New Chemicals Program Decision Framework for Hazard Identification of Eye Irritation and Corrosion

Purpose

This document provides a decision framework for use by human health assessors within EPA's New Chemicals Program (NCP) for identification of eye irritation or corrosion hazards for new chemical substances based on prioritization of reproducible, human-relevant data.

Introduction

Multiple test methods are available to assess the eye irritation or corrosion potential of new chemical substances. It has been demonstrated that the in vivo eye irritation test in rabbits lacks reproducibility, particularly in the mild to moderate range of irritancy (1), which questions the relevance of the in vivo data to humans. When available in chemico, in vitro, ex vivo and in vivo eye irritation/corrosion test methods were assessed for their reproducibility and relevance to mechanisms of human eye irritation/corrosion, these methods were found to perform as well as or better than the in vivo rabbit eye irritation test (1) (2). Many of these in chemico, in vitro and ex vivo methods have been formally validated and accepted internationally and their accuracy, specificity and sensitivity have been assessed in relation to historical in vivo rabbit eye irritation/corrosion data and the eye irritation/serious eye damage classifications derived from those data as described in the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) (3).[1]

To identify new chemicals that are not irritating to eyes, NCP prefers the use of methods that use human cells/tissues known to be sensitive, which carry a high degree of confidence when generating nonirritating predictions (e.g., OECD TG 492 (1)). To identify new chemicals that are irritating or corrosive to eyes, NCP prefers the use of methods that use human cells/tissues with the potential to assess the full range of severity (e.g., (OECD TG 492B (2)) and/or other reproducible and relevant in chemico, in vitro or ex vivo methods. NCP does not encourage the prospective use of the in vivo eye irritation test using live rabbits (e.g., the Draize test).

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[1] Although EPA's New Chemicals Program does not classify or label chemicals according to GHS categories, this document adopts GHS principles to guide the evaluation of eye irritation/corrosion hazards of new chemicals under Section 5 of the Toxic Substances Control Act (TSCA).

Decision Framework Overview

To predict the eye irritation or corrosion potential of new chemical substances,[2] a decision framework based on the prioritization of reproducible and human-relevant data has been developed and is presented below. Full details of how the existing data are prioritized are provided in Figure 1 and in the 'Decision Framework'. Briefly:

- Data from the new chemical substance of interest are reviewed for scientific quality and applicability to evaluating human eye irritation or corrosion potential and prioritized. Where data from the new chemical substance are unavailable, data from the most appropriate analogues are used.[3]
    - Data from new chemical or analogue eye irritation/corrosion test methods are prioritized in the following order:
        1. Data from human cell/tissue test methods that have been demonstrated to be reproducible and relevant to eye irritation/corrosion.
        2. Data from in chemico, in vitro and/or ex vivo test methods that have been demonstrated to be reproducible and provide information on the mechanisms of toxicity relevant to eye irritation/corrosion.
        3. Data from in vivo test methods.
    - Where no data from eye irritation/corrosion test methods are available, data from test methods that assess skin irritation/corrosion potential may be considered, following the same prioritization order as eye irritation/corrosion data. However, categorization is only possible in this scenario if skin irritation/corrosion data predicts the substance of interest to be irritating or corrosive.
- If eye and skin irritation/corrosion data are not available, physicochemical properties or other information such as structural alerts, other relevant test data or chemical category conclusions from the TSCA New Chemicals Program Chemical Categories document may be considered (4). The variety of information that EPA may consider is varied and broad and outside the scope of this framework. The complete absence of any relevant data or structural alert information for the new chemical substance may preclude a hazard determination.
- Any relevant human data will be evaluated and incorporated on a case-by-case basis. Details about that evaluation are outside the scope of this framework, however guidance developed by other international entities is available (5).

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[2] This eye irritation/corrosion hazard identification does not apply to nanomaterials.
[3] Methods for evaluating analogues for suitability are outside the scope of this framework.

NCP uses three categories to identify new chemicals for their eye irritation potential: corrosive, irritating and nonirritating. Whereas some in chemico/in vitro/ex vivo test methods provide only binary outcomes (e.g., to discriminate between nonirritants and those chemicals that cause ocular damage), outcomes from several in chemico/in vitro/ex vivo test methods are capable of assessing the full spectrum of eye irritation/corrosion and provide identification of all three categories (Table 3).

EPA maintains a List of Alternative Test Methods and Strategies (or New Approach Methodologies [NAMs]) that includes representative NAMs that EPA may consider. Test methods specified throughout this framework and presented in the EPA NAMs list are representative and are not meant to be an exhaustive list of NAMs that could be used for TSCA decisions. NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) also maintains a list of Alternative Methods Accepted by US Agencies. Data from studies not approved by OECD, EPA or NICEATM will be evaluated on a case-by-case basis for quality, reliability and relevance to mechanisms of human eye irritation/corrosion.[4] The applicability domain for some established test methods can be found in Table 3.

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[4] The review process for data from studies not approved by OECD, EPA or NICEATM is outside the scope of this framework.
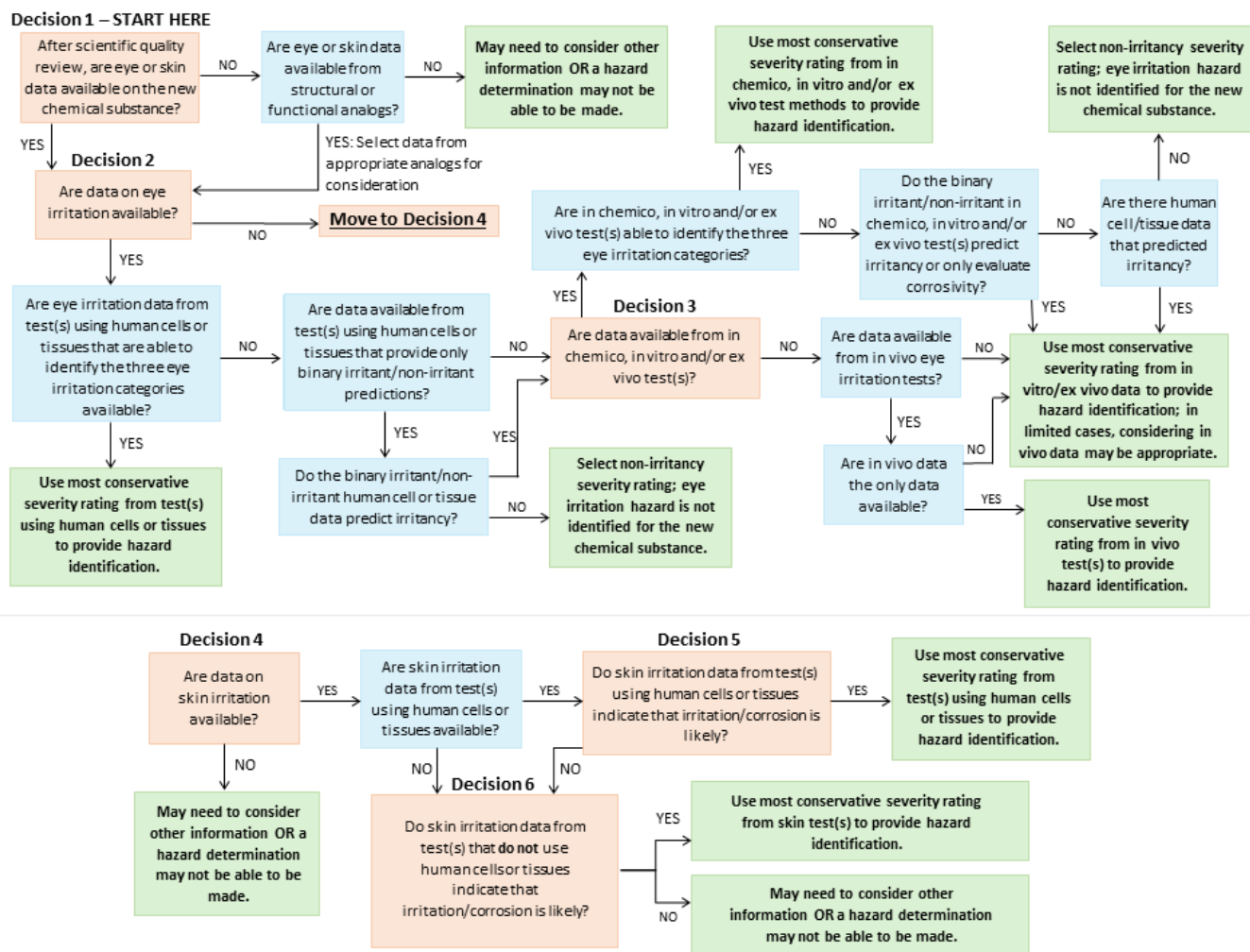
Decision Framework

Figure 1: Flowchart demonstrating the basic parameters and prioritization order of data within the decision framework. Further details and references are provided within the text. Orange boxes indicate major decisions within the framework, blue boxes represent sub-decisions and green boxes indicate outcomes.

Decision 1: Prioritize data

➢ Complete scientific quality assessment of all available data. This may include, but is not limited to, protocol adherence to test guidelines, inclusion of acceptable controls, sufficient sample size and/or appropriately applied statistical approaches. In vitro assays should be specifically evaluated for compliance with applicability domains (Table 3). Data from studies not approved by OECD, EPA or NICEATM must undergo a thorough review for quality, reliability and relevance to mechanisms of human eye irritation/corrosion. These data will be evaluated on a case-by-case basis for consideration; the review process for those studies is outside the scope of this framework. Data that do not meet scientific quality criteria or were gathered from in vitro assays that are not appropriate for the test substance type should not be further evaluated.[5]

➢ If eye or skin irritation/corrosion data are available for the new chemical substance, move to **Decision 2** to evaluate these data.

➢ If eye or skin irritation/corrosion data are not available for the new chemical substance, data from analogues may be considered.
   o If eye or skin irritation/corrosion data from analogues are available, collate data on the most appropriate structural or functional analogues and move to **Decision 2** to evaluate these data.

➢ If eye or skin irritation/corrosion data of sufficient quality as determined in Decision 1 for the new chemical substance or appropriate analogues are not available, physicochemical properties or other information such as structural alerts, other relevant test data or chemical category conclusions from the TSCA New Chemicals Program Chemical Categories document (4) may be considered. The variety of information that EPA may consider is varied and broad and outside the scope of this document. **Hazard identification for new chemical substances with a complete lack of any appropriate new chemical substance or analogue eye or skin irritation/corrosion test data is outside the scope of this document.** The complete absence of any relevant data or structural alert information for the new chemical substance may preclude a hazard determination. **STOP HERE.**

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[5] Further details regarding scientific quality considerations are outside the scope of this framework.

Decision 2: Evaluate prioritized data, either from new chemical substance or from one or more appropriate analogues, for relevance to eye irritation/corrosion endpoint and to humans.

➢ If eye irritation/corrosion data are available, then assess them for their relevance to humans: Are the data from a test that uses human cells or tissue (2)?

    o **All human | All in vitro**
If eye irritation/corrosion data are from test(s) that use human cells or tissues, then determine whether the test method(s) are able to identify the three eye irritation categories (corrosive, irritating and nonirritating) or whether the test method(s) only provide binary outcomes (i.e., nonirritating vs irritating/corrosive) (Table 1**:**).

Table 1: Hazard identification capability for test methods that use human cells or tissues

Hazard identification capability	Test Method[6]
• Able to identify all three eye irritation categories	OECD TG 492B (6)
• Provides binary outcomes (i.e., nonirritating vs irritating/corrosive)	OECD TG 492 (7) OECD TG 494 (8)

        ▪ If the data are from test method(s) that are able to identify the three eye irritation categories, then select the most conservative severity rating. **STOP HERE.**

        ▪ If the test(s) are only able to provide a binary identification and the data predicts nonirritancy, then select the nonirritating severity rating. **STOP HERE.**

        ▪ If the test(s) are only able to provide a binary identification and the data predicts irritancy/corrosivity, then select the most conservative severity rating. For example, in absence of any additional data, a result of irritating/corrosive from OECD TG 492 (or, as stated in the test guideline: "further information is required" (7)) would result in identifying a concern for corrosion. **STOP HERE.**

    o **Mixed human and nonhuman | Mixed human in vitro, nonhuman in vitro/ex vivo and/or in vivo**
If the eye irritation/corrosion data are from multiple species and type of assay, including tests that use human cells or tissues, then assess whether human cell or tissue method can identify the three eye irritation categories.

        ▪ If the test(s) are able to identify the three eye irritation categories, then select the most conservative severity rating. **STOP HERE.**

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[6] Assays listed in this table are representative test methods. Data from studies not approved by OECD, EPA or NICEATM will be evaluated on a case-by-case basis by EPA prior to acceptance for use in identifying eye irritation hazard. As new test methods are approved or established, this list may change.

- If the test(s) are only able to provide a binary identification and the data predict nonirritancy, then select the nonirritating severity rating. **STOP HERE.**
- If the test(s) are only able to provide a binary identification and the data predict irritancy/corrosivity, then assess data available from other in chemico/in vitro/ex vivo tests, moving to **Decision 3.**

- **All nonhuman | All in vitro/ex vivo**
  If none of the eye irritation/corrosion data are from test(s) that uses human cells or tissues, but are from in chemico, in vitro or ex vivo methods, then move to **Decision 3**.

- **All nonhuman | Mixed in vitro/ex vivo and in vivo**
  If the eye irritation/corrosion data are from a test(s) that do not use human cells or tissues, but there are multiple tests from in chemico, in vitro/ex vivo and in vivo sources, then move to **Decision 3**.

- **All nonhuman | All in vivo**
  If none of the eye irritation/corrosion data are from test(s) that use human cells or tissues but are from a nonhuman in vivo method (usually using rabbits), then select the most conservative severity rating associated with the in vivo methods. **STOP HERE.**

➢ If no eye irritation/corrosion data are available (i.e., only skin irritation/corrosion data are available), then move to **Decision 4.**

Decision 3: Evaluate nonhuman tests, including in chemico, in vitro and ex vivo eye irritation/corrosion data, many of which are more reproducible than the in vivo rabbit eye irritation test (1) (2). Only data from EPA-, OECD- or NICEATM-approved test methods or data from other test methods that passed scientific quality assessment in Decision 1 should be considered in this step.

➢ If the data are from in chemico, in vitro and/or ex vivo test(s) developed to assess for irritation or corrosion and the chemical tested (new chemical or analogue) is not excluded from the applicability domain of the test(s) then assess whether the in chemico, in vitro and/or ex vivo test(s) are able to identify all three categories.

Table 2: Hazard identification capability for some in chemico, in vitro and/or ex vivo test(s)

Hazard identification capability	Test Method[6]
• Able to identify all three eye irritation categories	OECD TG 467 (9) OECD TG 437: within an IATA or WOE approach (10) (11) OECD TG 438: within an IATA or WOE approach (12) OECD TG 491: within an IATA or WOE approach (13)
• Provides binary outcomes (e.g., nonirritating vs irritating/corrosive or evaluates for corrosivity only)	OECD TG 437: as a standalone (10) (11) OECD TG 438: as a standalone (12) OECD TG 491: as a standalone (13) OECD TG 460 (14) OECD TG 496 (15) Cytosensor Microphysiometer (16) OptiSafe (17) (18) Isolated/Enucleated Rabbit Eye (19) (20) Neutral Red Release (21) (22) Ex Vivo Eye Irritation Test (23)
• Identifies reversibility	Porcine cornea opacity/reversibility assay (24) (25)

○ If the data are from test method(s) that are able to identify the three eye irritation categories, then select the most conservative severity rating from the discriminating in chemico, in vitro or ex vivo test method. **STOP HERE.**

○ If the in vitro/ex vivo test(s) are only able to provide a binary identification and the data predicts irritancy, then select the most conservative severity rating. If the data are not able to discern between irritation and corrosion, select corrosivity. If the data are only able to evaluate corrosivity (e.g., Isolated/Enucleated Rabbit Eye assay (19) (20)), then select the most conservative severity rating. In limited cases, considering supporting in vivo data

may be appropriate. For example, if in vitro data rules out corrosivity <u>only</u>, evaluating in vivo data may be warranted. **STOP HERE.**

- o If the in vitro/ex vivo test(s) are only able to provide a binary identification and the data predicts nonirritancy, determine if human cell or tissue data is available:
    - If human cell or tissue data are not available, then select the nonirritating severity rating. **STOP HERE.**
    - If in Decision 2, human cell or tissue data were evaluated that are only able to provide binary identification and the test data predicted irritancy, then select the most conservative severity rating. If the data are not able to discern between irritation and corrosivity, select corrosivity. **STOP HERE.**

Decision 4: Evaluate available skin irritation/corrosion data.[7]

- ➤ If skin irritation/corrosion data are available, then assess whether they are relevant to the species of interest: Are the data from a test that uses human cells or tissue?
    - o **All human**
      If the data are from a test(s) that uses human cells or tissue, then move to **Decision 5**.
    - o **Mixed human and nonhuman**
      If the skin irritation/corrosion data are from multiple sources, including a test(s) that uses human cells or tissue, then move to **Decision 5**.
    - o **All nonhuman**
      If the data are from skin irritation/corrosion tests that do not use human cells or tissue, then move to **Decision 6.**
- ➤ If skin irritation/corrosion data from analogues are not available, physicochemical properties or other information may be considered. Hazard identification for these new chemical substances is outside the scope of this framework. The complete absence of any relevant data or structural alert information for the new chemical substance may preclude a hazard determination. **STOP HERE.**

Decision 5: Evaluate data from skin irritation/corrosion tests that use human cells or tissue.

- ➤ If the data available are from skin irritation/corrosion tests that use human cells or tissue and indicate that irritation or corrosion to the skin is likely, then use the data to select a severity rating. **STOP HERE.**
- ➤ If the data available are from skin irritation/corrosion tests that use human cells or tissue and do not indicate that irritation or corrosion to the skin is likely, then move to **Decision 6**.

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[7] International entities have concluded that skin irritation and/or corrosion data are sufficient to identify eye irritation and/or corrosion hazard in the absence of route-specific data (3) (26).

Decision 6: Evaluate data from skin irritation/corrosion tests that do not use human cells or tissue.

- ➢ If there are data available from skin irritation/corrosion tests that do not use human cells or tissue and indicate that irritation or corrosion to the skin is likely, then use the data to select a severity rating. **STOP HERE.**
- ➢ If there are data available from skin irritation/corrosion tests that do not use human cells or tissue and that do not indicate that irritation or corrosion to the skin is likely, physicochemical properties or other information may be considered. Hazard identification for these new chemical substances is outside the scope of this document. The complete absence of any relevant data or structural alert information for the new chemical substance may preclude a hazard determination. **STOP HERE.**

Table 3: Test principles, applicability domains and categorization potential and outcome type for a number of OECD test guidelines to assess eye irritation/corrosion potential (2). This list omits test methods not approved by OECD and is not intended to be an exhaustive list of NAMs that could be used for TSCA decisions.

METHOD OR APPROACH	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN AND LIMITATIONS[8]	CATEGORIZATION AND OUTCOME TYPE
OECD TG 437: Bovine Corneal Opacity and Permeability (BCOP) test method with histopathology option (10)	Test substance is directly applied to cow eyes obtained as by-products from abattoirs. Corneal opacity (measured quantitatively as the amount of light transmission through the cornea) and permeability (measured quantitatively as the amount of sodium fluorescein dye that passes across the full thickness of the cornea) are measured. One of two opacitometers can be used: OP-Kit and LLBO. Optional histopathology can be conducted for additional information.	• Applicable to substances and mixtures and to solids, liquids, semisolids, creams and waxes. • For substances with oxidizing or reactive components, data from histopathology should be consulted. • Alcohols and ketones risk overprediction.	Outcome type: Full spectrum As standalone, per OECD TG 437: • Severe/corrosive (GHS Cat 1) • Nonirritating (GHS No Category) Can identify all three categories within an Integrated Approach to Testing and Assessment (IATA), or weight of evidence (WoE) assessment: • Severe/corrosive (GHS Cat 1) • Irritating (GHS Cat 2) • Nonirritating (GHS No Category)
OECD TG 438: Isolated Chicken Eye (ICE) test (12)	Test substance is directly applied to chicken eyes obtained as by-products from abattoirs. Corneal swelling, opacity and fluorescein retention are assessed.	• Applicable to soluble or insoluble solids, liquids, emulsions and gels. • Alcohols risk overprediction • Histopathology was found to be a useful additional endpoint to decrease the false negative rates when used to identify UN GHS Category 1 nonextreme pH (2 < pH < 11.5) detergents shown to induce mainly persistent nonsevere effects in vivo and surfactants.	Outcome type: Full spectrum As standalone, per OECD TG 438: • Severe/corrosive (GHS Cat 1) • Nonirritating (GHS No Category) Can identify all three categories within an IATA or WoE assessment: • Severe/corrosive (GHS Cat 1) • Irritating (GHS Cat 2) • Nonirritating (GHS No Category)

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[8] Published OECD Test Guidelines provide additional details in the "Initial Considerations and Limitations" section.

OECD TG 460: Fluorescein Leakage (FL) Test Method for Identifying Ocular Corrosives and Severe Irritants (14)	Epithelial monolayer Madin-Darby canine kidney (MDCK) cells are cultured on permeable inserts. The test chemical is applied for 1 minute and then removed; next, the nontoxic, highly fluorescent sodium fluorescein dye is added and the amount of dye that passes through the cell layer is measured spectrofluorometrically and used to predict toxicity.	• Applicable to water-soluble chemicals or mixtures. Limitations for colored or highly viscous substances (predictivity is improved by increasing the number of wash steps). • Not applicable to strong acids and bases, cell fixatives or highly volatile substances. • Limitations: strong acids and bases, cell fixatives, highly volatile test chemicals, colored and viscous test chemicals, solid chemicals suspended in liquid that have a tendency to precipitate.	Outcome type: Binary Cannot discriminate between irritants and nonirritants: • Severe/corrosive (GHS Cat 1)
OECD TG 467: Defined Approach 2 for neat and diluted nonsurfactant liquids (DAL2) (9)	DAL2 combines information from two sources: Results from test substance tested in OECD TG 437 BCOP assay using the laserlight-based opacitometer (LLBO) and in OECD TG 491 Short Time Exposure (STE) assay.	• Applicable to neat and diluted nonsurfactant liquids and solids dissolved in water. • Additionally, see applicability domains of OECD TG 437 and OECD TG 491.	Outcome type: Full spectrum Can identify all three categories: • Severe/corrosive (GHS Cat 1) • Irritating (GHS Cat 2) • Nonirritating (GHS No Category)
OECD TG 491: Short Time Exposure (STE) in vitro test (13)	Measures cell viability (MTT assay) of Statens Seruminstitut Rabbit Cornea (SIRC) corneal epithelial cells in 96 well plates. As compounds are generally cleared from human eyes in 1 to 2 minutes and from rabbit eyes in 3 to 4 minutes, this test requires a 5-minute exposure.	• Applicable to substances that are soluble in saline, DMSO or mineral oil • Risk of under-prediction for substances that are insoluble in water or with high vapor pressure.	Outcome type: Full spectrum As standalone, per OECD TG 491: • Severe/corrosive (GHS Cat 1) • Nonirritating (GHS No Category) Can identify all three categories within an IATA, or WoE assessment: • Severe/corrosive (GHS Cat 1) • Irritating (GHS Cat 2) • Nonirritating (GHS No Category)
OECD TG 492: Reconstructed Human Cornea-like Epithelium (RhCE) test (7)	Test substance is applied to reconstructed tissue from human cells, which have been cultured to form a stratified, highly differentiated squamous epithelium that is morphologically similar to that found in the human cornea. Cell viability (MTT or WST-8 assay) is used to predict toxicity.	• Applicable to substances and mixtures and to soluble or insoluble solids, aqueous or nonaqueous liquids, semisolids and waxes. • Substances absorbing light in the same range as formazan dye (FD) and substances able to directly reduce the vital dye MTT to FD may interfere with the tissue viability measurements and need the use of adapted controls for corrections.	Outcome type: Binary Cannot discriminate between irritants and corrosives: • Severe/corrosive (GHS Cat 1) • Nonirritating (GHS No Category)

OECD TG 492B: RhCE Test Method for Eye Hazard Identification (Time-to-Toxicity (ET50) protocols) (6)	Test substance is applied to reconstructed tissue from human cells (as in OECD TG 492). Depending on whether the test substance is a liquid or a solid, cell viability is assessed at three or two exposure times, respectively.	• Applicable to substances and mixtures and to soluble or insoluble solids, aqueous or nonaqueous liquids, semisolids and waxes. • Risk of under-prediction for solid chemicals with poor water solubility (< 0.014 mg/mL). • Substances absorbing light in the same range as FD and substances able to directly reduce the vital dye MTT to FD may interfere with the tissue viability measurements and need the use of adapted controls for corrections.	Outcome type: Full spectrum Can identify all three categories: • Severe/corrosive (GHS Cat 1) • Irritating (GHS Cat 2) • Nonirritating (GHS No Category)
OECD TG 496: In vitro Macromolecular Test Method Ocular Irritection® (15)	Test substance is directly applied to an in chemico macromolecular matrix model composed of lipids, proteins, glycoproteins, carbohydrates and low molecular weight substances that model the cellular biochemical components of the human corneal epithelium. An increase in optical density is used to predict the ocular hazard effects of chemicals.	• Applicable to solids (may be soluble or insoluble in water) and liquids (may be viscous or non-viscous) whose 10% solution/dispersion has a pH in the range 4 ≤ pH ≤ 9 and mixtures. • Some limitations for intensely colored chemicals, chemicals that cause salting-out precipitation, high concentrations of some surfactants and highly volatile chemicals. • Risk of under-prediction for substances that are insoluble in water or with high vapor pressure.	Outcome type: Binary Cannot discriminate between irritants and corrosives: • Severe/corrosive (GHS Cat 1) • Nonirritating (GHS No Category)

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