Eliminating Pain and Distress in Ocular Safety Testing: Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints

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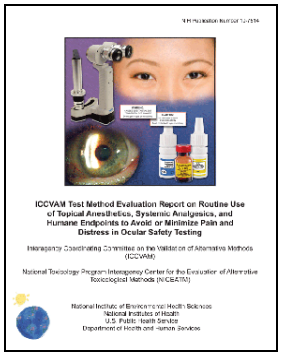
Introduction

- U.S. Public Health Service Policy (PHS 2002) and U.S. Department of Agriculture (USDA 2008) regulations on pain and distress in laboratory animals state that more than momentary or slight pain and distress:
  - Must be limited to that which is unavoidable for the conduct of scientifically valuable research or testing
  - Must be conducted with appropriate pain relief medication unless justified in writing by the principal investigator
  - Will continue for only the necessary amount of time
  - Animals suffering severe or chronic pain or distress that cannot be relieved should be humanely killed after or, if appropriate, during the procedure.
- Current U.S. and international test guidelines for the rabbit eye test allow for the use of topical anesthetics, but only when the user demonstrates that such pretreatments do not interfere with the test results (EPA 1998; OECD 2002).
  - In contrast, the U.S. Consumer Product Safety Commission (CPSC) has recommended preapplication of tetracaine ophthalmic anesthetic for all rabbit eye testing for over 25 years (CPSC 1984).

ICCVAM Evaluation of Topical Anesthetic, Systemic Analgesics, and Humane Endpoints

- ICCVAM evaluated the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during ocular safety testing.
  - ECVAM, Health Canada, and JaCVAM participated in the evaluation and peer review process.
- The ICCVAM evaluation process included scientific peer review by an international independent panel, review by the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and multiple public commenting opportunities.
- ICCVAM recommendations were published in September 2010 (ICCVAM 2010).



- ICCVAM recommendations were accepted in March 2011 by U.S. Federal agencies.

Background: Routine Use of Topical Anesthetics and Systemic Analgesics

- In 2005, ICCVAM, NICEATM, and ECVAM organized an international symposium titled “Minimizing Pain and Distress in Ocular Toxicity Testing”, which discussed the use of topical anesthetics and systemic analgesics during the conduct of the rabbit eye test.
  - Scientific experts at the workshop recommended:
    - Routine pretreatment with topical anesthetics and systemic analgesics, and
    - Treatment with systemic analgesics when ocular lesions associated with painful conditions and/or clinical signs of pain and distress are observed
- NICEATM also evaluated the effects of pretreatment with tetracaine hydrochloride (0.5% w/v) on the ocular irritancy potential of 97 formulations (ICCVAM 2010).
- Topical anesthetic pretreatment had little or no impact on:
  - Hazard classification severity category
  - Nature of the ocular irritation responses
  - Number of days for ocular lesions to clear
- An independent international scientific peer review panel reviewed all available relevant information and data, and recommended the routine use of topical anesthetics and systemic analgesics to prevent and minimize pain and distress during *in vivo* ocular safety testing (ICCVAM 2009).

Options for Pain Relief in Animals

Topical Anesthetics

- The two most commonly used topical ocular anesthetics are proparacaine and tetracaine (Wilson 1990; Bartfield et al. 1994).
- Although a range of onset times have been reported, proparacaine typically provides fast and effective anesthesia within 30 seconds following administration of a single dose. In contrast, tetracaine reportedly exhibits a slower onset of action of approximately 5-10 minutes (Bryant 1969; Bartfield et al. 1994; CPSC Report B [date unknown]; Webb 2009).
- For studies where both anesthetics were evaluated, tetracaine generally provided a longer duration of action than proparacaine (Bryant 1969; Nomura et al. 2001; Webb 2009).
- For tetracaine, animals are treated with two applications (10-15 minutes apart) prior to instilling the product to the eye (CPSC 1984). For proparacaine, animals are treated with one application prior to instilling the product in the eye.

Systemic Analgesics

- Buprenorphine has been effective in managing pain in rabbits and other small animals (Roughan and Flecknell 2002; Sawyer 2008).
  - Wide safety margin in rabbits, causes minimal sedation, and provides a long duration of analgesia (6–12 hours) (Flecknell 1984; Flecknell and Liles 1992; Roughan and Flecknell 2002)
  - Recommended dose range in rabbits is 0.01–0.05 mg/kg (Flecknell 1984, 1995; Flecknell and Liles 1990; Dobromylskyj et al. 2006)
- Meloxicam has been used for postoperative or chronic pain in human and veterinary medicine for over 10 years (Akarsu et al. 2004; Aoki et al. 2006).
  - Its effectiveness has been demonstrated in rabbits (Sawyer 2008; Cooper et al. 2009).
- A well-tested approach to balanced analgesia is to use an opioid (e.g., buprenorphine) in combination with a nonsteroidal anti-inflammatory drug such as meloxicam (Roughan and Flecknell 2002; Sawyer 2008; Cooper et al. 2009).

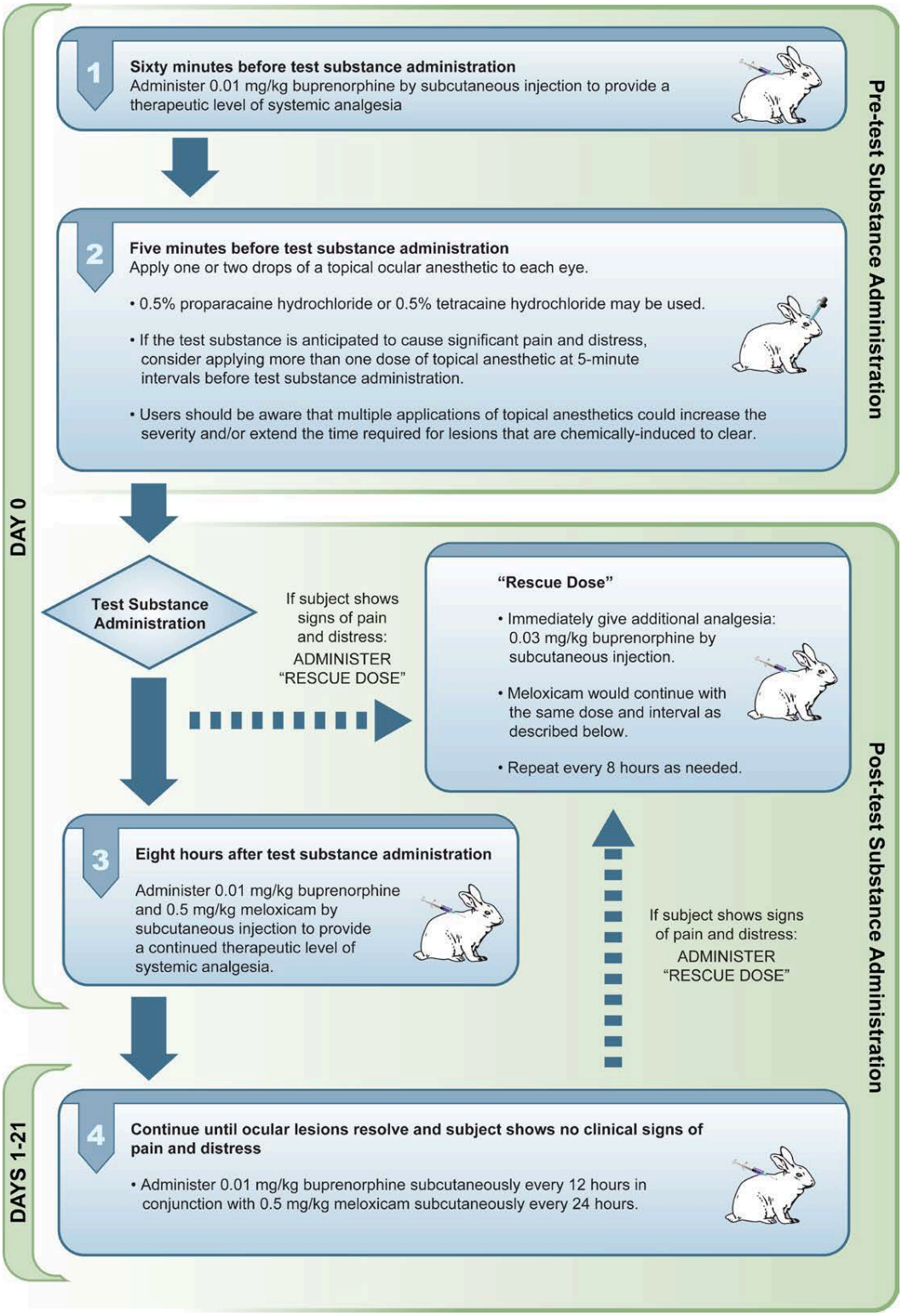
ICCVAM Recommendations for Routine Use of Topical Anesthetics and Systemic Analgesics

- ICCVAM concluded that preemptive pain management procedures should always be used to avoid or minimize pain and distress when it is necessary to conduct the rabbit eye test for regulatory safety purposes.
- This pain management should include:
    1. Pretreatment with a topical anesthetic and systemic analgesic prior to test substance administration
    2. Routine post-treatment with systemic analgesics, with additional treatments as necessary
    3. Scheduled observation, monitoring, and recording of animals for clinical signs of pain and distress
    4. Scheduled observation, monitoring, and recording of the nature, severity, and progression of all eye injuries
- Alternative pain management procedures may also be considered that provide as good or better analgesia and anesthesia than the recommended pain management plan.

ICCVAM Recommendations for Pain Management Procedures

- Pre-test substance administration, day 0
  - 60 minutes before test substance administration:
    - Administer 0.01 mg/kg buprenorphine subcutaneously to provide a therapeutic level of systemic analgesia
  - 5 minutes before test substance administration:
    - Apply one or two drops of a topical ocular anesthetic to each eye (e.g., 0.5% proparacaine hydrochloride or 0.5% tetracaine hydrochloride)
- Post-test substance administration, day 0
  - Additional pain management via systemic analgesics only; no topical drugs
  - If an animal shows signs of pain and distress post-test substance administration:
    - Administer rescue dose (i.e., 0.03 mg/kg buprenorphine and 0.5 mg/kg meloxicam subcutaneously)
  - 8 hours after test substance administration:
    - 0.01 mg/kg buprenorphine and 0.5 mg/kg meloxicam subcutaneously to provide a continued therapeutic level of systemic analgesia
- Days 1-21
  - Continue pain management with systemic analgesics until ocular lesions resolve and subject shows no clinical signs of pain and distress:
    - 0.01 mg/kg buprenorphine subcutaneously every 12 hours
    - 0.5 mg/kg meloxicam subcutaneously every 24 hours

ICCVAM-Recommended Pain Management Procedures for *In Vivo* Ocular Safety Testing



ICCVAM Recommendations for Future Studies: Routine Use of Topical Anesthetics and Systemic Analgesics

- Detailed ocular injury and pain response data should be collected during required regulatory testing and evaluated to assess the adequacy of the recommended pain management procedures.
- Where possible, eyes should be collected for histopathology to more thoroughly evaluate depth and area of ocular damage, as well as to provide a reference against which to compare effects produced *in vitro*.
- Digital photographs of observed lesions should be collected for reference and to provide a permanent record of the extent of ocular damage.
- Studies should be conducted to investigate topical anesthetics and systemic analgesics that might provide longer duration of action or other advantages.
- Users are encouraged to provide data generated using the recommended pain management procedures to validation organizations in order to further characterize the usefulness and limitations of such procedures for avoiding or minimizing pain and distress in ocular safety testing.

Background: Use of Humane Endpoints

- Humane endpoints are criteria that can be used as the basis for ending a test procedure early in order to avoid further pain and distress, or ideally, criteria that can be used to end a procedure before the onset of animal pain and distress.
- The following ocular lesions are considered predictive of severe irritant or corrosive injuries, are not expected to fully reverse by the end of the 21-day observation period after treatment, and can be used as earlier endpoints to terminate a study (OECD 2002):
  - Corneal opacity score of 4 (i.e., opaque cornea, iris not discernable through the opacity) that persists for 48 hours
  - Corneal perforation or significant corneal ulceration
  - Blood in the anterior chamber of the eye
  - Absence of light reflex (i.e., iris score of 2) that persists for 72 hours
  - Ulceration of the conjunctival membrane
  - Necrosis of the conjunctiva or nictitating membrane
  - Sloughing (separation of necrotic tissue from the living structure)
- During the 2005 symposium “Minimizing Pain and Distress in Ocular Toxicity Testing,” panelists recommended early adverse responses that could serve as humane endpoints to terminate a study including:
  - Endpoints currently accepted for study termination (OECD 2002; see above)
  - Vascularization of the corneal surface (i.e., pannus)
  - No diminishment in area of fluorescein staining and/or increase in depth of injury increased over time
  - Lack of re-epithelialization 5 days after application of the test substance
  - Depth of injury to the cornea (routinely using slit-lamp and fluorescein staining) in which corneal ulceration extends beyond superficial layers of the stroma
- An independent international scientific peer review panel reviewed all available relevant information and data, and concluded that the current and proposed (see below) humane endpoints should be routinely used (ICCVAM 2009).

ICCVAM Recommendations for Use of Humane Endpoints

- In addition to those endpoints currently accepted for study termination (OECD 2002; see above) ICCVAM recommends that the following additional ocular lesions can be used as humane endpoints to terminate studies before the end of the scheduled 21-day observation period:
  - Severe depth of injury (e.g., corneal ulceration extending beyond the superficial layers)
  - Destruction of more than 50% of the limbus, as evidenced by blanching of the conjunctival tissue
  - Severe eye infection (i.e., purulent discharge)
- While they should not be used alone, a combination of the following additional endpoints may be useful in clinical decisions on study termination:
  - Vascularization of the cornea surface (i.e., pannus)
  - Area of fluorescein staining not diminishing over time based on daily assessment
  - Lack of re-epithelialization 5 days after test substance application

ICCVAM Recommendations for Future Studies: Use of Humane Endpoints

Fluorescein Staining

- Wound healing data should be collected to help identify additional criteria that may be useful as earlier humane endpoints to terminate studies.
- Guidelines should be developed for (1) the frequency of fluorescein staining that can be conducted without impacting wound healing or hazard classification and (2) the progression/regression of fluorescein staining on area/intensity for identifying specific hazard classification categories based on new studies.

Other Recommendations

- Data should be collected during current testing that may help support the identification of other earlier endpoints (e.g., pannus).
- Improved guidance on clinical signs of pain and distress should be developed; routine pain assessment training should be provided to relevant personnel.
- Users are encouraged to provide validation organizations with data and observations from ocular safety testing to (1) further characterize the usefulness and limitations of proposed humane endpoints, and (2) identify potential new endpoints.

ICCVAM Recommendations for Changes to Ocular Safety Testing Protocols

- When conducting ocular safety testing for those regulatory authorities still requiring the use of live animals, testing should be conducted using the ICCVAM-recommended modifications to the current rabbit eye test (EPA 1998; OECD 2002).
- Comprehensive evaluations for the presence or absence of ocular lesions should be conducted one hour after test substance administration, 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 distress at least twice daily with a minimum of 6 hours between observations, or more often if necessary. Examples of relevant clinical signs include (Wright et al. 1985; NRC 2008, 2009):
  - Repeated pawing or rubbing of the eye
  - Excessive blinking
  - Excessive tearing
- Study termination based on humane endpoints should ensure that reversal is not expected and that no further useful information can be obtained from the study.
- A written record of all observations should be kept for determinations on the progression or resolution of ocular lesions.
- A slit-lamp biomicroscope should be used when considered appropriate (e.g., assessing depth of injury when corneal ulceration is present).
- Digital photographs should be taken to document ocular lesions and help assess their severity, progression, and resolution.

Conclusions

- ICCVAM recommends that preemptive pain management procedures should always be used to avoid or minimize pain and distress when it is necessary to conduct the rabbit eye test for regulatory safety purposes.
- ICCVAM also recommends additional clinical signs and ocular lesions that are considered predictive of an ocular corrosive or severe irritant response and, therefore, can be routinely used as humane endpoints to end studies early when deemed appropriate.
- Adoption and implementation of these recommendations will effectively eliminate animal pain and distress for most ocular safety testing, thereby refining animal use for this purpose.
- A proposal to update OECD TG 405 (Acute Eye Irritation/Corrosion, OECD 2002) with the ICCVAM-recommended pain management plan is currently being considered by OECD member countries.

References

References for all citations in this poster can be found in:

ICCVAM. 2010. Test Method Evaluation Report: Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing. Available at: http://iccvam.niehs.nih.gov/methods/ocutox/OcuAnest-TMER.htm

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