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PERFORMANCE STANDARDS FOR THE ASSESSMENT OF PROPOSED SIMILAR OR MODIFIED VITRIGEL-EYE IRRITANCY TEST METHOD FOR IDENTIFYING CHEMICALS NOT REQUIRING CLASSIFICATION AND LABELLING FOR EYE IRRITATION OR SERIOUS EYE DAMAGE

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Foreword

This document describes the Performance Standards (PS) for the assessment of proposed similar or modified methods to the Vitrigel Eye Irritancy Test Method included in TG 494. The PS are intended for the developers of new or modified similar test methods.

TG 494 was adopted in 2019, on the basis of a project led by Japan, who also developed the present Performance Standards, with the collaboration of the OECD expert group on eye irritation. The PS were circulated to the Working Group of the National Coordinators of the Test Guidelines Programme (WNT), together with the draft Test Guideline 494, for review and comments in August 2018; they were revised accordingly.

The WNT approved the Performance Standards at its 31st meeting in April 2019. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to the declassification of the Performance Standards on 20 June 2019. This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology

PERFORMANCE STANDARDS FOR THE ASSESSMENT OF PROPOSED SIMILAR OR MODIFIED VITRIGEL-EYE IRRITANCY TEST METHOD FOR IDENTIFYING CHEMICALS NOT REQUIRING CLASSIFICATION AND LABELLING FOR EYE IRRITATION OR SERIOUS EYE DAMAGE

INTRODUCTION

1. The purpose of Performance Standards (PS) is to provide a basis by which proposed similar or modified test methods, both proprietary (i.e., copyrighted, trademarked, or registered) and non-proprietary, can be deemed to be structurally and mechanistically similar to a Validated Reference Method (VRM) as well as can be shown to be scientifically valid, with sufficient reliability and relevance for the specific testing purposes described in Guidance Document No. 34. (1) PS are based on valid and accepted test methods and can be used to evaluate the reliability and relevance of proposed test methods that are based on similar scientific principles or that are used to measure or predict the same biological or toxic effect. (1) Such methods are referred to as similar, or "me-too," test methods. Moreover, PS may be used to evaluate modified test methods that propose potential improvements to an approved version of the test method. In such cases, PS should be used to determine the effect of the proposed changes on test method performance and the extent to which such changes affect other information available from the validation process, such as the definition of essential test method components. Depending, however, on the number and nature of the proposed changes as well as the data and documentation available in relation to these changes, modified test methods could be found unsuitable for PS-based validation when the changes are so substantial that the method is no longer sufficiently similar to the PS. In such cases, the modified test method should be subjected to the same validation process as any proposed test method. (1) Mutual Acceptance of Data (MAD) will only be guaranteed for test methods validated according to Performance Standards, if those test methods have been reviewed and included in the respective Test Guideline (TG) by the OECD. Proposed similar (me-too) or modified test methods considered similar to the VRMs according to these PS should therefore be submitted to the OECD for adoption and inclusion into TG *** before being used for regulatory purposes.

2. Proposed similar (me-too) or modified test methods proposed for use under TG *** should be evaluated prior to their use for regulatory purposes to establish their similarity to the VRMs and to determine their reliability and relevance to the identification of chemicals not requiring classification for serious eye damage or eye irritation according to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS). (2) The reliability and relevance of proposed similar (me-too) or modified test methods should be determined using a set of Reference Chemicals (Table 2) that represent the full range of the TG 405 in vivo serious eye damage or eye irritation responses: namely, Serious Eye Damage (UN GHS Category 1), Eye Irritation (UN GHS Categories 2A and 2B), and Not Classified (UN GHS No Category). (2)(3) The proposed similar or modified test methods should demonstrate reliability and predictive capacity at least as good as the defined minimum values derived from paragraph 26 of the Vitrigel™ Eye Irritancy Test (EIT) method (4)(5). The PS comprise (i) Essential Test Method Components, (ii) Minimum List of Reference Chemicals, and (iii) Defined Reliability and Accuracy Values that the proposed test method should meet or exceed. (1)

ESSENTIAL TEST METHOD COMPONENTS

3. The Essential Test Method Components consist of essential structural, functional, and procedural elements of the VRM that should be included in the protocol of a proposed, mechanistically and functionally similar or modified test method. These components include unique characteristics of the test method, critical procedural details, and quality control measures. Adherence to essential test method components will help to assure that a similar or modified proposed test method is based on the same concepts as the corresponding VRMs. (6)(7)(8) The essential test method components are described in detail in the following paragraphs.

Culture of human corneal epithelial cells

4. Human corneal epithelium-derived cells (e.g., HCE-T cells, RCB No. 2280, obtained from RIKEN BioResource Center (Tsukuba, Japan) (9)) should be used to fabricate the HCE model.

5. In the VRM, HCE-T cells are maintained in a culture medium comprising a 1:1 mixture of Dulbecco's modified Eagle medium and nutrient mixture F-12 supplemented with 5% heat-inactivated fetal bovine serum, 5 μg/mL recombinant human insulin, 10 ng/mL recombinant human epidermal growth factor, 0.5% dimethyl sulfoxide, 100 units/mL penicillin and 100 μg/mL streptomycin (e.g., commercially available culture medium for fabricating corneal models from Kanto Chemical Co., Inc. (Tokyo, Japan)). Cells are grown at 37°C in a humidified atmosphere of 5% CO2 in air.

Fabrication of a human corneal epithelium model

6. The HCE model should be prepared in a chamber accompanying a membrane that is composed of high density collagen fibrils equivalent to connective tissues in vivo (e.g., a collagen vitrigel membrane (CVM)). (10)(11)(12)(13)(14)

7. In the VRM, ad-MED vitrigel purchased from Kanto Chemical Co., Inc. is used for fabricating the HCE model. The chamber is set in the well of a 12-well plate. Then, the collagen xerogel membrane is immersed for 10 min in the culture medium by pouring 1.5 mL outside and 0.5 mL inside the chamber in the well to convert the xerogel into vitrigel immediately before use. The culture medium outside the chamber in the well of a 12-well plate is replaced with 1.5 mL of a fresh medium. The medium inside the chamber is removed and 0.5 mL of the HCE-T cell suspension in a culture medium at a density of 1.2 × 105 cells/mL is poured onto the CVM in the chamber and cultured for 2 days at 37°C. Subsequently, the cells are cultured for 4 days at the air–liquid interface to fabricate the HCE model after removing the inside medium and changing the outside medium to a fresh medium. The medium outside the chamber is changed on the third day of culture at the air–liquid interface.

Histomorphology

8. The HCE model should show a human corneal epithelium-like structure including about 6 layers of viable epithelial cells with a superficial layer of non-keratinized cells.

Barrier function

9. The HCE model should possess sufficient robustness equivalent to human corneal epithelium in order to avoid rapid disruption after chemical exposure. The barrier function

of each HCE model is checked by measuring its trans-epithelial electrical resistance (TEER) value. Adequate ranges should be provided for any proposed similar or modified test method.

10. In the VRM, the quality of HCE models is checked as follows. First, 500 μL of a fresh culture medium is poured in the chamber of the HCE models and the temperature of the culture medium is adjusted to 28±2°C. Next, the longer electrode of a TEER Measuring System (Refer to the section "Measurement of TEER value in a human corneal epithelium model.") is set into the culture media outside the chamber, and the shorter electrode is set into the culture media inside the chamber, after which the TEER value of each HCE model is measured. Only HCE models with a TEER value between 140 Ω·cm2 and 220 Ω·cm2 are acceptable for the following chemical exposure test performed on the same day.

Reproducibility

11. The results of the positive, negative, and reference controls of the test method should demonstrate reproducibility over time. (Refer to the section "Preparation of Control Substances.")

Measurement of TEER value in a human corneal epithelium model

12. TEER values of the HCE model should be measured by using an electrical resistance meter with low-voltage and alternating current. General specifications of the instrument are an alternating current of 50–1,000 Hz and a measuring range of at least 0.1–3 kΩ (e.g., TEER Measuring System from Kanto Chemical Co., Inc.). Schematic illustrations of the TEER measuring system are shown in Figure 1. The inner electrode is positioned inside the chamber, and the outer electrode is positioned outside the chamber. The distance between the inner and outer electrode must be consistent, because this distance affects the electrical resistance value obtained. Also, during resistance measurement, the depth to which the electrodes are submerged in the medium or buffer solution inside and outside of the chamber must also be consistent. The electrical resistance value of the HCE model fabricated in a CVM chamber (Rmodel) and that of its blank, an empty CVM chamber (Rblank) are measured. The TEER value of the HCE model is calculated as follows:

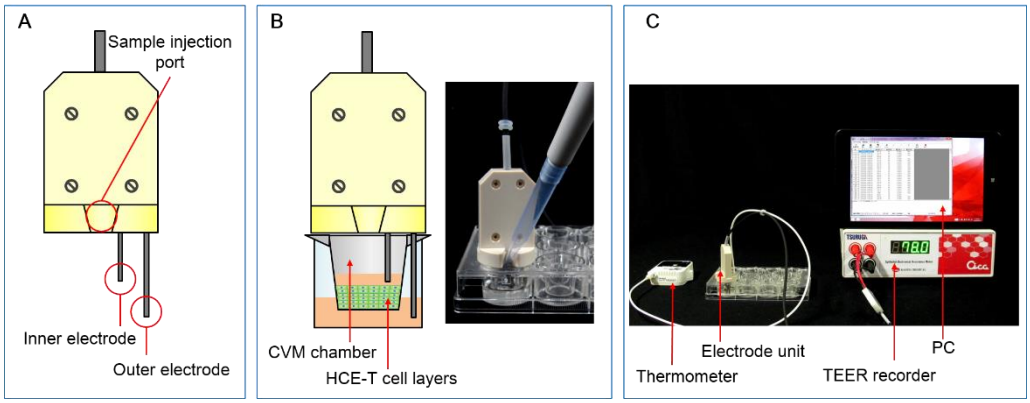
$$\text{TEER value of the HCE model } (\Omega\cdot\text{cm2}) = \{\text{Rmodel } (\Omega) - \text{Rblank } (\Omega)\} \times \text{effective surface area (cm2)}$$

The sensitivity of the TEER Measuring System in any proposed similar or modified instruments should be checked before testing, and adequate ranges should be provided.

13. In the VRM, the pre-operation check of the TEER Measuring System is performed as follows. The individual CVM-free chamber (ad-MED Vitrigel without a CVM) is set for the two wells of a 12-well plate, and subsequently one well is filled with 3.0 mL of 0.90% NaCl aqueous solution and another well is filled with 0.45% NaCl aqueous solution at 25±5°C. Then, the TEER values in both wells are measured using the TEER Measuring System. The TEER measurement is functioning normally when the measured TEER values satisfy the following conditions.

$$(\text{TEER value of 0.45\% NaCl aqueous solution}) - (\text{TEER value of 0.9\% NaCl aqueous solution}) \geq 60\ \Omega\cdot\text{cm2}$$

Figure 01. 1**igure 1. Schematic illustrations as well as photographic images of the TEER measurement electrodes for the HCE model and TEER recorder**



Note: Figure A shows the electrode unit, Figure B the electrode unit applied to the culture media via HCE model, and Figure C the TEER recorder system

Preparation of Control Substances

14. An appropriate positive control, negative control, and reference control should be tested for each run to ensure adequate performance of the HCE model. Each control substance should be dissolved in an electrically conductive solution suitable for the measurement of TEER values. Also, the concentration of each control substance solution should be adjusted so as not to affect measurement of TEER values.

15. The VRM specifies the use of saline as a negative control, benzalkonium-chloride as a positive control, and ethanol as a reference control. Control substance solutions are prepared in the culture medium at a concentration of 2.5% (weight/volume) by adding 0.1–0.2 g of the control substance to a 15-ml tube, pouring an appropriate volume of the culture medium into the tube, and mixing until dispersed uniformly. As long as the proper concentration is maintained for each control solution, the actual quantity is unimportant.

Preparation of Test Chemicals

16. A test chemical should be well dissolved or homogeneously dispersed in an electrically conductive solution that is suitable for measuring TEER values. Also, the concentration of each test chemical solution should be adjusted so as not to affect measurement of TEER values. The parameters (time, temperature, intensity, etc.) for preparing the test chemical solutions should be adjusted to accommodate the physiochemical properties of each test chemical.

17. The VRM stipulates that a test chemical solution is prepared in the culture medium at a concentration of 2.5% (weight/volume), and that each test chemical solution be suitable for measuring TEER values without undue influence from the electrical resistance of the test chemical itself. The test chemical is manually mixed in the medium until dissolved or for a maximum of one minute. If the test chemical does not dissolve readily, use one of the following techniques, which are listed here in order of preference:

a) mix mechanically for a maximum of one minute using a vortex mixer,

b) sonication for a maximum of 20 minutes, or

c) heating to a maximum temperature of 70°C.

18. After mixing, the temperature of the test chemical solution is adjusted to 28±2°C, and the solubility of the test chemical is checked. Move on to the next step only if the test chemical solution is well dissolved or homogeneously dispersed. Test chemicals that prove to be insoluble or immiscible using the above techniques are to be prepared as a homogeneous suspension by vortexing the test chemical in the medium for up to 1 minute immediately before use. The pH level of each 2.5% test chemical solution is measured using universal pH test paper from ADVANTEC (Tokyo, Japan).

Application of the Test Chemicals and Control Substances

19. The HCE models that pass the quality check are suitable for use in a chemical exposure test. At least three HCE models should be used for each control substance and each test chemical in each run. A sufficient amount of control substance solution or test chemical solution should be applied to uniformly cover the apical surface of the HCE model and soak a consistent length of electrodes in the medium or buffer solution during the chemical exposure test. The temperature of the control substance solutions, the test chemical solutions, and the HCE models should be kept constant, because temperature affects the measurement of electrical resistance.

20. The VRM stipulates that the medium inside the chamber of the HCE model be replaced with 500 μL of a test chemical solution, and Rmodel values be measured at intervals of 10 seconds for a period of 3 min after exposure to the test chemical solution. Three runs are made for each test chemical and the new HCE model is used in each test. To ensure reproducibility, it is essential that measurements begin between 2 to 5 s after adding the test chemical solution. A minimum of a two-second wait before beginning measurements is necessary, because the liquid around the electrode is often unstable for up to 2 s after adding the test chemical solution. Also, the TEER value of the HCE model has already been changed by adding the test chemical for over 5 s. The temperature of the HCE models and the test chemical solutions should be maintained at 28±2°C during the chemical exposure tests. This can be done using a hot plate, a water bath, or an air conditioner. The temperature of the HCE model can be confirmed by measuring the actual temperature of culture medium outside the HCE model.

Calculating eye irritancy of test chemicals

21. The TEER values of the HCE model after exposure to a test chemical is calculated using the formula given above in the section "Measurement of TEER value in a human corneal epithelium model." The mean TEER values for all three tests are analyzed by using the following three indexes: time lag (t1), intensity ($-$ [P2 $-$ P1] / [t2 $-$ t1]), and plateau level (100 $-$ P2), as shown in Figure 2. Time lag (t1) is defined as the maximum time at which a profile is maintained at $0 \geq dP/dT > -0.03\%$/second. The starting time of plateau level (t2) is defined as the initial time at which the profile is maintained at $0 \geq dP$ (P3 $-$ P2)/dT (t3 $-$ t2) $> -0.03\%$/s after the profile is maintained at $dP/dT \leq -0.03\%$/second for a particular period. The time (t3) is represented in the equation (t3 = t2 + 30 seconds) because the plateau level is evaluated by the profile for 30 seconds. P1, P2, and P3 are the percentages against the initial TEER value at t1, t2, and t3 after exposure to the test chemical. Subsequently, the eye irritation potential of a test chemical is predicted to be either irritant or non-irritant in accordance with the criteria for each index shown in Table 1. Suitable criteria should be established for any proposed similar or modified test method.

Figure.2. Graph showing an analysis of a TEER profile after exposure of a model to a test chemical.

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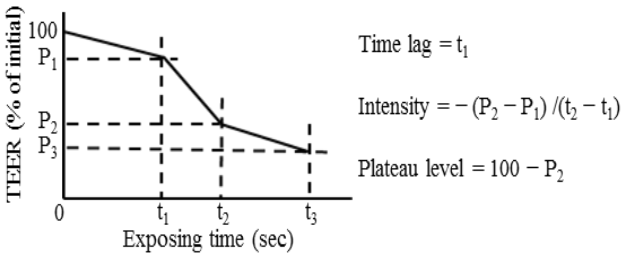

Note: t1 represents time lag, and t2 represents the start of the plateau level. t3 is defined as t2 + 30 s. P1, P2, and P3 indicate percentages at t1, t2, and t3, respectively, relative to the initial TEER value

Table 1. . Eye irritancy criteria

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Criteria	Prediction
Time lag = 180 or Intensity = 0.05 or Plateau level >5.0	1/2A/2B
Time lag > 180 and Intensity < 0.05 and Plateau level = 5.0	No category

Acceptance Criteria

22. Test results are judged to be acceptable when the following criteria are all satisfied:

a) Negative control: The plateau level is 5% or less of the TEER value at 0 seconds.

b) Positive control: The plateau level is 40% or more of the TEER value at 0 seconds.

c) Reference control: The plateau level is 10% or more of the TEER value at 0 seconds.

Applicability domain

23. It was reported that epithelial tissues such as isolated rabbit esophageal mucosal epithelium and normal human bronchial epithelial cell layers displayed increased TEER values when exposed to weak acidic solutions. (15)(16) Similarly, the TEER values of the HCE models were increased from their initial values after exposure to acidic test chemicals that yielded false-negatives. On the other hand, water-insoluble solids that were easily separated from the culture medium tend to yield false-negatives. The VRM stipulates two restrictions to the applicability domain in consideration of above:

- All chemicals that have a pH level of 5 or less in solution are excluded from the applicability domain.

- All solids that have both a logP value of 2.5 or more and a density of either less than 0.95 g/cm3 or greater than 1.10 g/cm3 are excluded from the applicability domain.

MINIMUM LIST OF REFERENCE CHEMICALS

24. Reference Chemicals are used to determine if the reproducibility and predictive capacity of a proposed similar or modified test method that is structurally and functionally similar to or comprises only a minor modification of the VRM are at least as good as the minimum values stipulated in the VRM. The 20 Reference Chemicals listed in Table 2 were selected from the chemicals used in validation studies of the VRM. (4) Insofar as possible, they include chemicals that

(i) include different physical states,

(ii) cover the full range of serious eye damage or eye irritation responses based on high quality results from the reference in vivo rabbit eye test (OECD TG 405) (2)(17) and Categories 1, 2A, 2B, or No Category of the UN GHS, (2)

(iii) cover the various in vivo drivers of classification, (18)(19)

(iv) are representative of the chemical classes used in the validation studies, (4)

(v) represent a wide range of organic functional groups,

(vi) have chemical structures that are well-defined,

(vii) include acidic chemicals or insoluble chemicals,

(viii) cover the full range of in vitro responses based on high quality Vitrigel™ EIT data,

(ix) are commercially available, and

(x) are not prohibitively expensive either to acquire or to dispose of.

25. The 20 Reference Chemicals listed in Table 2 include chemicals representing different chemical classes (i.e., chemical categories based on organic functional groups) and are representative of the full range of TG 405 in vivo responses. The list includes six chemicals from UN GHS Category 1, four from Category 2A, four from Category 2B, and six No-Category chemicals. These are the minimum number of chemicals that should be used to evaluate the reproducibility and predictive capacity of any proposed similar or modified test method able to identify chemicals not requiring classification and labelling for serious eye damage or eye irritation according to the UN GHS. (2) Nevertheless, the development or optimization of proposed similar or modified test methods should avoid using these Reference Chemicals exclusively and to the full extent possible all additional chemicals used for determining exposure times, establishing prediction models, or any other aspect of test method development should be reported when submitting a PS-based validation study. In situations where a listed chemical is unavailable or cannot be used for other justifiable reason, it should be substituted with another chemical that was used in the validation of a VRM or otherwise fulfills the criteria described in paragraph 22 above. To further evaluate the accuracy of the proposed test method, additional chemicals representing other chemical classes and for which adequate in vivo reference data are available may be tested in addition to the Reference Chemicals listed in Table 2.

Table 2. Minimum list of Reference Chemicals for determination of Reliability and Predictive Capacity for modified test methods

		Result in VRM[1]								
	Chemical Name	CASRN	Organic Functional Group	Physical State	Time lag (s)	Intensity (%/s)	Plateau level (%)	Prediction[2]	AD1(Acidic)	AD2) insoluble)
	In vivo category 1[3]									
1	Captan	133-06-2	Heterocyclic compound, Sulfur compound (organic)	Solid	>180	-0.01	0	No categoly	No	Yes
2	3-(2-Aminoethylamino) propyl]trimethoxysilane	1760-24-3	Silicon compound	Liquid	0	0.41	73	1/2A/2B	No	No
3	Tetraethylene glycol	17831-71-9	Acrylate, Ester	Liquid	0	0.20	35	1/2A/2B	No	No
4	Sodium salicylate	54-21-7	Organic salts	Solid	0	0.35	38	1/2A/2B	No	No
5	*m*-Phenylenediamine	108-45-2	Amines	Solid	0	0.42	74	1/2A/2B	No	No
6	Imidazole	288-32-4	Hetrocyclics	Solid	80	0.31	33	1/2A/2B	No	No
	In vivo category 2A[3]									
7	gamma-Butyrolactone	96-48-0	Heterocyclic compounds, Ketones	Liquid	10	0.23	42	1/2A/2B	No	No
8	Cyclopentanol	96-41-3	Alcohols	Liquid	0	0.28	51	1/2A/2B	No	No
9	Methyl acetate	79-20-9	Esters	Liquid	0	0.18	32	1/2A/2B	No	No
10	Dibenzyl phosphate	1623-08-1	Organophosphorus compound	Solid	0	0.39	59	1/2A/2B	Yes	No
	In vivo category 2B[3]									
11	2-Methyl-1-pentanol	105-30-6	Alcohols	Liquid	0	0.26	48	1/2A/2B	No	No
12	1-(2-Propoxy-1-methylethoxy)-2-propanol	29911-27-1	Alkoxylated alcohols	Liquid	0	0.21	39	1/2A/2B	No	No
13	Ethyl-2-methylacetoacetate	609-14-3	Esters	Liquid	10	0.19	34	1/2A/2B	No	No
14	Camphene	79-92-5	Hydrocarbons	Solid	>180	-0.03	0	No category[4]	No	No
	In vivo no category[3]									
15	*iso*-Octyl acrylate	29590-42-9	Acrylates	Liquid	>180	-0.01	0	No category	No	No
16	2-(*n*-Dodecylthio) ethanol	1462-55-1	Alcohol, Ether, Sulfur compound	Liquid	>180	0.00	0	No category	No	No
17	*iso*-Octylthioglycolate	25103-09-7	Thiocompound, Ester	Liquid	>180	-0.02	0	No category	No	No
18	2,4-Pentanediol	625-69-4	Alcohols	Liquid	130	0.12	8	1/2A/2B	No	No
19	Gluconolactone	90-80-2	Lactone	Solid	0	0.31	9	1/2A/2B	Yes	No
20	Potassium tetrafluoroborate	14075-53-7	Inorganic salt	Solid	0	0.47	14	1/2A/2B	No	No

Abbreviations: CASRN, Chemical Abstracts Service Registry Number; UN GHS, United Nations Globally Harmonized System of Classification and Labelling of Chemicals; VRM, Validated Reference Method; AD1, Applicability domain 1, i.e., Exclude all test chemicals that have a pH level of 5 or less in solution; AD2, Applicability domain 2., i.e., Exclude all solids that have both a logP value of 2.5 or more and a density of either less than 0.95 g/cm3 or over 1.10 g/cm3.

[1] Based on results obtained during validation of the Vitrigel-EIT method. (4)

[2] When discordant results were obtained within and/or between laboratories in the validation study, the prediction of the VRM indicated in the table is based on the mode of all predictions. (See footnote 4.) False-positive and false-negative predictions from VRM are underlined.

[3] Based on results from the in vivo rabbit eye test (OECD TG 405) (3)(17) and using the UN GHS. (2)

[4] The VRM prediction is based on the mode of all predictions obtained in the validation study. Discordant results were obtained at one of three laboratories.

Definitions of Calculated Values for Reproducibility and Predictive Capacity Used to Assess Reliability and Relevance

26. In order to assess the reliability and relevance of proposed similar or modified test methods, all 20 Reference Chemicals listed in Table 2 should be tested by at least three laboratories. Each test should comprise three independent runs and be performed with the HCE models from different manufacturing batches. Each run should comprise at least three HCE models for each test chemical, negative control substance, positive control substance and reference control substance, all of which are tested concurrently.

27. The calculation of values for within-laboratory reproducibility (WLR) and between-laboratory reproducibility (BLR) as well as the three components of predictive capacity—namely, sensitivity, specificity, and accuracy—should be performed according to the following rules to ensure the use of a predefined and consistent approach.

1. WLR should be calculated based on concordance of predictions made using only qualified tests results obtained from Reference Chemicals for which at least three qualified tests are available.

2. BLR should be calculated based on concordance of predictions made using only qualified tests results obtained from Reference Chemicals for which at least one qualified test per laboratory is available.

3. Sensitivity, specificity, and accuracy should be using all qualified test results obtained from each Reference Chemical for which at least one qualified test per laboratory is available. The calculations should be based on the individual predictions made for each qualified test of each Reference Chemical in each laboratory. The calculations should never be based on the arithmetic mean values of TEER values across multiple tests, the mode of all predictions obtained, or any other procedure used to summarize multiple test results into a single prediction per Reference Chemical. The predictive capacity should be assessed using a weighted calculation in which the final outcome of each individual qualified test obtained for each Reference Chemical from all laboratories participating in the validation study is captured as an independent prediction in the calculations and correction factors are applied so that all Reference Chemicals exert an equal weight in the calculations, even in cases where it was not possible to obtain the same number of qualified tests for all Reference Chemicals during the validation study. In summary, the prediction for each Reference Chemical obtained at each laboratory participating in the study should be divided by the total number of available predictions to determine the number of correct, over-, and under-predictions for each Reference Chemical and these should be used to calculate sensitivity, specificity, and accuracy in a manner that all chemicals exert an equal weight in the calculations.

28. The repeatability and predictive capacity of proposed similar or modified test methods should be at least as good as that stipulated in the VRM. (4) Assessments of WLR and BLR should show that predictions for the 20 Reference Chemicals listed in Table 2 and based on the results of different, independent tests conducted at each laboratory demonstrate a concordance of at least 90% as well as a sensitivity of 90%, a specificity of 50%, and an accuracy of 75%. Furthermore, no UN GHS Category 1 Reference Chemicals

should be under-predicted by a majority of qualified tests at the laboratories participating in the validation study.

LITERATURE

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