Comments to Public Consultation on Proposal for Harmonised Classification and Labelling

Substance: 2-ethylhexyl (2E)-3-(4-methoxyphenyl)acrylate
EC Number: 629-661-9
CAS Number: 83834-59-7

Submitter: [redacted]

In response to the public consultation, [redacted] would like to submit the following comments also on behalf of [redacted] to the proposal for Harmonised Classification and Labelling submitted by BAuA, the Dossier Submitter (DS). We strongly disagree with the basis of the arguments and conclusion of the proposed hazard categories. Our rationale and comments are summarized below and developed in further detail within this document.

Key comments

1. The reliability of the different studies reviewed was not assigned in line with the latest recommendations of ECHA's "Guidance on information requirements and chemical safety assessment. Chapter R.4: Evaluation of available information" and some assigned **Klimisch scores seem to have been established inconsistently**.

2. **The two studies selected as key to determine the environmental hazard classification and categories are unreliable and therefore, cannot be used for Classification and Labelling purposes**. In some cases, the DS indicated that the methods used were NOT reviewed at all, therefore the reliability of those studies could not have been assessed appropriately.

3. During the **Substance Evaluation process, the studies considered as key in the CLH report were considered as not sufficiently reliable** (and those same studies are considered as unreliable and disregarded in the registration dossier accordingly) and new studies were specifically required to cover these endpoints. However, in this new process those studies remain unconsidered although it is acknowledged that their reliability is superior.

4. **The fully reliable studies (Klimisch 1 as assigned by the CLH dossier submitter (DS)) available indicate that no classification is warranted for acute aquatic hazards and that in the case of the chronic aquatic classification, a category 2 is the most appropriate.**

Detailed comments

All references are cited as in the CLH report submitted by Baua.

1. **Acute aquatic hazard**

ECHA's Guidance on information requirements and chemical safety assessment. Chapter R.4: Evaluation of available information (December 2011) includes a section describing how the relevance and reliability of the information should be assessed.

The Dossier Submitter (DS) assigned a Klimisch score of 2 to all results available from the publication of Paredes et al. (2014). The Guidance document indicates the following about the Klimisch score of 2:

'2 = reliable with restrictions: "studies or data [...] (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable."'

Additionally, the Guidance on IR&CSA, Chapter R.4 section R.4.2 (page 4/5) on reliability says:

"*The following are key points that an assessor should consider when evaluating data reliability:*

- *The proven ability of the laboratory to perform the test method;*
- *The purity/impurities and origin of the test substance, as well as the reference substances, must be reported;*
- *The availability of the raw data from the study;*
- *There must be an adequate description of the study e.g. a complete test report, or a sufficiently detailed description of the test procedure, which must be in accordance with generally accepted scientific standards. In these cases, the information may be considered reliable;*
- *When the test procedure used to generate the test data is found to differ significantly from that described by the recognised test method or generally accepted scientific standards, or the reliability of the data cannot be established fully, the assessor must decide if and how the information can be used, e.g. as supporting information where a reliable study already exists.*

  The following factors, inter alia, can be used to support the view that these data may be acceptable for use in meeting the requirements of REACH:
  - *there are other studies or calculations available on the substance, and the data under consideration are consistent with them,*
  - *other studies are available, for example on isomers with similar structure activity profile, homologues, relevant precursors, breakdown products or other chemical analogues, and the data under consideration are consistent with them,*

- *an approximate value is sufficient for taking a decision on the endpoint of interest for the conclusion required by REACH;*

- *Where critical supporting information is not reported (e.g. species tested, substance identity and dosing procedure) the test data should be considered to be unreliable for the purposes of REACH.*

In principle, the same criteria apply to test data reported in the published literature; the extent of the information provided will provide the basis for deciding upon the reliability of the data reported. In general, publications in peer-reviewed journals are preferable to those which are not. High-quality reviews, summaries or abstract publications may be used as supporting information" (emphasis added).

While it is acknowledged that this guidance relates to the information requirements under REACH and not necessarily classification and labelling under CLP, it makes sense that the same evaluation is made as the purpose of filling the data requirements under REACH is to establish the classification and labelling of a substance in accordance with the CLP to be able to assess the risk to Human Health and the Environment (Annex I, Articles 1.0.1., 2.1. and 3.0.1. of REACH). Therefore, it would not be understandable if a study considered as unreliable under REACH would still be considered acceptable in the context of the CLP. This would also lead to legal uncertainty and incoherent assessment under different pieces of European legislations, while the two under consideration here are intrinsically interlinked.

In the light of the above, the result highlighted as "relevant for classification and labelling" in Table 8 of the CLH report, i.e., the 72h-EC50 value of 0.075 mg/L reported for *Isochrysis galbana* by Paredes et al (2014) presents a number of elements indicating that this study cannot be assigned a reliability score of 2 due to not meeting the scientific standards required. These elements are:

- Lack of standardized guideline and no positive or negative control data to prove the ability of the lab to perform the test.
- Unclarity on how many test concentrations were used, and why only a part of them were analytically verified.
- There is no information on purity of the material that was used and/or on the impurity profile.
- No description on how the stock solutions were prepared. The OECD GD 23 has a detailed list of options to deal with poorly-water soluble substances to make sure they are properly solubilized in solutions, but there is no reporting of the method applied in this study.
- Complete loss of the substance over the duration of the study.
- The Statistical analysis section mentions that "All data were corrected by the control response". If that is the case, the subsequent comparison with the control data seems biased.
- Figure 1 of Paredes et al (2014) raises a number of questions:

- It includes 7 data points for the Substance concerned but only three concentrations were tested (point above), therefore it is unclear what served as a basis for the curve and the determination of the EC50. The method applied to determine the EC50 is not provided. And if more concentrations were tested than those for which analytical verification is available, considering the losses observed and the requirement of the OECD GD 23 to proceed with analytically measured concentrations in these situations, the data points used to determine the EC50 cannot be adequate.
- The Y-axis mentions % of growth rate and the X-axis mentions log (D µg/L+1) without defining what D is. If one assumes that D is the concentration, and the Y-axis is the % of growth rate compared to the control, the visual interpretation of the figure leads to the conclusion that the highest concentrations lead to the highest growth rate, i.e., there is a positive effect of the Substance (labelled as EHMC in the publication) on the growth rate.
- The standard deviation of the 5th data point spans almost the entire range of the Y-axis also raising questions about the data. And the standard deviations of the first and last data points are cut out of the chart.

- No information on the validity criteria or controls is provided in the paper, including no indication of a solvent control to consider the possible effects of DMSO.
- No information on the concentration of DMSO in the final solutions with indications that seem to point to different concentrations in the different treatments.

The lack of analysis and the lack of information about validity criteria were considered as issues with the study from Sieratowicz et al (2011) which was considered unreliable (Klimisch 3) by the DS. The same information is missing in Paredes et al (2014) on top of other major key points missing as cited above. Moreover, the EC50 value reported is above the water solubility limit (in water but also in media used for ecotoxicological studies). This outcome was also considered as sufficient to conclude that a study was unreliable (Klimisch 3) by the DS (e.g., Notox B.V., 2000c). Therefore, it is unclear why this study would be considered as reliable with restrictions. It should be concluded as unreliable (Klimisch 3) in line with the assessment of the other studies by the DS and therefore, not used for Classification and Labelling purposes.

Nevertheless, the Guidance mentions that a study which lacks information could be considered <u>if other data are available and the results are consistent</u>. However, Table 11 of the CLH report (Summary of available toxicity data for algae) clearly shows that no other study shows the same range of effect on algae and that ALL EC50s (without any clarification on whether they relate to growth rate or not, in particular for Rodil et al (2009)) are above the water solubility limit of the substance (0.051 mg/L). This is further supported by the only two studies investigating the acute effects of the substance considered as reliable without restrictions (Reliability 1) by the DS (Fort Environmental Laboratories, 2021b (aquatic plants) and BASF AG, 2003 (invertebrates)) which show that there is no toxicity at the limit of solubility of the substance. Therefore, this further supports that the results of Paredes et al

(2014) are unreliable and inadequate for classification and labelling, the effects seen could be the results of inadequate procedures as described above.

As a matter of fact, a third study investigating the acute aquatic effects of the substance should be considered as reliable without restrictions, the study cited as Notox B.V., 2000a. A reliability score of 3 was assigned to the study in the CLH report due to the number of fish used per concentration. However, the DS assessed the study as if it was a full OECD TG 203 study whereas it was clarified (and acknowledged by the DS, page 9) that the test was a combined range-finder/limit test. Therefore, only the highest dose and the control had the required number of fish (7) and the test meets the test guidelines requirements as a limit test. The additional concentrations were informative as part of the range-finder. The limited number of fish (3) in the 0.1, 1 and 10 mg/L do not constitute deviations from the guideline as they are part of the range-finding test. The definitive test was the limit test using the 100 mg/L loading rate which had 7 fish as required. Therefore, the score of 3 is not justified and a reliability of 1 is indeed applicable as in the REACH dossier. This score is also supported by the fact that the substance identity, the proven ability of the lab to perform the test, the detailed description of the test, the species, the dosing procedure, etc. were all provided and available.

With this re-evaluation of the reliability scores, fully reliable and compliant data are available for all three trophic levels, fish (Notox B.V., 2000a), invertebrates (BASF AG, 2003), and aquatic plants (Fort Environmental Laboratories, 2021b). All three indicate that in studies conducted according to OECD test guidelines & quality control frameworks in place (e.g. GLP), respecting all considerations of the OECD GD 23 for poorly-water soluble substances, with analytical measurements of the concentrations, in labs with proven record of capability, with a defined test material, the Substance (OMC) shows no effect at the limit of its water solubility and hence no acute aquatic classification is warranted.

Moreover, the reliability score of 2 for some other experiments extracted from Paredes et al. (2014) raise questions. Indeed, for example, in the case of the study conducted with *Siriella armata*, the DS states the following (page 10 of the CLH report): "*The test was performed according to a method described in another reference (Pérez et al., 2010a), which has not been reviewed by the Dossier Submitter. [...] The paper contains limited information about the test methods, validity criteria and controls. [...] As the study documentation is acceptable for assessment, the reliability was rated with Klimisch 2*" (emphasis added). It is difficult to understand how the DS can conclude on the reliability of the study at all when the DS states that the method was not reviewed and when they acknowledge that some of the most critical information (e.g., validity criteria) is missing. The same applies to the study conducted with *Paracentrotus lividus* and *Mytilus galloprovincialis* for which the methods were also not reviewed and where the results indicate a significant misalignment (page 11 of the CLH report – *M. galloprovincialis "method [...] has not been reviewed [...] NOEC is above the EC10 [...]"; P. lividus "[...] method [...] which has not been reviewed [...] NOEC that is markedly higher than the EC50 [...]"*. The fact that the different experiments conducted by Parades et al. (2014)

show distinct flaws in the procedures used further casts doubt on the results obtained for *Isochrysis galbana*.

The uncertainty about the results from Paredes et al (2014) was further highlighted by ECHA and the eMSCA (the UK at the time) in the context of the first decision related to the Substance Evaluation (Decision of April 6th 2018[1], page 13) of the Substance: "*ECHA is uncertain whether these data are of sufficient quality to use for a definitive PNEC, for example they lack measured substance concentrations*".
To clarify the toxicity of the Substance to algae, in the first decision a new algae test was requested (page 14, "*Overall ECHA does not consider that an aquatic PNEC can be derived with confidence using the current data, but there are concerns that the value of the PNEC may be much lower than you currently estimate. Therefore, as a starting point, ECHA requires you to provide new ecotoxicity data: a 21-d Daphnia magna reproduction toxicity study and an algal growth inhibition study. Together these will provide reliable aquatic ecotoxicity information for these two endpoints, and the results can be used to determine an aquatic PNEC (together with information for fish toxicity requested in this decision*", emphasis added). Furthermore, the SEv Decision says (page 14): "*ECHA notes the difficulties in maintaining the concentration of the test substance, particularly in the available algal study included in the registration dossiers. This may be due (at least in part) to the methodology used for that test. However, if you find that concentration maintenance in the study is not feasible, you may alternatively choose to perform a 7-d Lemna growth inhibition test (OECD TG 221), since this can also be performed using semi-static or flow-through conditions.*"

This new test on aquatic plants (with *Lemna minor*) using a semi-static test design, an option put forward as a better alternative to allow for a relevant exposure in the SEv Decision, was conducted and made available (in addition to the two other tests requested, i.e., the long-term toxicity studies on *Daphnia magna* and fish).

The doubts surrounding the results of Paredes et al. (2014) were reiterated in the Substance Evaluation Conclusion and Evaluation Report[2] (eMSCA DE) in which it is stated "*Without better information on the controls and validity criteria, it is not possible to use the data as they stand for firm PNEC derivation*".

In light of this, it is unclear why the *Lemna minor* study which was conducted specifically to answer to the doubts related to the Paredes et al. (2014) study is not used as the key study to conclude on the aquatic hazards. If the Paredes et al. (2014) results are considered unreliable to conduct a risk assessment, they should not be considered acceptable to conclude on the hazard assessment either.

[1] https://echa.europa.eu/documents/10162/88e0cc0b-d64c-1b9e-d706-61c0cce731da
[2] https://echa.europa.eu/documents/10162/9a3674a2-1ba3-626d-3628-21024ce704d2

In view of the examples shown above (and other cases within the CLH report not mentioned here), it is clear that the reliability assessment of the different studies was not applied consistently and transparently. Before moving further with a harmonized classification and labelling for OMC, it would be of importance to re-do the assessment of the different studies based on the objective criteria of the Guidance on IR&CSA, Chapter R.4 and to have an objective, clear and transparent assessment of each study to obtain a reliable and repeatable weight-of-evidence conclusion for this substance.

The Substance Evaluation process requested new information specifically to address the shortcomings of the studies which are now put forward as key studies to conclude on the hazard assessment. It is confusing that the studies required are now not considered in this new process. All the fully reliable studies indicate that no classification for acute aquatic hazard is warranted. These studies are the key studies to conclude that no acute aquatic hazard classification is warranted. The publication by Paredes et al. (2014) is not reliable and is not fit-for-purpose to conclude on Classification and Labelling.

2. Long-term aquatic hazard

The DS relies on the study by Zhou et al. (2019b) to conclude on the long-term aquatic toxicity of the Substance. In the CLH report, the DS reports that the concentrations were not analytically verified (page 16) but that one could assume that the concentrations were maintained as reported by another study by the same authors. Considering that this is an assumption by the DS, the reliability score of 2 assigned by the DS can be questioned.

Moreover, the hatching rates were unaffected in another study included in the CLH report (Fort Environmental Laboratories Inc., 2020b) conducted with the same species, according to a standard test with a fully detailed report which was rated as Klimisch 1, hence more reliable than that of Zhou et al. (2019b). In view of this, the results of the latter which were obtained with a non-standard test, and which lacks information on the exact methods applied, the measured concentrations, the proven ability of the lab to perform the test, the validity criteria, etc. should be re-assessed and balanced with the other information available for the same species as required in a weight-of-evidence approach. The authors of the publication themselves note that there were technical issues in their experiment as they state that the Substance was detected in the control fish and that could have been due "*to the incomplete cleaning of the instrument or the mistakes in the operation*". Therefore, it is unclear if other mistakes could have impacted the results. Finally, no descriptors (EC50, NOEC, LOEC) were reported in the publication and they are assumptions from the DS.

Furthermore, while the authors themselves did not mention conducting the study according to the OECD TG 234, the DS highlighted that when the data is compared to that guideline, the validity criteria when it came to the hatching rates of the controls was met. Therefore, the DS considers that the OECD TG 234 is relevant and that parameters of the publications

should be compared to it. In that context, there were a number of parameters for which the study did not respect the recommendations of that same guideline when conducting the study with *Danio rerio* (zebrafish), the species considered here:

- the volume of water was significantly lower than that recommended in the OECD TG 234. Indeed, the publication reports a volume of solution of 50 mL when the OECD TG requires a minimum of 7 L (7000 mL).
- The guideline also requires a minimum of 120 eggs per treatment whereas only 50 in total for all 5 treatments (control, solvent control, 1, 10 and 100 µg/L; 3 replicates per treatment) were used which results in only 3 eggs per replicate, 9 eggs per treatment. The impact of such a low number of eggs used could be not negligible also considering the "*mistakes in the operation*" reported above. A mistake could have resulted in a strong impact.
- There were also only 3 replicates per treatment instead of 4.
- Finally, the OECD TG 234 includes other test acceptability criteria such as a wet weight of more than 75 mg and a length of more than 14 mm for the controls, both criteria are not met in the present study.

In the CLH report two other studies were considered as Klimisch 3 due to missing replicates and/or what was considered as a number of organisms lower than requested by the TG (Notox B.V., 2000a and Sieratowicz et al. 2011). Therefore, for consistency, this study should also be considered unreliable as there were not enough replicates AND not enough eggs tested, and other issues as reported above. The publication of Zhou et al (2019b) would therefore be assigned a Klimisch score of 3, rendering it disregarded for Classification and Labelling purposes.

Finally, the eMSCA in its Conclusion Document in the Substance Evaluation[2] process, which is the same entity as the DS, considered that the key value from Zhou et al (2019b) was a NOEC of 0.01 mg/L whereas in the CLH report, the key value is now a NOEC of 0.001 mg/L. It is unclear why that has changed as no specific value is reported in the publication.

Consequently, considering the number of issues related to the way the study by Zhou et al (2019b) was conducted, the reliability of 2 is inappropriate. It should be assigned a score of 3 and not be used for Classification and Labelling.

In view of the shortcomings above, a review of the reliability scores should be undertaken and the results observed in the different studies reconsidered with more caution based on the number of issues in the experimental designs. There are also issues with other studies such as that of Sieratowicz et al. (2011) where a reliability of 2 is assigned although the DS underlines that there is an issue with the solvent from which an impact cannot be excluded.

Following the initial decision of the Substance Evaluation, a fully compliant OECD TG 234 study (Fort Environmental Laboratories Inc. 2020b) was conducted according to the OECD

test guideline and with quality control frameworks in place (e.g. GLP), respecting all considerations of the OECD GD 23 for poorly-water soluble substances, with analytical measurements of the con**centrations, in a lab with proven record of capability, with a defined test material, the Substance (OMC) showed a LOEC of 46.9 µg/L which leads to a classification as Aquatic Chronic Category 2. This study was recognized as fully reliable (Klimisch 1) by the DS. This study should be used for concluding on the hazard assessment of the Substance. There are also two other studies considered as Klimisch 1 studies in the CLH report investigating long-term aquatic toxicity: Fort Environmental Laboratories Inc, 2020a (OECD TG 231) and 2021a (OECD TG 211). Those two studies showed no toxicity at the limit of solubility under the test conditions.**

3. Conclusion

Based on the above, and as submitted as part of our registration dossier, the appropriate and relevant classification for OMC is Aquatic Chronic Category 2.