Committee for Risk Assessment

RAC

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

1 December 2022

CLH-O-0000007217-74-01/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: ***tert*-butyl 2-ethylperoxyhexanoate**

EC Number: **221-110-7**

CAS Number: **3006-82-4**

The proposal was submitted by **France** and received by RAC on **11 January 2022.**

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

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **28 February 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **29 April 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Agnes Schulte**

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 **1 December 2022** 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	No current Annex VI entry										
Dossier submitter's proposal	TBD	*tert*-butyl 2-ethylperoxyhexanoate	221-110-7	3006-82-4	Repr. 1B Skin Sens. 1B	H360FD H317	GHS08 GHS07 Dgr	H360FD H317			
RAC opinion	TBD	*tert*-butyl 2-ethylperoxyhexanoate	221-110-7	3006-82-4	Repr. 1B Skin Sens. 1	H360FD H317	GHS08 GHS07 Dgr	H360FD H317			
Resulting Annex VI entry if agreed by COM	TBD	*tert*-butyl 2-ethylperoxyhexanoate	221-110-7	3006-82-4	Repr. 1B Skin Sens. 1	H360FD H317	GHS08 GHS07 Dgr	H360FD H317			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Tert-butyl 2-ethylperoxyhexanoate (hereafter abbreviated as TBPEH) is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 1 000 to < 10 000 tonnes per annum.

This substance is used in polymers and plastic products by consumers, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing (ECHA, 2021).

The industrial uses reported are the following:

- Industrial use of organic peroxides as polymerisation initiators, cross linking agents or curing agents
- Other industrial uses of organic peroxides
- Use of reactive processing aid at industrial site (no inclusion into or onto article)
- Industrial use of chemicals for polymer processing
- Industrial use of coatings and paints
- Industrial use as polymerisation initiator and cross-linking agent
- Use of reactive process regulators in polymerisation processes at industrial site (inclusion or not into /onto article)

On the ECHA website, no public registered data are noted for widespread uses by professionals but various PROCs are indicated in the registered dossier, with PROC 5, 8a, 8b, 9, 10, 11, 13, 15, 19 and 28 (ECHA dissemination, 2021).

Regarding consumer uses, the substance is according to publicly available data on the ECHA website used in adhesives and sealants (PC1), coating and paints, thinners, paint removers (PC9a) and fillers, putties, plasters, modelling clay (PC9b) (ECHA dissemination, 2021).

The lead registrant and members of the Organic Peroxides Consortium stated in their comments during consultation, that they don't support that there are any consumer or professional uses for TBPEH, and that it is only used under industrial settings, without widespread use.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The dermal sensitisation potential of TBPEH was evaluated in a Buehler assay according to OECD TG 406 (Unnamed, 1996, Klimisch 2). Ten male and ten female Guinea pigs were topically treated with 25 % w/v TBPEH in mineral oil, once per week, for 3 consecutive weeks. Following a two-week rest period, challenge exposure with 5 % w/v TBPEH in mineral oil was performed. As the group mean dermal scores were similar in the test animals compared to the challenge control animals, a re-challenge exposure with 2 % w/v TBPEH in mineral oil was performed following another week of rest period. A valid positive control with hexylcinnamaldehyde was performed concurrently. The study had a reliability of 2 according to Klimisch score. According to the CLH dossier, following rechallenge with 2 % w/v TBPEH in mineral oil, dermal scores of 1 were noted in 5/19 (26 %) test animals at the 24-h scoring interval; however, the dermal responses did not

persist to the 48-hour scoring interval. Therefore, the Dossier Submitter (DS) considered that TBPEH (in mineral oil) was positive in the Buehler test (≥ 15 % to < 60 % animals responding at > 0.2 % to ≤ 20 % topical induction dose[1]) and proposed classification as Skin Sensitisation Category 1B for TBPEH.

Comments received during consultation

The lead registrant on behalf of the Organic Peroxides Consortium agreed with the proposed classification for Skin Sensitisation Category 1B.

The proposed classification of Category 1B was supported by one Member State Competent Authority (MSCA). Another MSCA proposed to consider Category 1 without sub-categorisation since for topical induction concentrations of TBPEH ≤ 0.2 % were not tested and therefore a strong potency of TBPEH cannot be excluded.

The latter MSCA pointed to the observation that positive reactions in 3/10 (30 %) control animals after 24 h and in 2/10 (20 %) after 48 h may be due to irritating properties at 5 % challenge concentration whereas no response in control animals was seen at 2 % concentration for re-challenge.

Assessment and comparison with the classification criteria

RAC agrees that the rate of 26 % responsive animals (5/19; dermal score of 1) at a 2 % w/v re-challenge concentration of TBPEH at the 24-h scoring interval warrants a classification for skin sensitising properties. It is noted that the dermal score of the remaining animals was 0 and observed responses in test animals did not persist until the 48-h scoring interval.

Based on the results from the re-challenge with 2 % w/v TBPEH, the criteria for Skin Sens. 1B are fulfilled with ≥ 15 % (26 % in the study) animals responding at > 20 % (i.e., at 25 % w/v TBPEH) topical induction dose. Based on these results and according to Table 3.8 of the Guidance on the Application of the CLP criteria (CLP guidance, ECHA, 2017), TBPEH is of moderate potency. However, as a topical induction concentration of ≤ 0.2 % TBPEH has not been tested, a higher grade of potency and thereby a classification as Skin Sens. 1A cannot be excluded. Thus, insufficient information is available to appropriately assess the sub-category. RAC concludes that **classification for Skin Sens. 1, H317 (May cause an allergic skin reaction), without a sub-categorisation** is warranted.

RAC evaluation of reproductive toxicity

ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY

Summary of the Dossier Submitter's proposal

For assessing the adverse effects on sexual function and fertility of TBPEH, the DS cited 4 studies:

[1] Note that the DS referred to (> 0.2 % to ≤ 20 %). In this context related to the induction concentration of 25 %, the range cited in brackets is not appropriate.

- A reproduction/developmental toxicity screening test according to OECD TG 421 (Anonymous, 2008; Klimisch 1) with TBPEH (purity not specified) which did not show clear effects on sexual function and fertility up to 1 000 mg/kg bw/d. Increases in post-implantation loss were reported, but the DS considered this rather as a developmental effect, although the DS could not exclude that it is secondary to adverse fertility effects. The DS indicated that no full study report was available.
- An Extended One-Generation Reproduction Toxicity Study (EOGRTS) according to OECD TG 443 (Anonymous, 2020; Klimisch 1) with TBPEH (purity not specified) in the rat according to OECD TG 443 (including production of a second generation), in which impairment of fertility characterised by a decrease of reproduction performance and reproductive index in parental (P0) animals and Cohort 1B animals was observed. A second mating showed that the male parental animals (P0), which did not fertilise the treated females of the same dose group, mated successfully with non-treated females, suggesting that the decrease in fertility of treated animals originates from an alteration of reproductive function of the females. Other findings that can be related to effects on sexual function and fertility included irregular oestrous cycles at the high dose in P0 animals, histopathological effects in ovaries in pregnant and non-pregnant P0 females (decreased number of developing follicles and an increased (mean) number of follicular atresia), a lower copulatory index in F1 males and females, and a slightly longer mean duration of pregnancy. No abnormalities were observed in male reproductive organs or on sperm counts, morphology, or motility.
  General toxicity was reported to be slight and particularly restricted to males exposed to TBPEH at 1 000 mg/kg bw/d. It consisted of lower (absolute) mean body weight (up to -13 %) mainly in males; increased relative organ weights of liver and kidneys in both parental generations, chronic progressive nephropathy observed only in P0 males (necropsy on day 153-156), higher urine volume and lower urine pH and reductions of thyroid hormones T3 and T4. At 300 mg/kg bw/d, there were changes in relative organ weights (histopathology not evaluated).
- A 28-d oral (gavage) study with TBPEH (purity not specified) in rats according to OECD TG 407 (Anonymous, 2009), in which no effects on reproductive organs were reported at tested doses up to 1 000 mg/kg bw/d.
- A 90-d oral (gavage) study with TBPEH (purity not stated) in rats according to OECD TG 408 (Anonymous, 2013), in which no effects on reproductive organs, oestrous cycle or sperm parameters were reported at tested doses up to 450 mg/kg bw/d.

The DS concluded that the fertility effects observed in the EOGRTS are consistent between the generations and cannot be considered secondary to general toxicity. Hence, the DS considered that classification of TBPEH as Repr. 1B; H360F is warranted.

Comments received during public consultation

Two MSCAs supported the classification of TBPEH as Repr. 1B, H360F. The lead registrant on behalf of the Organic Peroxides Consortium also agreed with the proposed classification as Repr. 1B, H360F.

Assessment and comparison with the classification criteria

RAC agrees that the results of the available EOGRTS (according to OECD TG 443; Anonymous, 2020) with TBPEH (unknown purity) in rats (Cohort 1B animals were mated to produce a second

(F2) generation) showed clear adverse effects on sexual function and fertility induced by TBPEH treatment, without evidence of severe general systemic toxicity.

The adverse effects included:

Oestrus cycle:

- P0 females: a statistically significantly lower percentage of females with regular oestrus cycle (54 % versus 83 %; effect was dose-dependent), a lower mean number of days in pre-oestrous (1.5 days versus 3 days; effect was dose-dependent and gained significancy at 1 000 mg/kg bw/d) and a higher number of females in prolonged oestrous (29 % versus 0 %; effect was dose-dependent) at 1 000 mg/kg bw/d.
- F1 females: slightly higher number and percentage in prolonged oestrous at the high dose (15 % versus 0 %); irregular oestrus cycle observed in all groups, including controls.

Reproductive performance:

- P0 animals: decreased reproductive index (67 % pregnant females versus 91 % in controls) at 1 000 mg/kg bw/d.
- F1 animals: lower copulatory index at 1 000 mg/kg bw/d (males: 90 % versus 100 % in controls; females: 95 % versus 100 % in controls).
- F1 females: a dose-dependently lower reproductive index at 300 mg/kg bw/d and 1 000 mg/kg bw/d (80 % and 56 % pregnant females, respectively, versus 95 % in controls); statistically significant at 1 000 mg/kg bw/d.
- A second mating showed that the male parental animals (P0) exposed at 1 000 mg/kg bw/d that did not fertilise the treated females of the same dose group, mated successfully with non-treated females (n = 8), suggesting that the decrease in fertility of treated rats originate from alterations of female reproductive functions.

Female histopathology of reproductive organs:

- P0 females: decreased number of developing follicles.
- P0 females: increased mean number of follicular atresia in pregnant and non-pregnant animals at 1 000 mg/kg bw/d (13.2 versus 5.3 in controls at quantitative evaluation of ovaries).
- P0 females: follicular cyst in ovaries at 1 000 mg/kg bw/d, one or both sides (3/24 animals).
- F1 females (Cohort 1A): significantly increased number of primordial and primary follicles in all treatment groups.

Pregnancy:

- P0 females: slightly, but statistically significantly, longer mean duration of pregnancy (22.37 versus 21.97 days in controls) at 1 000 mg/kg bw/d (value reported is at upper limit of the historical control data (HCD) range (21.8-22.3 d; 13 studies, no further details on HCD)).

General toxicity:

- P0 and F1 animals: slight reduction in mean body weight gain and mean body weight (bw) at 1 000 mg/kg bw/d, particularly in males (P0 males: throughout the study duration starting at pre-mating day 28, max. of -13 % bw at day 152 (bw gain days 0-152: -28.6 % compared to controls); F1 males: throughout the study duration, max. -11.3 % at day 154 (bw gain not reported); P0 females: no effects on bw, except for lactation day 21 (+5.5 %), slightly increased bw gain at days 0-69 (+2.4 %, n.s.) of the premating period; no effects on bw gain during gestation; statistically significant increase in bw gain during lactation (lactation days 0-21: +82.9 %); F1 females: statistically significant decrease in bw only up to postnatal day (PND) 36, max. at day 22: -8.9 % bw, no

significant effects on F1 female bw and bw gain afterwards, except for a higher mean bw gain at GD0-7 and lactation days 0-4).

- P0 and F1 animals: some changes in various organ weights including kidney (relative weight; P0: +51 % in males and +10 % in females; F1: +44 % in males and +15 % in females) and liver (relative weight; P0: up to +18 % in males and +25 % in females; F1: no data reported) at 1 000 mg/kg bw/d with no supporting pathological alteration at histopathology evaluation, except for P0 males that presented chronic progressive nephropathy. At 300 mg/kg bw/d, there were only changes in organ weights (kidney: relative weight; P0: +16 % in males; F1: +13 % in males and +10 % in females and liver: relative weight; P0: +6 % in males; F1: no data reported) without associated histopathological findings. No further details reported.
- P0 males: reduction in thyroid hormones (% free T3 and % free T4, but no effect on TSH; no weight or histopathology effects in thyroid glands)
- F1 animals: reduction in % free T3 in males at ≥ 300 mg/kg bw/d and % free T4 in males and females at 1 000 mg/kg bw/d; the latter was significantly increased in males at 100 and 300 mg/kg bw/d; TSH below detection limit at all doses; no histopathology effects in thyroid glands; dose-dependent increase in relative thyroid to brain weight.
- P0 and F1A males and females: effects on urinalysis (higher volume, lower pH) at 1 000 mg/kg bw/d.

Other relevant studies performed with TBPEH, including a reproduction/developmental toxicity screening test (OECD TG 421), a 28-d and a 90-d oral toxicity study (OECD TG 407 and 408, respectively), did not result in adverse effects on male or female fertility and/or female oestrus cycling. However, exposure during pre-mating is much shorter in a screening study (14 days) and lower doses were used in the 90-day oral toxicity study (≤ 450 mg/kg bw/d) compared to the EOGRTS (exposure for ten weeks pre-mating, ≤ 1 000 mg/kg bw/d).

Overall, RAC concludes that treatment with TBPEH induced adverse effects on sexual function and fertility that cannot be considered secondary to other non-specific toxic effects. TBPEH treatment led to reduced number of pregnant females, changes in oestrous cycle and histopathological effects in the ovaries, as well as a slightly prolonged pregnancy period in rats without concurrent severe systemic toxicity. In conclusion, RAC agrees with the DS that **classification of TBPEH as Repr. 1B; H360F, is warranted.**

ADVERSE EFFECTS ON DEVELOPMENT

Summary of the Dossier Submitter's proposal

The DS considered that the criteria for classification in Category 1B for adverse effects on development (Repr. 1B; H360D) are fulfilled for TBPEH, as the developmental effects observed in two pre-natal developmental toxicity (PNDT) studies (according to OECD TG 414) in rats and rabbits, respectively, in a reproductive screening test (OECD TG 421) and an EOGRTS (OECD TG 443) in rats are consistent between the studies, species and generations. In addition, effects occurred in the absence of overt maternal toxicity and were hence not considered as secondary, non-specific consequence of other toxic effects.

In the PNDT study in Wistar rats (Anonymous, 2013; Klimisch 1), animals were exposed via oral gavage to doses of 0, 200, 400, 1 000 mg/kg bw/d (purity confidential), on gestation days (GD) 5-19.

Effects observed in this study included a slight but statistically significant decrease in mean foetal body weight at 1 000 mg/kg bw/d (6 %; said to be within HCD of the laboratory, no details provided), as well as an increase in the incidence of combined skeletal variations (32 % at 1 000 mg/kg bw/d versus 6 % in controls, significant for the sum of foetuses and non-significant for litter means: 6 per litter versus 3.7 per litter in controls): incomplete ossification of the skull bones (13 % versus 0 % in controls; marked = three bones or more in 7 % of pups versus 0 % in control pups) and incomplete ossification of metacarpal/metatarsal, less than three ossified (6 % versus 0 % in controls).

In addition, non-significant increase in the incidence of bilateral hydroureter (1, 0, 1 at 200, 400, 1 000 mg/kg bw/d, respectively, versus 0 in controls) or with unilateral hydroureter with dilated renal pelvis (0, 0, 2 at 200, 400, 1 000 mg/kg bw/d, respectively, versus 1 in the controls) were observed; the bilateral and unilateral hydroureters combined were 1, 0, and 3 at 200, 400, 1 000 mg/kg bw/d, respectively, versus 1 in controls. Based on the hydroureter findings, the percentage of visceral variations (4 % at 1 000 mg/kg bw/d versus 1 % in control and low dose groups) were significantly increased. No maternal toxicity was observed in this study. No effect on corrected body weight and body weight gain was observed at any of the doses. During the first days of treatment there was a transient reduction in food consumption (GD5-8: -22 %, GD8-11: -14 %) and body weight gain (GD5-8) at the dose level of 1 000 mg/g bw/d followed by increased body weight gain on GD8-11 (no effect on absolute body weight).

In the PNDT study in New Zealand White rabbits (Anonymous, 2018; Klimisch 1), animals were exposed to 0, 30, 100 and 300 mg/kg bw/d (purity confidential) on GD6-27.

In this study, 8 dams (32 %) out of 25 pregnant aborted and 4 additional moribund dams (16 %) were found at 300 mg/kg bw/d. Bleeding from the vagina (associated to abortion or post-implantation loss) and other clinical signs of maternal toxicity (gastro-intestinal tract findings, weakness, reduced activity) were observed at this dose. In dams of this treatment group, lower body weight (significant on GD9-24), transient body weight loss (significant at GD6-12 and GD15-18), lower body weight gain (GD6-12 and 15-18) and lower food consumption (significant from GD6 to 21) compared to controls were seen, but terminal body weight and body weight gain (throughout the study) were according to the CLH report not affected when corrected for gravid uterine weight. At 100 mg/kg bw/d, reduced food consumption, lower body weight gain (significant on GD15-18) and 2 animals (10 %) that aborted were observed. Abortion in one dam (4 %) was seen at 30 mg/kg bw/d versus none in the control group. Single incidences of blood in the bedding were observed for dams at 30 and 100 mg/kg bw/d, both aborted.

An increase of early embryonic death (36 (15 %) at the dose level of 300 mg/kg bw/d versus 13 (6 %) in controls) and post-implantation loss (41 (17 %) at 300 mg/kg bw/d versus 20 (8 %) in controls (statistically significant for both number and percentage of resorptions) at this dose was noted, and a decrease in absolute number of viable foetuses (sum: 91 viable foetuses (38 % of sum of implantations) at 300 mg/kg bw/d versus 191 viable foetuses in control group (81 % of sum of implantations); reported as not statistically significant) was observed as well.

There were 4 dams with total post-implantation loss in the 300 mg/kg bw/d group and the number of viable foetuses per litter was non-significantly lower than in controls (6.5 compared to 8.3 in controls). Foetal weight (-15.6 %) and crown-rump length (-6.7 cm (-7.3 %) in mean crown-rump-length (male + female) compared to controls) were significantly lower at 300 mg/kg bw/d. The number of foetuses with retarded body weight and crown rump length was significant higher (14 % each versus 2 % and 3 %, respectively, for both effects). Higher incidence in numbers of foetuses with skeletal variations due to increased incidences of delayed ossified proximal and middle phalanges (18 % of foetuses affected versus 7 % in controls, not significant

on a litter basis), misaligned and fused sternebrae (2 % of litters affected versus 0 % in controls, no effect on the numbers of foetuses with this effect) and multiple malformed ribs and vertebrae (3 % of foetuses versus 0 % in controls, significant also on a litter base in 2 litters (20 %) versus none in controls) were observed at 300 mg/kg bw/d. No significant or dose-related effects were observed at 100 mg/kg bw/d. No effect on the ureters (compared with some hydroureters in the rat PNDT study) were seen.

In the OECD TG 421 study in Wistar rats (Anonymous, 2008; Klimisch 1), 0, 100, 300 and 1 000 mg/kg bw/d of TBPEH (purity not specified) was administered orally (by gavage) for 14 days during the pre-pairing, pairing, gestation, and lactation period until PND3. At 1 000 mg/kg bw/d, a slight reduction in mean body weight (no details whether significancy was reached) from day 3 until necropsy and significantly decreased food consumption during the first pre-mating week only was reported in parental males. Decreased food consumption and lower body weight gain was seen in the lactation phase (sacrifice on day 4 post-partum). Clinical signs (fur and/or bad general health condition) was noted during the last two gestation days (seven dams) and the first two days of the lactation period (four dams). No treatment-related effects were seen on the fertility index, number of corpora lutea, implantation rate or gestation length. Treatment at 1 000 mg/kg bw/d was associated with an increase in post-implantation loss (4.9 % versus 1.5 % in the control group, statistically significant), post-natal loss (5/10 dams affected, statistically significant) and a reduction in live pups (43 dead and 20 pups missing) until day 4 post-partum. The mean body weight of pups was reduced at the high dose, up to PND4. Study authors reported that post-implantation losses and effects on pup viability and weight were considered as test-item related. No further quantitative details are available.

In the OECD TG 443 study in Wistar rats (Anonymous, 2020; Klimisch 1), TBPEH at doses of 0, 100, 300 and 1 000 mg/kg bw/d (purity not specified) did not cause overt general toxicity in the P0 or F1 (Cohort 1B) dams up to the highest tested dose but interfered with development of the offspring. At 1 000 mg/kg bw/d, higher post-natal mortality rates than in controls were reported in F1 (2 % on PND0; 9 % on PND0-21) and F2 offspring (12 % on PND0, 15 % on PND0-4). The mean body weight (PND0-21 in F1 pups, PND0-4 in F2 pups) and litter weight gain (PND4-21 in F1 pups, PND0-4 in F2 pups) was significantly lower at the high dose level. There were also effects on absolute anogenital distance (AGD) (significant reduction in absolute but not normalised AGD) and eye opening on PND14 (significant lower percentage of animals) at this dose. At the mid dose of 300 mg/kg bw/d, lower mean body weight in F2 pups (no effect in F1 pups) was also noted.

The mean body weight remained significantly lower during the offspring's development on PND22-90 in Cohort 1A males and on PND22-42 in Cohort 1A females. Food consumption during this period was unaffected in females or transiently lower in males.

Parental general toxicity, on the other hand, was slight and particularly restricted to P0 and F1 Cohort 1A males exposed to TBPEH at 1 000 mg/kg bw/d (lower body weight, up to -13 % versus controls, mainly in males; histopathological effects in kidney only in P0 males). At 300 mg/kg bw/d, changes in organ weights (in particular relative increase of kidney and liver weights) in both sexes were noted, without associated histopathological findings. Historical control data are reported to be available and used for the evaluation. However, no details on the source of HCD and on means/medium values and their ranges were presented.

Comments received during consultation

One MSCA supported classification of TBPEH as Repr. 1B; H360D. Another MSCA noted that the study descriptions lack a number of quantitative data that are considered necessary for an

assessment of the reproductive toxicity on development of TBPEH. A more detailed reporting in the dossier would have been appreciated by this MSCA and a recommendation for a more thorough evaluation (e.g., of maternal toxicity, post-natal loss, etc.) was suggested in order to show why classification for developmental toxicity is warranted. The DS acknowledged that the level of details is limited for the OECD TG 421 study since there was no access to the full study report but that detailed results of the EOGRTS are available in Annex I of the dossier and that RAC will have access to this information. The DS further highlighted the effects reported in the EOGRT study: "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 AGD). These effects are consistent between F1 and F2 offspring and more pronounced in the F2 generation. Effects on parents are detailed in section 11.8.2 and all numerical data are available in Annex I". Overall, the DS concluded that there are clear and severe developmental effects reported in three different studies that cannot be linked to maternal toxicity, justifying a classification as Repr. 1B; H360D.

One comment from a Company/Manufacturer was received, in which the classification of TBPEH as Repr. 1B; H360D was questioned, and an additional weight of evidence consideration based on all of the available studies was provided (see the RCOM document for responses by RAC). In their analysis, the commenter argued that the effects upon which the proposed classification is based are secondary to a fertility alteration and/or occurred at doses in presence of maternal toxicity. In their view, no classification for development is warranted.

Assessment and comparison with the classification criteria

RAC agrees with the DS that the results of the PNDT study and the EOGRTS in rats showed clear adverse effects on the development that were induced by TBPEH treatment without evidence of severe general systemic toxicity.

With regard to developmental toxicity and malformations the observed adverse effects were:

PNDT study in rat:

- Developmental effects: A slight but statistically significant decrease in mean foetal body weight and a higher incidence of skeletal variations (delayed ossification of skull, metacarpal and metatarsal bones) and visceral alterations (hydroureter) at 1 000 mg/kg bw/d were seen but reported as being within HCD of the laboratory.
- Maternal toxicity: A transient reduction in food consumption (GD5-11) and body weight gain (GD5-8) was observed at 1 000 mg/kg bw/d followed by increased body weight gain on GD8-11. Apart from that, there was no indication of maternal toxicity.
- In conclusion, it cannot be excluded that the observed skeletal variations are secondary to lower pup weight. The observed effect on the pup weight is slight (6 % lower than control values). The pup weights were reported to be within the HCD range, which were not given in the study report. This effect could be considered as treatment related as no maternal toxicity was observed. While the effect on pup weight is consistently seen in other rat studies (OECD TG 421 and the EOGRTS), no increase in post-implantation losses was observed in this study at the dose level of 1 000 mg/kg bw/d. The incidence of the hydroureter is significantly increased, but the overall increase in the incidence of this variation is low (4 % in total compared to 1 % in controls). Data on HCD are not available.

PNDT study in rabbit:

- Developmental effects: Early embryonic death[2] (15 % versus 6 % in control) and post-implantation loss (17 % versus 8 % in control) (relative to sum of implantations) were increased at the highest dose of 300 mg/kg bw/d. It is likely that these effects resulted in lower absolute number of viable foetuses compared to the control group.

  Foetal weight and crown-rump length were significantly lower at 300 mg/kg bw/d; the relative number of affected foetuses was higher.

  At 300 mg/kg bw/d a higher incidence in numbers of foetuses with skeletal variations were seen. No significant or dose-dependent effects were seen at 100 mg/kg bw/d.
- Maternal toxicity: Moribundity (16 %), weakness, reduced activity and gastro-intestinal tract findings were signs of severe maternal toxicity in rabbits at 300 mg/kg bw/d. Also the high rates of abortion (32 %) and vaginal bleeding were considered to be signs of maternal toxicity in this study (see discussion below). The 10 % incidence of abortion at 100 mg/kg bw/d was also considered indicative of maternal toxicity in this study (with significantly lower food consumption on GD 9-12). A significant and markedly lower food consumption (with very low mean food consumption reduced to a level equivalent of about 30 % of the food consumption of controls) on GD6-21, linked to lower body weight gain on GD6-18 and lower body weight on GD9-24 (causing a bw loss on GD9-21) was observed at the high dose. The corrected body weight at study termination, although reported as comparable to the controls, was only assessed for those 10 dams that did not show abortions, total post-implantation losses or early embryonic deaths.
  Considering the mean corrected body weight and bw gain on GD28 (reflecting the entire duration of pregnancy) for these 10 dams, the mean and median values were non-significantly lower than for controls. However, a reduced food consumption, although less severe than for those dams with abortion, moribundity, etc., was also noted in these 10 dams during the early phase of pregnancy. The median values for the bw gain on GD6-9 and GD9-12 were reduced (significant for GD6-9), concordantly significantly lower food consumption was seen on GD6-9 and GD9-12. Lower foetal weight and crown-rump length, reported for pups of these 10 dams, are likely to be secondary to lower food consumption and bw gain in the early pregnancy.
- In conclusion, due to the high incidence of dams at 300 mg/kg bw/d that aborted or were in a moribund health condition it cannot be excluded that the post-implantation losses and effects on pup development are secondary effects of the maternal toxicity. No treatment-related increase of effects on pup development were seen at 100 mg/kg bw/d or lower. No treatment-related malformations were identified.

OECD TG 421 screening study in rat:

- Developmental effects: Treatment at 1 000 mg/kg bw/d was associated with an increase of post-implantation loss (4.9 % versus 1.5 % in the control group, no details were given in the Annex I to the CLH report), post-natal loss (5/10 dams affected) and a reduction in live pups (43 dead and 20 pups missing until PND4). The mean body weight of the pups was reduced at the high dose, up to PND4.
- Maternal toxicity: At 1 000 mg/kg bw/d there were no signs of general toxicity in the dams during the gestation period. Slightly reduced food consumption was transiently

[2] Nishimura, 2001: In NZW rabbits implantation occurs on GD 6-9 depending on the region of the uterus blastocysts are attached to; early embryonic deaths may in rabbits (unlike rats) be seen as developmental effects; organogenesis progresses in parallel.

observed during the first week of premating period. Mean body weight of the dams at 1 000 mg/kg bw/d was slightly increased during gestation, while their mean body weight gain was statistically significantly decreased during lactation (sacrificed on PND4) (no details presented). 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. These effects are considered as likely to be attributable to the high number of dead pups (and missing pups that could either be post-implantation losses or stillborn pups cannibalised by their dams).

- In conclusion, the observed pup effects could not be attributed to maternal toxicity and are considered as substance-related developmental effects.

Extended one-generation study in rat:

- Developmental effects: Higher post-natal mortality than in controls were reported in F1 (2 % on PND0 versus 0 % in controls; 9 % on PND0-21 versus 1 % in controls) and in F2 pups (12 % PND0 versus 1 % in controls; 15 % on PND0-4 versus 3 % in controls).

  A reduced body weight on PND0 (-6.5 % in F1 and -5 % in F2 pups) and body weight gain (-8.6 % on PND0-21 in F1 pups; -12 % on PND0-4 in F1 pups; -13 % on PND0-4 in F2 pups) were observed in offspring at 1 000 mg/kg bw/d. Lower pup body weight was also reported at 300 mg/kg bw/d (-3.2 % for F1 and -3.4 % for F2 pups on PND0). Clinical signs, such as no milk in the stomach and cold pups, were reported in the treated groups.

  The mean body weight remained significantly lower during the offspring development on PND22-90 in Cohort 1A males (-12 % on PND90) and on PND22-42 (-6 % on PND42) in Cohort 1A females.

  No effect on post-implantation loss in P0 dams up to 1 000 mg/kg bw/d.

  No detailed information on HCD available.

- Maternal toxicity: No signs of overt general toxicity were observed in the dams up to the highest tested dose of 1 000 mg/kg bw/d.
- In conclusion, the observed pup effects could not be attributed to maternal toxicity and are considered as substance-related developmental effects. The observed effects were considered as treatment-related as no data on laboratory HCD ranges/mean values are available.

RAC concludes on the basis of the PNDT study and the EOGRTS in the rat, supported by evidence from the OECD TG 421 screening study, that TBPEH induced developmental toxicity which was not secondary to maternal toxicity. At oral doses of 1 000 mg/kg bw/d, TBPEH consistently caused lower pup body weight at PND0 (PNDT, EOGRTS) or PND0-4 (OECD TG 421 study) and/or body weight gain (PND0-21 in F1 and PND0-4 in F2 pups, EOGRTS) were seen in the three rat studies. Reduction in mean body weight continued on PND22-90 in Cohort 1A males and on PND22–42 in Cohort 1A females. Lower body weight gain was also noted in F1 and F2 pups at 300 mg/kg bw/d (EOGRTS).

Increased pup mortality rates were seen at 1 000 mg/kg bw/d TBPEH in the OECD TG 421 study (PND0, PND0-4) and the EOGRTS (PND0 in F1 and F2 pups; PND0-21 in F1 pups and PND0-4 in F2 pups).

Increases in post-implantation losses were observed in the OECD TG 421 study. However, only limited quantitative data are available and information on HCD is lacking in the study report summary. An increased rate of 4.9 % post-implantation losses at 1 000 mg/kg bw/d TBPEH versus 1.5 % in controls was reported in the RCOM document. This effect was also seen in the PNDT on rabbits, but not in the PNDT or EOGRTS in rats. In the OECD TG 421 study, the reporting on a reduction in live pups (43 dead and 20 pups missing until PND4) raises uncertainties about

the cause of dead or missing pups. Due to the lack of numerical data, the uncertainties on the dead/missing pups and the lack of HCD, the study report is less informative.

With regard to the PNDT rabbit study, RAC considers that it cannot be excluded that the effects at 300 mg/kg bw/d were due to the severe maternal toxicity observed in pregnant rabbits at this dose level. Beyond the abortions, moribundity, vaginal bleeding, weakness, reduced activity, and gastro-intestinal tract findings, the affected dams at this dose level consumed significantly less food from GD6 until GD21 (around 30 % of the control values). Literature data show that a markedly lower food consumption alone may cause abortions and post-implantation loss in dams and may have effects on pup weight and viability depending on the level of caloric restriction and the onset/duration of the anorexic period in pregnancy (Matsuoka et al., 2006; Matsuzawa et al., 1981). Reduced crown-rump length and foetal weight in the offspring of those 10 dams that did not show abortions, post-implantation losses and moribundity were considered as likely related to less severe, but still significantly lower food consumption throughout the early phase of pregnancy (GD6-12). Based on the uncertainty whether a treatment-relationship for the observed effects in rabbits up to 300 mg/kg bw/d are secondary to the maternal toxicity, this study is given less weight.

Historical control data were reported to be available (indicated in Annex I to the CLH report) for all studies except the OECD TG 421 study. However, no detailed information on the ranges/mean/medium values, the number of studies and animals and the use of relevant strains and time windows were given.

No treatment-related increase in foetal malformations was observed.

Taking all available data into account, developmental toxicity was consistently seen in pups in the available rat PNDT and EOGRTS at the high dose of 1 000 mg/kg bw/d, including significantly reduced pup weight. In F1 and F2 pups of the EOGRTS, post-natal mortality rates were significantly higher at PND21 in the F1 and at PND0 and PND4 in the F2 generation. In addition, the body weight gain was consistently lower at PND21 (F1 pups) and PND4 (F1 and F2 pups). Moreover, the pup body weight remained significantly lower up to PND42 (Cohort 1A, female pups) and PND90 (Cohort 1A, male pups). Increased rates of post-implantation losses and supporting evidence on lower body weight and post-natal loss comes from the less informative study report on the OECD TG 421 study in rats.

A non-significantly reduced pup body weight was also seen at 300 mg/kg bw/d in F1 and F2 pups of the EOGRTS. Due to the dose selection, no information is available for doses between 300 and 1 000 mg/kg bw/d in the EOGRTS and between 400 and 1 000 mg/kg bw/d in the PNDT rat study.

Overall, RAC agrees with the DS that **classification for TBPEH as Repr. 1B; H360D is warranted**.

ADVERSE EFFECTS ON OR VIA LACTATION

Summary of the Dossier Submitter's proposal

There are no experimental data specifically related to adverse effects on or via lactation. However, some information can be derived from the OECD TG 421 study and the EOGRTS (see sections above). Post-natal losses and decreased body weight of pups were found in both the OECD TG 421 study and the EOGRTS.

In the EOGRTS, an inadequate nursing behaviour of dams may be suggested considering the lower pup body weight during lactation and the higher extra-uterine mortality including the fact

that some pups did not have milk in the stomach. However, as these observed adverse effects occurred already at PND0, they may be due to exposure during gestation.

Overall, the DS stated that it cannot be clearly distinguished if the observed adverse effects were caused by gestational exposure and/or by lactation exposure. Hence, classification of TBPEH for adverse effects on or via lactation is considered not warranted.

Comments received during consultation

One MSCA agreed that data are insufficient for concluding on classification for adverse effects on or via lactation for TBPEH.

Assessment and comparison with the classification criteria

There are no experimental data specifically related to adverse effects on or via lactation. Information from the OECD TG 421 study and the EOGRTS (see sections above) may indicate an inadequate nursing behaviour of TBPEH treated dams, as post-natal losses, and decreased body weight of pups, as well as pups with no milk in the stomach were found in one or both studies.

In the OECD TG 421 study in rats, treatment at 1 000 mg/kg bw/d was associated with an increase of post-natal loss and a reduction of live pups (until day 4 post-partum). The mean body weight of pups was also reduced at this dose, up to day 4 post-partum.

In the EOGRTS in rats, a higher percentage of offspring showed signs such as no milk in the stomach, cold, found dead, missing and alopecia at 1 000 mg/kg bw/d that could have contributed to the higher extra-uterine mortalities on PND0 and between PND0 and PND4 (F2 pups) and between PND0 and PND21 (F1 pups). Mortality was accompanied by statistically significant lower body weight at PND0 and PND4 (F1 and F2 pups) and in F1 pups through PND21. Similarly, statistically significant reductions in body weight gain (PND0-21 in F1 pups; PND0-4 in F2 pups) were observed at that dose.

At 300 mg/kg bw/d, F1 and F2 pups had a lower body weight on PND0 and F2 pups showed a lower body weight (-3 %) on PND4 as well. At this dose, F1 pups also had a statistically significantly lower body weight gain between PND7 and PND14 due to a reduced body weight gain in female F1 pups.

Taking the available data into account, RAC agrees with the DS that it cannot be clearly distinguished if the observed adverse effects were caused by gestational exposure and/or by lactation exposure. Hence, **classification of TBPEH for adverse effects on or via lactation is not warranted due to inconclusive data**.

Additional references

Matsuoka, T, Mizoguchi Y, Serizawa K, Ishikura T, Mizuguchi H, Asano Y (2006) Effect of stage and degree of restricted feeding on pregnancy outcome in rabbits, Journal of Tox Sciences. 2006; 31 (2) 169-175. https://doi.org/10.2131/jts.31.169

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Nishimura, M. (2001), Timing of implantation in New Zealand White rabbits. Congenital Anomalies, 2001; 41: 198-203. https://doi.org/10.1111/j.1741-4520.2001.tb00833.x

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).