Helsinki, 16 December 2020

Addressees

Registrant(s) of 6,6'-di-tert-butyl-4,4'-butylidenedi-m-cresol listed in the last Appendix of this decision

Registered substance subject to this decision (the Substance)

Substance name: 6,6'-di-tert-butyl-4,4'-butylidenedi-m-cresol
EC number: 201-618-5
CAS number: 85-60-9

Decision number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)

DECISION ON SUBSTANCE EVALUATION

Under Article 46 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below:

A. Information required to clarify the potential risk related to reproductive toxicity and Endocrine disruption

1. Sub-chronic toxicity study (90-day), oral route (test method OECD TG 408; request A1) in rats with the Substance, modified to include liver specific analysis and reproductive parameters; Specific parameters are described in details in Appendix A

Deadline

The information must be submitted by **21 September 2022.**

Conditions to comply with the information requested

To comply with this decision, you must submit the information in an updated registration dossier, by the deadline indicated above. The information must comply with the IUCLID robust study summary format. You must also attach the full study report for the corresponding study in the corresponding endpoint of IUCLID.

You must update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You will find the justifications for the requests in this decision in the Appendices entitled ''Reasons to request information to clarify the potential risk'.

You will find the procedural steps followed to reach the adopted decision and some technical guidance detailed in further Appendices.

Appeal

Applicable only for the adopted ECHA decision: This decision may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information**.**

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Applicable only for the adopted ECHA decision:

Authorised[1] by Christel Schilliger-Musset, Director of Hazard Assessment

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Basis for substance evaluation

The objective of substance evaluation under REACH is to allow for the generation of further information on substances suspected of posing a risk to human health or the environment ('potential risk').

ECHA has concluded that further information on the Substance is necessary to enable the evaluating Member State Competent Authority (MSCA) to clarify a potential risk and whether regulatory risk management is required to ensure the safe use of the Substance.

The ECHA decision requesting further information is based on the following:

(1) There is a potential risk to human health or the environment, based on a combination of hazard and exposure information;
(2) Information is necessary to clarify the potential risk identified; and
(3) There is a realistic possibility that the information requested would allow improved risk management measures to be taken.

The Appendices entitled 'Reasons to request information' describe why the requested information are necessary and appropriate.

ECHA
EUROPEAN CHEMICALS AGENCY

Appendix A – Reasons to request information to clarify the potential risk related to reproductive toxicity and Endocrine disruption

1. Potential risk

1.1 Potential hazard of the Substance

Following its assessment of the available relevant information on the Substance, the evaluating MSCA and ECHA have identified the following potential hazards which must be clarified.

a) Potential specific target organ toxicity (repeated)

The substance causes adverse effects on liver, potentially leading to classification for specific target organ (liver) toxicity after repeated exposure according to the CLP Regulation (EC) No 1272/2008.

The available information shows that the substance induces clear effects on liver:

- In a 28d study available in the registration dossier that has been performed in rats, exposed in the diet at 0, 1000, 2500, 5000 and 10 000 ppm (equivalent to 0, 60, 150, 300 and 600 mg/kg bw/day for males and 0, 50, 125, 250 and 500 mg/kg bw/day), a significant decrease in relative and absolute liver and a discoloration was seen from the lowest dose tested i.e. 1000 ppm (equi. to 50-60 mg/kg bw/day). Histopathological examination shows hepatocellular vacuolation in all exposed dose groups and a hepatocellular degeneration/necrosis at exposure levels from 2500 ppm onward.

- In a 28d study (Yamasaki *et al*., 2008) rats are exposed by gavage to 5, 25 or 125 mg/kg bw/d of the Substance. The relative liver weights is significantly ($p < 0.05$) increased at mid dose (males) and highest dose (males and females). Centrilobular hepatocyte hypertrophy was seen in all rats at the highest dose. Lipid droplets were seen in hepatocytes in one male rat at 5 mg/kg bw/d, in 10 males and 4 females at 25 mg/kg bw/d, and in all rats at the highest dose. There is a significant increase of AST, ALT and ALP in both sexes at 125 mg/kg bw/d.

- In a study by Takahashi and Oishi (2006) conducted in male mice exposed during 2 months to 0.25% of the Substance (equi. to 340-450 mg/kg) in the diet, a significant relative increase of liver weight was observed with fatty liver.

- In the same study (Takahaski and Oishi, 2006) male F344 rats are exposed to a dose equivalent to 159-230 mg/kg of the Substance in the diet, relative liver

weights are approx. 50% higher than the controls and all treated rats had fatty livers ($p < 0.01$).

- In a non-GLP 90d study available in the registration dossier performed in rats, in which animals are exposed to the Substance in the diet, an increased liver weight and microscopic liver and lymph node changes are seen at 50 mg/kg bw/d with altered levels of SGPT and SGOT. For females only, a slight increase is seen at 25 mg/kg bw/d. No effects are seen at the lowest dose (5 mg/kg bw/day).

- In another 90d rat study, animals exposed to the Substance in the diet, at 25-30 mg/kg bw/d, animals had yellowish livers, increased relative liver weight and some fatty infiltration. At the highest dose (250-300 mg/kg bw/day) increased relative liver weight, yellowish livers, fatty infiltration and additionally necrosis was seen.

b) Potential reproductive toxicity

The substance may cause adverse effects on reproduction, potentially leading to classification for reproductive toxicity according to the CLP Regulation (EC) No 1272/2008.

The available information suggest that the substance may have an effect on male reproductive organs, potentially leading to impairment of sexual function and fertility in males.

- The potential hazard is derived from a 2-month study (Takahashi and Oishi, 2006) in which male mice exposed in the diet to 0.06% or 0.125% (equivalent to 80.4-95.4 and 205-206 mg/kg bw/day) of the Substance. The weights of the male sex accessory organs were higher than controls for most organs (absolute weights of epididymides, seminal vesicles, prostate glands, and preputial glands). A statistical significance was reached at 0.125% for testes ($p < 0.05$), seminal vesicles with coagulation glands ($p < 0.05$), prostate glands ($p < 0.01$) and epididymides ($p < 0.01$). At 0.125%, 2 animals exhibited exfoliation of seminiferous tubules and 3 had dilatated lumens; at 0.06%, one animal had presence of giant cells and 2 had dilatated lumens (not significant). Additionally mice exhibited dose-related decreases in daily sperm production (DSP) and DSP/g testis when evaluated ($p < 0.01$).

  In the another experiment conducted by the same authors, mice were exposed to 0.25% (equivalent to 340-450 mg/kg bw/day) of the Substance for 2 months. Relative weights of seminal vesicles with coagulation glands, prostate glands and preputial glands were all lower than controls ($p < 0.05$); at the same dose, absolute

weights of these same organs plus epididymides were also decreased ($p < 0.01$ for prostate glands, $p < 0.05$ for the other organs). Severe exfoliation of seminiferous tubules was observed in 75% of the mice ($p < 0.01$); sloughing of seminiferous tubules was seen in 50% of mice ($p < 0.05$); dilatated lumen of the seminiferous tubules was seen in 75% of the mice ($p < 0.01$) and finally two mice exhibited proliferation of Leydig cells (not statistically significant). The DSP was not measured in this experiment.

- In a second study in the same publication (Takahashi and Oishi, 2006) male F344 rats (8 rats/group) were administered the Substance (purity > 95%) in the diet at 0.06 or 0.25% (~ 40.7-57.7 or 159-230 mg/kg-bw/day) for 2 months. Separate experiments (with two different control groups) were conducted for each dietary concentration.

  At 0.25%, relative testicular weight was significantly increased ($p<0.05$) whereas relative weights of seminal vesicles with coagulation glands and ventral/dorsolateral prostate glands were decreased ($p < 0.01$). Absolute weights of these organs (seminal vesicles, prostate glands, preputial glands, epididymides) were also decreased at this dose ($p < 0.05$ or < 0.1).

  At 0.06%, however, absolute (and some relative) weights of the reproductive organs tended to be higher (instead of lower) than controls, although none were statistically significant. One rat in each of the doses had vacuolated Sertoli cells. Exfoliation of seminiferous tubules was seen in 1 and 7 animals at 0.06 and 0.25%, respectively ($p < 0.01$ at the high dose only). At 0.25%, sloughing of seminiferous tubules was seen in 6 animals ($p < 0.01$), and 5 rats exhibited disappearance of germ cells. DSP and DSP per gram testis was slightly decreased at 0.25% ($p < 0.05$).

- You provided a study conducted according to OECD TG 421 in which Wistar rats were exposed to the Substance via gavage with 0, 100, 300 and 1000 mg/kg bw/d. No reproductive effects were observed except one female at 300 mg/kg bw/d died during delivery with dilatation and prolapsed vagina and nodule on clitoral gland and 2 fetuses found in the right uterine horn. Additionally, the ratio testes/bw was reported to be significantly lower at 300 mg/kg bw/d due to one animal.

- A substance that can be considered as an analogue of the Substance, the 6,6'-di-tert-butyl-2,2'-methylenedi-p-cresol (DBMC) was evaluated by Denmark in 2016. The substance evaluation was concluded in 2017 and classification was adopted by

the RAC in June 2019. DBMC is now classified as Repro 1B H360F based on one reproductive/developmental toxicity screening study conducted according to OECD TG 421 and GLP in rats, and seven repeated dose toxicity studies in rodents not performed according to OECD Test Guidelines, and with only one performed according to GLP. In the repeated dose toxicity studies (six studies in rats, one study in mice and one study in dogs) the exposure ranged from 28 days to 18 months and male reproductive endpoints including testes histopathology and/or sperm parameters were assessed. The reproductive/developmental toxicity screening study in rats and the repeated dose toxicity studies in rats and mice consistently showed dose-related adverse effects on male sexual function and fertility following exposure to DBMC. These included severely reduced testes and epididymis weights, testis tubules atrophy, spermatogenic arrest and changes in sperm motility, viability and morphology. These effects were reported in rat studies ranging from 28 day to 18 months exposure at dose levels from approx. 40 mg/kg bw/d.

Similar effects were also reported in the mouse study following a 2-month exposure to one dose of DBMC (mean dose of 414 mg/kg bw/d). The adverse effects in rats on male sexual function and fertility following DBMC exposure were reported from 40-88 mg/kg bw/d. At these dose levels no to moderate general toxicity (reduction in body weights of 0-9% across the studies and relative liver weight increases of 0% to 30%) were reported. The effects observed in male reproductive parameters at these doses were considered therefore considered not to be secondary or non-specific consequences of other toxicity. It was concluded that DBMC directly affected male sexual function and fertility.

These effects seen in an analogue substance are considered to emphasise the concern for reproductive toxicity identified for the Substance evaluated.

- Regarding another possible analogue, 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol, evaluated by Austria in 2015, it was concluded that testis and spermatogenic tissue is a target of the substance and that the underlying mode of action has not been clarified, but an endocrine mode of action cannot be excluded.

The effects on reproductive organs observed following exposure to the Substance are of concern. However, the available data report them only in the presence of severe steatosis. There is no data available to evaluate if the reprotoxicity observed is secondary or independent of steatosis. Therefore, the available and current information is not sufficient to draw a conclusion on the hazard. Exposure to the Substance leads to effects on

reproductive organs. However, these effects are described only in presence of severe steatosis, or in studies in which effects on liver are not assessed. Further information is then needed to evaluate in detail if effects on reproduction can occur before or at lower doses than liver toxicity.

c) Potential endocrine disrupting properties

According to IPCS/WHO (2002), "*An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations*".

Based on this definition, the Substance may be an endocrine disruptor if the following conditions are met:

- it shows adverse effects(s) in (an intact) organism, or its progeny, or (sub)population;
- it shows endocrine activity, *i.e.* it has the potential to alter the function(s) of the endocrine system; and
- there is a biologically plausible link between the adverse effects and the endocrine activity, *i.e.* the Substance has an endocrine disrupting mode of action (ED MoA).

The available information suggests that the potential adverse effects on reproduction, as described in 1.1 above may result from endocrine activity and therefore the Substance may have an ED MoA.

Evidence of endocrine activity based on in vitro studies

- Assays investigating estrogenic, androgenic and steroidogenesis activity

The Substance was tested (Takahaski *et al*., 2006) in a test to assess the binding to ERalpha using a competitive binding with E2. IC_{50} of the Substance was 7.3 x 10^{-6} M. It is a high value although it is twice lower than the one measured with BPA (1.4 x 10^{-5} M)..

Additionally, in the framework of the United States Environmental Protection Agencies' Toxicity Forecast (Toxcast) (US EPA, 2019) the Substance was tested in 24 *in vitro* tests relevant for (anti-)estrogenic, (anti-) androgenic activity or activity on steroidogenesis in the US EPA ToxCast Program[2]. 11 tests out of 12 were positive for ER, 8 tests out of 10

[2] https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID8029602#invitrodb-bioassays-toxcast-data

for AR and 2 out of 2 for steroidogenesis but only at doses above cytotoxicity. These data are therefore considered irrrelevant.

When running the Danish QSAR database and the OECD QSAR toolbox it appears that there is a very strong binding to the ER receptor.

- Assays investigating thyroid activity

Positive results were seen in three out of 6 *in vitro* studies relevant for thyroid activity, conducted in the US EPA ToxCast Program. These tests investigated thyroid stimulating hormone receptor (TSHR) activity and thyroid hormone receptor (TR).

Evidence of endocrine activity based on in vivo studies

- In a uterotrophic assay performed by Takahashi and Oishi (2006), ovariectomised adult female mice (8 mice/dose) were administered the Substance at 0.06 and 0.25% (approximately 62.4-78.6 and 375-383 mg/kg-day) in the diet for 2 months. Mice were killed and uteri were dissected and weighed at termination of feeding. Body weight and food consumption were reported. In addition, radiation body temperature was measured at 3 weeks, and parametrial fat was weighed at necropsy. Absolute and relative uterine weights were increased at 0.25% ($p < 0.01$ or < 0.05, depending on statistical test). Absolute and relative parametrial adipose tissue weight was decreased at the high dose ($p < 0.01$), and body temperatures were decreased at both doses ($p < 0.05$).
  Ovariectomised adult female mice were exposed in a second experiment, by sub-cutaneous route, to 60 or 300 mg/kg bw/day of the Substance. Uterine wet weight was dose dependently increased after exposure to the Substance and the difference with control became statistically different in the 300 mg/kg bw/day group.

- In a study by Yamazaki *et al.* (2008) Sprague-Dawley rats (Crj: CD) (10/sex/concentration) were administered the Substance (98.5% purity) via gavage at 0, 5, 25 and 125 mg/kg-day for at least 28 days. At 125 mg/kg-day, serum thyroid-stimulating hormone was increased (both sexes) and serum triiodothyronine were decreased in males and thyroxin in both sexes ($p < 0.05$). Thyroid weights were increased in female rats at 125 mg/kg-day (and slightly increased in males at this dose; significance not stated). Follicular epithelial cell hypertrophy was seen in thyroids in 4 females at 125 mg/kg-day.

- Regarding the 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol (the analogue substance evaluated in 2015 by Austria), it was tested in two guideline studies: A rat OECD TG 407 study (Anonymous, 1995) and a rat OECD TG 421 study (registration dossier, 2010). Both was rated Klimisch 2 by Austria due to minor deficiencies and due to insufficient reporting.

  Further, the substance was investigated in the US National Toxicology Program in 1994 (NTP, 1994) resulting in a 15 day, a 13 week and a 2-year chronic study in mice and rats each. Another rat chronic study, with 3 and 6 months exposure, was conducted at the however, reporting was rather poor (e.g. the date of the study is not available) publication date unclear). No fertility study is available.

  Several mechanistic studies and analyses are available including two 14-day studies in female mice: Munson *et al.* (1988) evaluated the general toxicity of the substance and Holsapple *et al.* (1988) investigated several immunotoxicity parameters. The two studies should be considered together, as they were carried out at the same laboratory, more or less at the same time period. Takahashi & Oishi (2006) made a detailed analysis of male reproductive parameters and several uterotrophic assays.

  6,6'-di-tert-butyl-4,4'-thiodi-m-cresol was one of 81 substances included in an evaluation exercise intended to evaluate and improve the Hershberger assay within the OECD frame work, however, information on the exact mandate of the study is missing and no detailed information on the study is available (the report only contains a list of the substances indicating whether (anti-)androgenic activity was observed or not (METI, -2010).

  Further there are two published reviews of NTP studies. Yoshizawa *et al.* (2005) focused on the analysis of atrial thrombosis, which was seen in 13 substances of 500 investigated in the NTP program; one of these 13 substances was 6,6'-di-tert-butyl-4,4'- thiodi-m-cresol. Some information on the dose response curve and the degree of the effect can be read from this review, Another review of NTP studies (Morrissey, 1988) focused on parameters related to male and female fertility (sperm parameters, vaginal cytology and reproductive organ weights). Unfortunately, the review only reports whether an effect was seen or not, with no information on degree or at which dose the effect occurred.

  Overall Austria concluded that the database was rather old, leaving many questions open. No comprehensive modern high quality study is available and especially the information on reproductive toxicity is scarce, and therefore an EOGRTS was requested for this analogue substance 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol.

Taken together the available information suggest that the adverse effects on reproduction may be a consequence of endocrine (hormonal) activity. However, these effects are also reported in presence of severe steatosis. Based on all the information available it is difficult to conclude if the reproductive effects seen could be secondary to the effects observed on liver.

The available information is not sufficient to conclude on the potential hazard. Therefore, a well-conducted 90-day study is needed to be able to properly discriminate between the effects on liver and the one on reproductive function and fertility.

In a later stage, additionnal test(s) may be requested depending on the results of the 90–day study if some uncertainties remain regarding first the effects on reproduction but also on the thyroid activity.

1.2 Potential exposure

According to the information you submitted in the registration dossiers, the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of 100 – 1000 tonnes per year.

You also reported that among other uses, the Substance is used ain articles by consumers, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing processes.

The Substance is used in coating products, polymers, adhesives and sealants. The substance is used in building & construction work, formulation of mixtures and/or re-packaging areas.

Release to the environment of this substance can occur from industrial uses: industrial abrasion processing with low release rate (e.g. [redacted]). Other release to the environment of this substance is likely to occur from: indoor use in long-life materials with low release rate (e.g. [redacted]) and outdoor use in long-life materials with low release rate (e.g. [redacted]). This substance can be found in complex articles, with no release intended: [redacted]. This substance can be found in products

with material based on: plastic [redacted].

Therefore, significant exposure to workers, consumers, industrial workers and to a lower rate environment cannot be excluded.

1.3 Identification of the potential risk to be clarified

Based on the weight of evidence of all the available data, there is sufficient evidence the Substance may cause adverse effects on liver and on reproduction (sexual function and fertility), but the effects on reproduction may be secondary to liver effects. The endocrine disruptor (ED) concern cannot be evaluated based on the available information.

The information you provided on manufacture and uses demonstrates a potential for exposure of industrial workers, professionals and consumers.

Based on this hazard and exposure information the substance poses a potential risk to human health.

As explained in Section 1.1 above, the available information is not sufficient to conclude on hazard. Consequently, further data is needed to clarify the potential risk related to specific target organ toxicity after repeated exposure, reproductive toxicity and endocrine disruption.

1.4 Further risk management measures

Based on the adverse effects observed on liver the evaluating MSCA will analyse the options to manage the risks and whether the substance should be classified for specific target organ toxicity after repeated exposure according to the CLP Regulation.

The requested study will allow to evaluate the interlink between the hepatotoxicity and the reproductive effects observed in the available information. If the adverse effects on reproductive function and fertility are confirmed, independently of the liver effects, the evaluating MSCA will analyse the options to manage the risks and whether the substance should be classified for reproductive toxicity according to the CLP Regulation.

Since the Substance does not currently have any harmonised classification, the possible classification would result in stricter risk management measures such as improved measures at manufacturing sites and revised instructions on safe use, if appropriate.

If the study allows to show that the Substance interacts with hormones in such a way that explain the adverse effects observed elsewhere, the Substance would then be considered as an endocrine disruptor according to the IPCS/WHO definition (IPCS/WHO 2002). The evaluating MSCA will then:

- assess the need for further regulatory risk management in the form of identification as a substance of very high concern (SVHC) under Article 57 of REACH, and
- consider the need of a subsequent authorisation or a restriction of the Substance. This would lead to stricter risk management measures than those currently in place.

2. How to clarify the potential risk

2.1 Development of the testing strategy

As explained is Section 1.1 above, the available information is not sufficient to conclude on the potential hazard. Therefore in order to conclude if the reproductive effects seen could be secondary to the effects observed on liver or on a potential ED MoA, further information is needed. Therefore, a well-conducted 90-day study is needed to be able to properly discriminate between the effects on liver and the one on reproductive function and fertility. Additional parameters have been added (see section 2.2b) to also be able to assess the effect on thyroid and evaluate how endocrine system is impacted by the substance.
In a later stage, additional test(s) may be requested depending on the results of the 90–day study if some uncertainties remain regarding first the effects on reproduction but also on the thyroid activity.

2.1 Request A.1: Repeated Dose 90-Day Oral Toxicity Study in Rats (test method: OECD TG 408)

a) Aim of the study

As detailed in Section 1.1 above, information on specific target organ toxicity after repeated exposure, endocrine activity and adverse effects on reproduction (potentially endocrine mediated) is required to conclude on the potential hazard. In particular, the information resulting from Request A.1 will provide information on the link between these effects and if some are the consequence of the others. Therefore a study is required that investigates both types of effects (hepatotoxicity and reprotoxicity) at the same time and enables also the establishing of the ED MoA.

The evaluating MSCA will review the information you submitted as an outcome of this study, and evaluate whether further information is still needed to clarify the potential risk for reprotoxicity and/or endocrine disruption.

The OECD TG 408 is a standard information requirement at Annex IX and Annex X, Section 8.6.2 of REACH that may be subject to a compliance check under Article 41 of REACH. Since you have registered the Substance at the Annex IX level, the study is a standard information requirement for which a compliance check could have been launched. Nevertheless, the study requested in this decision includes specific parameters which do not enter in the scope of a compliance check. Additionally, the information requested aims at clarifying the potential risk that the Substance poses. These two reasons led the eMSCA to request the study of interest under the current substance evaluation process.

b) Specification of the requested study

Route of exposure

The substance must be administered orally by gavage and dissolved in a relevant solvent because of its very low solubility.

Dose level setting shall aim to induce systemic toxicity at the highest dose level

The top dose or concentration must be sufficiently high to give clear systemic toxicity in order to ensure that a wide range of exposures (high to low) is tested.

In order to be compliant and not to be rejected due to too low dose levels, the highest dose level shall induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the effects on liver. A descending sequence of dose levels should be selected in order to demonstrate any dose-related effect and to establish NOAELs. The selection of doses should be justified (meaning that You have to provide a justification with your study results that demonstrates that the dose level selection meets the conditions described above) and based on a range-finding study, conducted prior to the main study.

The range-finding results have to be reported with the main study.

Additional parameters

- As all available dose-repeated studies performed with the Substance induce severe effects in liver, specific parameters should be measured in order to assess and quantify the effect on liver. Therefore the following analysis should be included:
    - A red oil coloration in the histology analysis in order to evaluate the extend of the fatty liver
    - A measurement of the enzymatic activity of UGT, SULT and deiodinases
    - A measurement of the expression of CAR PXR and PPAR LXR
- In order to properly assess the fertility impairment and the effects on reproduction for both sexes the following additional parameters should be included:
    - Analysis of the oestrus cycles
    - In addition to total T4, T3 and TSH the following hormones should also be measured:
        - estradiol, FSH and LH during the diestrus phase in order to limit the influence of the oestrus cycle on their concentration
        - testosterone
    - Regarding testes:
        - weight of the right testis and measurement of the daily sperm production (DSP)
        - General histological aspect of the left testis including the percentage of the area occupied by the seminiferous tubules
        - Histology of the seminiferous tubules : thickness of the seminiferous epithelium, diameter of the lumen, exfoliation, number of giant cells, percentage of each of the 12 stages of the spermatogenic cycle
    - Regarding epididymis:
        - In caput epididymis: Weight, Spermatozoa counting, Spermatozoa morphology (percentage of abnormal forms), Round cells counting.
        - In caudal epididymis: Weight, Spermatozoa counting, Spermatozoa morphology (percentage of abnormal forms), Spermatozoa mobility.
    - Weight of all male accessory organs: ventral prostate, seminal vesicles (plus fluids and coagulating glands), levator ani-bulbocavernosus muscle, paired Cowper's glands, glans penis.

- In order to assess the impairment of the thyroid function, the following analysis should be performed:
    - Histology of the thyroid gland
    - A specific measurement of i.v. of radiolabelled T4 in order to measure the clearance rate of T4.

Request for the full study report

You must submit the full study report, which includes:

- a complete rationale of test design and
- interpretation of the results
- access to all information available in the full study report, such as implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.

This will enable the evaluating MSCA to fully and independently assess all the information provided, including the statistical analysis, and to efficiently clarify the potential hazard for the Reproductive toxicity and endocrine disruptive properties of the Substance.

c) Alternative approaches and how the request is appropriate to meet its objective

The request for a Repeated Dose 90-Day Oral Toxicity Study in Rodents (Test method: OECD TG 408) is:

- Appropriate, because it will provide information which will clarify potential adverse effects on liver, reproduction (sexual function and fertility) and related potential endocrine activity. This will enable the evaluating MSCA to conclude on potential classification for toxicity on specific target organ after repeated exposure, on reproduction and on a potential ED MoA;
- The least onerous measure, because there is no equally suitable alternative method available to obtain the information that would clarify the potential hazard, without using test on vertebrate animals.

2.2 References relevant to the requests

Anonymous (1995). Study report.

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immunotoxicological evaluation of 4,4¢-thiobis-(6-tbutyl-m-cresol) in female B6C3F1 mice, 2, Humoral and cellmediated immunity, macrophage function, and host resistance. Fundam Appl Toxicol 10:701–716.

National Toxicology Program (NTP) (1994) Technical report on the toxicology and carcinogenesis studies of 4,4¢-Thiobis(6-t-butylm-cresol) in F344/N Rats and B6C3F1 Mice (Feed studies) (Technical Report Ser. no. 435). Public Health Service, NC.

Morrissey RE, Schwetz BA, Lamb JC 4th, Ross MD, Teague JL, Morris RW. Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies. Fundam Appl Toxicol. 1988 Aug;11(2):343-58.

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Takahashi and Oishi (2006). Male reproductive toxicity of four bisphenol antioxidants in mice and rats and their estrogenic effect. Archives of Toxicology. 80:225-241.

The Ministry of Economy Trade and Industry (Japan METI) 2010, OECD Test Guideline Programme animal testing (Uterotrophic assay, Hershberger assay, enhanced 407 Test Guideline, in utero and lactational exposure study, two-generation reproductive toxicity study).

Yamasaki *et al*. 2008. Uterotrophic assay, Hershberger assay, and subacute oral toxicity study of 4,4'- butylidenebis(2-tert-butyl-5-methylphenol) and 3-(dibutylamino)phenol, based on OECD draft protocols. Arch. Toxiocol. 82:301-311.

Yoshizawa K., Kissling E. G., Johnson J.A., Clayton N. P., Flagler N. D., Nyska A. (2005), Chemical-Induced Atrial Thrombosis in NTP Rodent Studies, Toxicologic Pathology, 33:517–532, 2005.

Appendix B: Procedure

This decision does not imply that the information you submitted in your registration dossier(s) are in compliance with the REACH requirements. ECHA may still initiate a compliance check on your dossiers.

12-month evaluation

- Due to initial grounds of concern for PBT/vPvB, reprotoxicity and for Endocrine disruption, the Member State Committee agreed to include the Substance (EC No 201-618-5, CAS RN 85-60-9) in the Community rolling action plan (CoRAP) to be evaluated in 2019. The Competent Authority of France is the evaluating member state appointed to carry out the evaluation.
- In accordance with Article 45(4) of REACH, the evaluating MSCA carried out its evaluation based on the information in the registration dossier(s) you submitted on 6,6'-di-tert-butyl-4,4'-butylidenedi-m-cresol and on other relevant and available information.
- The evaluating MSCA completed its evaluation considering that further information is required to clarify the following concerns: Specific target organ toxicity (repeated), reprotoxicity and Endocrine disruption
- Therefore, it submitted a draft decision (Article 46(1) of REACH) to ECHA on 19 March 2020.

Decision-making

ECHA notified you of the draft decision and invited you to provide comments.

The decision making followed the procedure of Articles 50 and 52 of REACH as described below. For the purpose of the decision-making, this decision does not take into account any updates of your registration dossier after the end of the 12-month evaluation period i.e. after 19 March 2020.

(i) Registrant(s)' commenting phase

ECHA did not receive any comments from you by the end of the commenting period.

(ii) Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposal for amendment to the draft decision and modified the draft decision (see Appendix A).

ECHA referred the draft decision to the Member State Committee.

ECHA invited you to comment on the proposed amendment.

You did not provide any comments on the proposed amendment(s).

(iii) MSC agreement seeking stage

The Member State Committee reached a unanimous agreement in its MSC-72 written procedure and ECHA took the decision according to Article 52(2) and Article 51(6) of REACH.

After the deadline set in this decision has passed, the evaluating MSCA will review the information you will have submitted and will evaluate whether further information is still needed to clarify the potential risk, according to Article 46(3) of REACH. Therefore, a subsequent evaluation of the Substance may still be initiated after the present substance evaluation is concluded.

Appendix C: Technical Guidance to follow when conducting new tests for REACH purposes

Test methods, GLP requirements and reporting

Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[3].

Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

1. *Selection of the Test material(s)*

   The Test Material used to generate the new data must be selected taking into account the following:

   - the variation in compositions reported by all members of the joint submission,
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

[3] https://echa.europa.eu/practical-guides

2. *Information on the Test Material needed in the updated dossier*

   a) You must report the composition of the Test Material selected for each study, under the 'Test material information' section, for each respective endpoint study record in IUCLID.

   b) The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested, in this case.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual "How to prepare registration and PPORD dossiers"[4].

[4] https://echa.europa.eu/manuals