

新規試験法提案書

改訂 OECD TG No.405：ウサギを用いる眼刺激性試験法

平成26年 1 月

国立医薬品食品衛生研究所

新規試験法提案書

平成 26 年 1 月 20 日

No. 2013-04

改訂 OECD TG No.405：ウサギを用いる眼刺激性試験法 に関する提案

平成 25 年 10 月 21 日に東京、国立医薬品食品衛生研究所にて開催された新規試験法評価会議（通称：JaCVAM 評価会議）において以下の提案がなされた。

提案内容： ウサギを用いる眼刺激性試験法は、麻酔薬の使用および人道的エンドポイントの設定等を行っても、評価結果が基本的には変わらないことから、行政上での利用の可能性は、従来試験法の場合と同様である。

この提案書は、OECD (Organisation for Economic Co-operation and Development) Revised Test Guideline No. 405 および ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) Test Method Evaluation Report: Recommendations for Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing をもとに、眼刺激性試験評価委員会によりまとめられた文書を用いて JaCVAM 評価会議が評価および検討した結果、その有用性が確認されたことから作成された。

以上の理由により、行政当局の安全性評価方法として「改訂 OECD TG No.405：ウサギを用いる眼刺激性試験法」の使用を提案するものである。

吉田武美 

JaCVAM 評価会議 議長

西川秋佳 

JaCVAM 運営委員会 委員長

JaCVAM 評価会議

- 吉田武美 (日本毒性学会) : 座長
浅野哲秀 (日本環境変異原学会)
五十嵐良明 (国立医薬品食品衛生研究所 生活衛生化学部)
一鬼 勉 (日本化学工業協会) *
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黒澤 努 (日本動物実験代替法学会)
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牧 栄二 (日本免疫毒性学会)
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吉村 功 (座長推薦)
渡部一人 (日本製薬工業協会)

任期：平成 24 年 4 月 1 日～平成 26 年 3 月 31 日

*: 平成 25 年 4 月 1 日～平成 26 年 3 月 31 日

JaCVAM 運営委員会

- 西川秋佳 (国立医薬品食品衛生研究所 安全性生物試験研究センター) : 委員長
川西 徹 (国立医薬品食品衛生研究所)
小川久美子 (国立医薬品食品衛生研究所 安全性生物試験研究センター 病理部)
菅野 純 (国立医薬品食品衛生研究所 安全性生物試験研究センター 毒性部)
倉持憲路 (厚生労働省 医薬食品局 化学物質安全対策室) *
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高木篤也 (国立医薬品食品衛生研究所 安全性生物試験研究センター 毒性部
動物管理室)
広瀬明彦 (国立医薬品食品衛生研究所 安全性生物試験研究センター 総合評価研
究室)
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光岡俊成 (厚生労働省 医薬食品局 審査管理課)
山本順二 (厚生労働省 医薬食品局 化学物質安全対策室) *
小島 肇 (国立医薬品食品衛生研究所 安全性生物試験研究センター 薬理部
新規試験表評価室) : 事務局

* : 平成 25 年 8 月 1 日より

**JaCVAM statement
on revised Test Guideline No. 405: Acute Eye Irritation/Corrosion**

At the meeting concerning the above method, held on 21 October 2013 at the National Institute of Health Sciences (NIHS), Tokyo, Japan, the members of the Japanese Center for the Validation of Alternative Methods (JaCVAM) Regulatory Acceptance Board unanimously endorsed the following statement:

The Acute Eye Irritation/Corrosion revised by OECD (Organisation for Economic Co-operation and Development) in 2012 is considered to be able to predict eye irritating substances as it always has been in the past by the use of anesthetics.

Following the review of the results of OECD Revised Test Guideline No. 405 and ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) Test Method Evaluation Report: Recommendations for Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing, it is concluded that revised Acute Eye Irritation/Corrosion such as irritation testing are clearly beneficial.

The JaCVAM Regulatory Acceptance Board has been regularly kept informed of the progress of the study, and this endorsement is based on an assessment of various documents, including, in particular, the evaluation report prepared by the JaCVAM ad hoc peer review panel for eye irritation testing.



Takemi Yoshida
Chairperson
JaCVAM Regulatory Acceptance Board



Akiyoshi Nishikawa
Chairperson
JaCVAM Steering Committee

20 January, 2014

The JaCVAM Regulatory Acceptance Board was established by the JaCVAM Steering Committee, and is composed of nominees from the industry and academia.

This statement was endorsed by the following members of the JaCVAM Regulatory Acceptance Board:

Mr. Takemi Yoshida (Japanese Society of Toxicology): Chairperson
Mr. Norihide Asano (Japanese Environmental Mutagen Society)
Mr. Tsutomu Ichiki (Japan Chemical Industry Association)*
Mr. Yoshiaki Ikarashi (National Institute of Health Sciences: NIHS)
Mr. Tsutomu Miki Kurosawa (Japanese Society for Animal Experimentation)
Mr. Eiji Maki (Japanese Society of Immunotoxicology)
Mr. Mitsuteru Masuda (nominee by Chairperson)
Mr. Akiyoshi Nishikawa (NIHS)
Mr. Yasuo Ohno (nominee by Chairperson)*
Mr. Hiroshi Onodera (Pharmaceuticals and Medical Devices Agency)
Ms. Mariko Sugiyama (Japan Cosmetic Industry Association)
Ms. Tomoko Tanita (Pharmaceuticals and Medical Devices Agency)*
Mr. Takashi Yamada (National Institute of Technology and Evaluation)*
Mr. Hiroo Yokozeki (Japanese Society for Dermatoallergology and Contact Dermatitis)
Ms. Midori Yoshida (NIHS)
Mr. Isao Yoshimura (nominee by Chairperson)
Mr. Kazuto Watanabe (Japan Pharmaceutical Manufacturers Association)

Term: From 1st April 2012 to 31st March 2014

*: From 1st April 2013 to 31st March 2014

This statement was endorsed by the following members of the JaCVAM steering Committee after receiving the report from JaCVAM Regulatory Acceptance Board:

Mr. Akiyoshi Nishikawa (BSRC, NIHS): Chairperson
Mr. Akihiko Hirose (Division of Risk Assessment, BSRC, NIHS)
Mr. Masamitsu Honma (Division of Genetics and Mutagenesis, BSRC, NIHS)
Mr. Jun Kanno (Division of Cellular and Molecular Toxicology, BSRC, NIHS)
Mr. Toru Kawanishi (NIHS)
Mr. Kenji Kuramochi (Ministry of Health, Labour and Welfare)*
Mr. Toshinari Mitsuoka (Ministry of Health, Labour and Welfare)
Ms. Kumiko Ogawa (Division of Pathology, BSRC, NIHS)
Mr. Kazuyuki Saito (Pharmaceutical & Medical Devices Agency)
Mr. Masahiro Sasaki (Ministry of Health, Labour and Welfare)
Ms. Yuko Sekino (Division of Pharmacology, BSRC, NIHS)
Mr. Atsuya Takagi (Animal Management Section of the Division of Cellular and Molecular Toxicology, BSRC, NIHS)
Mr. Junji Yamamoto (Ministry of Health, Labour and Welfare)*
Mr. Hajime Kojima (Section for the Evaluation of Novel Methods, Division of Pharmacology, BSRC, NIHS): Secretary

* Arrival at post day: 1st August 2013

改訂 OECD TG No.405 : ウサギを用いる眼刺激性試験法

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経済協力開発機構（OECD）のウサギを用いた眼刺激性試験改訂ガイドライン（TG405）
の評価会議報告書

JaCVAM 評価会議

平成 25 年 10 月 21 日

JaCVAM 評価会議

- 吉田武美 (日本毒性学会) : 座長
浅野哲秀 (日本環境変異原学会)
五十嵐良明 (国立医薬品食品衛生研究所 生活衛生化学部)
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以上

経済協力開発機構（OECD）のウサギを用いた眼刺激性試験改訂ガイドライン（TG405, 2012年10月2日改訂）については眼刺激性評価委員会からの報告を受け、以下の10項目について評価したので報告する。

本ガイドラインは過去にも動物福祉の観点から改訂が行われているが、今回の改訂で動物福祉の更なる充実が図られた。ウサギを用いた眼刺激性試験における動物の痛みと苦痛を軽減するために、局所麻酔薬及び全身的鎮痛薬による定常的処置、痛みと苦痛症状及びすべての眼傷害の観察についての具体的方法、並びに、人道的エンドポイントの設定とその判断の際の実験動物獣医師などの関与が規定された。

<審議内容>

1. 当該試験法は、どのような従来試験法を代替するものか。または、どのような毒性を評価あるいは予測するものか。

これまで OECD が眼刺激試験（急性刺激性・腐食性試験）ガイドラインとして公表していたドレイズ試験を代替するものである。評価あるいは予測する毒性は、従来の OECDTG405 と同様である。

2. 当該試験法と従来試験法の間にはどのような科学的なつながりがあるか。

従来試験法における動物福祉への対処として、動物の痛みと苦痛をより軽減する試験方法が規定されている。例えば、被験物質投与前の局所麻酔薬、全身性鎮痛薬の投与、投与後の全身性鎮痛薬の投与、動物の痛みと苦痛の症状観察と試験からの除外、ならびに人道的エンドポイントの設定である。

3. 当該試験法とそのデータは、透明で独立な科学的評価を受けているか。

米国 ICCVAM の国際的な科学的ピアレビュー委員会（International Scientific Peer Review Panel）において科学的評価を受けている。

4. 当該試験法は、従来試験法の代替法として、どのような物質又は製品を評価することを目的としているか。

従来試験法で想定されていた物質又は製品を評価することを目的としている。評価対象の物質又は製品は、従来の OECDTG405 と同様である。

5. 当該試験法は、ハザード評価あるいはリスク評価のどちらに有用であるか。

従来試験法と同様、ハザード評価に有用であり、被験物質の濃度を調整することによりリスク評価にも使用可能である。

6. 当該試験法は、目的とする物質又は製品の毒性を評価できるか。その場合、当該試験法の適用条件が明確になっているか。

従来試験法と同様、目的とする物質又は製品の毒性（眼刺激性）を評価可能であり、適用条件も明確である。

7. 当該試験法はプロトコルの微細な変更に対して頑健であるか。

プロトコルの微細な変更に対しての頑健性は、従来試験法と同様である。

8. 当該試験法の技術習得は、適切な訓練と経験を経ている担当者にとって容易なものであるか。試験法の実施に特殊な設備が必要か。

技術習得は従来試験法を経験している担当者にとって容易である。試験法の実施に局所麻酔薬、全身性鎮痛薬の投与が必要であり、例示されている薬剤はいずれも医薬品としての使用が想定されており、実験動物獣医師の関与が必要となる。それ以外の特殊な設備は不要である。

また、試験中に動物に苦痛が生じた場合には選任獣医師、実験動物医学専門医、その他の動物の疾病を熟知している者への相談が記載されていることから、これまで特に必要とはされていない、専門家の関与が必要となる。

9. 当該試験法は、従来試験法と比べて時間的経費的に優れているか。

麻酔薬、鎮痛薬の投与が必要な事から、従来試験法よりも若干の時間的および経費増となる。またこれら医薬品の扱い、ならびに苦痛の判定と試験からの除外に専門家の関与が記載されており、経費は増している。

10. 当該試験法は、動物福祉の観点及び科学的見地から、目的とする物質又は製品の毒性を評価する代替法として、行政上利用することは可能か。

麻酔薬の使用および人道的エンドポイントの設定等を行っても、評価結果が基本的には変わらないことから、行政上での利用の可能性は、従来試験法の場合と同様に可能である。

参考文献

- 1) JaCVAM眼刺激性評価委員会：経済協力開発機構（OECD）改訂ガイドライン（Test Guideline No.405） 改訂ウサギを用いた眼刺激性試験の評価報告書
- 2) OECD Test Guideline, Revised TG405（2012）

経済協力開発機構（OECD）改訂ガイドライン（Test Guideline No.405）
ウサギを用いた眼刺激性試験の評価報告書

2013年10月

眼刺激性試験代替法評価委員会

委員名：

小坂 忠司（残留農薬研究所）

吉村 功（東京理科大学）

山本 直樹（藤田保健衛生大学）

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細井 一弘（参天製薬株式会社）

加藤 雅一（株式会社 J-TEC）

簾内 桃子（国立医薬品食品衛生研究所）

増田 光輝

1. 序 論

OECD試験ガイドラインは、最適の科学技術が反映されるように定期的に見直されてきた。その中で、これまでの改訂では不必要な動物実験を回避するための改善が行われてきた。今回はウサギを用いた急性刺激性・腐食性試験ガイドライン¹⁾について、動物福祉への配慮を目的とした改訂が行われた。

本改訂に先立ち、米国ICCVAMの国際的な科学的ピアレビュー委員会 (International Scientific Peer Review Panel)²⁾ において、局所麻酔薬と全身性鎮痛薬の使用により、試験成績に影響を与えることなく動物の痛みと苦痛を回避できるか、そしてその際の局所麻酔薬と全身性鎮痛薬の処方及び人道的エンドポイントについて議論された。本改訂により、ウサギを用いた眼刺激性試験において、試験成績に影響を与えることなく動物の痛みと苦痛が軽減されると考えられた。

今回の改訂の要点を次に示す。

- 1)被験物質投与前では、局所麻酔薬（例：プロパラカイン、テトラカイン）と全身性鎮痛薬（例：ブプレノルフィン）による定常的前処置。
- 2)被験物質投与後では、全身性鎮痛薬（例：ブプレノルフィン及びメロキシカム）による定常的処置。
- 3)動物の痛みと苦痛の症状の計画観察，モニタリングおよび記録。
- 4)全ての眼傷害の（性質，程度および進行状況）計画観察，モニタリング，記録および人道的エンドポイント設定。

2. ウサギ眼刺激性試験手順

2.1. 供試動物

健康な若齢成熟の白色ウサギを供試動物として使用する。試験開始前24時間以内に供試動物の両眼を検査する。眼の刺激，眼傷害，または角膜損傷が認められた動物は使用しない。

動物は個別飼育する。動物飼育室の温度は $20\pm 3^{\circ}\text{C}$ ，相対湿度は50%～60%とし，照明は12時間明期，12時間暗期とする。

2.2. 局所麻酔薬及び全身性鎮痛薬の適用

眼刺激性試験の実施にあたり，動物の痛み及び苦痛を回避ないし最小限にすることを目的とする。

被験物質投与の60分前に，ブプレノルフィン0.01 mg/kg（鎮痛薬として治療に用いられる用量）を皮下投与する。

被験物質投与5分前に，両眼に局所麻酔薬（例えば，0.5%塩酸プロパラカインあるいは0.5%塩酸テトラカイン）を1，2滴点眼する。また，市販局所麻酔点

眼薬中の防腐剤が試験結果に影響する可能性を回避するため、防腐剤が添加されていない局所麻酔点眼薬の使用を推奨する。対照眼には被験物質は投与せず、局所麻酔薬のみを投与する。被験物質投与が顕著な苦痛や痛みを誘起すると予期される場合には、通常 *in vivo* 試験を実施すべきではない。しかしながら、刺激性が不明な場合や試験実施が必要な場合には、被験物質投与前に5分間隔で局所麻酔薬の追加点眼の実施を考慮すべきである。その際、局所麻酔薬の複数回使用によって眼刺激の程度がわずかに高くなり、回復が遅くなる可能性に留意しなければならない。

被験物質投与8時間後にブプレノルフィン0.01 mg/kg及びメロキシカム0.5 mg/kg を皮下投与する。なお、メロキシカムが1日1回皮下投与で眼に対する抗炎症性作用を示すというデータはないが、被験物質投与の8時間後まではメロキシカムを投与すべきではない。

被験物質投与8時間以降は、眼所見や痛み及び苦痛が消失するまで、ブプレノルフィン0.01 mg/kgを12時間間隔で皮下投与し、メロキシカムを0.5 mg/kg 24時間間隔で皮下投与する。

先行投与の鎮痛薬及び局所麻酔薬の効果が不十分な場合には、被験物質投与直後に鎮痛薬の投与を実施する。試験期間中に動物が痛みや苦痛を示した際には直ちに追加の鎮痛薬（緊急用量として0.03 mg/kg ブプレノルフィン、皮下投与）を投与し、必要に応じて8時間毎に緊急用量を投与する。0.5 mg/kg のメロキシカムは緊急用量のブプレノルフィンと併用し、24時間間隔で皮下投与する。

2.3. 被験物質の適用

下眼瞼を穏やかに引き、各動物の片眼の結膜囊内に被験物質を投与する。被験物質の流失を防ぐため、約1秒間上下の眼瞼を静かに合わせ、閉じたままにしておく。

2.3.1. 洗浄

被験物質が固体の場合及び投与直後に刺激性・腐食性作用を示す場合を除いては、被験物質投与後少なくとも24時間は被験動物の眼を洗浄しない。24時間後に適切と考えられる場合は、洗浄してもよい。

科学的に妥当性がない限り、洗浄効果を調査するための衛星群の使用は推奨できない。もし、衛星群が必要な場合は2例のウサギを使用する。洗浄の条件、例えば洗浄時間、洗浄液の組成、温度、使用時間、量及び速度などを記録する。

2.3.2. 用量

(1) 液体の試験

液体の場合は、投与量は0.1 mL とする。被験物質を眼へ投与するために、ポンプスプレーは使用しない。液体のスプレー用検体では予め容器に試料を採取し、そこから0.1 mLを採取し、眼に滴下する。

(2) 固体の試験

固体、ペースト及び粒子状物質の場合は、投与量は容量として0.1 mL、あるいは重量で100 mg以下の量とする。被験物質は摩砕して微粉末化する。固体物質の容量は容器を軽くたたいて詰めた後に測定する。固体物質が投与後1時間の観察時点で生理的機構によって眼から除去されていない場合には、眼を生理食塩液または蒸留水で洗浄してもよい。

(3) エアゾールの試験

ポンプスプレー及びエアゾール製品の場合は、予め内容物を採取し眼に適用することが推奨される。ただし、内容物が気化するために加圧エアゾール容器に入れられている被験物質の場合は例外であり、その場合は眼を開き、眼の直前10 cm の距離から約1秒間単回噴射して被験物質を眼に適用する。この距離は、スプレーの射出圧力及びその含量に応じて変化してもよい。スプレーの射出圧力で眼を損傷しないように注意する。エアゾールからの投与量は、以下のシミュレーションによって概算の投与量を推定する。被験物質を秤量用紙の直前に置いたウサギの眼のサイズの穴を通してスプレーし、秤量用紙の重量増加から眼にスプレーされる量を概算する。揮発性物質の投与量については、被験物質をスプレーする前と後の容器重量を秤量することにより推定する。

2.4. 初回試験（動物1匹を用いる *in vivo* 眼刺激性・腐食性試験）

段階的試験戦略として、*in vivo*試験では、まず1匹の動物を用いて実施する。確認試験に移る前に、刺激性の重度と可逆性の有無を観察する。この観察結果により、被験物質が眼に対して腐食性または強い刺激性を有すると判断される場合には、確認試験は実施しない。

2.5. 確認試験（追加動物を用いる *in vivo* 眼刺激性試験）

初回試験において腐食性ないし強い刺激性作用が観察されない場合は、1匹ないし2匹の追加動物で刺激反応の有無を確認する。先の試験で中等度の刺激性が認められた場合は、2匹の追加動物を同時に投与するよりも、1匹ずつで確認試験を段階的に実施することが推奨される。そして2匹目の動物において、腐食性または強い刺激性作用が認められた場合は試験を継続しない。また2匹目の動物の結果でハザード分類に十分と判断される場合、その後の試験を継続しない。

2.6. 観察期間

観察期間としては、観察された作用の程度及び可逆性を十分に評価できる期間とする。しかし、動物が重度の苦痛または障害を示した場合は、その時点で実験を終了する。作用の可逆性を決定するには、被験物質投与後、通常21日間観察する。可逆性であることが21日以前に確認できた場合は、実験をその時点で終了する。

2.7. 臨床観察及び眼刺激性スコア（評点）

投与後1時間及びその後数日間毎日観察し、眼の損傷の有無を包括的に評価する。投与後3日間は1日に数回観察し、試験中止時期を適宜決定する。試験動物は試験期間中定期的に苦痛及び痛み（例；触れるか擦る、過度の瞬きをする、過度の流涙など）を少なくとも1日2回、6時間以上の間隔で観察し、また必要に応じてそれ以上の頻度で観察する。フルオレセイン染色は定期的に行うべきであり、必要と考えられた場合は細隙灯生体顕微鏡を使用し、眼の損傷域の検出及び計測を適切に判断する（例；角膜潰瘍が認められる時の損傷の深さの検出）。またこれら方法は人道的安楽殺処分のための制定されたエンドポイント要件を満たすかの評価にも使用される。眼のダメージの範囲を恒久的に記録する目的で、参考資料として傷害部位をデジタルカメラも用いて撮影する。確実なデータが得られたのちは、不必要に長く動物を維持してはならない。動物が重篤な苦痛を示した場合は遅滞なく人道的安楽殺処分しなければならず、被験物質の評価は以下のように行う。

投与後、次のような眼傷害を起こした動物は安楽殺を実施する。角膜の穿孔やぶどう腫（Staphyloma）を含む角膜の重度の潰瘍形成、前眼房の出血、程度4の角膜混濁、72時間継続する対光反射の消失（虹彩反応；程度2）、結膜の潰瘍、結膜または瞬膜の壊死、脱落が挙げられる。このような病変は一般的に不可逆性であることによる。さらに、21日間の観察終了までに次の眼病変が認められた場合には人道的エンドポイントとして試験中止と判断することを推奨する。それらの眼病変は、深い損傷（例：角膜実質層に達する角膜損傷）、角膜輪部の損傷が50%以上（結膜組織の蒼白化により評価）、重度の眼感染（化膿性の分泌物）であり、これらの眼病変は21日間の観察終了時までに完全に回復することが望めない、あるいは腐食性または強い刺激性を示す損傷を引き起こすと考えられるからである。また、角膜表面への血管新生（パンヌス）、フルオレセインによる染色域（毎日の観察によって縮小しない）、あるいは被験物質投与後5日以降に見られる角膜上皮再生の欠落などの複合的所見もまた早期試験終了の観察項目として考えられる。

しかしながら、上記眼所見の1つ1つでは早期試験終了の判断材料として十分

ではない。強度の眼への影響が認められた場合、早期試験終了すべきかどうかの判断のため、選任獣医師あるいは資格のある実験動物獣医師、あるいは臨床兆候を確認できるよう訓練された者に獣医学的試験に関して助言を求めねばならない。眼の刺激性反応（結膜、角膜及び虹彩）は被験物質投与後1, 24, 48及び72時間に採点する。眼の傷害が認められない時は、投与後3日以内に試験を終了させてはならない。軽度から中等度の刺激性が明瞭に認められる場合は投与後21日まで観察し、その時点で試験を終了する。観察の実施及び記録は少なくとも投与後1, 24, 48, 72時間, 7, 14及び21日に損傷の状態を評価して、可逆性か非可逆性かを判断する。試験動物が人道的な配慮から安楽殺処分にされるべきか、否定的な結果により試験から除外するべきかについては、より頻繁な観察によって判断すべきである。

眼刺激性のスコア（評点）を各試験で記録する。その他の眼の所見（例えばパンヌス、染色、前眼房の変化）や全身性の有害作用もまた記録する。反応の検査は、双眼ルーペ、手持ち式細隙灯生体顕微鏡または他の適切な器具の使用によって円滑に実施できる。24時間目の観察を記録後、フルオレセインを用いて眼をさらに検査してもよい。眼刺激性のスコア（評点）は主観的になりやすく眼刺激性反応評価の一致を促進させるため、また試験施設及び実験者が観察の実施及び理解を容易とするため、採点方法について観察者の適切な訓練が必要である。

3. 結果の評価

眼刺激性スコアを採点し、その性質及び重症度、ならびにその可逆性の有無についてGHSの基準に従い評価する。また、個別のスコアは刺激性評価の絶対的な標準とはみなさない。

4. 眼刺激性及び腐食性評価のための段階的試験戦略（試験ガイドライン405の付属文書）

科学的妥当性及び動物福祉の実施を目的として、動物の眼に重度の刺激性を誘起する可能性が高い化学物質の眼刺激性試験を回避あるいは最小限にして、不必要な動物の使用を避けることが重要な問題である。ウサギを用いた*in vivo*試験の実施前に、段階的試験戦略の一部として化学物質の潜在的刺激性・腐食性に関するすべての情報を収集し、評価する必要がある。以下の段階的試験戦略による情報を評価して、強度の刺激性や腐食性が認められる場合には*in vivo*試験を実施すべきではない。

(1) ヒトまたは動物の既存データ及び国際的に承認された方法による*in vitro*

試験データの評価

- (2) 構造活性相関 (SAR) の分析
- (3) 物理化学的特性及び化学反応性 (例えば, 2.0以下または11.5以上の極端な pHを示す物質, 強い局所作用の可能性)
- (4) 上記以外の既存情報 (皮膚経路からの全身毒性についての利用可能なすべての情報)
- (5) 皮膚腐食性の評価 (皮膚腐食性及び強い刺激性影響; OECD試験ガイドライン404あるいは国際的に承認された*in vitro* 皮膚腐食性試験法での評価)
- (6) *in vitro*または*ex vivo*試験からの結果 (国際的に承認された*in vitro*または*ex vivo*試験での腐食性ないし強い刺激性)

5. 眼刺激性評価委員会の見解

本委員会はわが国も本改訂試験ガイドラインを受け入れるべきであると考えた。しかしながら, ガイドラインの適用に際しては次の点を考慮すべきである。

(1) 本試験ガイドラインで例示されている局所麻酔薬はわが国では医薬品として流通していない。それゆえ, わが国で入手可能な局所麻酔薬を用いるべきである。防腐剤を添加していない局所麻酔点眼薬として, 「オキシブプロカイン塩酸塩ミニムス点眼液0.4%「センジュ」(千寿製薬株式会社)」がある。また, 局所麻酔薬原体を用いて, 0.5%塩酸プロパラカインあるいは0.5%塩酸テトラカインを調製する場合には, 麻酔薬に関する情報を熟知した獣医師あるいは薬剤師の指導の下で無菌的に実施する必要がある。

(2) 本ガイドラインに記載されている実験動物獣医師は, 欧米では実験動物福祉を担保するために定義されているが, 我が国の関連法令指針にはその定義がない。それゆえ, 実験動物獣医師と同等な獣医師あるいはそれに準ずる実験動物の生理, 疾患, 症状等に熟知した者が獣医学的試験を行い人道的エンドポイントの判定を行うものとすべきである。

(3) 本ガイドラインに記載されている段階的試験戦略 (上記4項) では, 化学物質の潜在的な眼刺激性・腐食性に関するすべての情報, 皮膚刺激性試験情報及び眼刺激性*in vitro*または*ex vivo*試験情報を収集・評価し, これらの情報及び試験情報により強度の刺激性や腐食性が認められると判断される場合には, ウサギを用いる*in vivo*試験 (OECDガイドラインTG405) を実施すべきではないと推奨している。本眼刺激性評価委員会は, ウサギを用いた眼刺激性試験の実施にあたり上記段階的試験戦略を十分考慮した上で当該眼刺激性試験を実施すべきであると判断した。

6. 文献

- 1) OECD (2012), Test No. 405: Acute Eye Irritation/Corrosion. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
- 2) ICCVAM (2010), ICCVAM Test Method Evaluation Report: Recommendations for Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing, NIH Publication No. 10-7514, Research Triangle Park, NC, USA.

参考資料

用語解説

選任獣医師：試験研究機関において使用されるすべての動物の健康と福祉に対して責任を有する。この責任を遂行するため研究機関から十分な権限と獣医学的ケア計画を運用するための資源（人的、物的、資金的）を与えられている。

実験動物獣医師（**Laboratory Animal Veterinarian**）：我が国では、国際実験動物医学専門医協会に属する日本実験動物医学専門医協会が「実験動物医学専門医」という資格を認定している。これは実験動物の病気、治療、処置に熟知した獣医師である。

臨床兆候を確認できるよう訓練された者：我が国では、獣医師以外に該当する認定資格はない。現段階では、動物看護師統一認定機構が認定した「認定動物看護師」や日本実験動物技術者協会で所定の教育訓練を受けた者などが、これに該当する例として挙げられる。

人道的エンドポイント（**Humane Endpoint**）：動物実験における強い痛み、苦痛、感染症罹患、または瀕死期を判断するための評価指標。

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 that they be developed through application of sequential testing (2) (3). The testing strategy includes the performance of validated and accepted *in vitro* tests and is provided as a Supplement to the Guideline. 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.

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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 (see Supplement to Guideline 405: A Sequential Testing Strategy for Eye Irritation and Corrosion). 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

<u>Cornea</u>	<u>Grade</u>
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	
 <u>Iris</u>	
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	
 <u>Conjunctivae</u>	
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	
 <u>Chemosis</u>	
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 corrosion/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]

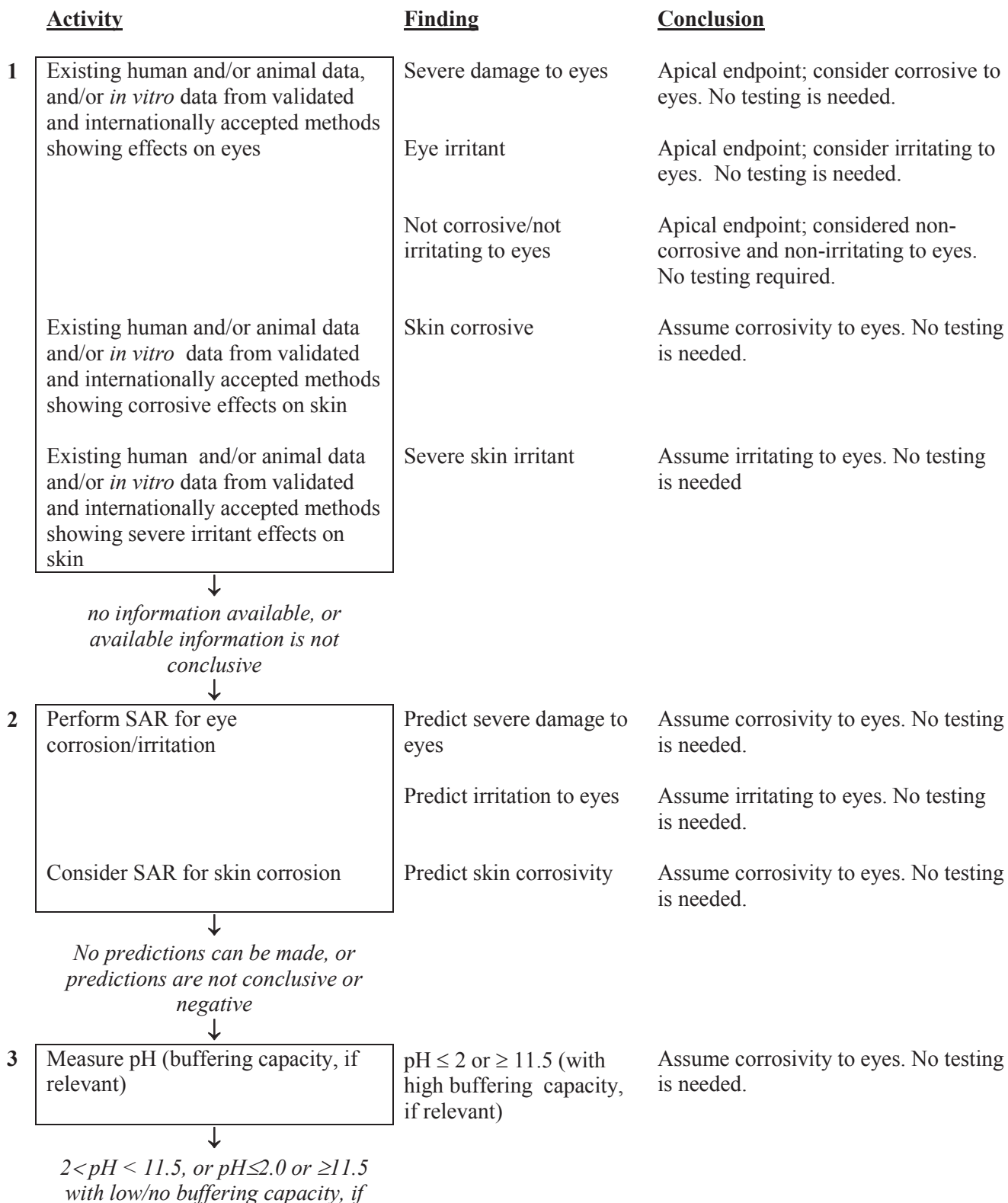
LITERATURE

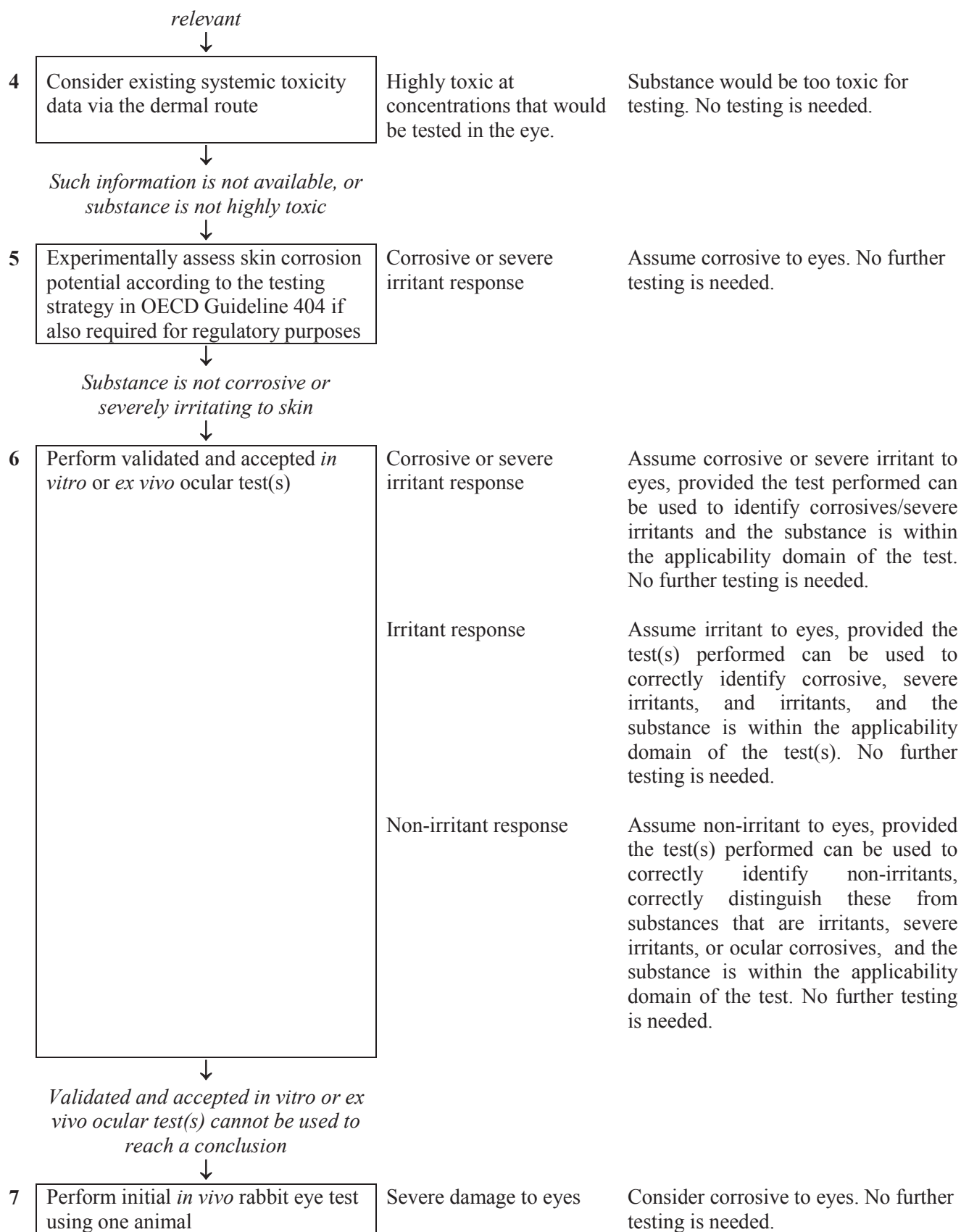
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FIGURE

TESTING AND EVALUATION STRATEGY FOR EYE IRRITATION/CORROSION





↓
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.

**ICCVAM Test Method Evaluation Report:
Recommendations for Routine Use of Topical Anesthetics,
Systemic Analgesics, and Humane Endpoints to Avoid or
Minimize Pain and Distress in Ocular Safety Testing**

**Interagency Coordinating Committee on the
Validation of Alternative Methods**

**National Toxicology Program Interagency Center for the
Evaluation of Alternative Toxicological Methods**

**National Institute of Environmental Health Sciences
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List of Abbreviations and Acronyms

°C	Degrees centigrade
AHT	Animal Health Technologist
BRD	Background review document
CPSC	U.S. Consumer Product Safety Commission
CV	Coefficient of variation
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
ESAC	European Centre for the Validation of Alternative Methods Scientific Advisory Committee
EU	European Union
FDA	U.S. Food and Drug Administration
FR	<i>Federal Register</i>
g	Gram
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practice
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILS	Integrated Laboratory Systems, Inc.
IS	Irritation score
JaCVAM	Japanese Center for the Validation of Alternative Methods
kg	Kilogram
LVET	Low volume eye test
MAS	Maximum average score
MeSH	Medical Subject Headings
mg	Milligram
mL	Milliliter
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NSAID	Nonsteroidal anti-inflammatory drug
NTP	U.S. National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	ICCVAM Ocular Toxicity Working Group
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SC	Subcutaneous
SRD	Summary review document
TG	Test guideline
TSA	Test substance administration
UN	United Nations

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Preface

Eye injury is a leading cause of visual impairment in the United States with 40,000 to 50,000 new cases of impaired vision reported each year.¹ Many eye injuries occur due to contact with workplace or household products or chemicals. Accidents involving common household products (e.g., oven cleaner and bleach) cause about 125,000 eye injuries each year.² These products often result in chemical burns and emergency room visits.³ Each day about 2,000 U.S. workers have a job-related eye injury that requires medical treatment. Although the majority of these eye injuries result from mechanical sources, chemical burns from industrial chemicals or cleaning products are common.⁴

To prevent eye injuries, regulatory agencies require testing to determine if chemicals and products may cause eye damage. This testing information is used to classify the ocular hazard and determine appropriate labeling to warn consumers and workers of the potential hazard. Appropriate labeling tells users how to avoid exposure that could damage the eye and what emergency procedures should be followed if there is accidental exposure. Nearly all ocular safety testing has been conducted using the Draize rabbit eye test, although *in vitro* methods can now be used to identify whether substances cause severe irritation or permanent eye damage. The Draize rabbit eye test (Draize et al. 1944) involves instillation of 0.1 mL of the test substance into the conjunctival sac of one eye. The other eye serves as the untreated control. The eye is examined at least daily for up to 21 days. The presence and severity of any injuries to the cornea, conjunctiva, and the iris (tissues inside the eye) are scored, and the duration that the injuries persist is recorded.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid or minimize pain and distress during ocular safety testing. As a part of this evaluation, ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) requested the submission of data and experience with topical anesthetics and systemic analgesics to alleviate pain and distress in rabbits during eye irritation testing (72 FR 26396).⁵

ICCVAM carefully compiled and assessed all available data and arranged an independent international scientific peer review. ICCVAM and the Ocular Toxicity Working Group (OTWG) solicited and considered public comments and stakeholder involvement throughout the evaluation process. As part of their ongoing collaboration with ICCVAM, scientists from the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) served as liaisons to the OTWG. ICCVAM, NICEATM, and the OTWG prepared (1) a draft background review document (BRD) on the use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during ocular safety testing and (2) draft test method recommendations for their usefulness and limitations. ICCVAM released this document to the public for comment on March 31, 2009. ICCVAM also announced a meeting of the independent international scientific peer review panel (Panel) (74 FR 14556).⁶

The Panel met in public session on May 19–21, 2009, to review the ICCVAM draft BRD for completeness and accuracy. The Panel then evaluated (1) the extent to which the draft BRD addressed established validation and acceptance criteria and (2) the extent to which the draft BRD supported

¹ Available at http://www.preventblindness.org/resources/factsheets/Eye_Injuries_FS93.pdf

² Available at <http://www.geteyesmart.org/eyesmart/injuries/home.cfm>

³ From the CPSC NEISS database, 2007

⁴ Available at <http://www.cdc.gov/niosh/topics/eye/>

⁵ Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_8898.pdf

⁶ Available at <http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-7220.pdf>

ICCVAM's draft test method recommendations. Before concluding their deliberations, the Panel considered written comments and comments made at the meeting by public stakeholders. The Panel prepared a report summarizing their conclusions and recommendations.⁷

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the Topical Anesthetics/Systemic Analgesics/Humane Endpoints draft BRD and draft test method recommendations, the Panel report, and all public comments for discussion at their meeting on June 25–26, 2009, where public stakeholders were given another opportunity to comment. A detailed timeline of the evaluation is included with this report.

ICCVAM solicited and considered public comments and stakeholder involvement throughout the test method evaluation process. ICCVAM considered the SACATM comments, the conclusions of the Panel, and all public comments before finalizing the ICCVAM test method recommendations. The recommendations and the BRD, which is provided as an appendix to this report, are incorporated in this ICCVAM test method evaluation report. As required by the ICCVAM Authorization Act, ICCVAM will forward its recommendations to U.S. Federal regulatory agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving the ICCVAM test method recommendations. ICCVAM recommendations are available to the public on the NICEATM–ICCVAM website, and agency responses will also be made available on the website as they are received.

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⁷ Available at http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPrept2009.pdf

Executive Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid or minimize pain and distress during *in vivo* ocular safety testing. This test method evaluation report provides ICCVAM's recommendations. The report also includes (1) ICCVAM's recommended changes to the protocol for the Draize rabbit eye test and (2) a final background review document (BRD) on the use of topical anesthetics, systemic analgesics, and earlier humane endpoints in the Draize rabbit eye test.

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), ICCVAM, and ICCVAM's Ocular Toxicity Working Group prepared a draft BRD on the use of topical anesthetics, systemic analgesics, and earlier humane endpoints to minimize pain and distress in ocular safety testing. The BRD is based upon published studies and forms the basis for the draft ICCVAM test method recommendations. NICEATM provided the draft BRD and ICCVAM recommendations to an independent international scientific peer review panel (Panel) and the public for comment. A detailed timeline of the ICCVAM evaluation process is appended to this report.

The Panel met in public session on May 19–21, 2009, to discuss its review of the ICCVAM draft BRD and to provide conclusions and recommendations on these proposed changes to the Draize rabbit eye test protocol. The Panel also reviewed how well the information in the draft BRD supported ICCVAM's draft test method recommendations. In finalizing this test method evaluation report and the BRD, which is included as an appendix, ICCVAM considered (1) the conclusions and recommendations of the Panel, (2) comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods, and (3) public comments.

Routine Use of Topical Anesthetics and Systemic Analgesics in the Draize Rabbit Eye Test

Specific ICCVAM Test Method Recommendations

Balanced preemptive pain management should be provided whenever the Draize rabbit eye test is conducted for regulatory safety testing. Pain management should include (1) treating the animals with a topical anesthetic and a systemic analgesic before applying test substances; (2) following a routine schedule of systemic analgesia after applying test substances; (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. ICCVAM further recommends that ocular safety testing protocols include a pain management procedure and schedule.

Changes to Ocular Safety Testing Protocol to Include the Routine Use of Topical Anesthetics and Systemic Analgesics

When required for regulatory safety assessment of potential ocular hazards (EPA 1998; OECD 2002), the current Draize rabbit eye test should be conducted with the following changes unless pain response monitoring is required (e.g., pharmaceutical tolerability testing). Alternative pain management procedures may be considered if they provide analgesia and anesthesia as good or better than the following pain management procedure:

- Sixty minutes before test substance application (TSA), provide a therapeutic level of systemic analgesia by administering 0.01 mg/kg buprenorphine by subcutaneous injection.
- Five minutes before applying the test substance, apply one or two drops of a topical ocular anesthetic (e.g., 0.5% proparacaine hydrochloride or 0.5% tetracaine)

hydrochloride) to each eye. For each animal, the eye that is treated with topical anesthetics and no test substance will serve as a control. If the test substance is anticipated to cause significant pain and distress, consider applying more than one dose of topical anesthetic at 5-minute intervals before TSA. Be aware that multiple applications of topical anesthetics could increase the severity of chemically induced lesions and/or extend the time required for them to heal.

- If a test subject shows signs of pain and distress during the test interval, immediately give additional analgesia (i.e., a “rescue” dose of 0.03 mg/kg subcutaneous buprenorphine). Repeat 0.03 mg/kg buprenorphine every 8 hours (+/- 30 minutes) instead of 0.01 mg/kg subcutaneously every 12 hours. Continue meloxicam with the same dose and interval described below. If preemptive analgesia is inadequate, give the “rescue” analgesia immediately after TSA.
- Eight hours (+/- 30 minutes) after TSA, administer 0.01 mg/kg buprenorphine and 0.5 mg/kg meloxicam subcutaneously to provide a continued therapeutic level of systemic analgesia.
- If ocular lesions and/or clinical signs of pain and distress are present following the buprenorphine and meloxicam treatment that was administered 8 hours after TSA, continue to administer 0.01 mg/kg buprenorphine subcutaneously every 12 hours (+/- 30 minutes) in conjunction with 0.5 mg/kg meloxicam subcutaneously every 24 hours. If the “rescue dose” described above is needed, administer buprenorphine at 0.03 mg/kg every 8 hours instead of 0.01 mg/kg every 12 hours.

Future Studies on the Routine Use of Topical Anesthetics and Systemic Analgesics

ICCVAM recommends routinely observing and recording lesions and clinical signs during ocular safety studies in order to evaluate the effectiveness of pain management and to determine if the enhanced “rescue” analgesia procedure should be implemented. These data should be reviewed to determine whether adjustments are needed to (1) improve the effectiveness of analgesia before and after treatment and (2) optimize dosages and treatment intervals. Data should be analyzed periodically to determine the effectiveness of the pain management procedures for specific types of lesions and clinical signs of pain and distress associated with ocular safety testing.

To support the development of improved pain management strategies, ICCVAM recommends evaluating detailed animal injury and pain response data collected from animals used for regulatory safety testing. This could help gauge the adequacy of the recommended pain management procedures and help identify the need for modifications to dosages and dosing intervals for anesthetics and/or analgesics. Additionally, where possible, ICCVAM recommends that the eyes of test animals 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*. ICCVAM emphasizes that new animal studies should be considered only when absolutely necessary in developing new pain management strategies for testing.

Use of Earlier Humane Endpoints — Test Method Usefulness and Limitations

ICCVAM recognizes that current ocular testing guidelines include criteria for study termination in the case of certain types of severe ocular injuries or evidence of severe pain and distress (EPA 1998; OECD 2002). There is also international guidance on general humane endpoints that can be used as the basis for ending an experiment (OECD 2000). In addition to these currently accepted endpoints, and consistent with the recommendations of the Panel, ICCVAM recommends that the following ocular lesions 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)
- Destruction of more than 50% of the limbus, as evidenced by blanching of the conjunctival tissue
- Severe eye infection (purulent discharge)

A combination of the following endpoints may be useful in clinical decisions on study termination. However, these endpoints cannot be used individually to justify early 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 emphasizes that, once severe ocular effects have been identified, a qualified laboratory animal veterinarian should perform a clinical exam to determine if the combination of these effects warrants early study termination.

Changes to the Ocular Safety Testing Protocol to Include the Use of Humane Endpoints

The current protocol for the Draize rabbit eye test, as used for regulatory safety testing (EPA 1998; OECD 2002), should be updated to incorporate ICCVAM's recommended use of humane endpoints. ICCVAM recommends that test animals be comprehensively evaluated for the presence or absence of ocular lesions one hour after 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 promptly. ICCVAM also recommends that test animals should be routinely evaluated for clinical signs of pain and/or distress at least twice daily with at least 6 hours between observations. Examples of relevant clinical signs include (Wright et al. 1985; NRC 2008, 2009)

- repeated pawing or rubbing of the eye
- excessive blinking
- excessive tearing

Decisions to end a study based on humane endpoints should ensure that reversal of the clinical signs is not expected or that no further useful information can be obtained from the study. A written record of all observations should be kept, including evidence of an infection and/or pain and distress. Such records can facilitate decisions on the progression or resolution of ocular lesions. ICCVAM emphasizes that fluorescein staining should be used routinely. A slit-lamp biomicroscope should also be used, when considered appropriate (e.g., assessing depth of injury when corneal ulceration is present), to help detect and measure ocular endpoints. Digital photographs should be taken to document ocular lesions and to help assess their severity, progression, and resolution.

Future Studies on the Use of Humane Endpoints

ICCVAM recommends that additional data should be collected on the use of fluorescein staining to monitor wound healing. These data should be evaluated to identify criteria that may be useful as humane endpoints to terminate studies.

ICCVAM encourages users to provide NICEATM with detailed data and observations collected in ocular safety studies that can be used to create a database to (1) further characterize the usefulness and limitations of proposed humane endpoints and (2) identify potential new endpoints. Such data submissions will contribute to efforts to find ways to further prevent and minimize pain and distress in ocular safety assessments.

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1.0 Introduction

Current U.S. Environmental Protection Agency (EPA) and Organisation for Economic Co-operation and Development (OECD) test guidelines for the Draize rabbit eye test provide for the use of topical anesthetics only when the user demonstrates that such pretreatments do not interfere with the test results (EPA 1998; OECD 2002).⁸ Topical anesthetics are seldom used because a separate study would likely be necessary to meet this requirement. EPA (1998), European Union (EU 2001), and the Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007) regulatory guidelines recognize and accept certain humane endpoints for ocular hazard assessment. These include (1) severe and enduring signs of pain or distress and (2) eye lesions considered to be irreversible. However, current testing guidelines underemphasize the routine use of such endpoints.

Consequently, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid or minimize pain and distress during *in vivo* ocular safety testing.

The ICCVAM Authorization Act of 2000 (Public Law 106-545, 42 United States Code 2851-3) charged ICCVAM with coordinating the technical evaluations of new, revised, and alternative test methods with regulatory applicability. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) administers ICCVAM and provides scientific support for ICCVAM activities. The ICCVAM Ocular Toxicity Working Group (OTWG) worked with NICEATM in evaluating alternative methods and testing strategies. Drs. João Barroso, Tom Cole, and Valerie Zuang were the European Centre for the Validation of Alternative Methods (ECVAM) liaisons, and Dr. Hajime Kojima was the Japanese Center for the Validation of Alternative Methods (JaCVAM) liaison to the OTWG.

To facilitate peer review, the OTWG and NICEATM prepared a comprehensive draft background review document (BRD). The BRD provided information and data from published and unpublished data on the use of topical anesthetics, systemic analgesics, and humane endpoints in ocular safety testing.

ICCVAM and NICEATM requested the submission of data and experience with topical anesthetics and systemic analgesics for alleviating pain and distress in rabbits during ocular safety testing (72 FR 26396).⁹ One individual provided comments supporting the use of anesthetics to minimize pain and distress in rabbit eye irritation studies. No additional data were received.

On April 4, 2008, NICEATM published a *Federal Register* notice (73 FR 18535)¹⁰ requesting relevant data and nominations of individuals to serve on an independent international scientific peer review panel (Panel). The request was also disseminated via the ICCVAM electronic mailing list and through direct requests to over 100 stakeholders. Twenty individuals were nominated as potential panelists for consideration. No additional data were received (see **Section 6.0**).

The BRD forms the basis for these ICCVAM test method recommendations. The ECVAM and JaCVAM liaisons to the OTWG provided input and contributed throughout the evaluation process. A detailed timeline of the ICCVAM evaluation is provided in **Appendix A**. The ICCVAM-recommended test method protocol and final BRD are provided in **Appendices B** and **C**, respectively.

⁸ OECD Test Guideline 405 states: “The type, concentration, and dose of a local anesthetic should be carefully selected to ensure that differences in reaction to the test substance will not result from its use.” Similarly, EPA (1998) states that “The type and concentration of the local anesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use.”

⁹ Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_8898.pdf

¹⁰ Available at <http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E8-6969.pdf>

On March 31, 2009, ICCVAM announced the availability of the ICCVAM draft BRD. ICCVAM also announced a public Panel meeting to review the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints in ocular safety testing (74 FR 14556). The ICCVAM draft BRD and draft test method recommendations were posted on the NICEATM–ICCVAM website (<http://iccvam.niehs.nih.gov/>). All of the information provided to the Panel and all public comments received before the Panel meeting were made available on the NICEATM–ICCVAM website.

The Panel met in public session from May 19–21, 2009, to review a proposal for the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints in ocular safety testing. The Panel also reviewed the completeness and accuracy of the ICCVAM draft BRD. They then evaluated (1) the extent to which the draft BRD addressed established validation and acceptance criteria and (2) the extent to which the BRD supported ICCVAM’s draft test method recommendations. Public stakeholders were provided opportunities to comment at the Panel meeting. The Panel considered all comments during their deliberations. On July 13, 2009, ICCVAM posted the final report of the Panel’s recommendations (**Appendix D**) on the NICEATM–ICCVAM website for public review and comment (announced in 74 FR 33444).¹¹

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the draft BRD, draft test method recommendations, the Panel report, and all public comments. SACATM discussed this material at their meeting on June 25–26, 2009. Public stakeholders were given another opportunity to comment.

After the SACATM meeting, ICCVAM and the OTWG considered the SACATM comments, the Panel report, and all public comments before finalizing the ICCVAM test method evaluation report and the BRD, provided as an appendix to this report. As required by the ICCVAM Authorization Act, ICCVAM will make this test method evaluation report and the accompanying final BRD available to the public and to U.S. Federal agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving ICCVAM test method recommendations. Agency responses to the ICCVAM test method recommendations will be made available to the public on the NICEATM–ICCVAM website at <http://www.iccvam.niehs.nih.gov> as they are received.

¹¹ Announcement available at <http://niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E9-16388>; report available at http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPrept2009.pdf

2.0 ICCVAM Recommendations for the Routine Use of Topical Anesthetics and Systemic Analgesics to Avoid or Minimize Pain and Distress in Ocular Safety Testing

2.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

ICCVAM recommends that balanced preemptive pain management should always be provided when the Draize rabbit eye test is conducted for regulatory safety testing. 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/or distress; and (4) scheduled observation, monitoring, and recording of the nature, severity, and progression of all eye injuries. ICCVAM further recommends that ocular safety testing protocols include a pain management plan and schedule consistent with that outlined below.

When required for ocular safety testing, the Draize rabbit eye test protocol currently used for regulatory safety assessments of potential ocular hazards (EPA 1998; OECD 2002) should be conducted with the following modifications unless there is a requirement for monitoring the pain response (e.g., pharmaceutical tolerability testing). Alternative pain management procedures may also be considered that provide as good or better analgesia and anesthesia than the recommended pain management procedure below:

- Sixty minutes before test substance administration (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) is 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 more than one application of 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 chemically induced lesions 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) is given immediately and repeated every 8 hours,¹² instead of 0.01 mg/kg SC every 12 hours. Meloxicam would continue with the same dose and interval described below. The “rescue” analgesia should be given immediately after TSA if preemptive analgesia is inadequate.
- Eight hours post-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.
- After the initial 8-hour 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 (0.03 mg/kg every 8 hours if the “rescue” dose is needed), in conjunction with meloxicam 0.5 mg/kg SC every 24 hours.

Independent Peer Review Panel Conclusions and Recommendations

Following the Panel’s review of the BRD and draft recommendations developed by ICCVAM, the Panel proposed an alternative preemptive pain management protocol for rabbits used for ocular safety

¹² Time intervals are +/- 30 minutes.

testing. This protocol (hereafter, the alternative protocol or the Panel's protocol) was proposed by the Panel to be applied to all *in vivo* rabbit ocular safety tests intended for regulatory safety testing, unless there is a requirement for monitoring the pain response (e.g., pharmaceutical tolerability testing). The only differences in the ICCVAM-recommended plan and the Panel's protocol are that the ICCVAM-recommended plan (1) allows for either tetracaine or proparacaine as a topical anesthetic and (2) recommends only one dose of topical anesthetic unless there is reason to believe that this will be insufficient to relieve pain and distress, at which time additional pre-TSA applications can be considered. The basis for these differences arise from previous studies showing that multiple doses of proparacaine can result in significant differences in hazard classification due to the increased severity and/or prolonged appearance of ocular lesions.

2.2 ICCVAM Recommendations: Test Method Protocol for the Routine Use of Topical Anesthetics and Systemic Analgesics

When required for ocular safety testing, the Draize rabbit eye test protocol currently used for regulatory safety assessments of potential ocular hazards (EPA 1998; OECD 2002) should be conducted with the modifications as outlined in **Section 2.1** unless pain-response monitoring is required (e.g., pharmaceutical tolerability testing).

Independent Peer Review Panel Conclusions and Recommendations

The Panel considered its proposal (**Section 2.1**) more appropriate in terms of the type and frequency of dosing for topical anesthetics and systemic analgesics.

The Panel noted that the available guidance on measuring fluorescein staining as presented in the draft ICCVAM recommendations is not adequate for laboratories to obtain consistent results, and the method of fluorescein staining will have to be standardized in order to be useful. In addition, the guidelines lack details about potential preservatives in the dye, anesthesia requirements, or physical restraint that may need to be considered.

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

The routine observation and recording of lesions and clinical signs is recommended during ocular irritation safety studies to evaluate the effectiveness of pain management and to determine if the enhanced "rescue" analgesia procedure should be implemented. Furthermore, periodic retrospective reviews of these data should be performed to determine if adjustments are needed to improve the effectiveness of pretreatment and post-treatment analgesia and to optimize dosages and treatment intervals. Ideally, data collected during routine safety testing should be analyzed periodically to determine the effectiveness of the pain management plan for specific types of lesions and clinical signs of pain and distress associated with ocular irritation/corrosivity testing.

ICCVAM recommends the following studies and activities to support the development of improved pain management strategies, recognizing that some involve research that would be conducted independent of regulatory safety testing.

- New animal studies should be considered only when absolutely necessary in developing new pain management strategies for testing.
- Detailed ocular injury and pain response data should be collected from animals used for required regulatory testing and evaluated to assess the adequacy of the recommended pain management procedures. This data will help identify the need for modifications to dosages and dosing intervals for anesthetics and/or analgesics.

- 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 determine whether the timing and dosing of systemic analgesics together with topical anesthetics might alter the ocular defense sufficient to change the classification of test substances.
- Studies should be conducted to investigate other topical anesthetics that might provide longer duration of action or other advantages.
- Studies should be conducted to evaluate the impact of using other systemic analgesics that might provide longer duration of action, improved analgesia, or other advantages.
- ICCVAM encourages users to provide data generated using the recommended pain management procedures to NICEATM to create a database that can be periodically evaluated to further characterize the usefulness and limitations of such procedures for avoiding or minimizing pain and distress in ocular safety assessments.

Independent Peer Review Panel Conclusions and Recommendations

The Panel agreed with the draft ICCVAM recommendations for future studies related to the routine use of topical anesthetics and systemic analgesics. The Panel also recommended a number of additional studies, which have been incorporated into the ICCVAM recommendations listed above.

3.0 Validation Status: Routine Use of Topical Anesthetics and Systemic Analgesics in Ocular Safety Testing

Since 1984, the U.S. Consumer Product Safety Commission has recommended preapplication of tetracaine ophthalmic anesthetic in all rabbit ocular safety studies. However, current EPA and OECD test guidelines for the Draize rabbit eye test provide for the use of topical anesthetics only when the user demonstrates that such pretreatments do not interfere with the test results (EPA 1998; OECD 2002).¹³ Topical anesthetics are seldom used because a separate study would likely be necessary to provide the necessary information.

In 2005, a symposium entitled “Minimizing Pain and Distress in Ocular Toxicity Testing” evaluated the use of topical ophthalmic anesthetics and/or systemic analgesics during the conduct of the Draize rabbit eye test. ICCVAM, NICEATM, and the European Centre for the Validation of Alternative Methods (ECVAM) organized the symposium. Experts acknowledged that a single treatment with a topical anesthetic to anesthetize the surface of the cornea before application of the test article could cause slight physiologic changes. However, the consensus was that such changes in the irritant response would be slight if any. Furthermore, the predominant view was that if there were any effect on the irritant response, it would tend to slightly increase the severity of the response.

Participants recommended routine use of topical anesthetics. The anesthetics at least prevent the discomfort caused by installation of the test article on the eye. They also temporarily prevent or minimize pain and distress that might result from immediate ocular damage.

NICEATM recently evaluated the effects of pretreatment with tetracaine hydrochloride (0.5% w/v) on the ocular irritancy potential of 97 formulations. The results indicate that such pretreatments have no statistically significant impact on the hazard classification severity category of observed ocular irritation (**Annex II of Appendix C**). For most of the formulations tested, topical anesthetic pretreatment had little or no impact on:

- The hazard classification severity category of observed ocular irritation
- The variability in ocular irritation responses among animals treated with the same test article
- The number of days required for an ocular lesion to clear

When a difference in ocular irritation response was observed, the more severe response was usually observed in the animals pretreated with topical anesthesia. However, none of the observed differences was statistically significant. Differences included both increases and decreases in the irritancy level, which suggests that they are related to the inherent inter-individual biological variability of response rather than topical anesthetic pretreatment.

Scientific experts at the 2005 workshop also recommended (**Annex I of Appendix C**) that animals be routinely pretreated with topical anesthetics and systemic analgesics to prevent pain. Animals that show signs of pain or distress and those with ocular lesions associated with painful conditions should be treated with systemic analgesics. Similarly, a recently convened independent international scientific peer review panel recommended the routine use of topical anesthetics and systemic analgesics to avoid or minimize pain and distress during *in vivo* ocular safety testing. The Panel recommended a protocol that includes pretreatment with systemic analgesics in conjunction with

¹³ OECD Test Guideline 405 states: “The type, concentration, and dose of a local anesthetic should be carefully selected to ensure that differences in reaction to the test substance will not result from its use.” Similarly, EPA (1998) states that “the type and concentration of the local anesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use.”

topical anesthetics prior to test substance administration. The protocol also includes treatment with systemic analgesics after test substance administration.

A therapeutic analgesic protocol conducted before the onset of pain is referred to as preemptive pain management (Polomano et al. 2008). The Panel recommended a balanced preemptive pain management protocol for all animals used for ocular safety testing. For routine safety testing, the Panel considered proparacaine preferable to tetracaine because the initial application to the eye is less painful (Bartfield et al. 1994). The relative merits of proparacaine and tetracaine are detailed in **Annex III** of **Appendix C**. Multiple applications of topical anesthetics before test substance administration maximize effective penetration of the epithelial layer (Sasaki et al. 1995). A 5-minute interval between the last topical anesthetic dose and test substance administration minimizes the possibility of any volume dilution (Maurice 1995).

The Panel recommended buprenorphine as the systemic analgesic of choice. Buprenorphine is an opioid agonist–antagonist analgesic that has been effective in managing pain in rabbits and other small animals (Roughan and Flecknell 2002; Sawyer 2008). It has a 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). Increasing buprenorphine dose rates in rabbits has little effect on the maximum degree of analgesia produced (Flecknell and Liles 1990). For this reason, the recommended dose range in rabbits is 0.01–0.05 mg/kg (Dobromylskyj et al. 2006; Flecknell 1984, 1995; Flecknell and Liles 1990).

The Panel recommended treatment with systemic analgesics after test substance administration to maintain the prior level of analgesia. A well-tested approach to balanced analgesia is to use an opioid (e.g., buprenorphine) in combination with a cyclooxygenase-sparing nonsteroidal anti-inflammatory drug such as meloxicam (Cooper et al. 2009; Roughan and Flecknell 2002; Sawyer 2008). Meloxicam has been used for postoperative or chronic pain in humans (Akarsu et al. 2004; Aoki et al. 2006) and dogs for over 10 years. Its effectiveness has been demonstrated in rabbits (Cooper et al. 2009; Sawyer 2008). The Panel recommended a low dose of meloxicam once daily in conjunction with the buprenorphine.

4.0 ICCVAM Recommendations for the Use of Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing

4.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

ICCVAM recognizes that current ocular testing guidelines include criteria for study termination in the case of certain types of severe ocular injuries or evidence of severe pain and distress (EPA 1998; OECD 2002). These include:

- Draize corneal opacity score of 4 that persists for 48 hours
 - Corneal score of 4 is defined as: Opaque cornea, iris not discernable through the opacity
- Corneal perforation or significant corneal ulceration including staphyloma
- Blood in the anterior chamber of the eye
- Absence of a light reflex (iridial response grade 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)

There is also international guidance on general humane endpoints that can be used as the basis for ending an experiment (OECD 2000). In addition to these currently accepted endpoints and consistent with the recommendations of the Panel, ICCVAM recommends that the following ocular lesions 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)
- Destruction of more than 50% of the limbus, as evidenced by blanching of the conjunctival tissue
- Severe eye infection (purulent discharge)

The following endpoints, in combination, may be useful in clinical decisions on early study termination:

- Vascularization of the corneal 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

However, these endpoints cannot be used individually to justify early study termination. ICCVAM emphasizes that, once severe ocular effects have been identified, a qualified laboratory animal veterinarian should perform a clinical exam to determine if the combination of these effects warrants early study termination.

Conclusions and Recommendations of the Independent Peer Review Panel

The Panel concluded that the current and proposed humane endpoints should be used routinely as humane endpoints. The Panel considered them predictive enough of irreversible or severe effects (i.e., EPA Category I, GHS Category 1, EU R41) that a study should be terminated as soon as they are observed. To ensure that termination decisions are made promptly, the Panel recommended that test animals be examined at least daily and the presence or absence of these lesions recorded. For the first three days, test animals should be examined at least twice daily, or more often if necessary. The Panel

emphasized the need for a slit-lamp examination to ensure accurate measurement of most of the ocular endpoints.

The Panel did not consider some of the endpoints adequate for early study termination when taken individually (e.g., pannus, area of fluorescein staining, lack of re-epithelialization). They can, however, be considered together. With this in mind, the Panel emphasized that decisions to terminate a study should be based on multiple endpoints when possible. Only very severe endpoints (e.g., corneal perforation) would be adequate alone to terminate a study.

4.2 ICCVAM Recommendations: Changes to the Ocular Safety Testing Protocol to Include the Use of Humane Endpoints

Ocular safety assessment studies should be conducted using the ICCVAM-recommended modifications to the current Draize eye test protocol for regulatory safety assessments of potential ocular hazards (EPA 1998; OECD 2002). ICCVAM recommends that test animals be comprehensively evaluated for the presence or absence of ocular lesions 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. ICCVAM also recommends that test animals be routinely evaluated for clinical signs of pain and/or 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. ICCVAM emphasizes that fluorescein staining should be used routinely to help detect and objectively measure ocular endpoints. 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.

4.3 ICCVAM Recommendations: Future Studies for the Use of Humane Endpoints

ICCVAM recommends that additional data should be collected on the use of fluorescein staining to monitor wound healing. These data should be evaluated to identify criteria that may be useful as humane endpoints to terminate studies. ICCVAM recommends that guidelines should be developed for (1) the frequency of fluorescein staining that can be conducted without significant impacts on wound healing that would affect classification categories and (2) the usefulness of the area, intensity, and progression/regression of fluorescein staining for identifying specific hazard classification categories.

ICCVAM also recommends the following:

- Studies should be conducted to identify earlier, more predictive endpoints such as those quantifying area and intensity of fluorescein staining.
- Data should be collected during current testing to support the identification of potential earlier endpoints and to facilitate development of a database that can be used to identify useful earlier endpoints.
- Data should be collected to further evaluate pannus as a potential earlier humane endpoint. (ICCVAM did not consider the BRD data sufficient to determine the adequacy of pannus as a recommended humane endpoint for terminating a test.)

- Improved guidance should be developed on clinical signs of pain and distress in rabbits. Pain assessment training is also an important part of an effective pain management program and should be routinely provided to relevant personnel.
- Users should provide NICEATM with detailed data and observations collected from ocular safety studies that can be used to create a database to (1) further characterize the usefulness and limitations of proposed humane endpoints and (2) identify potential new endpoints. Such data submissions will contribute to efforts to find ways to further avoid or minimize pain and distress during ocular safety assessments.

Independent Peer Review Panel Conclusions and Recommendations

The Panel agreed with the draft ICCVAM recommendations for future studies related to the routine use of humane endpoints to avoid or minimize pain and distress in ocular safety testing. The Panel also recommended a number of additional studies, which have been incorporated into the ICCVAM recommendations listed above. The Panel emphasized that Animal Health Technologist (AHT) training requirements are an important part of a successful humane endpoint program.

5.0 Validation Status of the Use of Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing

Public Health Service policy and U.S. Department of Agriculture regulations on pain and distress in laboratory animals state that more than momentary or light pain and distress (1) must be limited to that which is unavoidable for the conduct of scientifically valuable research or testing, (2) must be conducted with appropriate pain relief medication unless justified in writing by the principal investigator, and (3) will continue for only a necessary amount of time. These regulations also state that animals suffering severe or chronic pain or distress that cannot be relieved should be humanely killed after or, if appropriate, during the procedure. Finally, Institutional Animal Care and Use Committees must ensure that the principal investigator complies with the requirements. Of the animals reported to the Department of Agriculture as experiencing unrelieved pain and distress, the majority are justified by regulatory testing requirements.

The OECD published a guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety assessment tests (OECD 2000). According to this document, guiding principles for humane endpoints include:

- Designing studies to minimize any pain, distress, or suffering, consistent with the scientific objective of the study
- Sacrificing animals at the earliest indication of severe pain, distress, or impending death, and avoiding severe pain, suffering, or death as endpoints
- Terminating animal studies once study objectives are achieved or when it is realized that these objectives will not be achieved
- Including knowledge about the test substance in the study design
- Defining in the protocol or standard operating procedure the conditions under which authorized personnel should intervene to alleviate pain and distress by humane killing

Accordingly, humane endpoints recognized and accepted by current EPA (2003), Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007), and EU (2001) regulatory guidelines for ocular hazard assessment include severe and enduring signs of pain or distress or eye lesions considered to be irreversible.

A recent report of the National Research Council Committee on Recognition and Alleviation of Pain in Laboratory Animals emphasized the need for increased efforts to identify appropriate humane endpoints (NRC 2009).

During the 2005 symposium “Minimizing Pain and Distress in Ocular Toxicity Testing,” panelists recommended early adverse responses that could serve as early humane endpoints to terminate animals on a study. Among the invited participants were human and veterinary ophthalmologists and anesthesiologists, scientific experts in ocular hazard testing, research scientists, and industrial toxicologists. The following ocular lesions are predictive of maximal severity, that of a severe irritant or corrosive with irreversible effects, including EPA Category I (2003) GHS Category 1 (UN 2007), and EU Category R41 (2001). They could be used routinely as humane endpoints to terminate a study.

- Endpoints currently accepted for study termination (OECD 2002)
 - Draize corneal opacity score of 4 that persists for 48 hours
 - Corneal perforation or significant corneal ulceration including staphyloma
 - Blood in the anterior chamber of the eye
 - Absence of light reflex that persists for 72 hours
 - Ulceration of the conjunctival membrane

- Necrosis of the conjunctiva or nictitating membrane
- Sloughing
- Vascularization of the corneal surface (i.e., pannus)
- Destruction of more than 75% of the limbus
- No diminishment in area of fluorescein staining and/or increase in depth of injury 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

The Panel discussed other endpoints that might allow for early termination of a study. These included destruction of the limbus and the relationship to re-epithelialization of the cornea, and positive results in Shirmer's test. Shirmer's test measures moisture content of the corneal tear film. A positive result in Shirmer's test suggests that conjunctival redness is likely to return to normal within 21 days. After these discussions, the endpoints described above were recommended for routine use. As discussed in **Section 4.0**, the Panel also recommended many of these endpoints (see the Panel's full report at <http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm>).

6.0 ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process provides numerous opportunities for public stakeholder involvement, including submission of written comments and oral comments at ICCVAM independent peer review panel and SACATM meetings. **Table 6-1** lists the nine different opportunities for public comments that were provided during the ICCVAM evaluation of the validation status of alternative ocular safety testing methods and approaches. The number of public comments received in response to each of the opportunities is also indicated. Thirty-seven comments were received. Comments received in response to or related to the *Federal Register* notices are accessible on the NICEATM–ICCVAM website. The following sections, delineated by *Federal Register* notice, briefly discuss the public comments received.

Table 6-1 Opportunities for Public Comment

Opportunities for Public Comment	Date	Number of Public Comments Received
70 FR 13512: Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel	March 21, 2005	0
72 FR 26396: Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for <i>In Vivo</i> Eye Irritation Testing	May 9, 2007	1
72 FR 31582: Request for Ocular Irritancy Test Data From Human, Rabbit, and <i>In Vitro</i> Studies Using Standardized Testing Methods	June 7, 2007	0
73 FR 18535: Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data	April 4, 2008	12
74 FR 14556: Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRD); Request for Comments	March 31, 2009	8
74 FR 19562: Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)	April 29, 2009	2
Independent Scientific Peer Review Panel Meeting: Alternative Ocular Safety Testing Methods	May 19–21, 2009	12
SACATM Meeting, Arlington Hilton, Arlington, VA	June 25–26, 2009	2
74 FR 33444: Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of Availability and Request for Public Comments	July 13, 2009	0

**6.1 Public Comments in Response to 70 FR 13512 (March 21, 2005):
Request for Data on Non-Animal Methods and Approaches for Determining
Skin and Eye Irritation Potential of Antimicrobial Cleaning Product
Formulations; Request for Nominations for an Independent Expert Panel**

NICEATM requested (1) submission of data that would assist in evaluating the validation status of non-animal methods and approaches used for determining the skin and eye irritation potential of AMCP formulations to meet regulatory hazard classification and labeling purposes and (2) nominations of expert scientists to serve as members of an independent peer review panel.

No data or nominations were received in response to this *Federal Register* notice.

**6.2 Public Comments in Response to 72 FR 26396 (May 9, 2007):
Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for
In Vivo Eye Irritation Testing**

NICEATM requested submission of (1) data and information on the use of topical anesthetics and systemic analgesics for alleviating pain and distress in rabbits during eye irritation testing and (2) information about other procedures and strategies that may reduce or eliminate pain and distress associated with *in vivo* eye irritation methods.

Public Response

NICEATM received one comment in response to this *Federal Register* notice.

Comment:

The commenter supported the use of anesthetics to minimize pain and distress in rabbit eye irritation studies and offered assistance in the evaluation. However, the commenter noted that data from their studies involving the use of local anesthetics could not be shared without permission of its sponsors.

ICCVAM Response:

ICCVAM encourages users to provide data that are generated from future studies, as they could be used to further characterize the usefulness and limitations of topical anesthetics and systemic analgesics for avoiding or minimizing pain and distress in ocular safety assessments.

**6.3 Public Comments in Response to 72 FR 31582 (June 7, 2007):
Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro*
Studies Using Standardized Testing Methods**

NICEATM requested data on substances tested for ocular irritancy in humans, rabbits, and/or *in vitro* to be used to:

- Review the state of the science in regard to the availability of accurate and reliable *in vitro* test methods for assessing the range of potential ocular irritation activity, including whether ocular damage is reversible or not
- Expand NICEATM's high-quality ocular toxicity database. *In vitro* test methods for which data are sought include but are not limited to (1) the bovine corneal opacity and permeability test, (2) the isolated rabbit eye test, (3) the isolated chicken eye test, and (4) the hen's egg test–chorioallantoic membrane.

No data or information was received in response to this *Federal Register* notice.

**6.4 Public Comments in Response to 73 FR 18535 (April 4, 2008):
Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for
Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an
Independent Expert Panel and Submission of Relevant Data**

NICEATM requested the following:

- Nominations of expert scientists to serve as members of an independent peer review panel
- Submission of relevant data and information on AMCPs or related substances obtained from (1) human testing or experience, including reports from accidental exposures, and (2) rabbit testing using the standard eye test or the LVET
- *In vitro* ocular safety test methods such as the bovine corneal opacity and permeability test method, the Cytosensor[®] Microphysiometer test method, and the EpiOcular test method, including data supporting the accuracy and reproducibility of these methods

In response to this *Federal Register* notice, NICEATM received 12 comments, including nominations of 20 potential panelists. The nominees were included in the database of experts from which the Panel was selected. No additional data were received.

**6.5 Public Comments in Response to 74 FR 14556 (March 31, 2009):
Announcement of an Independent Scientific Peer Review Panel on Alternative
Ocular Safety Testing Methods; Availability of Draft Background Review
Documents (BRD); Request for Comments**

NICEATM requested public comments on the draft BRDs, SRDs, and draft ICCVAM test method recommendations that were provided to an independent scientific peer review panel meeting (May 19–21, 2009). These documents summarized the current validation status of several test methods and testing strategies for identifying potential ocular irritants. The test methods and testing strategies included the following:

- A testing strategy that proposes the use of three *in vitro* test methods to assess the eye irritation potential of AMCPs
- Four *in vitro* test methods for identifying moderate (EPA Category II, UN Globally Harmonized System of Classification and Labelling of Chemicals [GHS] Category 2A) and mild (EPA Category III, GHS Category 2B) ocular irritants and substances not classified as ocular irritants (EPA Category IV, GHS Not Classified)
- The *in vivo* LVET
- A proposal for the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid and minimize pain and distress during *in vivo* ocular irritation testing

NICEATM received 20 comments in response to this *Federal Register* notice. Eight written comments were received before the Panel meeting, and 12 oral comments were provided at the Panel meeting.

No written comments were relevant to the use of topical anesthetics, systemic analgesics, or earlier humane endpoints to minimize pain and distress in ocular safety testing.

None of the 12 oral public comments provided at the Panel meeting was relevant to the use of topical anesthetics, systemic analgesics, or earlier humane endpoints to avoid or minimize pain and distress in ocular safety testing.

**6.6 Public Comments in Response to 74 FR 19562 (April 29, 2009):
Meeting of the Scientific Advisory Committee on Alternative Toxicological
Methods (SACATM)**

NICEATM announced the SACATM meeting (June 25–26, 2009) and requested written and public oral comments on the agenda topics.

Public Response:

NICEATM received four comments. Two written comments were received before the meeting, and two oral comments were provided at the SACATM meeting.

SACATM Response:

In general, SACATM was pleased with the Panel report. One SACATM member expressed the need for harmonization in the assessment of performance standards. Another SACATM member said the focus should be on the GHS system because it will ultimately be adopted. Another SACATM member expressed concern regarding the availability of the Cytosensor[®] Microphysiometer.

**6.7 Public Comments in Response to 74 FR 33444 (July 13, 2009):
Independent Scientific Peer Review Panel Report: Evaluation of the Validation
Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of
Availability and Request for Public Comments**

NICEATM requested submission of written public comments on the independent scientific peer review panel report.

No public comments were received.

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