Appendix B

ICCVAM-Recommended Protocol:

Revised OECD Test Guideline 405 (Draize Test for Acute Eye Irritation/Corrosion)

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DRAFT PROPOSED REVISIONS TO GUIDELINES FOR OCULAR SAFETY TESTING

Boldface, underlined text represents ICCVAM's draft proposed revisions to Test Guideline 405.

Acute Eye Irritation/Corrosion

INTRODUCTION

Various national and international guidelines exist for acute eye irritation and corrosion testing. These guidelines are periodically reviewed to ensure that they reflect the best available science. ICCVAM and an independent international scientific peer review panel recently reviewed the usefulness and limitations of routinely using topical anesthetics, systemic analgesics, and humane endpoints during required *in vivo* ocular irritation safety testing (15). Based on this review, ICCVAM recommends that national and international guidelines be updated to require the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid and minimize pain and distress during acute eye irritation and corrosion testing. Proposed revisions to ocular test guidelines are provided as tracked changes in this document.

Balanced preemptive pain management should always be provided when the Draize rabbit eye test is conducted for regulatory safety testing and hazard classification and labeling purposes. The pain management should include (1) routine pretreatment with a topical anesthetic (e.g., proparacaine or tetracaine) and a systemic analgesic (e.g., buprenorphine), (2) a routine post-treatment schedule with systemic analgesia and a nonsteroidal anti-inflammatory drug (e.g., meloxicam), (3) scheduled observation, monitoring, and recording of animals for clinical signs of pain and/or distress, and (4) 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.

It was also recommended that test animals be comprehensively evaluated for the presence or absence of ocular lesions one hour after test substance administration (TSA), followed by at least daily evaluations. Animals should be evaluated once daily for the first 3 days, or more often if necessary to ensure that termination decisions are made in a timely manner. ICCVAM also recommends that test animals be routinely evaluated for clinical signs of pain and/or distress (e.g., repeated pawing or rubbing of the eye, excessive blinking, excessive tearing [Wright et al. 1985; NRC 2008, 2009]) at least twice daily, with a minimum of 6 hours between observations, or more often if necessary. This is necessary to (1) 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 (2) 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 (see paragraph 26). ICCVAM also recommends that 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 objective measurement of ocular endpoints, and to evaluate the extent that established criteria for humane euthanasia have been addressed.

Definitions of acute eye irritation and corrosion are set out in the Annex to the Guideline.

INITIAL CONSIDERATIONS

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 will include evidence from existing studies in humans and/or laboratory animals, evidence of 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 irritation (6)(7). The studies may have been conducted prior to, or as a result of, a weight-of-the-evidence analysis.

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 vivo* dermal effects of the substance should be conducted first and evaluated in accordance with Testing Guideline 404 (8). The application of a weight-of-the-evidence analysis and the sequential testing strategy should decrease the need for *in vivo* testing for eye corrosivity/irritation of substances for which sufficient evidence already exists from other studies. If a determination of eye corrosion or irritation potential cannot be made using the sequential testing strategy, even after the performance of an *in vivo* study of dermal corrosion and irritation, an *in vivo* eye corrosion/irritation test may be performed.

A preferred sequential testing strategy, which includes the performance of validated *in vitro* or *ex vivo* tests for corrosion/irritation, is included as a Supplement to this guideline. The strategy was developed at, and unanimously recommended by the participants of, an OECD workshop (9), and has been adopted as the recommended testing strategy in the Globally Harmonized System for the Classification of Chemical Substances (GHS) (10). 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 should 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

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.

Animals showing continuing 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** 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 (11).

PREPARATIONS FOR THE *IN VIVO* TEST

Selection of species

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

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

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. For feeding, conventional laboratory diets may be used with an unrestricted supply of drinking water.

TEST PROCEDURE

Use of topical anesthetics and systemic analgesics

The following modified Draize rabbit eye test pain management procedures are to be used 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.

Five minutes pre-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. 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, consideration should be given to additional applications of the topical anesthetic at 5-minute intervals pre-TSA. Users should be aware that multiple applications of topical anesthetics could increase the severity and/or extend the time required for lesions that are chemically induced to clear.

If a test subject shows signs of pain and distress during the test interval, additional analgesia (i.e., a "rescue" dose of 0.03 mg/kg SC buprenorphine) would be given immediately and repeated every 8 hours, instead of 0.01 mg/kg SC every 12 hours. Meloxicam would continue with the same dose and interval as described below. The "rescue" analgesia should be given immediately post-TSA if pre-emptive analgesia and topical anesthesia is inadequate.

After the initial 8 hrs post-TSA treatment, if ocular lesions and/or clinical signs of pain and distress are present, buprenorphine 0.01 mg/kg SC should be administered every 12 hours (8 hours if the "rescue" dose is needed), in conjunction with meloxicam 0.5 mg/kg SC every 24 hours.

Application of the test substance

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

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 16), and in case of immediate corrosive or irritating effects. At 24 hours a washout may be used if considered appropriate.

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

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

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

It is recommended that all pump sprays and aerosols be collected prior to installation 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.

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)

As articulated in the sequential testing strategy (Supplement to Guideline), it is strongly recommended that the *in vivo* test be performed initially using one animal.

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)

If a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals. If a severe irritant effect is observed in the initial test indicating a possible strong (irreversible) effect in the confirmatory testing, 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. Additional animals may be needed to confirm weak or moderate irritant responses.

Observation period

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 continuing signs of severe pain or distress (9). 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

The eyes should be **comprehensively evaluated for the presence or absence of ocular lesions one hr post-TSA, followed by at least daily evaluations. Animals should be evaluated once daily for the first 3 days, or more often if necessary, to ensure that termination decisions are made in a timely manner. Test animals should be routinely evaluated for clinical signs of pain and/or distress (e.g., repeated pawing or rubbing of the eye, excessive blinking, excessive tearing [Wright et al. 1985; NRC 2008, 2009]) at least twice daily, with a minimum of 6 hours between observations, or more often if necessary. 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 objective measurement of ocular endpoints. Digital photographs of observed lesions should be collected for reference and to provide a permanent record of the extent of ocular damage. A written record of all observations should be made to facilitate and document decisions on the progression or resolution of such ocular lesions.** Animals should be kept on test no longer than necessary once definitive information has been obtained. Animals showing continuing severe pain or distress should be humanely killed without delay, and the substance assessed accordingly.

Animals with the following eye lesions post-instillation should be humanely killed: corneal perforation or significant corneal ulceration including staphyloma; blood in the anterior chamber of the eye; grade 4 corneal opacity which persists for 48 hours; absence of a light reflex (iridial response grade 2) which persists for 72 hours; ulceration of the conjunctival membrane; necrosis of the conjuctivae or nictitating membrane; or sloughing. This is because such lesions generally are not reversible. **Furthermore, it is recommended that the following ocular lesions should also be used as earlier 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 after treatment: 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). Used in combination, vascularization of the cornea surface (i.e., pannus), area of fluorescein staining not diminishing over time based on daily assessment, and lack of re-epithelialization 5 days after test substance application should be considered as potentially useful criteria to influence the clinical decision on early study termination. However, there are insufficient data to use these endpoints individually to justify early study termination. ICCVAM emphasizes that once severe ocular effects have been identified, an attending or qualified laboratory animal veterinarian should be consulted for a clinical examination to determine if the combination of these effects warrants early study termination.**

Draize scores are obtained and recorded at 1, 24, 48, and 72 hours following test substance application. Animals that do not develop ocular lesions may be terminated not earlier than 3 days post instillation. Animals with mild to moderate lesions should be observed until the lesions clear, or for 21 days, at which time the study is terminated. Observations should be performed **and recorded daily until** 21 days in order to determine the status of the lesions, and their reversibility or irreversibility.

The grades of ocular reaction (conjunctivae, cornea and iris) should be recorded at each examination (Table I). Any other lesions in the eye (e.g. pannus, staining, **anterior chamber changes**) or adverse systemic effects should also be reported.

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.

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

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

The test report must 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. CAS number, source, purity, known impurities, lot number);
- physical nature and physicochemical properties (e.g. pH, volatility, solubility, stability, reactivity with water);
- in case of a mixture, composition and relative percentages of components;

- if local anaesthetic is used, identification, purity, type, dose, and potential interaction with test substance.

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.

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

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.

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

LITERATURE

(1) Barratt, M.D., Castell, J.V., Chamberlain, M., Combes, R.D., Dearden, J.C., Fentem, J.H., Gerner, I., Giuliani, A., Gray, T.J.B., Livingston, D.J., Provan, W.M., Rutten, F.A.J.J.L., Verhaar, H.J.M., Zbinden, P. (1995). The Integrated Use of Alternative Approaches for Predicting Toxic Hazard. ECVAM Workshop Report 8. ATLA 23, 410 - 429.

(2) de Silva, O., Cottin, M., Dami, N., Roguet, R., Catroux, P., Toufic, A., Sicard, C., Dossou, K.G., Gerner, I., Schlede, E., Spielmann, H., Gupta, K.C., Hill, R.N. (1997). Evaluation of Eye Irritation Potential: Statistical Analysis and Tier Testing Strategies. Food Chem. Toxicol 35, 159 - 164.

(3) Worth A.P. and Fentem J.H. (1999). A general approach for evaluating stepwise testing strategies ATLA 27, 161-177.

(4) Young, J.R., How, M.J., Walker, A.P., Worth W.M.H. (1988). Classification as Corrosive or Irritant to Skin of Preparations Containing Acidic or Alkaline Substance Without Testing on Animals. Toxicol. *In Vitro*, 2, 19 - 26.

(5) Neun, D.J. (1993). Effects of Alkalinity on the Eye Irritation Potential of Solutions Prepared at a Single pH. J. Toxicol. Cut. Ocular Toxicol. 12, 227 - 231.

(6) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Edsail, D.J., Holzhutter, H.G. and Liebsch, M. (1998). The ECVAM international validation study on in vitro tests for skin corrosivity. 2. Results and evaluation by the Management Team. Toxicology in Vitro 12, pp.483 – 524.

(7) EU (2000). Official Journal of The European Communities L136/91 of 8 June 2000, Method B.40 Skin Corrosion.

(8) OECD (2000). Test Guideline 404. Acute Dermal Irritation/Corrosion.

(9) OECD (1996). OECD Test Guidelines Programme: Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods. Held in Solna, Sweden, 22 - 24 January 1996 (http://www.oecd.org/ehs/test/background.htm).

(10) OECD (1998). Harmonized 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, November 1998 (http://www.oecd.org/ehs/Class/HCL6.htm).

(11) OECD (2000). Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. OECD Environmental Health and Safety Publications. Series on Testing and Assessment No. 19 (http://www.oecd.org/ehs/test/monos.htm).

(12) Wright EM, Marcella KL, Woodson JF. 1985. Animal pain: evaluation and control. Lab Animal. May/June:20-36.

(13) National Research Council (NRC). 2008. Recognition and Alleviation of Distress in Laboratory Animals. Washington, DC:The National Academies Press.

(14) National Research Council (NRC). 2009. Recognition and Alleviation of Pain in Laboratory Animals. Washington, DC:The National Academies Press.

(15) ICCVAM. 2009. Independent Scientific Peer Review Panel Report. Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf.

TABLE: GRADING OF OCULAR LESIONS

Cornea

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. Eye irritation is the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.

2. Eye corrosion is the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.