Helsinki, 23 March 2017

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DECISION ON SUBSTANCE EVALUATION PURSUANT TO ARTICLE 46(1) OF REGULATION (EC) NO 1907/2006

For 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol, CAS No 118-82-1 (EC No 204-279-1)

Addressees: Registrant(s)[1] of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol (Registrant(s))

This decision is addressed to the Registrant(s) of the above substance with active registrations pursuant to Article 6 of the REACH Regulation on the date on which the draft for the decision was first sent for comments. If Registrant(s) ceased manufacture upon receipt of the draft decision pursuant to Article 50(3) of the REACH Regulation, they did not become addressee(s) of the decision. A list of all the relevant registration numbers of the Registrant(s) that are addressees of the present decision is provided as an Annex to this decision.

Based on an evaluation by Umweltbundesamt GmbH on behalf of the Competent Authority of Austria (evaluating MSCA), the European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 52 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

This decision is based on the registration dossier(s) on 10 July 2015, i.e. the day until which the evaluating Member State granted an extension for submitting dossier updates which it would take into consideration.

This decision does not imply that the information provided by the Registrant(s) in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossier(s) of the Registrant(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

I. Procedure

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of Austria has initiated the substance evaluation for 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol, CAS No 118-82-1 (EC No 204-279-1) based on registration(s) submitted by the Registrant(s) and other relevant and available information and prepared the present decision in accordance with Article 46(1) of the REACH Regulation. The substance 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol is hereafter abbreviated as TBMD.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to Environment/Suspected PBT/vPvB, Potential endocrine disruptor, Suspected CMR, Suspected sensitiser, Exposure/Wide dispersive use, Consumer use,

[1] The term Registrant(s) is used throughout the decision, irrespective of the number of registrants addressed by the decision.

Exposure of workers, Exposure of environment, TBMD was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2014. The updated CoRAP was published on the ECHA website on 26 March 2014. The Competent Authority of Austria was appointed to carry out the evaluation.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding Environment/terrestrial toxicity and soil toxicity.

The evaluating MSCA considered that further information was required to clarify the following concerns: Suspected CMR, Potential endocrine disruptor, Environment/ Suspected PBT/vPvB, Environment/ terrestrial toxicity and soil toxicity. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 26 March 2015. No data are requested for the other concerns at this stage: Suspected sensitiser, Exposure/Wide dispersive use, Consumer use, Exposure of workers, Exposure of environment, as they will be reassessed after receipt of the data requested. Further data requests cannot be excluded after provision and evaluation of requested data at this stage.

On 6 May 2015 ECHA sent the draft decision to you and invited you pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

Registrant commenting phase

By 12 June 2015 ECHA received comments from you of which it informed the evaluating MSCA without delay. The evaluating MSCA considered the comments received from you.

On basis of this information, the deadline in Section II was amended and the Statement of Reasons (Section III) was changed accordingly.

Commenting by other MSCAs and ECHA

In accordance with Article 52(1) of the REACH Regulation, on 8 September the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, five Competent Authorities of the Member States and ECHA submitted proposals for amendment (PfAs) to the draft decision.

On 14 October 2016 ECHA notified you of the proposals for amendment to the draft decision and invited you pursuant to Articles 52(2) and 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

The evaluating MSCA reviewed the proposals for amendment received and amended the draft decision accordingly.

Referral to Member State Committee

On 24 October 2016 ECHA referred the draft decision to the Member State Committee.

By 14 November 2016, in accordance to Article 51(5), you provided comments on the proposals for amendment. In addition, you provided comments on the draft decision. The

Member State Committee took your comments on the proposals for amendment into account. The Member State Committee did not take into account your comments on the draft decision that were not related to the proposals for amendment made and are therefore considered outside the scope of Article 51(5).

After discussion in the Member State Committee meeting on 12–16 December 2016, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 16 December 2016. ECHA took the decision pursuant to Article 52(2) and Article 51(6) of the REACH Regulation

Following your comments and Committee discussions the requests for the Amphibian Metamorphosis Assay, terrestrial- and sediment toxicity testing were removed. However, in the follow-up process the need for further testing will be considered once the results of the currently requested tests are available.

II. Information required

Pursuant to Article 46(1) of the REACH Regulation you shall submit the following information using the indicated test methods (in accordance with Article 13 (3) and (4) of the REACH Regulation) and the registered substance subject to the present decision:

Concerns on endocrine disruption and reproductive toxicity

1. **Extended One Generation Reproduction Toxicity Study (OECD TG 443) in rats (oral route), specified as follows:**
   - i. **At least two weeks premating exposure duration for the parental (P0) generation;**
   - ii. **Dose level setting shall aim to induce some toxicity at the highest dose level;**
   - iii. **Cohort 1A (Reproductive toxicity);**
   - iv. **Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation;**
   - v. **Cohorts 2A and 2B (Developmental neurotoxicity); and**
   - vi. **Cohort 3 (Developmental immunotoxicity).**

Concern on persistency, bioaccumulation and toxicity (PBT)

2. **Soil simulation testing (test method: Aerobic and anaerobic transformation in soil, EU C.23/OECD 307)** (as further specified in Section III.2) using radioactively ^{14}C ring-labelled test substance. The kinetic part of the test shall be conducted at 12°C. For the identification of potential metabolites 20°C shall be used.

Deadline for submitting the required information

Pursuant to Article 46(2) of the REACH Regulation, you shall submit to ECHA by **1 July 2019** an update of the registration(s) containing the information required by this decision[2], including robust study summaries and, where relevant, an update of the Chemical Safety Report.

[2] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

III. Statement of reasons

1) Concerns on endocrine disruption and reproductive toxicity

Extended One Generation Reproduction Toxicity Study (OECD TG 443) (request 1)

The Concern(s) Identified

This study is requested to clarify the observed effects on the endocrine system and concerns for reproductive toxicity.

In a good quality 28-day toxicity study in rats ([redacted]) increased thyroid weight and thyroid hyperplasia, as well as increased liver, ovary and kidney weights were reported. The observed effects indicate that TBMD induces hormonal imbalance and affects thyroid homeostasis. As concluded by the study authors the observed thyroid effects could be caused by a negative feedback mechanism ascribable to reduced circulating thyroid hormone. The observed increase in liver weight supports this theory as the reduced thyroid hormone levels might result from liver enzyme induction leading to increased hormone catabolism. Some liver associated serum parameters were also affected and sporadic decrease in serum total cholesterol levels were reported in this study.

It should be noted that in males the absolute thyroid weight was still statistically significantly increased after the recovery period (+10.4%), the relative weight was also increased (+13.9%) but not statistically significant. Relative and absolute thyroid weights in females were comparable to controls after the recovery period. Absolute and relative ovary weights were still increased after the recovery period (absolute + 24.6%; relative + 22.4%) but not statistically significant.

Despite some recovery of some organ weights, the considerable ovary weight increase at the two highest doses (200 & 1000 mg/kg bw/day) tested (ranging from 34% to 41%) represents a significant effect on this hormone dependent tissue.

The effects seen in the 28-day rat study are supported by further repeated dose toxicity studies in rats and dogs. In a 2-year dog study ([redacted]) of restricted reliability significant increase of liver weights and ovary weights were reported (at the 6 months time interval at the highest dose of 75 mg/kg bw/day the ovary weight increased as high as 196% and 139% for the absolute weight and relative weight, respectively).

It should be noted that all test groups in this study consisted of 3 dogs per sex each, which is less than recommended in the OECD 409, where at least 4 dogs per sex per group are recommended (OECD 409 details the 90 day dog study, which is also relevant for conducting a chronic dog study as stated in OECD TG 453, chronic toxicity/carcinogenicity studies in rodents). At the 6 months time interval in the [redacted] study no dogs had died and the groups consisted of 3 dogs each. At this time the significant ovary weight increase was seen in the high dose females. At 2 years an increase in ovary weight (not statistically significant) was seen in the mid dose females, but not in the high dose females, where only 2 dogs were left (one had died after 14 months).

According to OECD 409 the animals should be between 4 and 6 months of age but not older than 9 months when dosing is started. In this study the animals were between 10 and 11 months of age. Although the tested animals were too old for the study to be in line with OECD 409 it can be assumed that all animals have been sexually mature by the 6 months time interval, and comparison of organ weights was therefore meaningful.

In this study also histopathological changes of the liver (fatty inclusions, fibrotic changes, cirrhosis), effects on liver associated blood biochemical parameters (after 2 years exposure)

and a dose and time dependent decrease of serum cholesterol values were detected. At 75 mg/kg bw/day serum protein bound iodine was lowered, indicating reduced thyroid hormone levels. Also some microscopic findings were reported in the thyroid. At the 6 month sacrifice the following microscopic effects were reported for the thyroid: moderate lymphocytic infiltration in 1/6 animals in the low and mid dose groups and 1/6 animals had moderate struma lymphomatosa in the high dose. After 2 years (or 27 months) struma lymphomatosa was seen in 1/4 of the animals. No microscopic findings were reported in the females.

Another 90-day study in dogs ( ) and a 90-day and a 2-year study in rats ( ) are of poor quality, but also in these studies effects on the liver (rat and dog) and reduced cholesterol levels (dog, in the two rat studies cholesterol levels were not determined) were reported. These observations further support the relevance of effects seen in the 28-day rat study ( ) and the 2-year dog study ( ).

Five studies addressing the endpoint toxicity to reproduction are part of the registration dossiers: A good quality developmental/reproductive toxicity screening test in rats (OECD 421,), a developmental/reproductive toxicity screening test in rats of restricted reliability ( ), a rat and a rabbit developmental toxicity study of restricted reliability and and a poor quality three generation study in rats ( ).

In contrast to you the evaluating MSCA classifies the three generation study by as not reliable (Klimisch 3 instead of Klimisch 2). The reason for this is that the study has several deficiencies. Among others, it was not conducted according to GLP, the animals (including control animals) were affected by disease (reported in terms of occasional anorexia, loss of body weight and rales in the lungs; several animals died from pneumonia), several parameters were not evaluated which are required nowadays (no ano-genital distance, no nipple retention, no preputial separation/vaginal patency, no serum T4/TSH, no functional observation battery, only gross-pathological analysis of reproductive tract - but no histopathological analysis, no organ weight, no sperm parameters, no estrous cycle analysis) and generally the study results are poorly reported. For example it is mentioned that 3 dams died from dystocia, but it is not reported at which doses or at which generation these effects occurred and no dose response can be derived. Further it is not mentioned whether dystocia also occurred in other dams, but at least in one dam difficulties in giving birth are reported. Dystocia can be caused by a multitude of reasons (including hormonal imbalance and overt toxicity). In case hormonal imbalance is the underlying cause for the effects this should be considered as adverse effect on reproduction. However, the cases of dystocia were not reported adequately and given the poor quality of the study it is not possible to draw any reliable conclusions on mode of action.

In the course of the present substance evaluation the evaluating MSCA noticed that TBMD was tested for its potential use as hypocholesterolemic drug in the 1970-ies, based on a reference contained in the 2-year dog study ( ) TBMD was found to be an effective hypocholesterolemic agent in humans at 7 mg/kg bw/day. Based on this reference the evaluating MSCA asked you for possibly available human data. In turn you submitted two human 28 day studies ( ) and a 42 day study in rhesus monkey ( ). While the cholesterol levels were clearly reduced in the human studies, no such effect was noticed in the non-human primate study. It should be noted, that in one of the human studies ( ) liver toxicity (i.e. increased serum glutamic pyruvic transaminase (SGPT) levels) was reported at a dose corresponding to ~7mg/kg bw/day.

Several *in vitro* data were generated for TBMD and published in the open literature (Blair et al, 2000; Miller et al., 2001; Kitagawa et al., 2003) and the US EPA selected TBMD as a screening substance in their ToxCast programme (see US EPA,

http://www.epa.gov/actor/ToxCastDB; for description of ToxCast see e.g. also Judson et al. 2014).

Particularly, several tests regarding the estrogenic activity were performed, which were negative. Also a test for thyroid hormone receptor binding was negative. In the ToxCast programme 159 *in vitro* tests covering a broad range of different mechanisms of action were carried out for TBMD. Of these, only 4 were positive. These results could indicate that TBMD is a rather inactive substance, but it cannot be ruled out that the substance was not sufficiently soluble and available to the cells to give positive results in these tests as TBMD has a very low water solubility (33 ng/L) and is highly adsorptive (Koc > 42700 L/kg, ([redacted]). Nevertheless, TBMD produced positive results for pregnane X receptor (PXR) activity, pregnane X receptor response element activity and for retinoid X receptor (RXR) activity which are relevant for the thyroid axis.

The pregnane X receptor activity for which two in vitro tests were positive is known to activate the UDP-glucuronosyl transferase (UDP-GT) and sulfotransferases (SULT) which results in an increase in the rate of metabolism and excretion of T4 and T3. The Retinoic X Receptor beta (RXRb) activity may be a relevant target for thyroid effects due to heterodimerisation of RXRb inter alia with thyroid hormone receptor and consequent downstream effects, e.g. in fetal brain development.

Additionally, a test for steroid receptor coactivator-1/ farnesoid X receptor (FXR) heterodimerisation was positive. FXR regulates lipid metabolism in the liver.

The effects on thyroid, liver and ovary seen in the 28 day rat study ([redacted]) and the 2-years dog study ([redacted]) indicate a possible endocrine Mode of Action. As mentioned in the original study report of [redacted] a negative feedback mechanism ascribable to reduced circulating thyroid hormone could explain the observed thyroid effects. However, no serum levels of thyroid hormones (T3, T4 or TSH) were determined in any of the available studies. The effects on ovaries and potential adverse effects on the birth process (dystocia) seen in a low quality 3-generation study indicate that TBMD could interfere with steroidogenesis. On the other hand, induction of liver enzymes might also lead to increased degradation of steroid hormones, which might also interfere with the HPG axis.

In response to the initial draft decision you made further studies available to the evaluating MSCA. These studies seem to have been generated in the course of the FDA application for the use of TBMD as hypocholesterolemic drug. You indicated that these studies were outdated and not relevant for the assessment of TBMD.

The evaluating MSCA examined the newly submitted studies and identified the following studies as relevant for the current substance evaluation of TBMD:

A series of *in vitro* studies using liver slices and homogenates of dogs investigated the interference of TBMD (solved in Tween 80) with the cholesterol synthesis pathway (Pre-clinical & clinical information, 1966). They propose that the pathway is interrupted at the pre-mevalonate level.

In ovariectomised rats a dose dependent positive uterotrophic response was observed (subcutaneous route; 26 days; five animals per group): At 100 mg/kg/bw/day the absolute and relative uterus weight was increased by 26% and 38%, respectively. At 300 mg/kg/bw/day the absolute and relative uterus weight was increased by 34% and 52%, respectively, indicating estrogenic activity of TBMD. Pituitary and adrenal weights as well as vaginal histopathology appeared not affected (Pre-clinical & clinical information, 1966). In the same animals a dose dependent decrease in cholesterol levels was measured.

Findings suggesting an oestrogenic mode of action (i.e. positive uterotrophic response in rats) are also considered as concern for immune modulation (e.g. Adori et al., 2010).

It is noted that hydroxylated forms of cholesterol as well as other molecules involved in the cholesterol synthesis pathway are important regulators of immune function (Cyster et al., 2014).

Why new information is needed

The available information on TBMD consists of only two studies of high quality: a rat 28 day study (OECD 407) and a rat developmental/reproductive toxicity screening test (OECD 421). While no adverse effects were seen in the OECD 421 study, effects indicating interference with the HPT axis and the HPG axis were reported. These findings were supported by some of the lower quality studies in rats and dogs. Also some in vitro results (activation of PXR and RXR receptors) and mechanistic investigations (demonstration of interference of TBMD with cholesterol synthesis) add to the conclusion that clarification of the observed results is needed.

Currently the substance has no harmonised classification. The results of an extended one-generation reproductive toxicity study (EOGRTS) can elucidate Human Health endocrine disruptor (ED) adverse effects. This could lead to a classification as reprotoxic and/or to an identification of the substance as Substance of very high concern (SVHC) (Reprotoxic and/or ED for Human Health) and possible inclusion in Annex XIV of the REACH Regulation (for Human Health).

Considerations on the test method

The concerns for this substance need to be solved in a comprehensive study with high statistical power including all relevant endpoints assessing reproductive toxicity and adverse endocrine effects.

Besides endpoints of relevance for endocrine disruption, the EOGRTS will address reproductive toxicity as well as developmental neurotoxicity and immunotoxicity.

The extended one-generation reproductive toxicity study (EOGRTS) is requested with the following study design:

1) Inclusion of the extension of Cohort 1B

The triggers for extension of Cohort 1B to produce the F2 generation are listed in REACH Annex IX and are further explained in the ECHA guidance (Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 5.0. December 2016). Exposure of consumers and professionals and wide dispersive use and indications of relevant mode(s) of action related to ED (from *in vivo* studies, with some support from *in vitro* studies) and bioaccumulation properties of TBMD trigger the inclusion of the extension of Cohort 1B to produce the F2 generation.

According to the current registration data, the substance is used in lubricants and greases (e.g. lubricants, greases, metal working-, hydraulic-, and heat transfer fluids), washing and cleaning products, industrial coatings and inks, plant protection products and as intermediate. It is used by industrial workers, professionals and consumers. Moreover, the substance is also used in [redacted]. Typical technical function of the substance is use as antioxidant and stabiliser. Based on these identified uses, the statements of wide dispersive use, exposure of consumers and professionals are considered to be demonstrated. You confirmed wide dispersive use.

Available data indicate that TBMD induces hormonal imbalance and affects thyroid homeostasis.

- In a good quality 28-day toxicity study in rats ([redacted]) a statistically significant increase in ovary weight at the two highest doses (200 & 1000 mg/kg bw/day) tested (ranging from 34% to 41%) was observed, representing a considerable effect on this hormone dependent tissue.

- A significant increase of absolute and relative thyroid weight in females at 200 (abs. 28%, rel. 22%) and 1000 (abs. 29%, rel. 27%) mg/kg bw/day and diffuse thyroid hyperplasia in two of five females at 200 mg/kg bw/day and in four of five females at 1000 mg/kg bw/day indicates that the thyroid axis is affected.
- The effects seen in the 28-day rat study are supported by findings in a two year dog study ([redacted], Klimisch 2). A significant increase of ovary weights was reported (at the 6 months time interval at the highest dose of 75mg/kg bw/day the ovary weight increased as high as 196% and 139% for the absolute weight and relative weight, respectively).

  Though there was no statistically significant effect on thyroid weight, the high dose animals had decreased levels of serum protein bound iodine, an indication for lowered amounts of thyroid hormone in the blood and there we and cases of lymphatic infiltration, thyroiditis and struma (after 6 months: 1 in 6 animals at the low and the mid dose with lymphatic infiltration of the thyroid, 1 in 6 animals at the high dose with struma, after 2 years: 1 in 4 animals at the high dose with thyroiditis). This indicates that the thyroid axis is also a target in dogs.
- A three generation study by [redacted] is classified as Klimisch 3. Nevertheless, it should be highlighted here, that 3 dams died from dystocia, but it is not reported at which doses or at which generation these effects occurred. Further it is not mentioned whether dystocia also occurred in other dams, but at least in one dam difficulties in giving birth are reported. Nevertheless, dystocia, if caused by hormonal imbalance, has to be considered as adverse effect on reproduction. This effect has further to be regarded as a rare finding and even low numbers can be of relevance. A study with high statistical power, including the F2 generation for confirmation of this effect, is therefore requested.
- A 4 week subcutaneous study with 5 ovariectomised rats per group was submitted after finalization of the draft decision (obviously generated in the course of the FDA application for the use of TBMD as hypocholesterolemic drug). In this study a dose dependent positive uterotrophic response was observed: At 100 mg/kg/bw/day the absolute and relative uterus weight was increased by 26% and 38% respectively and at 300 mg/kg/bw/day the absolute and relative uterus weight was increased by 34% and 52% respectively (Pre-clinical & clinical information, 1966). This indicates estrogenic activity of TBMD.
- TBMD produced positive results in two *in vitro* studies assessing PXR activation, one *in vitro* study assessing RXR activation and one *in vitro* study assessing FXR activity (US EPA, http://www.epa.gov/actor/ToxCastDB; for description of ToxCast see e.g. also Judson et al. 2014).

2) Inclusion of Cohort 2A/2B (developmental neurotoxicity)

Regarding the inclusion of a DNT cohort it should be noted that there is a well-known link between thyroid and neurodevelopment (ECHA, 2015; WHO-UNEP, 2012; Menard et al., 2014).
In the ECHA guidance (Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 5.0. December 2016, Appendix R.7.6–2, EOGRTS Study Design) information on specific hormonal mechanisms/modes of action with clear association with the developing nervous system, such as estrogenicity (Fryer *et al.*, 2012) is a trigger for inclusion of cohorts 2A/2B.

Hence, as effects on the thyroid axis were seen in rats and dogs and an estrogenic activity can be derived from a positive uterotrophic response in rats (see Point 1 *Inclusion of the extension of Cohort 1B* above for details on these studies) inclusion of cohort 2A/2B is especially warranted for TBMD.

3) Inclusion of Cohort 3 (developmental immunotoxicity)

In the ECHA guidance (Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 5.0. December 2016, Appendix R.7.6-2, EOGRTS Study Design) information on hormonal mechanisms/modes of action with clear association with the immune system, such as estrogenicity (Adori *et al.*, 2010), is a trigger for inclusion of Cohort 3.

Hence, inclusion of the DIT cohort is warranted here due to a possible estrogenic mode of action of TBMD, supported by a positive uterotrophic response in rats (see Point 1 *Inclusion of the extension of Cohort 1B* above for details of this study).

The request for the DIT cohort is further supported by TBMD's interference with cholesterol synthesis (TBMD was investigated for its use as hypocholesterolemic drug in the 1960s/1970s and interference of cholesterol synthesis at the pre-mevalonate level has been demonstrated in a mechanistic study, Pre-clinical & clinical information, 1966). Hydroxylated forms of cholesterol as well as other molecules involved in the cholesterol synthesis pathway are important regulators of immune function (Cyster et al., 2014).

4) Premating exposure duration

According to the ECHA guidance (Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 5.0. December 2016), the pre-mating exposure duration shall be 10 weeks in order to cover the full period of spermatogenesis and folliculogenesis. However, due to the request of the extension of cohort 1B, ECHA concludes that the pre-mating exposure period can be reduced to 2 weeks, as these development periods will be covered in the F1 generation.

5) Dose level setting

The choice of appropriate doses for the EOGRTS with TBMD is not straight forward: In the two most recent studies conducted by [redacted] (OECD 407 and OECD 421) doses up to 1000 mg/kg bw/day did not result in severe toxicity in rats. While in the 28-day study in Crj: CD(SD) rats a NOAEL of 40 mg/kg bw/day was derived (based on changes in several organ weights and histopathological changes in the thyroid at doses 200 and 1000 mg/kg bw/day) no adverse effects were seen in the OECD 421 study in Sprague-Dawles rats treated with doses up to 1000 mg/kg bw/day. In a 90-day study in CF albino rats a NOAEL of 700 (m) / 800 (f) mg/kg bw/day could be derived based on liver effects at 1400 (m) / 1625 (f) mg/kg bw/day (liver cell degeneration and considerable weight increase), though the study is poorly reported (Klimisch 3). In contrast, in a chronic study (Klimisch 3) in Crj: CD(SD) rats a NOAEL of 3,6 (m)/4,4 (f) mg/kg bw/day was derived based on increased incidence and severity of hepatic lesions, i.e. fatty liver degeneration and bile duct multiplication, compared to controls at 18 (m)/22 (f) and 107 (m) / (f) 138 mg/kg bw/day.

Given that animals are exposed for a longer time period in the EOGRTS compared to the OECD 407 or OECD 421 studies, a lower dose of 750 mg/kg bw/day instead of the high dose of 1000 mg/kg bw/day, which was used as high dose in the [redacted] studies, is requested. The requested doses are therefore: 8, 40, 200 and 750 mg/kg bw/day.

The use of four doses instead of the standard of three doses is justified, as the higher doses are intended to induce some toxicity, but no death and the lower doses are needed to optimize assessment of endocrine disrupting properties of TBMD. At the same time the dose intervals should not be too high (OECD 443 states that intervals of 2- to 4-fold would be optimal, but a fourth group would be preferable if the interval would be more than a factor of 10) and therefore, in line with OECD 443, an additional dose is requested.

6) Route of exposure

The test substance shall be dissolved in olive oil and applied via gavage, essentially as done in the 28 day rat study by [redacted]. Controls shall be treated with olive oil alone.

Alternative approaches and proportionality of the request

The request for the above defined EOGRTS protocol is suitable and necessary to obtain information that will allow to clarify whether there is a risk for human health. More explicitly, there is no equally suitable alternative way available of obtaining this information. Where the data, once obtained, confirm that there is risk for endocrine disrupting effects and/or neuro/immune developmental effects, it will allow authorities to consider further regulatory risk management such as SVHC identification (authorization procedure). ECHA notes that there is no experimental method available at this stage that will generate the necessary information without testing on vertebrate animals.

Consideration of your comments on the original draft decision

You disagreed with the request of an EOGRTS because you are of the view that there is insufficient evidence of adverse effects and of animal welfare reasons. You stated that the only significant finding in reproductive organs/tissues across a large data base of animal studies would be an increase in the ovary weights (without histopathological findings) in a single 28 day study. Similarly the majority of the available studies show no adverse effects in the thyroid, according to your comments. The only significant but reversible finding in the thyroid across a large data base of animal studies comes from a single 28 day study. You argue further that the relatively extensive available published literature on this substance also does not demonstrate any significant alert for direct acting estrogen or androgen receptor-mediated endocrine effects in in vitro studies. In addition two publications (Princz 2013 and Fang 2001) would highlight that the four t-butyl groups prevent H-bond interactions between the phenolic rings of TBMD and the estrogen or androgen receptor. Therefore you do not regard TBMD as a close structural analogue to Bisphenol A.

ECHA is of the opinion that several adverse effects were noted on hormone homeostasis and on reproductive organs in several studies and not only in the 28-day study.

Beside increased organ weights (liver, thyroid, ovaries, uteri of ovariectomised rats) also histopathology (liver, thyroid) and blood biochemistry (mainly liver but also thyroid associated parameters) was affected. Though the described cases of dystocia cannot be unambiguously linked to TBMD treatment, dystocia, which can be caused by hormonal imbalance, as such has to be regarded as adverse effect on reproduction.

Based on the above described effects on thyroid and ovaries in two species, a dose-dependent positive uterotrophic response in the rat and the occurrence of some cases of dystocia in the rat, ECHA concludes that TBMD causes hormonal imbalance.

Overall, the available studies are considered insufficient to assess the reproductive toxicity of TBMD because either they were of poor quality or they did not cover all relevant endpoints.

You agree in your comments that further investigation is warranted, but you argue that the scope of such investigation should be in line with the principle of proportionality: You propose to undertake a new OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. This would serve to reinvestigate the findings from the previous 28-day study and the previous OECD 421 study. You propose that this study will include several of the additional parameters that the evaluating MSCA

had foreseen for the EOGRTS in the draft decision sent to you for commenting to investigate any potential mode of action with regard to thyroid or steroid hormone mediated endocrine effects

In your opinion the qPCR analyses in liver, originally proposed by the evaluating MSCA, will not add any useful information to the decisions around mechanism based adversity in this study

You further argued that if the results of such an enhanced OECD 422 protocol would provide convincing evidence of endocrine or other adverse effects such that the need for further testing is indicated, then the study can serve as dose range finding study for an EOGRTS.

If the proposed study would not substantiate any of the isolated findings of the subacute study, you assume that all concerns with regard to reproductive toxicity and endocrine disruption are resolved and no additional testing was required at the tonnage level of up to 1000 t/a

ECHA is of the opinion that the concerns for this substance need to be clarified in a comprehensive study with high statistical power including all relevant endocrine and reproductive endpoints. Considering that there are already a well conducted 28-d and an OECD 421 screening study, it would not be a suitable use of animals to repeat this study in a combined protocol, which despite the proposed enhancement, would still miss relevant parameters. The Extended One Generation Reproduction Toxicity Study is a statistically powerful test, which will provide reliable data of good quality.

The EOGRTS protocol recommends the use of 20 animals per group, which is high enough to give results with high statistical power, whereas the OECD 422 protocol recommends the use of 10-12 animals per group:

- In this regard it is important to note that some effects like e.g. dystocia might need large enough group sizes to be detected, as was e.g. concluded for thiacloprid (RAC opinion, 2015).
- For TBMD some cases of dystocia were reported in a poor quality 3-generation study with 20 animals per group, while no such cases were seen in the two available reproductive screening assays (one using 10-12 animals per group, one using 5 animals per group).

The EOGRTS protocol covers all relevant aspects of reproduction:

- The parameters included in the EOGRTS protocol are specifically designed to assess hormonal imbalance, as observed in the available studies for TBMD.
- For TBMD it is important to investigate the whole reproduction cycle, which is covered by OECD test Guideline 443, but not by OECD test guideline 422 which has only a 2 week pre-mating exposure and does not investigate effects up until maturation of F1 animals.
- Possible effects on fertility and on development after extended exposure, are not detectable with OECD 421 or 422 as the premating exposure is only for 2 weeks. The extension to include F2 is especially important to properly evaluate toxicity to ovaries. The extension to include F2 is further supported by the bioaccumulative properties of TBMD.

An OECD 421 study of good quality is already available for TBMD, however, it should be noted that this guideline, like the OECD 422 guideline, generates limited information concerning the effects of a test chemical on male and female reproductive performance such

as gonadal function, mating behavior, conception, development of the conceptus and parturition. It is not an alternative to, nor does it replace the existing Test Guidelines 414, 415, 416 or 443.

The added value of the DNT and DIT cohorts is well established for substances with potential endocrine disrupting properties (Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 5.0. December 2016; WHO-UNEP, 2012; Menard et al., 2014; Adori et al., 2010) and therefore also asked for TBMD (for more details see section on the inclusion of cohorts 2A/B and 3).

It is concluded that an EOGRTS including a F2, as well as the inclusion of DNT and DIT cohorts is necessary to sufficiently clarify the identified concerns in the most efficient way.

Further in the case of TBMD full account should be taken of the available studies (toxicological studies as well as toxicokinetic data and information on physicochemical data). In the sense of animal welfare no additional range finding study is needed.

Regarding the mechanistic parameters originally requested in addition to the EOGRTS protocol ECHA agrees with you that such a request may be disproportionate.

Therefore, in contrast to the evaluating MSCAs initial proposal to include additional parameters (among others liver enzyme activities) in the EOGRTS protocol, it was decided to not include these parameters now. Although these parameters would clarify the underlying modes of action, they are not required to demonstrate the potential endocrine disrupting properties of TBMD when an EOGRTS study, including cohorts 2 and 3 is conducted. The proposed parameters are not sufficient to fully clarify human relevance of this substance and would increase the complexity of the EOGRTS protocol.

Referring to the absence of evidence for TBMD to bind to hormone receptors the evaluating MSCA wants to emphasise that it has not been convincingly demonstrated that TBMD does not interact with hormone receptors. The *in vitro* tests might be misleading because of the very low water solubility and the adsorptive nature of TBMD. It can be assumed that these properties hinder the performance of *in vitro* studies. In this regard it is important to note that a positive uterotrophic response in ovariectomised rats was reported. Additionally it is important to note that hormonal imbalance cannot only be induced by receptor binding, but also through interference with hormone synthesis or metabolism (see e.g. Craig et al., 2011).

In your comments you also refer to a Board of Appeal (BoA) case citing that ECHA failed in this case to ensure testing on vertebrate animals was a last resort and that a test using the minimum number of vertebrate animals would be used.

ECHA assumes that you are referencing to the case A-005-2011: This BoA decision is targeting a compliance check, which deals with information requirements according to Section 8.6.4. of Annex X of REACH. In contrast, the present draft decision is dealing with substance evaluation, for which the most adequate test is asked to clarify several concerns. Animal welfare reasons are considered: The concerns are so fundamental that a screening test prior to an EOGRTS would be an unnecessary sacrifice of animals.

Regarding *in vitro* tests you state that in literature no significant *in vitro* alert for direct acting estrogen, androgen or thyroid receptor-mediated endocrine effects were seen and that the only four out of 159 *in vitro* tests performed under ToxCast are consistent with modulation of liver metabolism and subsequent thyroid response. Those *in vitro* results would not call for an EOGRTS. You further state that due to lack of direct action regarding

the estrogen and the androgen receptor TBMD cannot be regarded as a structural analogue to Bisphenol A.

ECHA is of the opinion that the low number of positive *in vitro* results cannot be taken as a sign for inactivity. In contrast, the very low water solubility and the adsorptive nature of the substance have to be considered and it can be assumed that these properties hinder the performance of *in vitro* studies. Despite these difficulties positive results were obtained in 4 *in vitro* tests. These results could be an indication for increased liver activity of metabolising liver enzymes with a potential link to the observed thyroid effects.

The EOGRTS is mainly requested because of the effects seen *in vivo*. Some of these results are also in line with positive *in vitro* findings

Consideration of proposals for amendment and your comments

You also note the proposal of an MSCA to conduct the OECD 231 prior to the OECD 443, and understand its expectation here is that the results of the OECD 231 would be used to inform on the design of the OECD 443. With regard to this point, you agree that a preliminary testing is absolutely necessary to optimise the study design and minimise the numbers of animals used. However, you strongly disagree that the OECD 231 would provide useful information in this regard. Such a study would provide neither information to inform appropriate dosing range/intervals nor unique insight to effects to be investigated. Rather, as discussed below and in your comments on the original draft decision you propose to use a firmly scientific approach that starts with an enhanced OECD 422 study. You argue that the enhanced OECD 422 study proposed is designed to provide specific information to inform such a need including, as appropriate, the design of an OECD 443.

You submit that, with regard to animal welfare, undertaking an appropriate dose range finder is necessary to optimise the study design whilst keeping the numbers of animals used as low as possible.

ECHA notes that a recent OECD 421 study ([redacted]) of good quality is already available for TBMD. This guideline, like the OECD 422 guideline, is designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behavior, conception, development of the conceptus and parturition. It is not an alternative to, nor does it replace the existing OECD Test Guidelines 414, 415, 416 or 443. ECHA is of the opinion that there is no need for another reproduction screening assay like OECD 422, but there is a need for a study with high statistical power, covering all relevant endpoints to assess reproductive toxicity and possible endocrine disrupting modes of action, as is OECD 443.

Regarding the request for the DIT cohort, you agree with the argumentation of a MSCA that the observed cases of pneumonia in rats, guinea pigs and humans are not relevant to support the inclusion of a DIT cohort.

In response to your argument and the PfA received from a MSCA the argumentation for the inclusion of a DIT cohort does not any longer refer to the observed cases of pneumonia, but to other findings indicating possible effects on the immune system, i.e. interference with cholesterol synthesis as well as possible ED Mode of Action. Both were described in the draft decision. As it is known that TBMD is a cholesterol lowering agent and the likely Mode of Action is interference with the cholesterol synthesis, it is worth investigating potential immune effects.

Moreover, as there is a concern for endocrine disruption the inclusion of a DIT cohort is justified. There are several indications that TBMD induced hormonal imbalance and based

on the observed positive uterotrophic response oestrogenic activity can be concluded. Oestrogenic endocrine disruptors may modulate the immune system (Adori et al., 2010) and thus inclusion of the DIT cohort is requested.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the registered substance subject to this decision:

Extended One Generation Reproduction Toxicity Study (OECD TG 443) in rats, (oral route) with the registered substance, specified as follows:

i. **At least two weeks premating exposure duration for the parental (P0) generation;**
ii. **Dose level setting shall aim to induce some toxicity at the highest dose level;**
iii. **Cohort 1A (Reproductive toxicity);**
iv. **Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation;**
v. **Cohorts 2A and 2B (Developmental neurotoxicity); and**
vi. **Cohort 3 (Developmental immunotoxicity).**

2) Concerns on persistency, bioaccumulation and toxicity (PBT)

Based on the evaluation of all relevant information submitted on TBMD and other relevant available information the evaluating MSCA concludes that further information is required in order to clarify the PBT concern.

Regarding PBT/vPvB assessment the Registrant(s) provided data on the P-, B- and T-properties, which were assessed by the evaluating MSCA.

At the current stage evaluating MSCA evaluates the PBT properties to clarify if the substance is PBT/vPvB. Once the information currently requested becomes available, the need for further data will be considered in the follow-up process.

Bioaccumulation (B/vB – properties)

Based on two BCF studies (Blankinship et al. 2009, amended 2010; [redacted]), it is concluded that the BCF value of TBMD is ≥ 5,000 L/kg. According to these data TBMD meets the bioaccumulative (B) and the very bioaccumulative (vB) criterion according to Annex XIII of REACH. This conclusion is supported by a dietary study in fish, which yielded a BMF value of 0.95, which was higher than that of hexachlorobenzene used within the same study (Chemicals Evaluation and Research Institute Japan, Report, 2009). No further tests are required to clarify the B status. Supportive, Japanese and Swedish monitoring data show the occurrence of TBMD in wildlife and fish (NITE, 2010, National Screening, 2003).

In your comments you disagreed with the conclusion of the evaluating MSCA that TBMD meets the B and vB criteria. You were of the opinion it is inconclusive at this stage whether TBMD meets or does not meet the B/vB criteria.

In response to your comments on the derivation of the BCF values the following clarification is provided: In the bioconcentration study (Blankinship et al. 2009, 2010), the growth rate constant is close to the overall depuration rate constant indicating that growth dilution is the main "depuration" process. Due to the significant fish growth, the kinetic BCF corrected for growth is preferred over the steady-state BCF. The growth-corrected and lipid-standardized BCF value is around 14,100 L/kg based on ^{14}C-analysis. Approximately 60% of the ^{14}C in

fish at steady-state is parent compound; this implies a revised BCF for the parent substance of TBMD of 8,640 L/kg (lipid- and growth-corrected). In another study, the steady-state bioconcentration factor (BCFss) were determined from the mean tissue concentrations at apparent steady-state divided by the average water concentration and revealed at 0.1 µg TBMD/L a BCFss value of 4,600 and at 0.01 µg TBMD/L a BCFss value of 9,200 ([redacted]). The conclusion that TMBD is fulfilling the B/vB criteria is further supported by a dietary study yielding a BMF value of 0.95, which is higher than the BMF for hexachlorbenzene used within the same study. The BCF may be derived from the dietary data resulting in BCF values > than 5,000 (Chemicals Evaluation and Research Institute, Japan, Report, 2009).

You confirmed to provide additional bioaccumulation data on terrestrial organisms (Princz, 2014), a newly available *in vitro* study on fish hepatocytes and a study by [redacted]. However, the applicability of *in vitro* studies is limited, due to the lack of standardized protocols and limited validation. Furthermore, the B criterion is currently not based on terrestrial data.

Based on the available data it is concluded that TBMD clearly fulfils the B/vB criteria.

Persistency (P/vP - properties):

TBMD is not readily biodegradable ([redacted]; MITI, 2007). In wastewater primary degradation has been shown. Several potential persistent metabolites with unknown identity were arising ([redacted]). TBMD is therefore considered to be potentially (very) persistent. Based on the currently available data, no conclusion if TBMD meets the P/vP-criterion can be drawn for the substance itself or as appropriate on its transformation products (metabolites) yet to be determined.

Soil simulation testing (OECD 307) (request 2)

You waived the soil simulation test as no direct exposure of soil was expected from the uses as hydraulic fluids, lubricants, fillers, washing and cleaning products, and metal working fluids and as the substance undergoes rapid primary degradation. TBMD is considered to be potentially (very) persistent, highly hydrophobic, immobile and poorly water soluble and will therefore partition to soils, sediments and sewage sludge in the environment.

No water, soil or water-sediment simulation test is currently available for TBMD. Submitted biodegradation studies show that TBMD is not readily biodegradable ([redacted]; MITI, 2007). In wastewater fast primary degradation has been shown. Several major potential persistent metabolites with unknown identity were arising ([redacted]). Three metabolites (Smiles Codes:
CC(C)(C)c1cc(Cc2cc(c(O)c(c2)C(C)(C)CO)C(C)(C)C)cc(c1O)C(C)(C)C;
CC(C)(C)c1cc(Cc2cc(c(O)c(c2)C(C)(C)C=O)C(C)(C)C)cc(c1O)C(C)(C)C;
CC(C)(C)c1cc(Cc2cc(c(O)c(c2)C(C)(C)C([O-])=O)C(C)(C)C)cc(c1O)C(C)(C)C) of TBMD were predicted by the EAWAG-BBD Pathway Prediction System (prediction performed by evaluating MSCA). Whether these metabolites in fact occur in the respective environmental compartments is unknown.

The estimated half-life for TBMD is high for soil and sediment and the estimated percentage of TBMD in soil and sediment is 49 for each compartment (calculated using EPI Suite 4.1 by the evaluating MSCA) making both soil and sediment compartments relevant for testing. Due to the very low water solubility of TBMD soil simulation testing was considered the relevant choice of simulation test compared to water simulation testing (OECD 309).

In a study conducted by Ritchie *et* al. 2013, TBMD showed some evidence that it could be persistent in soil throughout the test which ranged from 14 days to 63 days in duration; on day 63 still 85 ± 2.5% were recovered. The information on persistency given by Ritchie et al., 2013 is important, but preliminary as half-lives of TBMD and the identity and also the rate of formation and decline of transformation products are missing.

So far, the data provided by you are not sufficient to conclude on the P/vP-status. Therefore further testing is needed. Based on the intrinsic properties of the substance (e.g. low water solubility, high apolarity, high log K_{OC}), soil is a compartment of concern. Therefore, a soil simulation test according to OECD 307 is considered to be the most appropriate test to investigate degradation. Depending on the outcome of this test, the evaluating MSCA will consider the need for further testing in sediment in the follow-up stage.

The test material shall be the purest form containing no transformation products of the registered substance, to ensure that at test start no degradants are present. The homogeneity of dosing shall be checked analytically. The soil shall not contain any stones. Although OECD test guideline 307 recommends a maximum test duration of 4 months, it is recommended that the test is allowed to run at least for 6 months (longer if possible), to give sufficient time for any transformation product to appear, unless it can already be concluded after a test duration of 4 months that the substance will meet the P- or vP-criterion.

The kinetic part of the study shall be performed under aerobic conditions at a constant temperature of 12°C, since this is a representative temperature for EU soils. Relevant environmental conditions include 12°C, The transformation pathway and the rates of transformation should be determined in accordance with the test method. For the identification of potential metabolites 20°C shall be used. ^{14}C-labelled test material shall be used.It is possible or even likely that the substance may form non-extractable residues (NER) in soil. You are therefore requested to justify scientifically that the extraction procedure/solvent chosen is appropriate to completely extract the non-irreversibly bound fraction of the substance/its metabolites from the soil matrix. Strong extractions, such as soxhlet-extraction with apolar solvents, should be used in order to conclude that the remaining part should be considered as irreversibly bound fraction. A complete mass balance analysis shall be conducted to trace the various fates of TBMD properly. FOCUS software shall be used for calculation of degradation rates. Further, you shall justify the number of sampling intervals, and monitor for volatiles/mineralisation products if considered relevant. Transformation products formed at levels of 1% or more of the test substance shall be assed, with reasonable attempts made to quantify these down to 0.1%.

In your comments you agreed to conduct the requested OECD 307 study. However, you stated that the requested % level of identification of transformation products is unlikely to be achieved.

In response to this, you shall try to quantify transformation products with reasonable attempts down to 0.1%. Degradation products might be formed very slowly, so attempts shall be made, as far as technically possible to quantify these down to 0.1%, otherwise a justification shall be provided, why it was not technically feasible. Technically feasible means, that it has been demonstrated within allocation of reasonable efforts to develop suitable analytical methods and other test procedures to accomplish testing in soil so that reliable results can be generated.

The requested level of 0.1% is also referred to in the ECHA Guidance (Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment Version 2.0 November 2014) which states: "The PBT/vPvB assessment shall be

performed on each relevant constituent, impurity and additive. It is not possible to draw overall conclusion if, e.g., the assessment of persistence has been concluded for one constituent and the assessment of bioaccumulation or toxicity for another constituent. Constituents, impurities and additives are relevant for the PBT/vPvB assessment when they are present in concentration of ≥ 0.1% (w/w). This limit of 0.1% (w/w) is set based on a well-established practice rooted in a principle recognised in European Union legislation. Individual concentrations < 0.1 % (w/w) normally need not be considered."

You stated that TBMD is likely to be inherently biodegradable in water, undergoes rapid primary degradation and that photolysis may be an important pathway and proposed further to investigate abiotic degradation processes (photolysis and hydrolysis). The evaluating MSCA is of the view that due to the missing functional groups susceptible to hydrolysis and because TBMD is highly insoluble in water, a hydrolysis study does not need to be conducted. As photolysis in water requires also dissolvation in the aqueous phase, this degradation pathway is also considered to be hampered under environmentally relevant conditions. If TBMD is released to water, TBMD is expected to quickly partition to suspended particles and finally to sediment. It might be therefore expected that only a very small fraction of the total TBMD present in the aquatic environment would have the potential for hydrolysis and photolysis. For soil, the abiotic degradation may be limited by sorption to soil particles and subsequent shielding, light attenuation by humic or other substances. Although such studies might provide some evidence for potential hydrolysis or phototransformation, the studies cannot be used to conclude on the extent and rate of the transformation in the environment, as these degradation pathways are not considered to be relevant due to the low water solubility of the substance.

In your comments on a PfA you questioned the requirement to perform the soil simulation study at 12°C as ECHA PBT Guidance would refer to this temperature in relation to water simulation studies only ECHA notes that REACH Annex XIII indicates that "*the information used for the purposes of assessment of the PBT/vPvB properties shall be based on data obtained under relevant conditions*". The Guidance on information requirements and chemical safety assessment R.7b (version 3.0, February 2016) specifies that simulation tests "*attempt to simulate degradation in a specific environment [...], and a typical temperature that represents the particular environment*". The Guidance on information requirements and chemical safety assessment Chapter R.16 on Environmental Exposure Estimation, Table R.16-8 (Version 3.0 October 2016) indicates 12°C as the average environmental temperature for the EU (for each compartment including soil).

You also disagreed that strong extraction techniques should be used to completely extract non-irreversible bound fractions, as the data of Ritchie et al., 2013 show that the substance was extractable and stable. In addition you are concerned that this is likely to lead to an overestimation of the bioavailable fraction.

In response to your comments it is noted that non extractable residues (NER) should be characterised and differentiated in re-mobilisable and in an irreversibly bound fraction with justified extraction techniques. According to Eschenbach et al., soxhlet extraction techniques account for NER-type I, which are potentially remobilisable (Eschenbach, 2013). Based on the intrinsic properties of the TBMD, NER formation cannot be completely ruled out for the four different soil types included in the requested soil simulation study.

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the registered substance subject to this decision:

Soil simulation testing. Test method: Aerobic and anaerobic transformation in soil, EU C.23/OECD 307) (as further specified above) using radioactively ^{14}C ring-

labelled test substance. The kinetic part of the test shall be conducted at 12°C. For the identification of potential metabolites 20°C shall be used.

IV. Adequate identification of the composition of the tested material

In relation to the required experimental studies, the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation. Finally, the test(s) must be shared by the Registrant(s).

V. Avoidance of unnecessary testing by data- and cost-sharing

In relation to the experimental studies the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). Registrant(s) are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:
https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

Further advice can be found at http://echa.europa.eu/regulations/reach/registration/data-sharing.

If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrants to perform the studies on behalf of all of them.

VI. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52(2) and 51(8) of the REACH Regulation. Such an appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://www.echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised[3] by Leena Ylä-Mononen, Director of Evaluation

Annex: List of registration numbers for the addressees of this decision. This annex is confidential and not included in the public version of this decision.

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

Annex:

List of registration numbers for the addressees of this decision

EC number: 204-279-1
CAS number: 118-82-1
Public name: 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol

This decision is addressed to the Registrant(s) of the above substance with active registrations according to Article 6 of the REACH Regulation on the date on which the draft for the decision was first sent for comments. If Registrant(s) ceased manufacture upon receipt of the draft decision in accordance with Article 50(3) of the REACH Regulation, they did not become addressee(s) of the decision. A list of all the relevant registration numbers of the Registrant(s) that are addressees of the present decision is provided below.

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