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Peer Review Report on Inclusion of KeraSkin Phototoxicity Assay as a me-too method in Test Guideline 498 on In Vitro Phototoxicity

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This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank, Basel, Rotterdam and Stockholm Conventions and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Foreword

The Foreword and Acknowledgements were added to this publication by the OECD Secretariat.

This document contains the Peer Review Report on Validation Study of KeraSkin™ Phototoxicity Assay. KeraSkin™ Phototoxicity Assay was validated by Korea Center for the Validation of Alternative Methods (KoCVAM) from 2023 to 2024, and Korea submitted a project proposal to OECD Test Guideline Programme to be included as a “me-too” method in Test Guideline 498 on In Vitro Phototoxicity Assay in 2025.

The peer review was organized by the OECD secretariat from May to July 2025 with peer review panel consisting of four experts from OECD Expert Group on Phototoxicity and according to charge questions based on OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. The outcome of the peer review, which was reviewed and discussed by the OECD Expert Group on Phototoxicity, supported inclusion of KeraSkin™ Phototoxicity Assay as a “me-too” method in TG 498. The Peer Review Report was endorsed by the Working Party of the National Coordinators of the Test Guideline Programme at its 38th meeting in April 2026.

This report was developed under the Working Party of the National Coordinators of the Test Guidelines Programme.

Acknowledgements

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

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1 Summary

1. In April 2024, WNT approved the Standard Project Submission Form (SPSF) submitted by Korea on inclusion of KeraSkin™ Phototoxicity Assay as a me-too test method in Test Guideline (TG) 498: *In Vitro* Phototoxicity – Reconstructed Human Epidermis (RhE) Phototoxicity Method (OECD, 2026[1]). As a result, the proposal was included as Project 4.169 in OECD TG Work Plan.

2. The KeraSkin™ Phototoxicity Assay was developed by the Korean Center for Validation of Alternative Methods (KoCVAM), who also served as the lead laboratory in the validation study. Four participating laboratories in the validation study were from Korean Ministry of Food and Drug Safety (MFDS), Biosolution Co., Ltd, CORESTEMCHEMON, and Korea Testing & Research Institute (KTR).

3. The validation study of KeraSkin™ Phototoxicity Assay was presented to the OECD Expert Group on Phototoxicity during a teleconference in September 2024. The Expert Group was supportive of the project to proceed with a peer review before the draft updated TG 498 with inclusion of KeraSkin™ Phototoxicity Assay is prepared

4. The OECD Secretariat organized an independent Peer Review with a panel comprised of four members of the OECD Expert Group on Skin and Eye Irritation and phototoxicity during May – July 2025. The results from the Peer Review were put together for a Peer Review Report (PRR) contained in this document.

2 Background

5. The KeraSkin™ Phototoxicity Assay is an *in vitro* phototoxicity test method using KeraSkin™, a reconstructed human epidermis model (RhE), which is similar to the Validated Reference Method (VRM), Epiderm™ in OECD TG 498 (OECD, 2026[1]). The RhE-based assay in TG 498 is used to predict phototoxicity of a chemical by quantifying the cell viability of the RhE model, followed by MTT assay using standard absorbance or an HPLC/UPLC-spectrophotometry. Based on the VRM of TG 498, the Performance Standards was developed (OECD, 2023[2]), which describes essential test method components, procedural conditions, a minimum list of reference chemicals, defined reliability and predictive capacity values that similar methods should fulfil to be considered and adopted as a “me-too” method in TG 498.

3 The peer review process

6. The Peer Review Panel (PRP) for KeraSkin™ Phototoxicity Assay was established in January 2025 (See Annex). The PRP was comprised of four experts from OECD Expert Group on Phototoxicity and supported independent peer review of the KeraSkin™ Phototoxicity Assay for its inclusion as a "me-too" method in OECD TG 498. The kick-off teleconference of the PRP took place in May 2025, where the PRP was introduced to the validation study of KeraSkin™ Phototoxicity Assay and to the OECD Peer Review process according to the Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (OECD, 2005).

7. For the Peer Review of KeraSkin™ Phototoxicity Assay contained in this report, the PRP was provided with the following materials, all available on the restricted OECD community site for expertise in skin/Eye irritation and phototoxicity:

- Validation Report
- Project Plan
- Standard Operating Procedure (SOP)
- Raw data for proficiency chemicals
- Transferability report including raw data from all participating laboratories
- Reproducibility data from all participating laboratories
- Chemical selection report and raw data on additional test chemicals
- Statistical Analysis Report
- QC Report

The PRP was requested to review all the materials and to provide written feedback to six charge questions based on the peer review evaluation principles in OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (OECD, 2005). Table 1 shows the evaluation principles and the associated charge questions that were used for the peer review of KeraSkin™ Phototoxicity Assay.

Table 1 Evaluation Principles and Charge Questions for the Peer Review of KeraSkinTM Phototoxicity Assay based on OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (OECD, 2005)

Evaluation Principle	Charge Question
Study objective and test method purpose	Were the objective and the purpose of the test method adequately described?
Comparison of the test method with the essential test method components and performance as described in the Performance Standards for TG 498	Are the essential test method components, the performance, and the accuracy and reliability value of the KeraSkin™ Phototoxicity Assay relevant to the Performance Standards for TG 498? In case of deviations from the Performance Standards, please describe your assessment of such deviations in considering KeraSkin™ as a me-too method (e.g., whether such deviations affect the merits of KeraSkin™ as a me-too method for TG 498)
Biological and mechanistic relevance	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?
Test method protocol	Is a detailed protocol for the test method available in a Standard Operating Procedure (SOP)? Are all descriptions sufficient and clear?
Appropriateness of the validation study conducted according to the principles and criteria documented in the OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment	Was the validation study conducted according to the principles and criteria documented in the OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment ?
All data supporting the assessment of the validity of the analysis should be available for expert review	Do you consider that all the data supporting the assessment of the validity of analysis are easily available for expert review?

8. The PRP reviewed the supporting materials for KeraSkin™ Phototoxicity Assay from May 20th to June 17th and provided their feedback. The secretariat compiled the feedback and summarized the outcome of the peer review, which was reviewed by the PRP for endorsement to produce the Peer Review Report contained in this document.

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?*

9. Overall, the PRP agreed that the objective and the purpose of the test method was adequately described. The purpose of the validation study is clearly defined as assessment of the KeraSkin™ Phototoxicity Assay as a me-too method for OECD TG 498. The importance of and the need for this method was presented, for example, to meet requirements of Regulations on the Examination of Functional Cosmetics and Regulation on Pharmaceuticals Approval, Notification and Review for Photosafety Evaluations.

10. The KeraSkin™ Phototoxicity Assay addresses the same biological endpoint and follows similar protocol steps as the VRM of TG 498, the Epiderm™ RhE method. The KeraSkin™ assay was presented similarly to the VRM in OECD TG 498 in that (i) it can be a stand-alone test for photoirritation testing, (ii) it can be part of a tiered testing approach (in combination with other methodologies such as TG 495 (OECD, 2019[1]) and TG 432 (OECD, 2019[2])), and (iii) it may overcome one of the limitations of the 3T3 NRU PT testing (OECD, 2019[2]) with regards to solubility of the test materials. Inclusion of KeraSkin™ Phototoxicity Assay is justified in the context of expanding availability of 3D reconstructed epidermis models for phototoxicity testing, and also in the unique benefits or technical differences of KeraSkin™ vs. EpiDerm™ (e.g., cost, local/international availability, tissue availability).

11. As a remark on a practical issue, the PRP noted lack of discussion on the availability of the test method outside and within Korea. Reconstructed tissue models may need to be produced within the country where the use is intended because of import barrier or due to the potential issues prohibiting the ability to receive the tissue models (with finite life spans) in timely manners.

5 Evaluation Principle 2. Comparison of the test method with the essential test method components and performance as described in the Performance Standards for TG 498

Charge Question 2: *Are the essential test method components, the performance, and the accuracy and reliability value of the KeraSkin™ phototoxicity assay relevant to the Performance Standards for TG 498? In case of deviations from the Performance Standards, please describe your assessment of such deviations in considering KeraSkin™ as a me-too method (e.g., whether such deviations affect the merits of KeraSkin™ as a me-too method for TG 498)*

12. Overall, the PRP agreed that the essential method components, the performance, and the accuracy and reliability value of KeraSkin™ Phototoxicity Assay is relevant to the Performance Standards for TG 498 (OECD, 2023[2]).

13. The KeraSkin™ Phototoxicity Assay meets the performance benchmarks as outlined in the OECD TG 498 Performance Standards, with sensitivity, specificity, and overall accuracy each reported at 90%. The set of reference chemicals used closely matches the minimum recommended list. The method's alignment with essential test method components is satisfactory.

14. However, the PRP identified several issues from the validation report, as described below in paragraphs 16-21.

15. The PRP noted that the details on the tissue sensitivity against increasing doses of the UVA/VIS light, which are part of the transferability exercise, were not provided in the validation report. This information should be added to the validation report in a condensed form, to make the report complete.

16. The PRP noted that different concentration of MTT is used between the QC testing and the phototoxicity test. The concentration should be unified as the usual concentrations of MTT used for 3D RhE tissues. Using a lower concentration of MTT might modulate the QC range towards higher IC50 values.

17. The PRP noted that the direct MTT testing and colorant control calculations are different from those presented in TG 498, and a value of 5% would prompt additional calculations. There is no additional detail in the SOP to discuss how these calculations would be performed to correct the value for the viable tissues. Further, more information should be provided as to how the 5% value was selected, since TG 498 presents that all direct reducers or colorant test materials

would have calculation adjustments from the viable tissue results, regardless of OD values produced by the functional checks.

18. The PRP found that the SOP is slightly confusing regarding the terms NC and VC. A serious issue is that it borrows the QC criteria and ODs for NC from the QC testing done with different concentrations of MTT, which differs from the one used in the phototoxicity assay. The acceptance range for VC seems to be far too broad (e.g., it is based on three folds of standard deviation (SD), when ideally, it should be only two folds). As shown in Table 2 below, this value is particularly low for VC (e.g., 0.3) and this could impact assay sensitivity. EpiDerm™ or SkinEthic™ tissue with OD of 0.3 would not be accepted in any of the OECD tests.

Table 2 Acceptance Range for Vehicle Control (VC) and Negative Control (NC) for KeraskinTM RhE Model.

		Lower limit	Upper limit
KeraSkin™ RhE Model	Vehicle (Solvent) control (-Irr)	0.3	2.2
	Negative control (-Irr) QC	0.6	1.2

19. The PRP remarked that the lead laboratory should determine if they will incorporate borderline results into the interpretation, as in TG 498, since borderline results are those which may have lower confidence. This is important given this test method requires only a single run for evaluation of phototoxicity hazard and that it could be used as a stand-alone for phototoxicity hazard. As an example, for PABA described in page 2 of the validation report, results of 3 trials for lab 1 would be positive, borderline, and negative.

20. The PRP remarked un uncertainty on section 4.5 from the validation report, on Phototoxicity evaluation of seven additional test chemicals (Table 10). Two phototoxic materials, ketoprofen and protoporphyrin IX were predicted non-phototoxic but are known to have phototoxic potential. Also, oxytetracycline was predicted to be phototoxic, but is not based on *in vivo* studies. Except for protoporphyrin IX, which one could argue would be a technically challenging chemical in such an assay, the results are concerning.

6 Evaluation Principle 3. Biological and mechanistic relevance

Charge Question 3: *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?*

21. Overall, the PRP agreed that KeraSkin™ Phototoxicity Assay adequately addresses the toxicological mechanisms and the relationship between the test method endpoint with the biological effect as well as the toxicity of interest.

22. The KeraSkin™ Phototoxicity Assay addresses the key biological mechanism of phototoxicity – namely, the induction of cytotoxic responses in human skin following exposure to a test substance and UVA/VIS light. The method uses a RhE model, providing tissue-level complexity and metabolic competence relevant to dermal exposure. The endpoint is cell viability assessed by the MTT assay, which aligns with mechanisms of phototoxic damage such as mitochondrial dysfunction and loss of membrane integrity.

23. The use of chlorpromazine as a positive control demonstrates the model's sensitivity to compounds known to generate ROS under the UV/VIS light. Moreover, tissue sensitivity and biological responsiveness are supported by apparent OD/viability shifts in UV(+) versus UV(-) conditions across multiple test labs. The approach is consistent with OECD TG 498 principles and offers mechanistic continuity with existing *in vitro* and *in vivo* phototoxicity methods.

24. As for additional information needed, the PRP noted that it would be essential to know whether the model resists well to UVB part of the spectra, as the RhE models are frequently used to test UVB absorbing molecules that cannot be easily tested in the OECD TG 432 (OECD, 2019[2]).

7 Evaluation Principle 4. Test Method Protocol

Charge Question 4: *Is a detailed protocol for the test method available in a Standard Operating Procedure (SOP)? Are all descriptions sufficient and clear?*

25. Overall, the PRP agreed that the provided SOP (version 1.2) for the KeraSkin™ Phototoxicity assay is structured clearly and logically.

26. The provided SOP includes step-by-step procedures for tissue handling, pre-incubation, dosing, UV exposure, post-incubation, MTT assay, and optical density (OD) reading. Critical parameters are outlined in both text and tabular/graphical formats, including UV exposure settings, MTT concentrations, and acceptance criteria for tissue viability. The SOP also includes visual representations of workflow, controls, and plate layouts, which enhance clarity.

27. However, the PRP noted that the SOP should have further details as described in paragraphs 28 – 31.

28. While the SOP is in general well aligned with the information described in OECD TG 498, additional details on some of the procedures are needed. In particular, the preliminary testing assessments and surrounding calculations, and light source calibration and/or measurement of intensity is missing from the report. Given the artificial light source is the most important part of the assay, there should be a section that presents the approach used for testing (spectrum, irradiance) beyond SOP section (1) UVA-solar simulator condition.

29. Some key parameters (e.g., treatment of borderline results) should be elaborated further for complete regulatory robustness.

30. For appropriate calculation of relative viability, the applicable solvent (or vehicle) control should be included in each test run. A consequence of this was evident in the positive control% relative viability (up to ~180% in one run) in several of the test runs. The positive control is prepared in water and therefore, water should be included with every run. Accordingly, each test chemical solvent (or vehicle) should be included in its respective test run and used to calculate relative viability.

31. The lead laboratory provided detail on concentrations for testing and solvents for dilution for the 2 transferability chemicals, 6 proficiency chemicals, and 12 reference chemicals outlined in the performance standards. As such, the predictions showed high concordance with expected results. However, in the absence of this type of information (e.g., the 7 additional test chemicals) presented in supporting documents and in validation study published (Kang, et al., 2025) resulted in higher occurrences of false positive or false negative predictions. In some of those instances, this may have been due to concentrations selected for evaluation and/or insufficient testing to the maximum concentration (10%). This highlights potential issues, especially for naïve laboratories who may transfer the technology and have less familiarity with appropriate dose selection/assessment. Additional commentary or guidance may be needed to address this. It was

mentioned in one of the documents that a dose range finding assay may be needed initially to narrow on doses of likely (or potential) photoirritation.

8 Evaluation Principle 5. Appropriateness of the validation study conducted according to the principles and criteria documented in the OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment

Charge Question 5: *Was the validation study conducted according to the principles and criteria documented in the OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (also known as GD 34)?*

32. Overall, the PRP considered that the studies were conducted in accordance with the criteria outlined in GD 34.

33. The PRP recognized that (i) scientific rationale was available, (ii) the endpoints were addressed, (iii), a detailed SOP was available, (iv) limitations (solubility, UVB) were noted (v) and additional details could be provided for applicability domain and/or limitations, for examples regarding mixtures.

34. The PRP recognized that the studies included an initial transferability phase, an inter-laboratory study across three sites, and performance testing with a set of 20 reference chemicals. Several reference chemicals were used throughout the transfer, demonstrating proficiency and evaluation of the test method as described in OECD TG 498 and its Performance Standards. The RhE model has human relevance and the reference chemicals included *in vivo* and/or human prediction for evaluation of *in vitro- in vivo* concordance. The test materials were blind-coded and all the data were available for review. When possible, the data were obtained in GLP accredited facilities. Some lab(s) that were not clearly identified were still indicated to have the data quality check (QC). Further, each tissue batch had QC by the tissue manufacturer. The overall structure aligns with the modular validation framework recommended by the GD 34.

35. However, the PRP noted some weak points in the transferability phase, as described in paragraphs 36-39.

36. The PRP noted that only two test chemicals and proficiency chemicals were used, which limits the robustness of the transferability assessment. Bergamot oil, which has a strong smell, can be easily identified by the laboratories.

37. The PRP noted that all participating laboratories were located in South Korea, reducing evidence for international reproducibility and stability of the model when shipped over a longer distance.

38. The PRP noted that there was no qualitative feedback (e.g., on SOP clarity, ease of implementation) collected from receiving laboratories.

39. The PRP noted that the independence and prior experience of the receiving laboratories were not described in the transferability study, which reduces confidence in the objectivity of the transfer. For example, it was not clear if training was provided to each laboratory and/or if retraining was provided in cases where additional trials were required due to lack of concordance.

9 Evaluation Principle 6. All data supporting the assessment of the validity of the analysis should be available for expert review

Charge Question 6: *Do you consider that all the data supporting the assessment of the validity of analysis are easily available for expert review?*

40. Overall, the PRP considered all data supporting the assessment of the validity of the analysis were available for review.

41. Most of the data necessary to support the assessment of the validity of the KeraSkin™ phototoxicity assay were available and accessible in the validation report and supporting documents. These include raw OD values, mean viability results, inter-laboratory performance metrics, acceptance criteria outcomes, and control performance data. Statistical summaries are included and aligned with OECD TG 498 performance standards (OECD, 2023[2]). The documentation was overall sufficient to enable peer evaluation and regulatory consideration, and most critical components were addressed in a traceable manner.

42. However, the PRP noted a few points that could be further detailed, as described in paragraphs 45-47.

43. The test materials were blind coded, and an un-coding summary sheet could be helpful for connection.

44. In the validation report and project plan there were a few instances where the lab identifications (1-4) were inconsistent. This should be clarified.

10 Evaluation Principle 7. Additional Remarks

Charge Question 7: *Do you have any suggestions or remarks to share?*

45. The PRP made additional remarks as described in paragraphs 46-48.

46. The linear regression in the transferability report (Table 8) for the sensitivity experiment is not the statistical method that should have been chosen. The decrease is not linear, and the equation y = ax+b does not match well with the shape of the curve.

47. As noted previously, the approach for taking three folds of the standard deviation (SD) is leading to very broad acceptance ranges and values of ODs. In general, most of the labs have taken two folds of SD approach or some median approach towards setting the ranges of the acceptance.

48. It is recommended to reassess the acceptance criteria for the VC. Most of the VC control data verified in the datasheets were around 1.6 – 1.8.

49. The PRP made specific comments on the validation report as described in paragraphs 50-52.

50. Page 1: It presents technicians in some cases for each lab, but not the study directors.

51. Page 3, line 3: Does this imply ‘alternative to the 3T3 NRU PT (TG 432)’?

52. The concentrations and solvents for the proficiency chemicals were selected by the lead lab, resulting in correct predictions in all but 1 instance. When the 7 additional test materials were evaluated, several were not correctly predicted, even when tested in different labs (reference, Table 1, page 49). This highlights the importance of dose selection and solvent (vehicle). For example, Oxytetracycline (max concentration = 1%, resulting in Not Phototoxic (NPT) in one lab) may have been correctly predicted if concentrations up to max 10% was evaluated (as shown in the second lab). Also, ketoprofen correctly predicted in 1 lab vs another, using different concentrations in each lab. Evaluation up to 10% or until cytotoxicity is achieved can be further emphasized in the documents. These components (concentration range and solvent selection) are critical since users will be evaluating materials with unknown phototoxicity potential.

53. The PRP made specific comments on the project plan document as described in paragraphs 54 and 55.

54. PABA was evaluated only to 5%, and this concentration did not produce cytotoxicity. Evaluation up to 10% should have been considered for definitive classification of phototoxicity potential (page 22).

55. Page 30: preparation of test material included a solvent not used (mineral oil) and did not include a solvent that was used (ethanol).

56. The PRP made specific comments on the SOP as described in paragraphs 57-89.

57. Throughout the document, when irradiation intensity at mW or W/cm^2 or m^2 is mentioned, clarify if this is UVA measurement/intensity.

58. Throughout the document, ethanol is mentioned as a solvent used, but there is no specific discussion on the dosing volume to be used. Please specify (e.g., if this aligns with non-water soluble material dosing volume).

59. Page 1: add “simulated” to sunlight.

60. Page 7, section 6.4.1: Can UVB be evaluated with the KeraSkin™ model, as is the case for the EpiDerm™? In the peer review’s experience with UVB and the EpiDerm™ tissues, the UVB produces minimal, if any, cytotoxicity when used with UVB/UVA/visible light filter (e.g., H2 Honle). The ability to incorporate UVB into exposures provides an advantage over the 3T3 NRU PT where the cells are not as tolerate to UVB exposures and cytotoxicity would be expected. Also consider removing the statement that UVB causes cytotoxicity, as this may be a reason users could not use (or would reconsider use of) the KeraSkin™ model. Has a UVB sensitivity been conducted to see if KeraSkin™ tissues are tolerant to this exposure?

61. Page 12, section 6.5.4: Why is the optical density (OD) measurement not considered for the colorant control (e.g., SOP presents to visually observed)? In the peer reviewer’s experience, materials that may precipitate and persist after rinsing can also artificially impact OD measurement. Consider an alignment with the TG 498 approach.

62. Sections 6.5.4 and 6.5.5: The calculations present direct MTT reduction and color interference as “% cell viability”, but this is not % viability and could be confusing. Please present this information more accurately (e.g., % OD of control).

63. Sections 6.5.4 and 6.5.5: If test material is a direct MTT reducer or colorant, specify appropriate concentrations to evaluate for functional checks (e.g., all? Only the highest?)

64. Sections 6.5.4 and 6.5.5: How many tissues are needed for the functional checks? Duplicate?

65. Section 6.5.5: The test presents as incubation for 30 minutes and then check at 1 hour. Is it 30 minutes incubation and then 30 minutes later before you can evaluate? Please clarify.

66. Section 6.5.5: It is not described how the killed control tissues are created (e.g., is there a suggested method? Can they be purchased from tissue vendor?)

67. Section 6.5.5: Is a negative or vehicle control with the direct MTT test and/or function check included? How does one account for non-specific reduction by the non-viable tissue?

68. Page 13, section 7: Please correct burbles to bubbles.

69. Page 13, section 7: How is the kit to be stored between when it is received and until it is used? Please provide guidance.

70. Page 14, section 7.2: Are the tissues re-fed after initial pre-incubation period (and prior to test material application)? Please add this information.

71. Page 14, section 7.3: more guidance on “swirling of tissues”- this could be interpreted in many ways. The peer review would expect the user to handle the insert with forceps and move the insert (e.g., tapping on side with forceps or repositioning) to ensure complete coverage over surface of tissue.

72. Page 14, section 7.3: please add dosing volume for ethanol.

73. Page 15 – Are the tissues blotted before addition to clear DPBS to ensure medium removed (does the medium contain phenol red?)

74. Page 16, section 7.7: What are the conditions for the shaking (2-8 Deg.C, or room temperature)? Also specify whether the tissue should be fully submerged in the 2 mL isopropanol?

75. Page 16, section 7.7(5): Is the instruction “before collecting extract, mix on plate shaker for 15 minutes” after the inserts have been removed? If so, please provide sentences above to remove the inserts (and if there are any specific processes to do so, e.g., mix the insert pulling up and down and then decant extract back into respective well).

76. Page 16, section 7.8: This section is somewhat confusing – should the inserts remain in the 24-well plate of which they were extracted, or should the entire contents be removed to a new 24-well plate?

77. Page 16, section 7.8: Specify blanks plated in triplicate.

78. Page 16: TG 498 uses a single well per extract of the tissue. This SOP indicates duplicates per tissue. Would a single plated extract be sufficient?

79. Page 18: Please add guidance on calculation for the direct MTT reducer or colorant. If >5%, then do you subtract this from the mean cell viability?

80. Page 18 (2): On the second bullet about replicate tissues not being concordant – please add this as not a sub-headed comment, also the third run as not sub-headed.

81. Page 18 (2): If the third run is conducted, how would results be interpreted? As a mean of results? As concordant trials? Provide guidance.

82. Page 18, section 7.9.3 (3) – There is a comment that ethanol is cytotoxic in RhE models. At the volumes used for the assay, it should produce minimal, if any, cytotoxicity. This statement could provide confusion about use of ethanol as a solvent. In the experience of the Peer Reviewer, using in the RhE assay at a 25µL volume, the OD results are comparable to the HBSS or what solvent control results. Is the KeraSkin™ model more susceptible to cytotoxicity of ethanol?

83. Page 19, Table 6 (general) – The Peer Reviewer understands a lot of analyses were conducted to demonstrate OD value of 0.3 – 2.2 to be acceptable for this test method, but 0.3 is very low and may be of concern that accepting a result with OD as low as a control 0,3 could affect assay sensitivity (as an example to highlight this concern, in a killed control, in a killed control EpiDerm™ tissue, the OD values, which accounts for non-specific reduction, are ~0.1, which would be ~30% of the acceptable OD signal).

84. Appendix 1 on general solar simulator: How is the irradiance checked? Is that check done with a calibrated broadband meter with UVA sensor? A calibration factor for the irradiance determination is presented – if another use had a different meter to check, would that same factor apply? Is verification of each light bulb via spectral analysis report prior to use also performed? (as checked by an outside vendor, or internally)? More guidance would be useful here for users who are not as familiar with this type of equipment and necessary requirements for achieving appropriate conditions (that could also meet GLP requirements).

85. Page 28: What is MCTT HCE Eye Irritation Test referring to?

86. Page 31, Note 5: This specifies that 0.316% concentration was used for cytotoxicity – what if this concentration is not cytotoxic? Additional testing needed?

87. Page 32, Appendix 6: Where did this table originate? Please provide a reference.

88. Page 33, Appendix 7: “X”, missing table legend – what does this signify?

89. Other comments:

- Can HPLC be used for a strong direct reducer (in alignment with TG 498)?
- Please include details on the borderline results. Is the same range 30%±5% as in TG 498 considered?
- It is indicated that the irradiation exposures are done at 37◦C. Could room temperature exposure be used?
- Throughout the document, please check names of chemicals (e.g., chlorpromazine, 4-Aminbenzoic acid).
- For each test material or positive control, the appropriate solvent (or vehicle) control should be evaluated concurrently, and appropriate calculations of relative viability should be to the solvent (or vehicle) the material was prepared in. There were notes of this in other documents (e.g., why the positive control % viability is very high in some instances) which suggested only a single solvent (or vehicle) was used.
- Emphasis should be made that the maximum concentration for full evaluation of phototoxicity potential is 10% (or lower if cytotoxicity achieved). At least 1 reference material (PABA) was tested only to 5% and predicted as NPT, which is misleading because according to TG, it should have been evaluated to 10%. Although this will not likely change the prediction, naïve users should understand the implication of not evaluating to the maximum concentration and confidence in NPT prediction).

90. The PRP made specific comments on the Transferability report as described in paragraphs 91-100.

91. The report refers to the SOP v1.1, but with the PRP, the SOP v1.2 was shared. The availability of new version of SOP may be indicated.

92. Throughout the document, please revise ‘sunlight’ to ‘simulated sunlight.’

93. Throughout the document, please clarify UV measures/intensity (e.g., UVA)

94. Page 23: There were 7 experiments needed for one lab. Does the developer have insight as to why the predictions were not concordant with the expected, and how the laboratory finally achieved the proper prediction? Was a re-training needed or conducted? This may be helpful to indicate if a re-training is needed and how it could be important in the success of the method.

95. Lot KS23028 was used for one of the runs with QC negative control OD = 0.7. Biosolution lab = 1.459, 1.521; CORESTEM = 0.977, 0.958; KTR = 1.582, 1.605 for +Irr, -Irr, respectively. Is that range typically expected between tissue lots to different laboratories? Is this an example of different solvent (or vehicles)?

96. Proficiency Data for NIFDC lab: -Irr was >20% SD for G235; are results still accepted? This material also resulted in a positive (run 1), borderline (run 2) and negative (run 3) result.

97. Proficiency Data KTR lab: PC viability for -Irr was ~180%! This also occurred in a few additional instances. The peer reviewer believes this was due to use of ethanol as the vehicle control. Since water was used to prepare the positive control, a separate water vehicle control should have been tested to calculate % relative viability for the positive control. For each run,

each solvent (or vehicle) used should be also included in the test run for appropriate calculations of relative viability for the test material and positive control, respectively.

98. The PRP made specific comments on the Statistical Analysis Report as described in paragraphs 104-105.

99. In the tables, several values are slightly different than those in the validation report. The peer reviewer suspect that this is due to rounding or truncation, but ideally they should be consistent. Further, in one instance (anthracene, 0.01%, table 10), the mean viability value in the validation report is incorrect, while the value in the Statistical Analysis Report is correct (e.g., 79.65% vs. 73.65%), although the prediction is unaffected.

100. Page 49: The results for bergamot oil from KTR lab is borderline for run 2 and 3 according to TG 498.

101. The PRP made specific comments on the validation study reference (Kang, 2025), as described in paragraph 102.

102. As previously noted, information on the importance of concentration selection for appropriate prediction and that concentrations up to 10% need to be tested for full evaluation of phototoxicity potential is missing.

11 Conclusions and recommendations

103. Overall, the PRP agreed that the KeraSkin™ Phototoxicity Assay meets the essential requirements evaluated based on the six charge questions in this Peer Review Report as a me-too assay in TG 498.

104. However, the PRP also identified several important issues that need to be further clarified and elaborated by the developer. These issues were identified and noted according to their relevance to each charge question throughout this document. Following issues were considered as some of the main concerns:

- Some inconsistencies in the level of detail and specification of the light source used for the validation study throughout the documents submitted and lack of detailed information on the calibration of light source.
- The acceptance range for VC seems to be far too broad (e.g., it is based on three folds of standard deviation (SD), when ideally, it should be only two folds). The value of 0.3 is particularly low for VC and this could impact assay sensitivity. EpiDerm™ or SkinEthic™ tissue with OD of 0.3 would not be accepted in any of the OECD tests
- Lack of description on borderline ranges and whether they are aligned with the information contained in the TG 498.
- Several points in the SOP are described without full clarity which could lead to uncertainties and confusion for the end-users, especially in the naïve laboratories (see comments under additional remarks).
- Lack of description on whether there was any incident or need for re-training for the participating laboratories in the validation study.
- Lack of qualitative feedback on the SOP from the participating laboratories in the validation study.
- Absence of non-Korean laboratory participating in the transferability study, which could question the reliability for the transferability of the assay over a long distance, especially given the international context of use for OECD Test Guideline.

105. Therefore, while the conclusion of the Peer Review for KeraSkin™ Phototoxicity Assay supports its inclusion as a me-too assay in TG 498, the PRP recommends the developer to address the comments and questions contained in this Peer Review Report and to submit the revised documents along with this Peer Review Report to the OECD Expert Group on Phototoxicity for further discussions on the next steps to fulfil the level of detail and clarity of information needed for the method's inclusion in an OECD Test Guideline.

12 Literature

OECD (2026)[1], *Test No. 498: In vitro Phototoxicity - Reconstructed Human Epidermis Phototoxicity test method*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/7b2f9ea0-en.

OECD (2023)[2], *Performance Standards for the Assessment of Proposed Similar or Modified in Vitro Phototoxicity: Reconstructed Human Epidermis (RhE) Test Methods for Testing of Topically Applied Substances, as described in Test Guideline 498*, OECD Series on Testing and Assessment, OECD, Paris. https://doi.org/10.1787/4cceda6b-en

OECD (2005), *Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment*, OECD Series on Testing and Assessment, OECD, Paris. https://doi.org/10.1787/e1f1244b-en

OECD (2019)[1], *Test no. 495: ROS (Reactive Oxygen Species) Assay for Photoreactivity, OECD Guidelines for the Testing of Chemicals*, Section 4, OECD, Paris. DOI: https://doi.org/10.1787/915e00ac-en

OECD (2019)[2], *Test no. 432: In Vitro 3T3 NRU Phototoxicity Test*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD, Paris. DOI: https://doi.org/10.1787/9789264071162-en

Kang NH, Park CE, Kim SH, Gwak EJ, Kim SS, Go MG, Kim JH and Woo SW (2025) KoCVAM-led validation of KeraSkinTM Phototoxicity Assay for inclusion in OECD TG 498, ALTEX. DOI: 10.14573/altex.2407041

Annex. Peer Review Panel Composition

Allison Hilberer, M.S., DABT,	Senior Toxicologist and Study Director, Institute for In Vitro Sciences, Inc. U.S.
Helena Kandarova, PhD., ERT	Director of IEPT at CEM and a member of Executive Board, Centre of Experimental Medicine (CEM), Institute of Experimental Pharmacology and Toxicology (IEPT), Slovak Academy of Sciences (SAS), Slovakia
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