新規試験法提案書

2018年改定 OECD TG 438
ニワトリ眼球を用いた眼刺激性試験
(ICE法 : Isolated Chicken Eye Test)

令和元年11月

国立医薬品食品衛生研究所

新規試験法提案書

令和元年 11 月 18 日

No.　2019-03

2018 年改定 OECD TG 438 ニワトリ眼球を用いた眼刺激性試験
（ICE 法 : Isolated Chicken Eye Test）に関する提案

令和元年 11 月 14 日に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： 本試験法は、化学物質による眼刺激性を評価でき、トップダウン方式において重篤な眼の傷害を引き起こす物質ならびにボトムアップ方式において眼刺激物質として分類されない物質を識別するという用途の範囲において行政的利用は可能であると考える。

この提案書は、眼刺激性試験資料編纂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 2018 年改定 OECD TG 438 ニワトリ眼球を用いた眼刺激性試験（ICE 法 : Isolated Chicken Eye Test）の使用を提案するものである。

大野泰雄

JaCVAM 評価会議　議長

平林容子

JaCVAM 運営委員会　委員長

JaCVAM 評価会議

大 野 泰 雄　（公益財団法人　木原記念横浜生命科学振興財団）：座長
五十嵐良明　（国立医薬品食品衛生研究所）
石 井 雄 二　（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）
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岩瀬裕美子　（日本製薬工業協会）
久 保 文 宏　（独立行政法人　医薬品医療機器総合機構）
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西 川 秋 佳　（国立医薬品食品衛生研究所　病理部 / 済生会宇都宮病院）
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任期：平成 30 年 4 月 1 日～令和 2 年 3 月 31 日
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JaCVAM 運営委員会

平 林 容 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）：委員長
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束 野 正 明	（厚生労働省　医薬・生活衛生局　医薬品審査管理課　化学物質安全対策室）
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本 間 正 充	（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　変異遺伝部）
足利太可雄	（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　安全性予測評価部第二室）：事務局
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　安全性予測評価部第二室）：事務局

JaCVAM statement on the 2018 revision of OECD Test Guideline 438: Isolated Chicken Eye Test Method for Eye Irritation or Serious Eye Damage

At a meeting held on 14 November 2019 at the National Institute of Health Sciences (NIHS) in Kanagawa, Japan, the Japanese Center for the Validation of Alternative Methods (JaCVAM) Regulatory Acceptance Board unanimously endorsed the following statement:

Proposal: We propose the Isolated Chicken Eye Test Method to be useful in a regulatory context as part of a top-down approach to identifying chemicals that induce serious eye damage (Category 1) or as part of a bottom-up approach to identifying chemicals that do not require classification for eye irritation or serious eye damage under the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS).

This statement was prepared to acknowledge that the results of a review and study by the JaCVAM Regulatory Acceptance Board have confirmed the usefulness of this assay.
Based on the above, we propose the 2018 revision of OECD TG 438 as a useful means for estimating eye irritation by regulatory agencies.

Yasuo Ohno
Chairperson
JaCVAM Regulatory Acceptance Board

Yoko Hirabayashi
Chairperson
JaCVAM Steering Committee

November 18, 2019

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

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

Mr. Yasuo Ohno (Kihara Memorial Yokohama Foundation for the Advancement of Life Sciences): Chairperson
Ms. Yoko Hirabayashi (Center for Biological Safety and Research: CBSR, National Institute of Health Sciences: NIHS)
Mr. Morihiko Hirota (Japan Cosmetic Industry Association)**
Mr. Yoshiaki Ikarashi (NIHS)
Mr. Noriyasu Imai (Japanese Society for Alternatives to Animal Experiments)
Mr. Kunifumi Inawaka (Japan Chemical Industry Association)
Mr. Tomoaki Inoue (Japanese Society of Immunotoxicology)
Mr. Yuji Ishii (CBSR, NIHS)
Ms. Yumiko Iwase (Japan Pharmaceutical Manufacturers Association)
Mr. Fumihiro Kubo (Pharmaceuticals and Medical Devices Agency)
Mr. Kenichi Masumura (Japanese Environmental Mutagen Society)
Ms. Ruriko Nakamura (National Institute of Technology and Evaluation)
Mr. Akiyoshi Nishikawa (CBSR, NIHS/ Saiseikai Utsunomiya Hospital)
Mr. Jihei Nishimura (Pharmaceuticals and Medical Devices Agency)
Mr. Satoshi Numazawa (Japanese Society of Toxicology)
Ms. Mariko Sugiyama (Japan Cosmetic Industry Association)*
Mr. Hiroo Yokozeki (Japanese Society for Cutaneous Immunology and Allergy)

Term: From 1st April 2018 to 31st March 2020
*: From 1st April 2018 to 31st March 2019
**: From 1st April 2019 to 31st March 2020

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

Ms. Yoko Hirabayashi (CBSR, NIHS): Chairperson
Mr. Manabu Fuchioka (Ministry of Health, Labour and Welfare)
Mr. Osamu Fueki (Pharmaceuticals and Medical Devices Agency)
Mr. Akihiko Hirose (Division of Risk Assessment, CBSR, NIHS)
Mr. Masamitsu Honma (Division of Genetics and Mutagenesis, CBSR, NIHS)
Ms. Mie Ikeda (Pharmaceuticals and Medical Devices Agency)
Mr. Koji Ishii (National Institute of Infectious Diseases)
Mr. Yasunari Kanda (Division of Pharmacology, CBSR, NIHS)
Mr. Satoshi Kitajima (Division of Toxicology, CBSR, NIHS)
Mr. Yoshinobu Nosaka (Ministry of Health, Labour and Welfare)
Ms. Kumiko Ogawa (Division of Pathology, CBSR, NIHS)
Mr. Haruhiro Okuda (NIHS)
Mr. Atsuya Takagi (Animal Management Section of the Division of Toxicology, CBSR, NIHS)
Mr. Masahiro Takahata (Ministry of Health, Labour and Welfare)
Mr. Masaaki Tsukano (Ministry of Health, Labour and Welfare)
Mr. Takao Ashikaga (Division of Risk Assessment, CBSR, NIHS): Secretary
Mr. Hajime Kojima (Division of Risk Assessment, CBSR, NIHS): Secretary

2018年改定 OECD TG 438 ニワトリ眼球を用いた眼刺激性試験
（ICE法：Isolated Chicken Eye Test）

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評価会議報告書

2018 年改定 OECD TG 438
ニワトリ眼球を用いた眼刺激性試験
（ICE 法 : Isolated Chicken Eye Test）

JaCVAM 評価会議

令和元年（2019 年）9 月 30 日

JaCVAM 評価会議

大 野 泰 雄	（公益財団法人 木原記念横浜生命科学振興財団）：座長
五十嵐良明	（国立医薬品食品衛生研究所）
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ニワトリ眼球を用いた眼刺激性試験（ICE 法 : Isolated Chicken Eye Test）は、ニワトリから摘出した眼球に被験物質を曝露して生じる角膜の変化をもとに *in vivo* の眼刺激性を予測する試験であり、ウサギを用いた Draize 眼刺激性試験法（Draize 法）の代替法である。眼に重篤な損傷を引き起こす化学物質（UN GHS : United Nations Globally Harmonized System of Classification and Labeling of Chemicals [1)] 区分 1 物質）をトップダウン方式により検出する方法として、2009 年に OECD TG 438 として採択された [2)]。2013 年に採択された改定 TG 438 [3)]では、トップダウン方式により UN GHS 区分 1 物質を、ボトムアップ方式により UN GHS 区分に該当しない物質を判定する場合の基準が定められており、JaCVAM 評価会議において妥当性が確認されている [4)]。今回、ICE 法の再評価が行われ、2018 年改定 TG 438 [5)]が採択された。本改定では、ボトムアップ方式による UN GHS 区分に該当しない物質の判定基準が変更され、偽陽性率が改善された。また、トップダウン方式による区分 1 の判定において、これまで偽陰性率の高かった洗浄剤と界面活性剤の評価性能を向上させるため、病理組織学的検査の併用が追加された。JaCVAM 評価会議は、眼刺激性試験資料編纂委員会により作成された「2018 年改定 OECD TG 438 ニワトリ眼球を用いた眼刺激性試験（ICE 法 : Isolated Chicken Eye Test）」 [6)] を用いて、本試験法の妥当性について検討した。

１．試験法の定義

名称 : 2018 年改定 OECD TG 438 ニワトリ眼球を用いた眼刺激性試験（ICE 法 : Isolated Chicken Eye Test）

代替する対象毒性試験：Draize 眼刺激性試験法

試験法の概略：ICE 法では、ニワトリから摘出した眼球に被験物質を曝露し、その結果、眼球の角膜に生じる変性を、角膜の腫脹、混濁度およびフルオレセイン染色性の変化としてとらえ、個別のスコアに変換して得られる総合評価をもとに *in vivo* での眼刺激性を予測する。本試験では、被験物質群および陽性対照群は 3 眼球以上、媒体対照群および陰性対照群は 1 眼球以上を使用する。角膜の腫脹は、光学的厚度計を装着した細隙灯顕微鏡を用いて角膜の厚さを曝露後 240 分間測定し、経時的な変化率として定量的に求める。角膜の混濁度は、細隙灯顕微鏡にて角膜混濁度の経時的な変化を曝露後 240 分間観察し、結果を評点化する。フルオレセイン染色性は、細隙灯顕微鏡にて曝露 30 分後の角膜表面のフルオレセイン染色性を観察し、評点化する。得られた結果を項目毎に眼刺激性の最も弱いクラス I から最も強いクラス IV の 4 段階に分類し、それらの分類結果を総合して、被験物質の眼刺激性を判定する。

2018 年改定点：

1）UN GHS 区分に該当しない物質のボトムアップ方式における判定基準に「1 項目がクラス I に分類され、2 項目がクラス II に分類される」場合が加わった。この基準の下に、新たな物質を加えて再構築したデータにおいて正確性が再評価された結果、TG 438（2013）の判定基準と比較して、偽陰性率は低く抑えられたまま、偽陽性率が改善された。

2）トップダウン方式による UN GHS 区分 1 物質の判定に、病理組織学的検査が追加された。2<pH<11.5 の洗浄剤および界面活性剤について、トップダウン方式において偽陰性率が高いことから、標準的な ICE 法で陰性結果が得られた際には、病理組織学的検査を併用して判定することを推奨する。ICE 法に用いた眼球の病理組織標本を作製して、角膜上皮のびらん、空胞化および壊死、角膜間質の核濃縮の程度および線維の乱れ、ならびに角膜内皮の壊死の有無を基準に刺激性を評点化する。評点を区分 1 物質の評価基準に照らし、合致した場合に UN GHS 区分 1 と判定し、合致しない場合には予測不可とする。

3）ICE 法および病理組織学的検査における習熟度確認物質一覧が更新された。

２．評価に用いた資料および評価内容の科学的妥当性

本試験法は OECD にて TG 438（2009）、改定 TG 438（2013）を経て、改定 TG 438（2018）として採択された。今回、改定 TG 438（2018）においてボトムアップ方式の判定基準を改定するにあたり、新たな物質を加えて再構築したデータ（184 物質）において再評価が行われた[5]。その結果、Draize 法との比較では、ICE 法の正確度は 88%（161/184）、感度 97%（98/101）、特異度は 76%（63/83）、偽陰性率は 3 %（3/101）および偽陽性率は 24%（20/83）を示し、TG 438（2013）の判定基準と比較して、偽陰性率は低く抑えられたまま、偽陽性率は改善された。 また、トップダウン方式において偽陰性率が高かった洗浄剤および界面活性剤について、検出感度を向上させるため、病理組織学的検査の併用について検証が行われた [7][8]。これらの結果を受けて、2<pH<11.5 の洗浄剤および界面活性剤 29 物質について JaCVAM 眼刺激性試験資料編纂委員会が解析した結果、*in vivo* の判定との比較において、病理組織学的検査を併用することにより、感度が 33%から 71%に向上し、正確度も 52%から 76%に上昇した。評価会議では、評価資料は適切であり、TG 438 改定における評価方法の変更は科学的に妥当であると考える。

３．本試験法の有用性と適用限界

ICE 法は生きた動物を用いないため代替法として有用性がある。2018 年の改定 TG 438 に基づいて正確性が向上したことから、UN GHS の眼刺激性分類においてトップダウン方式における区分 1 物質の同定およびボトムアップ方式における UN GHS 区分に該当しない物質の同定に ICE 法を適用することは可能であると考える。なお、ボトムアップ方式で陽性結果が得られた場合、他の適切な試験法による確認が必要とされている。また、トップダウン方式においては、2<pH<11.5 の洗浄剤および界面活性剤については、陰性結果が得られた場合、病理組織学的検査を併用することが推奨される。

４．目的とする物質又は製品の毒性を評価する試験法としての、社会的受け入れ性および行政上の利用の可能性

社会的受け入れ性：

本試験法は、食用として屠殺されたニワトリの眼球を用いるため、Draize 法よりも社会的受け入れ性は高い。今回の改定においては、動物福祉の観点からの変更がないことから、本試験法の社会的受け入れ性は、改定前と変わらない。

行政上の利用性：

本試験法は、化学物質による眼刺激性を評価でき、トップダウン方式において UN GHS 区分 1 物質（重篤な眼の傷害を引き起こす物質）ならびにボトムアップ方式において UN GHS 区分に該当しない物質（眼刺激物質として分類されない）を識別するという用途の範囲において行政的利用は可能であると考える。

参考文献

1) United nations (UN) (2017). Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Seventh revised edition, UN New York and Geneva, 2017. Available at: [https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev07/English/03e_part3.pdf]
2) OECD Guidelines for The Testing of Chemicals, Isolated Chicken Eye Test for Identifying Ocular Corrosives and Severe Irritants, TG 438 (Adopted: 7 September 2009)
3) OECD Guidelines for The Testing of Chemicals, Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, TG 438 (Adopted: 26 July 2013)
4) JaCVAM評価会議 : 眼刺激性試験代替法の評価会議報告書　2013年改訂 OECD TG 438 ニワトリ眼球を用いた眼刺激性試験（ICE 法: Isolated Chicken Eye Test）（2014年10月28日）
5) OECD Guidelines for The Testing of Chemicals, Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, TG 438 (Adopted: 25 June 2018)
6) JaCVAM資料編纂委員会 : 眼刺激性試験代替法評価報告書　2018年改定 OECD TG 438 ニワトリ眼球を用いた眼刺激性試験（ICE 法: Isolated Chicken Eye Test）（2019年9月26日）
7) Cazelle E., Eskes C., Hermann M., et al. (2014). Suitability of Histopathology as an Additional Endpoint to the Isolated Chicken Eye Test for classification of non-extreme pH detergent. Toxicology In Vitro 28, 657-666.
8) Cazelle E., Eskes C., Hermann M., et al. (2015). Suitability of the Isolated Chicken Eye Test for Classification of Extreme pH Detergents and Cleaning Products. Toxicology In Vitro 29, 609-616.

評価報告書

2018 年改定 OECD TG 438
ニワトリ眼球を用いた眼刺激性試験
（ICE 法 : Isolated Chicken Eye Test）

眼刺激性試験資料編纂委員会

令和元年（2019 年）9 月 26 日

眼刺激性試験資料編纂委員会

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要旨

ニワトリ眼球を用いた眼刺激性試験（ICE 法：Isolated Chicken Eye Test）は、ニワトリから摘出した眼球に被験物質を曝露して生じる角膜の変化をもとに、 *in vivo* の眼刺激性を予測する試験であり、OECD TG 438 として試験法が標準化されている。2013 年に採択された改定 TG 438（2013）には、トップダウン方式により UN GHS 区分 1 物質を、ボトムアップ方式により UN GHS 区分に該当しない物質を判定する場合の基準が定められており、既に JaCVAM 評価会議において妥当性が確認されている。本報告では、2018 年 6 月に採択された改定 TG 438（2018）について改正点の概要を説明し、バリデーション研究報告書、第三者評価報告書および関連論文などをもとに、JaCVAM 眼刺激性試験資料編纂委員会としての意見をまとめた。

改定 TG 438（2018）では、ボトムアップ方式による区分に該当しない物質の判定基準が変更され、偽陽性率が改善された。また、トップダウン方式による UN GHS 区分 1 の判定において、これまで偽陰性率の高かった界面活性剤の評価を向上させるため、病理組織学的検査の併用が検証された。この併用により、2<pH<11.5 の洗浄剤および界面活性剤（29 物質）の感度は 33%から 71%に向上し、偽陰性率は 67%から 29%に抑制された。また、12 の界面活性剤を用いた 3 施設間の再現性は 83%であった。

以上より、本委員会は、ボトムアップ方式による判定基準の変更は妥当であり、また、2<pH<11.5 の洗浄剤および界面活性剤については、トップダウン方式により UN GHS 区分 1 を検出する際、病理組織学的検査を補助的に用いることができると結論した。

略語

BCOP: Bovine Corneal Opacity and Permeability
BRD: Background Review Document
CM: Cytosensor Microphysiometer
CV: Coefficient of Variation
EURL ECVAM: European Union Reference Laboratory for Alternatives to Animal Testing
GHS: Globally Harmonized System of Classification and Labeling of Chemicals
ICCVAM: Interagency Coordinating Committee on the Validation of Alternative Methods
ICE: Isolated Chicken Eye
JaCVAM: Japanese Center for the Validation of Alternative Methods
NICEATM: NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
OECD: Organization for Economic Co-operation and Development
SSD: Streamlined Summary Document
TG: Test Guideline
UN: United Nations

1．改定の背景

ICE 法は、ウサギを用いた Draize 眼刺激性試験法（Draize 法）の代替法であり、トップダウン方式により眼に重篤な損傷を引き起こす化学物質（UN GHS [1)] 区分 1 物質）を検出する方法として、2009 年に OECD で TG 438（2009）として採択された。その後、眼刺激性に関する UN GHS 分類 について、*in vivo* と *in vitro*、双方のデータベースが再評価され、トップダウン方式における区分 1 物質の検出のみならず、ボトムアップ方式で UN GHS 区分 1 物質及び眼刺激性を引き起こす化学物質（UN GHS [1)] 区分 2 物質）のいずれの区分にも該当しない物質も検出することが可能であると判断され、2013 年に改定 TG 438（2013）として採択された。

今回、ICE 法の再評価が行われ、 改定 TG 438（2018）[2)]が採択された。主な改正点は、ボトムアップ方式による区分に該当しない物質検出のための判定基準の変更と、トップダウン方式による UN GHS 区分 1 を識別するエンドポイントとして 2<pH<11.5 の洗浄剤および界面活性剤についての病理組織学的検査の併用である。本資料編纂委員会は、この TG 438（2018）の改定点について検討した。以下にその 結果を報告する。

2．標準的な ICE 法の概要

ICE 法では、ニワトリから摘出した眼球に被験物質を曝露し、その結果、眼球の角膜に生じる変性を角膜の腫脹、混濁度およびフルオレセイン染色性の変化としてとらえ、個別のスコアに変換して得られる総合評価をもとに *in vivo* での眼刺激性を予測する。

試験では、被験物質群および陽性対照群は 3 眼球以上、媒体対照群および陰性対照群は 1 眼球以上を使用する。角膜の腫脹は、光学的厚度計を装着した細隙灯顕微鏡を用いて角膜の厚さを被験物質曝露後 240 分間測定し、経時的な変化率として定量的に求める。角膜の混濁度は、細隙灯顕微鏡にて角膜混濁度の経時的な変化を曝露後 240 分間観察し、結果を評点化する。フルオレセイン染色性は、細隙灯顕微鏡にて曝露 30 分後の角膜表面のフルオレセイン染色性を観察し、評点化する。得られた結果を項目毎に眼刺激性の最も弱いクラス I から最も強いクラス IV の 4 段階に分類し、それらの分類結果を総合して、被験物質の眼刺激性を判定する。

3．改定点

3−1．ボトムアップ方式による UN GHS 区分に該当しない物質判定基準の変更

TG 438（2013）に制定された UN GHS 区分に該当しない物質のボトムアップ方式における判定基準に「1 項目がクラス I に分類され、2 項目がクラスⅡに分類される」場合が加わった（表 1）。

表 1.　被験物質を UN GHS 区分に該当しないと評価するための判定基準 [2)]

UN GHS 区分	3 評価項目の組み合わせ
区分外	3 項目ともクラス I に分類される 2 項目がクラス I に分類され、1 項目がクラスⅡに分類される 1 項目がクラス I に分類され、2 項目がクラスⅡに分類される

区分外：UN GHS による眼に対する重篤な損傷性および眼刺激性のいずれの区分にも該当しない。「用語」参照。

上記の基準の下に、新たな物質を加えて再構築したデータにおいて正確性が再評価された。その結果、表 2 に示すように、Draize 法との比較では、ICE 法の正確度は 88%（161/184）、感度 97%（98/101）、特異度は 76%（63/83）、偽陰性率は 3 %（3/101）および偽陽性率は 24%（20/83）を示した。TG 438（2013）の判定基準と比較して、偽陰性率は低く抑えられたまま、偽陽性率は改善された。なお、ボトムアップ方式で陽性結果が得られた場合、他の適切な試験法による確認が必要とされている。また、固体で陰性結果が得られた場合、3 眼球を用いて 2 回目の実験を行い、陰性結果を承認または棄却することが推奨されている。

表 2.　ボトムアップ方式における ICE 法の正確性－UN GHS 区分法での区分に該当しない物質の同定[2)]

ボトムアップ方式	No.	正確度		感度		偽陰性率		特異度		偽陽性率	
		%	No.	%	No.	%	No.	%	No.	%	No.
判定基準（2018）	184	88	161/184	97	98/101	3	3/101	76	63/83	24	20/83
判定基準（2013）	152	82	125/152	99	72/73	1	1/73	67	53/79	33	26/79

なお、トップダウン方式においても、新たな物質が加わったデータにおいて区分 1 物質を検出する場合の正確性が再評価され、これらの予測性は、更新前と同程度であった。

3−2. トップダウン方式による UN GHS 区分 1 物質の判定

3−2−1. 病理組織学的検査の追加

病理組織学的検査を行う場合、ICE 法に用いた眼球（被験物質を曝露した 3 眼球全て）の病理組織標本を作製して評価する。10%中性緩衝ホルマリン、4 %グルタールアルデヒドまたは 2.5%グルタールアルデヒドと 2 %ホルムアルデヒド混合液で固定した眼球（角膜）を常法に従い脱水、パラフィン包埋、薄切し、HE 染色あるいは PAS 染色等を施した病理組織標本を作製する。角膜上皮のびらん、空胞化および壊死、角膜実質（固有層）の核濃縮の程度および線維の乱れ、ならびに角膜内皮の壊死の有無を基準に刺激性を評点化する（表 3）。

表 3.　ニワトリ眼球の病理組織標本のための半定量的評点化システム [2)]

指標	判定	スコア	説明
上皮：びらん	ごく軽度	1/2	数個の細胞から最表層 1 層までの欠損
	軽度	1	3 層以下の欠損
	中等度	2	50%までの上皮層の欠損
	重度	3	基底膜に達する上皮層の欠損
上皮：空胞化 上皮を上、中、下の各層に分けてスコア化	ごく軽度	1/2	空胞化細胞が 1 個～数個散在性にみられる
	軽度	1	空胞化細胞の集簇または小空胞を有する細胞が単一層にみられる
	中等度	2	上皮の 50%まで空胞化細胞が占める
	重度	3	上皮の 50～100%を空胞化細胞が占める
上皮：壊死	正常範囲	-	細胞質が膨化した細胞 10 個未満
	ごく軽度	1/2	細胞質が膨化した細胞 10～20 個
	軽度	1	細胞質が膨化した細胞 20～40 個
	中等度	2	細胞質が膨化した細胞が多数認められるが上皮層の 50%未満を占める場合
	重度	3	細胞質が膨化した細胞が上皮層の 50～100%を占める場合
実質：核濃縮 上部または下部領域	正常範囲	―	5 個未満の細胞の核濃縮
	軽度	1	5～10 個の細胞の核濃縮
	中等度	2	10 個を超える細胞の核濃縮
実質の線維異常	あり	P	不規則な線維
内皮：壊死	あり	P	内皮は単層のため、グレード分けはしない

表 3 の刺激性のスコアを区分 1 物質の評価基準（表 4）に照らし、合致した場合に UN GHS 区分 1 と判定し、合致しない場合には予測不可とする。

表 4.　UN GHS 区分 1 物質の同定のため、ICE 法に加えて実施することが推奨される病理組織学的検査の評価基準 [2)]

組織層	眼に重篤な損傷を引き起こす可能性（UN GHS区分1）の評価基準
角膜上皮	・少なくとも 3 眼中 2 眼で中等度（スコア 2）以上のびらんを示す ・3 眼中 2 眼で角膜上皮の中層ないし下層におけるごく軽度の空胞化（スコア 1/2）を伴う ・3 眼中 1 眼で中程度（スコア 2）以上のびらんを示した場合、少なくともその他の 2 眼中 1 眼で角膜上皮の中層ないし下層におけるごく軽度の空胞化（スコア 1/2）を伴う ・少なくとも 3 眼中 2 眼で中等度（スコア 2）以上の壊死を示す

さらに、表 3 のうち、角膜の空胞化については、洗浄剤および界面活性剤による空胞化の好発部位を考慮し、形態的な背景値に対して表 5 の鑑別基準が示されている。

表 5.　背景値として認められる小さな空胞と真の空胞化を鑑別するための基準 [3)]

空胞の大きさ	発生部位	真の空胞化であるか?（Yes/No）
上皮細胞の核の大きさの 1/3 またはそれ以上	上皮細胞内（不特定）	Yes
上皮細胞の核の 1/3 よりも小さい	基底膜に接する部位	No
	上皮細胞内の基底膜側から数えて 1 列目の核の層よりも上	Yes
	基底膜と 1 列目の核の層との間	追加情報を基に判断*

*段階的評価：

1) 他 2 つの眼球標本を観察し、2 つとも作用が認められない場合は真の空胞化ではないと判断し、2 つのうち 1 つの眼球ではっきりした作用が認められた場合は真の空胞化と判断する。
2) 他 2 つの眼球標本を観察してもはっきりしない場合、例えば固形物による局所的な作用の可能性を考慮するといった標準的な ICE 法での観察結果も参考にする。

これら上皮細胞の空胞化の他、界面活性剤による組織変化の特徴として、曝露および培養によって角膜上皮細胞と基底膜との接着性が低下したことにより生じた空隙や、刺激物質によって脆弱となった細胞-細胞間に病理標本作製過程で間隙が生じることが知られている。

なお、組織標本の評価は検査担当者の見解の基に成り立っていることから、病理組織学的検査は、ICE 法の組織標本観察に習熟した病理専門家が行った上で、OECD GLP No.16 [4)] に準じて、別の病理学専門家によるピアレビューを実施することを定めている。

3−2−2．病理組織学的検査の追加結果

TG 438（2013）の検証 [5)]において偽陰性率が高かった洗浄剤および界面活性剤について、検出感度を向上させるため、病理組織学的検査の併用が検証された。検証では、まず洗浄剤（1 種類以上の界面活性剤を 3 %超含む洗浄用混合物、界面活性剤単体の希釈液は含まない）のうち、2<pH<11.5 の 30 物質 [6)]、pH≤2 の 9 物質および 11.5≤pH の 9 物質 [7)]について病理組織学的検査を併用し、重篤な眼の損傷を引き起こす可能性（UN GHS 区分 1）を評価した。その結果、2<pH<11.5 の洗浄剤では、病理組織学的検査の併用により感度が向上した（0%から 75%、n=8）[6)]。一方、pH≤2 または 11.5≤pH の洗浄剤では、病理組織学的検査の併用により、偽陽性率が高くなり（17%から 67%、n=12）、その結果、特異性が 83%から 33%に低下した [7)]。

これらの結果を受け、2<pH<11.5 の洗浄剤および界面活性剤に限り病理組織学的検査を併用することが検討され、最終的に 30 物質について検証された [8)]。このうちの 1 物質がアルカリ洗剤（pH=12.0）であったことから、本委員会では、それを除く 29 物質につい

て解析した（表 6）。

表 6.　最終評価に用いた 29 物質の内訳 [8)]

	区分 1	区分 2	区分外
界面活性剤	13	2	3
洗浄剤（2 < pH < 11.5）	8	2	1

区分外：区分１および区分２のいずれにも該当しない。

その結果、表 7 に示すように、*in vivo* の判定との比較において、病理組織学的検査を併用することにより、感度が 33%から 71%に向上し、正確度も 52%から 76%に上昇した。

表 7.　トップダウン方式において病理組織学的検査を併用し 2<pH<11.5 の洗浄剤および界面活性剤を判定した場合の正確性の検証結果 [2)]

病理組織学的検査	No.	正確度		感度		偽陰性率		特異度		偽陽性率	
		%	No.	%	No.	%	No.	%	No.	%	No.
併用あり（2018)	29	76	22/29	71	15/21	29	6/21	87	7/8	13	1/8
併用なし（2013）	29	52	15/29	33	7/21	67	14/21	100	8/8	0	0/8

さらに、UN GHS 区分 1 の 6 物質、区分 2A の 3 物質、区分に該当しない 3 物質の計 12 物質の界面活性剤について、3 施設で UN GHS 区分 1 物質であるか否かを判定した場合の再現性を確認した結果 [8)]、施設間再現性は 83%（10/12）となり、良好であった。

以上、2<pH<11.5 の洗浄剤および界面活性剤については、トップダウン方式において偽陰性率が高いことから、標準的な ICE 法で陰性結果が得られた際には、病理組織学的検査を併用して判定することを推奨する。

3−3．その他

3−3−1．習熟度確認物質の変更：

TG 438（2013）では、ICE 法の習熟度確認物質として 13 物質が推奨された。本改定版では、このうち 4 物質が変更（濃度のみの変更を含む）され、表 8 に示す 13 物質になった。

表 8.　ICE 法の習熟度確認物質 [2)]

化学物質	CAS 番号	分類	物理的性状	*In Vivo* UN GHS 区分	ICE UN GHS 区分
Benzalkonium chloride (10%)	8001-54-5	オニウム化合物類	液体	区分 1	区分 1
Chlorhexidine	55-56-1	アミン類 アミジン類	固体	区分 1	区分 1
Sodium hydroxide (10%)	1310-73-2	塩基	液体	区分 1	区分 1
Imidazole	288-32-4	ヘテロサイクリック化合物類	固体	区分 1	区分 1
Trichloroacetic acid (30%)	76-03-9	カルボン酸類	液体	区分 1	区分 1
2,6- Dichlorobenzoyl chloride	4659-45-4	アシルハライド類	液体	区分 2A	予測不可
Ammonium nitrate	6484-52-2	無機塩	固体	区分 2B	予測不可
Sodium hydroxide (1%)	1310-73-2	塩基	液体	区分 2B	予測不可
Dimethyl sulfoxide	67-68-5	有機硫黄化合物	液体	区分外	区分外
Ethyl trimethyl acetate	3938-95-2	エステル	液体	区分外	区分外
Methylcyclopentane	96-37-7	炭化水素（環状）	液体	区分外	区分外
n-Hexane	110-54-3	炭化水素（鎖状）	液体	区分外	区分外
Triacetin	102-76-1	脂質	液体	区分外	区分外

区分外：表 1 参照。

予測不可：エンドポイントのスコアの組み合わせが区分 1 および区分外のどちらにも該当しないため、ICE における区分が判定できない

3−3−2．病理組織学的検査における習熟度確認物質

今回、新たに ICE 法の病理組織学的検査における習熟度確認物質として、表 9 に示す 6 物質（同一物質の濃度違いを含む）が推奨された。

表 9．ICE 法の病理組織学的検査の習熟度確認物質 [2)]

化学物質	CAS 番号	界面活性剤の種類	物理的性状	UN GHS 区分		
				In Vivo	標準的 ICE	ICE 病理組織学的検査*
Benzalkonium chloride (5%)	8001-54-5	カチオン	液体	区分 1	区分 1	区分 1（びらん）
Benzensulphonyl-chloride	98-09-9	アニオン	液体	区分 1	区分 1	区分 1（壊死および空胞化）
Cetylpiridinium bromide (10%)	140-72-7	カチオン	液体	区分 1	予測不可	区分 1（空胞化）
Cetylpiridinium bromide (1%)	140-72-7	カチオン	液体	区分 2A	予測不可	予測不可
N-Lauroyl sarcosine Na salt (10%)	137-16-6	アニオン	液体	区分 2A	予測不可	予測不可
Cetylpiridinium bromide (0.1%)	140-72-7	カチオン	液体	区分外	予測不可	予測不可

*表 3 ～ 表 5 の基準に従って判定し、合致することを確認する。

区分外：区分に該当しない

4．結論

JaCVAM 眼刺激性試験資料編纂委員会は、TG 438（2018）に基づいた正確性と再現性の結果から、UN GHS の眼刺激性分類においてトップダウ ン方式における区分 1 物質の同定およびボトムアップ方式における区分に該当しない物質の同定に ICE 法を適用することは可能であると考えた。また、2<pH<11.5 の洗浄剤および界面活性剤については、トップダウン方式において陰性結果が得られた場合、病理組織学的検査を併用することを推奨する。

用語（TG 438 ANNEX 1, DEFINITIONS）[2)]

トップダウン方式：

TG 438（2018）では、眼に重篤な損傷を引き起こす疑いのある化学物質に適用する段階的な評価方式を指し、眼に重篤な損傷を引き起こす化学物質（陽性結果）と、それ以外の化学物質（陰性結果）を 識別することから始める。

ボトムアップ方式：

TG 438（2018）では、眼刺激性あるいは重篤な眼の損傷性はないと予測される化学物質に適用される段階的な評価方式を指し、眼刺激性物質に区分されない化学物質と、それ以外の化学物質を識別することから始める。

区分 1 物質:

UN GHS 分類体系 [2)]下、眼球表面へ適用することにより、眼球の組織損傷や重篤な視力低下を引き起こす化学物質。いわゆる「重篤な眼の損傷」や「眼に対する非可逆的な作用」を示し、その損傷は適用後 21 日を経ても十分には回復しない。

区分 2 物質:

UN GHS 分類体系下、眼球表面へ適用することにより、眼球の変化をもたらす化学物質。「眼刺激性」や「眼に対する可逆的な作用」を示し、その損傷は適用後 21 日以内に完全に回復する。

区分外または UN GHS 区分に該当しない物質:

UN GHS 分類体系下、GHS 区分 1 あるいは 2（2A または 2B）への分類を要求されるような刺激性を有しておらず、眼刺激性物質として区分されない化学物質。

引用文献

1) United nations (UN) (2017). Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Seventh revised edition, UN New York and Geneva, 2017. Available at: [https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev07/English/03e_part3.pdf]

2) OECD Guidelines for The Testing of Chemicals, Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, TG 438 (Adopted 25 June 2018)

3) OECD (2017) Guidance Document on "The Bovine Corneal Opacity and Permeability (BCOP) and the Isolated Chicken Eye (ICE) Test Methods: Collection of Tissues for Histological Evaluation and Collection of Data on Non-Severe Irritants. Series on Testing and Assessment No. 160, ENV/JM/MONO(2011)45/REV1. Available at: [https://www.oecd.org/ chemicalsafety /testing/series-testing-assessment-publications-number.htm]

4) OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 16 Advisory Document of the Working Group on Good Laboratory Practice -Guidance on the GLP Requirements for Peer Review of Histopathology, JT03362564 26-Sep-2014

5) OECD Guidelines for The Testing of Chemicals, Isolated Chicken Eye Test Method for

Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, TG 438 (Adopted: 26 July 2013)

6) Cazelle E., Eskes C., Hermann M., et al. (2014). Suitability of Histopathology as an Additional Endpoint to the Isolated Chicken Eye Test for classification of non-extreme pH detergent. Toxicology In Vitro 28, 657-666.
7) Cazelle E., Eskes C., Hermann M., et al. (2015). Suitability of the Isolated Chicken Eye Test for Classification of Extreme pH Detergents and Cleaning Products. Toxicology In Vitro 29, 609-616.
8) OECD (2019). Streamlined Summary Document Supporting OECD Test Guideline 438 on the Isolated Chicken Eye for Eye Irritation/Corrosion. Series on Testing and Assessment No 188. ENV Publications, OECD, Paris.

OECD/OCDE 438

Adopted:
25 June 2018

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Isolated chicken eye test method for identifying I) chemicals inducing serious eye damage and II) chemicals not requiring classification for eye irritation or serious eye damage

INTRODUCTION

1. The Isolated Chicken Eye (ICE) test method was evaluated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), in conjunction with the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Centre for the Validation of Alternative Methods (JaCVAM), in 2006 and 2010 (1) (2) (3). In the original evaluation, the ICE was endorsed as a scientifically valid test method for use as a screening test to identify chemicals (substances and mixtures) inducing serious eye damage (Category 1) as defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (1) (2) (4). A re-evaluation of the in vitro and in vivo dataset used in the validation study concluded that the ICE test method could also be used to identify chemicals not requiring classification for eye irritation and serious eye damage as defined by the UN GHS which led to the revised version of TG 438 adopted in 2013 (4) (5). Since then, the Decision Criteria used to identify chemicals not requiring classification according to the UN GHS Classification System, has been revised based on the latest acceptance standards (5) (6) (7) (8). Furthermore, histopathology has been shown to be a useful additional endpoint to identify UN GHS Category 1 non-extreme pH ($2 < Ph < 11.5$) detergents and surfactants (9) (10). This Test Guideline (adopted in 2009 and updated in 2013 and in 2018) includes the latest recommended uses and limitations of the ICE test method based on these evaluations.

2. It is currently generally accepted that, in the foreseeable future, no single in vitro eye irritation test will be able to fully replace the in vivo Draize eye test to predict across the full range of irritation for different chemical classes. However, strategic combinations of alternative test methods within a (tiered) testing strategy and/or Integrated Approaches to Testing and Assessment (IATA) may be able to

In accordance with the decision of the Council on a delegation of authority to amend Annex I of the decision of the council on the Mutual Acceptance of Data in the assessment of chemicals [C(2018)49], this Guideline was amended by the OECD's Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology by written procedure on 25 June 2018.

replace the Draize eye test (7)(11). The Top-Down approach is designed to be used when, based on existing information, a chemical is expected to have high irritancy potential, while the Bottom-Up approach is designed to be used when, based on existing information, a chemical is expected not to cause sufficient eye irritation to require a classification (7)(11). The ICE test method is an in vitro test method that can be used, under certain circumstances and with specific limitations as described in paragraphs 7 to 11 for eye hazard classification and labelling of chemicals. While it is not considered valid as a stand-alone replacement for the in vivo rabbit eye test, the ICE test method is recommended as an initial step within a testing strategy such as the Top-Down approach recommended within the OECD GD 263 (7) to identify chemicals inducing serious eye damage, i.e., chemicals to be classified as UN GHS Category 1 without further testing (4). The ICE test method is also recommended to identify chemicals that do not require classification for eye irritation or serious eye damage as defined by the UN GHS (No Category) (4), and may therefore be used as an initial step within a Bottom-Up testing strategy approach (OECD GD 263 (7). However, a chemical that is not predicted as causing serious eye damage or as not classified for eye irritation/serious eye damage with the ICE test method would require additional information to establish a definitive classification. Choice of the most appropriate test method(s) and use of this Test Guideline should be seen in the context of the OECD Guidance Document on an Integrated Approach on Testing and Assessment for Serious Eye Damage and Eye irritation (7). Furthermore, the appropriate regulatory authorities should be consulted before using the ICE test method in a Bottom-Up approach for classification schemes other than the UN GHS.

3. The purpose of this Test Guideline is to describe the procedures used to evaluate the eye hazard potential of a test chemical as measured by its ability to induce or not induce toxicity in the enucleated eyes of chicken. Toxic effects to the cornea are measured by (i) a qualitative assessment of opacity, (ii) a qualitative assessment of damage to epithelium based on application of fluorescein to the eye (fluorescein retention), (iii) a quantitative measurement of increased thickness (swelling), and (iv) a qualitative evaluation of macroscopic morphological damage to the surface of the treated eyes. The corneal opacity, swelling, and damage assessments following exposure to a test chemical are assessed individually and then combined to derive an Eye Irritancy Classification. Furthermore, histopathological observations may also be used as an additional endpoint to potentially improve the prediction of UN GHS Category 1 non-extreme pH ($2 < pH < 11.5$) detergents and surfactants (see paragraphs 8 and 56).

4. Definitions are provided in Annex 1.

INITIAL CONSIDERATIONS AND LIMITATIONS

5. This Test Guideline is based on the protocol suggested in the OECD Guidance Document 160 (12), which was originally adopted in 2011 and further updated in 2017 and 2018. The protocol is based on information obtained from published protocols (13) (14) (15) (16) (17).

6. A wide range of chemicals has been tested in the evaluation underlying this Test Guideline and the overall database currently amounts to 184 test chemicals including 75 substances and 109 mixtures (5). The Test Guideline is applicable to solids, liquids, emulsions and gels. The liquids may be aqueous or non-aqueous; solids may be soluble or insoluble in water. Gases and aerosols have not been assessed yet in a validation study.

7. The ICE test method can be used to identify chemicals inducing serious eye damage, i.e., chemicals to be classified as UN GHS Category 1 (4). When used for this purpose, the identified limitations for the ICE test method are based on the high false positive rates for alcohols and the high false negative rates for solids and surfactants (1) (3) (18). Moreover, test chemicals inducing persistent non severe effects in vivo may also risk underprediction (22). However, false negative rates in this context (UN GHS Category 1 identified as not being UN GHS Category 1) are not critical since all test chemicals that come out negative would be subsequently tested with other adequately validated in vitro test(s), or as a last option in rabbits, depending on regulatory requirements, using a sequential testing strategy in a weight-of-evidence approach. Furthermore, histopathology was found to be a useful additional endpoint to decrease the false negative rates when used to identify UN GHS Category 1 non-extreme pH ($2 < pH < 11.5$) detergents shown to induce mainly persistent non severe effects in vivo (and surfactants (9) (10) (19). Regarding solids, it should be noted that these may lead to variable and extreme exposure conditions in the in vivo Draize eye irritation test, which may result in irrelevant predictions of their true irritation potential (20). Investigators could consider using this test method for all types of chemicals, whereby a positive result should be accepted as indicative of serious eye damage, i.e., UN GHS Category 1 classification without further testing. However, positive results obtained with alcohols should be interpreted cautiously due to risk of over-prediction.

8. When used to identify chemicals inducing serious eye damage (UN GHS Category 1), the ICE test method (without use of histopathology) was found to have an overall accuracy of 83% (142/172), a false positive rate of 7% (9/127) and a false negative rate of 47% (21/45) when compared to in vivo rabbit eye test method data classified according to the UN GHS classification system (4) (5). When histopathology is considered as an additional endpoint to identify UN GHS Category 1 non-extreme pH ($2 < pH < 11.5$) detergents and surfactants, the false negative rate of the ICE test method and its accuracy are improved (from 64% to 27% false negatives (n=22) and from 53% to 77% accuracy (n=30)), whilst an acceptable false positive rate is maintained (from 0% to 12.5% false positives (n=8)) (10).

9. The ICE test method can also be used to identify chemicals that do not require classification for eye irritation or serious eye damage under the UN GHS classification system (4). The test method can be used for all types of chemicals, whereby a negative result could be accepted for not classifying a chemical for eye irritation and serious eye damage. However, on the basis of one result from the validation database, anti-fouling organic solvent-containing paints may be under-predicted (5).

10. When used to identify chemicals that do not require classification for eye irritation and serious eye damage, the ICE test method has an overall accuracy of 88% (161/184), a false positive rate of 24% (20/83), and a false negative rate of 3% (3/101), when compared to in vivo rabbit eye test method data classified according to the UN GHS (4) (5). When test chemicals within certain classes (i.e., anti-fouling organic solvent containing paints) are excluded from the database, the accuracy of the ICE test method is 88% (159/181), the false positive rate 24% (20/83), and the false negative rate of 2% (2/99) for the UN GHS classification system (4).

11. The ICE test method is not recommended for the identification of test chemicals that should be classified as irritating to eyes (i.e., UN GHS Category 2 or Category 2A) or test chemicals that should be classified as mildly irritating to eyes (UN GHS Category 2B) due to the considerable number of UN GHS Category 1 chemicals underclassified as UN GHS Category 2, 2A or 2B and UN GHS No Category chemicals overclassified as UN GHS Category 2, 2A or 2B. For this purpose, further information and if needed, additional testing with another suitable method may be required.

12. All procedures with chicken eyes should follow applicable geographical regulations and the test facility's procedures for handling of human or animal-derived materials, which include, but are not limited to, tissues and tissue fluids. Universal laboratory precautions are recommended (21).

13. Whilst the ICE test method does not directly address conjunctival and iridial injuries as evaluated in the rabbit ocular irritancy test method, it addresses corneal effects which are the major driver of classification in vivo when considering the UN GHS Classification. In this respect, it should be noted that effects on the iris are of lesser importance for classification of chemicals according to UN GHS (8) (22). Also, although the reversibility of corneal lesions cannot be evaluated per se in the ICE test method, it has been shown that histopathological observations can help in identifying test chemicals causing irreversible effects not linked with initial high level injury such as those caused by non-extreme pH ($2 < pH < 11.5$) detergents (9). Finally, the ICE test method does not allow for an assessment of the potential for systemic toxicity associated with ocular exposure.

14. This Test Guideline will be updated periodically as new information and data are considered. For example, further histopathology data may become available for test chemicals other than non-extreme pH detergents and surfactants. To evaluate this possibility, users are encouraged to preserve eyes and prepare histopathology specimens that can be used to develop a database and decision criteria that may further improve the accuracy of this test method. The OECD has developed Guidance Document 160 to be considered when using the ICE and BCOP in vitro ocular toxicity test methods, which includes detailed procedures on the collection and processing of histopathology specimens for evaluation (12).

DEMONSTRATION OF PROFICIENCY

15. For any laboratory initially establishing the standard ICE test method, the proficiency chemicals provided in Annex 2 should be used. A laboratory can use these chemicals to demonstrate their technical competence in performing the standard ICE test method prior to submitting ICE data for regulatory hazard classification purposes. For any laboratory willing to establish ICE histopathology for the regulatory hazard classification of non-extreme pH detergents and surfactants, the ICE Atlas and recommendations provided within the revised OECD GD 160 should be used (12). Consolidated training, transferability and proficiency appraisal are recommended to ensure harmonized, consistent and reproducible histopathological observations. Furthermore, an internal pathology peer review should be conducted in accordance with current recommendations (23) and according to the OECD advisory document n. 16 on GLP requirements for peer review of histopathology (24), and as described in paragraph 50. Such peer review process allows to verify and improve the accuracy and quality of pathology diagnoses and interpretations. Finally, the proficiency chemicals provided in Annex 3 should be used for a laboratory to demonstrate technical competence in scoring the ICE histopathology effects, prior to submitting ICE histopathology data for the regulatory hazard classification of non-extreme pH detergents and surfactants.

PRINCIPLE OF THE TEST

16. The ICE test method is an organotypic model that provides short-term maintenance of the chicken eye in vitro. In this test method, damage by the test chemical is assessed by determination of corneal swelling, opacity, and fluorescein retention. Furthermore, histopathology can be used to increase the sensitivity of the method for identifying UN GHS Category 1 non-extreme pH ($2 < pH < 11.5$) detergents and surfactants (10). Whilst measurement of corneal swelling provides for a quantitative assessment, corneal opacity, fluorescein retention and histopathological changes each involve a qualitative assessment. Each measurement is either converted into a quantitative score used to assign an ICE Class (I to IV), or assigned a qualitative categorization that is used to assign an in vitro ocular hazard classification, either as UN GHS Category 1 or as UN GHS No Category (see Decision Criteria). However, no prediction can be made for chemicals not identified as UN GHS Category 1 or as UN GHS No Category with the ICE test method (see paragraph 11); in these cases, the "No prediction can be made" result of the ICE test would require additional information for classification purposes [see (7) for guidance].

Source and Age of Chicken Eyes

17. Historically, eyes collected from slaughterhouse chickens killed for human consumption have been used for this assay, eliminating the need for laboratory animals. Only the eyes of healthy animals considered suitable for entry into the human food chain are used.

18. Although a controlled study to evaluate the optimum chicken age has not been conducted, the age and weight of the chickens used historically in this test method are that of spring chickens traditionally processed by a poultry

slaughterhouse (i.e., approximately 7 weeks old, 1.5 - 2.5 kg).

Collection and Transport of Eyes to the Laboratory

19. Heads should be removed immediately after humane stunning of the chickens and incision of the neck for bleeding. Humane stunning methods include electrical stunning and controlled atmosphere stunning, as long as it can be shown not to adversely impact the quality of the chicken eyes (see paragraph 21). A local source of chickens close to the laboratory should be located so that their heads can be transferred from the slaughterhouse to the laboratory quickly enough to minimize deterioration and/or bacterial contamination. The time interval between collection of the chicken heads and placing the eyes in the superfusion chamber following enucleation should be minimized (typically within two hours) to assure meeting assay acceptance criteria. All eyes used in the assay should be from the same group of eyes collected on a specific day.

20. Since eyes are dissected in the laboratory, the intact heads are transported from the slaughterhouse at ambient temperature (typically between 18°C and 25°C) in plastic boxes humidified with tissues moistened with isotonic saline.

Selection Criteria and Number of Eyes Used in the ICE

21. Eyes that have high baseline fluorescein staining (i.e., > 0.5) or corneal opacity score (i.e., > 0.5) after they are enucleated are rejected.

22. Each treatment group and concurrent positive control consists of at least three eyes. The negative control group or the solvent control (if using a solvent other than saline) consists of at least one eye.

23. In the case of solid materials leading to a GHS No Category outcome, a second run of three eyes is recommended to confirm or discard the negative outcome.

PROCEDURE

Preparation of the Eyes

24. The eyelids are carefully excised, taking care not to damage the cornea. Corneal integrity is quickly assessed with a drop of 2% (w/v) sodium fluorescein applied to the corneal surface for a few seconds, and then rinsed with isotonic saline. Fluorescein-treated eyes are then examined with a slit-lamp microscope to ensure that the cornea is undamaged (i.e., fluorescein retention and corneal opacity scores ≤ 0.5).

25. If undamaged, the eye is further dissected from the skull, taking care not to damage the cornea. The eyeball is pulled from the orbit by holding the nictitating membrane firmly with surgical forceps, and the eye muscles are cut with a bent, blunt-tipped scissor. It is important to avoid causing corneal damage due to excessive pressure (i.e., compression artefacts).

26. When the eye is removed from the orbit, a visible portion of the optic nerve should be left attached. Once removed from the orbit, the eye is placed on

an absorbent pad and the nictitating membrane and other connective tissue are cut away.

27. The enucleated eye is mounted in a clamp (stainless steel or suitable alternative) with the cornea positioned vertically, and avoiding too much pressure on the eye by the clamp (due to the relatively firm sclera of the chicken eye-ball, only slight pressure is needed to fix the eye properly). The clamp is then transferred to a chamber of the superfusion apparatus (25). The clamps should be positioned in the superfusion apparatus such that the entire cornea is supplied with the isotonic saline drip (3-4 drops per minute or 0.1 to 0.15 mL/min). The chambers of the superfusion apparatus should be temperature controlled at 32 ± 1.5°C. Annex 4 provides a diagram of a typical superfusion apparatus and the eye clamps, which can be obtained commercially or constructed. The apparatus can be modified to meet the needs of an individual laboratory (e.g., to accommodate a different number of eyes).

28. After being placed in the superfusion apparatus, the eyes are again examined with a slit-lamp microscope (e.g., Haag-Streit BP900) to ensure that they have not been damaged during the dissection procedure. Corneal thickness should also be measured at this time at the corneal apex using the depth measuring device on the slit-lamp microscope. Eyes with; (i), a fluorescein retention score of > 0.5; (ii) corneal opacity > 0.5; or, (iii), any additional signs of damage should be replaced. For eyes that are not rejected based on any of these criteria, individual eyes with a corneal thickness deviating more than 10% from the mean value for all eyes are to be rejected. For the Haag-Streit slit lamp BP900 fitted with depth-measuring device no. 1, the slit-width setting should be 9½ equalling 0.095 mm. Alternatively the slit-lamp BQ900 from Haag-Streit may be used as long as it can be mounted with the depth measuring device and a slit width of 0.095 can be applied (see also paragraph 53). Users should be aware that slit-lamp microscopes could yield different corneal thickness measurements if the slit-width setting is different.

29. Once all eyes have been examined and approved, the eyes are incubated for approximately 45 to 60 minutes to equilibrate them to the test system prior to dosing. Following the equilibration period, a zero reference measurement is recorded for corneal thickness and opacity to serve as a baseline (i.e., time = 0). The fluorescein score determined at dissection is used as the baseline measurement for that endpoint.

Application of the Test Chemical

30. Immediately following the zero reference measurements, the eye (in its holder) is removed from the superfusion apparatus, placed in a horizontal position, and the test chemical is applied to the cornea.

31. Liquid test chemicals are typically tested undiluted, but may be diluted if deemed necessary (e.g., as part of the study design). The preferred solvent for dilution of test chemicals is physiological (isotonic) saline. However, alternative solvents may also be used under controlled conditions, but the appropriateness of solvents other than physiological saline should be demonstrated.

32. Liquid test chemicals are applied to the cornea such that the entire surface of the cornea is evenly covered with the test chemical; the standard volume is 0.03 mL.

33. If possible, solid test chemicals should be ground as finely as possible in a mortar and pestle, or comparable grinding tool. The powder is applied to the cornea such that the surface is uniformly covered with the test chemical; the standard amount is 0.03 g.

34. The test chemical (liquid or solid) is applied for 10 seconds and then rinsed from the eye with isotonic saline (approximately 20 mL) at ambient temperature. The eye (in its holder) is subsequently returned to the superfusion apparatus in the original upright position. In case of need, additional rinsing may be used after the 10-sec application and at subsequent time points (e.g., upon discovery of residues of test chemical on the cornea). In general the amount of saline additionally used for rinsing is not critical, but the observation of adherence of chemical to the cornea is important.

Control Chemicals

35. Concurrent negative or solvent/vehicle controls and positive controls should be included in each experiment.

36. When testing liquids at 100% or solids, physiological (isotonic) saline is used as the concurrent negative control in the ICE test method to detect non-specific changes in the test system, and to ensure that the assay conditions do not inappropriately result in an irritant response.

37. When testing diluted liquids, a concurrent solvent/vehicle control group is included in the test method to detect non-specific changes in the test system, and to ensure that the assay conditions do not inappropriately result in an irritant response. As stated in paragraph 31, only a solvent/vehicle that has been demonstrated to have no adverse effects on the test system can be used.

38. A known ocular irritant is included as a concurrent positive control in each experiment to verify that an appropriate response is induced. As the ICE test method is being used in this Test Guideline to identify chemicals inducing serious eye damage, the positive control should be a reference chemical inducing responses that fulfil the criteria for classification as UN GHS Category 1 in this test method. However, to ensure that variability in the positive control response across time can be assessed, the magnitude of the severe response should not be excessive. Sufficient in vitro data for the positive control should be generated such that a statistically defined acceptable range for the positive control can be calculated. If adequate historical ICE test method data are not available for a particular positive control, studies may need to be conducted to provide this information.

39. Examples of positive controls for liquid test chemicals are 10% acetic acid or 5% benzalkonium chloride, while examples of positive controls for solid test chemicals are sodium hydroxide or imidazole.

40. Benchmark chemicals are useful for evaluating the ocular irritancy potential of unknown chemicals of a specific chemical or product class, or for

evaluating the relative irritancy potential of an ocular irritant within a specific range of irritant responses.

Endpoints Measured

41. Treated corneas are evaluated prior to treatment and at 30, 75, 120, 180, and 240 minutes (±5 minutes) after the post-treatment rinse. These time points provide an adequate number of measurements over the four-hour observation period, while leaving sufficient time between measurements for the requisite observations to be made for all eyes.

42. The endpoints evaluated are corneal opacity, swelling, fluorescein retention, and morphological effects (e.g., pitting or loosening of the epithelium). All of the endpoints, with the exception of fluorescein retention (which is determined only prior to treatment and 30 minutes after test chemical exposure) are determined at each of the above time points.

43. Photographs are advisable to document corneal opacity, fluorescein retention, morphological effects and, if conducted, histopathology.

44. After the final examination at four hours, users are encouraged to preserve eyes in an appropriate fixative (e.g., neutral buffered formalin) for possible histopathological examination in particular for non-extreme pH ($2 < pH < 11.5$) detergents and surfactants (see paragraphs 7, 14 and 56). If histopathology is conducted, eyes should be fixed, trimmed, embedded in paraffin wax, sectioned and stained according to the procedures described for the collection and processing of histopathology specimens within the OECD GD 160 (12).

45. Corneal swelling is determined from corneal thickness measurements made with an optical pachymeter on a slit-lamp microscope. It is expressed as a percentage and is calculated from corneal thickness measurements according to the following formula:

$$\left(\frac{corneal\ thickness\ at\ time\ t \quad - \quad corneal\ thickness\ at\ time = 0}{corneal\ thickness\ at\ time = 0} \right) \times 100$$

46. The mean percentage of corneal swelling for all test eyes is calculated for all observation time points. Based on the highest mean score for corneal swelling, as observed at any time point, an ICE Class is assigned for each test chemical (see paragraph 53).

47. Corneal opacity is evaluated by using the area of the cornea that is most densely opacified for scoring according to the observations described in Table 1. The mean corneal opacity value for all test eyes is calculated for all observation time points. Based on the highest mean score for corneal opacity, as observed at any time point, an ICE class is assigned for each test chemical (see paragraph 53).

Table 1. Corneal opacity scores

Score	Observation
0	No opacity
0.5	Very faint opacity
1	Scattered or diffuse areas; details of the iris are clearly visible
2	Easily discernible translucent area; details of the iris are slightly obscured
3	Severe corneal opacity; no specific details of the iris are visible; size of the pupil is barely discernible
4	Complete corneal opacity; iris invisible

48. Fluorescein retention is evaluated at the 30 minute observation time point only according to the scores shown in Table 2. The mean fluorescein retention value of all test eyes is then calculated for the 30-minute observation time point, and used to assign an ICE class for each test chemical (see paragraph 53).

Table 2. Fluorescein retention scores

Score	Observation
0	No fluorescein retention
0.5	Very minor single cell staining
1	Single cell staining scattered throughout the treated area of the cornea
2	Focal or confluent dense single cell staining
3	Confluent large areas of the cornea retaining fluorescein

49. Morphological effects include “pitting” of corneal epithelium, “loosening” of epithelium, “roughening” of the corneal surface and “sticking” of the test chemical to the cornea. These findings can vary in severity and may occur simultaneously. The classification of these findings is subjective according to the interpretation of the investigator.

50. If histopathology is conducted, the semi-quantitative scoring system described in Table 3 should be used. It is critical to distinguish, for example regarding epithelial vacuolation effects, the treatment-related effects from histopathological artefacts and/or background morphology. For this purpose the Atlas presented in Annex II of the OECD GD 160 should be carefully consulted (12). Furthermore, original slides (rather than photomicrographs) need to be used as some effects require a three-dimensional evaluation of the tissues. Only effects that are observed should be scored. No assumptions should be made (e.g., if the top layer of the epithelium is missing it will not be possible to score for vacuolation in that layer). Furthermore, effects/changes close to the limbus should be scored if the tissue architecture was preserved. However, effects/changes occurring within the limbus should not be scored due to effects not linked to the chemical exposure. An internal pathology peer review system should be conducted in accordance with current recommendations (23) and according to the

OECD advisory document n. 16 on GLP requirements for peer review of histopathology (24). In this process, a pathologist (with expertise on the tissues to be evaluated)peer-reviews a number of slides and pathology data (e.g., 1 out of 3 eyes) to assist the study pathologist in refining pathology diagnoses and interpretations. Such peer review process allows to verify and improve the accuracy and quality of pathology diagnoses and interpretations. Finally, consolidated training, transferability and proficiency appraisal are recommended to ensure consistent histopathological observations.

Table 3. Semi-quantitative histopathological scoring system used for isolated chicken eyes that were fixed, trimmed, embedded in paraffin wax, sectioned and stained

Parameter	Observation	Score	Description*
Epithelium: erosion	Very slight	½	Few single cells up to the entire single superficial layer
	Slight	1	Up to 3 layers are gone
	Moderate	2	Up to 50 % of the epithelial layer is gone*
	Severe	3	Epithelial layer is gone up to the basement membrane
Epithelium: vacuolation ***Separately scored for the top, mid, and lower parts of the epithelium*** [**]	Very slight	½	Single to few scattered cells
	Slight	1	Groups of vacuolated cells or single string of cells with small vacuoles
	Moderate	2	Up to 50% of the epithelium consists of vacuolated cells*
	Severe	3	50 – 100% of the epithelium consists of vacuolated cells
Epithelium:necrosis [***]	Normal	-	< 10 necrotic cells[†]
	Very slight	½	10 – 20 necrotic cells[†]
	Slight	1	20 – 40 necrotic cells[†]
	Moderate	2	Many necrotic cells but < 50% of the epithelial layer
	Severe	3	50 – 100% of the epithelial layer is necrotic.
Stroma: pyknotic nuclei [††; †††] ***In top or bottom region***	Normal	-	< 5 pyknotic nuclei
	Slight	1	5-10 pyknotic nuclei
	Moderate	2	> 10 pyknotic nuclei
Stromal disorder of fibres [†††]	Present	P	Irregular appearance of the fibres.
Endothelium:necrosis	Present	P	The endothelium consists of only one layer, so a grade is not relevant

Notes: Annex II of the OECD GD 160 (12) displays an Atlas with typical photomicrographs of untreated as well as treated Isolated Chicken Eyes illustrating the various possible histopathological effects described above.

[*]Over the entire cornea except in case of test chemicals (e.g. some solid chemicals) causing localized effects despite of the homogenous application of the test chemical as required within the OECD TG 438. In this case the evaluation should be based on the localized effects at the site(s) of exposure.

[**]Top, mid and lower parts represent equal one third parts of the epithelial layer each. If the top layer is missing, the mid layer does not become the 'new' top layer, but is still the mid layer (see Annex II of the OECD GD 160 for more details (12)).

[***] Only necrosis of attached cells/tissues.

[†] Necrotic cells are counted across the entire length of the cornea (there is no need for a specific fixed length to report cell counts because the entire length of the cornea is consistent on each slide as there is almost no variation in the size of the chicken eyes used and in the size of the samples evaluated microscopically). The scoring system uses absolute cell counts from 'normal' to 'slight', versus a percentage for 'moderate' and 'severe'. This is due to the way the evaluation is performed by the examiner: necrotic cells are seen as individual items. If there are more, they are usually scattered. Therefore the examiner counts them to get an impression of the amount of necrosis. This is in contrast to erosion, for which the first effect the examiner notices is that a part of the epithelium is missing, so it makes sense to use an estimated percentage of loss.

[††] The ICE test method already includes a precise measurement of the thickness of the cornea using a slit lamp microscope. Therefore, swelling of the stroma is not separately scored during the subsequent histopathological evaluation.

[†††] The stromal effects that are scored consist of (1) pyknotic nuclei, which originate from the scoring system used by Maurer (2001) based on his observations in corneas of rabbits after *in vivo* exposure (described as keratocyte loss/necrosis), and of (2) disorder of fibres. Regarding (1), the presence of pyknotic nuclei is observed only occasionally and the development of pyknotic nuclei is proposed to be dependent on the depth of injury and/or the inflammation process of the cornea *(in vivo)*. Furthermore, due to the elongated form of the stromal fibroblasts, normal nuclei could be misleadingly considered as pyknotic nuclei depending on the section orientation of cells . Regarding (2), the observation and scoring of disorder of fibres may be difficult because the stromal fibres already show a "natural" disorder. The processing of the cornea for microscopy can also contribute to an artificial disorder of stromal fibres. In both cases (pyknotic nuclei and disorder of fibres), these observations coincide with severe corneal effects already observed by the slit-lamp microscope observations, and with effects observed in the mid and/or lower epithelial layer.

52. The OECD TG 438 requires test chemicals to be homogenously distributed on the surface of the treated eyes. Based on such exposure, test chemicals usually cause homogenous effects in the cornea of the isolated chicken eyes, and the mean of histopathological effects over the entire slide should be scored. However, some test chemicals may cause focal or multifocal effects confined to certain spots despite their homogenous application (e.g., as for some solid test chemicals). If (multi)focal effects are observed during the performance of the ICE test method, the histopathologist should be informed and the histopathological scoring should be conducted based on the localized adverse effects observed where exposure to the test chemical occurred. Furthermore, if doubts remain (e.g. a discrepancy between the ICE results and the histopathological observations is noticed), additional slices may be prepared on other parts of the cornea to ensure the localized effects are present in the observed section.

DATA AND REPORTING

Data Evaluation

53. Results from corneal opacity, swelling and fluorescein retention should be evaluated separately to generate an ICE class for each endpoint. The ICE classes for each endpoint are then combined to predict the In Vitro Classification of each test chemical. Similarly, histopathology evaluation, if applicable, should be conducted separately and considered according to paragraphs 55 and 56.

Decision Criteria

54. Once each endpoint has been evaluated, ICE classes can be assigned based on a predetermined range. Interpretation of corneal swelling (Table 4), opacity (Table 5), and fluorescein retention (Table 6) using four ICE classes is

done according to the scales shown below. It is important to note that the corneal swelling scores shown in Table 4 are only applicable if thickness is measured with a Haag-Streit BP900 slit-lamp microscope (or alternatively a Haag-Streit BQ900 slit-lamp microscope) with depth-measuring device no. 1 and slit-width setting at 9½, equalling 0.095 mm. Users should be aware that slit-lamp microscopes could yield different corneal thickness measurements if the slit-width setting is different.

Table 4. ICE classification criteria for corneal swelling

Mean Corneal Swelling (%)*	ICE Class
0 to 5	I
>5 to 12	II
>12 to 18 (>75 min after treatment)	II
>12 to 18 (=75 min after treatment)	III
>18 to 26	III
>26 to 32 (>75 min after treatment)	III
>26 to 32 (=75 min after treatment)	IV
>32	IV

Note: Highest mean score observed at any time point.

Table 5. ICE classification criteria for opacity.

Maximum Mean Opacity Score*	ICE Class
0.0-0.5	I
0.6-1.5	II
1.6-2.5	III
2.6-4.0	IV

Note: *Maximum mean score observed at any time point (based on opacity scores as defined in Table 1). *Based on scores as defined in Table 2.

Table 6. ICE classification criteria for mean fluorescein retention.

Mean Fluorescein Retention Score at 30 minutes post-treatment*	ICE Class
0.0-0.5	I
0.6-1.5	II
1.6-2.5	III
2.6-3.0	IV

Note: Based on scores as defined in Table 2.

55. The in vitro classification for a test chemical is assessed by reading the UN GHS classification that corresponds to the combination of categories obtained for corneal swelling, corneal opacity, and fluorescein retention as described in Table 7.

Table 7. Overall in vitro classifications

UN GHS Classification	Combinations of the 3 Endpoints
No Category	3 x I
	2 x I, 1 x II
	2 x II, 1 x I
No prediction can be made	Other combinations
Category 1	3 x IV
	2 x IV, 1 x III
	2 x IV, 1 x II*
	2 x IV, 1 x I*
	Corneal opacity = 3 at 30 min (in at least 2 eyes)
	Corneal opacity = 4 at any time point (in at least 2 eyes)
	Severe loosening of the epithelium (in at least 1 eye)

Note: Combinations less likely to occur.

56. If histopathology is used for non-extreme pH ($2 < pH < 11.5$) detergents and surfactants, the decision criteria shown in Table 8 should be used. In addition, in case stromal pyknotic nuclei scores ≥ slight (score 1) in at least 2 out of 3 eyes are observed; or any endothelium effects are observed in at least 2 out of 3 eyes, such effects should be noted as observations to give indication on the severity of effects.

Table 8. Histopathology decision criteria to be used in addition to the standard validated ICE test method for the identification of UN GHS Category 1 non-extreme pH (2<pH<11.5) detergents and surfactants

Tissue layer	Effects triggering eye serious damage (GHS Category 1) identification
Epithelium	- erosion = moderate (score 2) in at least 2 out of 3 eyes
	- and/or, any vacuolation (= very slight, score ½) observed in the mid and/or lower parts in at least 2 out of 3 eyes
	- or, if erosion = moderate (score 2) in 1 out of 3 eyes + vacuolation = very slight in mid and/or low part (score ½) is observed in at least another eye out of the 3 eyes
	- and/or, necrosis = moderate (score 2) observed in at least 2 out of 3 eyes

57. Furthermore, the prediction model shown in table 9 should be used. The ICE histopathology criteria and the prediction model described in Tables 8 and 9,

respectively are applicable only to identify UN GHS Category 1 non-extreme pH (2 < pH < 11.5) detergents and surfactants.

Table 9. Prediction model for identification of non-extreme pH (2<pH<11.5) detergents and surfactants based on ICE histopathology evaluations

Standard ICE	ICE histopathology criteria described in Table 8	UN GHS Classification
No prediction can be made	Criteria met	UN GHS Category 1
	Criteria not met	No prediction can be made

Study Acceptance Criteria

58. A test is considered acceptable if the concurrent negative or vehicle/solvent controls and the concurrent positive controls are identified as GHS Non-Classified and GHS Category 1, respectively.

Test Report

59. The test report should include the following information, if relevant to the conduct of the study:

Test and Control Chemicals

- Chemical identification, such as IUPAC or CAS name(s), CAS registry number(s), SMILES or InChI code, structural formula, and/or other identifiers;
- Purity and composition of the test/control substance or mixture (in percentage(s) by weight), to the extent this information is available;
- In case of multi-constituent and UVCB: characterization as far as possible by e.g., chemical identity (see above), purity, quantitative occurrence and relevant physicochemical properties (see above) of the constituents, to the extent available;
- Physicochemical properties such as physical state, volatility, pH, stability, chemical class water solubility relevant to the conduct of the study;
- Treatment of the test/control chemical prior to testing, if applicable (e.g. warming, grinding);
- Storage conditions and stability to the extent available;

Information Concerning the Sponsor and the Test Facility

- Name and address of the sponsor, test facility and study director; where applicable, the study pathologist;
- Identification on the source of the eyes (e.g., the facility from which they were collected);

Test Method Conditions

- Description of test system used;
- Slit-lamp microscope and pachymeter used (e.g., model) and the instrument settings used;
- Reference to historical negative and positive control results and, if applicable, historical data demonstrating acceptable concurrent benchmark control ranges;
- The procedure used to ensure the integrity (i.e., accuracy and reliability) of the test method over time (e.g., periodic testing of proficiency chemicals)).
- The procedure used for tissues fixation in case histopathology is performed.

Eyes Collection and Preparation

- Age and weight of the donor animal and if available, other specific characteristics of the animals from which the eyes were collected (e.g. sex, strain);
- Storage and transport conditions of eyes (e.g., date and time of eye collection, time interval between collection of chicken heads and placing the enucleated eyes in superfusion chamber);
- Preparation & mounting of the eyes including statements regarding their quality, temperature of eye chambers, and criteria for selection of eyes used for testing.

Test Procedure

- Number of replicates used;
- Identity of the negative and positive controls used (if applicable, also the solvent and benchmark controls);
- Test chemical dose, application and exposure time used;
- Observation time points (pre- and post- treatment);
- Description of evaluation and decision criteria used including for histopathology if applicable;
- Peer-review system used for histopathological observations, if applicable;
- Description of study acceptance criteria used;
- Description of any modifications of the test procedure.
- Furthermore, if not included in the e.g. standard operating procedure (SOP), when available, the following information shall be included:
- Description of consolidated training and transferability;
- Fixative, dehydration and clarifying agents, and protocols used;
- Embedding material, infiltration solvents, and concentrations used;
- Thickness of tissue sections;
- Stain (in report) and the associated staining protocol used;
- Information on instruments used;

Results

- Tabulation of corneal swelling, opacity and fluorescein retention scores obtained for each individual eye and at each observation time point, including the mean scores at each observation time of all tested eyes;
- Description of any morphological effects observed;
- The highest mean corneal swelling, opacity and fluorescein retention scores observed (from any time point), and its relating ICE class.;
- Tabulation of histopathological semi-quantitative scoring observations and derived conclusions if applicable;
- If applicable, indication of use of localized effects for histopathological scoring;
- Description of any other effects observed;
- The derived in vitro GHS classification;
- If appropriate, photographs of the treated and control eyes
- If applicable, optional digital images or digital slide scans of the histopathology specimens;

Discussion of the Results.

Conclusion.

Literature

(1) ICCVAM (2007). Test Method Evaluation Report - In Vitro Ocular Toxicity Test Methods for Identifying Ocular Severe Irritants and Corrosives. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). NIH Publication No.: 07-4517. Available at: [http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm]

(2) ESAC (2007). Statement on the conclusion of the ICCVAM retrospective study on organotypic in vitro assays as screening tests to identify potential ocular corrosives and severe eye irritants. Available at: [http://ecvam.jrc.it/index.htm].

(3) ICCVAM (2010). ICCVAM Test Method Evaluation Report – Current Status of In Vitro Test Methods for Identifying Mild/Moderate Ocular Irritants: The Isolated Chicken Eye (ICE) Test Method. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). NIH Publication No.: 10-7553A. Available at: [http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm]

(4) United nations (UN) (2017). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Seventh revised edition, UN New York and Geneva, 2017. Available at: https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev07/English/03e_part3.pdf.

(5) OECD (2018). Streamlined Summary Document Supporting OECD Test Guideline 438 on the Isolated Chicken Eye for Eye Irritation/Corrosion. Series on Testing and Assessment No 188. ENV Publications, OECD, Paris.

(6) OECD (2018). Test Guideline 492. OECD Guideline for Testing of Chemicals. Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage. Organisation for Economic Cooperation and Development, Paris, France. Available at: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.

(7) OECD (2018). Guidance Document on an Integrated Approach on Testing and Assessment for Serious Eye Damage and Eye Irritation. Series on Testing and Assessment, No. 263. Environment, Health and Safety Publications, Organisation for Economic Cooperation and Development, Paris, France.

(8) Adriaens E., Barroso J., Eskes C., Hoffmann S., McNamee P., Alépée N., Bessou-Touya S., De Smedt A, de Wever B., Pfannenbecker U., Tailhardat M., Zuang V. (2014). Draize test for serious eye damage / eye irritation: importance of the endpoints evaluated with regard to UN GHS / EU CLP classification. Archives of Toxicology 88, 701-723.

(9) Cazelle E., Eskes C., Hermann M., Jones P., McNamee P., Prinsen M., Taylor H., Wijnands M.V.W. (2014). Suitability of histopathology as an additional endpoint to the isolated chicken eye test for classification of non-extreme pH detergent and cleaning products. Toxicology In Vitro 28, 657-666.

(10) Reproducibility and predictive capacity of the Isolated Chicken Eye Test for the identification of non-extreme pH detergents and surfactants causing serious eye damage. Manuscript in preparation

(11) Scott L, Eskes C, Hoffman S, Adriaens E, Alepee N, Bufo M, Clothier R, Facchini D, Faller C, Guest R, Hamernik K, Harbell J, Hartung T, Kamp H, Le Varlet B, Meloni M, Mcnamee P, Osborn R, Pape W, Pfannenbecker U, Prinsen M, Seaman C, Spielmann H, Stokes W, Trouba K, Vassallo M, Van den Berghe C, Van Goethem F, Vinardell P, Zuang V (2010). A proposed Eye Irritation Testing Strategy to Reduce and Replace in vivo Studies Using Bottom-Up and Top-Down Approaches. Toxicology In Vitro 24, 1-9.

(12) OECD (2018) Guidance Document on "The Bovine Corneal Opacity and Permeability (BCOP) and Isolated Chicken Eye (ICE) Test Methods: Collection of Tissues for Histological Evaluation and Collection of Data on Non-Severe Irritants. Series on Testing and Assessment No. 160. **Error! Hyperlink reference not valid.**Available at: http://www.oecd.org/env/ehs/testing/series-testing-assessment-publications-number.htm.

(13) Prinsen, M.K. and Koëter, B.W.M. (1993). Justification of the enucleated eye test with eyes of slaughterhouse animals as an alternative to the Draize eye irritation test with rabbits. Fd. Chem. Toxicol. 31, 69-76.

(14) DB-ALM (INVITTOX) (2009). Protocol 80: Chicken enucleated eye test (CEET) / Isolated Chicken Eye Test, 13pp. Available at: [http://ecvam-dbalm.jrc.ec.europa.eu/].

(15) Balls, M., Botham, P.A., Bruner, L.H. and Spielmann H. (1995). The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxicol. In Vitro 9, 871-929.

(16) Prinsen, M.K. (1996). The chicken enucleated eye test (CEET): A practical (pre)screen for the assessment of eye irritation/corrosion potential of test materials. Food Chem. Toxicol. 34, 291-296.

(17) Chamberlain, M., Gad, S.C., Gautheron, P. and Prinsen, M.K. (1997). IRAG Working Group I: Organotypic models for the assessment/prediction of ocular irritation. Food Chem. Toxicol. 35, 23-37.

(18) ICCVAM. (2006). Background review document: Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method. NIH Publication No.: 06-4513. Research Triangle Park: National Toxicology Program. Available at: [http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd_ice.htm]

(19) Cazelle E., Eskes C., Hermann M., Jones P., McNamee P., Prinsen M., Taylor H., Wijnands M.V.W. (2015). Suitability of the Isolated Chicken Eye Test for Classification of Extreme pH Detergents and Cleaning Products. Toxicology In Vitro 29, 609-616.

(20) Prinsen, M.K. (2006). The Draize Eye Test and in vitro alternatives; a left-handed marriage? Toxicology in Vitro 20,78-81.

(21) Siegel, J.D., Rhinehart, E., Jackson, M., Chiarello, L., and the Healthcare Infection Control Practices Advisory Committee (2007). Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. Available at: [http://www.cdc.gov/ncidod/dhqp/pdf/isolation2007.pdf].

(22) Barroso J., Pfannenbecker U., Adriaens E., Alépée N., Cluzel M., De Smedt A., Hibatallah J., Klaric M., Mewes K.R., Millet M., Templier M., McNamee P. (2017). Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: the Draize eye test Reference Database (DRD). Archives of Toxicology 91, 521-547.

(23) Morton D., Sellers R.S., Barale-Thomas E., Bolon B., George C., Hardisty J.F., Irizarry A., McKay J.S., Odin M., Teranishi M. (2010). Recommendations for Pathology Peer Review. Toxicol. Pathol. 38, 1118-1127.

(24) OECD (2014). Advisory document n. 16. Guidance on the good laboratory practice (GLP) Requirements for Peer Review of Histopathology. OECD Series on principles of good laboratory practice and compliance monitoring. Advisory document of the working group on GLP. ENV/JM/MONO(2014)30. Organisation for Economic Cooperation and Development, Paris. Available at: http://www.oecd.org/chemicalsafety/testing/oecdseriesonprinciplesofgoodlaboratorypracticeglpandcompliancemonitoring.htm.

(25) Burton, A.B.G., M. York and R.S. Lawrence (1981). The in vitro assessment of severe irritants. Fd. Cosmet. Toxicol. 19, 471-480.

(26) OECD (2017). Test Guideline 405. OECD Guideline for Testing of Chemicals. Acute eye irritation/corrosion. Organisation for Economic Cooperation and Development, Paris, France. Available at: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.

ANNEX 1: DEFINITIONS

Accuracy: The closeness of agreement between test method results and accepted reference values. It is a measure of test method performance and one aspect of "relevance." The term is often used interchangeably with "concordance", to mean the proportion of correct outcomes of a test method.

Benchmark chemical: A chemical used as a standard for comparison to a test chemical. A benchmark chemical should have the following properties; (i), a consistent and reliable source(s); (ii), structural and functional similarity to the class of chemicals being tested; (iii), known physical/chemical characteristics; (iv) supporting data on known effects; and (v), known potency in the range of the desired response.

Bottom-Up Approach: step-wise approach used for a chemical suspected of not requiring classification for eye irritation or serious eye damage, which starts with the determination of chemicals not requiring classification (negative outcome) from other chemicals (positive outcome).

Cornea: The transparent part of the front of the eyeball that covers the iris and pupil and admits light to the interior.

Corneal opacity: Measurement of the extent of opaqueness of the cornea following exposure to a test chemical. Increased corneal opacity is indicative of damage to the cornea.

Corneal swelling: An objective measurement in the ICE test of the extent of distension of the cornea following exposure to a test chemical. It is expressed as a percentage and is calculated from baseline (pre-dose) corneal thickness measurements and the thickness recorded at regular intervals after exposure to the test material in the ICE test. The degree of corneal swelling is indicative of damage to the cornea.

Detergents: a mixture (excluding dilutions of single surfactant) containing one or more surfactants at a final concentration of > 3%, intended for washing and cleaning processes. Detergents may be in any form (liquid, powder, paste, bar, cake, moulded piece, shape, etc.) and marketed for or used in household, or institutional or industrial purposes.

Eye Irritation: Production of changes in the eye following the application of test chemical to the anterior surface of the eye, which are fully reversible within 21 days of application. Interchangeable with "Reversible effects on the Eye" and with "UN GHS Category 2" (4).

False negative rate: The proportion of all positive chemicals falsely identified by a test method as negative. It is one indicator of test method performance.

False positive rate: The proportion of all negative chemicals that are falsely identified by a test method as positive. It is one indicator of test method performance.

Fluorescein retention: A subjective measurement in the ICE test of the extent of fluorescein sodium that is retained by epithelial cells in the cornea following exposure to a test chemical. The degree of fluorescein retention is indicative of damage to the corneal epithelium.

Hazard: Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub) population is exposed to that agent.

IATA: Integrated Approach on Testing and Assessment.

Irreversible effects on the eye: see "Serious eye damage" and "UN GHS Category 1".

Mixture: A mixture or a solution composed of two or more substances in which they do not react (4).

Negative control: An untreated replicate containing all components of a test system. This sample is processed with test chemical-treated samples and other control samples to determine whether the solvent interacts with the test system.

Not Classified: Test chemicals that are not classified for eye irritation (UN GHS Category 2) or serious damage to eye (UN GHS Category 1). Interchangeable with "UN GHS No Category".

Positive control: A replicate containing all components of a test system and treated with a chemical known to induce a positive response. To ensure that variability in the positive control response across time can be assessed, the magnitude of the severe response should not be excessive.

Reliability: Measures of the extent that a test method can be performed reproducibly within and between laboratories over time, when performed using the same protocol. It is assessed by calculating intra- and inter-laboratory reproducibility and intra-laboratory repeatability.

Reversible effects on the Eye: see "Eye Irritation" and "UN GHS Category 2".

Serious eye damage: Production of tissue damage in the eye, or serious physical decay of vision, following application of a test chemical to the anterior surface of the eye, which is not fully reversible within 21 days of application. Interchangeable with "Irreversible effects on the eye" and with "UN GHS Category 1" (4).

Slit-lamp microscope: An instrument used to directly examine the eye under the magnification of a binocular microscope by creating a stereoscopic, erect image. In the ICE test method, this instrument is used to view the anterior structures of the chicken eye as well as to objectively measure corneal thickness with a depth-measuring device attachment.

Solvent/vehicle control: An untreated sample containing all components of a test system, including the solvent or vehicle that is processed with the test chemical-treated and other control samples to establish the baseline response for the samples treated with the test chemical dissolved in the same solvent or vehicle. When tested with a concurrent negative control, this sample also demonstrates whether the solvent or vehicle interacts with the test system.

Substance: Chemical elements and their compounds in the natural state or obtained by any production process, including any additive necessary to preserve the stability of the product and any impurities deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition (4).

Surfactants: Also called surface-active agent, this is a substance and/or its dilution (in an appropriate solvent/vehicle), which consists of one or more hydrophilic and one or more hydrophobic groups, that is capable of reducing the surface tension of a liquid and of forming spreading or adsorption monolayers at the water-air interface, and/or of forming emulsions and/or microemulsions and/or micelles, and/or of adsorption at water-solid interfaces.

Top-Down Approach: step-wise approach used for a chemical suspected of causing serious eye damage, which starts with the determination of chemicals inducing serious eye damage (positive outcome) from other chemicals (negative outcome).

Test chemical: Chemical (substance or mixture) assessed in the test method.

Tiered testing strategy: A stepwise testing strategy where all existing information on a test chemical 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 chemical can be assigned based on the existing information, no additional testing is required. If the irritancy potential of a test chemical cannot be assigned based on the existing information, a step-wise sequential animal testing procedure is performed until an unequivocal classification can be made.

United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS): A system proposing the classification of chemicals (substances and mixtures) according to standardized types and levels of physical, health and environmental hazards, and addressing corresponding communication elements, such as pictograms, signal words, hazard statements, precautionary statements and safety data sheets, so that to convey information on their adverse effects with a view to protect people (including employers, workers, transporters, consumers and emergency responders) and the environment (4).

UN GHS Category 1: see "Serious damage to eyes" and/or "Irreversible effects on the eye".

UN GHS Category 2: see "Eye Irritation" and/or "Reversible effects to the eye".

UN No Category: Test chemicals that do not meet the requirements for classification as UN GHS Category 1 or 2 (2A or 2B). Interchangeable with “Not classified”.

Validated test method: A test method for which validation studies have been completed to determine the relevance (including accuracy) and reliability for a specific purpose. It is important to note that a validated test method may not have sufficient performance in terms of accuracy and reliability to be found acceptable for the proposed purpose.

Weight-of-evidence: The process of considering the strengths and weaknesses of various pieces of information in reaching and supporting a conclusion concerning the hazard potential of a chemical.

ANNEX 2: PROFICIENCY CHEMICALS FOR THE ICE TEST METHOD

Prior to routine use of a test method that adheres to this Test Guideline, laboratories should demonstrate technical proficiency by correctly identifying the eye hazard classification of the 13 chemicals recommended in Table 10. The ICE outcomes provided represent examples of the range of responses observed during the evaluation studies and that may be expected (5)(18).These chemicals were selected to represent the range of responses for eye hazards based on results from the in vivo rabbit eye test (TG 405) and the UN GHS classification system (i.e., UN GHS Categories 1, 2A, 2B, or No Category) (4)(26). Other selection criteria were, to the extent possible that these chemicals produced reproducible results in the ICE test method, are commercially available and have high quality in vivo reference data available. Reference data are available in the SSD (5). In situations where a listed chemical is unavailable or cannot be used for other justified reasons, another chemical fulfilling the criteria described above, e.g. from the chemicals used in the evaluation and validation of the ICE test method could be used (5) (18). Such deviations should however be justified.

Table 10. Recommended chemicals for demonstrating technical proficiency with ICE

Chemical	CASRN	Chemical Class[1]	Physical Form	*In Vivo* UN GHS Classification[2]	ICE UN GHS Classification[3,4]
Benzalkonium chloride (10%)	8001-54-5	Onium compound	Liquid	Category 1	Category 1
Chlorhexidine	55-56-1	Amine, amidine	Solid	Category 1	Category 1
Sodium hydroxide (10%)	1310-73-2	Alkali	Liquid	Category 1	Category 1
Imidazole	288-32-4	Heterocyclic	Solid	Category 1	Category 1
Trichloroacetic acid (30%)	76-03-9	Carboxylic acid	Liquid	Category 1	Category 1
2,6-Dichlorobenz-oyl chloride	4659-45-4	Acyl halide	Liquid	Category 2A	No predictions can be made [4]
Ammonium nitrate	6484-52-2	Inorganic salt	Solid	Category 2A[5]	No predictions can be made [4]
Sodium hydroxide (1%)	1310-73-2	Alkali	Liquid	Category 2B	No predictions can be made [4]
Dimethyl sulfoxide	67-68-5	Organic sulphur compound	Liquid	No Category	No Category
Ethyl trimethyl acetate	3938-95-2	Ester	Liquid	No Category	No Category
Methylcyclo-pentane	96-37-7	Hydrocarbon (cyclic)	Liquid	No Category	No Category
n-Hexane	110-54-3	Hydrocarbon (acyclic)	Liquid	No Category	No Category
Triacetin	102-76-1	Lipid	Liquid	No Category	No Category

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; ICE: Isolated Chicken Eye test; n.a.: not available; UN GHS = United Nations Globally Harmonized System of Classification and Labelling of Chemicals (4).

[1]Chemical classes were assigned to each chemical using a standard classification scheme, based on the National Library of Medicine Medical Subject Headings (MeSH) classification system (available at http//www.nlm.nih.gov/mesh)

[2]Based on results from the *in vivo* rabbit eye test (OECD TG 405) and using the UN GHS (4)(26).

[3]Based on results in ICE as described in table 7.

[4] Combination of ICE scores other than the ones described in table 6 for the identification of GHS no-category and GHS Category 1 (see table 7)

[5] Classification as 2A or 2B depends on the interpretation of the UN GHS criterion for distinguishing between these two categories, *i.e.* 1 out of 3 vs. 2 out of 3 animals with effects at day 7 necessary to generate a Category 2A classification. The *in vivo* study included 3 animals. All endpoints apart from conjunctiva redness in one animal recovered to a score of zero by day 7 or earlier. The one animal that did not fully recover by day 7 had a conjunctiva redness score of 1 (at day 7) that fully recovered at day 10.

ANNEX 3: PROFICIENCY CHEMICALS FOR THE ICE HISTOPATHOLOGY TO BE USED IN ADDITION TO THE STANDARD ICE TEST METHOD FOR THE LIMITED APPLICABILITY DOMAIN OF NON-EXTREME PH (2 < PH < 11.5) DETERGENTS AND SURFACTANTS

Prior to routine use of ICE histopathology in addition to the standard ICE test method for the limited use domain of non-extreme pH (2 < pH < 11.5) detergents and surfactants, laboratories should demonstrate technical proficiency by correctly identifying the eye hazard classification of the 6 chemicals recommended in Table 11. These chemicals were selected to represent the range of responses for eye hazards based on results from the in vivo rabbit eye test (TG 405) and the UN GHS classification system (i.e., UN GHS Categories 1, 2, or No Category) (4)(26). Other selection criteria were, to the extent possible that these chemicals produced reproducible results in the ICE histopathology, are commercially available and have high quality in vivo data available. In situations where a listed chemical is unavailable or cannot be used for other justified reasons, another chemical fulfilling the criteria described above, e.g. from the chemicals used in the evaluation of the ICE histopathology could be used (10). Such deviations should however be justified.

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; NPCM:

Table 11. Recommended chemicals for demonstrating technical proficiency with ICE histopathology

Chemical	CASRN	Surfactant type	Physical Form	*In Vivo* Classification[1]	Standard ICE UN GHS classification[2]	ICE Histopathology UN GHS classification[3]
Benzalkonium chloride (5%)	8001-54-5	Cationic	Liquid	Category 1	Category 1	Category 1 (erosion) in 3 out of 3 laboratories
Benzensulphonylchloride	98-09-9	Anionic	Liquid	Category 1	Category 1	Category 1 (necrosis and vacuolation) in 3 out of 3 laboratories
Cetylpiridinium bromide (10%)	140-72-7	Cationic	Liquid	Category 1	No predictions can be made	Category 1 (vacuolation) in 3 out of 3 laboratories
Cetylpiridinium bromide (1%)	140-72-7	Cationic	Liquid	Category 2A	No predictions can be made	No predictions can be made in 3 out of 3 laboratories
N-Lauroyl sarcosine Na salt (10%)	137-16-6	Anionic	Liquid	Category 2A	No predictions can be made	No predictions can be made in 3 out of 3 laboratories
Cetylpiridinium bromide (0.1%)	140-72-7	Cationic	Liquid	No Category	No predictions can be made	No predictions can be made in 3 out of 3 laboratories

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; NPCM: No Prediction Can Be Made

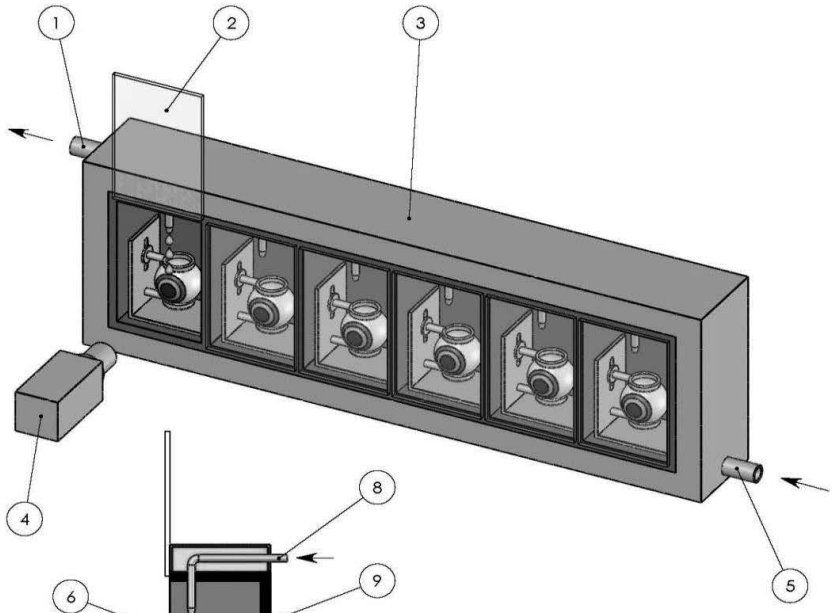
1Based on results from the in vivo rabbit eye test (OECD TG 405) and using the UN GHS (4)(26).

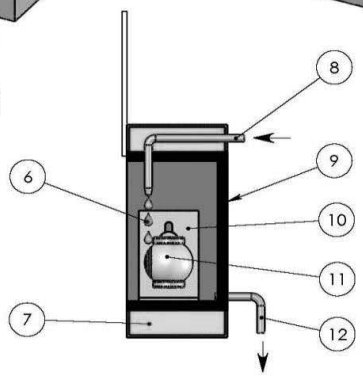
2Based on results in ICE as described in table 7.

3Based on ICE histopathology criteria as described in tables 8 and 9 and within the revised OECD GD 160 (12).

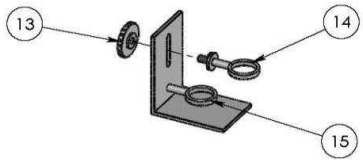
ANNEX 4

Figure 1. Diagrams of the ice superfusion apparatus and eye clamps





CROSS SECTION COMPARTMENT



EYE HOLDER

ITEM NO.	DESCRIPTION
1	OUTLET WARM WATER
2	SLIDING DOOR
3	SUPERFUSION APPARATUS
4	OPTICAL MEASURING INSTRUMENT
5	INLET WARM WATER
6	SALINE SOLUTION
7	WARM WATER
8	INLET SALINE SOLUTION
9	COMPARTMENT
10	EYE HOLDER
11	CHICKEN EYE
12	OUTLET SALINE SOLUTION
13	SETSCREW
14	ADJUSTABLE UPPER ARM
15	FIXED LOWER ARM

Note: See (25) for additional generic descriptions of the superfusion apparatus and eye clamp.