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Instructions for evaluating the reliability and relevance of ecotoxicity and nano-ecotoxicity studies using the CRED tool (Moermond et al. 2016), the NanoCRED tool (Hartmann et al. 2017), and the EthoCRED tool (Bertram et al. 2024) available at scirap.org.

Evaluating the study

Please use the respective Excel file available at www.scirap.org.

When evaluating the study, indicate how well each criterion is met by selecting an alternative from the drop-down menu to the right of each criterion. In the EVALUATION RESULT column (Fig. 1), choose between “Fulfilled”, “Partially fulfilled”, “Not fulfilled”, and “Not reported”.

No.	RELIABILITY	EVALUATION RESULT	COMMENT
Test setup			
1	Is a guideline method (e.g., OECD/ISO) or modified guideline used? (of minor importance for study reliability)	fulfilled	
2	Is the test performed under GLP conditions? (of minor importance for study reliability)	partially fulfilled	
3	If applicable, are validity criteria fulfilled (e.g. control survival, growth)?	not fulfilled	
4	Are appropriate controls performed (e.g. solvent control, negative and positive control)?	not reported	
Test compound			
5	Is the test substance identified clearly with name or CAS-number? Are test results reported for the appropriate compound?	REMOVE	
6	Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	fulfilled	
7	If a formulation is used or if impurities are present: Do other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	partially fulfilled	
Test organism			
8	Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clone, sex, if	REMOVE	

Fig. 1 Drop-down menu for the criteria in Reliability sections of the CRED tools.

Guidance from Moermond et al. (2016) for the CRED tool, Hartmann et al. (2017) for the NanoCRED tool, and Bertram et al. 2024 for the EthoCRED tool is provided by pointing to the criterion with the cursor (the criterion containing guidance has a red right corner, Fig. 2).

No.	RELIABILITY	EVALUATION RESULT	COMMENT
Test setup			
1	Is a guideline method (e.g., OECD/ISO) or modified guideline used? (of minor importance for study reliability)	fulfilled	
2	Is the test performed under GLP conditions? (of minor importance for study reliability)	partially fulfilled	
3	If applicable, are validity criteria fulfilled (e.g. control survival, growth)?	not fulfilled	
4	Are appropriate controls performed (e.g. solvent control, negative and positive control)?	not reported	
Test compound			
5	Is the test substance identified clearly with name or CAS-number? Are test results reported for the appropriate compound?	REMOVE	Guidance: In most test guidelines, validity criteria are provided to determine the validity of the test results. For instance, OECD guideline 201 on algal toxicity requires exponential growth in the controls and specifies criteria for the variation in growth rate within and between control replicates. For the Daphnia acute toxicity study, the validity criteria in the OECD 202 guideline include control mortality and oxygen concentrations. Besides this, control organisms should be from the same population as the treatment group(s), variability in the controls should fall within the same range as historical data, and attention should be given to natural fluctuations in results, such as fluctuations attributable to the age of the animals or seasonal influences. If a nonguideline test is performed with a guideline species, validity criteria as described in the relevant guideline should be met. If nonguideline species are used, expert judgment is needed to assess whether the test organism resembles the guideline test species enough to apply guideline validity criteria. Otherwise, expert judgment is needed to decide if control survival and/or other parameters are within the range of what is normal for the species and that other confounding (stress) factors can be ruled out. For guideline test species, however, complying with guideline criteria for validity (e.g., control survival, growth) is critical for a study to be reliable.
6	Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	fulfilled	
7	If a formulation is used or if impurities are present: Do other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	partially fulfilled	
Test organism			
8	Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clone, sex, if	not reported	
9	Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	not reported	
Exposure conditions			
10	Is the experimental system appropriate for the test substance, taking into account its physico-chemical characteristics?	REMOVE	
11	Is the experimental system appropriate for the test organism (e.g., choice of medium or test water, feeding, water characteristics, temperature, light/dark conditions, pH, oxygen content)? Have conditions been stable during the test?	fulfilled	

Fig. 2 Guidance for evaluating each criterion in the CRED tools.

Motivations and notes can be added in the "COMMENT" column (Fig. 3).

No.	RELIABILITY	EVALUATION RESULT	COMMENT
Test setup			
1	Is a guideline method (e.g., OECD/ISO) or modified guideline used? (of minor importance for study reliability)	fulfilled	
2	Is the test performed under GLP conditions? (of minor importance for study reliability)	partially fulfilled	
3	If applicable, are validity criteria fulfilled (e.g. control survival, growth)?	not fulfilled	
4	Are appropriate controls performed (e.g. solvent control, negative and positive control)?	not reported	WRITE A NOTE HERE!
Test compound			

Fig. 3 Writing a note in the "COMMENT" column.

Removing criteria

Criteria that are not applicable to the specific study or question being assessed may be removed from the evaluation by clicking "REMOVE" in the EVALUATION RESULT column. Motivations for removing criteria can be given in the comment fields. Please note that removing criteria will affect the colour profile and score, and this may be important to consider when comparing studies within the same study design.

Interpreting the results

The results of the study assessment are shown below the relevance section of the CRED tools. In the colour profile (Fig. 4), the evaluations of reliability and relevance are illustrated in bar charts, showing green for fulfilled criteria, yellow for partially fulfilled and red for criteria that were not fulfilled. Criteria that were "not reported" are shown as grey. The bar charts do not include criteria that have been removed.



Fig. 4 The evaluations of reliability and relevance are illustrated in bar charts.

Assigning the study to reliability and relevance categories

The result of the evaluation can be used, in combination with expert judgment, as basis for assigning studies into different reliability and relevance categories. The following categories are suggested:

a. Reliability categories – CRED and EthoCRED

- *Reliable without restrictions:* All critical reliability criteria for this study are fulfilled. The study is well designed and performed, and it does not contain flaws that affect the reliability of the study.
- *Reliable with restrictions:* The study is generally well designed and performed, but some minor flaws in the documentation or setup may be present. *Not reliable:* Not all critical reliability criteria for this study are fulfilled. The study has clear flaws in study design and/or how it was performed.
- *Not reliable:* Not all critical reliability criteria for this study are fulfilled. The study has clear flaws in study design and/or how it was performed.
- *Not assignable:* Information needed to make an assessment of the study is missing. This concerns studies that do not give sufficient experimental details and that are only listed in abstracts or secondary literature (books, reviews, etc.) or studies of which the documentation is not sufficient for assessment of reliability for one or more vital parameters.

b. Reliability categories - NanoCRED

- *Reliable without restrictions:* All critical and important reliability criteria are fulfilled or partially fulfilled. The study is well designed, performed and documented. Nanomaterial properties and behaviour in the test system is extensively documented. The experiment has been carried out according to methods that are considered scientifically appropriate for ecotoxicity testing of nanomaterials and where the physicochemical properties of the nanomaterial are considered in the test design. If (when) specific nanomaterial guidance or guidelines exist, the use of these may be considered favourable.
- *Reliable with restrictions:* Most critical and important criteria are fulfilled or partially fulfilled. The study is generally well designed, performed and documented, but some minor flaws in the documentation or setup may be present. Nanomaterial properties and behaviour in the test system is well documented. The experimental design and test method are considered scientifically appropriate for ecotoxicity testing of nanomaterials but may contain some minor flaws in documentation or setup.
- *Not reliable:* Not all critical reliability criteria are fulfilled or partially fulfilled. This mainly concerns studies which have clear flaws in study design and study conduction, and/or where the experimental design and test method are considered not to be scientifically appropriate for ecotoxicity testing of nanomaterials.
- *Not assignable:* Information needed to make an assessment of one or more critical and important criteria is missing. This concerns studies or data from the literature which do not give sufficient experimental details, or reports where the documentation is not sufficient for assessment of reliability for one or more critical parameters.

c. Relevance categories – all substances

- *Relevant without restrictions:* The study is relevant for the purpose for which it is evaluated.
- *Relevant with restrictions:* The study has limited relevance for the purpose for which it is evaluated.
- *Not relevant:* The study is not relevant for the purpose for which it is evaluated.
- *Not assignable:* Studies that do not give sufficient details since the result is presented in abstracts or secondary literature (books, reviews, etc.) or studies of which the documentation is not sufficient for assessment of relevance for one or more vital parameters.

Contact

For questions or comments, please contact Marlene Ågerstrand, Department of Environmental Science, Stockholm University, marlene.agerstrand@aces.su.se.

References

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