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Helsinki, 15 February 2019

Substance name: 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol
EC number: 202-525-2
CAS number: 96-69-5
Date of Latest submission(s) considered[1]: date
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressees: Registrant(s)[2] of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol

DECISION ON SUBSTANCE EVALUATION

1. Requested information

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on the registered substance 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol:

Endocrine disruption studies (human health and environment) including the human health endpoints reproductive toxicity, immunotoxicity and neurotoxicity using the registered substance 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol:

1. Extended One Generation Reproduction Toxicity Study (test method OECD TG 443) in rats, oral route, specified as follows: cohort 1A (reproductive toxicity); cohort 1B (reproductive toxicity); extension of cohort 1B to include F2 generation; cohorts 2A and 2B (developmental neurotoxicity); and cohort 3 (developmental immunotoxicity)

   If you can demonstrate that significant exposure of professionals and consumers can be excluded and update your Registration dossier accordingly, the extension of the cohort 1B for inclusion of the F2 generation is not required. In that case, it is required to extend the premating exposure period to 10 weeks as explained in Appendix 1.

2. Fish Sexual Development Test (FSDT) (test method OECD TG 234) using either Japanese Medaka *(Oryzias latipes)* or Zebrafish *(Danio rerio*) and five test concentrations.

Studies for assessment of persistency using the registered substance 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol:

[1] This decision is based on the registration dossier(s) at the end of the 12 month evaluation period.
[2] The terms Registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

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3. Simulation testing on ultimate degradation in surface water; test method: Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25./OECD TG 309, "pelagic test" – without additional suspended solids/sediment, kinetic part at a temperature of 12 °C, transformation part at a temperature of either 12 or 20°C; as further specified in Appendix 1.

You shall provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the Chemical Safety Report by **24 May 2021**.

Submission of the full study reports is required to allow the evaluating MSCA to conduct an independent assessment of the study results. The deadline takes into account the time that you, the Registrant(s), may need to agree on who is to perform any required tests.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

1. Who performs the testing

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the studies on behalf of all Registrant(s) within 90 days. Instructions on how to do this are provided in Appendix 3.

2. Appeal

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

Authorised[3] by Christel Schilliger-Musset, Director of Hazard Assessment

[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.

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Appendix 1: Reasons

Based on the evaluation of all relevant information submitted on 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol and other relevant available information, ECHA concludes that further information is required in order to enable the evaluating Member State Competent Authority (MSCA) to complete the evaluation of whether the substance constitutes unacceptable risk to human health and the environment.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested to clarify the concerns: endocrine disruption, reproductive toxicity, immunotoxicity, neurotoxicity, environmental/suspected PBT, exposure/wide dispersive use and consumer use in the follow-up process.

ENDPOINT 1: Concerns on endocrine disruption for human health and mammals living in the environment/ reproductive toxicity/ immunotoxicity/ neurotoxicity

Extended One Generation Reproduction Toxicity Study (EOGRTS) in rats, oral route, with the developmental neurotoxicity cohort (DNT) and the developmental immunotoxicity cohort (DIT) and the extension of cohort 1B for including of the F2 generation and an extended pre-mating period of 10 weeks (test method: OECD TG 443) including parameters clarifying mode of action.

The Concerns Identified

- Concerns on endocrine disruption for human health and mammals living in the environment/ Concerns on reproductive toxicity for human health
- Concerns on (developmental) immunotoxicity
- Concerns on developmental neurotoxicity

QSAR data

According to OECD Toolbox Version 3.1, 6,6´-di-tert-butyl-4,4´-thiodi-m-cresol is a very strong binder to the estrogen receptor, as the molecular weight of the substance is between 200 and 500 and with two non-impaired OH groups attached to two different 5 or 6 C-atoms ring.

In vitro data

Inhibitory activity against E2–ERα binding was determined using the ERα Competitor Screening Kit (Wako Pure Chemical Industries Ltd., Osaka) to know its binding activity to ERα (Takahashi and Oishi, 2006)[4]. 6,6´-di-tert-butyl-4,4´-thiodi-m-cresol competitively bound ERα against β-estradiol (E2): The 50% inhibitory concentration (IC50) of 6,6´-di-tert-butyl-4,4´-thiodi-m-cresol is 1.8×10^{-5} M while the IC50 of the positive control

[4] The Assay principle is to determine the amount of ligands between fluorescein-labelled E2 and plate-coated recombinant human ERa when each test compound is added. ERa-binding activity of the test compound is expressed in terms of the 50% inhibitory concentration (IC50) for the inhibition of E2–ERa binding.

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(Bisphenol A) was $1.4x10^{-5}$ M.

In METI(~2010), 6,6´-di-tert-butyl-4,4´-thiodi-m-cresol was positive in a human Androgen Receptor (AR) Binding Assay. Negative results were gained from a human Androgen Receptor (AR) Reporter gene assay testing agonistic as well as antagonistic activity.

In the framework of the United States Environmental Protection Agencies´ Toxicity Forecaster (ToxCast) (US EPA, 2015) 18 tests for 6,6´-di-tert-butyl-4,4´-thiodi-m-cresol are available. From these, 14 gave a positive result including estrogenic, anti-estrogenic, androgenic and anti-androgenic activity. Moreover, antagonistic activity for the thyroid hormone receptor (beta) and agonistic activity for the glucocorticoid receptor and PPARγ were observed. An overview on the ToxCast tests is presented in Table 1 below.

Table 1: Available in vitro data (ToxCast, US EPA, 2015)

Assays	**Out-come**	**Target**	**Detection mechanism**
Estrogen Receptor Assays			
ERa_LUC_BG1_Agonist	-	Nuclear receptor; Human, ovary, cell line: BG1, Regulation of gene expression	Luciferase induction, bioluminescence
ERa_BLA_Agonist_ratio	+	Nuclear receptor; Human, kidney, cell line HEK293T Regulation of gene expression	beta lactamase induction, fluorescence
ERa_LUC_BG1_Antagonist	+	Nuclear receptor; Human, ovary, cell line: BG1 Regulation of gene expression	Luciferase induction, bioluminescence
ERa_BLA_Antagonist_ratio	+	Nuclear receptor; Human, kidney, cell line: HEK293T Regulation of gene expression	beta lactamase induction, fluorescence
ERa_BLA_Antagonist_viability	+	Cell cycle Cell proliferation,	beta lactamase induction, fluorescence
Androgen Receptor Assays			
AR_LUC_MDAKB2_Agonist	-	Nuclear receptor, human, mammary gland /breast, cell line: MDAkb2 Regulation of gene expression	Luciferase induction, bioluminescence
AR_BLA_Agonist_ratio	+	Nuclear receptor, human, kidney, cell line: HEK293T Regulation of gene expression	beta lactamase induction, fluorescence
AR_LUC_MDAKB2_Antagonist	+	Target: Nuclear receptor, human, mammary gland /breast, cell line: MDAkb2 Regulation of gene expression	Luciferase induction, bioluminescence
AR_BLA_Antagonist_ratio	+	Nuclear receptor, human, kidney, cell line: HEK293T, Regulation of gene expression	beta lactamase induction, fluorescence
AR_BLA_Antagonist_viability	+	Cell cycle Cell proliferation,	beta lactamase induction, fluorescence
Thyroid Hormone Receptor Assay (beta)			
TR_LUC_GH3_Antagonist	+	Nuclear receptor, rat, pituitary gland, cell line GH3,	Luciferase induction,

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		TR transactivation; Block 6 assay according to OECD Scoping Document Nr.207 (2014): not a common site of interference of thyroid signalling by environmental toxicants	bioluminescence
Glucocorticoid Receptor Assays			
GR_BLA_Agonist_ratio	+	Nuclear receptor; regulation of transcription factor activity	beta lactamase induction, fluorescence
GR_BLA_Antagonist_ratio	-	Nuclear receptor; regulation of transcription factor activity	beta lactamase induction, fluorescence
GR_BLA_Antagonist_viability	+	cell cycle	beta lactamase induction, fluorescence
PPAR Assay			
PPARg_BLA_Agonist_ratio	+	Nuclear receptor, regulation of transcription factor activity	beta lactamase induction, fluorescence
ATPase Assay			
ELG1_LUC_Agonist	-	Hydrolase, ATPase family (ATAD5), regulation of transcription factor activity	Luciferase induction, bioluminescence
Mitochondrial Toxicity			
MitochondrialToxicity_viability	+	Cell cycle, Cell proliferation	
MitochondrialToxicity_ratio	+	Cell morphology, Mitochondrial damage	

In vivo data

6,6'-di-tert-butyl-4,4'-thiodi-m-cresol was tested in only two guideline studies: A rat OECD TG 407 study (Anonymous, 1995) and a rat OECD TG 421 study ([redacted] 2010). Both can only be rated Klimisch 2 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 [redacted], however, reporting was rather poor (e.g. the date of the study is not available) ([redacted] publication date unclear).

The developmental toxicity of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol was investigated in two studies: a prenatal developmental toxicity study (conducted in 1976) in rabbits, not according to standard protocol and with poor reporting and a post-natal development study in mice, not according to standard protocol and using only one dose, which resulted in considerable maternal as well as developmental toxicity [redacted] 1987). 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.

The substance 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 it can be concluded that the data base is rather old, leaving a lot of questions open. No comprehensive modern high quality study is available and especially the information on reproductive toxicity is scarce.

Potential for exposure
There is potential for exposure (see page 27 below).

Summary repeated dose toxicity and reproductive toxicity studies with 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol:

General toxicity:

At doses of about 200 to 300 mg/kg bw/day considerable reduction of body weight/body weight gain, reduced feed intake and diarrhea were frequently reported, at higher doses of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol mortality was increased. The degree of general toxicity induced by 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol differed between gavage and dietary studies and also between different rat and mouse strains. Rabbits were considerably more susceptible, though only pregnant animals were tested. In general dietary studies appeared to lead to more severe effects and at lower doses than gavage studies, indicating that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol might be better absorbed and better available systemically after dietary exposure. After gavage exposure retention of material in the stomach and reduced systemic up-take with increasing dose was observed.

Specific toxicity:

Overall it can be concluded that in most studies the liver, kidney and blood/bone marrow were the main target organs of systemic toxicity, but also local effects, i.e. irritation of the gastro intestinal tract were observed.

Changes in organ weights can be sensitive parameters for toxicity but are often influenced by general toxicity and effects on total body weight. The weights of several organs were affected after exposure to 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol, often at doses which induced considerable general toxicity, but some effects on organ weight were also seen without general toxicity and without changes in total body weight.

In most studies with 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol a dose dependent increase in relative liver weight was reported, but also kidney weights were affected, both are likely to be related to the observed toxicity in these organs.

Male reproductive organs:

Testis weight was not investigated or reported in each individual study, but in some studies increases in relative testis weight and decreases in absolute testis weight were reported, however, often together with effects on total body weight. It is therefore relevant to look at those doses where total body weight was not affected, as e.g. at the mid dose in the 13 week rat study (NTP, 1994), where the relative testis weight was increased by 14%. Morissey (1988) also reported effects on testis weight (relative weight increased) and on cauda and epididymis weight (absolute reduced, relative increased) in rat but not in mouse, but no information on the degree of change was presented. It was stated that these changes were partly seen together with changes in total body weight.

Effects on histology of testes/epididymides and sperm parameters were only investigated in Takahashi and Oishi (2006) and [redacted] (2010). Some information was extracted from the NTP (1994) study and published by Morissey (1988).

Takahashi and Oishi (2006) investigated the effects of two months exposure to 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol in rat and mouse. The weight of several reproductive organs was affected and histopathology revealed severe exfoliation of seminiferous tubules, sloughing of seminiferous tubules, vacuolization and proliferation of Leydig cells, dilated lumen of the seminiferous tubules and disappearance of adipose tissue. Decreases in daily Sperm Production (DSP) and DSP/g testis were reported. Disappearance of germ cells and degeneration of spermatids was also reported. The effects were mostly dose dependent and of considerable severity at higher doses. Some changes in testosterone levels were noted but it was not clear if this was induced by 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol treatment.

In the OECD TG 421 study ([redacted], 2010) a significantly lowered sperm forward motility index was seen in the high dose (500 mg/kg bw/day) compared to controls. Also at the lower doses some effects on sperm and reproductive organs were reported in individual animals (reduced forward motility and cell debris in the lumen of the epididymis).

In the 2 year NTP study in rats at the 15 month evaluation adenoma in testes (interstitial cell adenoma) occurred with higher frequency in treated animals compared to control (up to 57%), with the highest frequency at the mid dose (NTP, 1994). Further it was reported that granuloma sperm in the epididymis was increased, reaching 18% in the high dose group and a slight increase in hyperplasia of the epididymis was seen in the treated groups. Granuloma sperm was detected in one animal (10%) at the low dose, mineralisation of testes was seen in all treated groups, incidences ranking from 10% to 29%. Chronic active inflammation of the coagulating gland was detected in one animal at the low dose (this effect was only investigated in this single animal).

Overall it can be concluded that testis and spermatogenic tissue is a target of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol toxicity. The underlying mode of action has not been clarified, but an endocrine mode of action cannot be excluded.

Male fertility:

Male fertility was not affected in the only study investigating this parameter, the OECD TG 421, Klimisch 2 ([redacted] 2010). Though the study used 13 animals per dose (which

is more than 10 as required according to OECD TG 421) the statistical power was still lower than in a one- or two-generation study (OECD TG 443 or 416).

Sperm parameters and testis/epididymis were affected in this study (see previous section on male reproductive organs) but it should be noted that sperm parameters in the rat have to be affected considerably in order to adversely affect fertility, which is in contrast for instance to humans. In conclusion this means that the above described effects on sperm, testis and epididymis have to be considered as relevant effects also in the absence of adverse effects on male fertility in rats.

It should further be considered that effects on sperms might only evolve after a longer duration of exposure than was part of the present study design. The exposure duration of 42 days does not cover a complete spermatogenic cycle and the two weeks of exposure prior to mating makes the study design not particularly sensitive to detect effects on fertility, as is stated in the OECD TG 421 guideline itself. The duration of exposure could be especially short for substances with bioaccumulation potential, as might be the case for 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol.

Female reproductive organs:

Ovary weights were affected in one of three studies reporting this endpoint (28 day rat (Anonymous, 1995), 3 / 6 months rat [redacted], publication date unknown) and OECD TG 421 rat [redacted], 2010)). In the 28 day study ovary weight was increased in high dose animals, reaching statistical significance only for the right ovaries (relative weight: +29%, absolute weight: +35%). The increase was still visible after a 14 day recovery period. Total body weight was not affected in this study.

Uterus weight of intact mature animals was only assessed by [redacted] (publication date unclear) in the 3 / 6 months rat study. Decreases in uterus weight were reported both after 3 and 6 months reaching up to 30%. Total body weight was not affected in this study.

The results of 5 uterotrophic assays in immature and ovariectomised mice and rats via different exposure routes (diet, sc, ip) show predominantly estrogenicity via increased uterine weights, also anti-estrogenic effects (decreased uterine weight) were seen in one (ip) of the 2 rat uterotrophic assays (Takahashi and Oishi, 2006). Depending on differences in study design (dose, route) and target tissue either estrogenic or anti-estrogenic effects with one and the same substance can be elicited.

Some effects on reproductive organs in the 2 years chronic studies in rats and mice were interpreted to be not treatment-related by the study authors (NTP, 1994). However in the light of new knowledge of the modes of action of endocrine disruptors, which has grown considerably since 1994, the following effects are interpreted as treatment-related:

- A significant positive trend was seen for the occurrence of stromal polyps of the uteri of treated rats. However, while the incidences were clearly above the historical control average, they were well within the historical control range. The incidence in controls was reported to be unusually low compared to that in historical controls. Stromal sarcoma was also present in one low dose and one high dose female.
- Another finding was that the incidence of mammary fibroadenoma occurred with a negative trend in female rats (statistically significant at mid and high dose). A negative trend was also seen for mammary adenoma, carcinoma and fibroadenoma combined. No historical control data were found in the NTP-report.

Both, the uterus and the mammary gland represent hormone dependent tissues and it is well documented that hormones (especially estrogen) play an important role in normal

function as well as tumour formation in both organs. Both effects, the increase in uterus tumours and the decrease of mammary gland tumours, could be related to cresol treatment and an estrogenic mode of action in one organ (i.e. the uterus) and an anti-estrogenic mode of action in the other organ (i.e. mammary gland) are not considered to be contradictory.

Female fertility:

Female fertility was not affected in the only study assessing this parameter, the OECD 421 study in rat ([redacted] 2010). In this study no effects on reproductive organs and tissues, on estrus cycles, mating ability, gestation periods, birth index, number of corpora lutea, implantation number and index, delivery, and lactation were reported. According to the study authors no effects on reproduction up to a dose of 500 mg/kg bw/day were observed. However, a dose-dependent increase in sex-ratio was observed. It could be discussed whether this effect is regarded as an effect on fertility or reproduction, but it is considered a substance related effect.

Though the study used 13 animals per dose (which is more than 10 as required according to OECD TG 421) the statistical power was still lower than in a one- or two-generation study (OECD TG 443 or 416). Instead of keeping the animals on dose up to 13 days after delivery, the animals were dosed up to day 4 after delivery, and were sacrificed on day 5. Dosing was therefore only approximately for 54 days, in contrast to 63 days as required according to OECD TG 421. In most other aspects the study was carried out according to OECD TG 421, however, as the study was conducted in 2010 this was prior the up-date of the test guideline in 2016, where several parameters suitable to assess endocrine disruption potential were included. Further, it should be noted that OECD TG 421 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 Test Guidelines 414, 415, 416 or 443.

In a post-natal screening test ([redacted] 1987) - for details see following section on effects on development) - the duration of gestation was statistically significantly increased by 2.7% compared to control. Elongation of gestation can have several reasons, but hormonal imbalance can be one factor relevant with regard to fertility.

Effects on development:

In a post-natal screening test 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol was tested in CD1 mice ([redacted] 1987). In phase I and II of the study dose range finding was performed and an LD_{10} of 485 mg/kg bw/day was determined and taken forward as the single exposure concentration used in phase III, the post-natal screening assay. Animals were dosed from gestation day 6 to 15 and dams were allowed to litter. Some effects were reported, however, as considerable maternal toxicity was reported (22/50 died, all deaths related to toxicity, compared to control: 0/47), the relevance of the findings cannot be assessed. Maternal death and low statistical power may have masked possible effects on development. The authors suggested retesting at a lower dose in order to be able to draw conclusions on reproductive toxicity.

In an oral gavage prenatal developmental toxicity study in rabbits, daily dosages of 0.2, 2.0 and 20.0 mg/kg bw were administered during day 6 to 18 of gestation (13 does / group). Slight effects on body weight gain were seen at low and mid dose (dose dependent), considerable maternal toxicity was seen only in the high dose. The NOAEL for maternal toxicity was 2 mg/kg bw/day ([redacted] 1976). Pregnancy rate was not adversely affected by treatment. 6 animals aborted, one at 0.2 mg/kg bw/day, which

was excluded because of ill health (however, no "signs of ill health" are described under "signs and mortalities"), one at 2 mg/kg bw/day and four at 20 mg/kg bw/day. Difference between groups was statistically significant. No reason for these abortions was mentioned but as they occurred also at doses without considerable general toxicity (at and below the $NOAEL_{maternal}$ of 2 mg/kg bw/day), they indicate a concern. When considering all dams (including those with abortions) fetal loss was slightly increased at 2 mg/kg bw/day and markedly (above historical controls) increased at 20 mg/kg bw/day. There was a corresponding reduction in litter size at 20 mg/kg bw/day. Mean pup weights were lower than that of controls at mid and high dose. The effect was more pronounced at the mid dose, however, this lack of a dose response relation could be caused by the altered sample composition at the high dose (4 abortions). Skeletal anomalies were increased in all dose groups, but the difference from control was not statistically significant.

Overall, the study is considered not sufficient to conclude on teratogenicity and development, but it raises concern of potential adverse effects on development at low doses (reduced fetal survival, reduced pup weight).

Further an increasing tendency of dead embryos/fetuses at 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol doses ≥ 200 mg/kg bw/day was reported in the preliminary dose range finding test conducted prior to the OECD TG 421 study in rat [redacted] 2010). More information was not available on this observation.

Immune relevant parameters:

The lymphoid organs and tissues which inform on possible immunotoxicity are the spleen, the thymus, the bone marrow, lymph nodes and blood. Several of these organs have been affected in experimental animals upon treatment with 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol. While thymus weight was decreased considerably in several studies, indicating atrophy of this organ, no histopathological changes were reported (though histopathological investigations were not carried out in all studies available). In contrast, spleen weight was mostly increased which could be related to an increase in cell numbers. The increase in lymphocytes seen in several short term studies (14 days to 28 days) was mostly based on an increase in neutrophils without increase in immature forms and therefore not considered to be an inflammatory response, but rather a direct effect on the immune system. In studies with longer duration, this response was overshadowed by inflammatory responses (e.g. in the 13 week NTP studies) and in the 2 year studies these parameters were fluctuating, not allowing to discern a clear pattern.

Based on the observations from the 15 day NTP studies in rats and mice, Holsapple et al. (1988) decided to investigate the effects of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol in a broad panel of immune functional parameters, including humoral and cell mediated immunity, macrophage function and host resistance capabilities in female B6C3F1 mice. A number of parameters reflecting immune response were altered following exposure to 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol and changes in host resistance capabilities were altered. The observation that the various subpopulations of T lymphocytes were altered differentially by exposure to a xenobiotic had already been demonstrated previously for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; [redacted] 1978 in Holsapple et al. 1988).

In the study by Munson et al. (1988), which complemented the study by Holsapple et al. (1988) with an assessment of the effects of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol on general toxicity parameters, spleen weight was already increased at 10 mg/kg bw, the lowest dose tested. Besides the effects on the liver and hepatic microsomal parameters, the most pronounced changes were a dose related increase in the amount of hemoglobin

and a slight dose-related reticulocytosis. Because there was also a slight, but non-significant, increase in the number of erythrocytes, these results suggest a 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol-associated increase in erythropoiesis, perhaps related to an effect on the bone marrow. The authors concluded that the increased number of reticulocytes, increased number of bone marrow cells/femur and increased number of macrophage progenitors/femur indicate that exposure to 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol produces a generalized increase in bone marrow cellularity and suggest an increase in stem cells or stem cell activity. The increase in bone marrow cellularity is in contrast with the observed bone marrow depletion seen in several of the NTP studies. However, this depletion was seen at clearly higher doses and was considered a result of debilitation at these higher doses.

Holsapple et al. (1988) and Munson et al. (1988) concluded that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol induces changes in immune function not associated with destruction of lymphoid tissue. In the 14 day study in female mice (Munson et al., 1988) a dose dependent increase in peripheral blood leukocyte counts was reported (up to 41% in the high dose). All cell types were affected, but only lymphocytes and neutrophils were statistically significantly increased and only in the high dose. Holsapple et al. (1988) carried out a T- and B-cell enumeration and recorded statistically significant increases in spleen cell numbers at the high dose (200 mg/kg bw/day) which is in line with the splenomegaly observed by Munson et al. (1988). A dose related increase in lymphocytes was reported at 100 & 200 mg/kg bw/day, but no change in percentage of B-cells, indicating that the increase was due to an increase in T-cells.

Interference with the bone marrow was also seen in the form of myelofibrosis, which was reported in the 2 year mouse study (NTP, 1994). It was present in all groups of females with significant positive trend and the incidence in the high dose (100 mg/kg bw/day) was significant by pairwise comparison. Myelofibrosis was only seen in the 2 year mouse study, not in the 2 year rat study, the only other study, where exposure was equally long. However, mice could be more sensitive to this effect. An increase in platelets was mainly observed in male and female rats after 2 years exposure to 100/120 mg/kg bw/day (NTP, 1994). NTP interprets this effect as reactive thrombocytosis and mentions that this finding can be caused by, among others, inflammation and acute blood loss. However, inflammation and potentially resulting blood loss was not observed at doses of 100/120 mg/kg bw/day or at least was not severe enough to cause an increase in platelet counts. Behavior, general health appearance, feed consumption and survival was comparable to control at that dose. Body weight was hardly affected. Platelets were not affected in the 2 year mouse study, while, in the other NTP-studies (15 day and 13 week studies in rat and mice) platelets were not measured.

In the 3 / 6 months study by [redacted] (publication date unclear) increases in platelets up to 19 % were detected in males and females after 3 months and in high dose males after 6 months exposure, however, these increases were not statistically significant. No general toxicity was seen in this study and no effects on total body weight were reported. In this study a significant prolongation of the prothrombin time was reported at 500 mg/kg bw/day, both after 3 and 6 months. Prothrombin time was not affected in one other study 28 days study (Anonymous, 1995).

Also in the rat 28 day study (Anonymous, 1995) platelet counts were increased at the high dose of 250 mg/kg bw/day (14 % in males, 19 % in females), a dose which did not induce general toxicity or reductions in total bodyweight. In this study the platelets remained increased also after the recovery period of 14 days (11 % in males, 13 % in females).

In this regard it is important to note that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol was one of 13 chemical substances among 500 substances investigated in the NTP program for which atrial thrombosis was observed (Yoshizawa et al., 2005). In the rat 2 year study 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol induced an increase in atrial thrombosis in males adoses ≥ 20 mg/kg bw/day (12 %, 8 % & 6 % at 20, 40 & 100 mg/kg bw/day, respectively). Slight anemia (probably hemolytic) at mid and high dose and increases in platelet numbers (thrombocytosis) at the high dose were considered lesions that might be related to thrombosis induction. However, it has to be noted that atrial thrombosis was also increased in low dose males, where no anemia was seen and where platelets were not increased and that in females, although also anemia and increased platelet counts were observed, no increase of atrial thrombosis was reported.

It is known that, among other factors, progesterone or testosterone (Yoshizawa et al., 2005) are relevant factors in the regulation of platelet formation, though this process is quite complex (see also section "Comments of the Registrant(s) and Responses to Comments"). This indicates that hormonal imbalance might adversely affect platelet formation. The difference between the sexes regarding the formation of atrial thrombosis further supports that hormonal status might be relevant for the induction of this effect. In this regard it should also be considered that oral hormonal contraceptives are known to increase the risk of atrial thrombosis (e.g. Yoshizawa et al., 2005).

It can be concluded that the available data clearly indicate interference of 6,6'-di-tert-butyl-4,4'-thiodi-m-creso with the immune system.

Thyroid and thyroid axis:

Though in some in vitro tests indications for interaction of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol with the thyroid hormone receptor ß was observed, the results of apical tests do not indicate clear effects on the thyroid axis.

A slight dose dependent increase in C-cell adenoma with a significant positive trend was seen after 2 years in female rats, however, within historical control ranges. In contrast a decrease in C-cell hyperplasia was observed, though not statistically significant.

In the 28 day study by Anonymous (1995) an increase in heart weight was seen in male rats of the mid and high dose (7% at both doses) after 14 days recovery. Total body weight was not affected. However, as the heart weight increase was an isolated finding it is difficult to judge the relevance of this effect.

In the 14 day study by Munson et al. (1988) a dose dependent increase in body weight gain was reported in female mice. This finding is in contrast to the results of most other studies, where body weights at higher doses were decreased. However, the only study of comparable duration (NTP, 15 day study) used much higher doses than Munson et al. (1988).

Based on these observations no clear conclusion can be drawn.

Neurotoxicity:

Neurotoxicity tests were conducted in the 13 week and 2 year rat studies (NTP, 1994).

In the 13 week study doses of 60/70 and 165/170 mg/kg bw/day were tested. A statistically significant, dose related increase in forelimb and hindlimb grip strength was seen in males and females. All other investigated parameters were unaffected.

In the 2 year study after 3 months there was no effect on forelimb or hindlimb grip strength in the first 3 trials (3 trials are required according to the protocol of the conducted neurotoxicity test). However, 8 trials were conducted and the strength of

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control animals decreased with subsequent trials (possibly due to fatigue or habituation). Also in the exposed groups the grip strength decreased with trials but to a lesser extent. Thus the grip strength (especially of the forelimbs) in all exposed groups was greater than in controls. All other parameters were unaffected. No effect on grip strength was seen after 6 months.

Though not regarded as adverse the increase in grip strength seen in the 13 week and 2 year rat studies (NTP, 1994) has to be regarded as an effect on the peripheral nervous system.

The effects on brain weight are rather weak at doses without decreases in total body weight. There are also no related histopathological findings and the single case of brain abnormality in [redacted] 2010, OECD TG 421) is considered an isolated finding. Together with the effects in grip strength seen in the 13 week study and after 3 months in the 2 year study, which are not considered adverse, and in the absence of any other effects on the investigated parameters (motornerve excitability or conduction, neuromuscular transmission, muscle contractility, no microscopic lesions or effects on grip strength) we consider the support for direct toxicity on the nervous system as weak.

However, as there are strong indications for interference with the HPG axis, we still consider that the developing nervous system could be a target of the toxicity of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol and are of the view that the DNT-cohort is needed to adequately assess this substance.

Induction of hormonal imbalance:

Besides the available in silico and in vitro data providing indications for estrogenicity, and anti-estrogenicity as well as some indications for interactions with the thyroid beta, androgen, glucocorticoid and PPARy receptor in the 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol data base there are several findings which point towards interference with the hormonal balance in experimental animals. Ovary and uterus weight were not investigated in many studies, but uterus weight was considerably decreased in the 3 / 6 months rat study ([redacted], publication date unknown) and ovary weight was affected in the high dose of the 28 day rat study (Anonymous, 1995). This increase was still obvious after 14 days recovery (for details see section on female reproductive organs). Both organs consist of hormone dependent tissues and effects on their weight can be regarded as indicators of hormonal imbalance ([redacted], 2010).

While the decreases in uterus weight could be interpreted as anti-estrogenic effects it is noted that the majority of uterotrophic assays also conducted with 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol indicate estrogenicity, except for one assay using i.p. administration which resulted in reduced uterus weight (Takahashi and Oishi, 2006). In this regard it is, however, important to consider that one substance can act both estrogenic and anti-estrogenic depending on e.g. tissue, life stage, toxicokinetics and tissue-concentration (for details see section on female reproductive organs and response to comments of the registrant). Also the previously described dose related reduction in mammary gland tumours and the increase in uterus tumours could be related to (anti-) estrogenic effects of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol (see section on female reproductive organs and response to comments of the registrants). An in vivo androgen screening assay provided negative results (METI, 2010).

An increase in tumours was also seen in the liver, which is an organ not considered as hormone dependent per se, and the tumour increase was only seen in males not in females. In the case of endocrine effects, however, the presence of effects in only one sex does not necessarily weaken the importance of the finding, but could well be caused

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by different hormonal status between sexes in general. The liver tumours observed in male rats could also indicate interference with the endocrine system (see also section "Comments of the Registrant(s) and Responses to Comments").

Another effect that might be related to endocrine interference is the observed myelofibrosis, which was seen in female mice in the 2 year NTP study (NTP, 1994). For the occurrence of myelofibrosis estrogen regulation plays an important role as described e.g. by Schulze and Shivdasani (2005), though other hormones might also be involved.

Atrial thrombosis, which is rarely induced upon chemical exposure, was induced in male rats after 2 year exposure to 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol (NTP, 1994; Yoshizawa et al., 2005). The effect was seen in low, mid and high dose, but did not follow dose. The observed slight anemia does not seem to be the underlying cause of the observed atrial thrombosis as outlined previously in the section on immune relevant parameters.

As stated previously in the section on immune relevant parameters, several factors including progesterone or testosterone (Yoshizawa et al., 2005) are relevant factors in the regulation of platelet formation, though this process is quite complex (see also section "Comments of the Registrant(s) and Responses to Comments"). This indicates that hormonal imbalance might adversely affect platelet formation. The difference between the sexes regarding the formation of atrial thrombosis further supports that hormonal status might be relevant for the induction of this effect.

Effects from reproductive toxicity studies also indicate interference with hormonal balance. A successful pregnancy requires a complex interaction between several hormone systems. Also the duration of pregnancy depends on a proper interplay of different hormones. An increase in gestation length, as seen in the study by [redacted] (1987), might therefore be an indication for interference with hormonal balance. Also the dose dependent alterations in sex ratio which were seen in the OECD TG 421 study could be the result of interference with hormonal balance.

The effects on male reproductive organ weights (testes, epididymides, male accessory glands), the partly severe histopathological lesions in the seminiferous tubules, reductions in daily sperm production and lowered sperm forward motility might also be indications for a hormonal imbalance, though the underlying mechanisms have not been clarified (13 week rat study (NTP, 1994); Morrissey, 1988; Takahashi and Oishi, 2006; [redacted], 2010).

Due to the effects described above there are serious concerns for risks for human health regarding endocrine disruption, reproductive toxicity, developmental neurotoxicity and developmental immunotoxicity.

Why new information is needed

The available reproductive toxicity studies on 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol comprise a reproduction screening assay in rats which was essentially carried out according to OECD TG 421, a prenatal toxicity study in rabbits and a postnatal toxicity study in mice in which only one dose, which induced considerable toxicity in the dams, was tested. From the OECD TG 421 study the conclusion was drawn that no effects on reproduction were observed. Despite this conclusion effects on reproductive organs and endocrine interference were reported. These findings were supported by repeated dose toxicity studies in rats and mice (NTP, 1994) covering 14/15 days, 28 days, 13 weeks, 3 / 6 months and 2 years exposure periods. Also in vitro results (interference with the estrogen, androgen, thyroid, glucocorticoid and PPARγ receptor) and in vivo studies

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(level 3 OECD, uterotrophic assays and mechanistic studies related to male reproductive organs) add to the conclusion that clarification of the observed results is needed.

According to ECHA's Board of Appeal the Agency has a margin of discretion regarding the choice of procedure that it follows to request information that is a registration requirement. When requesting standard information for registration purposes under the substance evaluation procedure rather than the compliance check procedure, ECHA must, in addition to demonstrating a potential concern, show that the rights of all registrants of the substance concerned are not prejudiced by the Agency's choice of procedure. EOGRTS is a standard information requirement at Annex X, section 8.7.3 of the REACH Regulation. It is also a standard information requirement under Annex IX, section 8.7.3, in case of concern in relation with reproductive toxicity in the available repeated dose toxicity studies. Although you are of the opinion that the available repeated dose toxicity studies in rats and mice (NTP, 1994) do not show such concern, the evaluating MSCA noted that effects on reproductive organs and endocrine interference were reported in these studies as described above (pages 6-7). Therefore, an EOGRTS is a standard information requirement for both Annex IX and Annex X registrants of the substance. Your registrations are at these two Annex levels. Accordingly, this request for an EOGRTS under substance evaluation does not prejudice your rights.

What is the possible regulatory outcome

The results of the requested EOGRTS will, amongst other relevant and available information, be used by the evaluating MSCA to assess whether the Substance should be classified as Reprotoxicant 1 B or STOT RE 1 as defined in CLP Regulation (EC) No 1272/2008. The evaluating MSCA will also assess whether the substance should be proposed for identification as a substance of very high concern (SVHC) under Article 57 of REACH, which would lead to stricter risk management measures than those currently in place.

Considerations on the test method and testing strategy

The requested enhanced EOGRTS including the cohorts 1A, 1B, with extension to include the F2 generation, 2A, 2B and 3 is suitable and necessary to reveal the potential effects regarding endocrine disruption and reproductive toxicity as well as developmental immunotoxicity and neurotoxicity: This study is a long-term reproductive toxicity study which includes the parameters needed to cover the relevant aspects for endocrine disruption and reproductive toxicity as well as of developmental immunotoxicity and neurotoxicity with sufficient statistical power.

1) The extension of Cohort 1B to include the F2 generation

Exposure of consumers and professionals and wide dispersive use as well as indications of relevant mode(s) of action related to endocrine disruption (from in silico data, in vitro studies and in vivo studies) and bioaccumulating potential of 6,6´-di-tert-butyl-4,4´-thiodi-m-cresol trigger the inclusion of the extension of Cohort 1B to produce the F2 generation.

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

The request of inclusion of a DNT cohort (2A/2B) within the EOGRTS is justified by:

The suspected estrogenicity as well as anti-estrogenicity are suspected hormonal mechanisms/modes of action with clear association with the developing nervous system (ECHA, 2017a).

3) Inclusion of cohort 3 (developmental immunotoxicity)

The request of inclusion of a DIT cohort (3) within the EOGRTS is justified by:

Several immune relevant organs have been affected in experimental animals upon treatment with 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol treatment (see section on immune relevant parameters). Overall the effect on spleen weight, already observed at 10 mg/kg bw/day (Munson et al. 1988), is considered a direct effect of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol toxicity and has to be considered in relation to immune toxicity of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol. Further, changes in immune function involving innate (e.g. NK-cell function) or acquired immunity (e.g. cytotoxic T-cells and antibody production) have been observed in a short term study in female mice without remarkable general toxicity (Holsapple et al. 1988).

As a further support, there is evidence on estrogenicity which may affect the immune system.

The strong skin sensitisation properties of the substance (classification Skin Sens 1, H 317 by the Registrant(s)) provide further support.

Therefore it is requested to include the DIT cohort in the EOGRTS for further assessment of the immunotoxic potential of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol during developmental exposure.

4) Specific instructions for the requested EOGRTS

Dosing-Proposal:

Dose selection should be in line with the recommendations of OECD TG 443 (paragraph 20-24). As considerable effects were seen on immune parameters at a dose of 10 mg/kg bw/day in adult mice (Holsapple et al. 1988, Munson et al. 1988), there is a concern that this dose or even a lower dose could have effects on the developing organism of the rat. It is further noted, that exposure in Holsapple et al. and Munson et al. was only for 14 days, whereas an EOGRTS is of clearly longer duration. Inclusion of a dose lower than 10 mg/kg bw/day should therefore be considered. At the same time a dose high enough to induce clear maternal toxicity shall be included as well to allow for a comprehensively assessment the toxic potential of the substance. As two- to fourfold intervals should not be exceeded according to the OECD TG 443, the addition of a fourth test group could be preferable in this case.

Premating:

The premating period shall be prolonged to 10 weeks if the cohort 1B is not extended to include the F2 generation.

The extended premating period should assure that steady state is reached with this potentially bioaccumulating substance.

DIT:

Since the IgM endpoint (included in the EOGRTS) only reflects one aspect of immune function, inclusion of endpoints covering a broader functional entity such as IgG antibody levels (after SRBC and/or KLH booster), NK cell activity and host resistance shall be provided.

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DNT:

It is requested that the cohort 2A animals are subjected to a functional observational battery and an automated test according to OECD TG 443 (para 49).

Note for Consideration:

Further, it is recommended in line with paragraph 50 of the OECD TG 443 to include a test for spatial learning and memory. Further histopathological investigation of the hypothalamus as specified in paragraph 75 of OECD TG 443 is recommended. These parameters are known to be sensitive towards estrogenic effects during brain development. Care should be taken not to compromise the integrity of the other evaluations conducted in the study.

Analysis of milk:

Based on the pharmacokinetic properties of the substance, and the potential to bioaccumulate (e.g. log Kow 5.24) transfer to milk should be investigated, also if classification for effects on or via lactation is warranted.

Further parameters:

Specific caution should be given to haematological parameters: measurement of prothrombin time and platelet count.

Alternative approaches and Proportionality of the request

The requested EOGRTS study shall clarify the concerns on endocrine disruption for human health and mammals living in the environment, toxicity to reproduction and developmental immunotoxicity and neurotoxicity.

Currently there are no alternatives for this vertebrate test available. Nevertheless, regarding the numbers of animals needed and costs the EOGRTS is efficient as this test covers several concerns. The EOGRTS is a costly study, but it allows to evaluate the identified concerns for endocrine disruption in mammals as well as concerns regarding reproductive toxicity, immunotoxicty, neurotoxicity, myelofibrosis and atrial thrombosis in one study: Due to this approach the animals needed and costs are reduced in the end.

The EOGRTS with DNT/DIT cohorts is statistically very powerful and can therefore be expected to provide a sound basis for potential risk management measures. It is a level 5 test according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters providing comprehensive data on adverse effects on endocrine relevant endpoints covering most parts of the life cycle of the organism.

The request for an EOGRTS with DIT and DNT is suitable and necessary to obtain information that will allow to clarify whether there is a risk of endocrine disruption, toxicity to reproduction, to the developing immune and nervous system. More explicitly, there is no equally suitable alternative way available of obtaining this information on multiple endpoints. The data, once obtained, can confirm whether there is risk of endocrine disruption, reprotoxicity, developmental immunotoxicity and neurotoxicity and it will allow authorities to consider further regulatory risk management in form of classification, inclusion into the SVHC process or a proposal for a restriction.

Comments of the Registrant(s) and Responses to Comments

All of your comments on the draft decision were considered and are summarised and answered below.

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General toxicology

You comment that the general toxicity studies (2-weeks, 13-weeks, 2-years studies) do not raise concerns for carcinogenicity, immunotoxicity or neurotoxicity and that there is no evidence of toxicity to gonads or accessory organs. You claim that the effects seen are mostly related to severe toxicity and body weight loss and that the haematological findings are either inconsistent or stress related. Your comments discuss the various findings in great detail.

After a thorough analysis of the available data base and consideration of your comments the eMSCA identified several concerns, which were described in the previous sections of this document and which are briefly summarised below. Though the available studies on reproductive toxicity (fertility and developmental toxicity) are rather poor they give indications for adverse effects and further examination of reproductive toxicity is therefore necessary.

Several pieces of evidence from the available studies also indicate that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol disturbs the hormonal balance and an estrogenic/anti-estrogenic and androgenic/anti-androgenic mode of action might be involved. Differences in site of occurrence and strength of adverse effects seen between sexes provide further evidence for potential interference with the endocrine system.

Genetic toxicology

You comment on Genetic Toxicology that published data (as detailed within the registration dossier) indicates no evidence for genotoxicity which is supported by the lack of tumourigenic effects observed in the 2 year carcinogenicity studies in rats and mice.

Your conclusion that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol presumably has no genotoxic potential is supported, although it is noted that not a full genotoxicity test battery is available for 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol. The observed increases in C-cell thyroid tumours, uterine polyps and hepatocellular adenomas, and the decrease in mammary gland fibroadenomas seen in the 2-year rat studies might be mediated via an endocrine mode of action.

Reproductive toxicity

With regard to reproductive toxicity you comment that the Morrisey (1988) study derives data only from general toxicity studies and that a newer and well conducted reproductive screening assay [redacted] 2010) shows no effects on reproductive organs, tissues or functions. You state that this study also shows the unlikelihood for endocrine disruption, as impairment of reproduction would be a key consequence for this end point.

The eMSCA however is of the opinion that the effects observed in several studies including the [redacted] 2010 study indicate that exposure to 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol might lead to adverse effects on reproduction which might be caused by an endocrine mode of action, if tested in an adequate study. In males, the weights of testes or epididymides were significantly increased in rat studies and significantly reduced in a mouse study, as well as the weights of seminal vesicles, prostate glands and preputial glands. In the NTP study higher frequency of adenoma in testes was noted in the test substance groups after 15 months. In female rats, with higher doses a significant positive trend was noted for stromal uteri polyps and a significant negative trend for the incidence of fibroadenomas of the mammary gland.

Developmental toxicity

You also do not see a concern for developmental toxicity as there is no evidence for

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teratological effects in mouse and rabbit developmental toxicity studies, even in the presence of maternal toxicity.

In reply to this it is noted that the available studies are not sufficient for conclusion on developmental toxicity. Only one maternally toxic dose was tested in the mice study.

Abortions, fetal loss and reduced litter size as well as reduced pup weights were seen at the high dose, with clear maternal toxicity but also to a certain degree at the mid dose, the NOAEL for maternal toxicity. Though there are some deficiencies in this study it clearly supports the concern.

Conclusion on reproductive toxicity possibly triggered by an endocrine mode of action

You draw the overall conclusion that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol is not a suspected CMR.

In contrast, ECHA is of the opinion that the above described effects show the potential of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol to affect the reproductive system may be triggered by an endocrine mode of action.

To your view the effects detected in in vitro tests were not supported by in vivo studies and that changes (e.g. in male reproductive organs) were not considered to be an effect of treatment, because similar changes were not observed in the longer dosing period and at higher dose levels ([redacted] 2010).

There are however several pieces of information, which support effects on endocrine organs, such as an increase in uterine polyps with a significant positive trend seen in the 2 year rat study (NTP, 1994). This increase might also result from interference with hormonal balance in this hormone dependent tissue. The regulation of uterine tissue is modulated via estrogen and changes in estrogen levels have been related to tumours in this organ.

ECHA considers in that regard also the observation of increased incidence of atrial thrombosis which was observed in male rats in the NTP rat 2 year study (Yoshizawa et al., 2005). It is known that, among other factors, progesterone, testosterone (Yoshizawa et al., 2005) or estrogen (e.g. Khetawat et al., 2000, Bord et al., 2004; Schulze and Shivdasani, 2005) are relevant factors in the regulation of platelet formation, though this process is quite complex. This indicates that hormonal imbalance might adversely affect platelet formation. The difference between the sexes regarding the formation of atrial thrombosis further supports that the hormonal status might be relevant for the induction of this effect. In this regard it should also be considered that oral hormonal contraceptives are known to increase the risk of atrial thrombosis (e.g. Yoshizawa et al., 2005).

It should be noted that in the study report of [redacted], 2010 it is speculated that the absence of effects on the male reproductive organs which is in contrast to the Takahashi and Oishi, (2006) study might be caused by a considerably shorter study duration (42 days in [redacted] (2010) versus 61 days in Takahashi and Oishi (2006)).

It is concluded that the two weeks premating exposure might have been too short to cause adverse effects on male fertility. Further, changes in relevant organ weights were also reported in NTP (1994).

Other endocrine disruption related parameters

You state on androgenicity that in vitro and in vivo studies reveal negative results.

In reply to this comment it is noted that the cited data were already partly included into the draft decision: The in vitro data were included– together with positive results from a

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human androgen receptor binding assay – although the reporting is rather poor. The information, that the in vivo androgen screening test was negative was included in response to your comment and the reference name was updated to "METI (~2010)" instead of "Hershberger Assay, no date, study title or author names provided, last dated reference from 2001".

Your comment on other endocrine related parameters: The QSAR data uses an algorithm to provide suggestive evidence for ER binding.

ECHA agrees that QSAR data provide suggestive evidence for ER binding.

According to you the ToxCast toxicity screening (US EPA, 2015) provides no practical evidence for endocrine disruption, as the information of potency (strength) is not included and just "positive" or "negative" is mentioned.

In reply to this it is noted that though the cited ToxCast data (US EPA, 2015) provide only information on the "positive" or "negative" outcome but may be nevertheless considered a relevant indication for possible endocrine disruption in a weight of evidence approach.

You mentioned further that although antagonistic activity for the thyroid hormone receptor (beta) and agonistic activity for the glucocorticoid receptor and PPARγ were observed within the ToxCast (US EPA, 2015) but noted that there was no evidence of any untoward thyroid effect from the general toxicology e.g. weight change of the gland or histopathology and the liver weight increase appeared to be due to vacuolisation rather than any proliferative response.

It is stressed at this place that tumours of the thyroid (C-cell adenomas and carcinomas) occurred with a significant positive trend in female rats after two years, though the incidence was within historical control values. But it is agreed that no other related effects were seen in the thyroid gland.

Regarding the liver weight increase it is noted that foci were increased, as well as adenomas and carcinomas (males in 2 year NTP study). The large difference between the number of liver tumours in male and female human individuals could indicate hormonal influence on this tumour type (see e.g.: Shimizu at al., 2001).

You argue that the estrogenicity is in contrast with the negative trend resulting in a decreased incidence of mammary gland tumours in mid and high dose level rats (anti-estrogenicity) in the NTP 2-year study in rat which suggests no consistent endocrine effect.

ECHA does not consider the described effects as contradictory. It has to be noted that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol has the potential to elicit in vitro estrongenic and anti-estrogenic effects. While in vivo the results of 5 uterotrophic assays show predominantly estrogenicity, also anti-estrogenic effects were seen in one (ip) of the 2 rat uterotrophic assays the other uterotrophic assay in rat (sc) showed a tendency for estrogenicity. The decrease in mammary gland tumours observed in a 2 year study in rats (NTP, 1994) indicates anti-estrogenic activity as well.

Depending on differences in study design (dose, route) and target tissue it is not contradicting to induce either estrogenic or anti-estrogenic effects with one and the same substance. This is for example also described for triclosan in Gee et al. (2008) and for bis(2-ethylhexyl)phthalate (DEHP) in the Support Document for Identification of DEHP as Substance of Very High Concern because of its Endocrine Disrupting Properties (ECHA, 2014a): DEHP antagonized the androgen receptor in some assays (Takeuchi et al., 2005), but not in others (Krüger et al., 2008; Kim et al., 2010; Parks et al., 2000).

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These different results are therefore not considered contradictory but rather demonstrate estrogenic as well as anti-estrogenic effects of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol.

With regard to endocrine disruption you comment that the concern is based on a variety of sources including QSAR, in vitro and in vivo study results, but that there is no direct evidence of reproducible endocrine disruption in any of the general or reproductive toxicity studies.

In reply to this it is stressed that positive results of in vitro studies and effects detected in several in vivo studies described above do lead to the concern that 6,6´-di-tert-butyl-4,4´-thiodi-m-cresol might be an endocrine disruptor and this concern needs to be further clarified. The existing data are quite old and also, to a certain extent, of limited quality. The NTP study protocol does e.g. not document organ weights in endocrine organs of the 2 years studies (NTP, 1994). The reproduction screening assay, which showed effects on sperm and testes, sex ratio and lack of copulation in some animals, indicating interference with the endocrine system, may not be the appropriate tool for detecting more subtle effects ([redacted], 2010).

Immunotoxicity

You provide in your comments a working definition for immunotoxicity and comment that the toxicological assessment of the general toxicology does not lead to any evidence for conclusion of immunotoxicity and that several effects (gastrointestinal effects, effects on thymus and mesenteric lymph nodes) were caused by the substance's irritant properties.

You claim further that long term survival and health at dose levels up to a maximum tolerated dose is a practical demonstration of the lack of immunotoxic effects, neither immune-stimulatory nor immune-suppressive. The relevance of changes in relative organ weights is critically reviewed for relative bodyweight independent organs. Further, you refer to a conclusion made by [redacted], 2005) that does not consider the immune system as a critical target for the registered substance.

In reply to your comment it is stressed that immunotoxic substances may also have more subtle effects, which are however of high relevance for humans. One example thereof is the delayed immune response after vaccinations. Further it has to be considered that controlled environments used for animals in safety testing may limit the opportunity for robust antigenic stimulation or infectious agent challenge. As a result, the resting immune system of a laboratory animal in a controlled, relatively pathogen-free environment is an insensitive test system for evaluating chemically induced immune dysfunction (WHO, 2012).

ECHA agrees that effects on spleen which are accompanied by massive weight loss as observed in the 2-weeks NTP studies, are of minor relevance for the assessment of immunotoxicity. However, effects on spleen weight were observed in the 13 weeks and 2 year NTP studies at doses with no or no significant reduction in body weights and also in the Munson et al. (1988) study considerable increases in spleen weight were seen after 2 weeks exposure without decrease in total body weight (in contrast, increases in total bodyweight were seen in this study). Increases in spleen weight have to be considered as indications for immunotoxicity and were further underpinned by increased cell numbers in spleen (Munson et al., 1988).

In this regard it is also important to note that decreases in thymus weight were also reported (rat 13 week NTP study, without decrease in total body weight; mouse 13

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weeks NTP study, though not following dose; rat 3 / 6 months study ([redacted] publication date unknown), decreases after 3 and 6 months, but not statistically significant).

Referring to the conclusion by the Committee on Updating of Occupational Exposure Limits of the Netherlands (Health Council of the Netherlands; 2005) the eMSCA states that since 2005 the state of the science on endocrine disrupters has evolved significantly. Immune function and regulation involve the integration of the endocrine, immune and nervous systems; chemical exposure may produce a complex series of effects that result in stimulation of some parameters at one concentrations, while producing suppression it at other concentrations.

Further, it is replied that estrogenicity and anti-estrogenicity are accepted triggers for inclusion of the DIT cohort (ECHA 2017a). There are several indications from the 6,6′-di-tert-butyl-4,4′-thiodi-m-cresol data base that indicate these modes of action are relevant in this case (see previous responses to comments). In this regard it also has to be considered that the OECD TG 421 study protocol is only a screening study and does not finally evaluate all reproductive parameters (see also previous responses to comments).

In a weight of evidence approach it can be summarised that diverse immunological parameters have been affected by 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol treatment and need further clarification. Some effects were also seen with relevance for the nervous system, and it is well known that adverse effects on maternal immune function interfere with the foetal neurodevelopment (Smith et al., 2007).

With regard to immunotoxicity and neurotoxicity it should be considered that there is a well described link between adverse effects in these organ systems and (anti-)estrogenic and / or (anti-)androgenic activities (Fish, 2008; Adori et al., 2010; Robinson et al., 2011; Frye et al., 2012).

The demonstrated skin sensitisation potential also supports the inclusion of the DIT cohort of the EOGRTS, however, you stress that skin sensitization is an immunologically mediated cutaneous reaction to a substance and is not sufficient justification as a reason why an immunotoxicity study is required.

In reply to this it is noted that 6,6′-di-tert-butyl-4,4′-thiodi-m-cresol is a proven sensitiser and refers to the ECHA Guidance R7a which lists sensitisation as supporting factor of the particular concerns justifying the inclusion of the developmental immunotoxicity cohort into the EOGRTS testing scheme (ECHA, 2017a).

The request of the DIT cohort is justified and in line with the REACH guidance due to the following reasons: i) Information on hormonal mechanisms/modes of action with clear association with the immune system, such as (anti-)estrogenicity and (anti-)androgenicity as reported in several studies for 6,6′-di-tert-butyl-4,4′-thiodi-m-cresol; ii) Information on changes in immune function involving innate (e.g. NK-cell function, phagocytosis and oxidative burst) or acquired immunity (e.g. generation of immunological memory, cytotoxic T-cells and antibody production) as reported for 6,6′-di-tert-butyl-4,4′-thiodi-m-cresol and as a supporting factor the sensitising properties.

Because the immune system has a large functional reserve, toxic damage is likely to be measurable only after significant impairment (NRC, 1992). Nevertheless there is reason to believe that chemical-induced damage to the immune system might be associated with pathologic conditions, some of which could become detectable only after a long latency (NRC 1992). The WHO Guidance for Immunotoxicity Risk Assessment of Chemicals lists 25 potential target diseases and disorders for immunotoxicology-driven risk and 20 in category 1 (immune system is identified as critical factor). Recent epidemiology research demonstrates that certain substances interfere with the immune

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system and lead to e.g. delayed immune reactions after vaccination, reduced antibody response and increased risk of infections in children (Grandjean et al., 2012; Heilmann et al., 2006; Hertz-Piciotto et al.,2008; Glynn et al. 2008).

Subtle effects on the immune system will probably not be detected in a reproductive screening assay. Therefore, based on the severity of potential effects, the susceptibility of the developing organism and the long term consequences sufficient indication to integrate the DIT into the requested EOGRTS is seen.

Neurotoxicity

With regard to neurotoxicity, you comment that there is no neurotoxicity and that all of the neurotoxic findings cited by the eMSCA are considered to be toxicologically irrelevant. You claim that high level studies and additional trials including special neuro-histopathology did not show any evidence for any structural or functional effects. You consider therefore that the performance of the DNT cohort is unnecessary.

With regard to immunotoxicity and neurotoxicity it should be considered that there is a well described link between adverse effects in these organ systems and (anti-) estrogenic and / or (anti-)androgenic activities (Fish et al., 2008; Adori et al., 2010; Robinson et al., 2011; Frye et al., 2012).

There is strong indication for interference with hormonal balance (estrogenic/anti-estrogenic, androgenic/anti-estrogenic) and an appropriate study including the necessary parameters is therefore needed to assess whether these findings were only chance findings or true indications for thyroid interference. This takes into account the specific vulnerability of the developing organism towards thyroid affecting and neurotoxic chemicals.

Your conclusion

You comment that the justifications for the concerns have been considered very carefully and the basis for the concerns can be recognised looking at the lists of issues which could be taken to reflect target organ, or system dysfunction or modulation. However, much of the data is gathered from in silico or in vitro studies where the results or evidence cannot be concluded either way due to inconsistent findings. You conclude therefore that the overall weight of evidence indicates that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol is not of concern for reproduction effects, is not an endocrine disruptor, an immunotoxicant or a neurotoxicant.

In reply to this it is highlighted that the mechanistic in vivo (uterotrophic assays), in vitro and in silico data provide strong indications that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol has the potential to act as an endocrine disruptor. The data base is quite old and also inadequate to a certain extent: There is not a single Klimisch 1 reproductive toxicity or repeated dose study available. Despite the absence of high quality Klimisch 1 studies, there is evidence for endocrine disrupting and reprotoxic effects, as explained already above. As depicted in the Section Concerns identified: developmental toxicity was observed at doses which lack maternal toxicity. The available reproductive toxicity screening assay [redacted], 2010) which widely followed OECD TG 421 was conducted in 2010, which was prior the up-date of the test guideline in 2016, where several parameters suitable to assess endocrine disruption potential were included.

You acknowledge that further information could be supportive to the evaluation process. Currently, the lead registrant is preparing an OECD TG 422 study on a close structural analogue, the para derivative 6,6'-di-tert-butyl-2,2'-thiodi-p-cresol (EC 202-009-7; CAS 90-66-4). The OECD TG 422 screening study contains the endocrine disruption cohort

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required for the registered substance. It is proposed to ECHA that this study on a closely similar analogue could be used for potential read-across to provide further reassurance on the endpoints of concern and that if any valid residual concerns remain these could then be followed-up specifically on 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol using the most relevant studies mutually agreed between the parties.

In reply to this it is noted that the use of reference substances always needs to be substantiated by detailed read-across analyses. First, there needs to be structural similarity between substances resulting in the likelihood that the substances have similar physico-chemical or toxicological properties. Second, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach). Such a detailed analysis has not been performed for the referenced substances.

In your comments you argued that in the light of this assessment you do not consider that there is sufficient justification to conduct an OECD TG 443 in the first instance based on sound toxicological evaluation of the ECHA review and also based on animal welfare considerations balanced against the reasonable probability of obtaining no additional substantive findings.

These indications are based on all relevant information available for the eMSCA analysed in a weight of evidence. Considering all data together (including studies of higher as well as of lower quality) in a weight of evidence analysis the eMSCA comes to the conclusion that the identified concerns need to be clarified in a comprehensive study with high statistical power including all relevant endpoints assessing reproductive toxicity and adverse endocrine effects, i.e. an extended one generation study, including the DNT as well as the DIT cohort.

Comments on exposure

You state, that there is no exposure to consumers or the general public. Further, you state that the substance is contained within the final article matrix, and it is highly unlikely that the substance becomes bioavailable from the end use within rubber and plastic articles, as it is not known to migrate to any significant level from end use products. The substance is an approved indirect additive used in Food Contact Substances in both the US and the EU (where it is used as such).

You highlight, that the substance is included in the list of authorised substances in EU Regulation No. 10/2011. The Specific migration limit is: 0.48 mg/kg SML. No other restrictions and/or specifications are detailed.

In reply to your comments on exposure it is noted that according to the Registration dossier the substance is used in formulation of non-dusting blends, as anti-oxidant in medium/high voltage cross linked PE cables, as anti-oxidant/bleaching agent in adhesives and emulsifiers of rubber emulsion, for production of impact modified plastic and thermoplastic articles. The substance as manufactured is used industrially. The manufactured articles are used by professionals. Consumer uses of the substance as such are not specified in the Registration dossier. The registered uses cover activities like roller application or brushing, transfer of substance or preparations, etc.

Relevant contact of consumers and the general public can however not be excluded at this stage, considering, that the substance is still contained in the matrix of articles as such and releasable via migration or other potential processes. Furthermore, significant exposure of professionals cannot be excluded based on available information.

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In case you can demonstrate that there is no significant exposure of professionals, and consumers and update your Registration dossier accordingly, you are not required to extend the 1B cohort to include the F2 generation. In order to demonstrate that there is no significant exposure of professionals and consumers, you should for example: provide sufficient qualitative and quantitative details on the uses to be covered and refine the description of the risk management measures used.

Consideration of Proposals for Amendment and Registrant's comments on Proposals for Amendment

Based on a PfA, a paragraph was added explaining that you, all registrants of the substance, are not considered to be prejudiced by the choice of procedure (data request via compliance check or substance evaluation), as the requested EOGRTS is also considered to be a standard information requirement for Annex IX and Annex X registrants based on the concerns identified in relation with reproductive toxicity in the available repeated dose toxicity studies.

A new chapter "what is the possible regulatory outcome" has been introduced.

A new chapter "potential for exposure" has been included referring to the section discussing the concerns on exposure.

An MSCA made a PfA proposing to considerably shorten the draft decision and to only list the relevant studies supporting the specific cohorts, and to leave out all studies only describing general toxicity. It was also proposed to leave out all Klimisch 3 and 4 studies.

However, in this specific case it was considered necessary to have a comprehensive overview on the diverse effects of the substance and as the database is rather poor also Klimisch 3 and 4 studies were included, which were considered supportive on a weight of evidence basis and in order to include all pieces of information. This demonstrates that all pieces of information were considered and given the respective weight.

Two MSCAs stated in their PfAs that the inclusion of the DNT cohort was not sufficiently supported. They argued that the effects on the HPT axis were not sufficiently demonstrated and that the effects on brain weight and the reductions in grip strength were not sufficient, or not described in sufficient detail, to demonstrate neurotoxicity.

ECHA agrees that the effects on the HPT-axis were not clear cut. In addition, there are clear signs for possible interference of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol with the HPG-axis, in several in vitro and in vivo studies (including uterotrophic assays and different repeated dose toxicity studies). Steroid hormones govern normal sexual differentiation of the brain during the late gestational and early neonatal periods. Sexual differentiation of the brain is regulated by the action of fetal and maternal steroid hormones on the steroid hormone receptors in the brain. Testing possible effects on the developing nervous system is therefore clearly relevant.

It is noted that one of the two MSCAs referred to an unpublished report by ECHA, which failed to find any convincing evidence to link perturbation of sex hormone levels and developmental neurotoxicity. However, in reply to this PfA it is noted that there is a broad range of literature supporting a link between hormonal modes of action and brain development (e.g. Luttge et al., 1975, Isgor et al., 1998; Hotchkiss et al., 2002; Puts et al., 2006; Frye et al., 2012; Pallares et al., 2014; Gore et al., 2014; Fish et al.; 2008, Adori et al., 2010; Robinson et al., 2011,).

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An MSCA mentioned in a PfA that an extension of the EOGRTS design to include the F2 generation should be considered if the exposure is wide dispersive .As explained above there is wide dispersive use, according to your Registration dossier, therefore the study design was extended by requesting this extension.

Pre-mating exposure duration:

Though not clearly related to any PfA made by MSCAs, you commented on the length of the pre-mating period. You are in favour of reducing it from 10 weeks to 2 weeks, with the argument that a complete spermatogenic cycle would be covered by the total length of the EOGRTS and because the BAF value would indicate that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol has no bioaccumulation potential in mammals.

ECHA is of the view that exposure should cover a complete spermatogenic cycle prior to mating in order to be able to fully assess the effect of the substance on fertilisation of parental females. Reference was also made to ECHA guidance Chapter R7.a (ECHA, 2017a), which contains 10 weeks pre-mating exposure as standard recommendation. As already explained above, the premating period can be reduced to 2 weeks if a F2 generation will be included in the EOGRTS design.

In addition, 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol is considered to be potentially PBT/vPvB at this stage. Its expected bioaccumulation potential further supports the use of a 10 week pre-mating exposure. Regarding the BAF value mentioned by you, it is noted that it does not overrule the overall concern derived for bioaccumulation, based on, amongst others, toxicokinetic studies and that there exist currently no agreed standardized experiments on mammalian bioaccumulation to determine BAF-values.

In conclusion, the substance needs to be tested with a pre-mating exposure period of 10 weeks in order to cover all possible effects on spermatogenesis and to make sure that steady state is reached, for this potentially bioaccumulating substance.

Range finding study and dose selection:

You are of the view that a dose range finding study is needed prior to the foreseen EOGRTS. This is because you consider the available studies as not sufficient to derive appropriate doses because the studies were conducted in different species and using different routes of exposure. You also doubt that more than 3 doses, also covering the low dose range, would be needed.

The section on specification of test doses has been amended to clarify that more than 3 doses might be appropriate.

Inclusion of the DNT cohort (2A / 2B)

You are of the view that the available data do not indicate that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol is a neurotoxicant. You believe that the significant increase in grip strength seen in a 90 day study and after 3 months, but not after 6 months in a two year study, the slight effects on brain weight, without histopathological correlates and the isolated finding of a single case of brain abnormality in [redacted] (2010, OECD TG 421) and in the absence of any other effects on the investigated parameters (motornerve excitability or conduction, neuromuscular transmission, muscle contractility, no microscopic lesions or effects on grip strength) do not demonstrate neurotoxicity.

The same comment has already been provided by you during the commenting of the draft decision. A detailed response was provided.

ECHA agrees that the above described findings do not indicate that 6,6'-di-tert-butyl-

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4,4'-thiodi-m-cresol induces direct toxicity on the nervous system. However, as there are strong indications for interference with the HPG axis, the developing nervous system could be a target of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol toxicity and therefore the DNT-cohort is needed to adequately assess this substance.

Available read-across:

You presented an OECD TG 422 study for 6,6'-di-tert-butyl-2,2'-thiodi-p-cresol, which is structurally closely related to 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol. Based on a comparison of physico-chemical and available toxicological parameters you consider read-across as appropriate in this case.

As already pointed out in response to your previous comments , a read-across needs more profound justifications. Read-across from other substances needs to be based on comprehensive data on the structural analogue and extensive analyses of these data, in line with ECHA (2017c), would be necessary. Such data for clarifying the identified concerns are not considered to be available at this stage.

In addition a study according to OECD TG 422 is not sufficient to clear all concerns identified for 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol. A comprehensive study, as the EOGRTS, with a long enough exposure period to allow also observations at later developmental stages, with sufficient statistical power and with inclusion of relevant endpoints is therefore still necessary for the present substance.

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, , specified as follows:

1) Cohort 1A (Reproductive toxicity);
2) Cohort 1B (Reproductive toxicity)
3) Extension of cohort 1B to include the F2 generation
4) Cohorts 2A and 2B (Developmental neurotoxicity); and
5) Cohort 3 (Developmental immunotoxicity).

If you can demonstrate that significant exposure of professionals and consumers can be excluded and update your Registration dossier accordingly, the extension or the Cohort 1B for inclusion of F2 is not required. In that case, it is required to extend the premating exposure period to 10 weeks, as explained above.

ENDPOINT 2: Concerns for endocrine disruption for aquatic vertebrates

Fish Sexual Development Test (FSDT), test method OECD TG 234, using either Japanese Medaka *(Oryzias latipes)* or Zebrafish *(Danio rerio)* and five test concentrations

The Concern Identified and why new information is needed

The available QSAR data for 6,6´-di-tert-butyl-4,4´-thiodi-m-cresol predict that this substance is a very strong estrogen receptor binder (see endpoint 1). The available in vitro data show considerable hormone receptor activities e.g. regarding the estrogen and the androgen receptor (see endpoint 1).

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Data on rats, mice and rabbits show concerns for effects (see endpoint 1) including inter alia effects on organ weights of testes, epididymides, and accessory glands, serious lesions in seminiferous tubules, and decreased daily sperm production.

Five uterotrophic assays provide a data basis showing the ability of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol to act estrogenic and potentially also anti-estrogenic in vivo. (see endpoint 1 for a more detailed analysis.

No measured chronic aquatic vertebrate data are available.

The available QSAR data, in vitro data and in vivo data on rats, mice and rabbits indicate a potential for endocrine disruption also in aquatic vertebrate species.

What is the possible regulatory outcome?

The requested study will provide information on endocrine disruptive properties of the substance. Ultimately, this can lead to a confirmation of the suspected endocrine disruption properties according to the World Health Organisation/International Programme on Chemical Safety working definition and the evaluating MSCA will assess the need for further regulatory risk management in the form of identification as a substance of very high concern (SVHC) under Article 57(f) of REACH and subsequent authorisation or restriction of the substance.

Also a restriction based on adverse effects not attributable to endocrine disruption is possible. Moreover, adverse effects attributable to endocrine disruption or not attributable to endocrine disruption can lead to environmental classification including an M-factor.

Considerations on the test method and testing strategy

The FSDT assesses early life-stage effects and potential adverse consequences of putative endocrine disrupting chemicals (e.g. (anti-) estrogens, (anti-) androgens and steroidogenesis inhibitors) on sexual development and is therefore a good choice for the evaluation of potential endocrine effects in aquatic species.

Because of the possibility for genetic sex determination, it may be preferable to conduct the study on Japanese Medaka (*Oryzias latipes*). However the test guideline is also validated for Zebrafish (*Danio rerio*) and this species could be used instead.

The test shall be performed under flow-through conditions. Five test concentrations together with appropriate controls shall be used in order to obtain a robust concentration response and to increase the probability to derive a more precise NOEC/LOEC and/or EC_x to be used for further risk management considerations.

Further, in the uterotrophic assays predominantly an agonistic response is observed, but also an antagonistic response is elicited in one of the assays. These different responses might occur at different concentrations, which is one more reason to test a broader concentration range to cover both types of response.

Close attention should be paid to the analysis and presentation of actual measured concentrations of the substance. Histopathological examination of liver and kidney are recommended to allow identification of confounding systemic toxic effects when assessing ED-related endpoints. For the histopathological examination of liver and kidney

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the use of the Guidance Document on Medaka Histopathology Techniques and Evaluation for the Medaka Extended One-Generation Reproduction Test (OECD, 2015) is recommended where applicable.

The protocol of OECD TG 234 (OECD, 2011) is in principle an enhancement of test guideline 210: Fish, Early Life Stage Toxicity Test, where the exposure is continued until the fish are sexually differentiated: Therefore, the FSDT is also appropriate to assess long-term aquatic toxicity to fish.

Alternative approaches and Proportionality of the request

The FSDT will be used to clarify the concern: Endocrine disruption in aquatic vertebrates.

There are currently no better suited alternatives for this vertebrate test.

The FSDT is a Level 4 test according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters and is providing data on adverse effects on endocrine relevant endpoints.

As stated in the description of the FSDT results from this test can be used for hazard and risk assessment due to the population-relevant change in phenotypic sex ratio.

The request for an FSDT is suitable and necessary to obtain information that will allow to conclude whether the substance is an endocrine disrupter in aquatic wildlife species. There is no equally suitable alternative way available of obtaining this information without using a Level 5 test according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters, which would even need more animals.

Comments of the Registrant(s) and Responses to Comments

You argue that exposure is not likely as the main scope of exposure would be controlled within the workplace by appropriate risk management measures, good industrial hygiene and regulation under separate controls associated with the use of such substances within the workplace.

In reply it is stressed that emissions to the environment are also indicated based on your calculated exposure estimates for the environment. Regarding potential for emissions, the other stages of use (service-life and phase after service-life) are considered as relevant as the industrial processes.

The substance is not consumed during production of plastics and rubber products and is expected to remain as such in the matrix in most cases (e.g. as antioxidant). Therefore, release to the environment during and even after article service-life (disposal, if article is not combusted or recycled) might happen as well. The scope of plastics and rubber articles intended for professionals and consumers is not limited and could be broad based on the registration data. The intended use of articles is considered to be wide-dispersive.

Moreover, you refer to discussions under endpoint 1 and state that no direct evidence of reproducible endocrine disruption was observed. You are of the opinion that as of the 18 tests conducted with 6,6´-di-tert-butyl-4,4´-thiodi-m-cresol, 14 tests gave results for sex hormones indicating both estrogenic, anti-estrogenic and androgenic and anti-androgenic activity, the results are inconsistent and it is not possible to say whether this screening outcome provides any practical evidence for endocrine disruption or not.

In reply to these comments ECHA agrees that these studies alone do not provide clear evidence of the substance being an endocrine disrupter, but they do provide indications for endocrine activity as do the endocrine mediated adverse effects from the mammalian database. Therefore testing is required to clarify the ED concern for human health and

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the environment. The results from ToxCast (US EPA, 2015) are not deemed inconsistent: These data are usable in a weight of evidence approach. A substance can elicit estrogenic, anti-estrogenic and androgenic and anti-androgenic activity. Regarding discussions on QSAR, in vivo and further in vitro study results regarding ED indications see endpoint 1.

You argue that the substance is already classified in the highest classes for Aquatic Toxicity with Aquatic Acute 1 and Aquatic chronic 1, and that the risks are well understood and controlled. An FSDT would therefore be of no further value to further address the chronic fish toxicity concern. Nevertheless, you are amenable to further testing regarding endocrine disruption in aquatic vertebrates to assist in mitigating these concerns. As you consider that the available data raise only a low or moderate level of suspicion according to the Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, No. 150 (OECD, 2018), you propose an OECD TG 229 (Fish Short Term Reproduction Assay) or an OECD TG 230 (21-day Fish Assay,).

In reply to these comments it is acknowledged that the substance is self-classified as Aquatic Acute 1 and Aquatic chronic 1: Nevertheless, a higher M-Factor might be necessary to classify this substance adequately. At the moment, no chronic fish data are available, although acute data show that fish is a sensitive group of animals and QSAR data hint at high chronic fish toxicity. The possible endocrine Mode of Action in fish further increases the possibility to observe high toxicity leading to a need for a higher M-Factor.

Moreover, the confirmation of endocrine disruptive properties can lead to further risk management measures. Data from other vertebrate species like rats and mice indicate a concern for endocrine disruption and according to Guidance document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (OECD, 2018) endocrine systems with respect to hormone structure, receptors, synthesis pathways, hormonal axes and degradation pathways, are well conserved across vertebrate taxa especially in the case of estrogen, androgen, thyroid hormones and steroidogenesis.

Therefore, the aquatic toxicity cannot be considered to be adequately understood or controlled.

It is appreciated, that you are amenable to conduct further testing regarding aquatic ED testing although you propose screening tests only. Nevertheless, an FSDT is the more suitable option than a fish OECD Level 3 test in this case, as there is already a considerable amount of concern regarding endocrine disruption (see endpoint 1), already including positive OECD Level 3 tests (uterotrophic assays).

The FSDT provides information regarding both apical effects and MoA (OECD ED conceptual framework Level 4) as opposed to the alternative initial screening tests, the 21-day Fish Assay (OECD TG 230) or Fish Short Term Reproduction Assay (OECD TG 229) both of which are regarded as only providing information about sex hormone Mode of Actions (OECD ED conceptual framework Level 3). A further Level 3 test would not investigate the range of mechanistic and apical endpoints of a Level 4 test, nor show how these are linked. If positive endocrine disruption results were seen in the proposed Level 3 tests, then a higher tier test would still be required and this would not be in the interests of animal welfare. As there are sufficient in vitro and in vivo mammalian data already at Levels 2 and 3 of the OECD Conceptual Framework to indicate a plausible mechanistic endocrine mode of action regarding (anti-) estrogenicity and (anti-) androgenicity further testing at these lower Levels is not justified as the concern would remain.

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Moreover, the adverse endpoints measured in the FSDT are more adequate to be used for classification regarding Chronic Aquatic toxicity compared to the results obtained in an OECD TG 229 or 230 test.

Further, the FSDT is able to detect a broad variety of modes of actions, i.e. substances acting through (anti-)estrogenic, (anti-)androgenic and steroid synthesis disrupting mode of action (E, A and S modalities).

Consideration of Proposals for Amendments and Registrant's comments on Proposals for Amendments

Triggered by PfAs made by ECHA and MSCAs the following specifications for the FSDT were included:

- Use of test species (Medaka or Zebrafish with preference for Medaka)
- Use of five test concentrations and appropriate controls
- Attention to analysis and presentation of actual measured concentrations
- Recommendation for histopathological examinations of liver and kidney to allow identification of confounding systemic toxic effects when assessing ED-related endpoints. The use of the Guidance Document on Medaka Histopathology Techniques and Evaluation for the Medaka Extended One-Generation Reproduction Test (OECD, 2015) is recommended where applicable.

Following a PfA made by an MSCA the information regarding the concern for chronic fish toxicity including not valid QSAR predictions was deleted.

Following an ECHA PfA the chapter "*What is the possible regulatory outcome"* has been introduced.

You commented that you are amenable to this test, but you would request a lower number of test concentrations and the removal of any potential requirement for histopathological assessment of the liver and kidneys. Regarding the use of five test concentrations you state that it is difficult to use five test concentrations due to the low water solubility and that as it is understood that "chronic fish toxicity" itself is not a concern. You believe that three test concentrations, which is prescribed in the test guideline, is sufficient to conduct a risk assessment regarding the ED concerns.

In response to these comments it is specified that five test concentrations must be used to obtain a robust concentration response and to increase the probability to derive a precise NOEC/LOEC or ECx to be used for further risk management considerations.

Further, in the uterotrophic assays predominantly an agonistic response is observed, but also an antagonistic response is elicited in one of the assays. These different responses might occur at different concentrations, which is one more reason to test a broader concentration range to cover both types of response.

In reply to your argument referring to low water solubility it is stated that the substance has a water solubility of 27.7 µg/L: This value is considered to be high enough to conduct a test with five test concentrations using the spacing as foreseen in the test protocol of OECD TG 234.

Regarding the examination of the fish liver and kidney histopathology you consider that this request is beyond requirements of the test guideline, and furthermore, test fish in the FSDT are too young to be examined for histopathology in accordance with the

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MEOGRT guidance. It is difficult for you to accept this proposal, and you would question if it was definitely required.

In reply to this it is clarified that the examination of the fish liver and kidney histopathology is recommended to identify confounding systemic toxic effects when assessing ED-related endpoints. As far as possible the use of the Guidance Document on Medaka Histopathology Techniques and Evaluation for the Medaka Extended One-Generation Reproduction Test (OECD, 2015) is recommended.

Conclusion

Therefore, 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:

Fish Sexual Development Test (FSDT), test method OECD TG 234, using either Japanese Medaka *(Oryzias latipes)* or Zebrafish *(Danio rerio)* and five test concentrations.

ENDPOINT 3: Tiered approach for the PBT/vPvB concern

Simulation testing on ultimate degradation in surface water; test method: Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25./OECD TG 309, "pelagic test" – without additional suspended solids/sediment

The Concern(s) Identified

6,6'-di-tert-butyl-4,4'-thiodi-m-cresol is considered as potential P/ vP and potential B/ vB, based on estimated biodegradability, Log K_{OW}, Log K_{OA} and BCF values. The substance screens as potential P/vP based on BIOWIN v.4.10 (US EPA EPI-Suite, 2012) predictions (BIOWIN 2: 0.072, BIOWIN 3: 1.95, BIOWIN 6: 0.0079) and as potential B/vB based on the predicted Log K_{OW} of 8.24 (EPI Suite, EPI Web 4.1, KOWWIN v1.68) and the predicted Log K_{OA} of 15 (EPI Suite, EPI Web 4.1, KOAWIN v.1.10), indicating a bioaccumulation potential for aquatic and terrestrial organisms (ECHA, 2017). Bioaccumulation estimates using the predicted Log K_{OW} of 8.24 as input value were calculated using EPI Suite (BCFBAF v3.01) and resulted in a BCF of 1961. Further, there exists some evidence from animal studies that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol might accumulate in liver and adipose tissue. Estimated toxicity data show in addition, that the substance fulfils potentially the T-criterion. Based on the evaluation of all relevant information submitted on 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol the substance fulfils the screening criteria for the persistency, bioaccumulation and toxicity and ECHA deems that further information is required to conclude on the PBT/vPvB properties. The evaluating MSCA performed a simulation of the biodegradation pathway and two of the simulated transformation/degradation products screen as potential P/vP (BIOWIN v.4.10), potential B/vB (BCFBAF v4.01) and potential T (ECOSAR v1.11).

Why new information is needed

You provided screening data on the P – properties and concluded that the substance fulfils vP (and P) criteria, without having half-life data (ref. to CSR and ECHA dissemination site[5]). All studies (aerobic studies: [redacted], 1979; [redacted] 1980 and

[5] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16037/2/3/?documentUUID=0d763d4a-ef37-438d-9d33-1044709ed2b0

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anaerobic study: [redacted], 1980), except one screening study [redacted] 1980) are considered as not reliable by you. Your conclusion is based on BIOWIN predictions and on a screening biodegradability test with prolonged incubation and pre-adapted mixed source inoculums, which was assigned by you as similar to OECD TG 301B. You interpreted the results concluding that the substance is not readily and not inherently biodegradable. The lack of degradation (< 20%) was in your opinion sufficient to conclude on the persistency. Simulation tests were waived by you.

ECHA considers the data provided as not reliable, as the only study, [redacted], (1980), rated by you as reliable (Klimisch 2) is a non-guideline screening study and not sufficient for concluding on the P and vP criteria. In addition, it is not known whether inhibition by test substance occurred in this study (as there was no toxicity control) or whether the test substance concentration (20.7 mg/L), which is above the water solubility (27.7 µg/L at 20 °C) was limiting degradation and therefore not sufficient to finally conclude on the P and vP-criterion. ECHA rated the information submitted: According to the data no conclusion can be drawn, if 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol or potential transformation/degradation products meet the P/vP-criterion. QSAR estimates conducted by the evaluating MSCA with 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol indicate limited degradation indicative for persistence (VEGA – ready biodegradability model vers. 1.0.9, BIOWIN models 2, 3, and 6 from EPI-Suite (US EPA, 2012). PBT-profiler estimated high half-life for 6,6'-di-tert-butyl-4,4'-thiodi-m in soil (120 days) and sediment (540 days). According to Section 1.1.1. of Annex XIII to REACH Regulation, a substance is considered to fulfil the persistency criterion (P) if the degradation half-lives are above certain cut-off values in surface water, sediment or soil. The assessment of the persistency in the environment shall be based on available half-life data collected under the adequate conditions. However, simulation tests on degradation in order to definitely conclude on the P/vP properties are not available, but based on estimated data the substance screens as potential P/vP.

What is the possible regulatory outcome

Half-life data generated by the respective simulation test can either be used to relief the concern for P or the PBT-/vPvB assessment shall proceed. Information regarding PBT/vPvB properties can lead to identification as a substance of very high concern (SVHC) according to Article 57(d) and/or (e) of REACH.

Considerations on the test method and testing strategy

A simulation study in water (OECD TG 309) is to be performed and may allow you to conclude on the P- or vP-criterion. If the result of the OECD TG 309 test does not allow to conclude that the registered substance is persistent (P) or very persistent (vP) according to Annex XIII of the REACH Regulation, the evaluating MSCA might consider if further simulation testing needs to be requested in future Substance Evaluation decisions.

According to ECHA 2014b (part R.7.9) the compartment of concern is to be considered if new data is to be generated. 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol has a high adsorption potential (measured log K_{OC} 5.61) and therefore soil as well as sediment are considered to be the target compartments of the substance. Various distribution models (Level III fugacity model implemented in EpiSuite and PBT profiler) were used by the evaluating MSCA and resulted in the distribution of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol to soil and sediment. 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol is used as a non-staining hindered thiophenol anti-oxidant in e.g. adhesives, sealants, polymer preparations and compounds and based on the uses in Nordic Countries, the SPIN database assumes that

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waste water and soil are the most exposed compartments. Simple Treat model predicted that sewage sludge is the relevant compartment, which further can be indirectly spread to soil. Due to the high log K_{OC} value, 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol will adsorb to solid and suspended matter and will form high percentages of Non extractable Residues (NER). Interpretation of NER is challenging, but recently a publication to improve the interpretation of NER was published on the ECHA website[6]. ECHA guidance states that residues should be regarded, in the absence of systematic methodology, as non-degraded substance, unless, on a case-by-case basis, it can reasonably be justified or analytically demonstrated that a certain part of the residues can be considered to be irreversibly bound (ECHA 2017b, R.11.4.1.1.3). For relieving 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol from P suspicion it will need convincing evidence that degradation and not dissipation caused by adsorption is the relevant process.

A degradation simulation test in water (with surface water only "pelagic test") has the advantage of less bound residues formation compared to other simulation tests, which makes interpretation of the results more straightforward. Therefore, a test according to OECD TG 309 aerobic mineralisation in surface water in its "pelagic" version shall be conducted, if technically feasible, as in this test NER formation will be the lowest level among the simulation tests.

Sterile control flasks (prepared according to the OECD TG 309) must be included in the test to provide information for the contribution of abiotic degradation or other loss mechanisms.

The amount of suspended solids in the pelagic test should be representative of the level of suspended solids in EU surface water. The concentration of suspended solids in the surface water sample used should therefore be approximately 15 mg dw/L. Testing natural surface water containing between 10 and 20 mg SPM dw/L is considered acceptable.

Furthermore, if reporting NER in your test results you must explain and scientifically justify the extraction procedure and solvent used obtaining a quantitative measure of NER.

The test substance shall be the purest form containing no transformation/degradation products of the registered substance to ensure that at test start no such products are present. The test substance shall be ^{14}C-radiolabelled due to the low water solubility for an appropriate verification of the degradation and identification of potential transformation/degradation products. You must provide justification for the location of the radiolabel on the molecule.

As in all simulation tests several parameters will have to be considered together in interpretation of data, i.e. DT50, CO_2, transformation/degradation products and kinetics observed. Evidence and identification of transformation/degradation products and CO_2 will be crucial, since only their presence will prove that degradation processes occur. The eMSCA performed a simulation of the biodegradation pathway[7] and simulated some transformation/degradation products, which screen as potential P/vP (BIOWIN v.4.10),

[6] https://echa.europa.eu/documents/10162/13630/echa_discussion_paper_en.pdf/4185cf64-8333-fad2-8ddb-85c09a560f7c

[7] UM-BBD: Pathway Prediction; http://eawag-bbd.ethz.ch/predict/

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potential B/vB (BCFBAF v4.01) and potential T (ECOSAR v1.11). Degradation pathways should be modelled first by the OECD toolbox or Biocatalysis and Biodegradation Database, so that transformation/degradation products predicted and identified may be compared. Further, the test set-up should enable to check the mass balance (using radiolabelled 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol). Identification of transformation/degradation products relevant for PBT/vPvB assessment are recommended at a concentration of ≥ 0.1 % w/w unless it can be demonstrated, that this is technically not possible after reasonable attempts. 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 surface water so that reliable results can be generated (ECHA, 2017b).

The OECD TG 309 stipulates a test duration of 60 days, but it is recommended to be extended to a maximum of 90 days, if the provisions of Annex 3 of the guideline are fulfilled. The reasoning for the recommended extended test duration is to give sufficient time for any transformation/degradation product to appear, as the parent substance screens as potential P/vP and to facilitate the interpretation of the results and enhance comparison with the trigger values. As 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol is expected to adsorb rapidly a higher number of measurements (e.g. 11) is recommended to follow the adsorption process and to enhance kinetic modelling and data evaluation. ECHA recommends taking three samples on the first day (1, 6 and 12 hours after the start of the test). Another sample should be taken after 24 hours. It is recommended to take samples at day 7, 14 and day 28. The subsequent sampling times should be nearly evenly distributed in a four weeks interval. Rate and course of kinetics of parent and transformation/degradation products in the water phase should be compared with the respective results of the OECD TG 309 and considered in interpretation.

OECD TG 309 allows shaking or stirring with a shaking table or a magnetic stirrer to maintain particles and microorganisms in suspension. Based on Shrestha et. al (2016), shaking in OECD TG 309 experiments enhances degradation, whereas stirring reduced the bioavailability for biodegradation of the tested substances. Therefore you are recommended to shake flasks in OECD TG 309 studies to reduce NER formation during the experiment.

ECHA guidance R.11 (ECHA 2017b) and the Generic Guidance document for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registrations (FOCUS, 2014) shall be consulted to analyse simulation test results. The kinetic analyses should be performed with publcly available statistical software packages to allow independent re-calculation of results. Sufficient measurements (sampling times as explained above) shall be done to enhance kinetic modelling.

ECHA 2017b defines the average environmental temperature for the EU as 12°C and this is the reference temperature for the assessment of persistency in PBT/vPvB assessment. The kinetic part of the study must be performed at 12°C, since this is the relevant temperature for the EU. The transformation pathway part is recommended by the eMSCA to be performed with a higher test substance concentration and it is recommended to be performed at 20°C to increase the possibility to identify and quantify potential transformation/degradation products.

To assess persistence of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol it is necessary to differentiate between mere elimination and degradation processes (cf. ECHA 2014b, R 11.4.1.1).

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Alternative approaches and Proportionality of the request

Half-life data generated by the respective simulation test can either be used to relief the concern for P or the PBT-/vPvB assessment shall proceed. More explicitly, there is no equally suitable alternative way of obtaining half-life data. No vertebrate testing is needed at this stage.

Comments of the Registrant(s) and Responses to Comments

In your comments on the draft decision, you acknowledged the concerns with regard to the PBT status of the substance and that first the persistency will be investigated to avoid animal testing. Further you acknowledged that the substance may have persistency concerns. However, you stated that the substance had already the hazard statement H410, on the basis of the effects noted from the study data and as such you accept that the substance is P. You consider that the "P" studies requested are not necessary, as the substance will not be a PBT or vPvB substance on the basis of the lack of a bioaccumulation potential.

Regarding the persistency concern, it is replied that you concluded in the CSR and on the ECHA dissemination site[8] that the substance fulfils the vP (and P) criterion, without having reliable half-life data to base this conclusion upon. Your conclusion is based on BIOWIN predictions and on a screening biodegradability test [redacted] 1980) with prolonged incubation and pre-adapted mixed source inoculums, which was assigned by you as similar to OECD TG 301B. You accept that the substance is P. Results were interpreted that the substance is not readily and not inherently biodegradable. A lack of degradation (< 20%) in an inherent biodegradation test was in your opinion sufficient information to conclude on the persistency. Simulation tests were waived by you.

ECHA considers all submitted persistency studies as not reliable – [redacted] et al (1979); [redacted] (1980) and an anaerobic study by [redacted] (1980). Only one study ([redacted] 1980) was rated as reliable by you. In this study with acclimated inoculum only 1% CO_2 evolved after 35 days. No conclusion on the degradation can be drawn, as explained above (section "Why new information is needed"). Regarding your argumentation on classification as Aquatic chronic 1, it is noted that there is no link between your self-classification as Aquatic Chronic 1 and the P criterion. Your comment is therefore considered not relevant.

In addition, it is noted that a further "screening" biodegradation study is available on the Japanese NITE website[9] (online only), which is not included in the Chemical Safety report. The concentration of sludge in this study was indicated with 30 ppm, the duration was two weeks and the initial test substance concentration was 100 ppm. The observed degradation was indicated with 1.9% and 0.4% BOD after 28 days.

In general, according to the ECHA 2017b, a lack of degradation (<20% degradation) in an inherent test may provide enough information to confirm the fulfilment of the P-criterion. Confirmation of the vP-criterion solely based on a test equivalent to OECD TG

[8] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16037/2/3/?documentUUID=0d763d4a-ef37-438d-9d33-1044709ed2b0

[9] http://www.safe.nite.go.jp/jcheck/template.action?ano=1867&mno=3-1118&cno=96-69-5&request_locale=en

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302 is however not possible. The Japanese NITE biodegradation test[9] is not an inherent test and the biodegradation study by [redacted] (1980) was assigned by you as similar to OECD TG 301 B. Both studies are not equivalent to OECD TG 302 and moreover ECHA considers the study by [redacted],(1980) as not reliable. Therefore, none of the biodegradation studies can be used to confirm the P-criterion of the substance.

The eMSCA estimated the ready biodegradability with VEGA (Ready Biodegradability model,version 1.0.9). The prediction resulted in "non ready" biodegradable" for 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol. Similar substances were also predicted as "non-ready biodegradable". In addition, the estimates of the biodegradability of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol and similar substances using BIOWIN models 2, 3, and 6 from EPI-Suite (US EPA, 2012) also predicted the substances to be potentially persistent and very persistent. However, no final conclusion on the P/vP properties of the parent substance can be drawn on the basis of screening information (QSAR predictions) alone (ECHA 2017b). In addition, the transformation/degradation products (may have PBT/vPvB properties, thereby posing a concern for the environment.

Half-life data in soil, water-sediment or water are not available, and as already stated above, all biodegradability studies in the CSR are considered as unreliable. Therefore, the decision was not changed and simulation testing is still requested.

The request for the surface water and sediment simulation testing (the latter is not any longer requested) was triggered by the concern that the substance and/or the transformation/degradation products (may have PBT/vPvB properties, thereby posing a concern for the environment. Two different tests are requested, a kinetic test to derive suitable half-life data and a transformation study to identify transformation/degradation products. Half-life data are compared to the trigger values of Annex XIII. The kinetic part of the study shall be performed at 12°C, since this is the relevant temperature for EU. The transformation pathway to identify transformation/degradation products is recommended to be performed at 20°C. The higher temperature might facilitate the formation of relevant transformation/degradation products within the test duration and might increase their concentration to allow identification and to follow the relevant changes in concentration.

Simulation test(s) OECD TG 308/309 and the technical feasibility related to low water solubility:

In your comments you question the technical feasibility of the requested OECD TG 308/309 studies. You argue that the substance demonstrated a water solubility of 27.7 µg/L at 20°C and would be insoluble on the basis of the criteria detailed within IUCLID parameters "insoluble (< 0.1 mg/L). You would ascertain that it will not be possible to conduct the requested OECD TG 309 test, even when using radiolabelled material, due to the significant solubility problems associated with this material.

ECHA agrees that the water solubility of the test substance is low and is a crucial factor in determining the suitability of a simulation test, but the water solubility needs to be considered in combination with the detection limit / or limit of quantification of the analytical method for the test item.

The test substance concentration needs to be low enough to ensure biodegradation kinetics expected also in the environment. Therefore for the kinetic part of the study, it is important to ensure that the initial concentration of the substance in the test water

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does not exceed the water solubility and further that the decrease in water concentration can be followed over the test period (ref. to OECD TG 309).

According to ECHA 2017b, simulation studies on ultimate degradation in surface water are warranted unless the substance is highly insoluble in water. Further it is mentioned that it may not be warranted to conduct the study if the water solubility of the substance is well below 1 µg/L. The demonstrated water solubility of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol is 27.7 µg/L. OECD TG 309 stipulates to use two concentrations (differing by a factor of 5-10). Both concentrations should be below 100 µg/L and in the range of > 1 – 10 µg/L, which is beneath the water solubility of 27.7 µg/L.

The technical feasibility related to low water solubility is not expected to be challenged due to the water solubility of 27.7 µg/L. In addition, radiolabelling of the substances improves the ability to detect the parent substance and the transformation/degradation products.

Identification of transformation/degradation products:

In your comments, you question the technical feasibility to identify transformation/degradation products ≥ 0.1 %. You argue that, it is technically not possible and is unlikely applied in previous cases of evaluation.

For the registered substance, identification of transformation/degradation products ≥ 0.1 % is important, as two[10] out of in total four- simulated transformation/degradation products (UM-BBD, http://umbbd.ethz.ch/) screen as potentially P/vP (BIOWIN v.4.10), potentially B/vB (BCFBAF v4.01) and potentially $T_{ecotoxicity}$ (ECOSAR v1.11). At the same time, the formation of the transformation/degradation products from the parent is expected to be slow, because in screening tests with prolonged incubation and pre-adapted inoculum less than 20% degradation was found. Therefore the limit is recommended to be set to ≥ 0.1 % to enhance the possibility for identification of transformation/degradation products, which might cause a concern for the environment (ref. to table below).

Results from the persistency predictions BIOWIN v.4.10 (US EPA EPI-Suite, 2012) and the Danish biodegradation prediction[11] can be seen below:

	Results BIOWIN v.4.10 US EPA EPI-Suite, 2012							Danish QSAR database
CAS No.	Overall	BIOWIN 1	BIOWIN 2	BIOWIN 3	BIOWIN 4	BIOWIN 5	BIOWIN 6	
96-69-5	NO	0.55	**0.072**	**1.95**	2.97	0.026	**0.008**	NRB

[10] Transformation/degradation product 2: Cc1cc(O)c(cc1Sc1cc(c(O)c(O)c1C)C(C)(C)C)C(C)(C)C;
Transformation/degradation product 8: Cc1cc(O)c(cc1S(=O)(=O)c1cc(c(O)cc1C)C(C)(C)C)C(C)(C)C

[11] http://qsardb.food.dtu.dk/db/index.html

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No. 2	NO	0.502	**0.03**	**2.56**	3.7	0.130	**0.007**	
No. 8	NO	0.52	**0.05**	**1.86**	2.92	-0.07	**0.003**	

Note that BIOWIN 1 & 2 scores of < 0.5: predicted not rapidly biodegradable. BIOWIN 5 & 6 < 0.5: predicted not readily Biodegradable (NRB, according to MITI training set), BIOWIN 3 and 4 gives the ultimate and the primary timeframe for degradation (1 year, 2 months, 3 weeks, 4 days, 5 hours). The Danish prediction directly indicated whether RB or NRB. BIOWIN 5 and 6 and the DK QSAR DB predict that the substance will not degrade readily. The persistency screening algorithm: BIOWIN2 and/or BIOWIN 6 < 0.5 and BIOWIN 3 < 2.2 (-2.7), is fulfilled (ECHA, 2017).

Although the thioether-group is not considered quantitatively by BIOWIN 1-6, the estimates are considered as sufficiently reliable as aromatic ring structures are considered to be stable and potentially persistent based on the predictions and that there are no indications that the thioether-group is vulnerable to degradation.

Estimated BCFs (BCFBAF v4.01) of two simulated transformation/degradation products [10] are even higher (BCF=3372; BCF=5935) than the BCF predicted for the parent compound using the estimated Log K_{OW} (BCF=1961). From the parent to the transformation/degradation products the estimated water solubility (WSKOW v1.42) is increasing from 1.9 µg/L (parent) to 4 µg/L (transformation/degradation product 2) and 33 µg/L (transformation/degradation product 8).

ECHA 2017b states that transformation/ degradation products need to be subjected to the PBT/vPvB assessment. As the PBT/vPvB concern for these transformation/degradation products cannot be excluded, an analytical method to detect and identify them at a level ≥ 0.1 % is recommended to be established with sufficient sensitivity to follow relevant changes in concentration.

For the identification and quantification of transformation/degradation products higher test substance concentration may be used, if analytical limitations exist (e.g. OECD TG 309: > 100 µg/L and > 1mg/L). Recommended values are above the water solubility of the substance, but have been previously successfully applied under the biocides regime to identify major transformation/degradation products from active substances at concentrations above the water solubility. Simulated metabolites exhibit a higher predicted water solubility than the parent substances. If a sufficient low detection limit can be established for the parent and for the transformation/degradation products the identification of transformation/degradation products may be conducted with the parent substance at an initial concentration beneath the water solubility limit.

In reply to your argument that the identification level of 0.1% was unlikely applied in previous cases of evaluation it is mentioned that the identification of transformation/degradation products at a concentration ≥ 0.1% was applied in OECD TG 308 and OECD TG 309 tests in recent Substance Evaluation decisions[12,13] (e.g. on BMDB, CAS No. 70356-09-1; di-tertbutyl 3,3,5-trimethylcyclohexylidene diperoxide (CAS No. 6731-36-8)).

[12] https://echa.europa.eu/documents/10162/dfdb22a0-8c94-c8cf-79c5-d9ad8d856792
[13] https://echa.europa.eu/documents/10162/96623d2e-c8a3-40a6-8fe3-c4ee9b7b1240

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ECHA further notes that the draft decision stated "Further, the test set-up should enable to check the mass balance (using radiolabelled 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol) and the identification of transformation products[14] relevant for PBT assessment (at concentration of ≥ 0.1 % w/w) unless it can be demonstrated that this is technically not possible."

OECD TG 309 states, that transformation products[15] detected ≥ 10% of the applied concentration should be identified unless reasonably justified otherwise and that transformation products for which concentrations are continuously increasing during the study should also be considered for identification, even if their concentrations do not exceed the limit of ≥ 10%, as this may indicate persistence.

Thus, it is recommended to use a level of ≥ 0.1 % w/w, if technically feasible. If it is not technically possible, then it is recommended to explain the reasons in the study report.

Lack of bioaccumulation potential:

You question the fact that the substance is considered to be potentially B. You argue that in the dossier revision of 2015, various information was put forward to support the fact that the substance should not be considered potentially B. In detail, you included in the updated Registration dossier and in the Response to Draft Decision QSAR predictions using BCF Read-Across data (version 1.0.0, VEGA), and BCF models (US EPA EPI SuiteTM v4.11 model BCFBAF, the VEGA CAESAR BCF Model version 2.1.11, and the BCF Model Meylan version 1.0.0 VEGA) to predict BCF values for 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol. The QSAR predictions were used as supportive argument by you that the substance is not bioaccumulative and not very bioaccumulative as the BCF values were between 69and798.6. In addition, you used an analogous substance 6,6'-di-tert-butyl-2,2'-methylenedi-p-cresol (DBMC, EC: 204-327-1, CAS 119-47-1) as supportive argument that the substance is not bioaccumulative, as the BCFs are below 2000. You stated that "The overall conclusion for this analogous substance indicates that it is not deemed to be PBT and vPvB in nature". Further you cited the BCF study from 1979 ("[redacted] with BCF values from 1.3 to below 11. With regards to the potential for bioaccumulation in mammals, you argue that it seems unlikely that there is any significant difference (concerning first-pass metabolism and glucuronidation) in the accumulation potential or mechanism between mammals in the environment and fish in the BCF study and therefore the concern for accumulation potential in mammals, as for fish seems to be very low (Borghoff et al, 1988; Mulder et al., 1982). Further, you consider that the P studies requested are not necessary, as the substance will not be a PBT or vPvB substance on the basis of the lack of the bioaccumulation potential.

In response to your comment, ECHA considers your lines of information as either not applicable or not reliable enough to support your conclusion that the substance is unlikely to bioaccumulate.

Your QSAR predictions using BCF Read-Across (version 1.0.0, VEGA):

[14] The term "transformation product" was used in the same meaning as "transformation/degradation products".
[15] The term transformation/degradation products" in this decision is used in the same meaning as the term "transformation product" in the OECD 309 guideline.

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You predicted a BCF value based on five read-across substances (CAS: 119-47-1, CAS: 2668-47-5, CAS: 732-26-3, CAS: 4130-42-1, CAS: 128-37-0), which revealed a BCF value of 184 for 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol. 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol is a structure containing [redacted]. ECHA considers the substances obtained with VEGA as not "similar" (CAS: 119-47-1, CAS: 2668-47-5, CAS: 732-26-3, CAS: 4130-42-1, CAS: 128-37-0) as these substances contain no cSc-fragment and [redacted] (CAS: 732-26-3, CAS: 4130-42-1, CAS: 128-37-0). The presence of polar groups within structural similar substances increases hydrophilicity, related to lower values of BCF. The more aromatic rings and the more t-butyl groups linked to the aromatic ring and the less OH groups are present, the higher is the predicted BCF. ECHA does not consider substances containing only 1-ring (3 substances) as "similar" and therefore they cannot be used to calculate a read-across BCF value of the target substance. The remaining two substances with 2-rings (CAS: 119-47-1, CAS: 2668-47-5) contain no cSc- fragment, but revealed predicted BCF values higher than 2000 (US EPA EPI SuiteTM v4.11 model BCFBAF v3.01). Based on the used substance your Read-Across approach is considered as not valid.

Your QSAR predictions using US EPA EPI SuiteTM v4.11 model BCFBAF v3.01:

You estimated the BCF with BCFBAF v3.01 to be 798.6 As input value a user entered Log K_{OW} value of 5.24 was used, which is the measured value (with the HPLC-method). ECHA considers this measured value as underestimated, as all predicted Log P or Log K_{OW} values are > 7 [Log P=7.63 (ACD/Labs Percepta Platform); Log K_{OW}=8.24 (EPI Web 4.1, KOWWIN v.1.68) and Log P =7.23 (COSMO*therm*[16] *using* COSMO*conf* [17] & TURBOMOLE[18] and assuming wet octanol)], which are outside the applicability range of the HPLC method, which is 0 < Log Kow < 6. For substances with an expected Log K_{OW} between 5 and 8.2 the low-stirring method is the preferred method ([redacted] 2002; OECD TG 123). The evaluating MSCA used originally the predicted Log K_{OW} of 8.24, leading to an estimated BCF value (regression-based) nearly to 2000, which indicates together with the estimated Log K_{OW} or the now calculated Log P values that the substance is potentially B/vB. Using the novel Log P values estimated BCF values of 3920 and 6156 are caluclated (Epi Suite BCF/BAF, regression based method).

Your QSAR predictions using VEGA CAESAR BCF Model version 2.1.11:
As the substance is out of the applicability domain, ECHA considers the prediction as not reliable.

Your QSAR predictions using BCF Model Meylan version 1.0.0 VEGA:

As the substance is outside the applicability domain, ECHA considered this prediction as not reliable. You argued yourself, that it is not adequate to consider this model in assessing B.

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[16] COSMOtherm, C3.0, release 1601, COSMOlogic GmbH & Co KG, http://www.cosmologic.de

[17] COSMOconf, 4.0, COSMOlogic GmbH & Co KG, http://www.cosmologic.de

[18] TURBOMOLE 4.1.1 2015, a development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH, 1989-2007, TURBOMOLE GmbH, since 2007; available from http://www.turbomole.com

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Analogous substance 6,6'-di-tert-butyl-2,2'-methylenedi-p-cresol:

In response to your conclusion on the low bioaccumulation potential of the analogous substance DBMC (CAS No. 119-47-1), ECHA would like to state that the experimental BCFs results need to be treated with caution, as the two test substance concentrations were above the water solubility, indicating that the substance might have not been bioavailable for fish, resulting in very low BCF values. In detail, at a concentration of 0.1 mg/L the BCF was between 60 and125 and at the higher concentration of 1 mg/L the BCF was 23-37. In the OECD SIDS report[19], the water solubility is stated with 0.02 mg/L. On the ECHA website the water solubility is given with 0.027 mg/L (20°C, pH: 7.6 – 7.8, Klimisch 1 study). DBMC was evaluated in the former TEC-NES group and for this study it was concluded, that the substance could have adsorbed to the skin of the fish during the study and/or have adsorbed to the food during the study. Therefore the results should be treated with caution. But in the ECB-summary fact sheet[20] other BCF data from NITE are presented, at lower test concentration (0.2 and 2 µg/L), resulting in higher BCF values (320-780 and 400 – 840). *Cyprinus carpio*, a fast growing fish was used as test species. Growth dilution has not been considered within the study. Impact on feeding in Carp was discussed within the Substance Evaluation Decision[21] of bis (α, α-dimethylbenzyl)peroxide indicating that a daily growth of 2 – 4% per day would result in 3 to 9 times increase in weight. Kinetic BCFs were not reported in the study, but ECHA assumes that fish growth does play a significant role, especially with carp; therefore the "real" BCF values are expected to be higher. Summarising, as kinetic BCF values, and growth correction are missing, the reported value cannot be used as supportive argument that the target substance 6,6'-di-tert-butyl-2,2'-methylenedi-p-cresol is not bioaccumulative.

BCF study from 1979 ("Bioconcentration test of chemicals in fish and shellfish", Kanapogyo No. 5, Yakuhatsu No. 615, 49 Kikyoku No. 392) with BCF values from 1.3 to below 11:

Regarding to your conclusion that the substance should not be considered to be "B" based on the BCF study from 1979 ("Bioconcentration test of chemicals in fish and shellfish", Kanapogyo No. 5, Yakuhatsu No. 615, 49 Kikyoku No. 392), ECHA challenges the reliability of the study, as there is little or no information given in report on:

- The identity and purity of the substance is unknown: test item K-354 was used, the IR-spectrum of the test substance is mentioned, but missing.
- Steady-state of the substance was not indicated to be reached
- The number of fish used is unknown
- Weight of individual fish is not reported, only the mean body weight of 27.1 g and the mean body length was 10.3 cm reported, fish weight at the end of the test was not recorded.
- No control groups are reported

[19] http://www.inchem.org/documents/sids/sids/119471.pdf
[20] https://echa.europa.eu/documents/10162/4fbb0b56-ebe4-45b8-8ee7-4bf505ff7998
[21] https://echa.europa.eu/documents/10162/bec1b6f6-426a-eed9-fdd8-f81ffa5f0818

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- Reference items are missing
- Growth / behaviour and mortality of fish were not reported
- Test concentrations used were above the water solubility: 2 nominal test concentrations (30 and 300 µg/L) were used to expose fish (*Cyprinus carpio*) in a flow-through system at 25°C; both concentrations were above the water solubility of 27 µg/L. After 6 weeks uptake the BCFs are low and more or less the same for the high and low test concentrations: BCF = 4.2 (30 µg/L) and BCF = 6.7 (300 µg/L). Confidence in a BCF value above the aqueous solubility is low, as the substance might have not been available and taken up by fish. The predicted water solubility is 1.9 µg/L (EPI Suite, WSKOW v1.42) is lower than the measured water solubility of 27 µg/L (20°C, pH: 7.6 – 7.8, OECD TG 105). According to OECD TG 305 for a test to be valid the test substance concentration needs to be below its limit of solubility in water. ECHA 2017b states that the test concentrations should always be below the water solubility of the substance, this was not the case. Further, it is unknown if a steady- state was reached. Often BCF values differ between the low and high exposure concentration, which is not the case here. Uptake may have been limited by low exposure concentration, because of low water solubility, resulting in very low BCFs; at 30 µg/L, the BCF value ranged from 0.12 or below to 4.2 and at 300 µg/L, the BCF value ranged from 1.3 to 11.
- Length of the depuration phase is not mentioned in the report.
- As dispersant hydrogenated castor oil HCO-20 was used, the use of solubilising agents is not recommended by OECD.
- The lipid content of fish was not reported.

Low concern for accumulation potential in mammals and fish and the difference in the accumulation potential between mammals in the environment and fish is considered by you as unlikely.

For 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol information such as measured percentages of oral absorption, bioavailability, first-pass metabolism, Cmax, Tmax, AUC from animal studies were not available. ECHA agrees with you that concerning metabolism several studies showed that glucuronidation plays a major role in metabolism of 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol after oral and i.v. exposure (Birnbaum et at. 1983; Borghoff et al. 1988). Oral absorption was assumed by you with 50%. No percentage of oral absorption was calculated. However based on metabolites in feces, radioactivity in urine and tissue residues, 32% to 36% was assumed to be absorbed; higher absorption rates may occur for lower doses (Birnbaum et al., 1983). The characterisation of the retained radioactivity in the tissues was performed only after i.v. exposure and no comparison and characterisation of the radioactivity after i.v. and oral dosing was determined thus information on first-pass metabolism is lacking, that could have occurred. In a biliary secretion experiment enterohepatic circulation was suggested based on a comparison of the time course and metabolites in bile. While radioactivity in the bile consisted of 100% metabolites the amount in feces was around 80% indicating that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol-glucoronide is cleaved back to the substance and may be reabsorbed (Birnbaum et al., 1983). I.v. administered radioactive 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol was initially rapidly cleared with a half-life of 0.05 day (blood) followed by two

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slower elimination phases of (t1/2) 0.33 and 23.10 days. Levels in adipose tissue declined slowly with a half-life of 6.3 days. Also for skin and liver the biphasic elimination showed for the second phase half-lives of 2.1 and 8.7 days. Around 10% of the administered i.v. dose (radioactivity) can be expected to be slowly cleared from the major depots of liver, skin, and adipose tissue indicating a potential for bioaccumulation (cf. Birnbaum et al., 1983). Though there have been analytical difficulties in the characterisation of the distributed radioactivity in the tissues in the study conducted by Birnbaum et al. (1983), spiked control tissues have been used that indicate that the parent compound accounted for most of the slowly cleared radioactivity. No repeated dose administration study was done that would be suitable to more fully address the potential for accumulation. ECHA disagrees with you that based on arguments concerning first-pass metabolism and glucuranidation that it seems unlikely that there is any significant difference in the accumulation potential or mechanism between mammals in the environment and fish in the BCF study and therefor the concern for accumulation potential in mammals, as for fish, seems to be very low. First pass metabolism was not sufficiently investigated in the current data set for 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol. After i.v. dosing tissue half-lives in terms of % of applied radioactivity indicate that a potential for bioaccumulation cannot be excluded. Elimination rates and half-lives are acknowledged as useful metrics indicative of the bioaccumulation potential (ECHA, 2017).

No data on bioaccumulation in terrestrial organisms (soil dwelling organisms, vertebrates) were provided by you. As food is the main exposure route for non-aquatic organisms and it is demonstrated that bioaccumulation/biomagnification can also be higher in non-aquatic food webs compared to aquatic ones (Gottardo et al., 2014) we disagree with you that bioaccumulation in mammals is comparable to BCF fish. In addition homeotherms including mammals have higher energy requirements, feeding rates, trophic positions, longer life time and different biotransformation abilities than poikilotherms (including fish), hence extrapolation from fish-related bioaccumulation data to other organisms has a high uncertainty and should not be made according to Gottardo et al. (2014)..

ECHA considers the substance as potentially B/vB based on following indications:

- Estimated Log K_{OW} > 4.5: For the PBT and vPvB assessment a screening criterion has been established, which is Log Kow greater than 4.5. The measured Log K_{OW} of 5.24 is considered as underestimated, because the low stirring method and not the HPLC method is recommended for this Log K_{OW} range. The eMSCA used therefore the predicted Log K_{OW} of 8.24 (EPI Suite, EPI Web 4.1, KOWWIN v1.68). Based on the screening criterion Log Kow, the substance can be considered as potentially B/vB.

- Estimated BCF value of the substance = 1961: The eMSCA used for the calculation of the BCF (EPI Suite, EPI Web 4.1, BCFBAF v3.01) the predicted Log K_{OW} of 8.24. The estimated BCF is near the "B" threshold of 2000.

- Similar substances (e.g. CAS no. 96-66-2, 118-82-1, and 2668-47-5) with or without cSc-fragment show high estimated and/or experimental BCFs (BCF values > 2000) values.

- Other supportive information: In the acute toxicity study [redacted] (a time independent study)

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in a 14-day flow through toxicity test, it is stated in the original study report that "The LC_{50} values ranged from 0.21 mg/l on day 1 to 0.054mg/l on day 14. These data suggest this chemical is an accumulative toxin and is highly toxic to fathead minnows under the conditions of this test."

- Indications for terrestrial bioaccumulation: The estimated Log K_{OA} (EPI Suite, EPI Web 4.1, KOAWIN v.1.10) for the substance is 15, indicating also a bioaccumulation potential for terrestrial organisms

- Further, there is some evidence from animal studies that 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol might accumulate in liver and adipose tissue.

As no reliable experimental data on the aquatic and terrestrial bioaccumulation potential are available - the PBT / vPvB concern is still valid, and screening indicated that the substance is potentially B/vB. Further, 2 out of 4 simulated transformation/degradation products screen as potentially P/vP, potentially B/vB and potentially $T_{ecotoxicity}$ (ECOSAR v1.11).

Consideration of PfA(s) and Registrant's comments on PfA(s)

Following PfAs on specifications of the simulation test OECD TG 309 were made by ECHA and MSCAs and were accepted:

- Level of suspended solids (SPM) in EU surface waters was specified

- NER must be explained and the extraction method scientifically justified

- CO_2 was included to be considered for the interpretation of the data

- Sterile controls shall be included

- FOCUS, 2014 and ECHA guidance R.11 (ECH, 2017b) shall be consulted and publicly available software packages should be used

- Detailed recommendations on maintaining particles and microorganisms in suspension

PfAs from two MSCAs were submitted regarding the persistency testing strategy. The conditional OECD TG 308 test in sediment was removed following a PfA, but leaving it open to request further simulation tests in future, if deemed necessary.

A PfA from an MSCA was followed that a screening study on its own is not sufficient to conclude on the persistency. Another PfA was followed to add information on reliability of BIOWIN predictions.

Following an ECHA PfA the chapter "What is the possible regulatory outcome" has been introduced andAccording to another PfA the identification as SVHC according to Art. 57 (d) and/or (e) was included as a possible outcome.

A PfA from ECHA requesting the deletion of the text on the differences in bioaccumulation observed in aquatic and terrestrial species was not followed, as this information is directly related to a comment by you.

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Following a PfA made by ECHA several predicted log P/ log K_{OW} values were included to explain why the determined Log K_{OW} was underestimated by the HPLC method

You provided comments on PfAs that the lower acceptable SPM range in the OECD TG 309 (i.e. 10 mg SPM dw/L) should not be set and that the range is not appropriate. This comment was not followed, as this range is given in the PBT R.11 guidance (ECHA, 2017b) and stated in several final decisions.

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 studies using the registered substance subject to this decision:

Simulation testing on ultimate degradation in surface water; test method: Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25./OECD TG 309, "pelagic test" – without additional suspended solids/sediment, kinetic part at a temperature of 12 °C, transformation part at a temperature of either 12 or 20°C; as further specified above.

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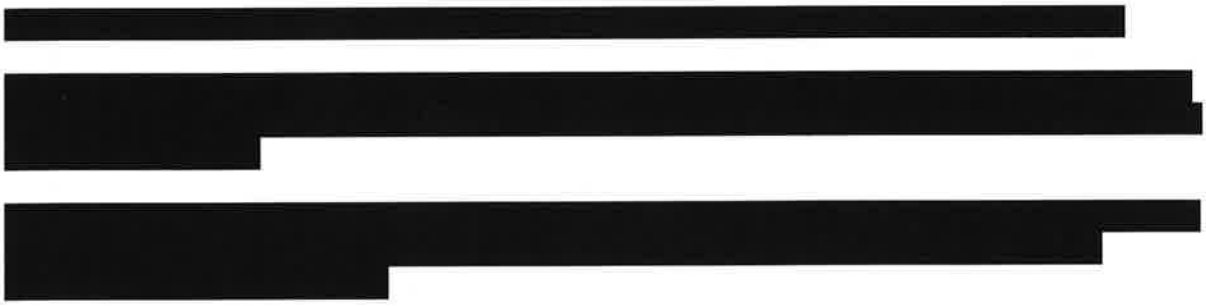

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Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to suspected CMR (reproductive toxicity), suspected sensitiser, immunotoxicity, suspected PBT, suspected endocrine disruptor, wide dispersive use, and consumer exposure, 6,6'-di-tert-butyl-4,4'-thiodi-m-cresol CAS No 96-69-5 (EC No 202-525-2) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2015. The updated CoRAP was published on the ECHA website on 17 March 2015. The competent authority of Austria (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

In accordance with Article 45(4) of the REACH Regulation, the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding developmental neurotoxicity.

The evaluating MSCA considered that further information was required to clarify the following concerns: endocrine disruption, reproductive toxicity, immunotoxicity, developmental neurotoxicity, and PBT/vPvB,. Therefore, it prepared a draft decision under Article 46(1) of the REACH Regulation to request further information. It subsequently submitted the draft decision to ECHA on 17 March 2016.

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

The initial request for an Activated sludge respiration inhibition test (OECD 209) has been withdrawn after your commenting period because this test was not considered necessary to investigate the PBT status of the substance. You have in your comments agreed to perform the test and if you still want to perform it, no testing proposal is needed under REACH.

Proposals for amendment by other MSCAs and ECHA and referral to 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(s) for amendment to the draft decision. They are reflected in the Reasons (Appendix 1).

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendment(s).

Any comments on the proposal(s) for amendment were taken into account by the Member State Committee and are reflected in the Reasons (Appendix 1). The Member State Committee did not take into account any comments on the draft decision as they were not related to the proposal(s) for amendment made and are therefore considered outside the scope of Article 52(2) and Article 51(5).

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The Member State Committee reached a unanimous agreement on the draft decision during its MSC-62 meeting and ECHA took the decision according to Article 52(2) and 51(6) of the REACH Regulation.

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Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(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.

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study/ies, 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 you. 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.

4. In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). You 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 stud(y/ies) on behalf of all of them.