EUROPEAN CHEMICALS AGENCY

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

For Oligomerisation and alkylation reaction products of 2-phenylpropene and phenol (EC No. 700-960-7), previously registered as Phenol, methylstyrenated, CAS No 68512-30-1 (EC No 270-966-8)

Addressees: Registrants of Oligomerisation and alkylation reaction products of 2-phenylpropene and phenol (EC No. 700-960-7), previously registered as Phenol, methylstyrenated (concerned registrants)

This decision is addressed to all Registrants of the above substance with active registrations on the date on which the draft for the decision was first sent, with the exception of the cases listed in the following paragraph. A list of all the relevant registration numbers subject to this decision is provided in Annex II to this decision.

Registrants meeting the following criteria are *not* addressees of this decision: i) Registrants who exclusively use the above substance as an on-site isolated intermediate and under strictly controlled conditions and ii) Registrants who have ceased manufacture/import of the above substance in accordance with Article 50(3) of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation) before the decision is adopted by ECHA.

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

This decision does not take into account any updates of the registrations of the concerned registrants after 1 August 2013, the date upon which the draft decision was circulated to the other Competent Authorities of the Member States and ECHA pursuant to Article 52(1) of the REACH Regulation.

This decision does not imply that the information provided by the concerned registrants in the registrations is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossiers of the concerned registrants at a later stage, nor does it prevent a new substance evaluation process once the present substance evaluation has been completed.

I. Procedure

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of Denmark has initiated substance evaluation for Phenol, methylstyrenated CAS No 68512-30-1 (EC No 270-966-8) based on registration dossiers submitted by the concerned registrants and prepared the present decision in accordance with Article 46(1) of the REACH Regulation.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds

for concern relating to potential PBT properties, potential endocrine disrupting properties, wide dispersive use and a high tonnage, Phenol, methylstyrenated was included in the Community rolling action plan (CoRAP) for substance evaluation pursuant to Article 44(2) of the REACH Regulation to be evaluated in 2012. The CoRAP was published on the ECHA website on 29 February 2012. The Competent Authority of Denmark was appointed to carry out the evaluation.

By 23 July 2013 the lead registrant submitted a registration dossier with new name and EC number following a targeted Compliance Check by ECHA on substance identity. In this regard the identifier and name of the substance was changed in REACH IT to "EC 700-960-7 Oligomerisation and alkylation reaction products of 2-phenylpropene and phenol". Following a proposal from ECHA within the 30 days commenting period, the heading of the draft decision was changed to reflect the new name and identifiers. However, the former name "Phenol, methylstyrenated" still appears in different sections of the draft decision and shall here be regarded as synonymous with the new name "Oligomerisation and alkylation reaction products of 2-phenylpropene and phenol".

The evaluating MSCA considered that further information was required to clarify the abovementioned concerns. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 28 February 2013.

On 5 April 2013 ECHA sent the draft decision to the concerned registrants and invited them pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

By 2 May 2013 ECHA received comments from concerned registrants of which it informed the evaluating MSCA without delay.

The evaluating MSCA considered the concerned registrants' comments received and did amend Section III of the draft decision.

In accordance with Article 52(1) of the REACH Regulation, on 1 August 2013 the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days.

Subsequently, ECHA submitted proposals for amendment to the draft decision.

On 6 September 2013 ECHA notified the concerned registrants of the proposals for amendment to the draft decision and invited them pursuant to Articles 52(2) and 51(5) of the REACH Regulation to provide comments on the proposals for amendment within 30 days of the receipt of the notification.

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

On 16 September 2013 ECHA referred the draft decision to the Member State Committee.

On 1 October 2013 the concerned registrants provided comments on the proposed amendments. The Member State Committee took the comments of the concerned registrants into account.

After discussion in the Member State Committee meeting on 4-8 November 2013, a unanimous agreement of the Member State Committee on the draft decision as modified at

the meeting was reached on 7 November 2013. ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

It is noted that a testing proposal has been submitted by the registrants for the substance subject to the present decision for the following endpoint: prenatal developmental toxicity study (OECD 414). This testing proposal will be examined by ECHA followed by respective decision making.

II. Information required

Pursuant to Article 46(1) of the REACH Regulation, the concerned registrants shall submit the following information using the indicated test methods on the **registered substance** subject to the present decision:

1. **Bioaccumulation in fish: dietary exposure (test method: OECD 305[1]).** The test design shall include quantitative measurements that enable an evaluation of the bioaccumulation potential for each of the four relevant groups of constituents in the substance (i.e. separation of dialkylated phenol, trialkylated phenol, dimers of C9 monomers and trimers of C9 monomers). The substance to be tested shall also include monoalkylated phenol since this constituent is relevant with regard to vitellogenin induction (see below), although it was concluded not to meet the criteria for B-/vB-.

   The test shall include additional measurements of vitellogenin induction in fish. Sampling and determination of vitellogenin shall follow the guidance for this parameter in OECD 229. Vitellogenin induction and sex-determination shall be assessed in individual fish at termination of the uptake phase. At least 16 additional fish (as specified in OECD 234), consisting of at least 10 male fish, shall be sampled for this purpose from each replicate (exposure and control groups) (compared to at least 4 fish at each sampling point in OECD 305).

   The test shall be conducted with one of the following fish species: Japanese medaka (*Oryzias latipes*), zebrafish (*Danio rerio*) or fathead minnow (*Pimephales promelas*).

2. **Combined Sub-chronic toxicity study (90 days) (test method EU B26/OECD 408) and Extended one-generation reproductive toxicity study in rats, oral route (test method: OECD 443) including Cohorts 2A and 2B for developmental neurotoxicity and Cohort 3 for developmental immunotoxicity.**

   The histopathological examinations shall be conducted on the F1-generation and shall include all of the organs listed in either OECD 408 or OECD 443.

   The premating exposure period for the P generation shall be extended to 10 weeks to increase the likelihood that steady-state exposure conditions in P males and females are achieved before mating. The post-weaning period of the F1-generation shall be adjusted, if necessary, to ensure that the animals are exposed for at least 90 days after weaning. A range finding test shall be conducted in order to determine relevant exposure levels for the combined study.

Pursuant to Article 46(2) of the REACH Regulation, the concerned registrants shall submit to ECHA by 24 August 2017 an update of the registration dossiers containing the information

[1] The test shall be conducted with dietary exposure as described in the newly revised OECD 305 that was adopted 2 October 2012.

required by this decision as well as updated Chemical Safety Reports addressing the new information.

At any time, the concerned registrants shall take into account that there may be an obligation to make every effort to agree on sharing of information and costs with other registrants.

III. Statement of reasons

Based on the evaluation of all relevant information submitted on Phenol, methylstyrenated and other relevant and available information, ECHA concludes that further information is required in order to enable the evaluating MSCA to complete the evaluation of whether the substance constitutes a risk to human health or the environment.

The information requested in Section II above constitutes the first tier in a testing strategy to clarify the concerns for potential PBT and endocrine disrupting properties of the registered substance.

Based on results from the requested dietary bioaccumulation test, a second tier of the PBT testing strategy may be triggered. If it is concluded that constituents of the registered substance meet the criteria for B- and/or vB- as specified in REACH, Annex XIII, then these constituents will need to be evaluated with regard to the criteria for persistency (REACH, Annex XIII). A degradation simulation test in surface water and/or in sediment (OECD 309/308) may be requested in a second draft decision. Specification of the test design including composition of the substance to be tested as well as choice of analytical methods depend on the outcome of the requested bioaccumulation study (OECD 305). Hence, the evaluating MSCA will review the information submitted by the concerned registrants as an outcome of this decision and evaluate if further information should be requested in order to clarify the PBT properties.

Based on results from the bioaccumulation test and the degradation simulation test, a third tier of the PBT testing strategy may be triggered. If constituents of the registered substance are concluded to meet the criteria for P- and B- as specified in REACH, Annex XIII, then these constituents will need to be evaluated with regard to the criteria for toxicity. The choice of test method and species to be tested depends on the outcome of other requested studies including the combined OECD 408/443 and the results of the assessment of vitellogenin induction in fish that has been included as an additional parameter in the requested dietary bioaccumulation study (OECD 305). Hence, the evaluating MSCA will review the information submitted by the concerned registrants as an outcome of tier 2 in the testing strategy and evaluate if further information should be requested in order to clarify the concern PBT properties.

For evaluation of the potential endocrine disrupting properties, further testing may be needed in order to clarify the indicated concerns if non-conclusive results are achieved in the requested tests.

1. Request for a dietary bioaccumulation study in fish with additional measurements of vitellogenin induction

Information on bioaccumulation is required in order to enable the evaluating MSCA to assess the properties of the substance and relevant constituents and to decide whether it is bioaccumulative in relation to the criteria for PBT assessment (REACH, Annex XIII). This information is thus needed to conclude on the suspected concern. Without the requested information it will not be possible to verify whether there remains an uncontrolled risk with the substance that should be subject to further risk management measures.

Based on an evaluation of all available information on bioaccumulation, ECHA has concluded that four out of five different groups of constituents of the registered substance potentially meet the criteria for B- and/or vB (according to REACH, Annex XIII) and that further testing is needed in order to derive a definitive conclusion on this property. The groups of constituents that need to be evaluated for their bioaccumulation potential are: dialkylated phenol, trialkylated phenol, dimers of C9 monomers and trimers of C9 monomers. A fifth constituent in the registered substance, monoalkylated phenol (CAS No 599-64-4), needs not to be assessed for its potential to bioaccumulate, since it was concluded that this constituent does not meet the criteria for B- by the evaluating MSCA.

It was concluded that the monoalkylated phenol (CAS No 599-64-4) does not meet the criteria for B- or vB (REACH, Annex XIII) based on a fish bioaccumulation study (equivalent to OECD 305) in *Cyprinus carpio* (NITE, 2003) with a BCF of 165 for the low exposure group and 69-190 for the high exposure group. In addition, this constituent has a measured log K_{ow} of 3.8.

Test data from an aqueous bioaccumulation test (equivalent to OECD 305) is available for the dimer of C9 monomers; 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl)bisbenzene (CAS 6362-80-7). BCF from this study is reported as 427-3330 for the high exposure group (10 µg/l) and 423-4410 for the low exposure group (1 µg/l) (NITE 2002). These BCF values have some degree of uncertainty due to the relatively high variation in the dataset and the use of a solvent in the study. Hence, it cannot clearly be concluded if the measured BCF indeed is above the B- criterion of 2,000.

No test data have been identified for the remaining constituents in Phenol, methylstyrenated. Therefore, QSAR estimates have been used to characterize the bioaccumulation potential for the following constituents: CAS No 2772-45-4 (representing dialkylated phenol); CAS No 30748-85-7 (representing trialkylated phenol); CAS No 3910-35-8, 6362-80-7 and 6258-73-7 (representing dimers of C9 monomers); CAS No 41906-71-2, 62604-62-0, 19303-34-5 (representing trimers of C9 monomers). The QSAR models predict the following ranges of BCF/BAF values for the constituents:

Dialkylated phenol: BCF predictions for three different QSAR models are 702 (Arnot-Gobas, upper trophic level, including biotransformation), 7724 (BCFWIN) and 101 (CAESAR). The substance is within the applicability domain of the Arnot-Gobas and the BCFWIN models but outside the applicability domain of the CAESAR model. Due to the lipophilic nature of the constituent, intake through diet may exceed intake over the gills. The Arnot-Gobas BAF estimate is 2911 (upper trophic level, including biotransformation rate estimates).

Trialkylated phenol: BCF predictions for three different QSAR models are 66.3 (Arnot-Gobas, upper trophic level, including biotransformation), 1479 (BCFWIN) and 10 (CAESAR). The substance is within the applicability domain of the Arnot-Gobas and the BCFWIN models but outside the applicability domain of the CAESAR model. Due to the highly lipophilic nature of the constituent, intake through diet will exceed uptake over the gills. The Arnot-Gobas BAF estimate is 10,120.

Dimers of C9 monomers: BCF predictions for three different QSAR models are: 1711-2482 (Arnot-Gobas, upper trophic level, including biotransformation), 3681-9201 (BCFWIN) and 1027-1225 (CAESAR). The substance is within the applicability domain of the Arnot-Gobas and the BCFWIN models but outside the applicability domain of the CAESAR model for two of the three constituents. Due to the hydrophobic nature of the constituent, intake through diet is likely to exceed uptake over the gills. The Arnot-Gobas BAF estimate is 7,388-21,760 (upper trophic level, including biotransformation rate estimates).

Trimers of C9 monomers: BCF predictions for three different QSAR models are: 28-113 (Arnot-Gobas, upper trophic level, including biotransformation), 722-1426 (BCFWIN) and 13-20 (CAESAR). The substance is within the applicability domain of the Arnot-Gobas and the BCFWIN models but outside the applicability domain of the CAESAR model for the three constituents. Due to the hydrophobic nature of the constituent, uptake through food may exceed uptake over the gills. The Arnot-Gobas BAF estimate is 743,900-177,000 (upper trophic level, including biotransformation rate estimates).

The above mentioned representative constituents in Phenol, methylstyrenated all have estimated log K_{ow} values above 6. According to the REACH PBT Guidance Document (Guidance on information requirements and chemical safety assessment, chapter R.11: PBT Assessment) and the newly revised test guideline on bioaccumulation in fish (OECD 305, Bioaccumulation in fish: Aqueous and Dietary Exposure, adopted 2 October 2012) dietary exposure is recommended for very hydrophobic substances such as the relevant constituents in Phenol, methylstyrenated.

In addition to the concern for potential PBT properties a concern has been identified with regard to potential endocrine disrupting effects for phenolic constituents in Phenol, methylstyrenated. Monoalkylated phenol (CAS No 599-64-4) has been shown to bind to the estrogen receptor and to exhibit estrogen activity *in vitro* and dialkylated phenol (CAS No 2772-45-4) has been shown to display juvenile hormone activity in crustaceans. In addition, QSAR models in the Danish QSAR database predict that monoalkylated phenol and dialkylated phenol will bind to the estrogen receptor (references to the relevant studies are given under Section III, 2 below).

For these reasons, it is requested that the dietary bioaccumulation study is modified to include additional measurements of vitellogenin induction in the fish. The induction of vitellogenin synthesis in fish is an effective and sensitive biomarker for exposure to environmental estrogens and the results from this added parameter will be used for evaluation of the potential endocrine disrupting properties of Phenol, methylstyrenated and, if relevant, for determination and specification of later tier tests in the testing strategy.

A procedure for measurements of vitellogenin induction in fish is described in the following test guidelines: OECD 229, OECD 230 and OECD 234. The three species that are validated for OECD 229 are also validated for the OECD bioaccumulation test (OECD 305). Hence one of the following species shall be used for the test: Japanese medaka (*Oryzias latipes*), zebrafish (*Danio rerio*) or fathead minnow (*Pimephales promelas*). Phenotypic sex-determination of adult fish should be possible for all three species (although at varying complexity).

The requested test only contains one exposure group and a dose-response relationship for vitellogenin induction can therefore not be derived. To achieve an adequate statistical power of the test the sampling shall include at least 16 fish from each replicate and control. This is identical to the minimum number of fish to be sampled for vitellogenin analysis in OECD 234. Since vitellogenin is naturally produced in female fish, interpretation of test results is more straightforward in male fish. For this reason, the 16 sampled fish from each replicate and control shall contain at least 10 male fish (by phenotypic determination).

For animal welfare reasons, the usual testing strategy for PBT assessment starts with persistency testing followed by bioaccumulation testing. However, in this case the testing strategy has been reverted to start with the bioaccumulation study. ECHA has considered the following reasons in favour for the proposed testing sequence:

If the UVCB substance itself is used as the test substance in the biodegradation simulation study, monitoring of the primary biodegradation (biotransformation) will be limited by the

procedures for extraction and analytical detection of representative constituents of the substance in the mixture. Obviously, it will be almost impossible to monitor the transformation of all the individual constituents in Phenol, methylstyrenated and, thus, the logical test design would be based on selection of one representative substance for each of the five main groups of constituents. Having made such a selection, the planning of the experimental part of the study should include thorough investigation of the extraction procedures and analytical detection limits. The result of this investigation might be that the initial concentration of the test substance needs to be much higher than the concentration which is normally considered environmentally realistic. With this complex mixture approach, only the primary biodegradation (biotransformation) of the selected substances representing the main groups of constituents may be determined, whereas supporting information on the formation of biotransformation products, which will probably be of a qualitative nature, might be obtained from the analysis and/or by use of an appropriate QSAR.

ECHA considers that a dietary bioaccumulation study performed with the registered substance is more likely to provide useful results. Constituents that are concluded to not meet the criteria for B- or vB- (REACH, Annex XIII) based on the results from this study, do not need to be investigated for their persistency potential (for the purpose of PBT assessment). Hence, it may be possible to reduce or totally eliminate the number of constituents to be assessed at tier two in the testing strategy. If only one or a few constituents are identified as B- or vB- it could be considered to test the individual constituents (instead of the registered substance) which may allow to design an analytical program to determine both the biotransformation of the test substance and the formation of major transformation products. In addition, this approach may make it possible that ^{14}C-labelled test substance can be manufactured within a reasonable time period and costs. The access to a ^{14}C-labelled test substance will add value to the biodegradation simulation test, because ultimate biodegradation (mineralization) can be determined and a low environmentally realistic concentration can be applied.

A second reason to first conduct the dietary bioaccumulation study, is that the additional included measurements of vitellogenin induction may enable the evaluating MSCA to assess the concern relating to endocrine disruption at an earlier stage in the process, which may be used for specifications of later tiers in the testing strategy. Finally, ECHA notes that a bioaccumulation study is a standard information requirement according to REACH, Annex IX. In the current version of the registration dossier this information has been filled by QSAR estimates from the CAESAR BCF model for the individual constituents. However, some of these QSAR estimates are outside the applicability domain of the applied model according to the VEGA program, which is a software tool developed to provide an applicability domain assessment for QSAR models, including the CAESAR BCF model. Therefore the presently provided QSAR estimates cannot adequately be used to fill the standard information requirement for bioaccumulation. This means that from a compliance perspective, the bioaccumulation study would probably need to be conducted anyway, irrespective of the testing sequence that has been included in this decision.

In their comments to the draft decision submitted by the evaluating MSCA the concerned registrants had expressed consent to provide the information on Bioaccumulation in fish.

Therefore, pursuant to Article 46(1) of the REACH Regulation, the concerned registrants are required to carry out the following study using the registered substance subject to this decision: Bioaccumulation in fish: dietary exposure (test method: OECD 305[2]) including additional measurements of vitellogenin induction in fish.

[2] The test shall be conducted with dietary exposure as described in the newly revised OECD 305 that was adopted 2 October 2012.

2. Combined Repeated dose 90-day toxicity study and Extended one-generation reproductive toxicity study in rats, oral route

Statement of reasons for Extended one-generation reproductive toxicity study
Information on reproductive toxicity is required in order to enable the evaluating MSCA to assess the properties of the substance and relevant constituents and to determine whether it has endocrine disrupting properties. In addition, hazard classes that are relevant for classification as toxic to reproduction have a direct link to the T criterion in PBT assessment (Annex XIII, Section 1.3. of the REACH Regulation). There is no information on reproductive toxicity in the registration dossier for Phenol, methylstyrenated. This information is thus needed to conclude on the suspected concern both with regard to endocrine disruption and PBT. Without the requested information it will not be possible to verify whether there remains an uncontrolled risk with the substance that should be subject to further risk management measures.

Studies of relevance to endocrine disrupting properties were identified for two of the constituents in Phenol, methylstyrenated (monoalkylated phenol; 4-(α,α-dimethylbenzyl)phenol, CAS No 599-64-4 and dialkylated phenol; 2,4-bis(1-methyl-1-phenylethyl)phenol, CAS No 2772-45-4):

Shibata *et al.* (2002) investigated the effects of BPA and 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4) on microsomal UDP-glucuronosyl-transferase activity toward sex hormones in adult rats. BPA treatment suppressed sex hormone glucuronidation but only in males. This suggests that BPA may disrupt the endocrine balance by effects on metabolism and excretion of endogenous hormones. 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4) had no effect on UGT activities towards sex hormones in this study.

Biggers and Laufer (2004) tested the juvenile hormone (JH) activity of different alkyl phenols in an assay based on their effects on the settlement and metamorphosis of larvae of the polychaete *Capitella*. In this sensitive assay the constituent 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4) showed high JH activity (EC_{50} of 3 µM). The constituent 2,4-bis(1-methyl-1-phenylethyl)phenol (CAS RN No 2772-45-4) also showed high JH activity (EC_{50} of 2 µM) whereas BPA showed very high activity (EC_{50} of 0.05 µM).

Terasaki *et al.* (2005) measured the estrogenicities of 10 compounds found as impurities in industrial grade bisphenol A (BPA) by yeast 2-hybrid assays incorporating the human estrogen receptor (hER) or the medaka fish (Oryzias latipes) estrogen receptor (mER). Five impurities showed greater activity than BPA itself in an agonist assay for hER. The constituent of Phenol, methylstyrenated 4-(α,α-dimethylbenzyl)phenol (CAS 599-64-4), was the most active of the impurities in the hER assay. It was 12 times as active as BPA in the assay incorporating the human estrogen receptor (hER) and 6 times as active in the assay incorporating the medaka estrogen receptor (mER).

Okuda *et al.* (2011) tested the estrogenic activity of BPA and eight BPA-related compounds in the yeast estrogen screening assay after incubation with rat liver S9 fraction in the presence of a NADPH-generating system. BPA and three of its analogues exhibited an increase of estrogenic activity after incubation with S9. In contrast, the estrogenic response was almost lost after incubation with S9 for the constituent in Phenol, methylstyrenated: 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4).

Matsushima *et al.* (2008) conducted an *in vitro* receptor binding assay and X-ray crystal structure analysis which demonstrated that 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4) strongly binds to the human estrogen-related receptor gamma (ERRgamma). 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4) had a similar binding affinity as BPA (tested in the same assay). Based on the results the authors suggest that it would be relevant to

examine whether or not 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4) cause low-dose effects similar to those reported for bisphenol A. This is particularly important because ERRγ is expressed very strongly in the mammalian fetal brain and also in the placenta, at sites that could have important outcomes for newborns.

Sanseverino *et al.* (2009) screened the estrogenic and antiandrogenic hormone activity in a bioluminescent yeast bioreporter assay. 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4) was found to display estrogenic activity with a higher relative potency (a factor of 3) than BPA compared with 17β-estradiol. No antiandrogenic activity was observed for 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4).

The LUMI-CELL ER (BG1Luc4E2) stably transfected estrogen receptor (ER) transcriptional activation (TA) assay uses the human ovarian cancer cell line, BG-1, that expresses both human hER-alpha and hER-beta to screen for substances that may induce or inhibit estrogenic activity *in vitro*. In this assay 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4) was found to display estrogen agonist activity (Casey *et al.*, 2010).

A combined repeated dose toxicity study/ reproductive toxicity screening study (OECD 422) is reported for 4-(α,α-dimethylbenzyl)phenol (CAS No 599-64-4) (Tyl *et al.*, 2005). The study exceeded the OECD 422 test design by following the F1 offspring to adulthood, with continued exposure and assessment of reproductive structures and functions. Systemic toxicity was observed at 300 mg/kg bw/day in the F0 males and females were observed. In addition, equivocal effects for body weight at 50 mg/kg bw/day for females and a slight decrease in uterine implantations at 300 mg/kg bw/day in females. A significant increase in testis weight (relative to body weight) was observed at 50 mg/kg bw/day for F0 but not for the F1 generation. It should be noted, that even though an extended study design was used this test is not considered to be sensitive for detection of endocrine disrupting effects (cf. OECD, 2012).

In addition, QSAR models in the Danish QSAR database predict that monoalkylated phenol (CAS No 599-64-4) and dialkylated phenol (CAS No 2772-45-4) will bind to the estrogen receptor. The predictions are judged to be within the structural applicability domain of the model (Danish QSAR Database, 2004).

The above mentioned studies represent lower tier screening tests/assays that provide an indication for an endocrine mediated mode of action for two constituents in Phenol, methylstyrenated; monoalkylated phenol (CAS No 599-64-4) and dialkylated phenol (CAS No 2772-45-4). They cannot, however, be used to derive conclusions on whether or not Phenol, methylstyrenated is an endocrine disrupter. A higher tier experimental study is needed in order to clarify the concern for endocrine disruption.

To clarify the indications of concern on endocrine disruption from non-animal approaches and the available OECD TG 422 screening study, an extended one-generation reproductive toxicity study (OECD 443) is the preferred test. This test is expected to provide relevant information on reproductive toxicity and systemic toxicity *in vivo* especially related to the indications of concern. OECD 443 includes parameters for adverse effects on reproduction and certain sensitive parameters to detect endocrine disrupting effects, including some adverse effects caused by an estrogenic mode of action which is indicated for some constituents in the registered substance. According to OECD (2013) the OECD 443 is a higher tier reproductive toxicity study, which is preferable for detecting endocrine disruption because it provides an evaluation of a number of endocrine endpoints in the juvenile and adult F1.

In this specific case, it is considered that the study design without extension of the Cohort 1B to assess F2 would be sufficient to address the identified concern and this would be in

line with general considerations made by Rorije *et* al. (2011) and Piersma *et* al. (2011). The conduct of an OECD 443 without extension of cohort 1B will furthermore save a significant number of experimental animals.

Existing knowledge for the chemical does not support an omission of the developmental neurotoxicity (DNT) and developmental immunotoxicity (DIT) cohorts (OECD 443, paragraph 2). In addition, as the concern is specifically on estrogenic effects, cohorts for developmental neurotoxicity (Cohort 2A and 2B) should be included to evaluate potential effects e.g. on the sexual dimorphic development of the brain which may be affected by estrogenic substances.

With regard to the DIT cohort, a scientific article suggests that estrogenic endocrine disruptors may modulate the immune system in mice (Calemine *et al.,* 2003). Based on the concern for an estrogenic mode of action of two of the constituents in Phenol, methylstyrenated, inclusion of the DIT Cohort is considered necessary. This is further supported by findings in two recent scientific papers (Tonk *et al.,* 2013; Tonk *et al.,* 2011), which indicate the susceptibility of the developing immune system to exposure to some chemical substances.

Since some of the constituents in the registered substance have potentially bioaccumulating properties (i.e. potentially a slow clearance rate in rat), and in absence of toxicokinetic information, the premating exposure period for the P animals shall be extended to 10 weeks to increase the likelihood that steady-state exposure conditions in P males and females are achieved before mating (c.f. also the general provisions of OECD 443 paragraph 26 stipulating the need to achieve steady state in the premating period).

Statement of reasons for Repeated dose 90-day study
Repeated dose toxicity testing is needed in order to determine if criteria for classification as STOT RE are fulfilled. This hazard class is a part of the $T_{mammalian}$- assessment under PBT evaluation. It is noted that Phenol, methylstyrenated contains potentially bioaccumulating constituents and that the available 28 days repeated dose toxicity study may be of too short duration for these constituents to have reached a steady state in the organism.

The repeated dose 90-day study is currently not available in the registration dossier for Phenol, methylstyrenated. The concerned registrant has proposed to adapt the information requirement of sub-chronic toxicity (Annex IX, Section 8.6.2. of the REACH Regulation) with the following explanation: "*In accordance with REACH Regulation 1907/2006, a repeated-dose study by the oral route - optionally required in Annex VIII, 8.6.1 - is not indicated according to 8.6.1, column 2, as firstly neither the acute oral nor the acute dermal studies gave evidence of overt and specific toxicity, secondly the existing dermal 28d study failed to reveal any substance-related adverse effects. This indicates that adverse effects are very unlikely to occur via repeated oral exposure.*"

However, ECHA notes that neither column 2 of section 8.6.2 nor general rules for adaptation in Annex XI include the possibility to adapt this standard information requirement on the basis of the argument made by the concerned registrant.

The justification by the concerned registrants most closely relates to the adaptation possibility of Annex IX, 8.6.2, Column 2 according to which no sub-chronic toxicity study needs to be conducted if "the substance is unreactive, insoluble and not inhalable and there is no evidence of absorption and no evidence of toxicity in a 28 day "limit test", particularly if such a pattern is coupled with limited human exposure."

The concerned registrants have, however, not claimed that the cumulative conditions of that

adaptation possibility are fulfilled. ECHA notes that the following conditions invalidate the proposed column 2 adaptation:

1. At least one of the major constituents in Phenol, methylstyrenated has been shown to be biologically reactive. CAS No 599-64-4 has been shown to bind to the estrogen receptor and to exhibit estrogen activity *in vitro*.
2. Slight dose related increases in relative liver weights were observed in the conducted 28-days dermal study with Phenol, methylstyrenated. This provides indirect evidence for systemic absorption of constituents of the substance.
3. A 28 days study with CAS No 599-64-4 - a constituent of Phenol, methylstyrentated - provides indirect evidence of absorption based on observed effects such as reduced body weight, increased kidney effects and renal tubular effects.
4. No evidence has been given of limited human exposure.

Based on the observations above, ECHA considers that information from a repeated dose 90-days study is needed.

Statement of reasons for requesting a combined OECD 443/408
The lead registrant for Phenol, methylstyrenated has proposed to include the parameters from OECD 408 in the test design of the requested OECD 443. This would reduce cost and use of experimental animals and hence be in accordance with Articles 1(1) and 13 of the REACH Regulation. Consequently, in their comments to the draft decision submitted by the evaluating MSCA the concerned registrants had expressed consent to provide the information of a combined study. This was reiterated by the concerned registrants when responding to the proposals for amendment made to the draft decision. ECHA based on the initial proposal by the evaluating MSCA supports this approach and is therefore requesting a combined OECD 443/408 which addresses the concerns that are being evaluated under substance evaluation.

A comparison of the standard examinations conducted under the two studies is summarised below. There is an overlap of many of the included parameters in the two tests. However, the histopathological examinations are slightly different in the OECD 408 compared to OECD 443.

Table 1. Comparison of examinations

Parameter	**90 day (OECD 408)**	**EOGRTS (OECD 443)**
Endpoint-targeted groups	One	P and F1 generation, F1 divided into sub-groups for • reprotox (320 animals) • neurotox (160 animals) • immunotox (80 animals)
Recovery/satellite groups	optional	none
Time	90 days	≥70 days
Clinical observation	daily	daily
Body weight	regular	regular
Blood biochemistry	Interim (optional) and at termination	At termination (P, F1)
Haematology	Interim (optional) and at termination	At termination (P, F1)
Neurobehaviour	included	included

Urinalysis	At termination	At termination (optional P, F1)
Ophthalmology	At termination (optional)	Not included
Gross pathology	At termination	At termination (P, F1)
Histopathology	At termination	At termination (P, F1)

Based on the comparison of histopathological examinations in the two tests, the following parameters are requested in the combined test: a full histopathological examination to cover all of the organs listed for full histopathological examinations in either OECD 408 or OECD 443.

It is possible to include the full histopathological examination on either the P-generation or the F1-generation in the OECD 443 study. Each approach has its strengths and weaknesses as outlined below.

- Additional histopathological examinations in the P-generation:

  Drawbacks: Females from the P-generation are likely to be different compared to the "virgin" females that are normally used in OECD 408 because they have been pregnant, have been giving birth and have been lactating which may lead to an increased variability in their toxicological dataset compared to data if OECD 408 was used.

  Another concern is that some ED related effects on the female reproductive organs may be obscured because of the pregnancy. Hence females exposed during pregnancy may have a different sensitivity. In addition, since some constituents in Phenol, methylstyrenated are lipophilic and potentially bioaccumulative, females may reduce their body burden by excretion through lactation which is not possible in OECD 408.

  A third drawback is that that the females do not give birth at the same time (and the termination of weaning does therefore also not take place exactly at the same time) for all rats and hence this may also increase variability between the females if account of this is not taken in the test design.

  To reduce this latter variability the study should be terminated at the same time after weaning of the pups, i.e. they should be sacrificed at approximately the same number of days after ceased lactation so that they have the same time for "normalization". This means that the exposure of the female P-generation will have to be prolonged for a couple of weeks after weaning and that the date of termination of the study for the females will be based on the date they give rise to birth. This means by other words that the exposure period will be slightly different between the female animals, since the onset of pregnancy will vary between the tested females and since the exposure of the P females starts at the same date.

  Advantage: The advantage of applying the full histopathological examinations to the P-generation is as regards the males that it is possible in most cases to discriminate between reproductive toxicity effects and general repeated dose toxicity.

  Furthermore there exist already an adopted approach for merging a repeated dose toxicity study and a reproductive toxicity study, i.e. OECD 422. Therefore it can be claimed that such a merged study as proposed here for a higher tier (i.e. merging of TG 408 & 443) has already been accepted for regulatory purposes at a lower tier. It is in this regard noted that REACH in Annex VIII 8.7.2. directly refers to a standard information requirement which specifically includes OECD 422.

- Additional histopathological examinations in the F1-generation:

  The test design should be modified to allow for exposure of F1 animals for 90 days after weaning, meaning that the duration of test on the F1 animals will be increased approximately with 30-40 days compared with a normal OECD 443 study (because weaning of rats takes place 22 days after birth and because the OECD 443 study normally only has a duration of 50-60 days after weaning of F1).

  Disadvantage: Animals from the F1-generation have also been exposed *in utero*. This means that the result may not be directly comparable to the results generated in OECD 408. In addition, it may be difficult in some cases to discriminate between reproductive effects and general repeated dose toxicity.

  Advantage: The advantage of applying the full histopathological examinations to the F1-generation is that this will be the most sensitive test design. In addition, the test females will not in contrast to using the P females be influenced by pregnancy, giving birth and lactation during the 90-days period and hence the disadvantages of the above approach will be avoided.

Taking the above mentioned arguments into consideration, ECHA based on the proposal by the evaluating MSCA is in favour of including the additional histopathological examinations on the F1-generation in the OECD 443. The post-weaning period shall be adjusted, if necessary, to ensure that the animals are exposed for at least 90 days.

A combined study may impose challenges with regard to classification and labelling for STOT and reproductive effects. However, this may be dealt with based on the effects observed and using weight of evidence approach. On the other hand, the combined study may give better data for risk assessment of both repeated dose and reproductive toxicity than two separate studies due to a better basis for evaluation across endpoints. Also, the increased dosing period including sensitive periods in the combined study might provide better data for risk assessment. The identified concerns that need to be addressed are potential PBT and endocrine disrupting properties for some of the constituents in the substance. In this regard, a NOAEL from the combined study may also provide the best possible basis for establishing a PNEC for secondary poisoning (due to reasons outlined above, i.e. increased dosing period including sensitive periods). Hence, in this specific case it is considered that the benefits of the combined study outweigh the potential risks/challenges.

Careful considerations should be given to the exposure levels in the combined study to ensure that adequate information is achieved for both reproductive and repeated dose toxicity effects. Currently, information is available from a 28 day repeated dose dermal toxicity study with the registered substance and a reproductive toxicity screening study on one of the constituents. However, no information is available for a reproductive toxicity screening study or 28 day repeated dose toxicity study using the registered substance and oral exposure. Therefore, the exposure levels would need to be established based on a carefully planned range finding study. This range finding study may also give useful information for the pre-natal developmental toxicity study (OECD 414), which has been proposed by the concerned registrants, provided that this study will be required by ECHA following the testing proposal examination and respective decision making.

Therefore, pursuant to Article 46(1) of the REACH Regulation, the concerned registrants are required to carry out the following study using the registered substance subject to this decision: Combined Repeated dose 90-day toxicity study (test method EU B26/OECD 408) and Extended one-generation reproductive toxicity study in rats, oral route (test method:

OECD 443) including the two cohorts DNT and DIT.

The histopathological examinations shall be conducted on the F1-generation and shall include all of the organs listed in either OECD 408 or OECD 443.
The premating exposure period for the P generation shall be extended to 10 weeks to increase the likelihood that steady-state exposure conditions in P males and females are achieved before mating. The post-weaning period of the F1-generation shall be adjusted, if necessary, to ensure that the animals are exposed for at least 90 days after weaning. A range finding test shall be conducted in order to determine relevant exposure levels for the combined study.

Initially the decision foresaw a 30 month deadline for submission of an update of the registration dossiers containing the information requested in this decision. This deadline has, however, been prolonged to 42 months in order to take into account the prolongation of the pre-mating period in the combined OECD 443/408 study as suggested in a proposal for amendment to the draft decision and the need for a range finding study, identified by the Member State Committee.

IV. Adequate identification of the composition of the tested material

The substance identity information submitted in the registration dossiers has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation. In relation to the required tests, the sample of substance used for the new studies shall have a composition that is within the specifications of the substance composition that are given by all concerned registrants. It is the responsibility of all the concerned registrants to agree on the tested materials to be subjected to the tests subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation. Finally, the studies must be shared by the concerned registrants.

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

Avoidance of unnecessary testing and the duplication of tests is a general aim of the REACH Regulation (Article 25). The legal text foresees the sharing of information between registrants. Since several registrants of the same substance are required to provide the same information, they are obliged to make every effort to reach an agreement for every endpoint as to who is to carry out the test on behalf of the other concerned registrants and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation.

If ECHA is not informed of such agreement within 90 days, it shall designate one of the concerned registrants to perform the tests on behalf of all of them. If a registrant performs a test on behalf of other registrants, they shall share the cost of that study equally and the registrant performing the test shall provide each of the others concerned with copies of the full study reports.

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/datasharing_en.asp.

VI. General requirements regarding Good Laboratory Practice

ECHA always reminds registrants of the requirements of Article 13(4) of the REACH Regulation that ecotoxicological and toxicological tests and analyses shall be carried out in compliance with the principles of good laboratory practice (GLP). National authorities monitoring GLP maintain lists of test facilities indicating the relevant areas of expertise of each facility.

VII. Information on right to appeal

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

[redacted]

Jukka Malm
Deputy Executive Director

Enclosures: Annex I: References

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

Annex I: References

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