#3181 | Evaluating New Chemicals in the US under the Toxic Substances Control Act: Application of New Approach Methods to Evaluate Eye Irritation

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Background

U.S. Environmental Protection Agency's Office of Pollution Prevention and Toxics, New Chemicals Division (NCD) is responsible for conducting risk assessments under the Toxic Substances Control Act (TSCA). In 2016, TSCA (amended under the Frank R. Lautenberg Act) directed EPA to "promote the development and implementation of alternative test methods and strategies to reduce, refine, or replace vertebrate animal testing and provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment".

To incorporate New Approach Methodologies (NAMs) data for eye irritation hazard identification, NCD is collaborating with colleagues from the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), Institute for In Vitro Sciences, Inc. and PETA Science Consortium International e.V. to develop a decision framework to evaluate eye irritation hazards for new chemicals under TSCA. This framework proposes to prioritize use of data from NAMs over in vivo studies in animal models for the prediction of eye irritation in humans.

Disclaimer: This poster presents a draft approach and does not reflect views or policies of the U.S. EPA.

General Approach

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 (Luechtefeld et al., 2016), particularly in the mild to moderate range of irritancy, which questions the relevance of the in vivo data to humans. When available in chemico, in vitro, ex vivo and in vivo eye irritation test methods were assessed for their reproducibility and relevance to mechanisms of human eye irritation, these methods were found to perform as well as or better than the in vivo rabbit eye irritation test (Clippinger et al., 2021). 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 data and the eye irritation classifications derived from those data as described in the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

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

Decision Framework Overview

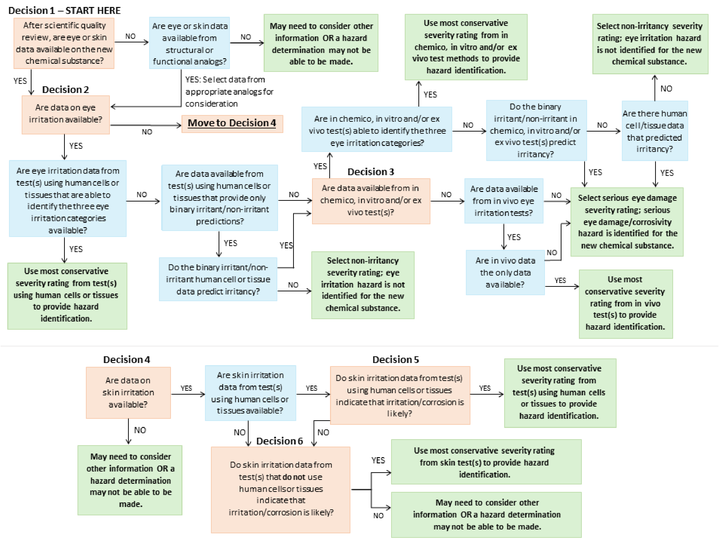
To predict the eye irritation or corrosion potential of new chemical substances, a decision framework based on the prioritization of reproducible and human-relevant data has been developed. This eye irritation hazard identification framework does not apply to nanomaterials, Microbial Commercial Activity Notices (MCANs) or TSCA Experimental Release Applications (TERAs).

- Data from the new chemical substance of interest are reviewed for scientific quality and applicability to evaluating human eye irritation potential and prioritized. Where data from the new chemical substance are unavailable, data from the most appropriate analogues are used.
  - From the collated data, data from eye irritation 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.
    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.
    3. Data from in vivo test methods.
- Where no data from eye irritation test methods are available, data from test methods that assess skin irritation potential may be considered, following the same prioritization order as eye irritation data. However, categorization is only possible in this scenario if skin irritation data predicts the substance of interest to be irritating or corrosive.
- If eye and skin irritation 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 (NCP) Chemical Categories document may be considered. The variety of information that EPA may consider is varied and broad and outside the scope of the 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 process are outside the scope of the framework.

Citations

- Luechtefeld T, Maertens A, Russo DP, et al. Analysis of draize eye irritation testing and its prediction by mining publicly available 2008-2014 REACH data. ALTEX. 2016;33:123–134.
- Clippinger AJ, Raabe HA, Allen DG, et al. Human-relevant approaches to assess eye corrosion/irritation potential of agrochemical formulations. Cutan Ocul Toxicol. 2021;40:145–167.
- Additional citations available upon request

Decision Tree



Example Application to New Chemical

- New chemical substance
  - No data
- Analogue 1 very close structural analogue
  - Health Extractions
    - Test 1: Test Guideline Not Specified (Human sensitization patch test): non-irritating
    - Test 2: Test Guideline Not Specified (Human sensitization patch test): non-irritating
  - ECHA Database:
    - Test 3: OECD 439 (In Vitro Skin Irritation: Reconstructed Human Epidermis): Category 2 skin irritant in an EPISKIN reconstructed epidermis model
    - Test 4: OECD 431 (In vitro skin corrosion: reconstructed human epidermis (RHE) test method): Not corrosive in an EPISKIN reconstructed epidermis model
    - Test 5: OECD 405 (Acute Eye Irritation/Corrosion): Category 2 eye irritant in rabbits
    - Test 6: OECD 437 (Bovine Corneal Opacity and Permeability Test Method): Not irritating in bovine cornea
    - Test 7: Non-Guideline Study (now OECD 492): Not irritating in a SkinEthic reconstructed human corneal epithelium model
- Analogue 2 very close structural analogue
  - Test 8: Test Guideline Not Specified (In vivo eye irritation): Mild corneal opacity and severe conjunctival irritation; mean irritation score of all three rabbits = 16
- Analogue 3 very close structural analogue
  - Test 9: Test Guideline Not Specified (In vivo skin irritation): Irritating to skin of rabbits under occlusive conditions
  - Test 10: Test Guideline Not Specified (In vivo eye irritation): Not irritating to eyes of rabbits

Decision 1: No eye data on the test chemical. Analogues 1-3 are equally good therefore evaluate data from Tests 1-10.

Decision 2: Tests 5, 6, 7, 8 and 10 provide eye irritation data. Test 7 is the only human relevant test, and provides a non-irritating severity rating, therefore select the severity rating associated with that test.

Final recommendation on example case: No concern for eye irritation or serious eye damage/corrosion identified

Representative Eye Irritation NAMs

METHOD OR APPROACH	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN AND LIMITATIONS	CATEGORIZATION AND OUTCOME TYPE
OECD TG 437: Bovine Corneal Opacity and Permeability (BCOP) test method with histopathology option	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, semi-solids, 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) • Non-irritating (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) • Irritant (GHS Cat 2) • Non-irritating (GHS No Category)
OECD TG 438: Isolated Chicken Eye (ICE) test	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 non-extreme pH (2 < pH < 11.5) detergents shown to induce mainly persistent non severe effects in vivo and surfactants	Outcome type: Full spectrum As standalone, per OECD TG 438: • Severe/corrosive (GHS Cat 1) • Non-irritating (GHS No Category) Can identify all three categories within an IATA or WoE assessment: • Severe/corrosive (GHS Cat 1) • Irritant (GHS Cat 2) • Non-irritating (GHS No Category)
OECD TG 460: Fluorescein Leakage (FL) Test Method for Identifying Ocular Corrosives and Severe Irritants	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 non-toxic, 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 non-irritants: • Severe/corrosive (GHS Cat 1)
OECD TG 467: Defined Approach 2 for neat and diluted non-surfactant liquids (DAL2)	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 non-surfactant 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) • Non-irritating (GHS No Category)
OECD TG 491: Short Time Exposure (STE) in vitro test	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) • Non-irritating (GHS No Category) Can identify all three categories within an IATA, or WoE assessment: • Severe/corrosive (GHS Cat 1) • Irritant (GHS Cat 2) • Non-irritating (GHS No Category)
OECD TG 492: Reconstructed Human Cornea-like Epithelium (RhCE) test	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 non-aqueous liquids, semi-solids 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) • Non-irritating (GHS No Category)
OECD TG 492B: RhCE Test Method for Eye Hazard Identification (Time-to-Toxicity (ET50) protocols)	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 non-aqueous liquids, semi-solids 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) • Irritant (GHS Cat 2) • Non-irritation (GHS No Category)
OECD TG 496: In vitro Macromolecular Test Method Ocular Irritection®	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) • Non-irritating (GHS No Category)