~~Confidential~~

Helsinki, 25 October 2023

Addressee
Registrant of JS_Valeraldehyde_110-62-3 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
03/09/2010

Registered substance subject to this decision ("the Substance")
Substance name: Valeraldehyde
EC/List number: 203-784-4

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***04 May 2026***.

Requested information must be generated using the Substance unless otherwise specified.

Information required from all the Registrants subject to Annex VII of REACH

1. In vivo mammalian alkaline comet assay or Transgenic rodent somatic and germ cell gene mutation assays (triggered by Annex VII, Section 8.4., column 2)

   Transgenic rodent somatic and germ cell gene mutation assay (test method: OECD TG 488) in transgenic mice or rats, oral route, on the following tissues: liver and glandular stomach; duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

   OR

   In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, or if justified, in other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum.

2. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3./OECD TG 201)

The reasons for the decision(s) are explained in Appendix 1.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressees of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

To comply with your information requirements, you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report,** where relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general requirements for testing and reporting new tests under REACH, see Appendix 4.

Appeal

This decision, when adopted under Article 51 of REACH, 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

Appendix 1: Reasons for the request(s)
Appendix 2: Procedure
Appendix 3: Addressees of the decision and their individual information requirements
Appendix 4: Conducting and reporting new tests under REACH

[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.

Appendix 1: Reasons for the request(s)

Contents

Reasons related to the information under Annex VII of REACH

1. In vivo mammalian alkaline comet assay or Transgenic rodent somatic and germ cell gene mutation assays

1 Further mutagenicity studies must be considered under Annex VII to REACH in case of a positive result (Section 8.4., column 2).

1.1. Triggering of the information requirement

2 Your dossier contains positive results for the *in vitro* mammalian cell gene mutation test (HPRT) on the Substance (1989).

3 In your comments to the draft decision, you stated that "*positive results in vitro were obtained in an UDS test in rat hepatocytes, an alkaline elution test in CHO cells and a HPRT-test in V79 cells* […] *further investigations to clarify the relevance of the existing positive in vitro results would be necessary*". In addition, you suggest to "*include a request for an in vivo comet assay (OECD TG 489) in the final decision, as* [you] *consider such an in vivo test as appropriate to clarify the relevance of the observed positive in vitro results*".

4 In your comments to the draft decision, you refer to the following study from your dossier:

- an *in vivo* micronucleus test (according to OECD TG 474) in mice with the analogue substance 3-methylbutanal (CAS 590-86-3) (2001)

5 You also refer to the following two studies:

- an *in vivo* micronucleus test (according to [redacted], 1993) in rats and mice with the analogue substance 2-methylpropanal (CAS 78-84-2) (1999)
- an *in vivo* bone marrow chromosomal aberration test (no guideline followed) in mice with the analogue substance 2-methylpropanal (CAS 78-84-2) (1999)

6 You consider that, as these studies on similar substances were all negative, they do not raise a concern for chromosomal aberration for the Substance.

7 ECHA agrees that further mutagenicity studies are necessary to address the gene mutation concern identified *in vitro*. Such positive *in vitro* study is sufficient to trigger the need for *in vivo* mutagenicity study as confirmed by the recent changes to the REACH Regulation aimed at clarifying it: Under Annex VII, Section 8.4., Column 2, an appropriate *in vivo* mammalian somatic cell genotoxicity study as referred to in Annex IX, point 8.4.4, must be performed in case of a positive result in any of the *in vitro* studies referred to in Annex VII, Section 8.4. The *in vivo* study must address the concerns raised by the *in vitro* study results, i.e., the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

1.2. Information provided and its assessment

8 Your dossier contains an *in vivo* micronucleus study (2001) on an analogue substance. This study addresses *in vivo* cytogenicity. Your dossier does not include any *in vivo* studies that address the gene mutation concern identified by the *in vitro* mammalian cell gene mutation test (HPRT) on the Substance (1989).

9 Therefore, the information requirement is not fulfilled.

1.3. Specification of the in vivo study design

1.3.1. Comet assay

10 In case you decide to perform the comet assay, according to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified (OECD TG 489, paragraph 23).

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

12 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, and from 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.

1.3.1.1. Germ cells

13 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, 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 in the comet assay, you should consider analysing 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.

14 In your comments to the proposal for amendment from a member state, you indicate that you do not consider the above analysis feasible since the test method OECD TG 489 is currently not optimised and validated for germ cell investigation. ECHA notes that the collection and further analysis of male gonadal cells is not requested from you, but put here for your consideration, as it would optimise the use of animals. Therefore, it is at your discretion to include it or not. Moreover, as specified in paragraph 10 of OECD TG 489, the inclusion of such examination may bring relevant information for the overall assessment of germ cell mutagenicity, for instance with respect to gonad exposure to the Substance and/or its metabolites. Furthermore, the feasibility of the analysis of cells from the gonads has been demonstrated in the literature (Speit et al, 2009[2]; Zheng and Olive, 1997[3]; Cordelli et al, 2003[4]; Dirven et al., 2023[5]) and a project on the "Update of TG 489 Comet Assay for gonadal cells to study germ cell specific genotoxic effects" is included in the current OECD work plan[6].

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[2] Speit, G, M. Vasquez, A. Hartmann (2009), The comet assay as an indicator test for germ cell genotoxicity, Mutation Research, Vol. 681/1, pp. 3-12

[3] Zheng, H., P.L. Olive (1997), Influence of oxygen on radiation-induced DNA damage in testicular cells of C3H mice, International Journal of Radiation Biology, Vol. 71/3, pp. 275-282

[4] Cordelli, E. et al. (2003), Evaluation of DNA damage in different stages of mouse spermatogenesis after testicular X irradiation, Journal of Radiation Research, Vol. 160/4, pp. 443-451

[5] Dirven, Y., Eide, D.M., Henriksson, E.W., Hjorth, R., Sharma, A.K., Graupner, A. et al. (2023) Assessing testicular germ cell DNA damage in the comet assay; introduction of a proof-of-concept. Environmental and Molecular Mutagenesis, 64(2), 88–104. https://doi.org/10.1002/em.22527

[6] OECD. Work plan for the Test Guidelines Programme (TGP). As of June 2022

1.3.2. TGR assay

15 In case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats.

16 Also, according to the test method OECD TG 488, the test substance is usually administered orally.

17 Based on OECD TG 488, you are requested to follow the 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals.

18 According to the test method OECD TG 488, the test must be performed by analysing tissues from liver, as slowly proliferating tissue and primary site of xenobiotic metabolism, and from glandular stomach and duodenum, as rapidly proliferating tissue and site of direct 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 mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum must be stored (at or below −70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed, only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

1.3.2.1. Germ cells

19 You may consider collecting the male germ cells (from the seminiferous tubules) at the same time as the other tissues, to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA to decide on the need for assessment of mutation frequency in the collected germ 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.

20 In your comments to the proposal for amendment from a member state you agreed with the collection and storage of male germ cells for the TGR.

2. Growth inhibition study aquatic plants

21 Growth inhibition study aquatic plants is an information requirement under Annex VII to REACH (Section 9.1.2.).

2.1. Information provided

22 You have provided the following information on the Substance:

(i) a growth inhibition study on algae according to EC Directive 79/831 EEC, Annex V, Section C (1990)

(ii) a growth inhibition study on algae according to OECD TG 201 (2003)

(iii) a growth inhibition study on algae according to OECD TG 201 (1998)

2.2. Assessment of the information provided

23 To fulfil the information requirement, a study must comply with OECD TG 201 and the requirements of OECD GD 23 (ENV/JM/MONO(2000)6/REV1) if the substance is difficult to test (Article 13(3) of REACH). Therefore, the following specifications must be met:

24 Technical specifications impacting the sensitivity/reliability of the test

a) for *Desmodesmus subspicatus*, the initial cell density is 2-5 x10^3 cells/mL;

However, for study i., you report that the initial cell density was 3.2 x10^4 cells/mL.

b) one of the two alternative growth medium (*i.e.* the OECD or the AAP medium) is used. Any deviations from recommended test media must be described and justified;

However, for study ii., you specified that the test medium used was AAM (algal assay medium). You have not provided a justification as to why a non-standard medium was used (including for instance the composition of the AAM medium)

c) if a solvent is used, its concentration is ≤ 100 µg/L;

For study i., you report that "*the stock solution of nominal 200 mg/l was prepared using 40 mg/l Cremophor RH 40 as a solvent*". The highest dose tested is 100 mg/L which corresponds to a solvent concentration of 20 mg/L.

25 Characterisation of exposure

d) the concentrations of the test material are measured at least at the beginning and end of the test:

- at the highest, and
- at the lowest test concentration, and
- at a concentration around the expected EC_{50}.

e) For volatile, unstable or strongly adsorbing test substances, additional samplings for analysis at 24 hour intervals is required.

f) the results can be based on nominal or measured initial concentration only if the concentration of the test material has been maintained within 80 to 120 % of the nominal or measured initial concentration throughout the test;

However, for study i., no analytical monitoring of exposure concentration was conducted. While, for study ii., you have reported that the concentration of the test material was determined only at t=0h and t=96h. At 96h, no quantifiable concentrations of n-valeraldehyde could be measured. No sampling at 24 hours intervals was conducted. For study iii., you report that it is not specified if an analytical determination of exposure concentration was conducted and you report no results of measured concentrations during the test.

26 Reporting of the methodology and results

g) the test design is reported (*e.g.*, number of replicates, number of test concentrations and geometric progression used);

However, for study iii., you have provided no information on the test design.

h) the test conditions are reported (*e.g.*, composition of the test medium, test temperature, test species, biomass density at the beginning of the test);

However, for study iii., you have provided no information on the test design. Furthermore for study i., you have not provided the test medium composition.

i) the method for determination of biomass and evidence of correlation between the measured parameter and dry weight are reported;

However, for study iii., this information is not reported.

j) the results of algal biomass determined in each flask at least daily during the test period are reported in a tabular form;

However, for study i. and iii., you have not provided this information. In addition, for study ii., you have only provided mean concentrations among replicates.

27 Other considerations

k) Algal biomass is determined based on dry weight per volume, or alternatively as cell counts or biovolume using microscopy or an electric particle counter. If an alternative method is used (*e.g.* flow cytometry, *in vitro* or *in vivo* fluorescence, or optical density), a satisfactory correlation with biomass must be demonstrated over the range of biomass occurring in the test.

For study i., you report that algal biomass was determined using a fluorimeter. However, you have not reported evidence of correlation between the measured parameter and dry weight;

28 Based on the above,

- the Substance is difficult to test due to its high volatility and there are critical methodological deficiencies resulting in the rejection of the study results. More, specifically, no analytical monitoring was performed in studies i. and iii. and the sampling frequency was insufficient for study ii. In addition, no indications on preventive action to avoid loss of the Substance during the test for any of these studies. Therefore you have not demonstrated that effect values can be reliably expressed based on nominal concentrations for study i. and iii. and that mean concentration measured at t=0h and t=96h in study ii. provide a reliable basis to express effect values.
- the reporting of the study is not adequate. More, specifically, as explained above the reporting of study iii. is insufficient to make an independent assessment of this study. In addition for study i., you have not demonstrated that the test medium and the method used for biomass determination were adequate. Finally you have either provided no reporting of measured biomass values (studies i. and iii.) or insufficient data (study ii.). Therefore, it cannot be verified whether the study meets the validity criteria of OECD TG 201 and provides a reliable basis to determine effect values.

29 Therefore, the requirements of OECD TG 201 are not met in any of these studies.

30 On this basis, the information requirement is not fulfilled.

31 In your comments to the draft decision, you state that you "*see the need to improve the database through a new guideline test*".

2.3. Study design

32 The Substance is difficult to test due to the high volatility (vapor pressure of 50 hPa). OECD TG 201 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. Due to the properties of Substance, it may be difficult to achieve and maintain the desired exposure concentrations. Therefore, you must monitor the test concentration(s) of the Substance throughout the exposure duration and report the results. If it is not possible to demonstrate the stability of exposure concentrations (i.e. measured concentration(s) not within 80-120% of the nominal concentration(s)), you must express the effect concentration based on measured values as described in OECD TG 201. In case a dose-response relationship cannot be established (no observed effects), you must demonstrate that the approach used

to prepare test solutions was adequate to maximise the concentration of the Substance in the test solution.

References

The following documents may have been cited in the decision.

Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)

Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

Guidance on data-sharing; ECHA (2017).
Guidance for monomers and polymers; ECHA (2012).
Guidance on intermediates; ECHA (2010).
All guidance documents are available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

RAAF, 2017 Read-across assessment framework (RAAF); ECHA (2017).
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs; ECHA (2017).

The RAAF and related documents are available online: https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

***OECD Guidance documents* (OECD GDs)**

OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

Appendix 2: Procedure

This decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 04 May 2021.

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

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

ECHA took into account your comments and amended the requests as follow:

- the request for Skin sensitisation (Annex VII, Section 8.3.) was removed
- the request for *In vitro* micronucleus study (test method: OECD TG 487) was removed
- the request for *In vivo* mammalian alkaline comet assay (triggered by Annex VII, Section 8.4., Column 2; test method: OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) was changed to In vivo mammalian alkaline comet assay (test method: OECD TG 489) or Transgenic rodent somatic and germ cell gene mutation assays (test method: OECD TG 488) (triggered by Annex VII, Section 8.4., column 2).

As you are no longer requested to conduct an *in vitro* micronucleus study prior to conducting the requested *in vivo* mutagenicity study, ECHA has amended the deadline from 36 to 30 months.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s) and referred the modified draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee unanimously agreed on the draft decision during its MSC-83 meeting. ECHA adopted the decision under Article 51(6) of REACH.

Appendix 3: Addressees of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

Registrant Name	**Registration number**	**Highest REACH Annex applicable to you**
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.

Appendix 4: Conducting and reporting new tests for REACH purposes

1. Requirements when conducting and reporting new tests for REACH purposes

1.1. Test methods, GLP requirements and reporting

(1) 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.

(2) 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.

(3) 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[7].

(4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design must ensure that the data generated are adequate for hazard identification and risk assessment.

1.2. 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
- 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.
- 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.

[7] https://echa.europa.eu/practical-guides

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 on How to prepare registration and PPORD dossiers[8].

[8] https://echa.europa.eu/manuals