Helsinki, 11 December 2023

Addressees
Registrant(s) of the Registered substance subject to this decision, listed in the last Appendix of this decision

Registered substance subject to this decision ("the Substance")
Substance name: Reaction Mass of 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one and 1-(1,2,3,4,6,7,8,8a-octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one and 1-(1,2,3,5,6,7,8,8a-octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (OTNE)

List number: 915-730-3

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

DECISION ON SUBSTANCE EVALUATION

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

A. Information required to clarify the potential risk related to Mutagenicity

1. ***In vivo* mammalian alkaline comet assay (test method: OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in mice, oral route, with the Substance.**
   - For the comet assay the following tissues must be analysed: liver, glandular stomach and duodenum.

B. Information required to clarify the potential risk related to PBT/vPvB

1. **Simulation testing on ultimate degradation in surface water (test method: EU C.25./OECD TG 309 - aerobic mineralisation in surface water-simulation biodegradation test), with the Substance, specified as follows:**
   - a pelagic test using EU representative surface water with a suspended particulate matter (SPM) concentration of approximately 15 mg dw/L must be conducted;
   - two concentrations must be tested and must not exceed the solubility limit of the Substance in the test medium;
   - the test material must be ^{14}C-radiolabelled with the radiolabel located in the most stable part of the molecule;
   - the test must be conducted with:
     - water sources taken from site without a history of agricultural, industrial or domestic inputs;
     - at an environmentally relevant test temperature of 12°C;
     - with sterile controls;
     - a reference control;

- the concentration of the test substance must be measured at appropriate intervals;
- the transformation and/or degradation products must be identified and quantified at every sampling time; at a concentration of ≥ 10% w/w, unless reasonably justified otherwise and at continuously increasing concentrations, if technically feasible;
- the test duration must be 60 days; if a longer duration is needed a semi-continuous procedure must be used;
- the total amount of non-extractable residues (NER) must be quantified and a scientific justification of the extraction procedures and solvents used must be provided, if technically possible.

For the above requests you must submit a full study report, as further specified in Appendices A (2.1.b) and B (2.1.b), respectively.

Deadlines
The information must be submitted by ***18 March 2027***.

Conditions to comply with the information requested

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

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

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

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

Appeal

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

Failure to comply

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

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment.

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

Basis for substance evaluation

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

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

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

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

ECHA
EUROPEAN CHEMICALS AGENCY

Appendix A – Reasons to request information to clarify the potential risk related to Mutagenicity

1. Potential risk

1.1 Potential hazard of the Substance

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

a) Potential mutagenicity

The available information shows that the Substance may have mutagenic properties.

Evidence based on in vitro data

- The Substance was not mutagenic in different Ames tests (Unpublished report, 1997a; RIFM, 1997b; NTP, 2016).
- The Substance exhibited negative results in a gene mutation assay in mouse lymphoma L5178Y cells (MLA TK) according to OECD TG 476 (1997). (Unpublished report, 2010).
- The Substance was tested in a mammalian chromosome aberration test (OECD TG 473, 1983) (Unpublished report, 1997b). Human lymphocytes cells were exposed to the Substance in the presence of metabolic activation for 3 hours followed by an incubation for a further 15 hours (after centrifugation and resuspension) or in the absence of metabolic activation for 18 hours. There was no short term treatment without metabolic activation, which was later recommended in the updated OECD TG 473 (2016). An additional sampling time at 32 hours was also performed with only one concentration: 75 µg/ml with metabolic activation and 15 µg/ml without metabolic activation.
  First experiment: with sampling time at 18 hours with metabolic activation, although not statistically significant, there were 4% of aberrant cells, gap damage excluded (versus 1.25% in the control group) at the highest concentration of 125 µg/ml, associated with a relative mitotic index (MI) of 40%. Also, there was no effect without metabolic activation.
  Second experiment: with sampling time at 18 hours in the absence of metabolic activation, there was a statistically significant increase in the number of aberrant cells, gap damage excluded (4% versus 0.75% in the control group) at the highest concentration of 30 µg/ml, associated with a relative MI of 58%. The study report indicates no dose-response relationship and that the value was inside the range of historical control data (HCD) (0-5.25%). However, very few details are available from the HCD (e.g. no information on distribution of the values, number of experiments). In addition, the mean value reported for these HCD (1.02%) is relatively low in regard to the minimum and maximum values. There was no effect with metabolic activation.
  Extra sampling time at 32 hours: No effect was reported in the experiment; only one concentration, inducing no or low cytotoxicity, was tested with metabolic activation (relative MI = 110%) and without metabolic activation (relative MI = 73%).

Based on the original results (Unpublished report, 1997b), and considering the criteria set in the current OECD TG 473 (2016), the outcome of the test is considered equivocal (i.e. statistically significant effect reported only at the highest tested dose in one experiment). Some shortcomings may have impacted the sensitivity of the overall results, in particular only 200 cells per replicate were analysed (which was the standard of the OECD TG 473 from 1997, instead of 300 recommended in the 2016 update) and

the choice of the top concentration analysed is questionable in some experiments (in particular the relative MI was above 50% without sign of precipitation in the experiments without metabolic activation). These shortcomings suggest that the test may not be sensitive enough to adequately identify mutagenicity.

In your comments on the draft decision, you agree that the mammalian chromosome aberration test is not fully compliant with the new OECD TG 473 and you agree with the shortcomings identified. However, you consider that the biological relevance of the significant increase observed is insufficient to justify repeating the test based on the following argumentations: the statistically significant result was only observed in one experiment, without dose-response relationship and within HCD, there was a steep dose-cytotoxicity relation and differences in chromosomal aberrations in the DMSO vehicle control with and without metabolic activation. Overall, you conclude that the methodology is somewhat less reliable, but sufficient for evaluation and that the test is negative.

ECHA agrees that the reported shortcomings are not sufficient, in themselves, to consider the mammalian chromosome aberration test as unreliable. However, ECHA disagrees with your conclusion that this test is negative. Since there was a statistically significant increase in the number of aberrant cells in the second experiment without metabolic activation, the result cannot be considered as "*clearly negative*" in regard to the criteria set in the OECD TG 473 (2016). Indeed, there was at least one of the test concentrations exhibiting a statistically significant increase compared with the concurrent negative control but the increase was not dose-related. Regarding HCD, as detailed above, very few details are available (e.g. no information on distribution of the values, number of experiments) preventing any independent interpretation. According to OECD TG 473 (2016): "*In case the response is neither clearly negative nor clearly positive as described above or in order to assist in establishing the biological relevance of a result, the data should be evaluated by expert judgement and/or further investigations*". In the absence of further information to clarify the biological relevance of the result, the outcome of the test is judged equivocal.

Evidence based on in vivo data

In a NTP dermal study similar to OECD TG 411 (NTP, 2016), the Substance was applied dermally without occlusion in F344/NTac rats and B6C3F1 mice at 6.25, 12.5, 25, 50 or 100% five days per week for 90 days, combined with a micronucleus analysis at the end of the study (equivalent to 31.25 to 500 mg/kg bw/day in rats and 125 to 2000 mg/kg bw/day in mice) (NTP, 2016). Blood from rats and mice (5/sex) was collected at the end of the study for micronucleus analysis. The effect of the 3-month exposure on micronucleus frequency and on erythropoiesis was examined in two experiments: a "multi-dose" experiment (doses from 6.25% to 50% OTNE compared to 95% ethanol vehicle control) and a "single-dose" experiment (comparison of 100% OTNE to an untreated control).

The NTP concluded to negative results in male and female rats. In particular, in the "multi-dose" test in male rats, the mean frequency of micronucleated polychromatic reticulocytes was statistically significantly ($p < 0.025$) elevated over the control in the two highest dose groups (25 and 50% OTNE). However, the magnitude of the increases was very small (lower than 2 times the control value) and the trend test was not significant. The response measured in the group that received 100% OTNE in the "single-dose" experiment was also lower than either of these two values.

The NTP reported equivocal results in male mice and positive results in female mice:

(i) in the 100% male mice group, statistically significant increases in the frequency of micronucleated normochromatic erythrocytes was observed ($p = 0.001$);
(ii) in female mice, there was a dose-dependent increase in micronucleated mature erythrocytes in the "multi-dose" experiment that reach statistical significance at the highest dose tested ($p < 0.001$ in the 50% tested animals) and there was a significant increase also in the "single-dose" experiment with 100% OTNE ($p < 0.001$). Micronucleated reticulocytes were also significantly increased ($p = 0.005$) in female mice exposed to 100% OTNE in the "single-dose" experiment.
(iii) Although all these differences were statistically significant, the level of the increases remained very small compared to vehicle or untreated controls (lower than 2 times the control values).

In your comments on the draft decision, you agree that the *in vivo* findings are difficult to evaluate but you consider the NTP test as unreliable based on the following arguments:

a) the increase, although reaching statistical significance, was very small and could be caused by the massive local tissues damage and the related stress of the animals, which was more pronounced in mice than in rats;

b) neither the historical control data nor positive control data were provided in the report;

c) the dermal exposure route in this test is not a route according to OECD TG 474 and the systemic exposure is unclear;

d) the highest dose is beyond the highest dose requirement of the OECD TG 474 when the exposure is more than 14 days (OECD TG 474: 33), and severe liver weight increase is seen indicating overloading of the metabolic pathway (OECD TG 474: 32) and therefore the results at 1000 mg/kg bw are not considered biologically relevant.

ECHA agrees that the study presents some methodological shortcomings compared to the OECD TG 474. However, ECHA disagrees that the study is unreliable:

a) As described above, ECHA also notes that the increases were very small but statistically significance was reached for different experiments (in male and female mice, in the "multi-dose" and/or "single-dose" experiment, at 50% or 100%, for reticulocytes or erythrocytes). The fact that the increase was not an isolated observation raises a particular concern. Additionally, currently there is no indication of a link existing between an increase of micronucleus in the blood (which is considered as a systemic effect) and local tissues damages. In particular the low systemic toxicity reported in animals in this study does not suggest a related stress. Moreover, the hypothesis was not further scientifically justified in your comment.

b) ECHA also notes the absence of positive control and historical control data to ensure laboratory proficiency. The study was performed by the NTP which has several years of experience in conducting these types of studies. Moreover, the result should be first analysed in the light of the concurrent control and significant effects were observed. The magnitude of the effects were rather small and mainly observed at the highest tested concentration. In absence of positive control and historical negative control data, ECHA agrees that the biological significance of the observed effect is unknown.

c) According to OECD guideline 474: "*The anticipated route of human exposure should be considered when designing an assay*." Thus, the oral route is not particularly

recommended. OTNE has widespread uses, among others, the Substance is used in fragrances and cosmetics or personal care products, and so dermal exposure is anticipated. You consider that systemic exposure is unclear. However, toxicokinetics data are available with OTNE showing dermal absorption, thus a systemic exposure is confirmed which in addition does not induce an excessive toxicity. The use of the dermal route is therefore considered as appropriate and relevant.

d) ECHA agrees that OECD TG 474 recommended that "*if the test chemical does not produce toxicity in a range-finding study or based on existing data, the highest dose for an administration period of 14 days or more should be 1000 mg/kg body weight/day*". This recommendation generally refers to an exposure by oral route. Systemic exposure is anticipated to be lower after dermal route in comparison with gavage and may justify the selection of higher doses by dermal route. This is confirmed by the NTP study reporting that OTNE is better absorbed after oral route than dermal administration (even if sites were uncovered) (NTP, 2016). Moreover, even if most of the statistically increases were observed at the top dose of 100 % (equivalent to 2000 mg/kg bw/d), there was also a dose-dependent increase in micronucleated mature erythrocytes in the "multi-dose" experiment that reach statistical significance at the dose of 50% in female mice (equivalent to 1000 mg/kg bw/d). ECHA finally notes that systemic toxicity of the Substance in this assay is rather low in this study. In female mice for which the NTP concluded to a positive mutagenicity result, there were some effects reported in particular in haematology parameters and in organ weight. There were no histopathological findings in the examined organs. Regarding specifically your comment on liver toxicity and metabolic overload, ECHA recognised that liver weight was increased (up to 89% in females) and associated with an increase in Cyp2e1 activity. However, despite the higher liver weight, the increase of Cyp2e1 is minimal (less than two-fold and not considered biologically relevant by the NTP) and there is no histopathological change in the liver of female mice. Thus, despite systemic exposure, the MTD is not exceeded as recommended in the guideline.

Overall, even if the methodology is somewhat less reliable, it is sufficient for raising a concern for the mutagenicity of the Substance since statistically significant increases in the frequency of micronucleated erythrocytes were reported in the different experiments.

Conclusion

The Substance induces an equivocal clastogenic response in an *in vitro* mammalian chromosome aberration test.

In an *in vivo* micronucleus test performed at the end of a 3-month study by dermal route in mice, equivocal results in male mice and positive results in female mice were reported by the NTP. However, in the absence of positive control and historical negative control data, the biological significance of the observed statistically significant effect is unknown. No firm conclusion on the mutagenic potential of the Substance can be reached but data raise a concern based on *in vivo* data in mice.

The available and current information is not sufficient to draw a conclusion on the hazard. Further information is needed to clarify the mutagenicity concern.

1.2 Potential exposure

According to the information you submitted in all registration dossiers, the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of 1 000 to 10 000 tonnes per year.

According to information in the registration dossiers the Substance is used in particular:

- by industrial workers in manufacture, formulation or re-packing and at industrial site in washing and cleaning products, perfumes and fragrances;
- by professionals in washing and cleaning products, polishes and waxes;
- by consumers in washing and cleaning products, biocides (e.g. disinfectants, pest control products), air care products, polishes and waxes, perfumes and fragrances and cosmetics and personal care products.

Therefore exposure to workers and consumers cannot be excluded.

1.3 Identification of the potential risk to be clarified

Based on all information available in the registration dossiers and information from the published literature, the Substance may cause genotoxic/mutagenic effects on somatic and/or germ cells.

The information you provided on manufacture and uses demonstrates that exposure of workers and consumers exists.

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

As explained in Section 1.1 above, the available information is not sufficient to conclude on hazard, and consequently, further data is needed to clarify the potential risk caused by the Substance.

1.4 Further risk management measures

If the mutagenicity of the Substance is confirmed, the evaluating MSCA will analyse the options to manage the risk(s).

In particular, as a further regulatory risk management measure, it may result in the harmonisation of the classification as germ cell mutagen as defined in the CLP Regulation. This would result in stricter risk management measures, such as improved measures at manufacturing sites, better waste management and revised instructions on safe use, if appropriate.

If classified as germ cell mutagen, the evaluating MSCA will also assess whether the Substance should be proposed for identification as a substance of very high concern (SVHC) under Article 57 of REACH, which would lead to stricter risk management measures than those currently in place.

2. How to clarify the potential risk

2.1 *In* vivo mammalian alkaline comet assay (test method: OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in mice, oral route, with the Substance.

a) Aim of the study

An *in vivo* micronucleus (MN) test combined with a comet assay will clarify the *in vivo* mutagenicity of the Substance.

Concerns are raised for chromosomal aberrations, from both *in vitro* and *in vivo* assays. However, the limitations associated with these tests do not allow to make a final conclusion on the genotoxicity of the Substance.

As the concerns only refer to chromosomal aberrations, the adequate follow-up could be a micronucleus (MN) test only. However, as one limitation of this test is that it only investigates the bone marrow, combination with the *in vivo* comet assay is appropriate to detect effects in both distant organs, such as the bone marrow or the liver, and at site(s) of contact, such as the glandular stomach and the duodenum (for the oral route of administration). Investigating several genotoxic endpoints and different tissues in a combined study is necessary to generate complementary information, provide a comprehensive overview of the genotoxic potential of a substance and allow to draw a conclusion on the mutagenic potential of the Substance.

Since the information request is based on a potential risk posed by the Substance, the request is necessary under the current substance evaluation.

In your comments on the draft decision, you consider the current OECD TG 473 test negative but you agree that the test does not comply with the most recent version of the TG. Moreover, you consider the current *in vivo* micronucleus test unreliable. You propose to perform an *in vitro* micronucleus test first before performing an *in vivo* mutagenicity test. You consider this stepwise approach fully in line with the REACH text and animal welfare provisions as well as with the most recent update on ECHA's guideline on mutagenicity testing.

ECHA considers that further investigation is mandatory since it cannot be concluded that the Substance demonstrates a clear negative result for Mutagenicity from the available studies. Despite methodological shortcomings, increases in chromosomal aberrations are observed both *in vitro* and *in vivo*, in different experiments and different studies. This thus raises a concern that needs to be clarified. In particular, it should be highlighted that the NTP, which is a well-recognised body, considered that OTNE is negative in rats, equivocal in male mice and positive in female mice in the MN assay. Overall, they concluded that "*this is the first study to suggest that repeated dermal application of OTNE may have genotoxic effects*". The fact that the effects occurred at high dose or at a low magnitude should not be used to totally dismiss the concern, since these considerations are not part of the criteria set in the OECD guideline to conclude on a clear negative result.

According to ECHA guidance R7a: "*The second in vivo test should only be performed if this test is required to make a conclusion on the genotoxicity of the substance under investigation*". ECHA considers that an *in vitro* assay would not be sufficient to firmly conclude on the mutagenicity of the Substance:

- if a new *in vitro* MN assay reveals a negative outcome, an *in vivo* testing will be required in any case as follow up since it will not be sufficient to dismiss the concern

raised by the *in vivo* micronucleus assay in addition to equivocal *in vitro* assay.
- even if a new *in vitro* MN would be positive, a follow up with *in vivo* testing would be required to be able to conclude on classification.
- The CLP criteria require a relevant *in vivo* positive result for a definitive classification. *In vitro* effects only are not sufficient to classify even for a classification as Muta. Cat. 2.

Hence, whatever the result of the proposed new *in vitro* test, an *in vivo* test will be needed as a follow-up in any case to clarify the concern. Therefore, as the least burdensome measure, it is more appropriate to request the *in vivo* testing in the first place to clarify the concern.

Moreover, in your comment, you consider that, as there is no concern for mutagenicity, a comet assay does not need to be performed. You add that, in case an *in vivo* mutagenicity test is requested, an *in vivo* micronucleus test in mice would be (according to ECHA guidance document Chapter 7a) the appropriate test.

ECHA reminds you that a comet assay detects primary DNA damage that may lead to structural chromosomal aberrations. In particular, and contrary to the MN assay, the comet assay is appropriate to investigate potential effects in site-of-contact tissues in particular clastogenic effects in this case. Therefore, the combined study provides a comprehensive overview of the genotoxicity of the Substance and can help reduce the number of tests performed and the number of animals used. Finally, the required protocol of a combined assay is in line with the approach agreed at the Member State Committee Meeting (MSC-78; June 2022) as an appropriate follow-up to investigate *in vivo* genotoxicity of potential clastogenic substances[2].

b) Specification of the requested study

Test material and concentration

Adequate doses of the Substance must be used to ensure a reliable interpretation of the results. Therefore, the choice of test concentrations must follow the recommendations set in the OECD TG 489 and 474[3] and the results from the existing micronucleus test performed with the Substance (NTP, 2016). Based on these data, a dose of 2000 mg/kg bw/day must be included, unless you can justify that this dose produces excessive toxicity in a range-finding study.

Route of exposure

Having considered the anticipated routes of human exposure and the need for adequate exposure of the target tissue(s), performance of the test by the oral route is appropriate.

Test species

The combined test (OECD TG 489 and OECD TG 474) must be performed in mice.

According to OECD TG 489, "*Rats are routinely used in this test. However, other species can be used if ethically and scientifically justified*". According to OECD TG 474: '*Mice, rats, or another appropriate mammalian species may be used*'. Considering the results of the NTP study (NTP, 2016), mice seem to be more sensitive to genotoxicity of the

[2] https://echa.europa.eu/documents/10162/11995235/minutes_msc-78_en.pdf/59402764-cb18-9ca8-1478-a1b511457d84?t=1656311969202

[3] "*If the test chemical does not produce toxicity in a range-finding study or based on existing data, the highest dose […] for administration periods of less than 14 days, [should be] 2000 mg/kg/body weight/day.*"

Substance. In this context, the required combined study must be performed in mice.

In your comments on the draft decision, you consider that the conclusion that mice are more sensitive than rats is flawed for the following reasons:

- "*Reaching statistical differences in one and not in the other species does not allow such a conclusion as the HCD data and thus the biological variation is not known, and the increase was very small in any case.*
- *Much more severe skin damage was noted in mice than in rats and the data at hand does not allow to assess the consequences of this.*
- *In the study ECHA refers to, the substance was administered repeatedly to the skin whereas the requested study requires the administration via the oral route. Thus, kinetics is not comparable and a prediction which species is more sensitive via oral administration is comprised*".

You also consider that without HCD for mice, the interpretation of the NTP findings is compromised and thus it does not justify to perform the assay in mice.

Finally, concerning the comet part of the request, you state that for the study design, Bowen *et al.* (2011) refers to rats and not mice. After consultation with several CROs, you state that there are no appropriate historical control data available for mice. Thus, you consider that a micronucleus test has to be performed using mice and the comet assay has to be performed using rats.

First of all, ECHA reminds that OECD TG 474 and/or 489 do not exclude the possibility to perform the tests in mice. In particular, according to the OECD TG 489, "*the choice of rodent species should be based on (i) species used in other toxicity studies (to be able to correlate data and to allow integrated studies), (ii) species that developed tumours in a carcinogenicity study (when investigating the mechanism of carcinogenesis), or (iii) species with the most relevant metabolism for humans, if known*". ECHA notes that there is no carcinogenicity study with the Substance and it is unknown if rats are more relevant for humans than mice in regards to metabolism. ECHA acknowledges that, at a same dose level in the NTP study, mice were exposed to higher internal concentrations of the Substance than rats. However, concern from the *in vivo* micronucleus assay was raised from experiments in mice. In addition, as detailed in section "aim of the study" of this decision, even if HCD is not available, the NTP has several years of experience in conducting these types of studies that ensures confidence in its interpretation of the result. Moreover, a result should be first analysed in the light of the concurrent control, and not the HCD which are useful to identify aberrant values but not to dismiss effects. Thus this result justifies that the study is required in this species. Finally, the lack of HCD cannot be used as a reason for not conducting a state of the art technique.

Somatic cells

In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Test design

The combination of the OECD TG 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al*. 2011).

In your comment, you consider the performance of an *in vivo* combined micronucleus/comet assay not justified. You consider that in case the evaluating MSCA/ECHA would request an *in vivo* mutagenicity test, an *in vivo* micronucleus test in male mice would be (according to ECHA guidance document Chapter 7a) the appropriate test. Indeed, you consider that the rationale for requesting the comet part is flawed as there are no indication for any mutagenic effect in vitro.

Based on the argumentations already set in section "aim of the study" of this decision, the combined assay is judged appropriate. In particular, ECHA reminds you that comet assay is not dedicated to identify gene mutations only but also chromosomal aberrations secondary to DNA damages and allow detection of effects at the site of contact. The required protocol of a combined assay is in line with the approach agreed at the Member State Committee Meeting (MSC-78; June 2022)[4].

Germ cells

You may consider to collect the male gonadal cells from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimize the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analyzing the slides prepared with gonadal cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

In your comments on the draft decision, you consider that a comet assay cannot be used to measure DNA strand breaks in mature germ cells, with reference to OECD TG 489 and ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a. To be able to do that, protocol modifications together with improved standardization and validation studies are needed as well as a longer exposure period. Furthermore, gonads contain a mixture of somatic and germ cells and for that reason, positive results in whole gonad (testis) are not necessarily reflective of germ cell damage and will only indicate that the Substance has reached the gonads when DNA strand breaks are observed. To study the effect on germ cells a new suitable test must be performed. Overall, the *in vivo* mammalian alkaline comet assay cannot be used for this purpose.

Considering your comments, the decision has been modified to include the collection and analysis of germ cells as a recommendation and not as a mandatory requirement, in order to be consistent with previous other ECHA decisions regarding this point. Such analysis is indeed not included yet in the validated guideline although the feasibility of the analysis has been demonstrated in the literature (e.g. Dirven *et al.*, 2023) and revision of the OECD TG 489 in this purpose is included in the OECD work plan[5].

[4] https://echa.europa.eu/documents/10162/11995235/minutes_msc-78_en.pdf/59402764-cb18-9ca8-1478-a1b511457d84?t=1656311969202

[5] OECD. Work plan for the Test Guidelines Programme (TGP). As of June 2022 https://www.oecd.org/chemicalsafety/testing/work-plan-test-guidelines.pdf

Request for the full study report

You must submit the full study report which includes:

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

This will enable the evaluating MSCA to fully and independently assess all the information provided, including the statistical analysis, and to efficiently clarify the potential hazard for the Mutagenicity for the Substance.

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

The request for a combined *in vivo* mammalian erythrocyte micronucleus test (OECD TG 474) and *in vivo* mammalian alkaline comet assay test (OECD TG 489) is:

- appropriate, because it will provide information which can clarify the suspected mutagenicity *in vivo*. The *in vivo* micronucleus test is the appropriate follow-up considering the concern raised both *in vitro* and *in vivo*, i.e. clastogenicity. However, in this protocol, only bone marrow is examined. The *in vivo* mammalian alkaline comet assay would also allow to identify chromosomal aberrations. Adding a comet assay to a MN assay will allow to assess many tissues including "site of contact" tissues (which avoids the uncertainty of exposure of the bone marrow to the parent compound if it would be tested only in an OECD TG 474 micronucleus test). This will enable the evaluating MSCA to conclude on possible classification for mutagenicity of the Substance (see section "aim of the study" for justification of the combined protocol).
- helping to reduce the number of tests performed and the number of animals used.

More explicitly, there is no equally suitable alternative way available for obtaining the information that would clarify the potential mutagenicity concern raised in *in vivo* test.

In vitro assay would not be sufficient to firmly conclude on the mutagenicity of the Substance. Indeed, in case of negative results, there will remain uncertainty based on the *in vitro* chromosomal aberration test and the *in vivo* NTP studies when taken as a weight of evidence. In case of positive result, an *in vivo* test would be required. For efficiency reasons and as the least burdensome measure, it is more appropriate to request the *in vivo* testing in the first instance to clarify the concern (see section "aim of the study" for justification of *in vivo* testing).

2.2. References relevant to the requests (which are not included in the registration dossier)

Bowen D.E. *et al*, 2011. Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. Mutation Research 722 7–19

Dirven *et al.,* 2023. Assessing testicular germ cell DNA damage in the comet assay; introduction of a proof-of-concept. Environ Mol Mutagen. 2023 Feb;64(2):88-104. doi: 10.1002/em.22527.

NTP, 2016. NTP Technical Report on the Toxicity Studies of Octahydro-tetramethyl-naphthalenyl-ethanone (OTNE) Administered Dermally to F344/NTac Rats and B6C3F1/N Mice. ISSN: 2378-8992

RIFM, Research Institute for Fragrance Materials, Inc., 1997b. Bacterial Mutation Assay with Iso E Super. Unpublished Report from IFF Incorporated, 8 October. Report Number 31711. (In: Scognamiglio J., Letizia C.S., Politano V.T., Api A.M., 2013. Fragrance material review on 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone (OTNE). Food and Chemical Tox. 62, Suppl. 1: S120-S132)

Appendix B – Reasons to request information to clarify the potential risk related to PBT/vPvB properties

1. Potential risk

1.1 Potential hazard of the Substance

Following its assessment of the available relevant information on the Substance, the evaluating MSCA identified the following potential hazard which must be clarified: Potential PBT properties (as defined in Annex XIII to REACH) of relevant transformation/degradation products of the Substance. Considering the available information, the Substance itself does not fulfil the bioaccumulation criteria (B/vB) in accordance with Annex XIII of REACH.

a) P/vP properties

If a substance fulfils the criteria in Section 1.1.1 or 1.2.1 of Annex XIII to REACH, it is considered that it has persistent (P) or very persistent (vP) properties.

For the purpose of the P/vP assessment and to check whether the criteria are fulfilled, the information listed in Section 3.2.1 to Annex XIII, including results from simulation tests, must be considered using a weight of evidence approach. If no such data are available, it is necessary to consider the screening information of Section 3.1.1 to Annex XIII, such as QSAR predictions. Annex XIII to REACH provides that the identification of PBT/vPvB substances must also take account of the PBT/vPvB properties of relevant transformation and/or degradation products.

(i) For the parent Substance:

Evidence based on modelling data on the parent Substance

The evaluating MSCA used the prediction models US EPA Biowin (version 4.1) 2, 3 and 6 for every main constituent of the Substance. The information indicates that main constituents are not readily biodegradable.

The prediction models indicate that the Substance is potentially P or vP, according to screening criteria specified in the ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.11: PBT/vPvB assessment.

Evidence based on experimental data on the parent Substance

As explained below, available studies on the Substance highlight the formation of transformation/degradation products which may be relevant for the PBT assessment.

Four screening experimental studies are available in the registration dossier. Two studies were conducted according TG OECD 301F and the others according to OECD TG 301C and TG OECD 302C, respectively. The results for the OECD 301C and 302C show neither ready biodegradation nor inherent biodegradation of the Substance. The OECD TG 301F studies show contradictory results: based on O_2 consumption, 2% of degradation after 28 days is observed in one study (Unpublished report, 1991) and 96.3% of degradation is observed after 28 days in the other study (Unpublished report, 2021). The contradictory results could be explained by the different origins of inoculum employed in the studies or the difference in the concentrations tested (18 mg/L in the second study and 100 mg/L in the first study). This is further discussed below under "*Potential persistence of the transformation/degradation products".*

The studies performed according to TG OECD 301F, OECD TG 301C and TG OECD 302C do not provide any information on the identity or potential PBT/vPvB properties of transformation/degradation products of the Substance.

No water, soil or water-sediment simulation test according to OECD standard test guidelines is currently available in the registration dossiers for the Substance. Nonetheless, a study (Unpublished report, 1999) is available in the registration dossier that investigates the fate of ^{14}C-radiolabelled Substance in three different environmental samples: a river sediment, a sludge amended soil (Soil 1) and an agricultural soil (Soil 2). The study was conducted according to the technical documents from the U.S. Food and Drug Administration for evaluating aerobic biodegradation of organic chemicals in soils (Environmental Assessment Technical Assistance Document 3.12, 1987) and is not under GLP compliance. The mass balance analysis provides the quantification of the parent substance and of CO_2 production on week 3, 6 and 12 in contrast to requirements in the OECD TG 308 and 307 (at least five-six sampling times included sampling at time zero). In addition, the number and types of samples tested are not according to the recommendations in the OECD TG OECD 308 and 307 (two and four different samples respectively).

- River sediment sample: based on nominal concentrations, 10%, 2.3% and 0.53% of parent substance remained in the microcosm at each time of analysis, respectively. The mass balance presents the distribution of the total radioactivity as CO_2 and for the different extractions (aqueous fractions, methanol extraction, hexane/acetone extraction, and humic fraction) of the overall system. From this mass balance, it is not possible to follow the dissipation of the Substance in both phases (water and sediment) or the formation of transformation/degradation products. In addition, the mass balance shows a low recovery of the radioactivity, around 59% at the end of the test. Regarding CO_2 production, some mineralization occurs, as 14% of ^{14}C was recovered as CO_2 on week 3 reaching up to 44% on week 12.

- Soil samples: for the Soil 1, 23%, 2.1%, and 0.28% of parent substance remained in the microcosm at each time respectively, and the total ^{14}C recovered as CO_2 reached up 61.7% on week 12. The recovery of the radioactivity was 78% and 89% at the end of the test. Similar results were reported for the Soil 2.

For all the samples tested, from the soxhlet extraction, transformation/degradation products were investigated using Thin Layer Chromatography (TLC) analyses. The results show the formation of more polar transformation/degradation products during the first four weeks of incubation. The authors concluded that the Substance is not persistent/very persistent in the sediment and soil samples tested as the half-lives calculated were far below the criteria for P/vP listed in Annex XIII to REACH. However, shortcomings were identified in the study: disappearance of the parent substance based on nominal concentrations (zero time sampling was not measured), missing information on the ratio water:sediment, the history of the samples, possible pre-adaptation of the samples to the Substance and missing physico-chemical characterisation of the samples. In addition, information on the quantification, identity and rate of formation and decline of transformation and/or degradation products were not provided in the study. The information available is therefore not sufficient to be able to conclude on the persistence of the Substance and its transformation/degradation products.

A supporting study (Unpublished report, 2006), equivalent to an OECD TG 314-Simulation Tests to assess the Biodegradability of Chemicals Discharged in Wastewater is provided in the registration dossier. In this study, the biodegradation of the

radiolabelled Substance was followed during 28 days in a mixture of river water with activated sludge. The results showed that the parent substance was significantly dissipated after 28 days of incubation, as no parent substance was measured in the test systems and 87% of the total radioactivity was associated with the formation of transformation/degradation products. The degradation/transformation products were not identified nor quantified by the authors.

Another study equivalent to an OECD TG 303A (Unpublished report, 2009) is available in the registration dossier. In this study, the removal of the ^{14}C-radiolabelled Substance was studied in a continuous flow activated sludge system (CAS). The results showed a total removal of 89.7% of the parent substance over the steady state (19 days) and detected four main degradation products. The identity of these degradation products was not provided in the study, thus it was not possible to conduct further QSAR estimations. Only information of their estimated log Kow is available. The main degradation product on the effluent (detected at day 39) exhibited a log Kow between 1.75 and 2.03. Additionally, on ECHA's dissemination website, it is indicated that the estimated log Kow for three other degradation products were lower than 2.1 and for one degradation product the log Kow was estimated to range between 2.1 and 6.5. No further information about the identity of degradation/transformation products is presented in the study and it is not possible to run model prediction for their PBT properties. However, the indication of formation of a degradation/transformation product which could exhibit a log Kow> 4.5, raises concern for the formation of a degradation/transformation product which can be considered as potential B. Indeed a log Kow> 4.5 is the screening criterion for 'B' indicated in the ECHA Guidance[6], chapter R.11.

For both studies (OECD TG 314 and 303A) the ECHA Guidance, chapter R.7b, indicates that these type of tests cannot be used on their own for the PBT/vPvB assessment and may only be considered as a part of a weight-of-evidence approach*, "because the study does not employ relevant environmental conditions for assessing the persistence of the substance in the compartments relevant for the PBT/vPvB assessment".* However, the studies highlight the formation of transformation/degradation products which may be relevant for the PBT assessment.

Information from literature (DiFrancesco *et al*. 2004 and Chen *et al.* 2009) provides some indications that dissipation of the Substance seems not to be as fast as it was observed in the studies described above. DiFrancesco *et al.* (2004) show in their mesocosm experiments, that after three months, the Substance was present in all spiked soils at concentrations ranging approximately from 15% to 40% of the initial concentrations. Another study (Chen *et al.,* 2009) estimated half-lives of the Substance in sludge reed bed systems in absence and in presence of three different macrophyte species. The authors, following first-order kinetics, estimated tentative half-lives for the registered substance for every container conditions ranging from 187 days to 204 days.

(ii) For the transformation/degradation products:

Prediction of transformation/degradation products

Three modelled transformation/degradation products from the University of Minnesota's Pathway Prediction System are available in the registration dossier. For these transformation/degradation products, the log Kow and water solubility values were estimated. Considering the log Kow estimated values (between 3.65-3.72), the degradation/transformation products proposed do not fulfil the screening criteria for B.

[6] ECHA Guidance on Information Requirements and Chemical Safety Assessment

However, (*i*) the modelling was only conducted for the beta isomer constituent, and (*ii*) the predictions do not include all the possible structures, such as the transformation/degradation products resulting from the break of the double bond.

Therefore, it is considered that other metabolites can be formed from the gamma and alpha isomers constituents. Considering that the prediction for the beta isomer indicates that the outcomes as not certain, it was not considered relevant to further run this model for the gamma and alpha isomers. Furthermore, the OECD TG 303 A (Unpublished report, 2009) indicated four main degradation/transformation products, including one with a log Kow>4.5. Consequently, considering all these uncertainties, it is not possible to exclude the formation of transformation/degradation products relevant for the PBT assessment.

Potential persistence of the transformation/degradation products

In the absence of specific information on the identity of the degradation products formed, it is not possible to further evaluate their P/vP potential.

Altogether, there are indications that the Substance may form transformation/ degradation products relevant for the PBT assessment of the Substance, and the available information is not sufficient to conclude on their PBT properties.

Further information on the identity and quantity of degradation/transformation products that can be formed in relevant environmental conditions is required.

According to your comments on the draft decision you indicate that ECHA has dismissed the OECD 301F (Unpublished report, 2021) performed with the Substance, because the inoculum was obtained from domestic water treatment. You disagree with the statement in the draft decision that it is not possible to exclude a pre-adaptation of the inoculum in the OECD 301F. You argue that the use of inoculum taken from domestic waste water is a standard approach in the OECD TG 301/310 series and this would also imply a pre-adaptation for all substances that are used and released in a wide dispersive manner. You add that since there is no defined threshold for what is considered wide dispersive use, there should be no threshold for excluding pre-adaptation of the inoculum in each study. Moreover, you indicate that the first OECD TG 301F study (Unpublished report, 1991) has been performed while the Substance was already under wide dispersive use for decades and the result does not support an adaptation of the inoculum.

ECHA recognises that a plausible explanation for the conflicting results can be also attributed to the difference in the concentrations tested (18 mg/L in the second study and 100 mg/L in the first study) but it is not possible to exclude differences in the origin of the inoculum. Ready biodegradability test are known to be highly variable due to the inoculum. According to ECHA guidance[7] it is acknowledged that pre-exposure of the degrading microorganisms may not be avoided for substances that are widely used and continuously emitted to WWTPs (waste water treatment), e.g. if they are ubiquitous in consumer products. This is the case for the Substance, which has consumer uses in cosmetics and personal care products (see Section 1.2).

More importantly, ECHA would like to stress that the recent OECD 301F (Unpublished

[7] ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7b: Endpoint specific guidance.
https://echa.europa.eu/documents/10162/13632/information_requirements_r7b_en.pdf/1a551efc-bd6a-4d1f-b719-16e0d3a01919

report, 2021) is not dismissed and the study is indeed considered in the overall weight of evidence approach used in the assessment of the persistence of the Substance. In addition, as described above, the concern which is identified relates to the formation of transformation/degradation products which may exhibit PBT/vPvB properties. The OECD 301F study (Unpublished report, 2021) does not provide information that disregards nor informs on this potential. Based on the overall database, further investigation on the degradation, especially on the formation and identity of degradation/transformation products of the Substance is needed.

b) B/vB properties

If a substance fulfils the criteria in Section 1.1.2 or 1.2.2 of Annex XIII to REACH, it is considered that it has bioaccumulative (B) or very bioaccumulative (vB) properties.
For the purpose of the B/vB assessment and to check whether the criteria are fulfilled, the information listed in Section 3.2.2 of Annex XIII must be considered, including bioconcentration factor (BCF) values.

(i) For the parent Substance:

Evidence based on modelling data

The predicted BCFs by the Arnot Gobas method shows that if biotransformation of the Substance is assumed, the constituent (beta-isomer) screens as potential B, conversely if absence of biotransformation is assumed, the BCFs estimated for every constituent screen as potential vB. In contrast, when the BCF are calculated by the regression method, the constituents do not appear as potential B/vB.

Evidence based on experimental data

An experimental study with *Lepomis macrochirus* is available in the registration dossier. The study was conducted according to OECD TG 305 and under GLP. Fish were exposed by flow-through aqueous exposure to two concentrations of radiolabelled Substance (technical grade - 1.3 and 13 µg/L) during 21 days. The total depuration was 14 days.

The BCFs based on the total radioactivity residue are calculated as 14C-ISO-E-SUPER (trade name) equivalents in water and fish tissues. The BCFs calculated by steady state approach after lipid normalization (5%) were 444 and 530 L/kg and by kinetics approach were 463 and 545 L/kg for both treatments respectively.

Information on the distribution of the total radioactivity residues (TRR) are available in fish and water aquaria for the sampling at 14 and 21 days. BCF values for the parent Substance by the steady-state approach can be calculated resulting to 699 L/kg and 793 L/kg for each concentration respectively. Regarding metabolites, the analyses of ^{14}C residue (TRR) distribution in fillet and viscera extracts at 14 and 21 days of sampling, shows the detection of 6 components including OTNE, which is the major radioactive residue. In fillet, between 84% and 74% of TRR was detected as OTNE. In fish viscera, the Substance represented also approximately between 54% and 46% of TRR (for both concentrations and sampling days) and two major polar metabolites (between 12% and 18%).

Considering the available information, the Substance itself does not fulfil the bioaccumulation criteria (B/vB) in accordance with Annex XIII of REACH.

(ii) For the transformation/degradation products:

Results from an OECD TG 303 A (Unpublished report, 2009) indicated the formation of four main degradation/transformation products, including one with a log Kow>4.5. According to ECHA Guidance[8], the indication of formation of degradation/transformation products which could exhibit a log Kow> 4.5, raises concern because it fulfils the screening criterion for 'B'.

Three transformation/degradation products identified using the University of Minnesota's Pathway Prediction System are available in the registration dossier. For these transformation/degradation products, their log Kow and water solubility values were estimated. Considering the log Kow estimated values (between 3.65-3.72), the degradation/transformation products predicted do not fulfil the screening criteria for B.

However, it is noted that (*i*) the modelling was only conducted for the beta isomer constituent, and (*ii*) the predictions do not include all the possible options, such as the transformation/degradation products resulting from the break of the double bond. Therefore, it is considered that other metabolites can be formed from the gamma and alpha isomers' constituents. Considering that the prediction for the beta isomer indicates the outcomes as not certain, it was not considered relevant to further run this model for the gamma and alpha isomers.

Considering the available information on the predicted transformation/degradation products of the Substance, some may fulfil the bioaccumulation criterion (B) in accordance with Annex XIII, section 1.1.2, of REACH. Further information on the identity and quantity of degradation/transformation products that can be formed in relevant environmental conditions is required.

c) Toxicity

If a substance fulfils the criteria in Section 1.1.3 of Annex XIII to REACH, it is considered that it fulfils the toxicity (T) criterion. For the purpose of the assessment of T and to check whether the criteria are fulfilled, the information listed in Section 3.2.3 of Annex XIII must be considered, such as results of long-term toxicity tests.

(i) For the parent Substance:

Concerning T properties of the Substance, no definitive conclusions can be made for human health. The available information shows that the Substance may have mutagenic properties and data is requested in this decision.

With respect to ecotoxicity information, short and long term experimental data are available for the three trophic levels. The most sensitive species was reported to be *Daphnia magna* in the long term test reproduction study (Unpublished report, 2002), where the results of this study showed a NOEC of 0.028 mg/L.

[8] ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment, https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f

(ii) For the transformation/degradation products:

You provided a QSAR model prediction (Ecosar v1.11) that was used to predict chronic aquatic toxicity values. Considering neutral organic equations (for fish, Daphnid and green algae), the ChV predicted are 0.09 mg/L - 2.31 mg/L. The interpretation of these predictions must be taken with caution because as it is indicated in the ECHA Guidance, chapter R.11. the QSAR models are not applicable for an unequivocal assessment of the T criterion, thus, it is not possible to exclude that some transformation/degradation products could exhibit T properties for aquatic organisms.

Moreover, as it is indicated in previous sections, only three predicted transformation/degradation products were provided. It is considered that other transformation/degradations products can be formed from the gamma and alpha isomers constituents that could be T.

In absence of specific information on the identity of the transformation/degradation products formed in experimental studies, it is not possible to further model their T properties.

d) Conclusion

The Substance may form relevant transformation/degradation products which may exhibit PBT/vPvB properties. In particular, a degradation product fulfils the screening B criteria but based on lack of information on the identity of degradation products, it is not possible to further evaluate its PBT/vPvB potential. The currently available information is not sufficient to draw a conclusion on this hazard.

Therefore, a well-conducted study under relevant environmental conditions is necessary. It will allow obtaining reliable information about the identification and quantification of relevant transformation/degradation products formed from the Substance. Subsequently, if formed, further information may need to be generated on these products to assess whether they meet the PBT/vPvB criteria of Annex XIII and to consider further risk management measures accordingly.

1.2 Potential exposure

According to the information you submitted in all registration dossiers, the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of 1 000 to 10 000 tonnes per year.

Furthermore, you reported that among other uses, the Substance is used in the following products: washing & cleaning products, air care products, polishes and waxes, perfumes and fragrances, cosmetics, personal care products, biocides (e.g. disinfectants, pest control products) by consumers, in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

The Substance can be released to the environment as emissions from manufacturing plants, emissions from industrial and professional facilities using the Substance and from consumer uses leading to emissions to municipal waste water treatment plants.

Based on the uses of the Substance as indoor use in long-life materials with high release rate (e.g. release from fabrics, textiles during washing, removal of indoor paints), indoor use in long-life materials with low release rate (e.g. flooring, furniture, toys, construction materials, curtains, foot-wear, leather products, paper and cardboard products,

electronic equipment), indoor use as processing aid and outdoor use as processing aid exposure to the environment is therefore likely.

Direct emissions to the environment can be expected from some consumer uses, in particular from cosmetics and personal care products.

Therefore exposure to environment cannot be excluded.

1.3 Identification of the potential risk to be clarified

Based on the weight of evidence of all information available in the registration dossiers and information from the published literature, there is sufficient evidence that the Substance may form transformation/degradation products that have potential PBT/vPvB properties.

The information you provided on manufacture and uses demonstrates a potential for environmental exposure.

Based on this hazard and exposure information, the Substance poses a potential risk to the environment.

As explained in Appendix B, Section 1.1 above, the available information is not sufficient to conclude on the hazard and in particular on the PBT properties. Consequently, further data is needed to clarify the potential risk related to PBT/vPvB properties.

1.4 Further risk management measures

If degradation/transformation products formed in the requested simulation study meet the P, B and T or vP and vB criteria, the Substance can be identified as a PBT/vPvB.

The evaluating MSCA will analyse the options to manage the risk(s) and will assess whether the Substances should be proposed for:

- further regulatory risk management, such as an identification as a Substance of very high concern (SVHC) under Article 57 of REACH due to its PBT or vPvB properties;
- a subsequent authorisation or a restriction of the Substance.

This would lead to stricter risk management measures than those currently in place, such as minimisation of emissions.

2. How to clarify the potential risk

2.1 Simulation testing on ultimate degradation in surface water (test method: EU C.25./OECD TG 309 - aerobic mineralisation in surface water-simulation biodegradation test)

a) Aim of the study

The aim of the required testing is to identify and quantify any relevant transformation/degradation products which are formed under relevant environmental conditions. Subsequently, if formed, further information may need to be generated on these products to assess whether they meet the PBT/vPvB criteria of Annex XIII.

The requested simulation test (OECD TG 309) including identification of degradation products is a standard information requirement at Annex IX and Annex X, Section 9.2.1.3 and 9.2.3 of REACH. It could therefore be requested under compliance check (Article 41

of REACH). However, since the quantification of the total amount of non-extractable residues, required in the study, is a non-standard parameter, and the information request is based on a potential risk posed by the Substance, the substance evaluation is considered as the appropriate process.

b) Specification of the requested study

Test conditions

You must conduct a pelagic test using EU representative surface waters with a suspended solids concentration of approximately 15 mg dw/L (but not outside the range of 10 to 20 mg dw/L), as explained in ECHA Guidance[9].

In your comments to the draft decision, you indicate that you understand the need to perform the study, but in order to confirm the biodegradation observed in the studies OECD TG 303 and OECD TG 314, you propose to conduct the OECD TG 309 test using similar water conditions than the one used in the available studies OECD TG 303 and OECD TG 314. You argue that OECD TG 303 and OECD TG 314 simulate conditions in wastewater treatment plants and the Substance is used in down-the-drain consumer products so will be treated in wastewater treatment plants before reaching the environment. You argue that changing the water conditions might lead to unrepresentative metabolites, or even different metabolites that might not increase the overall knowledge of the biodegradation fate of the Substance and its degradation products.

ECHA welcomes the agreement of the Registrant(s) to further clarify the degradation of the Substance in detail. However, ECHA highlights that the purpose of the requested test is to clarify if the Substance can form transformation/degradation products under relevant environmental conditions, which could exhibit potential PBT/vPvB properties.

The conditions applied in the OECD TG 309 test better reflect the potential biodegradation of organic substances under environmentally realistic conditions, as explained in ECHA Guidance[10]. ECHA highlights that, degradation simulation studies (such as OECD TG 309) performed in appropriate environmental media and at environmentally realistic conditions are the only tests that can provide a definitive degradation half-life value that can be compared directly to the persistence criteria as defined in REACH Annex XIII. OECD TG 309 uses a low test concentration of substance to ensure that the biodegradation kinetics obtained in the test reflect those expected in the environment.

ECHA points out that the OECD 303 and 314 tests use organic medium (such as synthetic sewage, domestic sewage or a mixture of both) in addition to the tested substance as sources of carbon and energy for the micro-organism in order to simulate sewage treatment plant (STP) conditions, contrary to the OECD TG 309 test which uses the Substance as the only carbon source to reflect the conditions in natural waters. If the water conditions in the simulation OECD 309 test is modified as you suggest, ECHA would not be able to solve the uncertainties regarding the formation of transformation/degradation products in natural waters. Direct emission of the Substance

[9] ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f

[10] ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f

to natural waters, resulting from uses, where contaminated waters are not subject to remediation measures, is possible as explained in Section 1.2 above. Therefore, ECHA considers that the water conditions used in the OECD 303 and 314 tests are not suitable to investigate the formation of degradation/transformation in the context of the PBT assessment.

Test material and concentration

The test must be conducted with two concentrations as recommended in the OECD TG 309 and you must ensure that all test concentrations are below the aqueous solubility of the Substance in the test medium. Moreover, the concentrations used must be appropriate to successfully identify and quantify the formed transformation and/or degradation products.

To identify transformation and/or degradation products relevant for PBT assessment, the test material must be ^{14}C radiolabelled and the radiolabel must located in the most stable part of the molecule. However, according to the OECD 309, the most stable part does not necessarily include the relevant functional moiety of the molecule (that can be related to a specific property such as toxicity, bioaccumulation, etc.). If this is the case, it may be appropriate to use a test substance, which is ^{14}C-labelled in the functional part in order to follow the elimination of the specific property.

In your comments on the draft decision you agree that the use of ^{14}C labelled material is the best approach to identify transformation/degradation products and you explain that considering that the Substance is a reaction mass, ^{14}C material synthesis for this kind of substance is more challenging and requires some time. Thus, you request to reconsider the deadline proposed to conduct the test.

ECHA welcomes the agreement of the Registrant(s) to use ^{14}C labelled material in the study. The timeline to conduct the OECD TG 309 study has been revised, as explained below.

Water samples

In order to reflect a range of natural water conditions, it is recommended to consider performing the test with more than one water source. In addition, to avoid pre-adaptation of the microorganisms to the Substance in water samples, the sampling sites for collection of the surface water should be selected taking into account the history of possible agricultural, industrial or domestic inputs. If it is known that an aquatic environment has been contaminated with the test substance or its structural analogues within the previous four years, it shall not be used for the collections of test water. This is because one aim of PBT/vPvB assessment is to protect pristine environments which have had no exposure to the Substance, as explained in ECHA Guidance R.11, page 11-12[11]. To this end, we recommend to use samples from, for example, smaller rivers that are less likely to have received effluent from industrial wastewater treatment plants or into which only smaller municipal wastewater treatment plants feed their effluent. In addition, it is more likely that water samples will contain adapted microorganisms if the water samples are collected below a treatment plant effluent instead of above. It is therefore recommended that the sampling location should be chosen suitable to eliminate

[11] ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment, https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f

such concerns. Your report shall justify and detail the arrangements you made to take non-adapted water samples.

In your comments on the draft decision, you mention that it is not clear if the study should be conducted with two sources of water tested separately or two sources of water mixed and then tested as one. ECHA wishes to clarify that if more than one water source is considered, they must be tested separately.

Temperature

The OECD TG 309 simulation test must be performed at environmentally relevant temperatures, i.e. by default at 12°C. This temperature is considered as the mean temperature of European surface waters (as per the ECHA Guidance, chapter R.7.9.4.1).

Sterile controls

Sterile controls must be performed and a justification of the method and procedure used for establishing the sterile controls must be provided. The inclusion of sterile controls is important to determine to what extent the decrease of the test material is due to potential contribution of abiotic losses.

Reference control

As indicated in the OECD TG 309, a substance, which is normally easily degraded under aerobic conditions (e.g. aniline or sodium benzoate) must be used as reference substance in order to demonstrate the viability of the system.

Measurement of test substance concentration and primary degradation

The concentration of the test substance must be measured at appropriate intervals during the study so that a primary degradation half-life can be determined, in addition to the half-life based on measurement of residual ^{14}C activity or the evolved $^{14}CO_2$. This is required for the following reasons:

- The measurement of the test substance concentration is important for the comparison between the active test and sterile controls to estimate the potential contribution of abiotic losses to the decrease in test substance concentration.
- Primary degradation half-life is important for the conclusion on the P/vP property of the parent substance in case that degradation half-life based on residual ^{14}C is above the P or vP criterion.
- Primary degradation half-life may be important for the estimation of the persistence of the degradation/transformation products.

Identification of transformation/degradation products

Transformation and/or degradation products present at a concentration of ≥ 10 % w/w must be identified and quantified at every sampling time, unless reasonably justified otherwise.

Furthermore, identification and quantification of transformation and/or degradation products whose concentrations are continuously increasing must also be considered, if technically feasible. Therefore, the concentrations used must be high enough to allow detection, with the applied analytical method, of the transformation and/or degradation products formed.

Therefore, you must attempt as far as technically possible, to quantify these products down to 1%. Otherwise you must provide a justification as to why it was not technically feasible. Technically feasible means that you have demonstrated within the allocation of reasonable efforts to develop suitable analytical methods and other test procedures to accomplish testing in soil, that reliable results can be generated.

The quantification of transformation and/or degradation products down to 1% can be achieved using LC-HRMS (liquid chromatography high resolution mass spectrometry).

In your comments on the draft decision you indicate that identification of metabolites may be very difficult as even identical masses could reflect different structures due to different position of the double bound and you add that a separation via HPLC may be challenging. Furthermore, you add that considering the complex chemistry of the Substance and the possible current limited capacity of CROs, the timeline proposed by ECHA of 18 months is not sufficiently long enough to be able to perform the test. Thus, you propose an extension of the deadline by at least 18 additional months (total of 36 months) in order to have a realistic chance to meet the deadline required by ECHA.

ECHA has reviewed the information you provided as a justification and recognises that the synthesis of ^{14}C-labelled material and the identification of degradation/transformation products can be challenging. Therefore, the original deadline is extended by 18 months and set to 36 months.

Test duration

The OECD TG 309 recommends a test duration of 60 days, and if a longer duration is needed a semi-continuous procedure should be used (See Annex 3 of OECD TG 309) to give sufficient time for any transformation and/or degradation product to appear.

Quantification of the total amount of non-extractable residues (NER)

As specified in ECHA Guidance, chapter R.7.9.4.1., the organic carbon (OC) concentration in surface water simulation tests is typically 2 to 3 orders of magnitude higher than the test substance concentration and the formation of NERs may be significant in surface water tests.

- You must explain and scientifically justify the extraction procedure and solvent used obtaining a quantitative measure of NERs.
- The total amount of NER must be quantified to demonstrate that all transformation and/or degradation products which have formed have been extracted and can be quantified. By default, total NER is regarded as non-degraded parent. However, if reasonably justified and analytically demonstrated a certain part of NER may be differentiated and quantified as irreversibly bound or as degraded to biogenic NER, such fractions could be regarded as removed when calculating the degradation half-life(s) (ECHA Guidance, chapter R.11.4.1.1.3.).

- You have the option to further characterise the types of NER to refine the P assessment. The Background note on 'Options to address NER in regulatory P assessment', published on the ECHA website[12] provides some suggestions on the further refinement.

[12] https://echa.europa.eu/documents/10162/13632/bg_note_addressing_non-extractable_residues.pdf/e88d4fc6-a125-efb4-8278-d58b31a5d342

Request for the full study report

You must submit the full study report which includes:

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

This will enable the evaluating MSCA to fully and independently assess all the information provided, including the statistical analysis, and to efficiently clarify the potential hazard for the PBT/vPvB properties of the Substance.

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

The request is appropriate, because the test is necessary to identify and quantify any relevant transformation/degradation products which are formed under relevant environmental conditions. In particular, testing in water is considered appropriate for the following reasons:

- Monitoring information shows that the Substance has been detected in European rivers at concentrations ranging from 10 to 100 ng/L for the Ruhr River and from 29 to 810 ng/L in Danube river (Bester *et al*., 2008). Klaschka *et al.* (2013) also reported concentrations of the Substance between <10 and up to 105 ng/L in surface water at 1 km downstream of STPs. Recently, higher concentrations of the Substance have been detected in Spain at concentrations up to 6540 ng/L in surface waters and up to 1285 ng/L in aquifers (Corada-Fernandez *et al.*, 2017). Water is therefore a relevant compartment for testing.
- Regarding technical issues, considering the water solubility of 2.68 mg/L measured for the Substance, a study conducted in water seems feasible.
- Additionally, conducting the test in the water compartment minimises the potential formation of non-extractable residues (NER), due to their adsorption capacity to particles (Koc of 12 600).
- A hydrolysis test (OECD TG 111) would not be appropriate for the identification of transformation/degradation products of the Substance relevant for PBT/vPvB assessment. The OECD TG 111 only measures degradation due to hydrolysis and does not include biotic degradation, but degradation studies on the Substance in the presence of microorganisms indicate the formation of transformation/degradation products. OECD TG 111 uses buffer solutions rather than natural surface water samples and the test is conducted in the dark for a shorter duration of 5 – 30 days.

2.2 References relevant to the requests (which are not included in the registration dossier)

Bester K. et al., 2008. Surface water concentrations of the fragrance compound OTNE in Germany – A comparison between data from measurements and models. Chemosphere 73(8): 1366-1372.

Chen X. et al., 2009. Removal of personal care compounds from sewage sludge in reed bed container (lysimeter) studies — Effects of macrophytes." Science of the Total Environment 407(21): 5743-5749.

Corada-Fernández C. et al., 2017. Effects of extreme rainfall events on the distribution of selected emerging contaminants in surface and groundwater: The Guadalete River basin (SW, Spain). Science of the Total Environment 605-606(Supplement C): 770-783.

DiFrancesco A. M. et al., 2004. Dissipation of Fragrance Materials in Sludge-Amended Soils. Environmental Science & Technology 38(1): 194-201.

ECHA dissemination website, 2017. Information on Chemicals. reaction mass of 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one and 1-(1,2,3,4,6,7,8,8a-octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one and 1-(1,2,3,5,6,7,8,8a-octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (EC 915-730-3). https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.144.093

Klaschka U. et al., 2013. Occurrences and potential risks of 16 fragrances in five German sewage treatment plants and their receiving waters. Environmental Science and Pollution Research 20(4): 2456-2471.

Appendix C: Procedure

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

12-month evaluation

Due to initial grounds of concern for PBT/vPvB, Suspected Reprotoxic, potential endocrine disruptor, exposure of environment, wide dispersive use and high RCR, the Member State Committee agreed to include the Substance (List No 915-730-3) in the Community rolling action plan (CoRAP) to be evaluated in year 2017. France is the competent authority ('the evaluating MSCA') appointed to carry out the evaluation.

In accordance with Article 45(4) of REACH, the evaluating MSCA carried out its evaluation based on the information in the registration dossier(s) you submitted on the Substance and on other relevant and available information.

Following a first evaluation, during which the environmental part only has been assessed, an initial draft decision was submitted to you for comments. Nevertheless, since data generation was ongoing for concerns relating to human health, to comprehensively evaluate the Substance, the decision-making process was suspended in order to wait for the ongoing studies (prenatal developmental toxicity and extended one generation reproductive toxicity studies).

When the extended one generation reproductive toxicity study was available and finally considered as compliant, the evaluation started again and the evaluating MSCA identified an additional concern for Mutagenicity.

The evaluating MSCA completed its evaluation considering that further information is required to clarify the following concerns: mutagenicity and PBT/vPvB.

Therefore, it submitted a draft decision (Article 46(1) of REACH) to ECHA.

Decision-making

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

The decision-making followed the procedure of Articles 50 and 52 of REACH as described below.

(i) Registrant(s)' commenting phase

ECHA received your comments and forwarded them to the evaluating MSCA.

The evaluating MSCA took your comments into account (see Appendices A and B). The request(s) and deadline(s) were amended.

In your comments you requested an extended timeline of 36 months to provide the requested information. Your justification is deemed plausible and the deadline is amended accordingly.

(ii) Notification to MSCAs

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

Subsequently, the evaluating MSCA received one proposal for amendment to the draft decision and made editorial modifications to the draft decision.

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

ECHA referred the draft decision, together with your comments, to the Member State Committee. ECHA invited you to comment on the proposed amendment(s).

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

(iv) Member State Committee agreement seeking stage

The Member State Committee reached a unanimous agreement in its MSC-84 written procedure and ECHA took the decision according to Article 52(2) and Article 51(6) of REACH.

(v) Follow-up evaluation

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

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

Test methods, GLP requirements and reporting

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

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

Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[13].

Test material

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

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

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

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

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

   a) You must report the composition of the Test Material selected for each study, under the 'Test material information' section, for each respective endpoint study record in IUCLID.
   b) The reported composition must include all constituents of each Test Material and their concentration values.

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

Technical instructions on how to report the above is available in the manual "How to prepare registration and PPORD dossiers"[14].

[13] https://echa.europa.eu/practical-guides
[14] https://echa.europa.eu/manuals