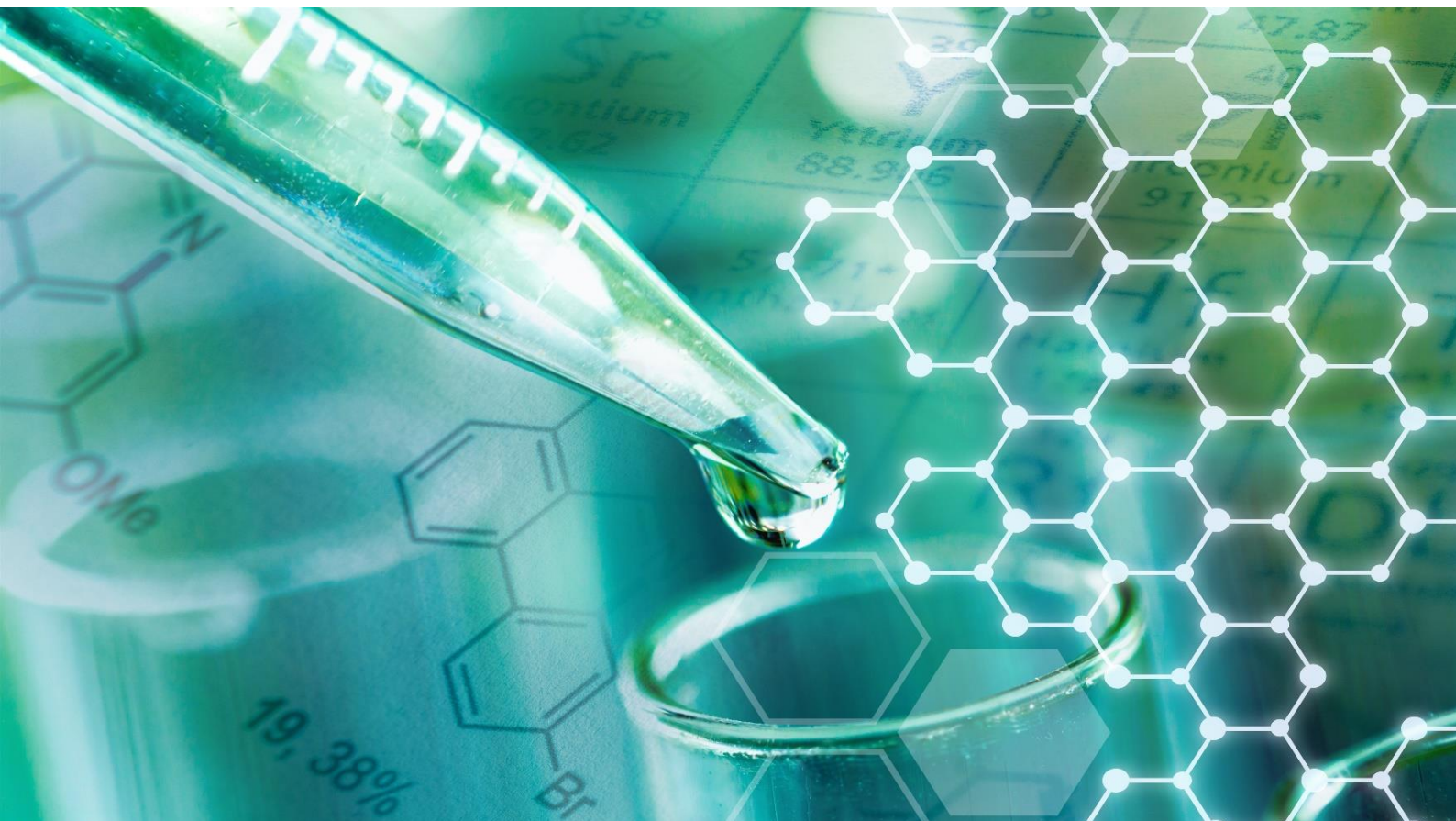




# Peer Review Report on Inclusion of OptiSafe Eye Irritation Test (EIT) in Test Guideline 496 on In Vitro Macromolecular Test Methods for Identifying the Eye Hazard Potential of Chemicals

Series on Testing and Assessment No. 400



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# Foreword

This document encloses the Peer Review Report (PRR) of the validation study for OptiSafe™ Eye Irritation Test (EIT) for its inclusion in OECD Test Guideline 496 on In Vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage (UN GHS Cat.1) and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage (UN GHS No Cat). The OptiSafe™ EIT is validated for identifying UN GHS No Cat.

The Peer Review was organized by the OECD Secretariat from November to December 2022 based on evaluation principles from OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment.

The peer review report was endorsed by the Working Party of the National Coordinators of the Test Guidelines Programme (WNT) at their 36<sup>th</sup> meeting in April 2024. This document is published under the responsibility of the Chemicals and Biotechnology Committee.

# Peer Review Report on inclusion of OptiSafe™ EIT in Test Guideline 496 on In Vitro Macromolecular Test Methods for Identifying the Eye Hazard Potential of Chemicals

## 1. Summary

In April 2022, WNT approved the Standard Project Submission Form (SPSF) submitted by the U.S on inclusion of OptiSafe™ as a me-too test method in TG 496: *In Vitro* Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals not Requiring classification for Eye Irritation or Serious Eye Damage.

The OptiSafe™ had been validated by the validated management team (VMT) comprised of experts within NICEATM, and the validation report was published in a peer-reviewed journal (1).

The OptiSafe™ Test Method was presented and discussed at the teleconferences of OECD Expert Group on Skin and Eye Irritation on July 15<sup>th</sup> and September 26<sup>th</sup>, 2022. The summary records from these teleconferences are available on the restricted community site for the OECD Expert Groups ([Link](#)).

The OECD Secretariat organized an independent Peer Review with a panel comprised of members of the OECD Expert Group on Skin and Eye Irritation during November – December 2022. The results from the Peer Review were put together for a Peer Review Report (PRR) contained in this document.

## 2. Background

The OptiSafe™ Test Method uses a similar test platform as the Validated Reference Method (VRM) in TG 496, which contains a macromolecular reagent composed of a mixture of proteins glycoproteins, carbohydrates, lipids, and low molecular weight components, that when rehydrated forms a complex macromolecular matrix which mimics the highly ordered structure of the transparent cornea (2).

The OptiSafe™ Test Method exhibits a few distinctions when compared with the VRM in TG 496. These deviations include a few differences in the test method components and protocols, and the hazard categories that the test method is intended for predictions. For the intended

hazard categories, the VRM is recommended for identifying chemicals that induce serious eye damage (i.e., UN GHS Category 1) and for chemicals that do not require classification (i.e., UN GHS No Category), while the OptiSafe™ is recommended for chemicals that are not UN GHS Category 1 (i.e., chemicals that do not cause serious eye damage) and for chemicals that do not require classification. Finally, the prediction model for the OptiSafe™ is presented for three hazard categories (i.e., UN GHS Category 1, Category 2 and No Category).

### 3. The peer review process

The secretariat solicited participations from the OECD Expert Group on Skin and Eye Irritation to serve as a member of Peer Review Panel for the OptiSafe™ Test Method in October 2022. The Peer Review Panel (PRP) was confirmed on 21 October 2022. The selected members of the Panel are listed in Annex I.

The secretariat drafted 8 Peer Review charge questions based on the validation principles outlined in OECD Guidance Document 34 (3). The intention of these questions was to evaluate i) the study objective and test method purpose, ii) the need and benefits of the test method in comparison to existing test methods, iii) the comparison of the test method with the essential test method components and performances as described in the Performance Standards for TG 496 (5), iv) the biological and mechanistic relevance, v) the test method protocol, vi) the appropriateness of the validation study management and conduct, and vii) the availability of all data supporting the assessment of the validity of the analysis. The final and eighth charge question asked for the panel's final remarks and additional comments on the proposal. The charge questions are listed in Annex II.

The developer of the OptiSafe™ test method had given two presentations at the OECD Expert Group teleconferences. As all members of the PRP were from the OECD Expert Group who attended the teleconferences, no additional presentation was given to the PRP prior to the review process.

Panel members were asked to perform their review based on following materials. All materials are available on the restricted community site for the OECD Expert Group ([Link](#)).

- a. The Performance Standards for TG 496 (5)
- b. The draft Appendix 2 on OptiSafe™ to TG 496
- c. Validation Report (1)
- d. Standard Operating Protocol
- e. Raw data for reference chemicals from performance standards
- f. Raw data for all chemicals in the database

The members of PRP reviewed the supporting materials for OptiSafe™, from November 7<sup>th</sup> to November 28<sup>th</sup>, 2022 and provided their feedback. The secretariat compiled the feedback and prepared this report.

## 4. Evaluation Principle 1. Study objective and test method purpose

**Charge Question 1:** *Were the objective and the purpose of the test method adequately described?*

2. Overall, the PRP does not consider the objective and the purpose of the test method to be adequately described. The draft Appendix 2 on OptiSafe™ for TG 496 proposes that the test method is recommended to identify chemicals that do not induce serious eye damage (i.e., Not UN GHS Category 1) and for chemicals that do not require classification (UN GHS No Category). Thus, the purpose and Prediction Model differ notably from both the purpose proposed in the SPSF and the VRM presented in TG 496. This then suggests that, in addition to identifying chemicals that do not require classification, OptiSafe™ can also identify chemicals causing eye irritation as GHS Category 2. The validation (1) was focused on validating the method for identifying chemicals that do not require classification, as a bottom-up test method.

3. With the very high over prediction rate of GHS category 2 materials predicted as GHS Category 1, it is clear that this test method is not particularly suited for discriminating between GHS category 1 and category 2 materials. Nonetheless, there is considerable confidence in a category 2 prediction, given the very low under prediction of materials as non-classified.

4. It is not clear from the available documentation whether criteria for accuracy, sensitivity and specificity for predicting category 2 materials have been prospectively defined. In the Table 2 of the draft Appendix 2, the performance of the OptiSafe™ test (presented with a 3 x 3 matrix) shows that for the 21 chemicals identified as category 2, four of the chemicals were category 1 materials that were under predicted, resulting in approximately 19% of the Cat.1 chemicals being identified as Cat.2, which in the absence of clear criteria for acceptance could readily be considered unacceptable from a regulatory safety perspective.

5. For the reasons described above and considering the absence of previously agreed and established acceptance criteria for evaluating a method's capacity to predict all three UNGHS eye hazard categories, the PRP suggests removing the method's protocol and performance regarding Category 2 prediction and update the draft Appendix 2 accordingly.

6. The PRP also made a few comments on the method's usage within a weight of the evidence (WoE) approach and on the applicability domain. It would be useful to include details and references on the mechanistic background of the method to help assessors to better understand how to use the results within a WoE approach, when to expect false-positive and false-negative results as well as high variability (i.e., usefulness and limitations as a result in a WoE assessment).

7. It would also be useful to add clarity on the chemicals resulting in "CNM" and being excluded from the applicability domain (i.e., which substances, chemical groups, pH, and physico-chemical properties are outside of the applicability domain of the method).

8. The PRP recommends deleting the following sections to avoid confusion:

- Improvements made to the OptiSafe™ method for when validation was conducted (4., page 1)

- Prediction models that go beyond the UN GHS categories, as these are not in scope for validation (5., page 2 and 8., page 3 and table 10 and 11, page 6)

## 5. Evaluation Principle 2. Need and benefits in comparison to existing test methods

**Charge Question 2:** *Were the need and benefits in comparison to existing test methods appropriately addressed in terms of (regulatory) purpose, IP rights, geographical availability, animal welfare, cost, analysis time, sample amount and other relevant aspects?*

9. Overall, the PRP considers that there are needs and benefits of OptiSafe™ in comparison to existing test methods.

10. The OptiSafe™ test method shares its founding lineage with the validated reference method (VRM) and as such has a very similar biochemical basis for evaluation of eye irritation as the VRM. However, improvements to the OptiSafe™ test method provide an expansion on the applicability domain, and address some of the limitations of the VRM. Otherwise the test methods presumably would be available in similar markets globally, and provide similar benefits in terms of animal welfare considerations, costs, expertise and equipment required in a laboratory. As both test methods are based on similar biochemical modelling in a macromolecular matrix, both have limited mechanistic relevance to ocular injury in comparison to cell-based or ex vivo ocular irritation test methods.

11. VRM for TG 496 can be used to identify UN GHS Cat.1 and GHS No Cat as a stand-alone method, while the OptiSafe™ can be used to identify UN GHS No Cat as a stand-alone method. Moreover, the OptiSafe™ shows an improved prediction performance for UN GHS Cat.2 and Cat.1 chemicals, which can help to increase robustness of WoE assessments when using positive results from the OptiSafe™ in addition to other information and/or testing to substantiate UN GHS Cat.1 or Cat.2 classifications. Additionally, the OptiSafe™ is an improved version of VRM in terms of biological and human relevance (ascorbic acid as antioxidant to mimic tear fluid).

12. In terms of IP rights, it is not clear whether a patent has been filed for reagents and/or kit required for the OptiSafe™. If OptiSafe™ can be used only with the kit (as it is currently written in the SOP), then the method becomes obsolete, once the kit is not commercially available anymore. There was also no information on availability, costs, analysis time, sample amount, etc.

## 6. Evaluation Principle 3. Comparison of the test method with the essential test method components and performances as described in the Performance Standards for TG 496

**Charge Question 3:** *Are the essential test method components, the performance, and the accuracy and reliability values of the OptiSafe™ relevant to the Performance Standards for TG 496? In case of deviations from the Performance Standards, please describe your assessment of such deviations in considering OptiSafe as a me-too method (i.e., whether such deviations affect the merits of OptiSafe as a me-too method for TG 496).*

13. Overall, the PRP considers the OptiSafe™ test method relevant as a me-too method for TG 496.

14. The proposed application of the OptiSafe™ test method differs from the VRM, which is validated and approved to identify UN GHS Cat.1 and No Cat substances. Thus it is not clear that the OptiSafe™ test method fully falls within a me-too method characterizations. Accordingly, evaluations to determine whether a me-too method performs the same as or performs better than the VRM may not be fully possible, in particular in discriminating between UN GHS Cat.1 and Cat.2 substances.

15. The surfactants (Sodium lauryl sulfate (3%) and Sodium lauryl glucose carboxylate (and) lauryl glucoside) as well as the alkylating agent Sodium chloroacetate seem to be overpredicted in the OptiSafe™. However, data is very limited to conclude on chemical groups that may be outside of the applicability domain of the method.

16. Nonetheless, the OptiSafe™ test method would indeed fit well within TG 496, particularly given that this test method is mechanistically the same as the VRM, but provides additional latitude in the applicability domain and addressing limitations. The community should always expect that technological improvements and innovations from otherwise similar test methods come about which meet the same regulatory requirements but perhaps with higher performance characteristics.

17. Based on the findings reported for the accuracy, sensitivity and specificity of the OptiSafe™ test method, as well as the inter- and intra-laboratory reproducibility, the OptiSafe™ test method would certainly be considered to perform as well as the VRM for identifying UN GHS Cat.1 and No Cat substances, and thus appear to meet or exceed the criteria established for the me-too method.

## 7. Evaluation Principle 4. Biological and mechanistic relevance

**Charge Question 4:** *Are the toxicological mechanism and the relationship between the test method endpoint(s) with the biological effect as well as the toxicity of interest adequately addressed?*

18. Overall, the PRP considers that the biological and mechanistic relevance of OptiSafe™ test method has similar limitations as the VRM in TG 496.

19. The test method provides a biochemically analogous endpoint of increase in turbidity and light scattering by irritant test chemicals in a plant-based matrix of proteins, lipids, carbohydrates and “low molecular weight components”; presumably modelling the denaturation of corneal proteins and solubilization of membrane lipids, etc. The test method validation publication (1) and the Performance Standards document for TG 496 (3) provide a reasonable description of the test system and the biological and mechanistic relevance of in vitro macromolecular test method. The draft Appendix 2 for OptiSafe™ does not provide any further information and does not appear to be warranted.

20. Whereas these biochemical events are reasonably modelled, much as a molecular initiating event might be envisioned for ocular and conjunctival injuries, it is not at all clear how this uniform matrix can model the complexity of the corneal epithelium, basal lamina, and corneal stroma in its ability to discriminate between reversible injuries typically limited to the corneal epithelium and upper stroma, and those that result in irreversible damage throughout the cornea and into the deep stroma (according to the Depth of Injury concept by Maurer and Jester (4)). Whereas the test method presumably responds to a continuum of toxic potencies by irritant chemicals, the cellular events which define the depth and degree of injuries associated with ocular injuries and discriminate between moderate reversible injuries and those more aggressive injuries resulting in irreversible corneal damage are simply not modelled. The specific barrier properties of the corneal epithelial layers, the epithelial basal lamina, and the stroma all play key roles and how different chemicals permeate into the cornea, and this is simply not modelled in the homogenous macromolecular matrix. In addition, the key cellular events associated with corneal damage, particularly where relevant to moderate and severe ocular injuries, are cell lysis, cell stress signalling and cell death, none of which are modelled by any of the macromolecular test methods.

21. As an added note, a more detailed mechanistic background of the method, especially with regards to ascorbic acid as antioxidant to mimic tear fluid, could be described in the draft Appendix 2 to help assessors to better understand how to use the results within a WoE approach to establish a final UN GHS classification and to ensure that the applicability domain is well defined for OptiSafe™.

## 8. Evaluation Principle 5. Test Method Protocol

**Charge Question 5:** *Is a detailed protocol for the test method (Standard Operating Procedure, or SOP) available?*

22. Overall, the PRP considers that the detailed protocol for OptiSafe™ is available.

23. The test method developer has provided a highly detailed protocol ([Link](#)) for review by the PRP, and this test method protocol appears sufficiently detailed to be able to accurately and precisely carry out the test method, presumably to a level that would support Good Laboratory Practice (GLP) compliance. The test method protocol provides details on the principle of the test, special handling procedures for specific chemicals, definition of the applicability of domain and considerations for limitations, a detailed list of components, reagents and equipment required, specific details to execute the work, and the data collection, data analyses, prediction models and reporting procedures. In summary the test method protocol requirement appears adequate.

24. However, the SOP is not sufficiently summarized in the draft Appendix 2 and the SOP (same as the draft Appendix 2) requires some more clarity with regards to data interpretation and reporting. For example, the draft Appendix 2 should contain the criteria for PCHP mentioned in the SOP and the terminology for the 3 pre-tests should be aligned throughout the SOP and the draft Appendix 2. The SOP should be referenced throughout the draft Appendix 2 where needed.

25. Additionally, the section in the SOP “Materials provided with the OptiSafe Kit” are not descriptive enough to obtain the materials without purchasing the kit. If the kit is not commercially available anymore, then the method becomes obsolete.

26. Finally, acceptance criteria are not very clear (different criteria for different stages) as well as the criteria to define the 5 dilutions of the test chemicals and when are dilutions based on neat vs. diluted test chemicals (e.g., Cetylpyridinium bromide, glycerol)

## 9. Evaluation Principle 6. Appropriateness of the validation study management and conduct

**Charge Question 6:** *Was the validation study conducted according to the principles and criteria documented in pages 20-23 of the OECD Guidance Document 34?*

27. Overall, the PRP considers that the validation study for OptiSafe™ was conducted according to the principles and criteria detailed in OECD Guidance Document 34.

28. The prospective validation of the OptiSafe™ test method, as presented in the validation study report (1), overall follows the basic principles and criteria for test method validation, as described in OECD guidance document 34 (3). Specifically the validation management team (VMT) was comprised of validation experts within NICEATM, who were charged with reviewing and approving the study design, work plan, test method protocol, study timeline, and deliverables. The VMT selected the chemicals for the validation study and coordinated the coding and distribution of the reference chemicals to the participating laboratories. The validation management team ensured that an initial training effort was executed and allowed for discussions among participating laboratory staff to occur only within the training phase. Thereafter, all communications by participant laboratories were required only to be directed to the VMT. During the testing phase, participant laboratories submitted data on a weekly basis to the VMT. At the conclusion of the study the VMT was responsible for data analyses and reporting of the validation outcome.

29. The VMT presented on the following aspects of the validation:

- a. the rationale for evaluating and ocular test method using a macromolecular matrix as a test system was presented,
- b. the relationship between the changes in the test system and relevance to the biochemical changes in ocular irritation was presented,
- c. a brief overview of the test method protocol using validation was presented. The validation report indicates that the detailed protocol can be obtained from the lead laboratory,
- d. the intra-and inter-laboratory reproducibility of the test method on 30 chemicals from Phase 2 was reported,
- e. test method performance was evaluated predominantly on reference chemicals appropriately within the applicability domain, as well as a few known to be outside of the applicability domain, thus evaluating the pre-screen methodology,
- f. the reference chemicals included *in vivo* classifications in both UN GHS and EPA categorization schemes,
- g. although no claims of full GLP compliance were made, the VMT report stated that the testing was conducted in accordance with the principles of OECD GLP. Note that the quality assurance personnel from the lead laboratory conducted the data audit and analyses from all of the participating laboratories. Although it is customary for data auditing to be conducted by independent personnel from the participating laboratories, often under contract by the VMT, according to the principles of OECD GLP's, the quality assurance personnel may be considered sufficiently independent in their abilities to execute their duties,

h. the VMT has provided Excel sheets with the representative data from the validation.

30. The VMT report included a narrative on the progress of the validation study through the phases, with commentary on modifications made to the protocol procedures as a result of discrepancies identified after training, as well as a result of Phase I test results. The VMT reported on the accuracy, sensitivity, specificity, false-negative and false-positive rates from the validation relative to the *in vivo* EPA and UN GHS categorization schemes.

31. One short coming was that not all validation studies were performed in compliance with GLP Principles and intra- and inter-lab as well as intra- and inter-study variability should be more specific for the actual measurement (OD<sub>400</sub> values) versus final classification outcome. This way, acceptance criteria can be defined with more clarity. In addition, the applicability domain needs to be defined in more detail (chemical resulting in “CNM” should be clearly defined as outside of the applicability domain).

## 10. Evaluation Principle 7. All data supporting the assessment of the validity of the analysis should be available for expert review

**Charge Question 7:** *Do you consider that all the data supporting the assessment of the validity of analysis are easily available for expert review?*

32. Overall, the PRP considers that the data supporting the assessment of the validity needs clarifications.

33. Data for control chemicals (QC1 and QC2) could not be found and data is not easily available for review. One data table with all raw data (versus data per sheet) would be preferred to review raw data, calculations, variability, and final classification results. The draft Appendix 2 and SOP should explain in more detail the relevance of and acceptance criteria for “a True Negative Checks” and “Depth of Migration (DM) Results”.

34. One reviewer posed the following question:

- a. In the EXCEL sheet OptiSafe™ Data Log\_Performance\_10.18.22.xlsx (Link), Linda Nguyen is often identified as “QA” and for a few chemical analyses also identified as “Technician” (see PS No.3, Run2). Are these roles for Linda Nguyen to be understood as operating Technician and as independent Quality Assurance auditor? If so, it does not appear that the lead lab quality assurance personnel were truly independent from the data generation and collection duties for work conducted in the lead lab.
- b. The test method submitter responded with an acceptable explanation that Nguyen did not perform as both Technician and QA on the same set of data and added that clarification to the data sheet will be made.

35. Additionally, one of the Peer Review Panellists was not able to follow all the data through to the analyses and did not conduct any audits of the data and the analyses in the Excel data sheets. It is not clear how to relate the data from individual chemicals in the Excel sheets with the analysis’s tables in the validation report. For example, in Table 2, chemical no. 3 (2-Ethyl-1-hexanol) (CAS 104-76-7) is presented as having been tested in the inter-laboratory study, but that chemical does not seem to appear in the Excel sheets.

## 11. Additional Remarks

### Charge Question 8: Do you have any suggestions or remarks to share?

36. Establishing acceptance criteria for test method performance at either extreme of the eye irritation continuum (i.e., in for either UN GHS Cat.1 or No Cat) has historically been achievable. However, establishing the acceptance criteria in 3 x 3 matrix to discriminate both between GHS Cat.1 and Cat.2, as well as between Cat.2 and No Cat has proven difficult, if for no other reasons than due to the variability of the animal data historically used as reference for performance standards and the data interpretation procedures used to assign GHS categories. The OptiSafe™ test method statistically appears to do well by being highly sensitive in predicting UN GHS Cat.1 materials, as well as high accuracy in identifying No Cat, not unlike the VRM. As has historically been observed in other alternative test methods the accuracy in the middle ground (predicting UN GHS Cat.2 with high confidence) is notably reduced, as both considerable over- and under prediction rates occur frequently. In the opinion of one of the PRP panellists, the OptiSafe™ test method is not likely to perform better than most other test methods given our assertion that the reference animal data are inherently variable. More importantly, the OptiSafe™ test method does not appear to adequately discriminate between UN GHS Cat.1 and Cat.2 materials as evidenced by the high over-prediction rate of the UN GHS Cat.2 materials and considerable under-prediction of UN GHS Cat.1 materials, and in the absence of any biological relevance to the cellular based mechanisms involved in discriminating between reversible and irreversible ocular injuries *in vivo*, the PRP would not consider this test method adequate as a stand-alone to identify UN GHS Cat.2 materials.

37. One of the PRP panellists provided additional comments and inquiries for clarifications, as described below in paragraphs 47 – 53,

38. As this is a relatively simple test method, the draft Appendix 2 as well as the SOP should describe in detail to allow labs to establish this method without the need to contact the test method developer for details.

39. Equations and illustrations would be helpful in the draft Appendix 2, if they deviate from the VRM (i.e., Annex 2b and paragraph 18, respectively) and the flow diagram from SOP would be helpful in the draft Appendix 2.

40. Some further background why OD<sub>400</sub> needs to be measured vs. OD<sub>405</sub> (VRM) would be helpful for labs when establishing the OptiSafe™ test method. Does this wavelength reflect the max absorbance of a specific ingredient in the OptiSafe™ reagent?

41. When a neat chemical tests positive, but the 5 dilutions do not show any dose-response, should the chemical be considered as outside of the applicability domain?

42. Would it be possible to predict different UN GHS classifications based on the 5 different dilutions applied per test chemical? Cetylpyridinium bromide is tested at different concentrations (6%, 1%, 0.1%) in accordance with the Proficiency Chemical data set. However, the OptiSafe™ requires 5 dilutions per test material and thus, the dilutions for Cetylpyridinium bromide 6%, 1%, 0.1% seem to overlap at least partly. Would it be possible to define different UN GHS classifications based on the 5 dilutions?

43. Some sections in the draft Appendix 2 and in the SOP (e.g., the test report section about results (page 7)) should be in line with the according section in the TG for the VRM, which is more detailed.

## 12. Conclusions and recommendations

44. Overall, the PRP considers that the OptiSafe™ meets the standards performance for TG 496 to be considered as a me-too method to the VRM, but suggests to revise the draft Annex 2 based on the recommendations made throughout the Peer Review Report contained in this document.
45. The PRP does not consider that the objective and purpose of the test method is adequately described. The PRP does not consider that information on the OptiSafe™'s performance for predicting UN GHS Cat.2 substances is adequate, and recommends removing information on the model and performance metrics intended for predicting three UN GHS eye hazard categories
46. The PRP considers that there are needs and benefits of OptiSafe™ in comparison to existing test method. The PRP considers that OptiSafe™ provides additional latitude in the applicability domain and address limitations of the VRM.
47. The PRP considers that the biological and mechanistic relevance of OptiSafe™ test method has similar limitations as the VRM in TG 496.
48. The PRP considers that the detailed protocol (SOP) for OptiSafe™ is available, with a suggestion to provide more detail on the sections for data reporting and interpretation, materials provided with an OptiSafe™ Kit, and for acceptance criteria.
49. The PRP considers that the validation study for OptiSafe™ was conducted according to the principles and criteria detailed in OECD Guidance Document 34, but whether the process was fully GLP compliant is not clear.
50. The PRP considers the data supporting and assessment of the validity of the method adequate in general. However, the PRP would have preferred to have a data table with all raw data, calculations, variability, and final classification results in order to evaluate the linkage between the raw data and the final classifications.

## 13. Acknowledgements

The OECD Secretariat thanks the Peer Review Panel for their review and valuable discussions and comments.

## 14. Literature

- (1) Choksi N., Lebrun S., Nguyen M., Daniel A., DeGeorge G., Willoughby J., Layton A., Lowther D., Merrill J., Matheson J., Barroso K., Yozzo K., Casey W., Allen D. (2020). "Validation of the OptiSafe™ eye irritation test." *Cutan Ocul Toxicol.* 39(3):180–192. doi:10.1080/15569527.2020.1787431
- (2) OECD. (2019). "Test No. 496: In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage." OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. Available at: <https://doi.org/10.1787/970e5cd9-en>.
- (3) OECD. (2005). "OECD Series on Testing and Assessment, Number 34. Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment." Available at: <https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-gd34.pdf>.
- (4) Jester J.V., Li H.F., Petroll W.M., Parker R.D., Cavanaugh H.D., Carr G.J., Smith B., Maurer J.K. (1998). Area and depth of surfactant-induced corneal injury correlates with cell death. *Investigative Ophthalmology & Visual Science* 39, 922-936.
- (5) OECD. (2019). "OECD Series on Testing and Assessment No. 312 Performance Standards for the assessment of proposed similar or modified in vitro macromolecular test method for identifying eye hazard potential as described in Test Guideline 496." Available at: [https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO\(2019\)32&docLanguage=en](https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2019)32&docLanguage=en)

## Annex 1. Peer Review Panel Composition

Hans Raabe

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NV Procter & Gamble Services Company SA  
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David Lehmann

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**Peer Review Manager**

Eugene Choi (OECD Secretariat)

## Annex 2. Charge Questions for the Peer Review of OptiSafe™ Test Method as a me-too assay for TG 496

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| <b>Charge Question 1:</b> <i>Were the study objective and the purpose of the test method adequately described?</i>   |
| <b>Charge Question 2:</b> <i>Were the need and benefits in comparison to existing test methods appropriately addressed in terms of (regulatory) purpose, IP rights, geographical availability, animal welfare, cost, analysis time, sample amount and other relevant aspects?</i>  |
| <b>Charge Question 3:</b> <i>Are the essential test method components, the performance, and the accuracy and reliability values of the OPTiSafe™ relevant to the Performance Standards for TG 496? In case of deviations from the Performance Standards, please describe your assessment of such deviations in considering OptiSafe™ as a me-too method. (i.e., whether such deviations affect the merits of OptiSafe™ as a me-too method for TG496)</i> |
| <b>Charge Question 4:</b> <i>Are the toxicological mechanism and the relationship between the test method endpoint(s) with the biological effect as well as the toxicity of interest adequately addressed?</i>   |
| <b>Charge Question 5:</b> <i>Is a detailed protocol for the test method available?</i>   |
| <b>Charge Question 6:</b> <i>Was the validation study conducted according to the principles and criteria documented in pages 20-23 of the OECD Guidance Document 34?</i>   |
| <b>Charge Question 7:</b> <i>Do you consider that all the data supporting the assessment of the validity of analysis are easily available for expert review?</i>   |
| <b>Charge Question 8:</b> <i>Do you have any suggestions or remarks to share?</i>  |

# **Peer Review Report on Inclusion of OptiSafe Eye Irritation Test (EIT) in Test Guideline 496 on In Vitro Macromolecular Test Methods for Identifying the Eye Hazard Potential of Chemicals**

**Series on Testing and Assessment No. 400**

This document encloses the Peer Review Report (PRR) of the validation study for OptiSafe™ Eye Irritation Test (EIT) for its inclusion in OECD Test Guideline 496 on In Vitro Macromolecular Test Methods. The OptiSafe™ EIT is validated for identifying chemicals that do not require classification for eye damage. The project was on the Test Guidelines Programme work plan until 2024.