 421), oral (gavage) exposure of TBPEH at 1000 mg/kg bw/d (high dose) resulted in a statistically significant increase in post-implantation loss (no numerical value available). Furthermore, post-natal loss was statistically significantly increased (5/10 dams were affected) with a reduction of live pups (until day 4 post-partum). Pup mean body weight was reduced at the high dose (up to day 4 post-partum). Mean body weight of the dams at 1000 mg/kg bw/d was slightly increased during gestation, while their mean body weight gain was statistically significantly decreased during lactation. During the last two days of gestation or the first two days of lactation, seven dams (gestation) and four dams (lactation), respectively, were noted periodically to have ruffled fur and/or a generally bad condition.
In an EOGRTS (OECD TG 443), oral (gavage) administration of TBPEH in rats resulted in an increased post-natal mortality at the high dose (1000 mg/kg bw/d; observed from PND 0, 0 (2 % versus 0 % in control of F1 pups; 12 % versus 0 % in F2 pups). Reduction of body weight in both F1 and F2 offspring relative to controls was observed (-6.5 % for F1 pups; -5 % for F2 pups on PND 0) at the high dose (1000 mg/kg bw/d). There were also effects on post-natal development regarding absolute, but not the normalised anogenital distance (in F1 males and females and F2 males), as well as on eye-opening at 1000 mg/kg bw/d. Mean body weight and body weight gain were comparable in the control and test item-treated female animals at 1000 mg/kg bw/day during the pre-mating, gestation and lactation periods.
In a prenatal developmental toxicity study (OECD TG 414) in rats, using 200, 400 and 1000 mg/kg bw/day of TBPEH, a slight but statistically significant reduction in mean body weight of male and female foetuses was observed. Furthermore, a significantly increased incidence of skeletal variations (delayed ossification of skull and metacarpal/metatarsal bones) at 1000 mg/kg bw was observed. There were no dose-related differences in the corrected body weight and corrected body weight gain of the dams in the experimental groups.
In a second study according to OECD TG 414 in rabbits, abortions occurred at the mid (100 mg/kg bw/d) and the high dose (300 mg/kg bw/d). Moribund animals and clinical signs were also reported. Mean body weight of the animals was lower until GD 24 and body weight gain decreased during GD 6 - 12 and GD 15 - 18. However, there was no statistically significant effect when corrected body weight and body weight gain were

considered. Food consumption was reduced up to GD 21. An increase of early embryonic death (15 % vs. 6 % in control) and post-implantation loss (17 % vs. 8 % in control) at 300 mg/kg bw was observed. Dose-related statistical significance ($p > 0.01$) was observed in the 300 mg/kg bw/day group due to an increased incidence of delayed ossification of proximal and middle phalanges in association with lower foetal body weight (-15.6 %) and crown-rump length (-7.3 %).
Effects on development observed in all available studies on toxicity to reproduction conducted with TBPEH include reduction in mean body weight of the foetus/offspring, post-natal mortality and delayed ossification (OECD TG 414 with rat and rabbit), all at the respective highest doses tested (at 1000 mg/kg bw/d in rats and 300 mg/kg bw/d in rabbits). Post-implantation loss was seen in a reproduction/developmental toxicity screening test in rats at 1000 mg/kg bw/d (OECD TG 421, high dose 1000 mg/kg bw/d; no numerical values available), however not confirmed in the EOGRTS in rats (high dose 1000 mg/kg bw/d), in which a much longer exposure during pre-mating occurred. Also, the relevance of delayed ossification as a skeletal variation should be discussed for classification.
There is only little evidence of maternal toxicity in the studies in rats. In rabbits, strong maternal toxicity was described, with an increased number of abortions and moribund animals occurring above the intermediate dose.
It is noted that the study descriptions lack a number of quantitative data necessary for an assessment of the reproductive toxicity on development of TBPEH. A more detailed reporting in the dossier would be appreciated. It is recommended to evaluate the effects more thoroughly (e.g. of maternal toxicity, post-natal loss, etc.) to show why a classification for developmental toxicity is warranted.

The German CA agrees that based on the available data a classification on or via lactation is not warranted for TBPEH.

Dossier Submitter's Response

Thank you for your supporting Repro. 1B for fertility.

Regarding your comment to add complementary information in the CLH report, Dossier Submitter (DS) reminds that CLH report is not revised after public consultation. As mentioned in the comment, the information is already provided in Annex I. Thus, RAC will have access to these data.

Classification for development:
DS acknowledges that the level of details are limited for the OECD 421 study since there was no access to the full study report but only to the disseminated study summary. However, information available supports developmental effects (in utero and post natal letality). Regarding parental toxicity, no mortality was observed but only a decreased body weight that cannot be adequately interpretated in regards to developmental effect without numerical value.

Results of EOGRTS show clear effects on development at the high dose: post-natal mortality (max 15% versus 3% in control), lower BW (max -13%) and delayed development (surface righting reflex, pinna detachment, eye opening and anogenital distance). These effects are consistent between F1 and F2 offsprings and more pronounced in F2 generation. Effects on parents are detailed in section 11.8.2 and all numerical data are available in Annex I. At the high dose, there was no mortality, body weight was slightly reduced (< 10%) but only for adult cohort 1 (not F0) and there was no histological lesion. Considering the severity and consistency of the developmental effects in the light of the very low maternal toxicity, developmental effects cannot be considered as a consequence of parental toxicity. Although, there is no clear mode of

action for the delayed development, it has to be noted that a decrease of T3/T4 is consistently found in adults of F0 and F1 generations, suggesting that the retardation on post-natal development might be linked, at least in part, to T3/T4 decreases.

Data from OECD 414 study in rat : ossification delays are reversible and do not support a classification. They might be linked with the decrease of thyroid hormons, as reported in EOGRTS.

Data from OECD 414 study in rabbit : increases of early embryonic death and post-implantation loss were reported at the highest tested dose of 300 mg/kg/d. On the one hand, there are 4 moribund females at this dose so an excessive maternal toxicity can be raised and on the other hand, there is no decrease in corrected maternal body weight, which is not in line with an excessive maternal toxicity. Concerning the observed abortions, it is difficult to say if they are due to maternal toxicity or a disruption in development (characterised by foetal mortality). The consistency of fetal mortality in 3 studies (OECD 421, EOGRTS and OECD 414) supports a direct developmental effect rather than a consequence of maternal toxicity

Overall, there are clear and severe developmental effects reported in 3 different studies that cannot be linked to maternal toxicity, justifying a classification as Repr. 1B for development.

RAC's response

The comment and the response of the DS (in particular regarding the evaluation of the rabbit PNDT study) are noted and the issues are reflected in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2022	Germany	United Initiators GmbH on behalf of the Organic Peroxides Consortium	Company-Manufacturer	5

Comment received

see attached

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to CLP Proposal TBPEH_2022-04-28.pdf

Dossier Submitter's Response

Dossier submitter considers that developmental toxicity is clear from the **OECD 421 study** based on the following:
- post implantation loss = 4.9% versus 1.5% in the control group (it is doubled)
- early resorptions and dead foetuses = 4% versus 0.3% in the control group
- late resorption = 2.3% versus 0.3% in the control group
- viable foetuses = 7.2% versus 13.2% in the control group.

The comment mentions that there is no dose response and a limited statistical analysis (10 animals/sex). However as reported above, there is a marked effect (> 2 times the values of the control group) at high dose that cannot be ruled out. Moreover, the argument indicating that the "protocol study that exclusively 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" cannot be used to dismiss the effect observed. Indeed, the

fact that these effects are observed in a study with a low statistical power support a clear developmental toxicity (severe and with high incidence).

The comment also underlines that the DRF study for the OECD 421 did not report similar effect on post-implantation. However, it has to be noticed that, in contrast, similar effects are reported in the prenatal developmental toxicity study in rabbits.

The comparison with historical control data (HCD) is not considered appropriate by DS. For example, there are no detailed values for the reference to MARTA HCD which moreover are not contemporary to the OECD 421 study. In addition, table 2 provided cannot be properly interpreted since there is no information on the date of performance of these HCD and no details on how much studies are compiled.

Concerning the comments on decreased body weight of pups in the OECD 421 study, the numerical mean values at birth provided shows a decrease being higher than 10% at the highest tested dose. This would support a biologically significant effect even if the values are inside the CRL HCD (Edimbourg) mentioned. The relevance of these HCD cannot be properly interpreted based on the link provided: the type and number of studies considered are not clear and the dates of the HCD are too wide (1996-2018) and thus not completely contemporary (within 5 years) to the study with TBPEH.
Moreover, DS wants to underline that the decreased body weight of pups are reported in the EOGRTS in rats (up to – 14%) and in the prenatal developmental toxicity study in rabbits (-15.6%).

One comment indicates that mother's nutritional status is altered at 1000 mg/kg bw/d (decrease in food consumption in dams) and could lead to the increase post-implantation loss. However, the decrease in food consumption is only observed during the first week of the pre-pairing period and during lactation but not during mating and gestation. Furthermore, it has to be noted that the mean body weight of parents are not adversely impacted during mating and gestation.

Overall the interpretation of all data concerning the influence of maternal behaviour on the altered developments of pups rather point to developmental effects as the cause of high post-implantaion losses.

Finally, it is to be reminded that the proposed classification for developmental effect is not only based on the OECD 421 study but based on consistent effects reported in 3 different studies with 2 species (OECD 421 study and EOGRTS in rats and prenatal developmental toxicity study in rabbits).

OECD 414 study in rats: DS acknowledges that theorically the delay in ossification may be secondary to maternal toxicity. However, there is no sign of toxicity in dams, as reported by mortality, clinical signs, corrected body weight and at necropsy. In particular, DS emphasizes that even if the body weight is reduced in dams, the corrected body weight is normal, so there is no direct maternal toxicity. Moreover, as indicated in the response to comment 4, this may be related to an alteration of T3/T4, as observed in the EOGRTS.
The reference to Charles River HCD (Horsham) provided in the comment is not usable as the type and number of studies considered are not clear and the dates of these studies are too wide (1996-2018) and thus not completely contemporary (within 5 years) to the study with TBPEH.

OECD 414 study in rabbits: the lower fetal weight is not to be considered as marginal in rabbits since the decrease is higher than -10%. The statement that the lower foetal body weight is within HCD is made without any further reference.

See also response to comment 4 for maternal toxicity.

Finally, DS wants to remind that the proposed classification for developmental effect is not only based on the OECD 414 study but based on consistent effects reported in 3 different studies with 2 species (OECD 421 study and EOGRTS in rats and prenatal developmental toxicity study in rabbits).

Results of EOGRTS:
Concerning the lower body weight in rats at high dose, the comparison to HCD is made without any reference.

The effect on eye opening, surface righting reflex and pinna detachment, are adverse neurodevelopmental effects. As indicated in response to comment 4, this may be related to an alteration of thyroid hormones.

In the comment, there is no reference to the higher post-natal mortality that is considered as a clear developmental effect induced by TBPEH exposure.

The effects observed such as decrease in viable foetuses, post-natal delayed development and decrease in body weight of foetuses may also be due to:
-altered mammogenesis / lactogenesis
-behavioral alteration linked to the nutritional quality of maternal milk and /or the suckling capacity of pups
-maternal metabolic disturbances
However, there are no measurements of quantity and quality of milk to support this potential mode of action.

Therefore, based on the overall dataset for TBPEH, the DS maintains that the proposed classification for Repr. 1B for development is justified.

RAC's response

(Following the summary of the comments received, RAC's responses are in italics)

The commenting party concluded that the findings do not justify classification as Repr. 1B for developmental toxicity due to the following reasons:

OECD TG 421

- Comment: Regarding the OECD TG 421 study, the commenter emphasised that the observed increase in post-implantation loss was only seen at the high dose (4.9%/litter) and did not follow any dose-response relationship. In fact, this parameter was lower in the low (1%/litter) and mid dose (1.3%/litter) when compared to the control group (1.5%/litter). Similarly, no-dose-response was observed in the corresponding DRF study.
  RAC response: RAC noted the lack of a dose-response with respect to the parameter post-implantation loss at the low and mid doses (100 and 300 mg/kg bw/d, respectively). Nevertheless, an increase in post-implantation loss seen only at 1 000 mg/kg bw/d can still be indicative of a substance-related adverse effect.

- Comment: It was further suggested by the commenter that the statistical analysis is rather limited in the case of an OECD TG 421 study, as only a limited number of animals are evaluated (n=10 parental animals) and variability is rather high.
  RAC response: RAC notes that the selected numbers of animals per group are in agreement with the provisions given in the OECD TG 421 to produce sufficient pregnant females and offspring to assure meaningful evaluation. It should also be mentioned that the performed statistical analyses are appropriate for a sample size of 10. The results of these analyses are, thus, considered reliable. The OECD TG 421 study states in this context (paragraph 6): "Due (amongst other reasons) to the relatively small numbers of animals in the dose groups, the selectivity of the end points, and the short duration of the study, this method will not provide evidence for definite claims of no effects". However, "positive results are useful for initial hazard assessment and contribute to decisions with respect to the necessity and timing of additional testing". Thus, the study can be used when evaluating the reproductive toxicity of a substance. As this is not the only study available, the number of animals in the study is not a reason to disregard the findings.

- Comment: The commenter refers to an average post-implantation loss in natural delivery studies of 6% and 8.8%.
  RAC response: RAC notes that the source and details of these historical data are unknown, also the cited publication (Marta and MTA, 1996). Whether it is related to the same rat strain used at the laboratory in an acceptable time period of 3-5 years before the study was conducted is uncertain. Published data from 1996 are unlikely to be relevant for a study published in 2008.

- Comment: The commenter provided historical control data (HCD) for the parameters total post-implantation loss (10.6 %/litter [5.8–14.1 %/litter]), early and late resorptions (8.2 %/litter [5.8–11.3 %/litter] and 1.1 %/litter [0.0–1.4 %/litter], respectively), dead and viable foetuses (1.3 %/litter [0.0–4.8 %/litter] and 10.2 %/litter [9.2–12.0 %/litter], respectively) and indicated that "there were no significant differences in the numbers of successful implantations between the historical controls and treatment groups".
  RAC response: RAC notes that the there are no details available on the source these data and whether the provided HCD on "HCD [Hsd. Brl. Han: WISTAR Rat]" stem from the same laboratory and time period as the OECD TG 421 study at hand. However, as both the OECD TG 421 and the OECD TG 443 study results are compared to the same HCD, it is assumed that the provided HCD are obtained from other sources/laboratories and may not be as reliable as indicated by the commenter. In addition, the parameters late resorptions and viable foetuses in the OECD TG 421 study are outside of the provided HCD ranges (late resorptions: 0.0-1.4 %/litter in provided HCD versus 2.3 %/litter in OECD TG 421; viable foetuses: 9.2-12.0 %/litter in provided HCD versus 7.2 %/litter in OECD TG 421).

- Comment: The commenter further emphasised that the "20 missing pups" at the high dose were not missing for reasons related to treatment and this means that "if these 20

missing pups have been used to evaluate the post-implantation loss, it is likely that the estimation of the post-implantation loss has been overestimated".
RAC response: RAC notes that in the Annex to the CLH report, as well as in the information obtainable on the ECHA website, it is reported that the missing pups at PND4 were "considered most likely test item related" and corresponded to the noted clinical signs and symptom in the dams. Therefore, RAC considers the inclusion of these missing animals in the statistical analysis appropriate. Missing pups could be due either to post-implantation losses or to the number of dead pups as mothers may show cannabilistic behaviour after birth of dead pups.

- Comment: The commenter, in addition, highlighted that "there was a complete absence of malformations" in the pups of the OECD TG 421 study.
RAC response: RAC notes that an OECD TG 421 study can be used "to provide initial information on possible effects on reproduction and/or development, either at an early stage of assessing the toxicological properties of chemicals, or on chemicals of concern". It, however, "does not provide complete information on all aspects of reproduction and development. In particular, it offers only limited means of detecting post-natal manifestations of pre-natal exposure, or effects that may be induced during post-natal exposure" (OECD TG 421, paragraph 6). For a detailed evaluation of pup development, including the assessment of potentially observed malformations, an OECD TG 414 is to be considered the study with the most appropriate study design.

- Comment: Moreover, it was stated by the commenter that the OECD TG 421 study was not performed in accordance with GLP.
RAC response: On the contrary, RAC notes that the provided information in the Annex of the CLH dossier, as well as the information obtainable on the ECHA website, in fact indicates that the study was very well performed in compliance with GLP.

- Comment: Overall, the commenter considered that the increase in post-implantation loss seen in the OECD TG 421 study does "not indicate an exquisite effect on morphogenesis and suggest that the maternal homeostasis and nutritional status may have resulted in this subsequent insult to the products of conception". The commenter was further of the view that "this finding cannot therefore be utilised to derive classification for the proposed deleterious and direct effects on prenatal development". In addition, the commenter stated that even ANSES has made the interpretation that "regarding the increase of post-implantation loss, this is rather considered as a developmental effect but it cannot be excluded that it is secondary to fertility alteration". The commenter, therefore, considers that in order to determine the real effects on prenatal development, other more relevant studies shall be considered and "not the apparent effects from an insufficient study whereby the evaluators have already determined the post-implantation loss to be secondary to a fertility alteration".
RAC response: RAC notes, as ANSES already reported in their evaluation, that the results of the OECD TG 421 study by themselves are for this substance not sufficient to draw a conclusion on classification for developmental toxicity. The data, however, support the concern that is consistently reported in all available and relevant studies and are therefore used as supporting information.

- Comment: It was further stated by the commenter that the CLH proposal noted a reduction in mean pup body weights at the top dose of 1000 mg/kg bw/d; however, a separate analysis of mean pup body weight by the commenter revealed no significant difference in any of the treatment groups. The difference in pup body weight in the top dose of 1000 mg/kg bw/d was considered slight (ca. 12% lower than controls) and without a clear dose-response relationship. Moreover, it was stated that the values were within the provided HCD range for the breed (Wistar rats) (CRL, Edinburgh). Thus, this effect on foetal body weight was considered to be a not adverse but rather transient effect, not adding to the weight of evidence for classification.
  RAC response: RAC notes that according to the Annex to the CLH report, no HCD was available for the OECD TG 421 study.

- Comment: The commenter stated that treatment at 1000 mg/kg bw/d was also associated with decreased food consumption in males and females during the first week of the pre-pairing period and during lactation, arguing that there may have been a fertility alteration but that direct prenatal developmental toxicity has not taken place.
  RAC response: RAC considers that the lack of mortality, a slight reduction in food consumption limited to the first week of the preparing week and the observation of slightly increased body weights during the gestation phase do not indicate parental toxicity. Clinical signs (ruffled fur/general bad condition) was observed in 7 dams only during the last two days of gestation. The reported clinical signs may indicate some general toxicity in dams that could either be indicative of maternal toxicity or be related to missing/dead pups on PND0. As no indication of maternal toxicity was observed before the two last days of gestation, it is considered as more likely that the bad conditions are due to stillbirths/dead pups. This assumption is supported by the conclusion by the study authors. As reported in the Annex to the CLH report, the missing pups at PND4 were "considered most likely test item related" and corresponded to the noted clinical signs and symptoms in the dams.

- Comment: The commenter stated that there was also a lack of similar effects on post-implantation loss in the dose range-finding study at the high dose level (1000 mg/kg bw/d).
  RAC response: RAC takes note of the results. As no details on the study design regarding number of animals and duration of treatment and no full study summary/report are available, the outcome of the DRF is of limited relevance for the interpretation of the main study.

- Comment: In the commenter's view "the presence of significant maternal toxicity signals that the healthy mother has been affected during the pre-pairing and lactation periods and this can be evidenced by the clinical signs present as well as decreased food consumption that has led to slight post-implantation loss so that the affected mother could protect the foetuses secondary to an alteration of her physiological balance and therefore fertility (see effects on p53-stress response gene related to systemic toxicity)".

RAC response: Regarding the lactation period (females were sacrificed on Day 4 post partum) RAC notes that effects during this period are not relevant for the prenatal period.

OECD 414 Prenatal Developmental Toxicity Study in Han Wistar rats (2013)

- Comment: The commenter considered that there were no lesions that indicated direct prenatal developmental toxicity from TBPEH. TBPEH did not increase the incidence of external and visceral variations significantly and caused no skeletal malformations.
  RAC response: RAC has noted the effects on pup weights and the significant increases in skeletal and visceral variations.

- Comment: In the commenter's view, TBPEH did not reveal any adverse effect on the pregnancy, the intrauterine mortality of the conceptuses, the number of viable foetuses or their sex distribution. However, it did show statistically significantly ($p<0.01$) reduced maternal body weight gain on the first three days of treatment and statistically significantly ($p<0.01$) reduced maternal food consumption in the first week of treatment in the 1000 mg/kg bw/d dose group. This decrease in maternal net body weight is an indicator of maternal systemic toxicity.
  RAC response: RAC finds it debatable whether a transiently reduced food consumption as a stand-alone finding (not as a consequence of bad general health status or moribund condition of the dams) should be considered an indication of systemic toxicity. The effect has to be taken into consideration, but here it is not a sign of maternal toxicity. According to the study report, as summarised in the Annex to the CLH report, there are no differences in the absolute body weights throughout the study in comparison to the control values and no other indications of toxicity, including clinical signs.

- Comment: The commenter stated that the decreased maternal body weight gain during the late gestational period is linked with reduced skeletal mineralisation. Thus, the lower mean body weight (19 grams on GD 5-11) and the increased incidence of foetuses with delayed ossification of skeletons (7-13%) in the 1000 mg/kg bw/d group might, according to the commenter, be attributed to the statistically significantly reduced body weight gain and the affected food intake between GD 5 and 11 of the dams at this dose level. This slight delay in ossification at this highest dose level of 1000 mg/kg bw/d was not considered adverse when the underlying cartilaginous structures are present and properly articulated, and considered a background finding. The values are also within expected HCD findings (up to 16% for incomplete ossification) (CRL, Horsham; Mylchreest et al., 2005). These findings imply a less targeted, more generalised response.
  RAC response: RAC notes that the maternal body weight gain was only reduced during GD5-8 and was increased on GD8-11. No effect on corrected body weight and body weight gain was seen at the end of the study. Although it cannot be excluded that the lower food consumption and body weight gain during the first days of treatment may have an effect on pup weight, a late gestational effect on maternal body weight gain is not evident. Thus, a link to the skeletal ossification during the late gestational period is unlikely. The study report did not include data on historical controls of the laboratory on

the same rat strain in a time range of about 5 year around the study conductionn (study report from 2013). The reference to HCD on delayed ossification in the publication of Mylchreest et al., 2005 is not relevant. The authors referred to a secondary source of data (Haskell Laboratories from 1995 to 2002) without details on the rat strain and numbers of animals and sex.

OECD 414 Prenatal Developmental Toxicity Study in NZ White rabbits (2018)

- Comment: There were no lesions that indicated direct prenatal developmental toxicity from TBPEH. The visceral development of the foetuses was not affected. There was only slightly lower foetal weight (mean of 29.71 g), which is within the HCD range of 40.5 (33.6–44.6) for NZW rabbits.
  RAC response: The relevance of the HCD data is not known to RAC. No details are given in the Annex to the CLH report.

- Comment: The CLH proposal stated that 'early embryonic death (15% versus 6% in control) and post-implantation loss (17% versus 8% in control) (data compared to number of implantations) were increased at 300 mg/kg bw/d. These effects are statistically significant if the number and percent of resorptions are evaluated, but more importantly these findings are found to be not statistically significant if the mean number and SD are calculated.
  RAC response: RAC finds the data here on mean numbers less informative. The non-significancy is likely to have resulted from high standard deviations (SD). Nevertheless, the mean post-implanation losses per litter are markedly higher (33.4 %, SD: 44.34) than in the control litters (7.4 %, SD: 10.2).

- Comment: The commenter acknowledged that it can be seen that the mean number of viable foetuses were decreased (sum: 91 viable foetuses in treated group versus 191 in controls; not statistically significant) in the 300 mg/kg bw/d dose group. However, abortion, post-implantation loss, and reduced foetal body weight observed in the 300 mg/kg bw/day group as well as abortion of two does at 100 mg/kg bw/day can be considered as secondary to the markedly reduced food consumption (means of 43.1 g at GD6-9, 52.2 g at GD9-12, 43.3 g at GD12-15 and 32.5 g at GD18-21).
  RAC response: RAC has included a discussion on the reduced food consumption in the CLH opinion.

- Comment: The commenter pointed to the finding that significantly reduced food consumption was observed from start of the treatment up to GD21 in the 300 mg/kg bw/d dose group ($p<0.01$ GD6-18 and $p<0.05$ GD18-21). The majority of the animals with total post-implantation loss at 300 mg/kg bw/d had minimal or zero food consumption during the in-life phase and the post-implantation loss observed can be attributed to the low food consumption seen and based on individual data.
  RAC response: RAC did not have access to the individual animal data.

- Comment: In the commenter's view, the body weight reductions of up to 6% in final body weight of females at 300 mg/kg/d are linked to the slight increase in post-

implantation loss. This indicates that the marginal increase of early embryonic death (15% versus 6%) and post-implantation loss (17% versus 8%) were linked to body weight changes and reduced food consumption, rather than a direct prenatal developmental effect. As no other signals of direct prenatal developmental toxicity exist in the studies, disruption of maternal homeostasis and nutritional status (and thus systemic toxicity to the mother) is seen here and this is the most likely cause of the post-implantation loss observed.
RAC response: One should be aware that a reduction of terminal body weight (uncorrected) may also be the result of pre- and post-implantation losses. A lower food consumption alone, to a certain extent, will not cause post-implantation losses. In this study there was a significant and markedly lower food consumption during GD6-21 that is linked to lower body weight gain from GD6-18 and lower body weight from GD9-24. The corrected body weight was comparable to the controls.

- Comment: The commenter considered the effect on crown-rump length observed at the highest dose of 300 mg/kg bw/d as well as the increase in skeletal variations, mainly delayed ossification, to be linked to the lower body weights and likely secondary to the severe maternal toxicity seen. They thus considered that direct prenatal developmental toxicity did not take place. According to the commenter, it can be evidenced that the effects on the dams are relevant as maternal body weight (means of -238.8 g at GD6-9, -85.8 g at GD9-12 and -101.3 g at GD15-18) and food consumption were reduced and clinical findings (GI tract findings, vaginal bleeding, lying down, weakness, reduced activity and sneezing) occurred in parallel at 300 mg/kg bw/d. The commenter pointed out that these are severe clinical signs of maternal systemic toxicity. They stated that this indicates that there is no exquisite effect on morphogenesis to the offspring and suggested that the mother is more sensitive at this high dose. In conclusion, the slight effects on development were in most cases not statistically significant and only seen at marked maternal toxicity, which does not justify a classification as Repr. 1B (development).
  RAC response: On the last points, please note the assessment of the findings in this rabbit study as laid down in the RAC opinion.

OECD TG 443 (EOGRTS) including cohorts 1A and 1B in Han Wistar rats (2020)

- Comment: In the EOGRTS, a lower body weight was also reported in pups at 300 mg/kg bw/d. However, the mean number of post-implantation losses, the mean number of total births, mean number of viable and live born pups as well as the live birth index (live pups/total births) were comparable in all groups. Delivery data of dams were not adversely affected at doses of 100, 300 or 1000 mg/kg bw/d (P0 and Cohort 1B). The number of F1 offspring with reduced body weight on PND0 (-6.5%) and body weight gain was suppressed on PND0-21 (-8.6%). The lower foetal weight was within the laboratory's HCD range for the breed (10.77 g), and therefore, this effect on foetal body weight was not considered adverse by the commenter. They argued that this indicates that the foetuses weighing 10.77 g at birth are able to survive and thrive and that the weight reduction was likely to be temporary.

RAC response: RAC agrees with the DS's conclusion that treatment-related effects on pup viability and body weight were seen at 1 000 mg/kg bw/d. Data on HCD were not available to RAC.

- Comment: The anogenital distance of each pup was determined on PND4. The anogenital distance was normalised to the cube root of the body weight. The individual body weight of pups was determined with an accuracy of 0.01 g on postnatal day 4. In the view of the commenter, the anogenital distances were not adversely affected in either male or female offspring at 100, 300 and 1000 mg/kg bw/d. Statistical significance was only detected at the shorter absolute anogenital distance of male and female pups at the highest dose of 1000 mg/kg bw/d. Once this data was normalised, the anogenital distances were found to be comparable with the control both in male and female offspring at 1000 mg/kg bw/d. Eye opening usually occurs within PND11 to 18 in rats, with the average acquisition around PND 13 to 14. The F1 offspring with a negative response for eye opening in the control, 100, 300 and 1000 mg/kg bw/d dose groups were found to be 42, 31, 32 and 69%, respectively. However, a negative response for eye opening was only shown to be statistically significant (69%) on PND14 at the highest dose of 1000 mg/kg bw/d (compared with 42% in the concurrent control group). According to the commenter, this finding implies a less targeted, more generalised response, and is not indicative of direct prenatal developmental toxicity. Thus, the effects noted in the high dose group are sufficiently covered by a classification with Repr. 1B (fertility) and an additional classification for development is not required.
  RAC response: On the last two points, RAC noted that the indications on a delay in the development of pups could be secondary to reduced body weight. For RAC the major effects relevant for the classification for development are the effects on viability, body weight and growth.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2022	Estonia		MemberState	6
Comment received				
The MSCA agrees with the DS proposal on classification of TBPEH as Skin Sens. 1B – H317 (May cause an allergic skin reaction). In the acceptable Buehler test (OECD 406), 26% animals responded following a topical induction with 25% TBPEH. Therefore, according to CLP classification criteria (≥ 15 % responding at > 20 % topical induction dose), classification as Skin Sens. 1B – H317 (May cause an allergic skin reaction) is warranted for TBPEH.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON TERT-BUTYL 2-ETHYLPEROXYHEXANOATE

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2022	Germany		MemberState	7
Comment received				
The classification of TBPEH as skin sensitising is supported. The skin sensitisation potential of TBPEH was investigated in a Buehler test (OECD TG 406). Topical induction with 25 % and challenge with 5 % TBPEH resulted in dermal reactions in 9/19 (47 %) test animals at the 24 h reading and 3/19 (16 %) test animals at the 48 h reading. However, positive reactions were also detected in 3/10 (30 %) control animals after 24 h and in 2/10 (20 %) after 48 h. After re-challenge with 2 % TBPEH, 5/19 (26 %) of the test animals showed positive reactions after 24 h and there were no reactions in the control animals. OECD TG 406 recommends performing a pre-test to determine the highest concentration that causes mild irritation. This concentration should be used for topical induction. The concentration used for the challenge exposure should be the highest non-irritating dose. It is reported that a pre-test was performed; however, the results were not specified. The number of negative control animals showing a positive reaction after challenge was quite high (3/10 animals). The Dossier Submitter may consider clarifying whether this weakens the value of the positive reaction triggered by TBPEH. It is presumed that a dose of 5 % of TBPEH as the challenge concentration was too high as it resulted in positive reactions in test animals, but also control animals, thereby decreasing the reliability of the results (not reliable). Based on the results from the re-challenge with 2 % TBPEH (with ≥ 15 % (26 %) of animals responding at > 20 % (25 %) TBPEH for topical induction), the criteria for classification of TBPEH in Category 1B are fulfilled. According to the CLP Regulation (Table 3.4.3), doses for topical induction ≤ 0.2 % resulting in ≥ 15 % responding animals in the Buehler test warrant classification in Category 1A. However, doses of TBPEH ≤ 0.2 % for topical induction were not tested and therefore a strong potency of TBPEH cannot be excluded. Therefore, a classification of TBPEH in Category 1 without sub-categorisation should be considered.				
Dossier Submitter's Response				
Thank you for your support. Concerning the comment on the positive reactions in control animals: DS considers that the results are still reliable. Positive reactions in controls are only observed after topical induction and challenge but not after re-challenge. In contrast, positive reactions in treated animals remain consistent after challenge and re-challenge. Considering the low sensitivity of the Buehler 3 inductions and the dose used for topical induction, DS agrees that a stronger potency of TBPEH cannot be completely excluded. Subcategorisation would be further discussed at the RAC level.				
RAC's response				
The RAC opinion discusses the subcategorisation.				

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2022	Germany	United Initiators GmbH on behalf of the Organic Peroxides Consortium	Company-Manufacturer	8
Comment received				
see attached				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON TERT-BUTYL 2-ETHYLPEROXYHEXANOATE

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Response to CLP Proposal TBPEH_2022-04-28.pdf
Dossier Submitter's Response
Thank you for your support.
RAC's response
Noted.

PUBLIC ATTACHMENTS

1. Response to CLP Proposal TBPEH_2022-04-28.pdf [Please refer to comment No. 2, 5, 8]