EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 1 June 2020

Addressee: [redacted]

Decision number: TPE-D-2114509566-45-01/F
Substance name: [1,3(or 1,4)-phenylenebis(1-methylethylidene)]bis[tert-butyl] peroxide
EC number: 246-678-3
CAS number: 25155-25-3
Registration number: [redacted]
Submission number subject to follow-up evaluation: [redacted]
Submission date subject to follow-up evaluation: 14 February 2020

DECISION TAKEN UNDER ARTICLE 42(1) OF THE REACH REGULATION

By decision TPE-D-2114344773-45-01/F of 7 October 2016 ("the original decision") ECHA requested you to submit information by 16 October 2017 in an update of your registration dossier.

Based on Article 42(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA examined the information you submitted with the registration update specified in the header above, and concludes that

Your registration still does not comply with the following information requirement:

Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species (rabbits), oral route using the registered substance

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

The respective Member State competent authority (MSCA) and National enforcement authority (NEA) will be informed of this decision. They may consider enforcement actions to secure the implementation of the original decision and exercise the powers reserved to them under Article 126 of Regulation No 1907/2006 (penalties for non-compliance) for the period during which the registration dossier was not compliant[1].

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.

Approved[2] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

[1] See paragraphs 61 and 114 of the judgment of 8 May of the General Court of the European Court of Justice in Case T-283/15 Esso Raffinage v. ECHA
[2] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

Pre-natal developmental toxicity study

You were requested to submit information derived with the registered substance for Pre-natal developmental toxicity study in second species, rabbit, oral route.

In the updated registration subject to follow-up evaluation, you have provided an oral pre-natal developmental toxicity (PNDT) study in rabbits, oral route, according to test guideline OECD 414 performed with the registered substance. The doses used in the study were 25, 100 and 200 mg/kg body weight/day. There was no maternal or developmental toxicity in the study and you considered a NOAEL for maternal and developmental toxicity to be equivalent to the highest dose tested (200 mg/kg).

ECHA notes that you first conducted an oral dose range finding study and main PNDT study in rabbits using corn oil as vehicle. That main study had to be terminated due to mortality in all dose groups.

Consequently, you performed a new dose range finding (DRF) study, using as vehicle corn oil in an aqueous solution of carboxy methyl cellulose and doses of 50, 250 and 500 mg/kg bw/day. Based on this dose range finding study you concluded: "*At dose level of 500 mg/kg/day, clinical signs such as reduced faeces were observed in most of the females. Body weight gain and food consumption were reduced during the course of the treatment. At dose level of 250 mg/kg/day, reductions in faeces, body weight gain and food consumption were also observed. At dose level of 50 mg/kg/day, no maternal toxicity was noted. At necropsy examination, no treatment related abnormalities were detected. At 500 mg/kg/day, one female aborted and higher incidence of post implantation loss was also noted. Total litter weight and uterine weight were significantly lower than the control group. The other reproduction parameters were unaffected by the treatment with the test item or did not follow a dose dependent pattern. In conclusion, the treatment with the test item at dose level of 500 mg/kg/day caused maternal and developmental toxicity and at 250 mg/kg/day signs of maternal toxicity were also evident. Based on these outcomes, the highest dose level for the subsequent main reproductive toxicity study should be lower than 250 mg/kg/day*."

As regards this second DRF study results ECHA observes that at the high dose there was slightly reduced body weight, body weight gain, one abortion, lower gravid uterus weight due to higher post-implantation loss (17.7 vs 1.4% in controls) and statistically significant reduction in litter weight by 31%. While the maternal toxicity was not very high (there was reduced faeces in 6 animals, lower body weight). At mid dose, there was no significant effect on body weight and only transient reduced body weight gain during gestational days 18 and 21 and some statistically non-significant signs of potential foetal toxicity: reduced litter weight by 23%. While this dose level was practically without meaningful maternal toxicity (3 animals with reduced faeces, lower body weight gain).

Based on the DRF you reduced the top dose of the main OECD 414 study from the DRF study top dose of 500 mg/kg to below the mid dose of 250 mg/kg and performed the main study with the highest dose of 200 mg/kg which did not result in maternal (or developmental toxicity). ECHA concludes that given the above DRF findings the doses used in the pre-natal developmental toxicity study were not selected with view to the principles of EU Test Method B.31, OECD TG 414 .i.e. *"the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering."*

In your comments you explain that you selected the dose levels of the main OECD TG 414 study based on the results of the new DRF study (vehicle: corn oil in an aqueous solution of carboxy methyl cellulose), but also taking into account the first DRF study (vehicle: corn oil) and the first OECD TG 414 study (vehicle: corn oil; study terminated due to mortality in all groups including control). Based on this information, you concluded that "*the dose levels of 500, 300 and 250 mg/kg/d were in excess of the maximal tolerated dose. The dose level of 250 mg/kg/d induced, whatever the vehicle used, clear signs of GI tract intolerance associated with a decrease of the body weight, food consumption, net carcass weight, uterus weight and/or litter weight.*" You suspect that the gastro-intestinal (GI) tract effects in the first DRF and OECD TG 414 studies could be at least partly secondary to the use of corn oil as vehicle. Therefore, to avoid this confounding effect of the high corn oil administration, the new DRF study was conducted with corn oil in an aqueous solution of carboxy methyl cellulose.

ECHA agrees that in the first DRF and OECD TG 414 studies (study terminated due to mortality in all groups including control), there was a confounding effect due to the high corn oil administration. Therefore, ECHA considers that this information cannot be used to reliably assess maternal or developmental toxicity. However, assuming that the control group expresses the effect of the vehicle, it may be possible to roughly estimate the potential contribution of the treatment to the GI tract-related effects. The result from the 1st main study indicates that the Substance did not contribute to the reduced faeces or soft faeces, but 20% of the cases for diarrhoea and emaciated appearance could be due to administration of the Substance at 250 mg/kg bw/day.

ECHA further notes that the effects potentially related to GI tract intolerance were very minor at 250 mg/kg bw/day in the new DRF study: reduced food consumption on GD 15-21 (by 23%) and as a consequence slightly reduced body weight gain on GD 18-21 and reduced faeces (3/8 animals). There was no soft faeces or diarrhoea. Net carcass weight was reduced only by 7%. Signs of developmental toxicity were seen as the litter weight was 23% lower than in controls and uterus weight was 20% lower (both statistically non-significant). Thus, ECHA considers that these effects seen at 250 mg/kg bw/day do not indicate that that this dose would be in excess for the purpose to induce toxicity at the highest dose as required in OECD TG 414.

You further state in your comments: "*Therefore, to not take the risk to jeopardise the outcome of the repeated OECD TG 414 study by an unexpected maternal toxicity or an exacerbation of the maternal toxicity due to the higher number of treated females, it was decided to set the top dose-level at 200 mg/kg/d, which is the dose level immediately lower than 250 mg/kg/d and which was expected to be the maximal tolerated dose for a developmental toxicity study in rabbits.*"

You have not defined what you mean by "*the maximal tolerated dose*". However, based on your argumentation and the selection of the NOAEL value, it seems that "*the maximal tolerated dose*" means no adverse effects to you.

However, OECD TG 414 defines the dose level setting in paragraph 14 as follows: "*At least three dose levels and a concurrent control should be used. [...] The dose levels should be spaced to produce a gradation of toxic effects. Unless limited by the physical/chemical nature or biological properties of the test chemical, the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering. At least one intermediate dose level should produce minimal observable toxic effects. The lowest dose level should not produce any evidence of either maternal or developmental toxicity. A descending sequence of dose levels should be selected with a view to demonstrating any dosage-related response and no-observed-adverse-effect level (NOAEL) or doses near the limit of detection that would allow the*

determination of a benchmark dose. Two- to four-fold intervals are frequently optimal for setting the descending dose levels, and the addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages."

In your comments, with regard to the dose level selection, you consider that "*The vague criteria given by the OECD TG give room to interpretations*"[3]. ECHA considers that the aim is to induce some developmental and/or maternal toxicity at the high dose, "*minimal observable toxic effects*" at the mid dose and then the lowest dose to derive the NOAEL, i.e. to demonstrate any possible dosage-related response. ECHA further notes that dose level setting for the highest dose level in OECD TG 414 is not based on "*in excess of the maximal tolerated dose*", "*maximal tolerated dose*", avoiding unexpected toxicity or higher number of affected animals due to higher number of treated animals.

As explained above, ECHA considers that the maternal toxicity seen at 250 mg/kg bw/day does not demonstrate that that the dose would be in excess for the purpose to induce toxicity at the highest dose as required in the OECD TG 414.

In reference to the main OECD TG 414 study, you conclude that "*even if the maternal and fetal effects were not considered to be adverse and the NOAEL for maternal and developmental toxicity was set at 200 mg/kg/day, the effects observed at 100 and 200 mg/kg/d demonstrated that the dams and the fetus were adequately exposed to the test item at a top dose which was the maximal tolerated dose.*" You justify this conclusion by referring to reduced food consumption, slightly reduced body weight gain on GD 6-29, and some body weight losses on GD 18 and/or GD 21. With reference to the new DRF, you present a food restriction study[4] which has shown that the development of the foetuses was affected in dams that consumed 60 g/day although maternal body weight remained stable, and that dams receiving 150 g/day did not show developmental effects.

ECHA notes that in the new DRF study, the food consumption did not drop to 60 g/day at 250 or 500 mg/kg bw/day. The mean food consumption on GD18 and GDs 23-29 seem to be lower than 150 g at 250 mg/kg bw/day. However, on GD 26 the control value is already close to 150 g (158.6 g) and on GD 29 already below 150 g (147.4 g) and the difference between the control value and value at 250 mg/kg bw/day is not large, largest on GD 18 (statistically significant). The lowest value (128.8 g) on GD 18 is only slightly (14%) below the 150 g. The only clinical sign seem to be reduced faeces in 3 out of 8 animals, which is likely to be due to reduced food consumption. Therefore this is not an indication of clear maternal toxicity or "*in excess of the maximal tolerated dose*".

Regarding maternal toxicity in the main OECD TG 414 study, ECHA notes that the food consumption was slightly below 150 g on study days 26-29 in all dose groups, including the controls. The only statistically significant finding was a slight reduction (<10 %) in food consumption on one day only (GD 9) at 100 and 200 mg/kg bw/day. The robust study summary describes that "*the significant and transient food consumption was not considered to be adverse*", "*No significant changes were recorded for the absolute weight gain*" and "*The frequency of the observed clinical signs did not indicate an adverse effect of the test item*". Regarding foetal exposure, in your comments, you agree that the decreased uterus and litter weights as well as number of live fetuses reflect the number of implantations (which take place before the treatment), rather than developmental toxicity. These conclusions do not demonstrate that the animals were adequately exposed, and do not reflect the dose level selection criteria in OECD TG 414. I.e. "*the highest dose should be chosen with the **aim** to*

[3] Beyer BK, et al. (2011) ILSI/HESI Maternal Toxicity Workshop. Summary: Maternal Toxicity and Its Impact on Study Design and Data Interpretation. Birth Defects Research (Part B), 92:36–51
[4] Matsuzawa T, et al. (1981) Dietary deprivation induces fetal loss and abortion in rabbits. Toxicology, 22:255-259

*induce **some developmental** and/or maternal toxicity **(clinical signs or a decrease in body weight)** but not death or severe suffering." (emphasis added)*

ECHA notes further that in the new DRF only external malformations and brain ventricles were investigated in foetuses, therefore the DRF does not inform on the relevance in a 2nd species of the observations in 1st species (rat) on anophthalmia (0, 1, 1, 2 foetuses at 0, 100, 300, 1000 mg/kg bw/day, respectively (OECD TG 414) and malpositioned pelvic girdle 3, 4, 7, 14% in rat foetuses at 0, 100, 300 and 1000 mg/kg bw/day, respectively with a maternal NOAEL of 300 mg/kg bw (OECD TG 414). Neither does it inform on the increased post-implantation loss and lower pup body weight that was observed at 300 and 1000 mg/kg bw/day in the rat OECD 421 study with a developmental NOAEL of 100 mg/kg bw/day (compared to maternal NOAEL based on lower body weight at 300 mg/kg bw/day).

In your comments, you consider that the anophthalmia observed in the rat OECD TG 414 study is incidental and not related to the treatment, because, although there were no cases in the recent historical controls of the labs, this abnormality is known to occur spontaneously up to 1.1% of the foetuses in the rat strain used in the study[5]. ECHA considers that the information on the concurrent control is the primary information to be relied on, followed by the historical controls from the laboratory conducting the study which may be used to help interpretation. In this case the total incidence is high, 4 animals in one study, and the observed malformations cause a concern which needs further clarification to be able to conclude on the developmental toxicity property. Malpositioned pelvic girdle, as you also indicate in your comments, is related to the treatment, but you consider this as non-adverse. Irrespective whether or not the finding is considered as adverse, it indicates changes in development and together with anophthalmia increases the concern for developmental toxicity.

In your comments, you refer to the new DRF study which, associated to "*severe maternal toxicity*", indicated increased post-implantation losses and reduced litter weights at 500 mg/kg bw/day, and that "*No effect was observed at 250 mg/kg/d.*" Based on the absence of such effects in the first DRF (vehicle: corn oil), you consider that the threshold dose level to induce an embryo-foetal toxicity in rabbits is between 300 and 500 mg/kg bw/day, dose level which is higher than "*the maximal tolerated dose*".

As explained above, ECHA considers that in the new DRF study the maternal toxicity seems not to be very high at 500 mg/kg bw/day and at 250 mg/kg bw/day there is practically no meaningful maternal toxicity. Furthermore, as explained above, information from earlier studies with high corn oil administrations cannot be used to assess maternal or developmental toxicity due to confounding effect of the oil administration. Therefore, ECHA cannot agree with your claim that doses between 300 and 500 mg/kg bw/day show severe maternal toxicity, and that these would be higher than the maximal tolerated dose.

In your comments you also question the relevance of a pre-natal developmental toxicity study conducted in rabbits to inform on the decreased pup body weights which were observed on post-natal day 4 in an OECD TG 421 study conducted in rats. ECHA notes that OECD TG 414 includes fetal body weight measurements and therefore, regardless of the species being tested, it gives relevant information on body weight development.

In conclusion, there is a concern for developmental toxicity stemming from the rat prenatal developmental toxicity study (foetuses with anophthalmia and malpositioned pelvic girdle), supported by findings from OECD TG 421 study (increased postimplantation loss and lower

[5] Noritake et al. (2013) Study for collecting background data on Wistar Hannover [Crl:WI(Han)] rats in embryo-fetal development studies--comparative data to Sprague Dawley rats. J Toxicol Sci., 38(6):847-54.

pup body weight). The available relevant dose range finding study (i.e. the new DRF) using less oily vehicle (Ceccatelli, 2018) does not clarify the concern and the relevant main study (Monetini, 2018) does not show any toxicity (adverse effects) and, thus, cannot clarify the concern due to too low dose levels. Furthermore, the results from the new DRF (Ceccatelli, 2018) reveal that the dose level setting for the main study is not according to OECD TG 414.

Consequently, there is still a concern over developmental toxicity that needs to be investigated with a pre-natal developmental toxicity study in a second species (rabbit) with dosing that follows the principles of TG OECD 414.

As detailed above, the request in the original decision was not met, and you are still required to provide a pre-natal developmental toxicity study in rabbits, oral route (test method: EU B.31/OECD 414) using the registered substance subject to the present decision and conforming to the dose selection principles of test guideline OECD 414.

Appendix 2: Procedural history

In accordance with Article 42(1) of the REACH Regulation, the Agency examined the information submitted by you in consequence of decision TPE-D-2114344773-45-01/F. The Agency considered that this information did not meet one or more of the requests contained in that decision. Therefore, a new decision-making process was initiated under Article 40 of the REACH Regulation.

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft of this decision was notified to the Member States Competent Authorities according to Article 51(1) of the REACH Regulation.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

ECHA took into account your comments and did not amend the request.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix 3: Further information, observations and technical guidance

1. This decision does not prevent ECHA from initiating further compliance checks on the present registration at a later stage.

2. The Article 42(2) notification for the original decision is on hold until all information requested in the original decision has been received.