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United States regulatory requirements for skin and eye irritation testing

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Abstract

Purpose: Eye and skin irritation test data are required or considered by chemical regulation authorities in the United States to develop product hazard labeling and/or to assess risks for exposure to skin- and eye-irritating chemicals. The combination of animal welfare concerns and interest in implementing methods with greater human relevance has led to development of non-animal skin- and eye-irritation test methods. To identify opportunities for regulatory uses of non-animal replacements for skin and eye irritation tests, the needs and uses for these types of test data at U.S. regulatory and research agencies must first be clarified.

Methods: We surveyed regulatory and non-regulatory testing needs of U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) agencies for skin and eye irritation testing data. Information reviewed includes the type of skin and eye irritation data required by each agency and the associated decision context: hazard classification, potency classification, or risk assessment; the preferred tests; and whether alternative or non-animal tests are acceptable. Information on the specific information needed from non-animal test methods also was collected.

Results: A common theme across U.S. agencies is the willingness to consider non-animal or alternative test methods. Sponsors are encouraged to consult with the relevant agency in designing

their testing program to discuss use and acceptance of alternative methods for local skin and eye irritation testing.

Conclusions: To advance the implementation of alternative testing methods, a dialog on the confidence of these methods to protect public health and the environment must be undertaken at all levels.

Keywords

eye irritation testing; skin irritation testing; alternative approaches; non-animal methods; regulatory requirements; corrosive

Introduction

A corrosive substance is any substance that causes destruction of a living tissue upon contact, while an irritant produces a local inflammatory reaction on immediate, repeated, or prolonged contact with living tissue [1]. Regulatory agencies require testing to identify substances that are corrosive or irritating to the skin or eye. The major regulatory need for skin and eye corrosivity and irritation testing data is for hazard classification and labeling of products, which is intended to alert handlers and consumers to potential injury hazards and indicate the level of personal protective equipment needed. Other needs include determining exposure limits and countermeasures that should be employed against exposures, estimating an acceptable topical dose to give to humans, and establishing a starting dose for long-term studies.

Historically, animal tests have been used to assign substances to toxicity categories that are associated with the hazard phrases included on product labels. A substance is applied to the skin or eye of a laboratory animal that is then observed over a period of time for lesions that are qualitatively scored. Studies suggest that the responses observed in animal studies are not always relevant to the response observed in humans [2, 3, 4].

One of the main goals of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), composed of representatives from U.S. Federal regulatory and research agencies, is to reduce, refine, or replace animal testing, where feasible. As part of an effort to increase confidence in alternative methods and improve their relevance to human health, ICCVAM developed a strategic roadmap for incorporating new approaches for evaluating the safety of chemicals and medical products [5]. The roadmap addresses development and evaluation of alternative approaches for acute toxicity tests as well as alternative approaches to eye and skin irritation testing. ICCVAM's efforts to establish alternatives to eye and skin irritation testing are led by the ICCVAM Ocular and Dermal Irritation Workgroup (ODIWG), which is comprised of experts from multiple member agencies and is supported by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The ODIWG's main activity is to evaluate and promote the use of alternative test methods for regulatory use in eye and skin irritation hazard assessments [6].

A key element in progress toward incorporation of replacements for animal tests is an understanding of what data are needed and how they are used by regulatory agencies. Seven ICCVAM member agencies use eye and skin irritation data to satisfy the research and regulatory functions delegated to them under federal laws: the U.S. Consumer Product Safety Commission (CPSC), the U.S. Environmental Protection Agency (EPA), the U.S. Food and Drug Administration (FDA), the Occupational Safety and Health Administration (OSHA), the U.S. Department of Transportation (DOT), the National Institute for Occupational Safety and Health (NIOSH), and the U.S. Department of Defense (DoD). This review summarizes (and where appropriate, contrasts) the current regulatory and non-regulatory needs of these agencies for skin and eye irritation testing in order to:

- Provide test method developers with clear targets for alternative method development by clarifying the regulatory requirements for skin and eye irritation testing for multiple U.S. regulatory agencies
- Describe how the different agencies are satisfying these needs while reducing or eliminating animal use
- Clarify the status of existing alternative methods as a starting point for future method development and validation efforts

Overview of U.S. regulatory testing requirements for skin and eye irritation

Table 1 lists statutory requirements, regulations, and regulated products relevant to five ICCVAM member agencies that use skin and eye irritation data for regulatory purposes. Some regulations do not call for the use or consideration of skin or eye irritation data specifically, but indicate that toxicity or safety assessments must be performed.

While DoD has no statutory requirements in this area, the agency generates and uses skin and eye irritation data to protect DoD personnel, including troops, who may be exposed to chemicals in their work activities. NIOSH also uses skin and eye irritation data to protect workers who may be exposed to chemicals in their work.

The following sections review the uses of skin and eye irritation data for ICCVAM member agencies that have needs for such data to protect human health and the environment from substances to which topical exposure could occur. The review addresses the following specific questions:

(1) What are the regulatory requirements for skin and eye irritation testing?

(2) Is there a specific requirement for animal data or is there flexibility to use alternative approaches?

(3) Does the agency track the number of tests conducted and whether animal or non-animal approaches were used? If so, how many *in vivo* and *in vitro* tests are being conducted for each agency?

Information was gathered through review of public web sites for each of the noted agencies. These data and information were collected and then forwarded to the respective ICCVAM agency representatives to the ODIWG for their review on the accuracy and completeness.

Regulatory uses of skin and eye irritation data by agency

U.S. Consumer Product Safety Commission

Regulatory requirements for skin and eye irritation data: The Consumer Product Safety Act (CPSA) [7] was enacted to: (1) protect the public against unreasonable risks of injury associated with consumer products; (2) assist consumers in evaluating the comparative safety of consumer products; (3) develop uniform safety standards for consumer products and to minimize conflicting state and local regulations; and (4) to promote research and investigation into the causes and prevention of product-related deaths, illnesses, and injuries. The CPSC administers the CPSA, the Poison Prevention Packaging Act (PPPA) [8], and the Federal Hazardous Substances Act (FHSA) [9], among other statutes. The FHSA requires cautionary labeling on household substances and mixtures of substances that are hazardous substances, as defined by the FHSA [9]. The FHSA also imposes a duty on manufacturers of household products to determine if their products are hazardous substances and to label them accordingly. The PPPA requires select household substances to be packaged in child-resistant packaging [8]. The CPSC does not have the authority to provide premarket approval of product labeling or to review irritation data.

The FHSA defines a corrosive and an irritant [15 U.S.C. §1261 (i) and (j)] and provides a supplemental definition in its implementing regulation available in Title 16 of the U.S. Code of Federal Regulations (C.F.R.) [1, 10]. CPSC defines a corrosive substance as any substance that comes in contact with living tissue and causes destruction of the tissue by chemical action. An irritant refers to a substance that is not identified as a corrosive, as defined above, but on immediate, repeated, or prolonged contact with living tissue produces a local inflammatory reaction [1]. Substances that meet the definition of an irritant or a corrosive and meet the remaining parts of the definition of ‘hazardous substance’ must bear cautionary labeling to warn about the hazard and to provide for safe handling and use of the product. Criteria for classification of chemicals as skin and/or eye irritants based on results from animal studies is provided in Tables 2 and 3.

Current requirements for animal testing and flexibility for the use of alternatives: CPSC requires that household products be labeled or packaged to reflect the hazards associated with the product, including potential skin or eye corrosion and/or irritation [1]. To determine if hazard labeling is necessary, CPSC needs information sufficient to classify substances and mixtures as irritants or corrosives. CPSC prefers data based on reliable human experience over animal test data [11], and prefers the use of existing data when possible. To meet FHSA requirements regarding labeling for toxicity and irritancy, and for exemptions from standards issued under the PPPA, the use of animal tests is acceptable for hazard determination when human data or existing animal data are not available.

Animal testing is not required for identification of a corrosive or an irritant by the CPSC. CPSC’s animal testing policy indicates that the Commission strongly encourage the use of existing data and alternatives to animal testing whenever possible [11, 12]. According to the CPSC’s animal testing policy, these alternatives include prior human experience (e.g., published case studies), *in vitro* or *in silico* test methods that have been approved by the

Commission, literature sources containing the results of prior animal testing or limited human tests (e.g., clinical trials or skin patch testing), and expert opinion (e.g., hazard assessment or structure-activity analysis). In the absence of other data, results from animal studies can be used for classification. Animal methods for determining local skin and eye irritation are provided in 16 C.F.R. §1500.41 and §1500.42, respectively [13, 14].

When determining when a product should be labeled as a hazardous substance, CPSC may also consider data generated using Organisation for Economic Co-operation and Development (OECD) test guidelines as a result of the Mutual Acceptance of Data (MAD) agreement [12]. Data from methods other than OECD test guidelines that are scientifically supported may be considered as well. However, neither the MAD agreement nor CPSC staff's decision to consider any test data guarantees that CPSC staff will accept data from any test that has not been previously approved by CPSC as adequate for purposes of classification and/or labeling. CPSC staff may ask for additional data.

Although CPSC toxicity categories are based on animal test results, CPSC recommends *in vitro* tests over *in vivo* skin and eye irritation tests or modifying traditional irritation tests to reduce the number of animals used whenever possible [11]. Acceptable alternatives to animal tests are provided on the CPSC website [12]. CPSC will also accept and review submissions of data from methods not previously approved, on a case-by-case basis. Manufacturers are encouraged to contact CPSC's Office of Compliance and Field Operations to discuss the use of such alternative tests prior to testing.

Number of skin and eye irritation tests submitted: The FHSA does not provide for pre-market approval; therefore, the agency does not know the actual extent of a manufacturer's use of *in vivo*, alternative, or non-animal tests to assign hazard labeling.

Department of Defense

Regulatory requirements for skin and eye irritation data: The DoD is not a regulatory agency and has no statutory requirements mandating the collection and use of skin or eye irritation data. However, certain military services within the DoD (i.e., Army, Navy, Air Force) generate and use acute toxicity data (including eye and skin irritation data) with the goal of protecting human health and the environment from acutely dangerous chemical exposures [15]. Prior to the acquisition and use of new potential toxicants, the Army requires a Toxicity Clearance, which typically involves, at a minimum, collecting data from acute systemic toxicity, skin and eye irritation, and skin sensitization tests for the assessment decision. Although the Navy and Air Force do not require a Toxicity Clearance for new substances, they follow the same principles for acute toxicity testing. However, they only conduct testing needed to develop exposure limits, which usually includes a limit test (acute oral or inhalation toxicity) and a skin irritation test, at a minimum. Based on the outcome of these tests, they may defer or waive further testing.

DoD regulations also require that a life cycle assessment and a Programmatic Environmental Safety and Health Evaluation be conducted for all new substances in the acquisition system. The Tri-Service Toxicology Consortium, an association of toxicologists from the Army,

Navy, and Air Force, conducts evaluations and provides recommendations on the use of potential toxicants within the DoD.

Military services within the DoD use toxicity data most often for chemicals that are operationally relevant in oral, skin, or inhalational exposure scenarios. This includes chemical warfare agents as well as propellants, fire suppressants, smokes, fuels, pyrotechnics, explosives, and munitions that armed services members may be exposed to on base, a ship, or in the field. These and all other available data, including skin and eye irritation test results, are used to develop Safety Data Sheets to determine when and what type of personal protective equipment is needed, and to identify what countermeasures should be used in the event of exposure.

Current requirements for animal testing and flexibility for the use of alternatives: Military components of the DoD currently rely on skin and eye irritation data generated using EPA or OECD test guidelines. Consistent with these guidelines, these services utilize a phased testing approach to determine if *in vivo* testing is considered necessary. Early in the development of a new substance, *in silico* data (e.g., quantitative structure activity relationships; QSARs) and *in vitro* tests, with or without guidelines, are utilized to provide a more comprehensive picture of the potential toxicity issues that may arise in later testing, or to determine whether the compound under evaluation should not move forward in the development and testing process [16, 17]. The Army, Navy, and Air Force conduct skin and eye irritation testing in animals during the testing and demonstration phase of the life cycle of new substances. Alternative *in vitro* and *in silico* approaches are also considered early in the research, development, testing, and evaluation of new systems.

DoD sponsored a National Research Council (NRC) report on modern non-animal approaches that could be used to predict chemical toxicity and protect its deployed personnel against chemical threats [15]. DoD is currently reevaluating its research and testing approach to consider the recommendations made in this report. If DoD implements the NRC recommendations, it will need to categorize chemical toxicity assessments as having high confidence of high toxicity, high confidence of low toxicity, and uncertain toxicity due to inadequate data [15]. The NRC evaluation notes that skin and/or eye irritation or corrosion may be assessed by the DoD using alternative methods.

The NRC recommendations for acceptable alternatives for skin irritation and corrosivity [15] include the *in vitro* membrane barrier test method for skin corrosion (OECD test guideline [TG] 435) [18] or the reconstructed human epidermis (RhE) test method (OECD TG 439), that includes EpiSkin™, SkinEthic™ RHE, Epiderm™, or LabCyte EPI-MODEL24 SIT skin irritation tests [19].

The NRC recommendations [15] include use of various *ex vivo* and *in vitro* assays as part of a tiered testing strategy. These include the bovine corneal opacity and permeability (BCOP; OECD TG 437) [20], the isolated chicken eye (ICE; OECD TG 438) [21], or the Epi-Ocular™ reconstructed human cornea-like epithelium (RhCE; OECD TG 492) [22] test methods.

Where alternative methods for generating new test data are not available, physicochemical properties could be used to predict physical hazard. Use of physicochemical and structural alert exclusion rules are used by the German Federal Institute for Risk Assessment (BfR). These rules are encoded in the OECD QSAR Toolbox and the European Commission Joint Research Centre Toxtree [23, 24]. Some components within DoD already employ a tiered testing strategy that includes the use of databases, high throughput screening assays, *in vitro* and *in silico* models, and other tools to predict toxicity while balancing accuracy and timeliness. The testing tiers progress from non-testing evaluations to high- and medium-throughput non-animal assays and then to animal testing, which is only done if DoD deems it necessary following analysis of existing data.

Since the DoD is responsible for protecting human health and the environment, non- animal tests that are able to predict both human and animal toxicity would be useful.

Number of skin and eye irritation tests conducted: DoD does not track the number of tests or methods used to generate skin or eye irritation data.

Department of Transportation

Regulatory requirements for skin and eye irritation data: The DOT Pipeline and Hazardous Materials Safety Administration (PHMSA) requires hazard labeling and special packaging on hazardous materials shipped within the United States under the authority of the Federal Hazardous Material Transportation Act [25]. Information on toxicity is used to classify substances as poisonous, which is one of the hazards that require categorization, labeling, packaging, and shipping papers. A ‘poisonous material’ is a substance that is known or presumed to be so toxic to humans as to afford a hazard to health during transportation.

Testing for eye irritation is not currently required by DOT as packaging information is based on skin corrosivity. For skin corrosion/irritation, DOT allows the use of OECD and EPA test guidelines for the purposes of categorizing transported substances. DOT defines a corrosive material (Class 8) as either a liquid or solid that causes full thickness destruction of human skin at the site of contact within a specified period of time, or a liquid (or solid that may become a liquid) that has a severe corrosion rate on steel or aluminum (49 C.F.R. §173.136) [26]. Whenever practical, data from the tests described in 49 C.F.R. §173.137 [27] should be used to determine corrosivity. PHMSA may revise its classification of a material if human experience or other data indicate that its hazard is greater or less than indicated by the results of approved test methods.

The packing group of a Class 8 hazardous material is indicated in Column 5 of the §172.101 Hazardous Materials Table [28]. When this table identifies more than one packing group for a Class 8 material, the packing group must be determined using data obtained from the OECD TG 435 [18] for an *in vitro* barrier test method for dermal corrosion or OECD TG 404, which describes the *in vivo* test for dermal irritation or corrosion [29]. A material that is determined not to be corrosive in accordance with OECD TG 430 or TG 431, which describe other *in vitro* dermal irritation test methods [30, 31] may be considered not to be corrosive to human skin without further testing. However, a substance determined to be

corrosive according to OECD TGs 430 or 431 must be further tested according to OECD TG 435 [18] or 404 [29] in order to assign definitive packing groups. The packing group assignments based on OECD TG 435 and 404 are:

- Packing Group 1: Materials that cause full-thickness destruction of intact skin tissue within an observation period of up to 60 minutes starting after the full exposure time of three minutes or less
- Packing Group 2: Materials other than those meeting Packing Group I criteria that cause full thickness destruction of intact skin within an observation period of up to 14 days starting after the exposure time of more than three minutes, but not more than 60 minutes
- Packing Group 3: Material, other than those meeting Packing Group I or II criteria – (1) That cause full thickness destruction of intact skin tissue within an observation period of up to 14 days starting after the exposure time of more than 60 min but not more than 4 hours; or (2) That do not cause full thickness destruction of intact skin tissue but exhibit a corrosion on either steel or aluminum surfaces exceeding 6.25 mm (0.25 inch) a year at a test temperature of 55° C (130 °F) when tested on both materials [27, 28]

Current requirements for animal testing and flexibility for the use of alternatives: DOT will accept data from OECD TG 430 [30] and OECD TG 431 [31], but may require additional testing as noted above. OECD TG 404 recommends that *in vivo* testing should not be undertaken until all available data relevant to the potential corrosivity/irritation of a test chemical have been evaluated in a weight-of-evidence analysis as described in the OECD Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Skin Irritation/Corrosion [32]. If *in vivo* testing is necessary, OECD TG 404 [29] recommends the use of a single rabbit to assess skin corrosivity. If a corrosive effect is observed, additional testing is not needed. If an irritant effect is observed in the single rabbit, confirmatory studies may be conducted in sequentially or concurrently tested animals.

DOT recommends that *in vitro* methods be used to determine whether a material is corrosive to skin [26]. Alternatively, historical data produced no later than September 30, 1995, using the procedures of Part 173 (49 C.F.R. §173 Appendix A) and in effect on September 30, 1995, may be used for corrosivity classification [26].

If neither human nor existing animal data are available, DOT recommends an authorized method of determining skin corrosivity such as OECD TGs 435, 430, or 431 as defined in 49 C.F.R. §173.137 [27]. Since 1993, PHMSA has been able to approve non-animal methods for skin corrosivity through a special permit application process (DOT-SP-10904).

Although DOT does not need or require eye irritation data, a person, manufacturer or shipper may choose to conduct such testing to provide initial preliminary information on the toxicity of a compound. Eye irritation data may provide useful information in safety evaluations of accidental exposures of chemicals or may be required for the Material Data Safety Sheets by the manufacturer or importer.

Number of skin and eye irritation tests conducted: DOT does not collect or keep records of the number of skin or eye irritation tests conducted or the method used.

Environmental Protection Agency (EPA)

Regulatory requirements for skin and eye irritation data: Eye and skin irritation data are utilized by EPA to administer two statutes: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA), recently amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act (130 Stat 448) [33, 34, 35].

The EPA Office of Pesticide Programs (OPP) regulates the sale and use of pesticides in the U.S. under FIFRA. Any distributor wishing to sell pesticides in the U.S. must register their product with OPP, with limited exemptions. Registration requires the applicant to demonstrate that the product does not cause unreasonable adverse effects to human health or the environment when used appropriately [36]. Under FIFRA, eye and skin irritation data are required for pesticide active ingredients and final products.

The EPA Office of Pollution Prevention and Toxics (OPPT) administers the amended TSCA, which regulates new and existing chemical substances that are manufactured or imported, processed, distributed, used and disposed of in the U.S TSCA requires the applicant to submit a pre-manufacture notice (PMN) to OPPT prior to either the manufacture or import of a new chemical substance or initiating a significant new use of an existing substance. Although TSCA does not require generation of data for eye and skin irritation or any other specific toxicity endpoint, any existing toxicity data in the possession or control of the submitter must be submitted to EPA with pre-manufacture notices. OPPT must evaluate the new chemical substances to make one of the following five determinations:

- the new chemical substance presents an unreasonable risk of injury to human health or the environment (TSCA §5(a)(3)(A)),
- the information on the new chemical substance is insufficient to make a reasoned evaluation of the health and environmental effects (TSCA §5(a)(3)(B)(i)),
- in the absence of sufficient information to make a reasoned evaluation the new chemical substance may present an unreasonable risk of injury to human health or the environment (TSCA §5(a)(B)(ii)(I),
- the new chemical substance is or will be produced in substantial quantities, and such substance either enters or may reasonably be anticipated to enter the environment in substantial quantities or there is or may be significant or substantial human exposure to the substance (TSCA §5(a)(B)(ii)(II), or
- the new chemical substance is not likely to present an unreasonable risk of injury to human health or the environment (§5(a)(3)(C)).

In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act amended TSCA to give OPPT authority to require the generation of hazard and/or exposure information on chemicals to make risk determinations for both new and existing chemicals. Furthermore, the new act requires that OPPT develop a Strategic Plan to promote the development and

implementation of alternative test methods for new and existing chemical substances that reduce, refine, or replace vertebrate testing; the Strategic Plan was recently published [37]. Examples of such methods include high- throughput screening methods, computational approaches, and *in vitro* studies (130 Stat 448) [33]. The EPA Strategic Plan describes core components to promote the development and implementation of alternative test methods and strategies and includes descriptions of near (up to 3 years), intermediate (3–5 years), long-term (>5 years) activities.

OPP requires that products regulated under FIFRA undergo a risk assessment and hazard assessment for classification into one of four eye and skin irritation categories to provide appropriate hazard information for product labels (see Table 4).

OPPT performs a risk assessment based on hazard and exposure when reviewing new chemical substances under TSCA. When the information provided for the risk assessment indicates that a chemical substance may or will present an unreasonable risk to human health or the environment, OPPT may require risk mitigation steps (e.g., changes to the safety data sheet for the substance, including requirements for personal protective equipment). In addition, specific engineering or administrative controls may be required, as appropriate, to adequately protect worker health and the environment. When information provided for the risk assessment is insufficient to make a risk determination, additional testing may be required. OPPT and OPP are interested in a future where models predict human responses.

Current requirements for animal testing and flexibility for the use of alternatives: OPP requires acute toxicity test data for oral, skin, and inhalation routes of exposure, primary eye/skin irritation, and skin sensitization (the so called ‘six-pack’ studies) for all pesticide active ingredients and all pesticide products that contain the active ingredients [38]. Under MAD, EPA accepts data generated using OECD test guidelines (e.g., OECD TG 437) [20]. OPP also accepts studies conducted using EPA test guidelines [39, 40]. OECD and EPA test guidelines for acute eye irritation testing (OECD TG 405 and EPA OPPTS 870.2400, respectively) recommend testing a single rabbit for eye irritation if marked effects are anticipated. Should the results of this test suggest that the test material is a severe irritant or corrosive to the eye, further tests may not need to be performed; otherwise eye irritation data from three tested animals should be provided [39, 41]. OECD and EPA test guidelines for acute dermal irritation testing (OECD TG 404 and EPA OPPTS 870.2500, respectively) recommend testing a single rabbit in case corrosivity or severe irritation is observed (in which case testing can be stopped and the pesticide labeled accordingly), followed by testing in a minimum of two additional rabbits [29, 40].

OPP allows flexibility in meeting the data requirements for pesticides; alternative approaches may be accepted. Skin irritation and corrosion harmonized OECD test guideline test methods that are accepted by OPP include OECD TGs 430, 431, 435, and 439 [18, 19, 30, 31]. Eye irritation test methods that have been adopted as harmonized test guidelines include OECD TGs 437, 438, 460, 491, and 492 [20, 21, 22, 42, 43]. Additionally, the cytosensor microphysiometer (CM) test method is a proposed OECD test guideline [44]. It should be noted that although OPP accepts such data for review, OPP may require additional information to fulfill U.S. pesticide data submission requirements.

For skin and eye irritation testing of new chemicals, OPPT recommends consideration of *in vitro* or *ex vivo* alternative test methods prior to using animal models available in the EPA OSCPP test guidelines [39, 40]. For skin and eye irritation testing alternatives, OPPT recommends all the currently accepted OECD TGs for these endpoints as mentioned above and currently on the TSCA List of Alternative Test Methods and Strategies (or New Approach Methodologies [NAMs]) [45].

OPP has developed a strategic vision for implementing the 2007 NRC of the National Academy of Science report on Toxicity Testing in the 21st Century [15, 46, 47]. One such application of the strategic visions is EPA's guidance, 'Use of an alternate testing framework for classification of eye irritation potential of EPA pesticide products'. The guidance describes a testing framework for assessing eye irritation potential of EPA- registered antimicrobial cleaning products using three *in vitro*/*ex vivo* assays (non- animal tests) [48]. OPP is currently considering expanding this testing strategy to other classes of pesticides and formulations, including antimicrobial pesticides other than those with cleaning claims, conventional pesticides, and biochemical pesticides.

As part of the implementation of the strategic vision, OPP developed a document called 'Guiding principles for data requirements' [49] that describes some of the key principles for moving towards smarter testing approaches. The document specifically notes the importance of requiring only data that adequately inform regulatory decision-making while avoiding the generation and evaluation of data that do not materially influence a regulatory decision. Adhering to these principles avoids unnecessary use of time and resources, data generation costs, and animal testing. Use of animals for eye and skin irritation testing required under FIFRA can be avoided under certain conditions. 'Guidance for Waiving or Bridging of Mammalian Acute Toxicity Tests for Pesticides and Pesticide Products' [50] provides guidance and criteria for submitting waiver requests and bridging toxicity data.

Waiving studies provides an opportunity to eliminate considerable testing and thus reduce animal use. Waivers are considered when a data endpoint is not relevant to the chemical (e.g., an eye irritation study may be waived when the product is determined to be corrosive to skin or the product design [e.g. large pellets] precludes testing in the eye) [50]. 'Bridging' refers to assessing the hazard of a chemical for which there is little or no existing data by using a read-across method to evaluate data for a structurally similar chemical, thus avoiding the need to generate new test data. OPP may accept bridging of data in situations including, but not necessarily limited to, those in which either the toxicity profile of a proposed product matches that of the product for which the cited data was submitted and demonstrates a reduced hazard potential or new product is essentially a water dilution of a registered product.

In 2016, OPP described its processes for establishing and implementing alternative approaches for acute effects testing (e.g., eye irritation testing) for regulatory use [51]. The document describes a stepwise process that includes phases for evaluation; proposal and public comment; and implementation of an alternative method. The evaluation phase determines the reliability of the alternative method and its utility for EPA's regulatory use and identifies uncertainties. During the proposal and public comment phase, EPA will accept

comments on the proposal for using the alternative method and incorporate the comments in the final proposal, if appropriate. During the implementation phase, the final policy for the alternative method will be published and EPA OPP will accept the alternative data in place of the *in vivo* data requirements.

Animal testing is not generally required by OPPT for pre-manufacture notices under TSCA. If no chemical-specific data are available, OPPT uses a read-across method, when feasible, to assess toxicity of the submitted new chemical substance. If available data are inadequate for read-across, new toxicity data may be requested for new chemical substances under Section 5 of TSCA or for existing chemical substances under Section 4 of TSCA. The amended TSCA mandates a statement of need under Section 4(a)(3) prior to requesting testing. Two of the main requirements for this include: (1) why the information is needed; and (2) how information reasonably available was evaluated. In addition, Section 4(a)(4) mandates that OPPT use a tiered approach to the gathering of new information; under which the results of screening tests are performed first and those results will determine the need for additional testing.

OPPT currently accepts and uses non-animal approaches for eye and skin irritation hazard assessments; however, OPPT may require additional information for these endpoints for quantitative risk assessments. Under Section 4(h) of the amended TSCA, OPPT was required to develop a Strategic Plan to reduce, refine, and replace the use of vertebrate animals in toxicity testing, including eye and skin irritation. The Strategic Plan was published on June 22, 2018 as required by law [52].

EPA is working with industry groups and non-governmental organizations to expand use of alternatives and acceptance of data generated using alternative methods for eye and skin irritation. In addition to the publication of the Strategic Plan, TSCA identifies many examples of achieving this goal, including the formation of an industry consortia [33]. The EPA also is engaging stakeholders for input on the Strategic Plan and the implementation of the new act, as well as for other activities.

Number of skin and eye irritation tests submitted: OPP does not conduct acute eye and skin irritation studies; registrants (i.e., sponsors) of pesticide products submit these studies. Annually, OPP receives approximately 250 submissions of acute toxicity ‘six-pack’ data. These submissions are for pesticide formulations and support pesticide active ingredient testing [47]. While the number of submissions that contain *in vitro* data are relatively small, the numbers are increasing. OPP is working towards implementing a transparent system to track the number of alternative approaches submitted.

From 2004 to 2012, approximately 15% of 1100 annual pre-manufacture notice submissions to OPPT contain health effects data; most of which are acute toxicity information which include irritation tests [53].

U.S. Food and Drug Administration Center for Biologics Evaluation and Research and Center for Drug Evaluation and Research

Regulatory requirements for skin and eye irritation data: The FDA Center for Drug Evaluation and Research (CDER) regulates over-the- counter and prescription drugs (including medicines, fluoride toothpaste, antiperspirants, dandruff shampoos, and sunscreens) and some therapeutic biological products. The FDA Center for Biologics Evaluation and Research (CBER) regulates biological products for human use (e.g., vaccines) other than those regulated by CDER. CBER and CDER's stated missions are to ensure the safety and efficacy of drugs and biologics used by humans.

CBER and CDER both reference the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidance, 'Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6(R1)' [54]. CBER and CDER also reference the ICH document, 'Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals M3(R2)' [55].

According to ICH M3(R2), it is preferred that local tolerance studies (i.e., skin and eye irritation) be conducted by the intended therapeutic route as part of general toxicity studies [55]. ICH S6(R1) also notes that, in some cases, potential adverse effects of a product can be evaluated during toxicity studies. This would eliminate the need to conduct separate local tolerance studies [54].

FDA regulations (under CDER) for an Investigational New Drug are provided in 21 §312.23 (a)(8). These regulations require that the sponsor submit the information supporting their conclusion that their product is reasonably safe to conduct the proposed clinical investigations [56]. If some of that information includes skin or eye irritation studies, then the sponsor should submit those studies. The regulations state that the type, duration, and scope of animal tests required vary with the duration and nature of the proposed clinical investigations. CBER and CDER would prefer that alternative methods predict human rather than animal toxicity, when possible.

Current requirements for animal testing and flexibility for the use of alternatives: CBER and CDER have no specific requirements for skin and eye irritation testing in animals. The FDA guidance document 'Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route' states that alternative approaches can be used for nonclinical evaluation of previously approved drug substances when a new formulation or a new route of administration for a previously approved formulation is proposed by the sponsor, provided that the new approaches satisfy the requirements of the applicable statutes and regulations [57]. For example, the FDA recommends using appropriate *in vitro* or *ex vivo* test methods to determine irritation potential rather than using the *in vivo* rabbit eye test method irritation method to determine the eye irritation potential of a topical dermal drug product [57]. Drug sponsors interested in submitting alternative data should discuss their proposal with CBER or CDER prior to data submission as part of the routine consultation process.

In 2018 the FDA released a roadmap to incorporate new technologies into regulatory review, as applicable [58]. The roadmap describes a comprehensive strategy to evaluate new methods and technologies to expand the FDA's toxicology predictive capabilities and potentially reduce the use of animal testing. The roadmap describes a six-part framework for new or enhanced FDA engagement in the science of toxicology.

Number of skin and eye irritation tests conducted: Neither CBER nor CDER requires or requests acute toxicity data. Therefore, neither center tracks the number or type of tests submitted.

U.S. Food and Drug Administration Center for Devices and Radiological Health

Regulatory requirements for skin and eye irritation data: The FDA Center for Devices and Radiological Health (CDRH) regulates the marketing of all medical devices under chapter 5 of the Federal Food, Drug, and Cosmetic Act (FFDCA) of 1938. Medical devices are categorized into one of three classes based on their safety risk, and testing requirements vary depending on the device classification.

Methods for evaluating the biocompatibility of medical devices are outlined in Part 11 of the International Organization for Standardization (ISO) document ISO 10993 'Biological Evaluation of Medical Devices: Tests for Systemic Toxicity.' CDRH provides guidance for industry and FDA staff in 'Use of International Standard ISO 10993–1, "Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process"' [59]. Biocompatibility testing is not requested by CDRH for all medical devices but depends on the device category (i.e., surface, external communicating, or implanted) and the nature and duration of its intended contact with the human body. CDRH recommends irritation data be submitted for all externally communicating and implanted devices, as well as surface devices that contact breached or compromised skin or have prolonged or permanent contact with mucosal membranes [59].

Current requirements for animal testing and flexibility in the use of alternatives: Eye irritation testing for CDRH under ISO 10993 should be considered if (1) safety data cannot be obtained by other ways and (2) the medical device will come in contact with the eye or eyelid. Skin irritation and corrosivity testing for CDRH under ISO 10993–10 [60] calls for *in vivo* testing of medical devices with the finished product and/or extracts thereof, but allows flexibility in the design and selection of test method(s) and lists several factors that may affect the results of irritation studies. These include (1) the nature of the device used in a patch test, (2) the dose of the test material, (3) the method of application of the test material, (4) the degree of occlusion, (5) the application site, (6) the duration and number of exposures, and (7) the techniques used in evaluating the test. Materials or products may be excluded from testing if previous dermal studies have demonstrated that the materials or products have the potential to cause corrosion or severe irritation. Additionally, materials that are skin irritants should not be tested for eye irritation. Materials with a pH ≤ 2.0 or ≥ 11.5 should not be tested for skin or eye irritation.

CDRH's guidance on the use of ISO 10993 states that an alternative approach can be used if it 'satisfies the requirements of the applicable statutes and regulations.' Sponsors who want to use an alternative approach are encouraged to contact CDRH prior to conducting any testing. New testing using animals may also be avoided if a sponsor provides evidence that a device is 'substantially equivalent' to a legally marketed device that has been approved for marketing.

ISO 10993–10:2010 states that skin and eye irritation testing can provide general information on health hazards that may result from exposure to the test substance. CDRH prefers human data over animal data when possible and would prefer that alternatives predict human toxicity.

In 'Biological evaluation of medical devices – Part 2: Animal welfare requirements' (ISO 10993–2), it is noted that scientifically validated tests conducted in preference of *in vivo* tests shall be considered.

Number of skin and eye irritation tests submitted: CDRH does not track the number or types of tests conducted.

U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition

Regulatory requirements for skin and eye irritation data: FDA Center for Food Safety and Applied Nutrition (CFSAN) regulates food ingredients, including food additives, color additives used in food, food contact substances, dietary supplements, and substances generally recognized as safe, under the 1958 Food Additives Amendment of the FFDCA. CFSAN does not require skin or eye irritation testing for making final decisions on the safety of direct food additives and color additives that are used in food. A hazard analysis is required to identify safety hazards that are likely to occur, but there are no specific testing requirements provided (21 C.F.R. §120.7) [61].

CFSAN also regulates cosmetics, which include products 'intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body…for cleansing, beautifying, promoting attractiveness, or altering the appearance' [FFDCA, sec. 201(i)]. Among the products included in this definition are moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial makeup preparations, cleansing shampoos, hair colors, and deodorants, as well as any substance intended for use as a component of a cosmetic product. CFSAN also regulates color additives intended for use in cosmetics.

CFSAN evaluates the safety of food ingredients according to the criteria presented in their guidance document 'Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food,' also known as Redbook 2000 [62]. The tests included in Redbook 2000 are not requirements; eye and skin irritation testing data are not currently required. Redbook 2000 is currently in the process of being updated; it was last updated in 2007.

CFSAN does not specifically require skin and eye irritation testing for cosmetics. It remains the responsibility of the manufacturer to substantiate the safety of both ingredients and finished cosmetic products prior to marketing.

Current requirements for animal testing and flexibility for the use of alternatives: CFSAN does not require eye or skin irritation data from animal testing. In general, Redbook 2000 [62] provides recommendations for toxicological testing approaches to evaluate the safety of ingredients added to foods. Manufacturers or sponsors who wish to collect data are urged to consult with the relevant divisions of CFSAN prior to testing to discuss acceptable alternative testing approaches that may utilize fewer animals or provide more cost efficiency.

The FFDCA does not specifically require the use of animals in testing cosmetics for safety, nor does the Act subject cosmetics to FDA pre-market approval. The agency has consistently advised cosmetic manufacturers to employ whatever testing is appropriate and adequate for substantiating the safety of their products. In all cases where animal testing is used, FDA advocates that research and testing derive the maximum amount of useful scientific information from the minimum number of animals and employ the most humane methods available within the limits of scientific capability. CFSAN also believes that prior to use of animals, consideration should be given to the use of scientifically valid alternatives to *in vivo* testing.

Although CFSAN does not need or require skin and eye irritation data, a manufacturer or sponsor may choose to conduct such testing. CFSAN would prefer that alternative methods for irritation testing predict human, rather than animal, responses.

Number of skin and eye irritation tests conducted: CFSAN does not require or request skin or eye irritation data, nor does CFSAN track the number or types of tests conducted.

Occupational Safety and Health Administration

Regulatory requirements for skin and eye irritation data: The Occupational Safety and Health Act of 1970 (29 U.S.C. § 651 et seq) gives OSHA the authority to protect customers of chemical manufacturers and importers and employees who may be exposed to hazardous chemicals in the workplace. Requirements for labeling and safety data sheets are specified in OSHA's Hazard Communication Standard (HCS)."

Criteria and hazard classifications for serious eye damage (irreversible effects) and eye irritation are specified in the HCS, 29 C.F.R. §1910.1200 [63] (Table 5). The classifications were revised in 2012 [64] to be consistent with the provisions of the United Nations (UN) Globally Harmonized System (GHS), Revision 3 [65]. Criteria for skin irritation and corrosivity hazards also are specified in the HCS, 29 C.F.R. §1910.1200 [63] (Table 6). Observation of toxic effects consistent with the criteria for GHS classification, whether seen in humans or animals, usually justifies classification [64]. Transmittal of information is to be accomplished by means of comprehensive hazard communication programs, which are to include container labeling and other forms of warning, safety data sheets, and employee

training. As with the eye irritation classification criteria, the classifications were revised to be consistent with the GHS.

Current requirements for animal testing and flexibility for the use of alternatives: For hazard labeling and safety data sheets, OSHA needs information that can classify chemicals into hazard categories that are associated with specific labeling requirements [64]. Hazard classification, as defined in Tables 6 and 7 for eye and skin corrosives or irritants, may result directly from study data that satisfy the criteria. OSHA requires that scientifically valid test methods be used to determine skin and eye irritation and corrosivity, but no specific methods are required [63, 64]. Any national and international test guidelines and protocols are acceptable, including those recommended by ICCVAM or published by ASTM or OECD. Data from OECD TGs, including TG 430, TG 431, TG 435, and TG 439 may be used for classification of skin irritants or corrosives [18, 19, 30, 31]. Data from OECD TGs, including TG 437 and TG 438 may be used for classification of eye irritants or corrosives [20, 21].

Where data are available from multiple studies or sources, classification of a chemical shall be determined on the basis of the total weight of evidence using expert judgment. This means that all available information bearing on the classification of the hazard shall be considered together, including the results of valid *in vitro* tests, relevant animal data, and human experience such as epidemiological and clinical studies and well- documented case reports and observations. When using a weight-of-evidence approach, reliable, high-quality human data should generally have precedence over other data. Where human and animal data conflict, the quality and reliability of the evidence from both sources shall be evaluated to resolve the question of classification. Therefore, positive results from well-conducted animal studies are not necessarily negated by the lack of positive human experience but require an assessment of the robustness, quality, and statistical power of both the human and animal data.

Validated alternatives are acceptable. OSHA would prefer that alternative skin and eye irritation test methods reliably predict human rather than animal toxicity. Because OSHA does not receive data, there is no formal ‘acceptance’ of alternative or standard approaches.

Number of acute toxicity tests submitted: OSHA does not require the submission of skin or eye irritation test data or the conduct of toxicity tests.

Discussion

Many agencies are flexible in their consideration of alternative methods. There are multiple sources for information on valid non-animal methods, including the websites of ICCVAM [66] and the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) [67] and the EURL ECVAM DB- ALM database [68]. Tables 7 and 8 summarize available non-animal approaches that are accepted by U.S. authorities for eye and skin irritation testing, respectively. Table 7 also includes non-animal approaches recommended by EPA OPP to pesticide sponsors for eye irritation testing. There are also a

number of reduction alternatives that still require animals, albeit in fewer numbers than the traditional *in vivo* test.

Important to the discussion of alternatives to animal testing is not only whether an alternative approach exists, but also whether the alternative approaches are being used by industry and accepted by government agencies. This understanding is critical to determine the best course of action to reduce animal use. A common theme across U.S. agencies is the willingness to consider alternatives and the recommendation for sponsors to consult directly with the relevant agency in designing their testing program. Communication is clearly essential to ensure that transparency is maintained in defining expectations from both industry and government agencies. National validation organizations (e.g., NICEATM, EURL ECVAM, the Japanese Center for the Validation of Alternative Methods, and the Korean Center for the Validation of Alternative Methods) and relevant non-governmental organizations could provide an important contribution in promoting existing alternative approaches to interested stakeholders.

However, communication within government agencies is also critical to facilitate the use of alternative methods. Training of regulatory reviewers on the usefulness and limitations of available alternative approaches is paramount. A regular training program at regulatory agencies will ensure that reviewers are up-to-date on non-animal approaches so that consistent and timely decisions are made on submissions using alternative approaches. For example, EPA OPP and OPPT have convened in-house training sessions on *in vitro* and *in silico* methods. Similarly, FDA CBER and CDRH engage in external site training visits to learn about industry operations and provide an open dialog between industry and the agencies. However, truly capturing the value of these efforts requires that adequate information exchange occurs within each agency to ensure that all interested parties share a common understanding.

Perhaps most notably, international harmonization impacts the extent to which alternatives are used, particularly with respect to global companies. It is impractical to conduct duplicate testing to fulfill differing regulatory requirements if a single test can suffice (i.e., if one or more agencies require the animal test, companies will have to conduct that test even if other agencies accept the alternative approach). International collaborations such as the International Cooperation on Alternative Toxicological Methods (ICATM) provide opportunities for dialog to increase harmonized approaches to global implementation of non-animal approaches.

Advances in science and technology have greatly enhanced the access to mechanistic information that can be used to establish eye and/or skin irritation/corrosion hazards. In order to take the next step in advancing these alternative approaches towards implementation, transparent dialog must occur to establish scientific confidence in their ability to protect public health and the environment. Such dialog must occur on all levels to realize success, necessitating discussions at the levels of intra- and inter- agency, corporate and governmental, and national and international levels. Regardless of the level of acceptance at the national level, global implementation of alternatives requires global harmonization.

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Table 1.

Current U.S. regulatory requirements for skin and eye irritation data

U.S. Agency	Statutes	Regulations	Substances Regulated
CPSC	Federal Hazardous Substances Act [9]	16 C.F.R. §1500.3	Hazardous household substances
	Poison Prevention Packaging Act [8]	16 C.F.R. §1700	Hazardous household substances
DOT PHMSA	Federal Hazardous Material Transportation Act [69]	49 C.F.R. §173.132 49 C.F.R. §173.137	Transported substances
EPA OPP	Federal Insecticide, Fungicide and Rodenticide Act [35] Food Quality Protection Act [70]	40 C.F.R §156 40 C.F.R. §158.500 40 C.F.R. §158.2140 40 C.F.R. §158.2230 40 C.F.R. §159.165	Pesticides
EPA OPPT	Toxic Substances Control Act [34]	40 C.F.R. §720.50	New and existing manufactured or imported chemicals
FDA CBER	The Federal Food, Drug, and Cosmetic Act [71] and its amendments Public Health Service Act [72]	No specific requirement for stand-alone assessment of local toxicity such as skin or eye; can also be assessed clinically; need determined by route of administration (see Guidance for Industry: Developing Medical Imaging Drug and Biological Products: Part 1: Conducting Safety Assessments [73]	Biologics other than those regulated by CDER, including allergenics, blood and blood products, cellular & gene therapies, tissue and tissue-based products, vaccines and xenotransplantation products
FDA CDER	The Federal Food, Drug, and Cosmetic Act [71] and its amendments	There is no specific regulation for stand-alone assessment of local toxicity such as skin or eye irritation; can also be assessed clinically. Standalone studies are generally not recommended for local tolerance [55] *.	All routes of administration for small molecule drugs, protein therapeutics, and monoclonal antibodies, including topical and ocular
FDA CDRH	Federal Food, Drug, and Cosmetic Act [71] and its amendments	21 C.F.R. §807.92 There are no specific regulations for local irritation studies. The need for such studies is determined by the site of device use. FDA CDRH provides guidance for use of the International Standard ISO-10993–1 'Biological Evaluation of Medical Devices Part 1: Evaluation and testing within a risk management process' [59].	Medical devices and radiation-emitting products
FDA CFSAN	The Federal Food, Drug, and Cosmetic Act [71] and its amendments	There are no specific regulations for skin or eye irritation testing; it is up to the manufacturer to ensure product safety 21 C.F.R. §170 21 C.F.R. §73 21 C.F.R. §74 21 C.F.R. §700 21 C.F.R. §701 21 C.F.R. §710 21 C.F.R. §720 21 C.F.R. §740	Food ingredients, including food additives and color additives, food contact substances, and generally recognized as safe substances; cosmetics
OSHA	Occupational Safety and Health Act [74]	29 C.F.R. §1910.1200	Workplace materials
OSHA	Occupational Safety and Health Act [74]	29 C.F.R. §1910.1200	Workplace hazards

* FDA guidance documents are not regulations, but are made available to assist in interpretation of FDA policy and to provide guidance for testing. International harmonization or standardization publications are provided to assist in the standardization of testing, where applicable.

Table 2.

CPSC classification criteria for skin irritants

Classification	Primary irritation score*	Skin reaction(s)	Observation time
Corrosive	-	Necrosis or other non- reversible effects (e.g., ulceration, scarring)	≤ 24 hours
Irritant	≥ 5	Erythema and/or eschar formation	24 and 72 hours post- occlusion

*Average primary irritation score grading and calculation are described in 16 C.F.R. §1500.41 [14].

Table 3.

CPSC classification criteria for eye

Positive response*	***In vivo* effect**
Corneal ulceration (other than fine stippling)	First Test – If 4/6 animals are positive, the test is positive. If ≤1/6 animal is positive, the test is negative. If 2/6 or 3/6 animals are positive, the test is repeated using a different group of six animals.
Corneal opacity ≥1	
Iritis ≥1	Second Test – If 3/6 animals are positive, the test is positive. If 0/6 animals is positive, the test is negative. If 1/6 or 2/6 animals are positive, the test is repeated using a different group of six animals.
Conjunctival swelling and/or redness ≥2	Third Test – If ≥1/6 animals are positive, the test is positive. If 0/6 animals are positive, the test is negative.

* As defined in 16 C.F.R. §1500.42 [13] and based on scoring system described in U.S EPA Test Guideline OPPTS 870.2400 [39].

Table 4.

EPA hazard classification categories

Category	*In Vivo* Effect for Eye Irritation Classification	*In Vivo* Effect for Skin Irritation Classification
I	Corrosive (irreversible) or corneal involvement or other eye irritation persisting for >21 days	Corrosive (tissue destruction into the dermis and/or scarring)
II	Corneal involvement or other eye irritation clearing in 8 to 21 days	Severe irritation at 72 hours (severe erythema or edema)
III	Corneal involvement or other eye irritation clearing in 7 days or less	Moderate irritation at 72 hours (moderate erythema)
IV	Minimal effects clearing in less than 24 hours	Mild or slight irritation at 72 hours (no irritation or slight erythema)

Table 5.

OSHA eye corrosion and irritation categories

Category	Classification Criteria
1 (irreversible effects on the eye)	Substance produces: a) in at least one tested animal, effects on the cornea, iris, or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or (b) in at least in 2 of 3 tested animals a positive response of: (i) corneal opacity ≥3#, and/or (ii) iritis >1.5 calculated as the mean scores following grading at 24, 48, and 72 hours after substance instillation
2 (reversible effects on the eye) *	Substance produces in at least 2 of 3 tested animals a positive response of: (i) corneal opacity ≥1; (ii) iritis ≥1; (iii) conjunctival redness ≥2; and/or (iv) conjunctival edema ≥2 calculated as the mean scores following grading at 24, 48, and 72 hours after substance instillation and the effects are fully reverse within an observation period of normally 21 days

* A substance is classified as an Eye Irritant Category 2B when the effects listed for Category 2 classification are fully reversible within 7 days of observation.

Lesion grading performed according to scoring defined in Draize et al. (1944) [75].

Table 6.

OSHA skin corrosion and irritation categories

Category	Classification Criteria*
1 (corrosive)	1: Destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least one of three tested animals after exposure ≤4 hours Subcategories 1A: Corrosive responses in at least one of three animals following exposure ≤3 min during an observation period ≤1 hour 1B: Corrosive responses in at least one of three animals following exposure >3 min and ≤1 hour and observations ≤14 days 1C: Corrosive responses in at least one of three animals after exposures >1 hour and ≤4 hours and observations ≤14 days
2 (irritant)	(1) Mean score of ≥2.3 and ≤4.0 for erythema/eschar or for edema in at least two of three tested animals based on gradings at 24, 48, and 72 hours after patch removal or, if reactions are delayed, from grades on three consecutive days after the onset of skin reactions; or (2) Inflammation that persists to the end of the observation period normally 14 days in at least two animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasic, and scaling; or (3) Positive effects in a single animal that are shown to be related to chemical exposure but are less than the criteria above, particularly in cases where there is pronounced variability of response among animals.

* Lesion grading performed according to scoring defined in Draize et al. (1944) [75].

Table 7:

Non-animal approaches accepted by U.S. authorities that reduce animal use for eye irritation/corrosivity testing

Alternative approach	**Relevant Testing Guideline/Recommendations***
Bovine Corneal Opacity and Permeability	Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage (OECD TG 437 [20])
Isolated Chicken Eye	Test No. 438: Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage (OECD TG 438 [21])
Fluorescein Leakage	Test No. 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants (OECD TG 460 [42])
Cytosensor Microphysiometer	CPSC Policy on Animal Testing [12] The Cytosensor Microphysiometer Test Method: An *In Vitro* Method for Identifying Ocular Corrosive and Severe Irritant Chemicals as Well as Chemicals not Classified as Ocular Irritants (Draft OECD TG [44])
Short time exposure	Test No. 491: Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage (OECD TG 491 [43])
Reconstructed human Cornea-like Epithelium	Test No. 492: Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage (OECD TG 492 [22])
Bovine Corneal Opacity and Permeability, EpiOcular, or Cytosensor Microphysiometer for testing antimicrobial cleaning products	Use of an Alternative Testing Framework for Classification of Eye Irritation Potential of EPA Pesticide Products [48]
Acute toxicity waivers or bridging for pesticides	Guidance for Waiving or Bridging of Mammalian Acute Toxicity Tests for Pesticides and Pesticide Products (Acute Oral, Acute Dermal, Acute Inhalation, Primary Eye, Primary Derma, and Dermal Sensitization) [50]
Open literature to support human health risk assessment	Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment; Procedures for Reviewing Relevant Effects Data Published in the Open Literature for Use in OPP's Human Health Risk Assessments [76]
Evaluation and implementation of alternative approaches to traditional *in vivo* acute toxicity studies for FIFRA	Evaluation and implementation of alternative approaches to traditional *in vivo* acute toxicity studies for FIFRA

*OECD test guidelines are accepted in all 35 OECD member countries, including European Union countries.

Table 8:

Non-animal approaches accepted by U.S. authorities that reduce animal use for skin irritation/corrosivity testing

Alternative approach	**Relevant test guideline***
Corrositex® test method for skin corrosivity testing	Test No. 435: *In Vitro* Membrane Barrier Test Method for Skin Corrosion (OECD TG 435 [18])
Rat Transcutaneous Electrical Resistance for skin corrosivity testing	Test No. 430: *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER) (OECD 430 [30])
Reconstructed Human Epidermis test methods for skin corrosivity testing	Test No. 431: *In Vitro* Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method (OECD TG 431 [31])
Reconstructed Human Epidermis test methods for skin irritation testing	Test No. 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method (OECD TG 439 [19])

*OECD test guidelines are accepted in all 35 OECD member countries, including European Union countries.