OECD/OCDE 405

Adopted:
9 October 2017

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Acute Eye Irritation/Corrosion

INTRODUCTION

1. OECD Guidelines for Testing of Chemicals are periodically reviewed to ensure that they reflect the best available science. In previous reviews of this Test Guideline, special attention was given to possible improvements through the evaluation of all existing information on the test substance in order to avoid unnecessary testing in laboratory animals and thereby address animal welfare concerns. This Test Guideline (adopted in 1981 and updated in 1987, 2002, and 2012) includes the recommendation that prior to undertaking the described *in vivo* test for acute eye irritation/corrosion, a weight-of-the-evidence analysis be performed (1) on the existing relevant data. Where insufficient data are available, it is recommended to follow the Guidance Document on an Integrated Approache~~s to~~ on Testing and Assessment for Serious Eye Damage and Eye irritation (21). Testing in animals should only be conducted if determined to be necessary after consideration of available alternative methods, and use of those determined to be appropriate. At the time of drafting of this updated TG 405, there are instances where using this Test Guideline is still necessary or required by some regulatory authorities.

2. The latest update mainly focused on the use of analgesics and anesthetics without impacting the basic concept and structure of the Test Guideline. ICCVAM and an independent international scientific peer review panel reviewed the usefulness and limitations of routinely using topical anesthetics, systemic analgesics, and humane endpoints during *in vivo* ocular irritation safety testing (12). The review concluded that the use of topical anesthetics and systemic analgesics could avoid most or all pain and distress without affecting the outcome of the test, and recommended that these substances should always be used. This Test Guideline takes this review into account. Topical anesthetics, systemic analgesics, and humane endpoints should be routinely used during acute eye irritation and corrosion *in vivo* testing. Exceptions to their use should be justified. The refinements described in this proposal will substantially reduce or avoid animal pain and distress in most testing situations where *in vivo* ocular safety testing is still necessary.

3. Balanced preemptive pain management should include (i) routine pretreatment with a topical anesthetic (e.g., proparacaine or tetracaine) and a systemic analgesic (e.g. buprenorphine), (ii) routine post-treatment schedule of systemic analgesia (e.g., buprenorphine and meloxicam), (iii) scheduled observation, monitoring, and recording of animals for clinical signs of pain and/or distress, and (iv) scheduled observation, monitoring, and recording of the nature, severity, and progression of all eye injuries. Further detail is provided in the updated procedures described below. Following test substance administration, no additional *topical* anesthetics or analgesics should be applied in order to avoid interference with the study.

This Guideline was adopted by the OECD Council by written procedure on 9 October 2017 [C(2017)97].

Analgesics with anti-inflammatory activity (e.g., meloxicam) should not be applied topically, and doses used systemically should not interfere with ocular effects.

4. Definitions are set out in the Annex to the Guideline.

INITIAL CONSIDERATIONS

5. In the interest of both sound science and animal welfare, *in vivo* testing should not be considered until all available data relevant to the potential eye corrosivity/irritation of the substance have been evaluated in a weight-of-the-evidence analysis. Such data include evidence from existing studies in humans and/or laboratory animals, evidence of eye corrosivity/irritation of one or more structurally related substances or mixtures of such substances, data demonstrating high acidity or alkalinity of the substance (4) (5), and results from validated and accepted *in vitro* or *ex vivo* tests for skin corrosion and eye corrosion/irritation (6) (13) (14) (15) (16) (17). The studies may have been conducted prior to, or as a result of, a weight-of-the-evidence analysis.

6. For certain substances, such an analysis may indicate the need for *in vivo* studies of the ocular corrosion/irritation potential of the substance. In all such cases, before considering the use of the *in vivo* eye test, preferably a study of the *in vitro* and/or *in vivo* skin corrosion effects of the substance should be conducted first and evaluated in accordance with the sequential testing strategy in Test Guideline 404 (7).

7. A preferred sequential testing strategy, which includes the performance of validated *in vitro* or *ex vivo* eye corrosion/irritation tests, is included as a Supplement to this Guideline. It is recommended that this testing strategy be followed prior to undertaking *in vivo* testing. For new substances, it is the recommended stepwise testing approach for developing scientifically sound data on the corrosivity/irritation of the substance. For existing substances with insufficient data on skin and eye corrosion/irritation, the strategy can be used to fill missing data gaps. The use of a different testing strategy or procedure, or the decision not to use a stepwise testing approach, should be justified.

PRINCIPLE OF THE *IN VIVO* TEST

8. Following pretreatment with a systemic analgesic and induction of appropriate topical anesthesia, the substance to be tested is applied in a single dose to one of the eyes of the experimental animal; the untreated eye serves as the control. The degree of eye irritation/corrosion is evaluated by scoring lesions of conjunctiva, cornea, and iris, at specific intervals. Other effects in the eye and adverse systemic effects are also described to provide a complete evaluation of the effects. The duration of the study should be sufficient to evaluate the reversibility or irreversibility of the effects.

9. Animals showing signs of severe distress and/or pain at any stage of the test or lesions consistent with the humane endpoints described in this Test Guideline (see Paragraph 26) should be humanely killed, and the substance assessed accordingly. Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of a separate Guidance Document (8).

PREPARATIONS FOR THE *IN VIVO* TEST

Selection of species

10. The albino rabbit is the preferable laboratory animal and healthy young adult animals are used. A rationale for using other strains or species should be provided.

Preparation of animals

11. Both eyes of each experimental animal provisionally selected for testing should be examined within 24 hours before testing starts. Animals showing eye irritation, ocular defects, or pre-existing corneal injury should not be used.

Housing and feeding conditions

12. Animals should be individually housed. The temperature of the experimental animal room should be 20°C (± 3°C) for rabbits. Although the relative humidity should be at least 30% and preferably not exceed 70%, other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. Excessive light intensity should be avoided. For feeding, conventional laboratory diets may be used with an unrestricted supply of drinking water.

TEST PROCEDURE

Use of topical anesthetics and systemic analgesics

13. The following procedures are recommended to avoid or minimize pain and distress in ocular safety testing procedures. Alternate procedures that have been determined to provide as good or better avoidance or relief of pain and distress may be substituted.

- Sixty minutes prior to test substance application (TSA), buprenorphine 0.01 mg/kg is administered by subcutaneous injection (SC) to provide a therapeutic level of systemic analgesia. Buprenorphine and other similar opiod analgesics administered systemically are not known or expected to alter ocular responses (12).
- Five minutes prior to TSA, one or two drops of a topical ocular anesthetic (e.g. 0.5% proparacaine hydrochloride or 0.5% tetracaine hydrochloride) are applied to each eye. In order to avoid possible interference with the study, a topical anesthetic that does not contain preservatives is recommended. The eye of each animal that is not treated with a test article, but which is treated with topical anesthetics, serves as a control. If the test substance is anticipated to cause significant pain and distress, it should not normally be tested *in vivo*. However, in case of doubt or where testing is necessary, consideration should be given to additional applications of the topical anesthetic at 5-minute intervals prior to TSA. Users should be aware that multiple applications of topical anesthetics could potentially cause a slight increase in the severity and/or time required for chemically-induced lesions to clear.
- Eight hours after TSA, buprenorphine 0.01 mg/kg SC and meloxicam 0.5 mg/kg SC are administered to provide a continued therapeutic level of systemic analgesia. While there are no data to suggest that meloxicam has anti-inflammatory effects on the eye when administered SC once daily, meloxicam should not be administered until at least 8 hours after TSA in order to avoid any possible interference with the study (12).
- After the initial 8-hour post-TSA treatment, buprenorphine 0.01 mg/kg SC should be administered every 12 hours, in conjunction with meloxicam 0.5 mg/kg SC every 24 hours, until the ocular lesions resolve and no clinical signs of pain and distress are present. Sustained-release preparations of analgesics are available that could be considered to decrease the frequency of analgesic dosing.
- "Rescue" analgesia should be given immediately after TSA if pre-emptive analgesia and topical anesthesia are inadequate. If an animal shows signs of pain and distress during the study, a "rescue" dose of buprenorphine 0.03 mg/kg SC would be given immediately and repeated as often as every 8 hours, if necessary, instead of 0.01 mg/kg SC every 12 hours. Meloxicam 0.5 mg/kg SC would be

administered every 24 hours in conjunction with the "rescue" dose of buprenorphine, but not until at least 8 hours post-TSA.

Application of the test substance

14. The test substance should be placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids are then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control.

Irrigation

15. The eyes of the test animals should not be washed for at least 24 hours following instillation of the test substance, except for solids (see paragraph 18), and in case of immediate corrosive or irritating effects. At 24 hours a washout may be used if considered appropriate.

16. Use of a satellite group of animals to investigate the influence of washing is not recommended unless it is scientifically justified. If a satellite group is needed, two rabbits should be used. Conditions of washing should be carefully documented, e.g., time of washing; composition and temperature of wash solution; duration, volume, and velocity of application.

Dose level

(1) Testing of liquids

17. For testing liquids, a dose of 0.1 mL is used. Pump sprays should not be used for instilling the substance directly into the eye. The liquid spray should be expelled and collected in a container prior to instilling 0.1 mL into the eye.

(2) Testing of solids

18. When testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 mL or a weight of not more than 100 mg. The test material should be ground to a fine dust. The volume of solid material should be measured after gently compacting it, e.g. by tapping the measuring container. If the solid test substance has not been removed from the eye of the test animal by physiological mechanisms at the first observation time point of 1 hour after treatment, the eye may be rinsed with saline or distilled water.

(3) Testing of aerosols

19. It is recommended that all pump sprays and aerosols be collected prior to instillation into the eye. The one exception is for substances in pressurised aerosol containers, which cannot be collected due to vaporisation. In such cases, the eye should be held open, and the test substance administered to the eye in a simple burst of about one second, from a distance of 10 cm directly in front of the eye. This distance may vary depending on the pressure of the spray and its contents. Care should be taken not to damage the eye from the pressure of the spray. In appropriate cases, there may be a need to evaluate the potential for "mechanical" damage to the eye from the force of the spray.

20. An estimate of the dose from an aerosol can be made by simulating the test as follows: the substance is sprayed on to weighing paper through an opening the size of a rabbit eye placed directly before the paper. The weight increase of the paper is used to approximate the amount sprayed into the eye. For volatile substances, the dose may be estimated by weighing a receiving container before and after removal of the test material.

Initial test (in vivo eye irritation/corrosion test using one animal)

21. It is strongly recommended that the *in vivo* test be performed initially using one animal (21). Observations should allow for determination of severity and reversibility before proceeding to a confirmatory test in a second animal.

22. If the results of this test indicate the substance to be corrosive or a severe irritant to the eye using the procedure described, further testing for ocular irritancy should not be performed.

Confirmatory test (in vivo eye irritation test with additional animals)

23. If a corrosive or severe irritant effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals. If an irritant effect is observed in the initial test, it is recommended that the confirmatory test be conducted in a sequential manner in one animal at a time, rather than exposing the two additional animals simultaneously. If the second animal reveals corrosive or severe irritant effects, the test is not continued. If results from the second animal are sufficient to allow for a hazard classification determination, then no further testing should be conducted.

Observation period

24. The duration of the observation period should be sufficient to evaluate fully the magnitude and reversibility of the effects observed. However, the experiment should be terminated at any time that the animal shows signs of severe pain or distress (8). To determine reversibility of effects, the animals should be observed normally for 21 days post administration of the test substance. If reversibility is seen before 21 days, the experiment should be terminated at that time.

Clinical observations and grading of eye reactions

25. The eyes should be comprehensively evaluated for the presence or absence of ocular lesions one hour post-TSA, followed by at least daily evaluations. Animals should be evaluated several times daily for the first 3 days to ensure that termination decisions are made in a timely manner. Test animals should be routinely evaluated for the entire duration of the study for clinical signs of pain and/or distress (e.g. repeated pawing or rubbing of the eye, excessive blinking, excessive tearing) (9) (10) (11) at least twice daily, with a minimum of 6 hours between observations, or more often if necessary. This is necessary to (i) adequately assess animals for evidence of pain and distress in order to make informed decisions on the need to increase the dosage of analgesics and (ii) assess animals for evidence of established humane endpoints in order to make informed decisions on whether it is appropriate to humanely euthanize animals, and to ensure that such decisions are made in a timely manner. Fluorescein staining should be routinely used and a slit lamp biomicroscope used when considered appropriate (e.g., assessing depth of injury when corneal ulceration is present) as an aid in the detection and measurement of ocular damage, and to evaluate if established endpoint criteria for humane euthanasia have been met. Digital photographs of observed lesions may be collected for reference and to provide a permanent record of the extent of ocular damage. Animals should be kept on test no longer than necessary once definitive information has been obtained. Animals showing severe pain or distress should be humanely killed without delay, and the substance assessed accordingly.

26. Animals with the following eye lesions post-instillation should be humanely killed (refer to Table 1 for a description of lesion grades): corneal perforation or significant corneal ulceration including staphyloma; blood in the anterior chamber of the eye; grade 4 corneal opacity; absence of a light reflex (iridial response grade 2) which persists for 72 hours; ulceration of the conjunctival membrane; necrosis of the conjunctivae or nictitating membrane; or sloughing. This is because such lesions generally are not reversible. Furthermore, it is recommended that the following ocular lesions be used as humane endpoints to terminate

studies before the end of the scheduled 21-day observation period. These lesions are considered predictive of severe irritant or corrosive injuries and injuries that are not expected to fully reverse by the end of the 21-day observation period: severe depth of injury (e.g., corneal ulceration extending beyond the superficial layers of the stroma), limbus destruction >50% (as evidenced by blanching of the conjunctival tissue), and severe eye infection (purulent discharge). A combination of: vascularization of the cornea surface (i.e., pannus); area of fluorescein staining not diminishing over time based on daily assessment; and/or lack of re-epithelialization 5 days after test substance application could also be considered as potentially useful criteria to influence the clinical decision on early study termination. However, these findings individually are insufficient to justify early study termination. Once severe ocular effects have been identified, an attending or qualified laboratory animal veterinarian or personnel trained to identify the clinical lesions should be consulted for a clinical examination to determine if the combination of these effects warrants early study termination. The grades of ocular reaction (conjunctivae, cornea and iris) should be obtained and recorded at 1, 24, 48, and 72 hours following test substance application (Table 1). Animals that do not develop ocular lesions may be terminated not earlier than 3 days post instillation. Animals with ocular lesions that are not severe should be observed until the lesions clear, or for 21 days, at which time the study is terminated. Observations should be performed and recorded at a minimum of 1 hour, 24 hours, 48 hours, 72 hours, 7 days, 14 days, and 21 days in order to determine the status of the lesions, and their reversibility or irreversibility. More frequent observations should be performed if necessary in order to determine whether the test animal should be euthanized out of humane considerations or removed from the study due to negative results

27. The grades of ocular lesions (Table 1) should be recorded at each examination. Any other lesions in the eye (e.g. pannus, staining, anterior chamber changes) or adverse systemic effects should also be reported.

28. Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, biomicroscope, or other suitable device. After recording the observations at 24 hours, the eyes may be further examined with the aid of fluorescein.

29. The grading of ocular responses is necessarily subjective. To promote harmonisation of grading of ocular response and to assist testing laboratories and those involved in making and interpreting the observations, the personnel performing the observations need to be adequately trained in the scoring system used.

DATA AND REPORTING

Evaluation of results

30. The ocular irritation scores should be evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. The individual scores do not represent an absolute standard for the irritant properties of a material, as other effects of the test material are also evaluated. Instead, individual scores should be viewed as reference values and are only meaningful when supported by a full description and evaluation of all observations.

Test report

31. The test report should include the following information:

Rationale for *in vivo* testing: weight-of-the-evidence analysis of pre-existing test data, including results from sequential testing strategy:

– description of relevant data available from prior testing;

- data derived in each step of testing strategy;
- description of *in vitro* tests performed, including details of procedures, results obtained with test/reference substances;
- description of *in vivo* dermal irritation / corrosion study performed, including results obtained;
- weight-of-the-evidence analysis for performing *in vivo* study

Test substance:

- identification data (e.g. chemical name and if available CAS number, purity, known impurities, source, lot number);
- physical nature and physicochemical properties (e.g. pH, volatility, solubility, stability, reactivity with water);
- in case of a mixture, components should be identified including identification data of the constituent substances (*e.g.* chemical names and if available CAS numbers) and their concentrations;
- dose applied;

Vehicle:

- identification, concentration (where appropriate), volume used;
- justification for choice of vehicle.

Test animals:

- species/strain used, rationale for using animals other than albino rabbit;
- age of each animal at start of study;
- number of animals of each sex in test and control groups (if required);
- individual animal weights at start and conclusion of test;
- source, housing conditions, diet, etc.

Anaesthetics and analgesics

- doses and times when topical anaesthetics and systemic analgesics were administered;
- if local anaesthetic is used, identification, purity, type, and potential interaction with test substance.

Results:

- description of method used to score irritation at each observation time (e.g., hand slitlamp, biomicroscope, fluorescein);
- tabulation of irritant/corrosive response data for each animal at each observation time up to removal of each animal from the test;
- narrative description of the degree and nature of irritation or corrosion observed;
- description of any other lesions observed in the eye (e.g., vascularization, pannus formation, adhesions, staining);
- description of non-ocular local and systemic adverse effects, record of clinical signs of pain and distress, digital photographs, and histopathological findings, if any.

Discussion of results.

Interpretation of the results

32. Extrapolation of the results of eye irritation studies in laboratory animals to humans is valid only to a limited degree. In many cases the albino rabbit is more sensitive than humans to ocular irritants or corrosives.

33. Care should be taken in the interpretation of data to exclude irritation resulting from secondary infection.

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TABLE 1: GRADING OF OCULAR LESIONS

Cornea	**Grade**
Opacity: degree of density (readings should be taken from most dense area)*	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible	1
Easily discernible translucent area; details of iris slightly obscured	2
Nacrous area; no details of iris visible; size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Maximum possible: 4	
* The area of corneal opacity should be noted	

Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect	1
Hemorrhage, gross destruction, or no reaction to light	2
Maximum possible: 2	

Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse,crimson colour; individual vessels not easily discernible	2
Diffuse beefy red	3
Maximum possible: 3	

Chemosis	
Swelling (refers to lids and/or nictating membranes)	
Normal	0
Some swelling above normal	1
Obvious swelling, with partial eversion of lids	2
Swelling, with lids about half closed	3
Swelling, with lids more than half closed	4
Maximum possible: 4	

ANNEX

DEFINITIONS

1. Acid/alkali reserve: For acidic preparations, this is the amount (g) of sodium hydroxide/100 g of preparation required to produce a specified pH. For alkaline preparations, it is the amount (g) of sodium hydroxide equivalent to the g sulphuric acid/100 g of preparation required to produce a specified pH (Young et al. 1988).

2. Non irritants: Substances that are not classified as EPA Category I, II, or III ocular irritants; or GHS eye irritants Category 1, 2, 2A, or 2B; or EU Category 1 or 2 (18) (19) (20).

3. Ocular corrosive: (a) A substance that causes irreversible tissue damage to the eye; (b) Substances that are classified as GHS eye irritants Category 1, or EPA Category I ocular irritants, or EU Category 1 (18) (19) (20).

4. Ocular irritant: (a) A substance that produces a reversible change in the eye; (b) Substances that are classified as EPA Category II or III ocular irritants; or GHS eye irritants Category 2, 2A or 2B ; or EU Category 2 (18) (19) (20).

5. Ocular severe irritant**:** (a) A substance that causes tissue damage in the eye that does not resolve within 21 days of application or causes serious physical decay of vision; (b) Substances that are classified as GHS eye irritant Category 1, or EPA Category I ocular irritants, or EU Category 1 (18) (19) (20).

6. Tiered approach: A stepwise testing strategy where all existing information on a test substance is reviewed, in a specified order, using a weight-of-evidence process at each tier to determine if sufficient information is available for a hazard classification decision, prior to progression to the next tier. If the irritancy potential of a test substance can be assigned based on the existing information, no additional testing is required. If the irritancy potential of a test substance cannot be assigned based on the existing information, a step-wise sequential animal testing procedure is performed until an unequivocal classification can be made.

7. Weight-of-the-evidence (process): The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.

SUPPLEMENT TO TEST GUIDELINE 405

A Sequential Testing Strategy for Eye Irritation and Corrosion

GENERAL CONSIDERATIONS

1. In the interests of sound science and animal welfare, it is important to avoid the unnecessary use of animals, and to minimise testing that is likely to produce severe responses in animals. All information on a substance relevant to its potential ocular irritation/corrosivity should be evaluated prior to considering *in vivo* testing. Sufficient evidence may already exist to classify a test substance as to its eye irritation or corrosion potential without the need to conduct testing in laboratory animals. Therefore, utilizing a weight-of-the-evidence analysis and sequential testing strategy will minimise the need for *in vivo* testing, especially if the substance is likely to produce severe reactions.

2. It is recommended that a weight-of-the-evidence analysis be used to evaluate existing information pertaining to eye irritation and corrosion of substances and to determine whether additional studies, other than *in vivo* eye studies, should be performed to help characterise such potential. Where further studies are needed, it is recommended that the sequential testing strategy be utilised to develop the relevant experimental data. For substances which have no testing history, the sequential testing strategy should be utilised to develop the data needed to evaluate its eye corrosion/irritation. The initial testing strategy described in this Supplement was developed at an OECD workshop (1). It was subsequently affirmed and expanded in the Harmonised Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, in November 1998 (2), and updated by an OECD expert group in 2011.

3. Although this testing strategy is not an integrated part of Test Guideline 405, it expresses the recommended approach for the determination of eye irritation/corrosion properties. This approach represents both best practice and an ethical benchmark for *in vivo* testing for eye irritation/corrosion. The Guideline provides guidance for the conduct of the *in vivo* test and summarises the factors that should be addressed before considering such a test. The sequential testing strategy provides a weight-of-the-evidence approach for the evaluation of existing data on the eye irritation/corrosion properties of substances and a tiered approach for the generation of relevant data on substances for which additional studies are needed or for which no studies have been performed. The strategy includes the performance first of validated and accepted *in vitro* or *ex vivo* tests and then of Guideline 404 skin irritation/corrosion studies under specific circumstances (3) (4).

DESCRIPTION OF THE STEPWISE TESTING STRATEGY

4. Prior to undertaking tests as part of the sequential testing strategy (Figure), all available information should be evaluated to determine the need for *in vivo* eye testing. Although significant information might be gained from the evaluation of single parameters (e.g., extreme pH), the totality of existing information should be assessed. All relevant data on the effects of the substance in question, and its structural analogues, should be evaluated in making a weight-of-the-evidence decision, and a rationale for the decision should be presented. Primary emphasis should be placed upon existing human and animal

data on the substance, followed by the outcome of *in vitro* or *ex vivo* testing. *In vivo* studies of corrosive substances should be avoided whenever possible. The factors considered in the testing strategy include:

5. Evaluation of existing human and/or animal data and/or *in vitro* data from validated and internationally accepted methods (Step 1). Existing human data, e.g. clinical and occupational studies, and case reports, and/or animal test data from ocular studies and/or *in vitro* data from validated and internationally accepted methods for eye irritation/corrosion should be considered first, because they provide information directly related to effects on the eyes. Thereafter, available data from human and/or animal studies investigating dermal corrosion/irritation, and/or *in vitro* studies from validated and internationally accepted methods for skin corrosion should be evaluated. Substances with known corrosivity or severe irritancy to the eye should not be instilled into the eyes of animals, nor should substances showing corrosive or severe irritant effects to the skin; such substances should be considered to be corrosive and/or irritating to the eyes as well. Substances with sufficient evidence of non-corrosivity and non-irritancy from previously performed ocular studies should also not be tested in *in vivo* eye studies.

6. Analysis of structure activity relationships (SAR) (Step 2). The results of testing of structurally related chemicals should be considered, if available. When sufficient human and/or animal data are available on structurally related substances or mixtures of such substances to indicate their eye corrrosion/irritancy potential, it can be presumed that the test substance will produce the same responses. In those cases, the substance may not need to be tested. Negative data from studies of structurally related substances or mixtures of such substances do not constitute sufficient evidence of non-corrosivity/non-irritancy of a substance under the sequential testing strategy. Validated and accepted SAR approaches should be used to identify the corrosion and irritation potential for both dermal and ocular effects.

7. Physicochemical properties and chemical reactivity (Step 3). Substances exhibiting pH extremes such as ≤2.0 or ≥11.5 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive or irritant to the eye, then its acid/alkaline reserve (buffering capacity) may also be taken into consideration (5)(6)(7). If the buffering capacity suggests that a substance may not be corrosive to the eye (i.e., substances with extreme pH and low acid/alkaline reserve), then further testing should be undertaken to confirm this, preferably by the use of a validated and accepted *in vitro* or *ex vivo* test (see paragraph 10).

8. Consideration of other existing information (Step 4). All available information on systemic toxicity via the dermal route should be evaluated at this stage. The acute dermal toxicity of the test substance should also be considered. If the test substance has been shown to be highly toxic by the dermal route, it may not need to be tested in the eye. Although there is not necessarily a relationship between acute dermal toxicity and eye irritation/corrosion, it can be assumed that if an agent is highly toxic via the dermal route, it will also exhibit high toxicity when instilled into the eye. Such data may also be considered between Steps 2 and 3.

9. Assessment of dermal corrosivity of the substance if also required for regulatory purposes (Step 5). The skin corrosion and severe irritation potential should be evaluated first in accordance with Guideline 404 (4) and the accompanying Supplement (8), including the use of validated and internationally accepted *in vitro* skin corrosion test methods (9) (10) (11). If the substance is shown to produce corrosion or severe skin irritation, it may also be considered to be a corrosive or severely irritant to the eye. Thus, no further testing would be required. If the substance is not corrosive or severely irritating to the skin, an *in vitro* or *ex vivo* eye test should be performed.

10. Results from *in vitro* or *ex vivo* tests (Step 6). Substances that have demonstrated corrosive or severe irritant properties in an *in vitro* or *ex vivo* test (12) (13) that has been validated and internationally accepted for the assessment specifically of eye corrosivity/irritation, need not be tested in animals. It can be presumed that such substances will produce similar severe effects *in vivo*. If validated and accepted *in vitro/ex vivo* tests are not available, one should bypass Step 6 and proceed directly to Step 7.

11. *In vivo* test in rabbits (Steps 7 and 8): *In vivo* ocular testing should begin with an initial test using one animal. If the results of this test indicate the substance to be a severe irritant or corrosive to the eyes, further testing should not be performed. If that test does not reveal any corrosive or severe irritant effects, a confirmatory test is conducted with two additional animals. Depending upon the results of the confirmatory test, further tests may be needed. [see TG 405]

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FIGURE

TESTING AND EVALUATION STRATEGY FOR EYE IRRITATION/CORROSION

	Activity	Finding	Conclusion
1	Existing human and/or animal data, and/or *in vitro* data from validated and internationally accepted methods showing effects on eyes	Severe damage to eyes	Apical endpoint; consider corrosive to eyes. No testing is needed.
		Eye irritant	Apical endpoint; consider irritating to eyes. No testing is needed.
		Not corrosive/not irritating to eyes	Apical endpoint; considered non-corrosive and non-irritating to eyes. No testing required.
	Existing human and/or animal data and/or *in vitro* data from validated and internationally accepted methods showing corrosive effects on skin	Skin corrosive	Assume corrosivity to eyes. No testing is needed.
	Existing human and/or animal data and/or *in vitro* data from validated and internationally accepted methods showing severe irritant effects on skin	Severe skin irritant	Assume irritating to eyes. No testing is needed
	↓ *no information available, or available information is not conclusive* ↓		
2	Perform SAR for eye corrosion/irritation	Predict severe damage to eyes	Assume corrosivity to eyes. No testing is needed.
		Predict irritation to eyes	Assume irritating to eyes. No testing is needed.
	Consider SAR for skin corrosion	Predict skin corrosivity	Assume corrosivity to eyes. No testing is needed.
	↓ *No predictions can be made, or predictions are not conclusive or negative* ↓		
3	Measure pH (buffering capacity, if relevant)	pH ≤ 2 or ≥ 11.5 (with high buffering capacity, if relevant)	Assume corrosivity to eyes. No testing is needed.
	↓		

2< pH < 11.5, or pH≤2.0 or ≥11.5 with low/no buffering capacity, if relevant

↓

4	Consider existing systemic toxicity data via the dermal route	Highly toxic at concentrations that would be tested in the eye.	Substance would be too toxic for testing. No testing is needed.

↓

Such information is not available, or substance is not highly toxic

↓

5	Experimentally assess skin corrosion potential according to the testing strategy in OECD Guideline 404 if also required for regulatory purposes	Corrosive or severe irritant response	Assume corrosive to eyes. No further testing is needed.

↓

Substance is not corrosive or severely irritating to skin

↓

6	Perform validated and accepted *in vitro* or *ex vivo* ocular test(s)	Corrosive or severe irritant response	Assume corrosive or severe irritant to eyes, provided the test performed can be used to identify corrosives/severe irritants and the substance is within the applicability domain of the test. No further testing is needed.
		Irritant response	Assume irritant to eyes, provided the test(s) performed can be used to correctly identify corrosive, severe irritants, and irritants, and the substance is within the applicability domain of the test(s). No further testing is needed.
		Non-irritant response	Assume non-irritant to eyes, provided the test(s) performed can be used to correctly identify non-irritants, correctly distinguish these from substances that are irritants, severe irritants, or ocular corrosives, and the substance is within the applicability domain of the test. No further testing is needed.

↓

Validated and accepted in vitro or ex vivo ocular test(s) cannot be used to

reach a conclusion

↓

7	Perform initial *in vivo* rabbit eye test using one animal	Severe damage to eyes	Consider corrosive to eyes. No further testing is needed.

↓

No severe damage, or no response

↓

8	Perform confirmatory test using one or two additional animals	Corrosive or irritating	Consider corrosive or irritating to eyes. No further testing is needed
		Not corrosive or irritating	Consider non-irritating and non-corrosive to eyes. No further testing is needed.