EUROPEAN CHEMICALS AGENCY

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

For 2-(4-tertbutylbenzyl)propionaldehyde, CAS No 80-54-6 (EC No 201-289-8)

Addressees: Registrants of 2-(4-tertbutylbenzyl)propionaldehyde (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 2 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 Swedish Chemicals Agency as the Competent Authority of Sweden (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 5 September 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 Sweden has initiated substance evaluation for 2-(4-tertbutylbenzyl)propionaldehyde, CAS No 80-54-6 (EC No 201-289-8) 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 human health effects for reprotoxicity and workers and consumers exposure and a wide dispersive use 2-(4-tertbutylbenzyl)propionaldehyde 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 Sweden was appointed to carry out the evaluation. In the course of the evaluation, the evaluating MSCA noted additional concerns regarding endocrine disrupting properties and developmental toxicity of the substanace.

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 22 February 2013.

Further information requirements related to evaluation of 2-(4-tertbutylbenzyl)propionaldehyde have been addressed to the relevant registrant in a separate confidential draft decision.

On 4 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 6 May 2013 ECHA received comments from concerned registrants of which it informed the evaluating MSCA without delay.

The MSCA considered the registrants' comments received and did amend Section II of the draft decision. The comments were reflected in Section III of the draft decision (Statement of Reasons).

In accordance with Article 52(1) of the REACH Regulation, on 5 September 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, Competent Authorities of the Member States and ECHA submitted proposals for amendment to the draft decision.

On 11 October 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 those proposals for amendment within 30 days of the receipt of the notification.

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

On 21 October 2013 ECHA referred the draft decision to the Member State Committee.

On 11 November 2013 the concerned registrants provided comments on the proposed amendments. The Member State Committee took into account the comments the concerned registrants made on the proposals for amendment. However, the Member State Committee did not consider the Registrants' comments that were not related to the proposals for amendment.

After discussion in the Member State Committee meeting on 10 to 13 December 2013, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 13 December 2013. ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

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 and specifications and the registered substance 2-(4-tertbutylbenzyl)propionaldehyde (pure grade compositions: [redacted], further called registered substance (pure grade)) subject to the present decision:

1. Fish Short Term Reproduction Assay; test method OECD TG 229 ; test species Fathead minnow with specifications as outlined in Section III; and

2. Extended one-generation reproductive toxicity study in rats, oral route (test method: OECD 443) including the extension of Cohort 1 B to mate the F1 animals to produce the F2 generation which shall be kept until weaning and the Cohorts 2 and 3 to assess developmental neurotoxicity (DNT) and immunotoxicity (DIT). In addition to the standard clinical biochemistry / haematological parameters required by TG 443, examination of acetyl cholinesterase activity in different compartments including plasma, erythrocytes, brain, peripheral neuronal system in parental animals F 0 and offsprings F 1 shall be undertaken.

Pursuant to Article 46(2) of the REACH Regulation, the concerned registrants shall submit to ECHA by 21 May 2016 an update of the registration dossiers containing the information required by this decision.

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 2-(4-tertbutylbenzyl)propionaldehyde 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.

1. Fish Short Term Reproduction Assay; test method OECD 229

The Registrant(s) failed to include existing and publicly available data on endocrine disruption (ED) properties of 2-(4-tert-butylbenzyl)propionaldehyde in the registrations and did not investigate the ED properties of 2-(4-tert-butylbenzyl)propionaldehyde further. The existing data (Charles and Darbre, 2009) correspond to level 2 of OECD (OECD GD150, OECD/ENV/JM/TG (2012)22) conceptual framework (CF) on identification of ED– in vitro assays providing mechanistic data and indicate estrogenic potential of 2-(4-tert-butylbenzyl)propionaldehyde (both agonist and antagonist responses have been observed).

Specifically, the results of the competitive binding to Estrogenic Receptor (ER) from MCF7 human cells show that 2-(4-tert-butylbenzyl)propionaldehyde at 3 000 000-fold molar excess inhibits the binding of 3H-oestradiol to ER alpha and ER beta. The extent of inhibition was greater for ER alpha and varied between the type of ER alpha, namely for ER alpha from MCF7 human cells cytosol inhibition was about 47% and for human recombinant receptor ER alpha 27%. For comparison the 2 500 000-fold molecular excess of methylparaben in the same assay system resulted in 43% inhibition of 3H-oestradiol binding. Furthermore 2-(4-tert-butylbenzyl)propionaldehyde at concentration $5x10^{-4}M$ induced 1.8-fold increase in expression of oestrogen-sensitive gene CAT in test with stably transfected reporter system (ERE-CAT). Control induction with $10^{-8}M$ of 17β–oestradiol resulted in 2-fold induction. The potential of 2-(4-tert-butylbenzyl)propionaldehyde to effect the oestrogen responsive genes was further confirmed by increased expression of

oestrogen-regulated gene pS2 mRNA in MCF7 cells. Treatment with 2-(4-tert-butylbenzyl)propionaldehyde had slightly smaller effect than the positive control exposure to 17β–oestradiol. In the following *in vitro* test 2-(4-tert-butylbenzyl)propionaldehyde increase the growth of oestrogen-dependent MCF7 human breast cancer cell, likely through the ER-mediated mechanism as the effect could be inhibited by the antioestrogen. Although growth of MCF7 cells over 7 days was lower than with the positive control exposure to 17β–oestradiol, the exposure for extended time with the tested substance lead to comparable cell density. Beside the oestrogen agonistic effects, small antagonist effect was observed in the MCF7 human breast cancer cell proliferation when cells were co-treated with 17β–oestradiol and 2-(4-tert-butylbenzyl)propionaldehyde (Charles and Darbre, 2009).

Further studies reported that alkylated non-phenolics resembling the structure of alkylphenols have the potential to act estrogenic in fish. Alkylated non-phenolic compounds interacted with the hepatic rainbow trout estrogen receptors (rtERs) with lower affinity than alkylphenols (Tollefsen and Nilsen (2008)). One of the metabolites of the 2-(4-tert-butylbenzyl)propionaldehyde, 4-tert-butylbenzoic acid (PTBBA), displayed both agonistic and antagonistic activity in vitellogenin production when co-exposed to 17β-estradiol in a rainbow trout hepatocyte assay, however no effect on vitellogenin in this assay was observed after PTBBA alone (Tollefsen et al. 2008).

The estrogenic activity is relevant for many species in the environment and might constitute an environmental risk. The substance is emitted and/or released to the environment.

The available information did not enable the evaluating MSCA to conclude on ED potential of the registered substance in aquatic species.

In order to clarify this concern further information on potential ED effects in aquatic species is required.

The potential to cause estrogenic effects in aquatic species may be further investigated using one of the *in vivo* test methods included in the Conceptual Framework (CF) for testing and assessment of endocrine disrupters levels 3 – 5. The CF is intended to provide a guide to tests that can provide information for ED assessment but is not intended to be a tiered testing strategy, i.e. for example a level 4 test may be chosen before a level 3 test if considered more appropriate. In the original draft decision, the FSDT OECD 234 was chosen by the evaluating MSCA as the most appropriate test.

a) Registrants' comments to the Draft Decision pursuant to Article 50(1):

The Registrants in the comments considered the *in vitro* study on human breast cells (Charles and Darbre, 2009) as not relevant for the assessment of environmental effects of 2-(4-tert-butylbenzyl)propionaldehyde and objected to the originally proposed fish (FSDT) test requirement. The following reasons were provided by the Registrants: i) expected acute toxicity in the originally proposed fish study (FSDT) at the test concentration anticipated based on the direct extrapolation of the threshold concentration for the ED effects from the existing *in vitro* study; ii) no likelihood of ED effects at concentrations lower than the threshold concentration of the *in vitro* study; iii) estimated environmental exposure concentrations of 2-(4-tert-butylbenzyl)propionaldehyde are orders of magnitude lower than the *in vitro* threshold concentration (due to the substance being readily biodegradable and has no bioaccumulation potential in fish according to the Registrants); iv) no evidence for ED mode of action in existing *in vivo* data.

The evaluating MSCA's response to "i" is that the approach of the direct extrapolation of the threshold exposure concentration for the ED effects from the existing *in vitro* study to the required fish study, as presented by the Registrants in the comment, is not valid. Several factors like species differences in the receptor affinity or possible ED effects of transformation products make such extrapolation highly uncertain. The responses to "ii" and "iii" are related to each other. It is considered that the statement "At lower test concentrations [than directly extrapolated from the *in vitro* study], however, no ED effects

will be observed as not even molecular processes that might lead to ED effects (such as receptor binding) takes place", is not adequately substantiated as no study exists examining ED effects of the substance on aquatic species in the range of sublethal effects exposure. In order to further support ECHA's reasoning that a threshold concentration from an *in vitro* study cannot be directly extrapolated to anticipate *in vivo* effect concentrations, ECHA provides the following example concerning another substance. Based on the results of the evaluation of p-tertbutylphenol (http://echa.europa.eu/documents/10162/13628/conclusion_4_tert_butylphenol_en.pdf), and the studies referred to therein, the substance showing relatively low estrogenic potency 0.3 – 5 million times lower than E2 in the in vitro MCF7 cells studies (Soto, A.M., et al. 1995), caused endocrine effects at 300 microg/L in the *in vivo* fish study (Krueger H.O. et al 2008).

In response to the statement "it can be demonstrated that there is no bioaccumulation potential in fish due to the high metabolism rate in the organism" it is noted that no experimental bioaccumulation data exist on the registered substance. The calculated BCF values range from 4.6 in the OASIS model (as provided in the Registrants' comment) to 349.8 in EPISuite (as provided in the registration database) and indicate low and moderate bioaccumulation potential, respectively. Information on concentration and properties of environmental and metabolic transformation products is missing. The available information on metabolism does not remove the concern of potential ED effects. It is considered that structurally similar metabolites may share properties of the parent substance contributing to the *in vivo* effects. One of the metabolites, 4-tert-butylbenzoic acid (PTBBA) displayed both agonistic and antagonistic activity in vitellogenin production when co-exposed to 17β-estradiol in a rainbow trout hepatocyte assay (Tollefsen et al. 2008). Based on the RCR provided by the Registrants exposure cannot be neglected (RCR close to 1 were reported).

In response to "iv" it is considered that the scope of examined toxicological endpoints in the existing *in vivo* data is not considered sufficient to detect effects from weak estrogenic substances.

The information provided by the Registrants in the comments is not sufficient to clarify the concern of potential ED effects in the environment.

b) Proposal for amendment and the Registrants' comments thereon:

A proposal for amendment (PfA) was received proposing to replace the OECD 234 test by an OECD 229 or OECD 230 study. In line with the reasoning based on consideration that OECD level 4 study may not be necessary at this step of evaluation, the following factors were listed: i) potential classification as Repro Cat 1B and STOT SE 2 may effect the use pattern and environmal exposure to be reduced to near zero; ii) lack of confidence whether the referenced *in vitro* study provides sufficient evidence to justify an OECD level 4 test.

ECHA considered the PfA and in particular the following: i) There is uncertainty with the outcome of classification for human health hazard at present, and it is unlikely that potential reclassification as Repro 1B would have the regulatory impact to reduce the environmental release and exposure to near zero; ii) However, ECHA found it possible that the screening study may, depending on the outcome and taken together with other available data, be sufficient to conclude on the concern. As there are doubts whether the OECD 229 or OECD 230 on the one hand may suffice, or whether the OECD 234 will ultimately be necessary, ECHA finds it in this case proportionate and in accordance with the OECD Fish Toxicity Testing Framework (Series on Testing and Assessment No. 171, Paris 2012) to request a screening study first. The OECD 229 and OECD 230 use a lower number of fish than the originally requested Fish Sexual Development Test (TG 234).

The OECD 229 includes in addition to the diagnostic endpoints of hormonal activity also the apical endpoint fecundity (indicating effects on reproduction). Although not endocrine specific, fecundity, due to its demonstrated sensitivity across known endocrine active substances, is an important endpoint to include because when it and other endpoints are

unaffected one is more confident that a compound is not likely endocrine active. However, when fecundity is affected it will contribute heavily in weight of evidence inferences. Therefore ECHA considered the TG 229 to be the more appropriate choice.

Specific attention should be given to the initial considerations and limitations given in the introduction of the test guideline when carrying out the test. The species used shall be the fathead minnow. The test design used for the fathead minnow, including four replicates per treatment level, should allow more power to the fecundity endpoint, compared to a test design with two replicates only. Furthermore, gonads shall be collected and preserved for optional histopathology examination depending on the outcome of the test. If either vitellogenin or secondary sex characteristics is positive the performance of gonadal histopathology may not need to be carried out.

Registrants did not support the suggestion made in the PfA to replace the initially proposed OECD 234 with the OECD 229, or OECD 230. Instead the Registrants suggested to reject the test as such. Therefore this comment does not constitute a comment within the scope of Article 51(5). Reasons brought forward by the Registrants pursuant to Article 50(1) have already been fully taken into consideration and reflected appropriately above.

c) Outcome

Therefore, pursuant to Article 46(1) of the REACH Regulation, the concerned registrants are required to carry out the following study: OECD 229 Fish Short Term Reproduction Assay using the registered substance (pure grade) subject to this decision and following the instructions as explained above.

d) Notes for consideration by the Registrants:

Further testing may be of relevance depending on the results obtained in the Fish Short Term Reproduction Assay (OECD 229). This may include a Fish Sexual Development Test (OECD 234). The evaluating MSCA will review the results submitted by the concerned registrants as an outcome of this decision and evaluate if further information needs to be requested in order to clarify the suspected endocrine disrupting properties of the registered chemical.

2. Extended one-generation reproductive toxicity study in rats, oral route (test method: OECD 443) including the examination of acetyl cholinesterase activity in different compartments in parental animals F0 and offsprings F1.

In the prenatal developmental toxicity study (gavage), an increased post implantation loss (15% as compared to 4.4 in controls), reduced fetal weight (19%) and increased incidences of skeletal variants indicating incomplete ossification were observed at the high dose level (45 mg/kg) in the presence of clear maternal toxicity (corrected bodyweight gain was 32% less than that of the controls). Effects on fetal weight (-8%) and signs of delayed ossification was also observed at the intermediate dose level in presence of only minimal maternal toxicity (only a transient decreased body weight gain between GD 6-8). In addition to effects on maternal body weights, increase in liver weights (10-20%), serum AChE inhibition (17-43%), erythrocytes AChE inhibition (9-16%) was apparent at both dose levels. Interestingly, a similar degree of decreased birth weight (19%), but in absence of clear effects on gestational maternal bodyweight gain, was also observed in the dietary one generation range finding study at a similar dose level (400ppm ~47 mg/kg, low dose in this study). Also in this study maternal serum AChE and erythrocytes AChE inhibition was decreased dose-dependently at all does levels and at the lowest dose (400 ppm) they were 49% and 13.5 %, respectively, less than in the controls. Both of these studies indicate that 2-(4-tert-butylbenzyl)propionaldehyde caused adverse effects on fetal development.

The inhibitory effect of of 2-(4-tert-butylbenzyl)propionaldehyde on plasma AChE, erythrocytes AChE and slight inhibitory effects on liver AChE were confirmed in the repeated treatment studies. No effect on the brain AChE and AChE in the erythrocytes were reported in one study investigating those endpoints however no explanation exists to why results of this study contradicts the results indicating inhibitory effects in erythrocytes AChE reported as maternal toxicity in the developmental and dose range finding studies. No effects on brain AChE activity in parental animals of the one-generation range finding study and the developmental toxicity study were reported. Evaluation of possible effects on brain AChE was hampered by methodological uncertainty (high SD). Inhibition of blood (plasma or erythrocyte) AChE may serve as an indicator for AChE inhibition in the CNS and/or PNS and indicate a potential for adverse effects on the nervous system (WHO (1990), Dorsey, L.C. (1997), Sette, W.F. (1997), JMPR (1998). However, no measurement of the effect on the AChE in the peripheral neuronal system was reported and no functional observations aiming at investigating neurotoxic effects were reported. In addition, no data on the effects on the prenatal and early postnatal organism AChE activity is available.

Furthermore in vitro assays providing mechanistic data on ED (Charles and Darbre, 2009) (level 2 of OECD conceptual framework) indicate estrogenic potential of 2-(4-tert-butylbenzyl)propionaldehyde. Specifically, the results of the competitive binding to ER from MCF7 human cells show that 2-(4-tert-butylbenzyl)propionaldehyde at 3 000 000-fold molar excess inhibit the binding of 3H-oestradiol to ER alpha and ER beta. The extent of inhibition was greater for ER alpha and varied between the type of ER alpha, namely for ER alpha from MCF7 human cells cytosol inhibition was about 47% and for human recombinant receptor ER alpha 27%. For comparison the 2 500 000-fold molecular excess of methylparaben in the same assay system resulted in 43% inhibition of 3H-oestradiol binding. Furthermore 2-(4-tert-butylbenzyl)propionaldehyde at concentration $5x10^{-4}M$ induced 1.8-fold increase in expression of oestrogen-sensitive gene CAT in test with stably transfected reporter system (ERE-CAT). Control induction with $10^{-8}M$ of 17β–oestradiol resulted in 2-fold induction. The potential of 2-(4-tert-butylbenzyl)propionaldehyde to effect the oestrogen responsive genes was further confirmed by increased expression of oestrogen-regulated gene pS2 mRNA in MCF7 cells. Treatment with 2-(4-tert-butylbenzyl)propionaldehyde had slightly smaller effect than the positive control exposure to 17β–oestradiol. In the following in vitro test 2-(4-tert-butylbenzyl)propionaldehyde increase the growth of oestrogen-dependent MCF7 human breast cancer cell, likely through the ER-mediated mechanism as the effect could be inhibited by the antioestrogen. Although growth of MCF7 cells over 7 days was lower than with the positive control exposure to 17β–oestradiol, the exposure for extended time with the tested substance lead to comparable cell density. Beside the oestrogen agonistic effects, small antagonist effect was observed in the MCF7 human breast cancer cell proliferation when cells were co-treated with 17β–oestradiol and 2-(4-tert-butylbenzyl)propionaldehyde (Charles and Darbre, 2009). However, the available information did not enable to conclude on the potential to cause ED mediated adverse effects *in vivo*.

The available information did not enable the evaluating MSCA to conclude on the potential of the substance to cause direct pre-natal developmental adverse effects and the effects in post-natal offsprings. Neither has it enabled to conclude about potential to cause neurotoxic effects in mature animals. Neither has it enabled to conclude on the potential to cause ED mediated adverse effects *in vivo*.

In order to clarify concerns regarding the potential of 2-(4-tert-butylbenzyl)propionaldehyde to cause adverse effects on the pre and postnatal development (ED and developmental neurotoxicity) and to establish a NOAEL for effects on pups weight that was not provided from the one generation dose range finding study further testing using the Extended one-generation reproductive toxicity study in rats, oral route (test method: OECD 443) is required. It is further required that the ED related observations follow the OECD 443

including Cohorts 2 and 3 and other OECD guidance as referred therein. In relation to the DNT cohort, there is no scientific reason to omit this cohort on the basis of the information on 2-(4-tertbutylbenzyl)propionaldehyde. Furthermore, inhibition of blood (plasma or erythrocyte) AChE indicate a potential for adverse effects on the nervous system. In addition to standard clinical biochemistry / haematological parameters required by OECD 443, the examination of AChE activity in different compartments including plasma, erythrocytes, brain (discrete regions or when unfeasible the whole brain), peripheral neuronal system in parental animals and offsprings (including surplus pups) is included. Surplus pups should be used to examine the activity at the early stage of postnatal development.

In relation to the DIT cohort, there is no scientific reason to omit this cohort on the basis of the information on 2-(4-tertbutylbenzyl)propionaldehyde. The susceptibility of the developing immune system to chemical disruption warrants the assessment of immune parameters in reproductive and developmental testing protocols such as OECD 443. Several papers have shown the sensitivity of immune parameters in this test design. These papers confirm the added value of immune parameters in the EOGRTS (Tonk et al. 2013; Tonk et al. 2011). Moreover, a paper suggests that estrogenic endocrine disruptors modulate the immune system in mice (Calemine et al 2003). There is an unresolved concern for an estrogenic mode of action of this substance and hence, an additional substance specific reason to include the DIT Cohort. The importance of the DIT is further substantiated by the fact that the aldehyde-structure of the compound gives reason to expect reactivity. Such reactivity could lead to immunotoxic effects. The classification as sensitizing category 1 supports this concern. Signs of hypertrophy of zona fasciculata in adrenal glands were reported in the repeated oral studies in females rats treated with 25 and 50 mg/kg bw/day 2-(4-tertbutylbenzyl)propionaldehyde.

Generation of data with the use of F 2 generation is required in this particular case to address the functional fertility and reproductive performance of the generation exposed already during prenatal development until sexual maturity, as justified by the concern related to endocrine disrupting potential together with the indication of reproductive toxicity after adult exposure.

The evaluating MSCA also notes the wide dispersive indoors and outdoors use and use profile indicating likely human exposure (including sensitive subpopulations) through the use of many products categories.

a) Registrants' comments to the Draft Decision pursuant to Article 50(1):

Following the Registrants' comments to DD, the DD has been amended for this endpoint in Section III as presented below.

The Registrants in the comments stated that they did not consider the rationale to perform the additional animal tests addressing endpoint reproductive toxicity as sufficient. The following reasoning was provided by the Registrants: i) available *in vivo* data (developmental toxicity study OECD TG 414, one-generation range finding study, repeated dose toxicity studies in different species) was considered by the Registrants to be sufficient to address the reproductive toxicity with the proposed self-classification as Repro 2 (fertility) H361f (EU 1272/2008); ii) identification of testicular toxicity, spermototoxicity and an impairment of male reproduction as leading toxicological effect based on existing *in vivo* data; iii) no effect on testosterone secretion in in vitro study on primary rat Leydig cells; iv) no evident indication of ED effects in the existing in vivo studies; v) no support for neurotoxic effects in existing data (no clinical signs indicative for neurotoxicity, no significant changes in the activity of brain cholinesterase); vi) technical aspects of performing required specific observations in the EOGRTS; vii) systemic human exposure regarded as very low.

In response to this comments further clarification of rationale and justification of performance an EOGRTS follows:

The standard data requirements for substances manufactured or imported in quantities of 1000 tonnes or more according to REACH Annex X, the production quantities range relevant for this substance includes two-generation reproductive toxicity study, with the adaptation possibility according to Annex X Collumn II. "If a substance is known to have an adverse effect on fertility, meeting the criteria for classification as toxic for reproduction category 1A or 1B: May damage fertility (H360F), and the available data are adequate to support a robust risk assessment, then no further testing for fertility will be necessary. However, testing for developmental toxicity must be considered." The self classification as Repr. Cat. 2 (based on male fertility effects) does not fulfil adaptation Column II condition. Based on the existing data classification as Repr. Cat. 1B (based on male fertility effects) should however be considered in the process of harmonised classification.

The existing data is not sufficient to conclude about developmental toxicity (including specific effects like ED and neurotoxicity) and neurotoxicity in adults, and the required EOGRTS is considered suitable and necessary for the following reasons and with following conditions:

(1) The TG 414 does not fully evaluate developmental toxicity effects. The TG 414 does not cover functional endpoint, and refers to the TG 416 for evaluation of functional endpoints. It should at this point be noted that the TG 414 dates January 22, 2001, thus, reference to the TG 443 could not have been done.

(2) The existing in vitro data Charles and Darbre (2009) indicate ED potential of the substance however is not sufficient for conclusion about *in vivo* effects. Evaluation of male and female reproduction after *in utero* exposure is missing and is considered essential. The ED cannot be excluded based on existing mechanistic study on primary Leydig rat cells and *in vivo* data. Estrogenic receptors are also expressed in the seminiferous epithelium and oestrogenic activity "appears to involve not only the classical genomic pathway, but also the rapid membrane receptor pathway and possibly non-classical nuclear ER-tethering pathways" (Carreau and Hess, 2010). Oestrogen is also important for the developing testis (Albrecht et al. 2009). The EOGRTS is the most developed method to identify effects from substances with ED properties.

(3) The existing data may suggest neurotoxic potential of the substance however is not sufficient for conclusion for this endpoint. Inhibition of AChE in plasma (30-70% after treatment with 25-50 mg/kg bw/day in 90-day study, and 17-43% after treatment with 15-45 mg/kg bw/ day for 14 days as maternal toxicity in developmental study) and AChE in erythrocytes (9-16% after treatment with 15-45 mg/kg bw/ day for 14 days as maternal toxicity in developmental study) not considered adverse by itself but indicative of potential to inhibit AChE in the neuronal systems were reported with NOEL at 5 mg/kgbw/day. No sufficient functional observation (no behavioral observation in 90-days study, solely cage side observation in 52 days study) to clarify the concern about the potential neurotoxic effects were reported. The EOGRTS entails a cohort for evaluation of neurodevelopmental (DNT) effects which addresses those data gaps. If neurotoxic effects are detected this may be the lead effect (NOEL for AChE < NOAEL fertility). In response to the Registrants' comment that the present data does not support neurotoxic potential and does not suffice to trigger for DNT according to OECD 443, it is considered that the DNT cohort (as well as DIT cohort) is the integral part of the test according to OECD 443 and no trigger is required. Conditions of possible omission were not fulfilled. For the description of justification on DNT cohort (and DIT cohort), please see above.

(4) Generation of data with the use of F 2 generation is required in this particular case to address the functional fertility and reproductive performance of the generation exposed already during development as justified by the concern related to endocrine

disrupting potential together with the indication of reproductive toxicity after adult exposure and the wide dispersive indoors and outdoors use and use profile indicating likely human exposure through the use of many products categories. No agreed criteria exist on how the systemic human exposure, regarded by the Registrant as very low, should be weight in. Based on the RCR provided by the Registrants exposure can not be neglected (RCR close to 1 were reported).

(5) There are some factors that have to be considered in the study design including:

- the fact that the substance is toxic to testis and gives maternal toxicity requires special attention in the selection of doses (number of and intervals between), which should ensure that a high enough number of pups are produced while still allowing reproductive effects to be identified;
- technical aspects to include additional AChE measurements in the Cohort 2A and 2B;
- protocol for assessment of AChE in PNS in the set of suitable peripheral tissues supported by the peer-review literature; e.g. analysis of AChE activity in peripheral tissues including diaphragm, skeletal muscle, and heart required four times higher concentration of the homogenate in comparison with brain samples homogenates in the method by Shih TM et al. 2005, Shih TM et al. 2010. The colorimetric detection method used in those studies, based on modified method by Ellman et al., 1961, required small volume samples (7microL). For further information see also US EPA 2000.
- protocol for assessment of AChE activity in the brain regions supported by the peer review literature together with the considerations of specific technical feasibility that could if justified lead to modification of requested test conditions. This could for instance include examination of the whole brain instead of discrete brain regions in cases where the expected amount of tissue does not suffice for analysis.

b) Proposal for amendment and the Registrants' comments thereon:

A proposal for amendment (PfA) was received proposing to reject the OECD 443 on the basis that sufficient information is available to enable hazard identification and risk management of this substance and that the conduct of the proposed EOGRTS would appear to be unnecessary.

In response ECHA considered that the wording of the PfA allowed it to be interpreted as being based on the premise that classification as Repr. Cat 1B (fertility) (acc. to regulation (EU) 1272/2008) is warranted based on already existing data. As already noted above, the substance is currently not classified as Repr. Cat. 1B. Furthermore, ECHA notes that although this would be a standard information requirement at the tonnage level of Registrants, no data for Annex X, 8.7.3. of the REACH Regulation and no justified adaptation is included in the registration dossiers for the endpoint. Specifically, it notes that the conditions of the adaptation according to Annex X, Column 2 are not fulfilled. Furthermore if the PfA reasoning is interpreted to refer to a weight of evidence approach, it is considered that the Registrants did not fulfill standard data requirement with the indicated weight of evidence (Annex XI, Section 1.2). No other data was available to the evaluating MSCA which would have added sufficient weight of evidence to the data available in the dossiers. The scope of presented toxicological endpoints does not cover functional and other postnatal developmental effects and it cannot be concluded if the substance has or has not a particular dangerous property for those endpoints. Furthermore Registrants did not account in a current risk assessment and management for the uncertainty of not investigating of above indicated property.

It is noted that the requested OECD 443 is required to investigate ED properties as explained above. Furthermore, the classification as Repro Cat 1B would not prevent the

substance to be used at low concentrations in consumer products and therefore may not remove the concern for potential ED mediated effects. This PfA has been therefore rejected.

To support the PfA the Registrants provided exposure arguments that could contribute to a weight of evidence argument. Specifically they referred to systemic human exposure, relevant routes of exposure, reported RCR, concentration in consumer products. With regards to this further argument ECHA notes that claims made in the comments are not supported by the information provided in the registrations or any other data available to evaluating MSCA and therefore cannot contribute to the weight of evidence argument.

In response to further Registrants' comments seeking to support this PfA ECHA refers to the reasons why the PfA was not accepted.

A further PfA was received proposing to reject the OECD 443 and request test OECD 426 with inclusion of specific investigations for cholinesterase inhibition. In response ECHA considered that the test proposed in the PfA has the benefit to more comprehensivly address the developmental neurotoxicity endpoint in comparison to DNT cohorts in the OECD 443, however does not investigate other potentially relevant reprotoxicity endpoints. In this context, the OECD 443 test has the advantage to investigate potential of ED, neurotoxic, immunotoxic effects leading to reprotoxicity. It is therefore requested as a first choice study. This PfA has been therefore rejected.

Further PfAs were received proposing inclusion of DIT cohort according to OECD 443. In response, ECHA considered this proposal justified and amended the decision accordingly.

In respect to the test design referring to F2 according to OECD 443, PfAs were received proposing omission of the F2, and other PfAs proposing amending of justification. In response the evaluating MSCA/ECHA considered inclusion of F2 justified in this case and the justification was amended taking into account the PfAs.

Registrants did not support PfAs proposing testing. ECHA refers to the above presented responses to PfAs.

While considering the PfAs, ECHA noted the following:

In response to the Registrants' comment referring to the practicability of the specific observations of AChE activity in the brain regions and PNS, it is acknowledged that this part of the request is outside of the scope of the standard protocols. A specific response is provided in the section concerning study design recommendations (point 5).

The Registrants are informed that the Registrants' comments to the draft decision have been considered by the evaluating MSCA and reflected above (see III.2.a). The MSC discussion reflected the PfAs and Registrants' comments on the PfAs.

Therefore, pursuant to Article 46(1) of the REACH Regulation, the concerned registrants are required to carry out the following study: Extended one-generation reproductive toxicity study in rats, oral route (test method: OECD 443) using the registered substance (pure grade) subject to this decision and following the instructions as explained above.

c) Notes for consideration by the Registrants:

Further testing may be of relevance to address the mode of action depending on the results obtained in the requested Extended One Generation Reproductive Toxicity Study (OECD 443). This may include testing for e.g.: Anti-androgenicity (OECD 441), Oestrogenicity (OECD 440) and/or in vivo study examining level of testosteron in the foetuses (modified OECD 414). Moreover a TG 456 (H295R Steroidogenesis Assay) may be relevant. The evaluating MSCA will review the results submitted by the concerned registrants as an outcome of this decision and evaluate if further information needs to be requested in order to clarify the suspected endocrine disrupting properties of the registered chemical.

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.

Two different grades, one with the compositions of [redacted] (referred to as the pure grade in the present decision) and another with the composition of [redacted], are registered. The information required by the present decision shall be generated using the pure grade of the registered substance. 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 of both [redacted]. 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 registrant(s) 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 report(s).

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 for the generation of information and Good Laboratory Practice

ECHA 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).

According to Article 13(3) of the REACH Regulation, tests that are required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the European Chemicals Agency as being appropriate. Thus, the Registrant shall refer to Commission Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 as

adapted to technical progress or to other international test methods recognised as being appropriate and use the applicable test methods to generate the information on the endpoints indicated above.

VII. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52 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://echa.europa.eu/appeals/app_procedure_en.asp. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

[redacted]

Jukka Malm
Deputy Executive Director

Annexes: 1. References
2. 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 1. References

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