COMPILED COMMENTS ON CLH CONSULTATION

Comments provided during consultation are made available in the table below as submitted through the web form. Please note that the comments displayed below may have been accompanied by attachments which are listed in this table and included in a zip file if non-confidential. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Last data extracted on 22.01.2024

Substance name: ethylene bis[3,3-bis(3-*tert*-butyl-4-hydroxyphenyl)butyrate]
CAS number: 32509-66-3
EC number: 251-073-2
Dossier submitter: Belgium

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2024	Germany	Clariant Plastics & Coatings (Deutschland) GmbH	Company-Manufacturer	1
Comment received				
Föll, Mecklenburg & Partner GmbH is a toxicology consulting service with expertise in Reproductive and Developmental Toxicology. Föll, Mecklenburg & Partner GmbH was commissioned by Clariant Plastics & Coatings (Deutschland) GmbH to provide an independent scientific evaluation including a discussion on the proposed ReproTox Cat 1B classification with Lactation of Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl) butyrate] (Trade Name Hostanox O3) submitted by the Belgian Member State Competent Authority. Please find herein a summary of an expert evaluation provided by <confidential>, her full evaluation of the available, relevant evidence is provided in the attached PDF. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 240119_Comments_CLH_EC_251-073-2_2.pdf				

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2024	Germany	<confidential>	Company-Manufacturer	2
Comment received				
<confidential> notes that classification as Reproductive Toxicant Category 1B (H360D) with Lactation (H362) according to CLP criteria is not scientifically justified based on the available evidence in the context of the regulatory criteria (REGULATION (EC) No 1272/2008). <confidential> requests that the RAC reconsider the recommendation made by the dossier submitter. In the following, the evidence available to the MSCA at the time of the review is summarized. Please note that the comments below are fully supplemented with a detailed argumentation and evidence summary in the attached PDF. The non-confidential attachment details study summaries, the rationale underpinning <confidential> position, and a discussion on the applicability of the available evidence for classification purposes. In the following, we summarize the findings of a comprehensive review of all relevant studies. To warrant classification in accordance with the CLP criteria, primary requisite is adversity of				

the observed effects, (CLP Annex I, section 3.7.2.1.1), in accordance with additional criteria specifying toxicological significance including the nature of the effects, severity, and incidence (CLP Annex I, section 3.7.2.3.1). Importantly, if potentially relevant effects coincide with other toxic effects, it needs to be demonstrated that "the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects". Consequently, conclusions on the inherent ability of a chemical to induce a specific adverse effect (CLP Annex I, section 3) should be based upon an assessment of all relevant data and considering the total weight of evidence (CLP Annex I, section 3.7.2.3.1) as opposed to unilaterally weighing isolated observations. Comprehensive assessment of the scientific studies available to the dossier submitter at the time of their review demonstrates the following basis of evidence for classification purposes:

1. Multiple reliable key studies unequivocally establish that Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl) butyrate] does not elicit adverse effects on reproduction, is not teratogenic, and there is no indication that the substance elicits an endocrine mode of action.
2. Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl) butyrate] exhibited considerable maternal systemic toxicity at mid- and high-doses concordant across two reliable, guideline- and GLP-conform studies (OECD 414 and ancillary mechanistic study). Observed systemic effects included significant reduction in maternal body weight gain, concurrent with decreased food consumption, alongside clinical chemistry and hematological alterations, and organ level changes (enlarged adrenal gland, small thymus) indicative of maternal stress. Moreover, test item-related microscopic changes in liver, adrenal glands, small intestinal segments (mainly jejunum, but also in duodenum and ileum), stomach, spleen, and thymus were documented. The NOAEL for systemic maternal toxicity was determined at 100 mg/kg bw/d.
3. Apparent developmental effects are limited to observations of late resorption and post implantation loss at mid and high doses in OECD 414 and ancillary mechanistic studies. Importantly, late resorption and post implantation loss strictly coincided with maternal systemic toxicity and was only observable following bolus administration of the test substance via oral gavage. In the absence of overt maternal toxicity, as demonstrated in multiple dietary studies, late resorption and post implantation loss do not occur even at doses up to and including the limit dose of 1000 mg/kg bw/d. These findings support the notion that increases in late resorption and post implantation loss are secondary to maternal stress and systemic toxicity exacerbated by bolus administration via oral gavage, and not primary developmental effects.
4. The MSCA's statement that "The effect on post-implantation loss and late resorptions in the mid dose and high doses, observed in the 2 more recent studies, cannot solely be attributed to maternal toxicity" is not supported by the available evidence, particularly taking into consideration ancillary data provided by the mechanistic study purposely conducted to evaluate maternal systemic toxicity in conjunction with the OECD 414 study, and as summarized in items 2 and 3 and discussed in further detail in this document.
5. A modified OECD 421 study with cross-fostering conclusively demonstrated that in utero exposure to Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl) butyrate] does not affect prenatal/ fetal development as pup body weight at birth was comparable between treatment and control groups. The study also demonstrated that in utero exposure has no effect on postnatal development, as pups exposed during pregnancy and cross-fostered to unexposed dams during lactation did not show any developmental alterations. The cross-foster satellite groups further demonstrated that at high dietary doses (exceeding the limit dose of 1000 mg/kg bw/d), pups can be exposed to the test substance via milk during lactation. Effects on pups were limited to significant, albeit transient and fully reversible decreases in body weight gain in (in prenatally not exposed) pups during late lactation, no other developmental observations were identified. It is plausible that the decreased body weight gain is at least partially attributable to effects on milk quality/quantity because of maternal

toxicity but systemic effects in pups (concordant with systemic effects in dams) following exposure through milk cannot be ruled out as a contributing factor based on the available data.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 240119_Comments_CLH_EC_251-073-2_3.pdf

Date	Country	Organisation	Type of Organisation	Comment number
18.01.2024	Germany		MemberState	3
Comment received				
On page 24 of the CLH report it is stated: "At necropsy, as observed in Table 26, the corrected bw was severely reduced at the highest dose and tend to decrease at the mid dose." We assume it should read "[…] the corrected bw gain was severely reduced […]". Could you please verify? Moreover, the corrected body weight of the mid-dose group is "149.91 g" according to table 26 on page 24 of the CLH report. We assume it should read "249.91 g". Could you please verify this as well?				

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2024	Germany	Clariant Plastics & Coatings (Deutschland) GmbH	Company-Manufacturer	4
Comment received				
Upon request of Clariant Plastics & Coatings (Deutschland) GmbH, <confidential> (DABT, ERT) has reviewed reproductive toxicity (OECD 414 & 421) and mode of action (14 days repeat dose & 414) study reports to determine the adverse effects of the test substance and whether the effects can account for Harmonized Classification and Labelling requirements. All the reports were reviewed, and a summary was prepared independently. Please find herein a summary of the expert evaluation provided by <confidential>, the full evaluation of the available, relevant evidence is provided in the attached PDF. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 240119_Comments_CLH_EC_251-073-2_1.pdf				

HEALTH HAZARDS – Reproductive toxicity

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2024	Germany	Clariant Plastics & Coatings (Deutschland) GmbH	Company-Manufacturer	5
Comment received				
Föll, Mecklenburg & Partner GmbH concluded that based on the REACH definition of				

reproductive toxicants, the presence of pronounced maternal toxicity, a strong relationship between maternal and offspring effects and the occurrence at only high exposure values, a classification of Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl) butyrate]as reproductive toxicant category 1B is not justified (pages 3-9). Regarding Classification as Lactation (H362) in the opinion of the expert, the qualitative clinical chemistry data from the mechanistic study with a dose-dependent decline of maternal plasma cholesterol and triglyceride levels, the quantitative data from the OECD 421 study on maternal BW and BWG decline in the timeframe of the highest milk production and the parallel development of maternal and offspring BW/BWG at this crucial time period form a physiologically plausible picture pointing toward a maternal problem with sufficient high-qualitative milk production. As these effects occurred above dose levels from a toxicologically limit test (1000 mg/kg/d) and nursing human mothers are unlikely to be exposed to such high exposures, the relevance for human health effects is not given. Therefore, a labelling as H362 (May cause harm to breast-fed children) is not deemed justified (for more details please refer to pages 9-12).

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 240119_Comments_CLH_EC_251-073-2_2.pdf

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2024	Germany	<confidential>	Company-Manufacturer	6

Comment received

Classification as Reproductive Toxicant Category 1B entails "clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects." <confidential> notes that the available evidence, as summarized above, does not support classification of Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl) butyrate] as a Reproductive Toxicant Category 1B according to criteria defined in CLP for the following reasons:

1. Late resorption and post implantation loss strictly coincide with overt maternal systemic toxicity observed in two oral gavage studies. A mechanistic study provides corroborating evidence underpinning the mechanistically plausible correlation between maternal effects and late resorption and post implantation loss. The fact that late resorption and post implantation loss observed in the two oral gavage studies was not reproducible in multiple dietary studies up to and including the limit dose, alongside less pronounced maternal systemic effects, further supports that these are not primary developmental effects.
2. Effects through lactation were limited to reversible, non-adverse reduction in pup body weight gain and observable at doses considerably exceeding the limit dose of 1000 mg/kg bw/d applicable in OECD guideline studies. <confidential> notes that a classification for lactation is questionable based on the high dosage utilized in the dietary study which exceeds dosages applicable for CLP purposes and should be considered only as a precaution.
3. The evidence available to the CLH dossier submitter at the time of the review conclusively demonstrates that Ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl) butyrate] does not affect any further reproductive or developmental endpoints, i.e., the substance does not elicit adverse effects on reproduction and prenatal fetal development, is not teratogenic, and there is no indication that the substance elicits an endocrine mode of action.

In the attached public, non-confidential PDF, <confidential> provides further detail and

evidence on the claims made above, and we hope the RAC will find this information helpful in their assessment of the proposal for harmonized classification.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 240119_Comments_CLH_EC_251-073-2_3.pdf.pdf

Date	Country	Organisation	Type of Organisation	Comment number
18.01.2024	Germany		MemberState	7

Comment received

a) Adverse effects on sexual function and fertility
We agree with the BE CA that the available data presented in sections 10.10.1 and 10.10.2 of the CLH report do not provide sufficient evidence for adverse effects on sexual function and fertility which would justify a respective proposal for classification of ethylene bis[3,3-bis(3-tert-butyl-4-hydroxy-phenyl)¬bu¬ty¬rate].

b) Adverse effects on development
We agree with the BE CA to classify ethylene bis[3,3-bis(3-tert-butyl-4-hydroxy-phenyl)¬bu¬ty-rate] for developmental toxicity in category 1B (Repr. 1B – H360D).
In a prenatal developmental toxicity study via gavage, significant developmental toxic effects were observed in Wistar rats starting from the mid dose (i.e. at 300 mg/kg bw/d: increase in incidences of dams with any resorptions, post-implantation loss and foetuses with anasarca). Maternal toxic effects were clearly present only in high-dose dams (i.e. at 1000 mg/kg bw/d: body weight loss, adrenal glands and thymus identified as target organs) (according to OECD TG 414, Anonymous 2017a).
In another study, in which pregnant Wistar rats were exposed to ethylene bis[3,3-bis(3-tert-butyl-4-hydroxy-phenyl)¬bu¬ty¬rate] via gavage at dose levels of 0, 100, 300, 600 mg/kg bw/d, post-implantation losses were also significantly increased at the highest dose level (two of six dams at 600 mg/kg bw/d had complete resorptions). Moreover, one foetus of the high-dose group also showed anasarca. Developmental toxicity occurred in the presence of maternal toxicity (i.e. body weight loss, adrenal glands and thymus as target organs) (Anonymous 2017b).
However, it is doubtful that maternal toxicity was causing the developmental toxic effects, because post-implantation loss and anasarca were statistically significantly increased also in the absence of marked maternal toxicity at 300 mg/kg bw/d in Anonymous (2017a).
Three feeding studies with Wistar rats including 2 - 4 weeks of exposure before mating did not demonstrate increased intrauterine resorptions, although two studies were conducted with doses comparable to those used in the studies with oral gavage (Anonymous (1976): up to 800 mg/kg bw/d; Anonymous (1979): up to 500 mg/kg bw/d; Anonymous (2019): up to 1320 mg/kg bw/d). Since ethylene bis[3,3-bis(3-tert-butyl-4-hydroxy-phenyl)¬bu¬ty¬rate] is a very lipophilic substance (Log Pow of 13.66, see CLH report p. 6), it is conceivable that the oral application of the test substance in corn oil might have enhanced its bioavailability.
Taken together, the data provided for developmental toxicity are conclusive and support the proposal to classify ethylene bis[3,3-bis(3-tert-butyl-4-hydroxy-phenyl)¬bu¬ty¬rate] for developmental toxicity in category 1B (Repr. 1B – H360D).

c) Adverse effects on or via lactation
There is some evidence which could support the classification proposed by the DS for the effects on or via lactation:
Based on the results of the modified reproductive/developmental toxicity screening study according to the OECD TG 421 including cross-fostered groups (Anonymous,2019), clear

evidence of adverse effects on the growth of the developing organism in the post-natal phase was convincingly demonstrated to be due to lactational exposure. Pups nursed by high-dose dams gained less weight during lactation than pups nursed by control dams. Statistically significantly reduced body weights in pups in the mid- and high-dose groups below the historical control range (-16.4% in mid-dose pups and -33.6% in high-dose pups) was duplicated in the control pups cross-fostered by the dams from the high dose group, causing severe depression of growth (by -44.6 % body weight) by PND 21.
At the same time, there is evidence of maternal toxicity, such as affected body weight development in lactating dams of the high-dose group compared to the control dams, reaching statistical significance on LD 13 and LD 21 (see CLH report p. 12, table 10):
-Lactation day 13: 289 g in controls vs. 255 g** at the high dose (-11.8%)
-Lactation day 21: 280 g in controls vs. 248 g** at the high dose (-11.4%)
This effect on the maternal body weight was concurrent to clearly lower feed consumption during lactation in high-dose dams compared to the control group (see Annex I to the CLH report p. 8, table 5):
-Mean of means over lactation period: 65 g food/animal/day in controls vs. 42 g food/animal/day in the high-dose group (-35.4%).
The reduced feed consumption by dams may be related to the poor palatability of the test substance, as in a subchronic feeding study with ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] all animals of the high-dose group (5.0% test substance in feed) died between exposure days 11 and 39 because "at this dose, practically no food had been consumed" (see CLH report p. 16, Anonymous, 1968).
Furthermore, the reduced feed intake cannot be attributed to the lower number of pups to be nursed by the high-dose dams, because the number of living pups at the first litter check as well as total post-natal loss were comparable between the respective groups (see CLH report p. 14, table 13 and Annex I to the CLH report p. 11, table 10).
This raises the question of whether reduced feed intake by the high-dose dams could lead to an unspecific reduction in milk production without causing marked overt systemic toxicity in lactating dams. In our opinion, the CLH report would have benefitted from a discussion on effects of reduced feed consumption on the lactation and on pup body weight development.
Furthermore, we would like to point out that the dams of the mid- and high-dose group of the study by Anonymous (2019) received dose levels clearly above 1000 mg/kg bw/d during lactation: according to the table 2 on page 7 of Annex I to the CLH report, the actual mean test item intake during lactation reached levels of 762, 1483 and 1859 mg/kg bw/d for the low-, mid- and high-dose groups, respectively. Based on the results of this study alone it may be questionable, whether the effects on body weight development of the offspring are still relevant for classification.
At the same time, we acknowledge that post-natal toxicity of the test substance is further supported by the evidence from two earlier feeding studies conducted at lower dose ranges (Anonymous (1976) and Anonymous (1979)), demonstrating reduced body weight of pups by the end of/during the lactation period in the high-dose groups of both studies, at 500 and 800 mg/kg bw/d respectively. However, based on the data available in the CLH report and its Annex, we cannot assess whether the above mentioned dose levels really correspond to the actual mean test item intake during lactation. In this context, we identified discrepancies between the studies which may give rise to doubts. For example: While pups developed in a normal way when nursed by dams receiving 762 mg/kg bw/d in the study by Anonymous (2019) (= actual mean test item intake during lactation; see CLH report p. 32, table 33), the study by Anonymous (1976) reports that mean pup weight was statistically significantly reduced by 21% on lactation day 21 in the high-dose group (i.e. at 800 mg/kg bw/d; uncertain if the dose represents the actual dose during intake). In Anonymous (2019), pup body weight development was statistically significantly reduced by 16.4% in the mid-dose group (i.e. at 1483 mg/kg bw/d = actual mean test item intake during lactation).

Taken together, the data provided could support the proposal to classify ethylene bis[3,3-bis(3-tert-butyl-4-hydroxy-phenyl)butyrate] for effects on or via lactation because
i) the body weight of nursed pups was statistically significantly reduced in three independent feeding studies (Anonymous, 1976, 1979 and 2019);
ii) additionally, one feeding study provides evidence that the reduced body weight of nursed pups from exposed dams fell below historical control ranges (Anonymous, 2019);
iii) it was convincingly proven by cross-fostered groups that body weight reduction in nursed pups arose from lactational exposure and not from in utero exposure (Anonymous, 2019);
iv) the test substance was detected in milk from exposed lactating dams (Anonymous, 2019); (however, this data is not further used in the CLH report in order to estimate the likely body burden in nursed pups and to compare it with the toxicity data in adult rats.)
Despite all these facts, we are of the opinion that two issues should still carefully be evaluated before concluding on a potential classification of ethylene bis[3,3-bis(3-tert-butyl-4-hydroxyphenyl)butyrate] for adverse effects on or via lactation:
i) the potential impact of reduced feed intake by lactating dams on lactation and on post-natal pup body weight development and
ii) the relevance of adverse effects on or via lactation when occurring at dose levels higher than 1000 mg/kg bw/d.

Date	Country	Organisation	Type of Organisation	Comment number
18.01.2024	France		MemberState	8
Comment received				
FR agrees that available data showing limited effects (rat and dog studies not following guidelines) on male reproductive system (some modifications in testes weight and alterations in spermatogenesis) are not sufficient to warrant classification 1B or 2 for fertility. Regarding developmental toxicity, FR agrees with the classification as Repr.1B H360D based on the increases in post-implantation loss, resorption, decreases in the number of total live pups and foetus/pup weight. FR suggests adding the effects of reddish discharge from vagina that occurred in the two embryo-foetal developmental toxicity studies at high dose to support the classification. Regarding the effects on or via lactation, FR agrees with the classification as Lact. H362 based on clear adverse effect as severe decrease in the body weight of pups from non-exposed dams cross-fostered for three weeks by exposed dams.				

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2024	Germany	Clariant Plastics & Coatings (Deutschland) GmbH	Company-Manufacturer	9
Comment received				
Based on a review of the above cited studies, it is evident that no effect on sexual function and fertility was observed. There was no direct effect on the development of the fetus. Due to the significant reduction in the maternal body weight gain and lowered food consumption, the decreased gravid uterus weight, and significant increase in late resorption and post				

implantation loss along with significant increase in any resorption and completed resorptions were considered as secondary in nature. In addition, data from the mechanistic toxicity study indicate that the pathological changes (hematology – increased neutrophils/monocytes; increased haptoglobin, A1 alpha glycoprotein, increased bilirubin, minimal increase in ALT/AST with lower ALP activity) including gross (adrenals/thymus) and histopathological changes in adrenal, thymus, liver, stomach were found to be systemic toxicity effects, hence it is concluded that no direct effect of reproduction was evidenced from these animal studies.

Based on the observations from the reproductive/developmental toxicity screening test, the developmental toxicity observed postnatally with evidence of cross-fostering groups, adverse effects in the offspring caused by the lactational transfer in breast milk as was confirmed by the results of bioanalysis in plasma and milk samples derived from the dams. Pups from non-exposed dams cross-fostered, the mean body weight for sexes combined was 45% (PND 21) lower compared to the mean body weight of pups from 11250 ppm females, cross-fostered to control females. On PND 13 and 21, mean body weight for male and female pups were reduced without cross-fostering group at 11250 ppm group (PND13- 19% and PND21- 34%) and at 7500 ppm group on PND 21 (16%). All high dose females (treated at 11250 ppm) used for cross-fostering were exposed to Hostanox O 3 P as confirmed by high concentrations of test item in the plasma samples (ranging from 1650 to 5660 ng/mL) on lactation Days 11 or 12. Comparable concentrations of test items were also measured in the milk of these dams (ranging from 1280 to 5990 ng/mL). This indicates that pups cross-fostered to high dose dams had been exposed to Hostanox O 3 P by lactational transfer. No other developmental parameters were affected such as gestation index, postnatal implantation index, litter size, live birth index, viability index, sex ratio, AGD areola/nipple retention, T4, and lactation index. No reproductive toxicity was observed in the reproduction/developmental toxicity screening test. Classification for effects via lactation is yet to be confirmed by assessing the quality of the milk; hence the above postnatal developmental toxicity could be due to effect on lactation.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 240119_Comments_CLH_EC_251-073-2_1.pdf

PUBLIC ATTACHMENTS
1. 240119_Comments_CLH_EC_251-073-2_1.pdf [Please refer to comment No. 4, 9]
2. 240119_Comments_CLH_EC_251-073-2_2.pdf [Please refer to comment No. 1, 5]
3. 240119_Comments_CLH_EC_251-073-2_3.pdf [Please refer to comment No. 2, 6]