ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 17 December 2020

Addressees
Registrant(s) of 1-phenylethanol listed in the last Appendix of this decision

Registered substance subject to this decision (the Substance)
Substance name: 1-phenylethanol
EC number: 202-707-1
CAS number: 98-85-1

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]

1. *In vitro* mammalian cell micronucleus assay (OECD 4TG 87/EU B.49), with fluorescence *in situ* hybridisation (FISH) or immunochemical labelling of kinetochores (CREST) in case of positive result (Request A.1).
2. Only if the results from Request (A.1) demonstrate that the Substance fulfils the criteria for positive *in vitro* genotoxicity according to REACH Annex VIII, a combined *in vivo* mammalian erythrocyte micronucleus test in bone marrow (OECD TG 474/B.12 EU) and *in vivo* mammalian alkaline comet assay test (OECD TG 489) in liver, gastro-intestinal tract (stomach and duodenum), kidney performed in rats via oral route using the test substance 1-phenylethanol, including full study report, as further specified in Appendix A (Request A.2).

Deadlines

A sequential testing strategy must be applied with multiple deadlines:
Request A.1: The requested information must be provided by 24 March 2022.
Request A.2: The requested information must be provided by 22 September 2023 only if the results from Request A.1 show a positive result.

Table 1 - Overview of requested studies and corresponding deadlines, reflecting the sequential testing strategy.

Requested information	Conditions when to perform test	Deadline
Request A.1 – OECD TG 487	None - always to be performed	12 months from the date of the decision
Request A.2 – combined OECD TG 489 and OECD TG 474	Only if results from Request A.1 show positive results (performance and reporting of such study takes 18 months)	30 months from the date of the decision

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 study/ies 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 Appendix/ces 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

Applicable only for the adopted ECHA decision: 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[2] by Christel Schilliger-Musset, Director of Hazard Assessment

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

The Appendices entitled 'Reasons to request information' describe why the requested information are necessary and appropriate.

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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, the evaluating MSCA and ECHA have identified the following potential hazard(s) which must be clarified.

a. Potential mutagenicity

The available information reported by the Registant(s) is not sufficient to clarify the identified concern. In particular, the available *in vitro* data were analysed using a weight of evidence approach. The results raised doubts about the ability of 1-phenylethanol to induce both chromosomal aberration in mammalian cell lines and/or gene mutation. The available *in vivo* data are considered inconclusive. Therefore a concern on potential mutagenicity of 1-phenylethanol cannot be excluded.

In vitro genotoxicity studies

Two key studies are reported in the registration dossier(s). Both studies are data owner reports. Only the summaries of the results are presented in the CSR, while the full study reports are not available.

1-phenylethanol was tested for the ability to induce gene mutations in *Salmonella typhimurium strains* TA 1535, TA 1537, TA 98, TA 100 and TA 102. The assay was performed in two independent experiments, both with and without liver microsomal activation. Based on the solubility and precipitation preliminary test, the main study was conducted with the following doses: 0, 0.0158, 0.050, 0.158, 0.501, 1.582 mg/plate. Negative results were reported both with and without metabolic activation.

The second key study reported by the Registrant(s) is a mammalian cell gene mutation assay performed according to OECD TG 476 in Chinese Hamster Ovary (CHO) cells. CHO cells were treated with 1-phenylethanol for three hours at the following concentrations: 0, 0.5, 1.0, 2.5, 5.0 mM with and without metabolic activation. The justification for the selection of the top concentration used (5 mM), probably due to precipitation, is not given in the available summary report. N-ethyl-N-nitrosurea was used as positive control. No mutagenicity was observed in any experimental condition, while the positive control gave a clear indication of mutagenicity, demonstrating the sensitivity of the test system. The Registrant(s) concluded that the test substance is not mutagenic in the experimental conditions used. The full study report was not provided by the Registrant(s).

As supporting information, the technical report number 369 (1990) of the National Toxicology Program (NTP) is also mentioned in the CSR. The following studies are reported:

- An *in vitro* gene mutation assay in Salmonella Typhimurium strains TA98, TA100, TA 1535 and TA 1537 with and without metabolic activation, where the highest concentration tested was 6.7 mg/plate: negative results were reported in all stains with and without metabolic activation. This result confirms the negative outcome of the key study reported in the CSR.
- An *in vitro* gene mutation assay in the mouse lymphoma cell line L5178Y/TK$^{+/-}$ only without metabolic activation. The highest tested dose was limited by toxicity and

solubility and did not exceded the 1.5 mg/ml (12 mM). In this conditions 1-phenyethanol was reported to be mutagenic.

- A chromosomal aberration assay in CHO cells, with and without metabolic activation. In this study 1-phenylethanol was tested up to 2 mg/ml without S9 and 3 mg/ml with S9. The test item was reported to induced chromosomal aberration only in the presence of metabolic activation. However no evaluation of the cytotoxicity was reported, therefore it is not possible to conclude if the observed effect is due to real genotoxicity or is the secondary effect of cytotoxicity.
- A Sister Chromatid Exchange (SCE) test in CHO cells, up to 0.33 mg /ml without S9 and 1 mg/ml with S9, that was negative in all the experimental conditions. Overall, induction of chromosomal aberrations in CHO cells in the presence of metabolic activation was reported in a NTP study, however the reliability of this result is questionable.

The potential induction of gene mutations by 1-phenylethanol is more controversial: the substance was consistently negative in Ames test, negative in a gene mutation assay in CHO cells (full study report not provided), but positive in a gene mutation assay in mouse lymphoma cell line L5178Y/$TK^{+/-}$ only without metabolic activation.

In vivo genotoxicity study

The Registrant(s) submitted a published *in vivo* micronucleus study (Engelhardt G., 2006). This study was performed in mouse bone marrow with oral administration (gavage) according to OECD TG 474. Cyclophosphamide was used as positive control substance for clastogenicity and was given in a single oral dose of 20 mg/kg body weight. Vincristine sulfate was used as positive control substance for aneugenic activity and was given once intraperitoneally in a dose of 0.15 mg/kg body weight. The dose 750 mg/kg body weight was considered to be the maximum tolerated dose in a dose-range finding study. The dose levels selected for this study were 750, 375 and 187.5 mg/kg body weight. For each animal, 2.000 polychromatic erythrocytes (PCEs) were scored and evaluated for the presence of micronuclei. The number of normochromatic erythrocytes (NCEs) both with and without micronuclei was recorded separately.

The results demonstrate that the number of polychromatic erythrocytes containing micronuclei at each dose was not significantly increased above the concurrent negative (solvent) control frequencies and was always within the historical negative control range (0.3–3.3‰ based on > 300 experiments) at each sampling time. Clearly increased numbers of micronucleated polychromatic erythrocytes were obtained using cyclophosphamide and vincristine, demonstrating the sensitivity of the experimental system. At 750 mg/kg body weight, all animals survived, but clinical signs of toxicity were observed (piloerection, irregular respiration and staggering), this dose was considered the maximum tolerated dose for the experiment. However, no indication of local cytotoxicity (i.e. alteration of PCE/NCE ratio) was reported in this study, therefore target cells exposure is not demonstrated. The reported clinical signs of toxicity are not sufficient to demonstrate target cell exposure, therefore no conclusion on the clastogenicity observed in the *in vitro* study can be made based on this *in vivo* study.

Supporting information

1-phenylethanol was tested for carcinogenicity by oral route in mice and rats in a two year study (NTP, 1990). Group of 50 rats or 50 mice of each sex were administered 0, 375, 750 mg/kg of 1-phenylethanol in corn oil by gavage, 5 days per week for 103 weeks.

The conclusions reported are: some evidence of carcinogenic activity for male rats, as

shown by increased incidences of renal tubular cell adenomas and adenomas or adenocarcinomas (combined); no evidence of carcinogenic activity was observed in female rats and in female and male mice. Moreover 1-phenylethanol is the major metabolite of ethylbenzene, a substance that causes increased tumour incidences in mice and in rats after inhalation exposure and is classified by IARC as a possibly carcinogenic to humans. 1-phenylethanol is a member of the benzylic acid series and has potential alkylating ability based on the benzyl carbonium ion (NTP 1990).

In conclusion, the available *in vitro* experimental data do not allow to conclude on the possible clastogenicity of 1-phenylethanol. Although data regarding gene mutation assays in cultured mammalian cells show conflicting outcomes, the negative results consistently reported in the Ames test indicate that the ability of 1-phenylethanol to induce gene mutation is unlikely.

Further information is therefore needed to clarify the mutagenic properties of the test substance.

In case of positive results in the *in vitro* micronucleus study, a combined *in vivo* mammalian erythrocyte micronucleus test in bone marrow (OECD TG 474) and *in vivo* mammalian alkaline comet assay test (OECD TG 489) in liver, gastro-intestinal tract (stomach and duodenum), kidney performed in rats via oral route is requested in a tiered-testing strategy.

1.2 Potential exposure

According to the information you submitted in chemical safety reports, the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of 1-10 tonnes per year.

Furthermore, you reported that among other uses, the Substance is used

- by industrial workers in formulation (i.e., use in compounding) and at industrial site as intermediate, pharma application - chiral building block, cleaning agents and maintenance products, washing and cleaning products;
- by professional workers in polishes and wax blends, in washing and cleaning products, in cosmetic products;
- by consumers in cleaning agents and maintenance products (i.e., air care products, polishes and waxes, washing and cleaning products) and cosmetic products (i.e., cosmetics and personal care products, perfumes and fragrances, pharmaceuticals).

Albeit, the low tonnage, the severity of the endpoints (mutagenicity, carcinogenicity) as well as the potential direct contact should be taken into account and 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 dossier 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 a potential for exposure of workers and consumers.

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 the potential hazard. Consequently further data are needed to clarify the potential risk related to mutagenicity of 1-phenylethanol.

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). The results of Request(s) will, amongst other relevant and available information, be used by the evaluating MSCA to assess whether the Substance should be classified as germ cell mutagen as defined in the CLP Regulation.

The potential classification of the Substance as germ cell mutagen (Cat. 1B or 2) based on Request A.2 would also have consequences for the classification of mixtures containing the Substance due to cut-off/concentration limits triggering classification and acceptability of consumer products.

If classified as germ cell mutagen Cat. 1B, 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 Development of the testing strategy

You must follow a tiered-testing strategy encompassing the requests below:

- *In vitro* mammalian cell micronucleus assay (OECD TG 487/ EU B.49), with fluorescence in situ hybridisation (FISH) or immunochemical labelling of kinetochores (CREST).
- In case of a positive result in *in vitro* mammalian cell micronucleus assay (OECD TG 487), a combined *in vivo* mammalian erythrocyte micronucleus *(in vivo* micronucleus*)* test (OECD TG 474/ EU B.12 EU) and an *in vivo* mammalian alkaline comet assay (OECD TG 489) performed in rats via oral route (by gavage) on tissues as specified below, on the Substance.

2.2 Request A.1 *In vitro* mammalian cell micronucleus test (OECD TG 487/EU B.49)

a. Aim of the study

As detailed in Section 1.1, information on genotoxicity/mutagenicity *in vitro* is required to clarify on the potential hazard of the test item.

b. Specification of the requested study

Test material and concentration: 1-phenylethanol

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 the *in vitro* MN (OECD TG 487) is:

- appropriate because it will provide information which will clarify the mutagenicity *in vitro* and to develop a strategy approach also to clarify the mutagenicity *in vivo*.
- this will enable the evaluating MSCA to conclude on potential classification for mutagenicity and on potential MoA of the carcinogenicity effects observed in rats.

2.3 Request A.2 A Combined in vivo mammalian erythrocyte micronucleus test (OECD TG 474/EU B.12 EU) and an in vivo mammalian alkaline comet assay (OECD TG 489) performed in rats via oral route (by gavage) on tissues as specified below

a. Aim of the study

In case of positive results in the *in vitro* micronucleus study requested, a new *in vivo* micronucleus test combined with a Comet assay will clarify the *in vivo* mutagenicity of the Substance as further specified below.

Moreover the requested *in vivo* study design is also suitable to inform on the potential mode of action of the positive results observed in the carcinogenicity study in rats (NTP, 1990). The information requested aims at clarifying the potential risk that the Substance poses. Therefore, it is requested under the current substance evaluation.

To address the missing information identified above, the OECD TG 489 required will provide information both on mutagenicity and on the mode of action of the carcinogenicity effect.

b. Specification of the requested study

The combination of OECD TGs 489 and 474 should not impair the validity of the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al*. 2011[3]).

Test material and concentration: 1-phenylethanol

Route of exposure

[3] 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

For the *in vivo* study, the oral route is the most appropriate to investigate local and systemic genotoxicity potential for this substance.

The following tissues must be investigated:
The bone marrow in the micronucleus *in vivo* study and the liver, gastro-intestinal tract (stomach and duodenum), kidney in the comet assay.

You may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al*.[4]) at the same time as the other tissues, as it would optimise 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, you should consider analysing the slides prepared with gonadal cells, using the comet assay. 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. As also reported in the OECD TG 489, "positive results in whole gonad are not necessarily reflective of germ cell damage, nevertheless, they indicate that tested chemical(s) and/or its metabolites have reached the gonad".

In case of positive results in any of the somatic tissues, it is then recommended to analyse the collected gonadal cells.

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 in bone marrow (OECD TG 474) and *in vivo* mammalian alkaline comet assay test (OECD TG 489) is:

- appropriate, because it will provide information which will clarify the mutagenicity *in vivo*. The micronucleus *in vivo* is the appropiate follow-up of the micronuleus *in vitro.* The *in vivo* mammalian alkaline comet assay ("Comet Assay", OECD TG 489) is suitable to follow up the positive *in vitro* result for gene mutation and chromosomal aberrations and can be applied in many tissues including "site of contact" tissues and gonadal cells. This will enable the evaluating MSCA to conclude on potential classification for mutagenicity and on potential MoA of the carcinogenicity effects observed in rats;
- the least onerous measure because there is no equally suitable alternative method available to obtain the information that would clarify the potential mutagenicity

[4] O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. J. Vis. Exp. (90), e51576, doi:10.3791/51576

hazard, in the *in vivo* tests. The TGR assay is a more expensive test and is able to detect only gene mutation *in vivo*;

- the least onerous measure because there is no equally suitable alternative method available to obtain the information that would clarify the potential mutagenicity hazard, combining the two *in vivo* tests.

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

- Technical report N°369 of the National Toxicology Program, 1990.
- Engelhardt, G. *In vivo* micronucleus test in mice with 1-phenylethanol. *Arch Toxicol* 80, 868–872, 2006.

Appendix B: 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 Carcinogenicity, Mutagenicity, consumer use, exposure of workers and wide dispersive use the Member State Committee agreed to include the Substance (EC No 202-707-1, CAS RN 98-85-1) in the Community rolling action plan (CoRAP) to be evaluated in 2019. Italy 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.

- The evaluating MSCA completed its evaluation considering that further information is required to clarify the following concerns: Mutagenicity

- Therefore, it submitted a draft decision (Article 46(1) of REACH) to ECHA on 19 March 2020.

Decision-making

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

For the purpose of this decision-making, dossier updates made after the date the draft of this decision was notified to you (Article 50(1) of REACH) will not be taken into account.

(i) Registrant(s)' commenting phase

ECHA did not receive any comments from you by the end of the commenting period.

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

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment. Subsequently, the evaluating MSCA received proposal for amendment to the draft decision and modified the draft decision (see Appendix A).

ECHA referred the draft decision to the Member State Committee.

ECHA invited you to comment on the proposed amendment. You did not provide any comments on the proposed amendment(s).

(iii) MSC agreement seeking stage

The Member State Committee reached a unanimous agreement in its MSC-72 written procedure and ECHA took the decision according to Article 52(2) and Article 51(6) of REACH.

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Appendix C: 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[5].

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 and other parameters relevant for the property to be tested.

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"[6].

[5] https://echa.europa.eu/practical-guides
[6] https://echa.europa.eu/manuals