新規評価法提案書

ディファインド アプローチによる
眼に対する重篤な損傷性／眼刺激性評価法

令和８年１月

国立医薬品食品衛生研究所

新規評価法提案書

令和 8 年 1 月 19 日

No.　2025-01

ディファインド アプローチによる眼に対する重篤な損傷性／眼刺激性評価法に関する提案

令和 7 年 12 月 25 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：本評価法は、物理化学的特性や既存の TG を組み合わせた方法であり、今回 TG 467 に含まれた DAL および DAS の予測性は、OECD 専門家会議が定めた採用基準を満たしていた。TG467 に準拠した DAL-1、DAL-2 および DAS は、区分 1、区分 2 の分類および区分に該当しない場合の判定を可能とする評価法であり、科学的妥当性の観点からは行政利用上大きな問題はないものと考えられる。ただし、それぞれの TG の最新バージョンを常に確認し、指定されている物理化学的特性を同定するための個々の方法の適用限界を理解した上での利用を推奨するものである。

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ディファインド アプローチによる眼に対する重篤な損傷性／眼刺激性評価法の使用を提案するものである。

西川秋佳

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

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　病理部/
名古屋徳洲会総合病院）：座長
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 病理部）
小 島 幸 一 （一般財団法人 食品薬品安全センター）
中村るりこ （独立行政法人 製品評価技術基盤機構）
西 村 次 平 （独立行政法人 医薬品医療機器総合機構）
西 村 拓 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）
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）
松 本 一 彦 （名古屋市立大学大学院）

任期：令和 6 年 4 月 1 日～令和 8 年 3 月 31 日

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
石 井 孝 司　（国立感染症研究所）
齋 藤 嘉 朗　（国立医薬品食品衛生研究所）
豊 田 武 士　（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）
諫 田 泰 成　（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）
杉 山 圭 一　（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 ゲノム安全科学部）
髙 橋 暁 子　（独立行政法人 医薬品医療機器総合機構）
髙 橋 祐 次　（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）
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）
西 村 次 平　（独立行政法人 医薬品医療機器総合機構）
林 亜 紀 子　（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）
増 村 健 一　（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）
宮 坂 知 幸　（厚生労働省 医薬・生活衛生局 医薬品審査管理課）
山 田 隆 志　（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）
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 ゲノム安全科学部 第四室）：事務局
大 野 彰 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 ゲノム安全科学部 第四室）：事務局
堀　　武 志　（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 ゲノム安全科学部 第四室）：事務局
安 彦 行 人　（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 ゲノム安全科学部 第四室）：事務局

JaCVAM statement on the Defined Approach for Serious Eye Damage/Eye Irritation

At a meeting held on 25 December, 2025, at the National Institute of Health Sciences (NIHS) in Tokyo, Japan, the Japanese Center for the Validation of Alternative Methods (JaCVAM) Regulatory Acceptance Board unanimously endorsed the following statement:

Proposal: This defined approach (DA) method combines physicochemical properties and existing test guidelines (TGs). The predictive performance of DAL and DAS included in TG 467 meets the acceptance criteria established by the OECD expert meeting. DAL-1, DAL-2, and DAS, in accordance with TG 467, enabled the determination of categories 1 and 2, and Not Classified, and these methods have no significant issues for regulatory use concerning scientific validity. However, these methods should be used while verifying the latest versions of the respective TGs and understanding the applicability domains of the individual methods to identify the specified physicochemical properties.

This statement was released following a review prepared by the eye irritation test JaCVAM Editorial Committee to acknowledge that the results of the review and study by the JaCVAM Regulatory Acceptance Board confirmed the usefulness of this evaluation method.

Based on the above, we propose the use of a DA for serious eye damage/eye irritation as a useful means for safety assessments by regulatory agencies.

Nishikawa Akiyoshi
Chairperson,
JaCVAM Regulatory Acceptance Board.

Hirabayashi Yoko
Chairperson,
JaCVAM Steering Committee.

January 19, 2026

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:

Nishikawa Akiyoshi (Division of Pathology, Center for Biological Safety and Research: CBSR, NIHS / Nagoya Tokushukai General Hospital) : Chairperson
Hirabayashi Yoko (CBSR, NIHS)
Ishii Yuji (Division of Pathology, CBSR, NIHS)
Kojima Koichi (Food and Drug Safety Center)
Matsumoto Kazuhiko (Nagoya City University)
Nakamura Ruriko (National Institute of Technology and Evaluation)
Nishimura Jihei (Pharmaceuticals and Medical Devices Agency)
Nishimura Takuya (Division of Cellular and Molecular Toxicology, CBSR, NIHS)

Term: From 1st April 2024 to 31st March 2026

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

Hirabayashi Yoko (CBSR, NIHS): Chairperson
Hayashi Akiko (Ministry of Health, Labour and Welfare)
Ishii Koji (National Institute of Infectious Diseases)
Kanda Yasunari (Division of Pharmacology, CBSR, NIHS)
Masumura Kenichi (Division of Risk Assessment, CBSR, NIHS)
Miyasaka Tomohiro (Ministry of Health, Labour and Welfare)
Nishimura Jihei (Pharmaceuticals and Medical Devices Agency: PMDA)
Saito Yoshiro (NIHS)
Sugiyama Keiichi (Division of Genome Safety Science, CBSR, NIHS)
Takahashi Akiko (PMDA)
Taquahashi Yuhji (Animal Management Section of Division of Cellular and Molecular Toxicology, CBSR, NIHS)
Toyoda Takeshi (Division of Pathology, CBSR, NIHS)
Tsukano Masaaki (Ministry of Health, Labour and Welfare)
Yamada Takashi (Division of Cellular and Molecular Toxicology, CBSR, NIHS)
Ashikaga Takao (Division of Genome Safety Science, CBSR, NIHS): Secretary
Hori Takeshi (Division of Genome Safety Science, CBSR, NIHS): Secretary
Ohno Akiko (Division of Genome Safety Science, CBSR, NIHS): Secretary
Yasuhiko Yukuto (Division of Genome Safety Science, CBSR, NIHS): Secretary

ディファインド アプローチによる眼に対する重篤な損傷性／眼刺激性評価法に関する提案

添付資料一覧

添付資料 1

評価会議報告書

ディファインド アプローチによる

眼に対する重篤な損傷性／眼刺激性評価法

JaCVAM 評価会議

令和 7 年(2025 年)7 月 31 日

JaCVAM 評価会議

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 病理部／名古屋徳洲会総合病院）：座長
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 病理部）
小 島 幸 一	（一般財団法人 食品薬品安全センター）
中村るりこ	（独立行政法人 製品評価技術基盤機構）
西 村 次 平	（独立行政法人 医薬品医療機器総合機構）
西 村 拓 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）
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）
松 本 一 彦	（名古屋市立大学大学院）

任期：令和 6 年 4 月 1 日～令和 8 年 3 月 31 日

略語

AOP： Adverse Outcome Pathway

BCOP： Bovine Corneal Opacity and Permeability

DA： Defined Approach

DAL： Defined Approach Liquids

DAS: Defined Approach Solids

DIP： Data Interpretation Procedure

KE： Key Event

LLBO： Laser Light-Based Opacitometer

OECD： Organisation for Economic Cooperation and Development

(Q)SAR： Quantitative Structure-Activity Relationship

RhCE： Reconstructed human Cornea Epithelium

STE： Short Time Exposure

TG： Test Guideline

UN GHS： United Nations, Globally Harmonized System of Classification and Labelling of Chemicals

JaCVAM 評価会議は、経済協力開発機構 (OECD) テストガイドライン (TG) 467 [1] および眼刺激性試験資料編纂委員会により作成された「評価報告書 ディファインド アプローチによる眼に対する重篤な損傷性／眼刺激性評価法」[2] をもとに本評価法の科学的妥当性、社会的受け入れ性および行政上の利用性について検討した。

1. 評価法の概要および科学的妥当性

評価法の概要：

当該評価法は、OECD TG 467 に収載の *in silico* モデルにより算出した物理化学的特性や既存の TG を組み合わせたディファインド アプローチ (Defined Approach: DA, 定義済み総合判定方式) による眼に対する重篤な損傷性／眼刺激性評価法 (DAL および DAS) である。本評価法は化学物質が UN GHS [3] の区分 1 や区分 2 への分類または区分に該当しない場合の判定を可能とする。

DAL には DAL-1 と DAL-2 の 2 種類の評価法がある。DAL-1 は界面活性剤を除く液体原体を適用物質とし、物質の物理化学的特性および OECD TG 492 ヒト再構築角膜上皮 (RhCE) 法 [4] で区分に該当しない物質を識別し、さらに OECD TG 437 ウシ角膜を用いる混濁度および透過性 (BCOP) レーザー光ベースのオパシトメーター (LLBO) 試験法 [5] の予測モデルを組み合わせたデータ解釈手順 (DIP) を用いて区分 1 または区分 2 に識別する評価法である。DAL-2 は液体原体、および液体・固体の水溶液を適用物質とし、OECD TG 491 短時間曝露 (STE) 法 [6] と OECD TG 437 (BCOP LLBO) の二つの試験法を組み合わせて、区分 1 または区分 2、もしくは区分に該当しないと識別する評価法である。DAS は液体には適用できず、原体の界面活性剤を除く固体物質に適用され、OECD TG 492 (RhCE) で区分に該当しない物質を識別し、さらに OECD TG 437 (BCOP LLBO) を用いて区分 1 または区分 2 に識別する評価法である。

科学的妥当性：

DAL および DAS に使用される各 *in vitro* 試験法については、OECD に収載されている検証済みの TG である。さらに、物理化学的特性や化学構造による *in silico* 試験としては TG467 で推奨されている OECD (Q) SAR バリデーション原則に基づいた計算モデル (OPERA [7] や T.E.S.T. [8] 等) を用いている。以上のことから、科学的に妥当な手法である。

2. 本評価法の社会的受け入れ性および行政上の利用性

社会的受け入れ性：

当該評価法は、生きた動物を用いない、すなわち動物実験の代替法であるという観点で 3Rs の精神に合致している。実施可能性については、日本国内において主要試験である BCOP LLBO 試験を実施できる施設がないことから、海外に依頼せざるを得ない状況である。そのため国内の試験法の実施体制を整えることが今後の課題となる。また、国内で実施できる試験法を組み合わせた新たな DA について早急に TG 467 に追加されることが望まれる。以上より、本評価法は、社会的受容性も高いと考えられるが、実施可能性については課題が残されている。

行政上の利用性：

本評価法は、物理化学的特性や既存の TG を組み合わせた方法であり、今回 TG 467 に含まれた DAL および DAS の予測性は、OECD 専門家会議が定めた採用基準を満たしていた。TG467 に準拠した DAL-1、DAL-2 および DAS は、区分 1、区分 2 の分類および区分に該当しない場合の判定を可能とする評価法であり、科学的妥当性の観点からは行政利用上大きな問題はないものと考えられる。ただし、それぞれの TG の最新バージョンを常に確認し、指定されている物理化学的特性を同定するための個々の方法の適用限界を理解した上での利用を推奨するものである。

参考文献（最終確認日：2025 年 7 月 31 日）

1) OECD (2024) Guideline for Testing of Chemicals No.467, Defined Approaches for Serious Eye Damage and Eye Irritation, Organisation for Economic Co-operation and Development, Paris. https://www.oecd.org/content/dam/oecd/en/publications/reports/2022/06/test-no-467-defined-approaches-for-serious-eye-damage-and-eye-irritation_236dd995/28fe2841-en.pdf

2) JaCVAM　眼刺激性試験資料編纂委員会 : 眼に対する重篤な損傷性／眼刺激性ディファインドアプローチ評価報告書（2024 年 12 月 27 日）

3) United Nations (UN) (2023), Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Tenth revised edition, New York and Geneva, United Nations Publications. https://unece.org/sites/default/files/2023-07/GHS%20Rev10e.pdf

4) OECD (2024). Guideline for Testing of Chemicals No. 492: 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 Co-operation and Development, Paris. https://www.oecd.org/en/publications/test-no-492-reconstructed-human-cornea-like-epithelium-rhce-test-method-for-identifying-chemicals-not-requiring-classification-and-labelling-for-eye-irritation-or-serious-eye-damage_9789264242548-en.html

5) OECD (2023). Guideline for Testing of Chemicals No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage. Organisation for Economic Cooperation and Development, Paris. https://www.oecd.org/content/dam/oecd/en/publications/reports/2023/07/test-no-437-bovine-corneal-opacity-and-permeability-test-method-for-identifying-i-chemicals-inducing-serious-eye-damage-and-ii-chemicals-not-requiring-classification-for-eye-irritation-or-serious-eye-damage_g1g34044/9789264203846-en.pdf

6) OECD (2023). Guideline for Testing of Chemicals No. 491: Short Time Exposure *In Vitro* Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage. Organisation for Economic Co-operation and Development, Paris. https://www.oecd.org/content/dam/oecd/en/publications/reports/2023/07/test-no-491-short-time-exposure-in-vitro-test-method-for-identifying-i-chemicals-inducing-serious-eye-damage-and-ii-chemicals-not-requiring-classification-for-eye-irritation-or-serious-eye-damage_g1g59945/9789264242432-en.pdf

7) Mansouri, K., Grulke, C.M., Judson, R.S. Williams A.J., OPERA models for predicting physicochemical properties and environmental fate endpoints. *J Cheminform* 10, 10 (2018). https://doi.org/10.1186/s13321-018-0263-1

8) User's Guide for T. E. S. T. (Toxicity Estimation Software Tool) Version 5.1. https://www.epa.gov/sites/default/files/2016-05/documents/600r16058.pdf

添付資料 2

評価報告書

ディファインド アプローチによる

眼に対する重篤な損傷性／眼刺激性評価法

眼刺激性試験資料編纂委員会

令和 7 年 (2025 年) 2 月 14 日

眼刺激性試験資料編纂委員会

山 本　直 樹　（委員長：藤田医科大学）
小 島　肇　（山口東京理科大学/国立医薬品食品衛生研究所）
佐々木 正 治　（アレクシオンファーマ合同会社）
竹 内　小 苗　（P&G イノベーション合同会社）*
波 多 野 浩 太　（ホーユー株式会社）
山 下　晴 洋　（アステラス製薬株式会社）

*：令和 6 年 3 月末まで

略語

BCOP： Bovine Corneal Opacity and Permeability
DA： Defined Approach
DAL： Defined Approach Liquids
DAS: Defined Approach Solids
DIP： Data Interpretation Procedure
IATA： Integrated Approaches to Testing and Assessment
LLBO： Laser Light-Based Opacitometer
LogP： Octanol-water Partition Coefficient
OECD： Organisation for Economic Cooperation and Development
OPERA： The Open (Quantitative) Structure-Activity/Property Relationship App
OP-KIT： Opacitometer Kit
PCA： Principal Component Analysis
QSAR： Quantitative Structure-Activity Relationship
QMRF： QSAR Model Reporting Format
RhCE： Reconstructed human Cornea Epithelium
ST： Surface Tension
STE： Short Time Exposure
TG： Test Guideline
T.E.S.T.： Toxicity-Estimation-Software-Tool-Test
VP： Vapour Pressure
UN GHS： United Nations, Globally Harmonized System of Classification and Labelling of Chemicals
UVCB： Substances of Unknown or Variable Composition, Complex Reaction Products or Biological Materials
WS： Water Solubility

添付資料

1. DAL-1 スキーム
2. DAL-2 スキーム
3. DAS スキーム
4. DAL-1 EpiOcular™ EIT と SkinEthic™ TTL の予測性を比較した被験物質リスト (37 物質)
5. DAL-1 SkinEthic™ EIT と SkinEthic™ TTL の予測性を比較した被験物質リスト (37 物質)
6. DAL-2 と SkinEthic™ TTL の予測性を比較した被験物質リスト (45 物質)
7. DAS と SkinEthic™ TTS の予測性を比較した被験物質リスト (71 物質)

要旨

経済協力開発機構 (Organisation for Economic Co-operation and Development: OECD) 試験法ガイドライン (Test Guideline: TG, 467) に掲載された眼に対する重篤な損傷性および眼刺激性ディファインド アプローチ (Defined Approach: DA, 定義済み総合判定方式) である DAL (Defined Approach Liquids) および DAS (Defined Approach Solids) は、物理化学的特性や既存の TG を組み合わせた眼刺激性評価方法である。開発者から提案のあった液体物質および固体物質に関する予測性に関しては、OECD 専門家会議が定めた採用基準を満たしていた。DAL および DAS に準拠して実施した場合、UN GHS (United Nations, Globally Harmonized System of Classification and Labelling of Chemicals) 区分 1、UN GHS 区分 2 への分類および区分に該当しない場合の識別を可能とする組み合わせであると本委員会は考える。

ただし、DAL-1、DAL-2 および DAS ともに主要試験であるウシ角膜を用いる混濁度および透過性 (Bovine Corneal Opacity and Permeability: BCOP) レーザー光ベースのオパシトメーター (Laser Light-Based Opacitometer: LLBO) 試験を実施できる施設が日本にはなく、海外に試験を依頼せねばならない。国内で実施できる試験法を組み合わせた新たな DA が早急に TG 467 に追加されるべきと本委員会は考える。

1. 背景および目的

化学物質の眼に対する重篤な損傷性および眼刺激性の評価は、従来、経済協力開発機構 (Organisation for Economic Co-operation and Development: OECD) の定めた白色ウサギを用いた Draize 眼刺激性試験法ガイドライン (Test Guideline: TG, 405) [1] により行われており、変化の程度および回復期間に基づいて UN GHS (United Nations, Globally Harmonized System of Classification and Labelling of Chemicals) [2] の基準により、分類がなされる。UN GHS 分類によると、眼に不可逆的な影響または眼に重篤な損傷を与える (21 日以内に完全に回復しない) ものを UN GHS 区分 1 (区分 1) と定義しており、UN GHS 区分 2 (区分 2) は、眼に対する可逆的な影響または刺激を与える (21 日以内に完全に回復する) ものと定義される。区分 2 は、21 日以内に完全に回復がみられる区分 2A と 7 日以内に完全に回復がみられる区分 2B に分けられる。区分 1 および区分 2 のいずれにも該当しない化学物質は分類を必要とせず、区分に該当しない物質となる。

重篤な眼傷害を引き起こす区分 1 の化学物質の特定、あるいは眼刺激性や重篤な眼傷害の危険性について区分に該当しない化学物質の特定を目的とした *in vitro* 試験については、OECD TG 437、TG 438、TG 460、TG 491、TG 492、TG 494 および TG 496 [3,4,5,6,7,8,9] など、多くの TG がすでに採用されている。ただし、単一の *in vitro* 試験法で *in vivo* のウサギ眼刺激性試験 (TG 405) を代替する場合、特に中程度の分類である区分 2 を予測することが困難である。そのため、刺激性のポテンシャルを評価するためには、個々の *in vitro* 試験法の長所を組み合わせた評価戦略が推奨されている (例：トップダウンまたはボトムアップアプローチ) 。

OECD では、これらの *in vitro* 試験データと物理化学的特性や化学構造による *in silico* データとの組み合わせによる評価方法として、試験と評価の統合的アプローチ (Integrated Approaches to Testing and Assessment: IATA) またはディファインド アプローチ (Defined Approach: DA, 定義済み総合判定方式) による評価が発表されている [10] 。DA による予測は、IATA をもとに開発され、または、UN GHS のような適用される法的基準に従って、単独あるいは追加情報とともに使用される。DA は、試験法を組み合わせて評価を行うことで、個々の独立した評価法による限界を克服し、より信頼性の高い結果を導くことを目的としている。

DA として開発された TG 467 [11] には、界面活性剤を除く液体物質に対する 2 つの DA が含まれており、それぞれハザードの特定 (区分 1、区分 2、区分に該当しない物質の 3 つの UN GHS 分類の識別 [2]) について記載されている。また、界面活性剤を除く固体物質には 1 つの DA が含まれている。

詳細な DA の評価結果については、「Supporting document to the Guideline on (DAs) Defined Approaches for Serious Eye Damage / Eye Irritation」(非公開) に記載されており、液体物質に関しては、多様な有機官能基 (79 種類) を持ち、幅広い用途とケミカルクラスをカバーする 86 の化学物質について、固体物質に関しては、幅広い用途とケミカルクラスをカバーする 109 の化学物質について、DA の予測、Draize 眼刺激性試験データおよび物理化学的特性などのデータセットが収集されている。同時期に OECD TG 492B (SkinEthic™ HCE TTT 法) が発出されており，この方法は本資料編纂委員会が「UN GHS 区分 1、区分 2 への分類および区分に該当しない場合の判定を可能とする試験法であるが，固体物質の UN GHS 区分の分類は慎重に評価されるべきである」と結論した *in vitro* 試験法である。これに対して，TG 467 は同様に UN GHS 区分 1、区分 2 への分類および区分に該当しない場合の判定を行うことができる物理化学的特性と既存の *in vitro* 試験の組み合わせによる評価法である。

本評価報告書では、TG 467 と DA の Supporting document を用いて、DAL (Defined Approach Liquids) および DAS (Defined Approach Solids) の科学的な妥当性を評価するとともに、TG 492B との比較も行った。

2. DA の性能基準とガイドラインに含まれる DA

In vivo 試験の試験内および試験間の変動を念頭に *in vivo* 試験の不確実性を考慮して、DA の評価には表 1 の性能基準を用いることが OECD の専門家会議で合意された。この表の中で、重要な基準は、区分 2 の一致度が 50%以上であること、区分 1 の一致度が 75%、区分外の一致度が 70%であることに加え、区分 1 の物質が区分外と判定される割合が 5%以下である。その他の数値には若干の許容範囲が含まれている。

表 1　DA 性能基準

UN GHS	DA		
	区分 1	区分 2	区分外*
区分 1	≥ 75%	≤ 25%	≤ 5%
区分 2	≤ 30%	≥ 50%	≤ 30%
区分外*	≤ 5%	≤ 30%	≥ 70%

* 区分外：区分に該当しない

2.1. DAL-1 の手順

DAL-1 は界面活性剤をのぞく液体 (単一物質) を適用物質とし、物質の物理化学的特性および OECD TG 492 ヒト再構築角膜上皮 (Reconstructed human Cornea Epithelium: RhCE) 法と OECD TG 437 ウシ角膜を用いる混濁度および透過性 (Bovine Corneal Opacity and Permeability: BCOP) レーザー光ベースのオパシトメーター (Laser Light-Based Opacitometer: LLBO) の予測モデルを組み合わせたデータ解釈手順 (Data Interpretation Procedure: DIP) を用いる。OECD TG 437 に収載されている 2 つの BCOP 試験のうち LLBO で角膜混濁度を測定する方法を用いる。

物理化学的特性および OECD TG 492 (RhCE) で区分に該当しない物質を識別し、そこで区分に該当しないと識別されなかった物質を OECD TG 437 (BCOP　LLBO) を用いて区分 1 または区分 2 に識別する (添付資料 1 のスキームを参考) 。

1)　区分に該当しない物質を識別

物理化学的特性または OECD TG 492 (RhCE) より、まず、区分に該当しない物質を識別する。どちらかで区分に該当しない物質と識別された場合、それ以上の試験は必要としない。物理化学的特性情報を最初に確認する Option 1 スキーム、RhCE 試験法を最初に確認する Option 2 スキーム、どちらを先に行っても構わない。

物理化学的特性

水溶性 (水に対する溶解度 water solubility: WS) 、または、オクタノール/水分配係数 (octanol-

water partition coefficient: LogP) と蒸気圧 (vapour pressure: VP) と表面張力 (surface tension: ST) の組み合わせを基にした表 2 に示す除外規定に当てはまる物質は区分に該当しない物質と識別する。

表 2　物理化学的特性による除外規定

除外規定		
WS < 0.02 mg/mL	または	LogP > 1 かつ VP > 3 mm Hg かつ ST < 30 dyne/cm

物理化学的特性のデータは、実験的に測定された値が最も優先されるが、OECD の (Q) SAR ((Quantitative) Structure-Activity Relationship) バリデーション原則に基づいたモデルから得られた予測値を使用することも可能である。試験法および予測モデルの一例を以下の表 3 にまとめた。

表 3　試験法および予測モデル

方法	LogP	VP	ST	WS
測定 OECD TG	TG 107 TG 117 TG 122	TG 104	TG 115	TG 105
予測 (Q)SAR	OPERA	OPERA	T.E.S.T. (EPA)	OPERA

OECD TG 492 (RhCE)

OECD TG 492 に収載されている試験法のうち EpiOcular™眼刺激性試験あるいは SkinEthic™ ヒト角膜上皮モデル眼刺激性試験を用いる。TG 492 に従って、平均細胞生存率が 60%を超えた物質は区分に該当しないと識別する。

2)　区分 1、区分 2 物質の識別

上記の物理化学的特性および OECD TG 492 (RhCE) で区分に該当しないと識別されなかった物質を OECD TG 437 (BCOP LLBO) を用いて区分 1 または区分 2 に識別する。

OECD TG 437 (BCOP LLBO)

TG437 の判定基準とは異なり透過性の値は使用せず、ここでは LLBO で測定した混濁度のみを用いて判定する。LIS (LLBO Irritation Score) の算出は必要としない。混濁度が 145 を超えた物質は区分 1、混濁度が 145 以下の物質は区分 2 と識別する。オパシトメーター (Opacitometer Kit: OP-KIT) で測定した結果を用いた場合の予測性も検討されたが、区分 1 の予

測性は性能基準の≥ 75%を満たさなかった。よって、OP-KIT は DAL-1 では使用できない。

2.2. DAL-2 の手順

DAL-2 は界面活性剤を除く液体 (単一物質) 、および、液体・固体の水溶液を適用範囲とし、OECD TG 437 (BCOP LLBO) と OECD TG 491 短時間曝露 (Short Time Exposure: STE) 法の組み合わせを用いる。DAL-1 同様、DAL-2 でも OP-KIT は使用できない。

OECD TG 491 (STE) で区分 1 物質および区分に該当しない物質を識別し、そこでどちらにも識別されなかった物質を OECD TG 437 (BCOP LLBO) を用いて区分 1 または区分 2 に分類する (Option1) 。あるいは、まず OECD TG 437 (BCOP LLBO) を用いて区分 1 物質を識別し、そこで区分 1 に識別されなかった物質を OECD TG 491 (STE) を用いて、区分 1、区分 2、および区分に該当しない物質に識別する (Option2) 。(添付資料 2 のスキームを参考)

OECD TG 437 (BCOP LLBO)

試験方法は DAL-1 の記載に準じる。

OECD TG 491 (STE)

OECD TG 491 (STE) に収載されている方法に従い、被験物質の 5%および 0.05%の希釈液の細胞毒性を判定に用いる。Option1 スキームでは、OECD TG 491 (STE) の予測モデル (平均細胞生存率 70%) に従い、区分 1 物質および区分に該当しない物質を識別する。分類ができなかった物質は OECD TG 437 (BCOP LLBO) の判定が必要となる。Option2 スキームでは、OECD TG 437 (BCOP LLBO) で区分 1 と分類されなかった物質を試験し、次いで OECD TG 491 (STE) の予測モデルに従い、区分 1 物質および区分に該当しない物質を識別する。どちらにも区分されなかった物質は区分 2 に識別される。

2.3. DAS の手順

OECD TG 492 (RhCE) で区分に該当しない固体物質を識別し、そこで区分に該当しないと識別されなかった物質を OECD TG 437 (BCOP LLBO) を用いて区分 1 または区分 2 に識別する (添付資料 3 のスキームを参考) 。DAL-1 および DAL-2 同様、DAS でも OP-KIT は使用できない。

1) 区分に該当しない物質を識別

OECD TG 492 (RhCE) より、まず、区分に該当しない物質を識別する。どちらかで区分に該当しない物質と識別された場合、それ以上の試験は必要としない。

OECD TG 492 (RhCE)

OECD TG 492 に収載されている試験法のうち SkinEthic™ヒト角膜上皮モデル眼刺激性試験 (SkinEthic™ HCE EITS) を用いる。TG 492 に従って、平均細胞生存率が 50%を超えた物質は区分に該当しないと識別する。

2) 区分 1、区分 2 物質の識別

SkinEthic™ HCE EITS で区分に該当しないと識別されなかった物質を OECD TG 437 (BCOP LLBO) を用いて区分 1 または区分 2 に識別する。

OECD TG 437 (BCOP LLBO)

OECD TG 437 に収載されている BCOP 試験のうち LLBO で角膜混濁度を測定する方法を用いる。LIS (LLBO Irritation Score) の算出は必要としない。LLBO で測定した混濁度および透過性の値 (OD 値) を用いて判定する。混濁度が 145 および／または OD 値が 2.5 を超えた物質は区分 1、それ以外の物質は区分 2 と識別する。

3. DA の予測性

新たな DA 開発の基準となる現時点での DA の予測性値を TG 467 より引用して、委員会の検討結果も含み、以下に示す。

3.1. DAL-1

3.1.1. 物理化学的特性データについて

DAL-1 ではステップ 1 で、物理化学的特性データを用いて区分に該当しない物質の識別を可能としている (除外規定) 。

Alépée らは 147 の液体物質について、分子量、オクタノール/水分配係数 (LogP) 、融点、蒸気圧 (VP) 、水溶性 (WS) 、表面張力 (ST) の 6 つの物理化学的特性 (実測値・予測値を含む) に関して主成分分析 (principal component analysis: PCA) を基に検討した [12)] 。その結果、WS が低い物質は区分に該当しない物質であることが多かった。また、LogP 値が高く、VP 値が高く、かつ、ST 値が低い物質は 区分に該当しない物質であることが多かった。これらを Classification Tree の予測因子として使用し、区分に該当しない物質と区分に該当する物質 (区分 1 および区分 2) を分ける最適な閾値を求めた。その結果、WS 単独または、LogP、VP、ST の 3 つの物理化学的特性の組み合わせの除外規定を組み入れることにより、ボトムアップ方式において区分に該当しない物質 (RhCE 法を単独で用いるよりも正確に) を同定することができると結論付けた。

物理化学的特性データは実測値が最優先されるが、その情報が得られない場合は *in silico* モデルから得られた予測値を用いることもできる。

DAL-1 の予測性の検討で用いられた 108 物質の実測値・予測値の利用状況は表 4 に示すとおりである。どの特性データも半数以上の物質が予測値を用いていた。LogP&VP&ST における除外規定の適用においては、およそ 30%の物質 (表 4 の最後の項目「LogP+VP+ST すべての 33 物質」) ですべての項目に予測値を用いていた[1]。

[1] 非公開資料である全データ一覧シートから、物理化学的特性データの source の列でフィルターをかけ、該当する 33 物質を確認した。
（フィルター条件 LogP：ECHA を除く、VP：ECHA を除く、ST：Jesper を除く）

表 4　物理化学的特性データの実測値・予測値を利用した物質数

特性項目	実測値	予測値	利用モデル
WS	41 (うち除外規定が適用された物質：9)	67 (うち除外規定が適用された物質：14)	OPERA (66) EPISuite (1)
LogP	39	69	OPERA (68) EPISuite (1)
VP	31	77	OPERA (76) EPISuite (1)
ST*	41	65	T.E.S.T. (53) ACD/Labs (12)
LogP+VP+ST すべて		33 (うち除外規定が適用された物質：3)	

* 2 物質はデータなし

表 5 では、除外規定が適用された物質だけを抽出し、物理化学的特性データそれぞれについて、実測値あるいは予測値が用いられた物質数を示している。

WS により除外規定が適用された物質が 23、LogP & VP & ST における除外規定が適用されていた物質が 20、うちどちらにも当てはまる物質が 6 物質であるので、23+20-6 であることから、37 物質となる。除外規定が適用され区分に該当しないと判断された 37 物質は、いずれも *in vivo* のデータより区分に該当しない物質であった。よって、本委員会は、DAL-1 において、物理化学的特性データ (実測値・予測値) を用いて、区分に該当しない物質を識別することは可能であると考えた。

表 5　除外規定が適用された物質数

除外規定	総数*	実測	予測	使用モデル
WS＜0.02	23	9	14	OPERA
LogP >1 かつ、 VP >3 かつ、 ST <30	20	LogP: 8 VP: 7 ST: 13	LogP: 12 VP: 13 ST: 7	LogP: OPERA VP: OPERA ST: T.E.S.T.

* 規定除外 WS＜0.02 と LogP >1& VP >3 & ST <30 のどちらにも当てはまる (重複する) 物質は 6 物質

3.1.2. DAL-1 の予測性

RhCE 法ごとに DAL-1 (EpiOcular™ EIT) および DAL-1 (SkinEthic™ EIT) の予測性を表 6 にまとめた。物理化学的特性情報を最初に確認する Option 1 スキーム、RhCE 試験法を最初に確認する Option 2 スキーム、どちらも、その予測性は同じであった。

DAL-1 の予測性は RhCE 法に EpiOcular™ EIT を用いた場合も SkinEthic™ EIT を用いた場合も、表 1 に示す性能基準を満たしていた。前述したように、OP-KIT は DAL-1 では使用できない。

表 6　DAL-1 予測性

UN GHS	DAL-1 (EpiOcular™ EIT) 94 物質			DAL-1 (SkinEthic™ EIT) 86 物質		
	区分 1	区分 2	区分外**	区分 1	区分 2	区分外**
区分 1	76.5% (13.0/17) *	23.5% (4.0/17)	0.0% (0.0/17)	76.5% (13.0/17)	23.5% (4.0/17)	0.0% (0.0/17)
区分 2	27.3% (6.0/22)	59.1% (13.0/22)	13.6% (3.0/22)	30.4% (7.0/23)	68.7% (15.8/23)	0.9% (0.2/23)
区分に該当しない	5.5% (3.0/55)	24.0% (13.2/55)	70.5% (38.8/55)	3.1% (1.4/46)	17.2% (7.9/46)	79.7% (36.7/46)

* *in vivo* との一致率 (%) は加重算出した。たとえば、ある被験物質について 3 回の試験結果があり、2 回は区分 1、1 回は区分 2 と識別された場合、区分 1 および区分 2 の予測はそれぞれ 0.66 (2/3)、0.33 (1/3) とした。

** 区分に該当しない

3.2. DAL-2 の予測性

表 7 に DAL-2 の予測性を、DAL-2 オプション 1 スキーム (STE＞BCOP　LLBO) を用いた場合についてまとめた。

表 7　DAL-2 予測性

UN GHS	DAL-2 164 物質		
	区分 1	区分 2	区分外**
区分 1	81.2% (13.8/17) *	17.6% (3.0/17)	1.2% (0.2/17)
区分 2	30.2% (7.2/24)	56.3% (13.5/24)	13.5% (3.2/24)
区分に該当しない	4.1% (5.1/123)	10.6% (13.0/123)	85.3% (104.9/123)

* *in vivo* との一致率 (%) は加重算出した。

** 区分に該当しない

DAL-2 の予測性は表 1 に示す性能基準を満たしていた。

3.3. DAS の予測性

表 8 に DAS の予測性を、DAS スキームを用いた場合についてまとめた。DAS の予測性は表 1 に示す性能基準を満たしていた。

表 8　SkinEthic™ HCE EITS と BCOP LLBO による DAS 予測性

UN GHS	DAS 109 物質		
	区分 1	区分 2	区分外**
区分 1	77.4% (24.0/31) *	22.6% (7.0/31)	0.0% (0.0/31)
区分 2	29.5% (5.3/18)	52.3% (9.4/18)	18.2% (3.3/18)
区分に該当しない	1.7% (1.0/60)	28.3% (17.0/60)	70.0% (42.0/60)

* *in vivo* との一致率 (%) は加重算出した。
** 区分に該当しない

3.4. TG 492B との比較

DA における区分 2 の利用に関しては、同時期に OECD より公表された区分 2 の利用を促す TG 492B (13) との比較を行った。以下に DAL-1 (EpiOcular™ EIT) および DAL-1 (SkinEthic™ EIT) と TG 492B 液体 (SkinEthic™ TTL) のみの比較結果を表 9 に示す。SkinEthic™ TTL における区分 2 の予測性は 79.8%であり DAL-1 より 10～20%高かった。

表 9　DAL-1 (11) と SkinEthic™ TTL (13) の一致率比較

UN GHS	DAL-1 EpiOcular™ EIT 94 物質			DAL-1 SkinEthic™ EIT 86 物質			SkinEthic™ TTL 70 物質		
	区分 1	区分 2	区分外 *	区分 1	区分 2	区分外 *	区分 1	区分 2	区分外 *
区分 1	76.5%	23.5%	0.0%	76.5%	23.5%	0.0%	85.4%	14.6%	0.0%
区分 2	27.3%	59.1%	13.6%	30.4%	68.7%	0.9%	20.2%	79.8%	0.0%
区分に該当しない	5.5%	24.0%	70.5%	3.1%	17.2%	79.7%	0.0%	20.8%	79.2%

* 区分に該当しない

また、同一の物質の結果のみを抽出して DA と SkinEthic™ TTL/TTS の一致率を比較した。

DAL-1 の EpiOcular™ EIT および SkinEthic™ EIT 並びに SkinEthic™ TTL における予測性を表 10 および表 11 に示す。なお、評価物質数は同じだが、評価物質種が異なるため、DAL-1 の EpiOcular™ EIT および SkinEthic™を同条件で評価することはできない。区分 1 の予測性は同等であるが、区分に該当しない物質の予測性は DAL-1 の EpiOcular™ EIT および SkinEthic™が高かった。区分 2 の予測性は SkinEthic™ TTL が高かった。

次に、表 12 に示すように DAL-2 と SkinEthic™ TTL を比較したところ、区分 1 の予測性は同等であり、区分 2 の予測性においては、SkinEthic™ TTL の方が高く、区分に該当しない物質の予測性については DAL-2 の方が高かった。

固体物質の予測性に関しては、表 13 に示すように DAS と SkinEthic™ TTS を比較した。区分 1 の予測性は、SkinEthic™ TTS の方が高かったが、区分 2 や区分に該当しない物質の予測性は同等であった。DAS と比較すると SkinEthic™ TTS は、区分 2 の予測では区分に該当しない物質と予測することが多く、区分に該当しない物質 (Gluconolactone) を区分 1 と分類した結果もあった (添付資料 7) 。なお、各試験法の結果と被験物質に関しては、添付資料 4～7 に記載している。

区分 2 の評価という点では SkinEthic™ TTL の方が DAL よりも予測性は高いが、SkinEthic™ TTS は DAS と同程度であった。なお、DAL-1、DAL-2 および DAS は、いずれも OECD の定めた性能基準を満たしていた。利用者は、各試験法の特徴や被験物質の特性を理解し、試験法の取捨選択を行う必要があると考える。

表 10　DAL-1 (EpiOcular™ EIT) と SkinEthic™ TTL との予測性比較 (37 物質) **

	DAL-1 EpiOcular™ EIT			SkinEthic™ TTL		
UN GHS	区分 1	区分 2	区分外*	区分 1	区分 2	区分外*
区分 1	83.3% (10/11)	16.7% (2/12)	0.0% (0/12)	83.3% (10/11)	16.7% (2/12)	0.0% (0/12)
区分 2	28.6% (4/14)	64.3% (9/14)	7.1% (1/14)	0.0% (0/14)	100.0% (0/14)	0.0% (0/14)
区分に該当しない	0.0% (0/11)	0.0% (0/11)	100.0% (11/11)	0.0% (0/11)	36.4% (4/11)	63.6% (7/11)

* 区分に該当しない

** 複数の試験結果があり、結果が一致していない物質は多数決とし、同数の場合には刺激性の低い区分に分類した

表 11　DAL-1 SkinEthic™ EIT と SkinEthic™ TTL との予測性比較 (37 物質) **

	DAL-1 SkinEthic™ EIT			SkinEthic™ TTL		
UN GHS	区分 1	区分 2	区分外*	区分 1	区分 2	区分外*
区分 1	83.3% (10/12)	16.7% (2/12)	0.0% (0/12)	83.3% (10/12)	16.7% (2/12)	0.0% (0/12)
区分 2	28.6% (4/14)	71.4% (10/14)	0.0% (0/14)	0.0% (0/14)	100.0% (0/14)	0.0% (0/14)
区分に該当しない	0.0% (0/11)	0.0% (0/11)	100.0% (0/11)	0.0% (0/11)	18.2% (2/11)	81.8% (9/11)

* 区分に該当しない

** 複数の試験結果があり、結果が一致していない物質は多数決とし、同数の場合には刺激性の低い区分に分類した。

表 12　DAL-2 と SkinEthic™ TTL との予測性比較 (45 物質) **

UN GHS	DAL-2			SkinEthic™TTL		
	区分 1	区分 2	区分外*	区分 1	区分 2	区分外*
区分 1	84.6% (11/13)	15.4% (2/13)	0.0% (0/13)	84.6% (11/13)	15.4% (2/13)	0.0% (0/13)
区分 2	40.0% (6/15)	60.0% (9/15)	0.0% (0/15)	6.7% (1/15)	93.3% (14/15)	0.0% (0/15)
区分に該当しない	0.0% (0/17)	5.9% (1/17)	94.1% (16/17)	0.0% (0/17)	17.6% (3/17)	82.4% (14/17)

* 区分に該当しない

** 複数の試験結果があり、結果が一致していない物質は多数決とし、同数の場合には刺激性の低い区分に分類した

表 13　DAS と SkinEthic™ TTS との予測性比較 (71 物質）**

UN GHS	DAS			SkinEthic™ TTS		
	区分 1	区分 2	区分外*	区分 1	区分 2	区分外*
区分 1	73.9% (17/23)	26.1% (6/23)	0.0% (0/23)	82.6% (19/23)	17.4% (4/23)	0.0% (0/23)
区分 2	31.3% (5/16)	50.0% (8/16)	18.8% (3/16)	18.8% (3/16)	50.0% (8/16)	31.3% (5/16)
区分に該当しない	0.0% (0/32)	31.3% (10/32)	68.8% (22/32)	3.1% (1/32)	25.0% (8/32)	71.9% (23/32)

* 区分に該当しない

** 複数の試験結果があり、結果が一致していない物質は多数決とし、同数の場合には刺激性の低い区分に分類した

3.4. 本委員会の DAL および DAS に関する見解

TG 467 に準拠した DAL-1、DAL-2 および DAS は、区分 1、区分 2 の分類および区分に該当しない場合の判定を可能とする試験法であると本委員会は考える。なお、DAL-1 は、物理化学的特性データのみで眼刺激性がないと評価できるスキームである。また、そのデータの取得に *in silico* の予測モデルの利用を可能とした。すなわち、TG 467 は *in silico* を用いて眼刺激性がないと評価できるスキームが示された初の公文書であり、区分に該当しないことを、眼刺激性試験を実施せずに判定できることから、有用性が高いと考える。

ただし、DAL-1、DAL-2 および DAS ともに必要である LLBO を用いた BCOP を実施できる施設が現時点では日本にはなく、海外に依頼せねばならない。国内で実施できる方法を組み合わせた新たな DA が早急に検討され、TG 467 に追加されることを期待する。

4. DAの留意点

DA の開発にあたり、留意する点は適用範囲とボーダーラインの設定、*in silico* の利用等が挙げられる。DAL-1 において *in silico* は、RhCE の偽陽性結果を補完して区分に該当しない物質

を判別する役割を果たしており、予測性能の向上に貢献している。

以下に適用範囲とボーダーラインについての留意点を記す。今後、新たな *in vitro* 試験や *in silico* を両アプローチに組み込む場合や新たな組み合わせを提案する場合には、以下の点に留意する必要がある。

4.1. 適用範囲

個々の情報源の結果から、それぞれのTGで詳述されているように、当該化学物質が適用範囲から外れている場合、このDAは使用できない。

DAL-1 は界面活性剤や固体物質には適用できない。DAL-1 は、液体原体に適用できる。ただし、混合物、UVCB[2]、多成分構成物質を除く。不純物が >5% かつ＜20% 濃度の場合には、不純物の物理化学的特性も考慮する必要があり、すべての成分が除外規定に当てはまる時のみ、液体は区分に該当しない物質と予測する。

DAL-2 は、界面活性剤や水に分散した固体物質には適用できない。DAL-2 は、液体原体、および液体・固体の水溶液に適用できる。

DAS は、液体には適用できない。原体の界面活性剤を除く固体物質に適用できる（すなわち、ピペッティング不可能な試験物質）。

ユーザーは、それぞれの TG に規定されている個々の *in vitro* 試験方法の限界を理解する必要がある。それらは新たなデータにより改訂されるので、定期的に確認すべきである。それぞれの TG の最新バージョンを常に確認する必要がある。ユーザーはまた、それぞれの TG に指定されている物理化学的特性を測定するための個々の方法の限界を理解する必要がある。

その他の試験法の組み合わせについては、今後の検討・承認を経て、本 TG に含まれる可能性がある。

4.2. ボーダーライン

1) DAL-1予測に関連したTGのボーダーラインの取り扱い法

それぞれの*in vitro*法 (TG 437、TG 492) のTGsに記載されている各試験法の適用範囲を以下に示す。別に考慮する事項として、適用範囲内物質であっても、試験結果は本質的にバラツキやすく、これらのバラツキは、特に (分類) カットオフ閾値に近い場合、すなわち境界範囲内にある場合、試験結果を不確実にする。それぞれのTGs内に記載されている不確実性の程度を制御するために、以下の手順が示されている。

- ✓ TG 492 (RhCE) : 少なくとも2つの組織モデルからなる単一の試験の結果が明白である場合、十分な結果となる。しかし、反復測定の非一致および/または組織モデルの平均生存率が60±5%にある境界結果の場合、第2の試験の実施、および2回の試験の間で不一致の場合には第3の試験を考慮するべきである。
- ✓ TG 437 (BCOP LLBO) : 角膜混濁 (Lux/7、平均混濁度>145) で区分1と予測される。最初の試験で混濁 (Lux/7) を持つ3つの角膜のうち1つが<130となるような境界線の結果が発生した場合は、第2の試験の実施、および 2 回の試験の間で不一致の場合は第3の試験を考慮する必要がある。

[2] UVCB：組成が未知または変化する物質、複雑な反応生成物または生物材料、Substances of Unknown or Variable Composition, Complex Reaction Products or Biological Materials

2) DAL-2予測に関連したTGのボーダーラインの取り扱い法

それぞれの*in vitro*法 (TG 437、TG 491) のTGsに記載されている各試験法の適用範囲を以下に示す。別に考慮する事項として、適用範囲内物質であっても、試験結果は本質的にばらつきやすく、これらのバラツキは、特に (分類) カットオフ閾値に近い場合、すなわち境界範囲内にある場合、試験結果を不確実にする。それぞれのTGs内に記載されている不確実性の程度を制御するために、以下の手順が示されている。

- ✓ TG 437 (BCOP LLBO) : 角膜混濁 (Lux/7、平均混濁度>145) で区分1と予測される。最初の試験で混濁 (Lux/7) を持つ3つの角膜のうち1つが<130となるような境界線の結果が発生した場合は、第2の試験を実施し、および 2 回の試験の間で不一致の場合は 第3の試験を考慮する必要がある。
- ✓ TG 491 (STE) : 独立した 3 回の反復試験から得られた最終的な細胞生存率の標準偏差は、被験化学物質の濃度 5%と 0.05%の両方で 15未満であること。標準偏差が 15以上の場合、その結果を用いず、さらに 3 回反復試験を実施する。

3) DAS予測に関連したTGのボーダーラインの取り扱い法

それぞれの*in vitro*法 (TG 437、TG 492) のTGsに記載されている適用範囲が使用できる各情報である。別に考慮する事項として、適用範囲内物質であっても、試験結果は本質的にバラツキやすく、これらのバラツキは、特に (分類) カットオフ閾値に近い場合、すなわち境界範囲内にある場合、試験結果を不確実にする。それぞれのTGs内に記載されている不確実性の程度を制御するために、以下の手順が示されている。

- ✓ TG 492 (RhCE) : 少なくとも2つの組織モデルからなる単一の試験の結果が明白である場合、十分な結果となる。しかし、反復測定の非一致および／または組織モデルの平均生存率が50±5%にある境界結果の場合、第2の試験の実施、および2回の試験の間で不一致の結果の場合に第3の試験を考慮するべきである。
- ✓ TG 437 (BCOP LLBO) : 角膜混濁 (Lux/7、平均混濁度>145) で区分1と予測される。最初の試験で混濁 (Lux/7) を持つ3つの角膜のうち1つが<130となるまたは平均ODが2.5を超える、あるいは3つの角膜のうち1つのODが2.0未満となるような境界線の結果が発生した場合は、第2の試験の実施、および 2 回の試験の間で不一致の場合は 第3の試験を考慮する必要がある。

4.3. 物理化学的特性データに予測モデルを用いる場合の留意点

TG 467 では、物理化学的特性データの実測値がない場合、OECD の (Q) SAR バリデーション原則に基づいた計算モデルから得られた予測値を使用することも可能としている。バリデーション原則は以下の 5 項目から成る。

- エンドポイントの定義
- 曖昧さのないアルゴリズム
- 適用範囲の定義
- 適合度、頑健性、予測性の適切な評価
- 可能ならば、メカニズムに関する解釈

本 TG467 で推奨されている OPERA (The Open (Quantitative) Structure-activity/property Relationship App) は、この OECD の (Q) SAR バリデーション原則に基づいて開発された QSAR モデルである。DAL-1 の除外規定に用いられる LogP&VP&WS の予測に関しては、QSAR モデルに関わる様式 (QMRF: QSAR Model Reporting Format) が作成されている [14]。予測値だけでなく、適用範囲指数や、調査物質が適用範囲内であるときにはさらに信頼水準指数など予測値の信頼性の指標となる情報も得られる。これらの情報を理解して、予測値を適切に評価し、本 TG467 に用いる必要がある。特に、予測値がカットオフ値近傍である場合は、その扱いは注意を要する。

DAL-1 の除外規定に用いられるもう一つの物理化学的特性である ST については T.E.S.T. (Toxicity Estimation Software Tool) が推奨されている [15]。T.E.S.T.ではいくつかの QSAR 方法論が用いられている。ST のユーザーガイドによると、4 つの QSAR の方法論 (Hierarchical clustering method, Single model method, Group contribution method, Nearest neighbor method) で得られた予測値の平均値を用いるコンセンサス法 (consensus method) により予測性と物質の適用対象においてもっともよい結果を得ることができるとしている。

In silico モデルの妥当性および予測結果の評価に関しては、OECD QSAR framework において、A guidance for the regulatory assessment of (Quantitative) Structure−Activity Relationship models, predictions, and results based on multiple predictions (定量的) 構造活性相関モデル、予測、および複数の予測に基づく結果の規制評価のためのガイダンスが開発され、2023 年 12 月に公開されている [16]。このように *in silico* QSAR モデルは今後も様々な発展があることが期待されるため、モデルを使用する者は上記ガイダンスを参考にして、各モデルの長所・短所を十分に理解したうえで、利用する必要がある。

4.4. 今後の DA 開発の留意点

新たな試験法の組み合わせや新たなモデルを TG 467 に加える場合には、性能基準値および DA の留意点をよく理解して開発に取り組むべきと考える。なお、DAL はケーススタディとして OECD IATA Case study でも公表されている [17]。

5. 結論

DA は、物理化学的特性や既存の TG を組み合わせた方法である。今回 TG 467 に含まれた DAL および DAS の予測性は、OECD 専門家会議が定めた採用基準を満たしていた。

TG467 に準拠した DAL-1、DAL-2 および DAS は、区分 1、区分 2 の分類および区分に該当しない場合の判定を可能とする試験法であると本委員会は考える。

ただし、DAL-1、DAL-2 および DAS ともに主要試験である BCOP LLBO を実施できる施設は日本にはなく、海外に依頼せねばならない。国内で実施できる試験法を組み合わせた新たな DA が早急に TG 467 に追加されるべきと本委員会は考える。

6. 参考文献

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4) OECD (2023). Guideline for Testing of Chemicals No. 438: Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification. Organisation for Economic Cooperation and Development, Paris.

5) OECD (2023). Guideline for Testing of Chemicals No. 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants. Organisation for Economic Co-operation and Development, Paris.

6) OECD (2023). Guideline for Testing of Chemicals No. 491: Short Time Exposure *In Vitro* Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage. Organisation for Economic Co-operation and Development, Paris.

7) OECD (2024). Guideline for Testing of Chemicals No. 492: 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 Co-operation and Development, Paris.

8) OECD (2021). Guideline for Testing of Chemicals No. 494: Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage. Organisation for Economic Co-operation and Development, Paris.

9) OECD (2024). Guideline for Testing of Chemicals No. 496: *In vitro* Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage. Organization for Economic Co-operation and Development, Paris.

10) OECD (2024). Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation, Third Edition. Series on Testing and Assessment No.263. ENV Publications, Organisation for Economic Cooperation and Development, Paris.

11) OECD (2024) Guideline for Testing of Chemicals No.467, Defined Approaches for Serious Eye Damage and Eye Irritation, Organisation for Economic Co-operation and Development, Paris.

12) Alépée N, Adriaens E, Abo T, Bagley D, Desprez B, Hibatallah J, Mewes KR, Pfannenbecker U, Sala A, Van Rompay AR, Verstraelen S, McNamee P. (2019). Development of a defined approach for eye irritation for serious eye damage for neat liquids based on cosmetic Europe analysis of *in vitro* RhCE and BCOP test methods, *Toxicology In Vitro* 59, 100-114. https://doi.org/10.1016/j.tiv.2019.04.011

13) OECD (2024) Guideline for Testing of Chemicals 492B. Reconstructed human Cornea-like Epithelium (RhCE) test method for Eye Hazard, Organisation for Economic Co-operation and Development, Paris.

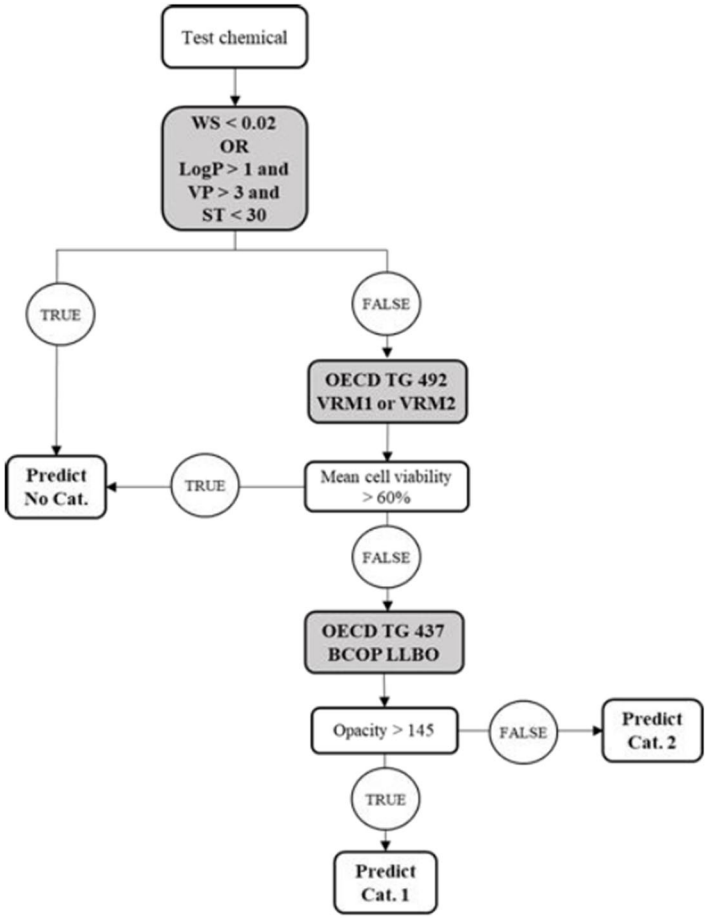
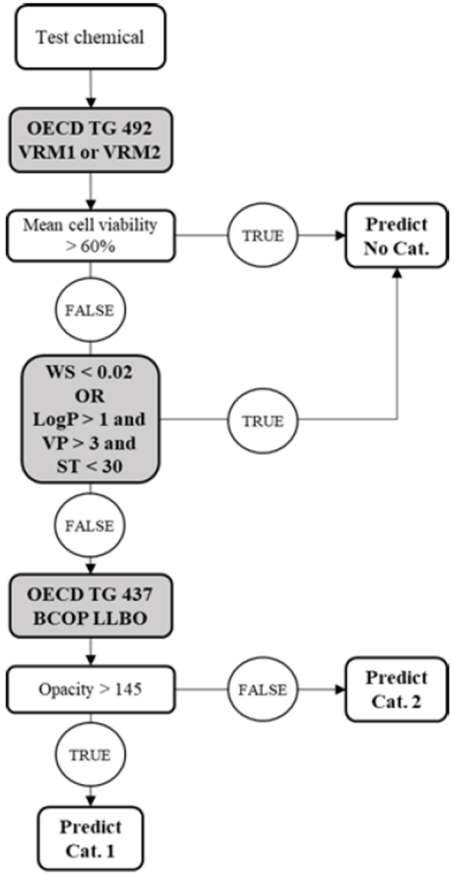
14) Mansouri, K., Grulke, C.M., Judson, R.S. *et al.* OPERA models for predicting physicochemical properties and environmental fate endpoints. *J Cheminform* 10, 10 (2018). https://doi.org/10.1186/s13321-018-0263-1

15) User's Guide for T. E. S. T. (Toxicity Estimation Software Tool) Version 5.1. https://www.epa.gov/sites/default/files/2016-05/documents/600r16058.pdf

16) OECD (2023) Series on Testing and Assessment No.386, (Q)SAR assessment framework: Guidance for the regulatory assessment of (Quantitative) Structure−Activity Relationship models, predictions, and results based on multiple predictions, Organisation for Economic Co-operation and Development, Paris.

17) OECD (2023) Series on Testing and Assessment No. 375, Case Study on the use of Integrated Approaches for Testing and Assessment for "Eye hazard identification" of "non-surfactant neat liquids" , Organisation for Economic Co-operation and Development, Paris.

添付資料 1　DAL-1 スキーム

Option 1	Option 2
	

添付資料 2　DAL-2 スキーム

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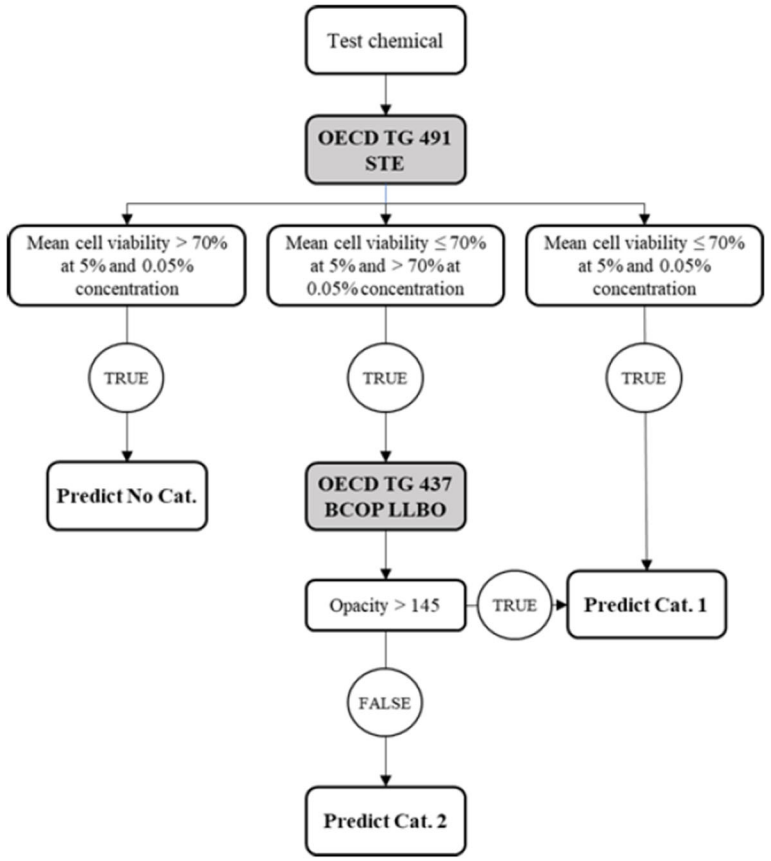
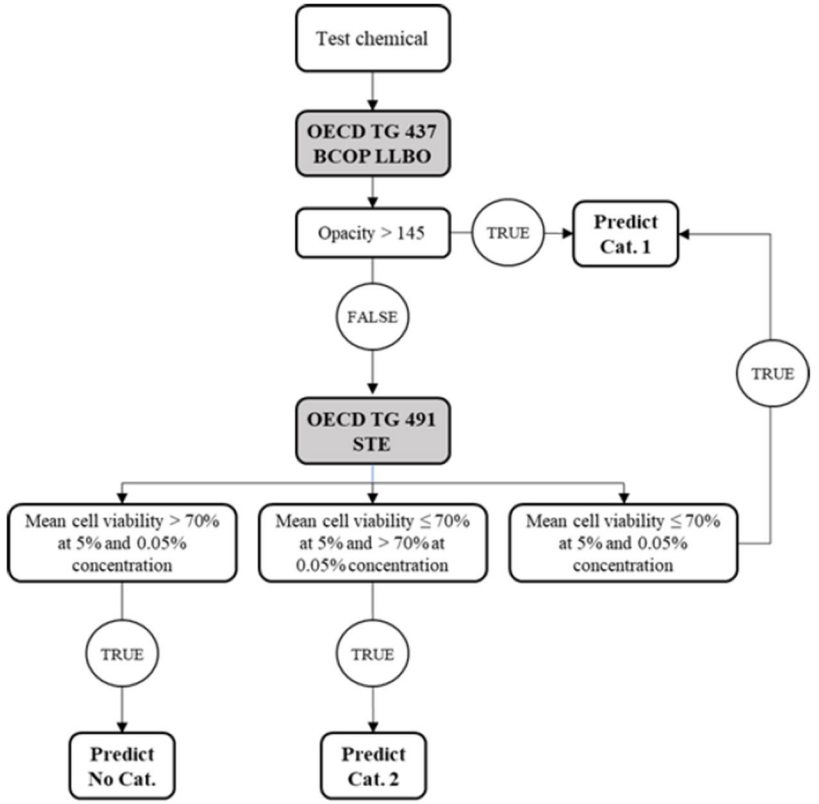
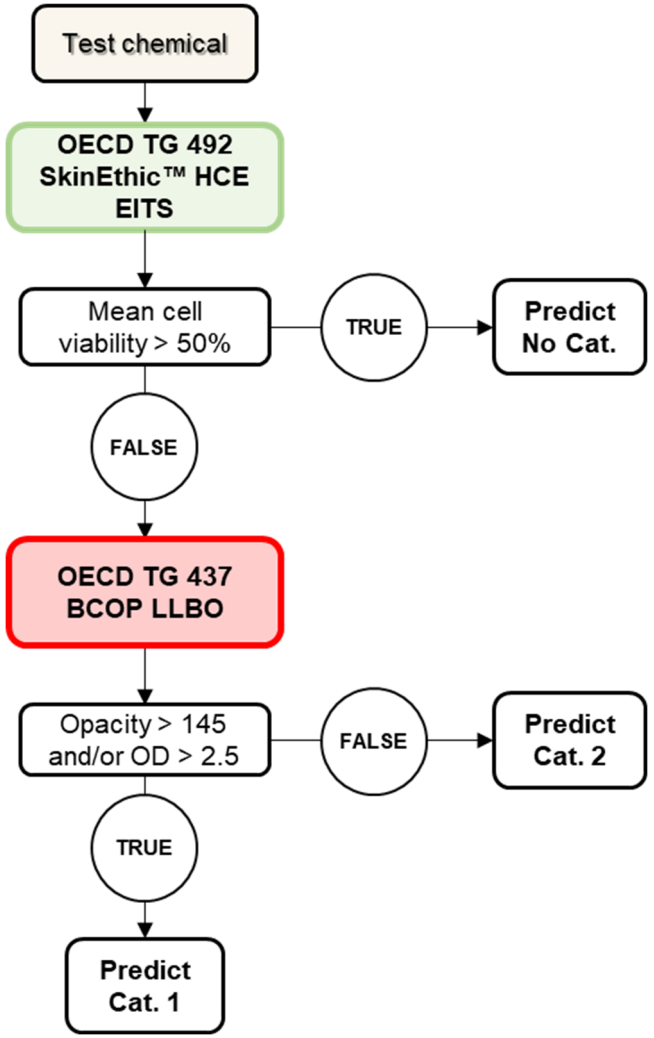
	

添付資料 3　DAS スキーム



添付資料４　DAL-1 EpiOcular™ EIT と SkinEthic™ TTL の予測性を比較した被験物質リスト (37 物質)

Chemical Name	CAS RN	UN GHS	DAL-1 EpiOcular™ EIT 結果	SkinEthic™ TTL 結果
2-Hydroxy iso-butyric acid ethyl ester	80-55-7	Cat 1	Cat 1	Cat 1
Benzensulphonylchloride	98-09-9	Cat 1	Cat 1	Cat 1
bis-(3-Aminopropyl)-tetramethyldisiloxane	2469-55-8	Cat 1	Cat 1	Cat 1
Cyclohexanol	108-93-0	Cat 1	Cat 2	Cat 1
Diethylethanolamine	100-37-8	Cat 1	Cat 1	Cat 1
Ethylhexyl acid phosphate ester	12645-31-7	Cat 1	Cat 1	Cat 1
Hydroxyethyl acrylate	818-61-1	Cat 1	Cat 1	Cat 2
Benzyl alcohol	100-51-6	Cat 1	Cat 2	Cat 1
(Ethylenediamine propyl)trimethoxysilane	1760-24-3	Cat 1	Cat 1	Cat 2
(3-Aminopropyl)triethoxy silane	919-30-2	Cat 1	Cat 1	Cat 1
Methoxyethyl acrylate	3121-61-7	Cat 1	Cat 1	Cat 1
n-Octylamine	111-86-4	Cat 1	Cat 1	Cat 1
2-Ethyl-1-hexanol	104-76-7	Cat 2	Cat 2	Cat 2
Acetone	67-64-1	Cat 2	Cat 1	Cat 2
Cyclopentanol	96-41-3	Cat 2	Cat 1	Cat 2
Methyl acetate	79-20-9	Cat 2	Cat 1	Cat 2
Methyl ethyl ketone	78-93-3	Cat 2	Cat 1	Cat 2
n-Octanol	111-87-5	Cat 2	Cat 2	Cat 2
Furfural	98-01-1	Cat 2	Cat 2	Cat 2

iso-Propanol	67-63-0	Cat 2	Cat 2	Cat 2
3-Chloropropionitrile	542-76-7	Cat 2	Cat 2	Cat 2
Butyl Dipropasol Solvent	29911-27-1	Cat 2	Cat 2	Cat 2
Ethyl-2-methyl acetoacetate	609-14-3	Cat 2	Cat 2	Cat 2
n-Butanal	123-72-8	Cat 2	Cat 2	Cat 2
2-Pseudoionone	141-10-6	Cat 2	No Cat	Cat 2
iso-Propyl acetoacetate	542-08-5	Cat 2	Cat 2	Cat 2
2,2-Dimethyl-3-pentanol	3970-62-5	No Cat	No Cat	Cat 2
Methyl iso-butyl ketone	108-10-1	No Cat	No Cat	Cat 2
n-Butyl acetate	123-86-4	No Cat	No Cat	No Cat
1,3-Di-iso-propyl benzene	99-62-7	No Cat	No Cat	No Cat
1-Methylpropylbenzene	135-98-8	No Cat	No Cat	No Cat
2,4-Pentanediol	625-69-4	No Cat	No Cat	No Cat
2-Methylpentane	107-83-5	No Cat	No Cat	No Cat
Bromo-2-butane	78-76-2	No Cat	No Cat	Cat 2
Dioctyl carbonate	1680-31-5	No Cat	No Cat	Cat 2
Dodecane	112-40-3	No Cat	No Cat	No Cat
Glycerol	56-81-5	No Cat	No Cat	No Cat

添付資料 5　DAL-1 SkinEthic™ EIT と SkinEthic™ TTL の予測性を比較した被験物質リスト (37 物質)

Test Chemical Name	CAS RN	UN GHS 区分	DAL-1 SkinEthic™ EIT 結果	SkinEthic™ TTL 結果
2-Hydroxy iso-butyric acid ethyl ester	80-55-7	Cat 1	Cat 1	Cat 1
Benzensulphonylchloride	98-09-9	Cat 1	Cat 1	Cat 1
bis-(3-Aminopropyl)-tetramethyldisiloxane	2469-55-8	Cat 1	Cat 1	Cat 1
Cyclohexanol	108-93-0	Cat 1	Cat 2	Cat 1
Diethylethanolamine	100-37-8	Cat 1	Cat 1	Cat 1
Ethylhexyl acid phosphate ester	12645-31-7	Cat 1	Cat 1	Cat 1
Hydroxyethyl acrylate	818-61-1	Cat 1	Cat 1	Cat 2
Benzyl alcohol	100-51-6	Cat 1	Cat 2	Cat 1
(Ethylenediamine propyl)trimethoxysilane	1760-24-3	Cat 1	Cat 1	Cat 2
(3-Aminopropyl)triethoxy silane	919-30-2	Cat 1	Cat 1	Cat 1
Methoxyethyl acrylate	3121-61-7	Cat 1	Cat 1	Cat 1
n-Octylamine	111-86-4	Cat 1	Cat 1	Cat 1
2-Ethyl-1-hexanol	104-76-7	Cat 2	Cat 2	Cat 2
Acetone	67-64-1	Cat 2	Cat 1	Cat 2
Cyclopentanol	96-41-3	Cat 2	Cat 1	Cat 2
Methyl acetate	79-20-9	Cat 2	Cat 1	Cat 2
Methyl ethyl ketone	78-93-3	Cat 2	Cat 1	Cat 2
n-Octanol	111-87-5	Cat 2	Cat 2	Cat 2
Furfural	98-01-1	Cat 2	Cat 2	Cat 2

iso-Propanol	67-63-0	Cat 2	Cat 2	Cat 2
3-Chloropropionitrile	542-76-7	Cat 2	Cat 2	Cat 2
Butyl Dipropasol Solvent	29911-27-1	Cat 2	Cat 2	Cat 2
Ethyl-2-methyl acetoacetate	609-14-3	Cat 2	Cat 2	Cat 2
n-Butanal	123-72-8	Cat 2	Cat 2	Cat 2
2-Pseudoionone	141-10-6	Cat 2	Cat 2	Cat 2
iso-Propyl acetoacetate	542-08-5	Cat 2	Cat 2	Cat 2
2,2-Dimethyl-3-pentanol	3970-62-5	No Cat	No Cat	Cat 2
n-Butyl acetate	123-86-4	No Cat	No Cat	No Cat
Propylidynetrimethanol, propoxylated	25723-16-4	No Cat	No Cat	No Cat
1,3-Di-iso-propyl benzene	99-62-7	No Cat	No Cat	No Cat
1,4-Dibromobutane	110-52-1	No Cat	No Cat	No Cat
2,4-Pentanediol	625-69-4	No Cat	No Cat	No Cat
Bromo-2-butane	78-76-2	No Cat	No Cat	Cat 2
Dodecane	112-40-3	No Cat	No Cat	No Cat
Glycerol	56-81-5	No Cat	No Cat	No Cat
Methyl tetraglycol	23783-42-8	No Cat	No Cat	No Cat
Silan 108 (Chemical name: Trimethoxyoctylsilane)	3069-40-7	No Cat	No Cat	No Cat

添付資料 6　DAL-2 と SkinEthic™ TTL の予測性を比較した被験物質リスト (45 物質)

Test Chemical Name	CAS RN	UN GHS 区分	DAL-2 結果	SkinEthic™ TTL 結果
2-Hydroxy iso-butyric acid ethyl ester	80-55-7	Cat 1	Cat 1	Cat 1
Benzensulphonylchloride	98-09-9	Cat 1	Cat 1	Cat 1
bis-(3-Aminopropyl)-tetramethyldisiloxane	2469-55-8	Cat 1	Cat 1	Cat 1
Cyclohexanol	108-93-0	Cat 1	Cat 2	Cat 1
Diethylethanolamine	100-37-8	Cat 1	Cat 1	Cat 1
Ethylhexyl acid phosphate ester	12645-31-7	Cat 1	Cat 1	Cat 1
Hydroxyethyl acrylate	818-61-1	Cat 1	Cat 1	Cat 2
Acetic acid (10%)	64-19-7	Cat 1	Cat 1	Cat 1
Benzyl alcohol	100-51-6	Cat 1	Cat 2	Cat 1
(Ethylenediamine propyl)trimethoxysilane	1760-24-3	Cat 1	Cat 1	Cat 2
(3-Aminopropyl)triethoxy silane	919-30-2	Cat 1	Cat 1	Cat 1
Methoxyethyl acrylate	3121-61-7	Cat 1	Cat 1	Cat 1
n-Octylamine	111-86-4	Cat 1	Cat 1	Cat 1
2-Ethyl-1-hexanol	104-76-7	Cat 2	Cat 2	Cat 2
Acetone	67-64-1	Cat 2	Cat 1	Cat 2
Cyclopentanol	96-41-3	Cat 2	Cat 1	Cat 2
Methyl acetate	79-20-9	Cat 2	Cat 1	Cat 2
Methyl ethyl ketone	78-93-3	Cat 2	Cat 1	Cat 2
n-Octanol	111-87-5	Cat 2	Cat 2	Cat 2

Furfural	98-01-1	Cat 2	Cat 2	Cat 2
iso-Propanol	67-63-0	Cat 2	Cat 2	Cat 2
Sodium hydroxide (1%)	1310-73-2	Cat 2	Cat 1	Cat 1
Butyl Dipropasol Solvent	29911-27-1	Cat 2	Cat 2	Cat 2
Ethyl-2-methyl acetoacetate	609-14-3	Cat 2	Cat 2	Cat 2
n-Butanal	123-72-8	Cat 2	Cat 2	Cat 2
2-Pseudoionone	141-10-6	Cat 2	Cat 2	Cat 2
iso-Propyl acetoacetate	542-08-5	Cat 2	Cat 2	Cat 2
Glycolic acid (10%)	79-14-1	Cat 2	Cat 1	Cat 2
2,2-Dimethyl-3-pentanol	3970-62-5	No Cat	No Cat	Cat 2
Furan	110-00-9	No Cat	No Cat	No Cat
Methyl iso-butyl ketone	108-10-1	No Cat	No Cat	Cat 2
n-Butyl acetate	123-86-4	No Cat	No Cat	No Cat
1,3-Di-iso-propyl benzene	99-62-7	No Cat	No Cat	No Cat
1,4-Dibromobutane	110-52-1	No Cat	No Cat	No Cat
1-Methylpropylbenzene	135-98-8	No Cat	No Cat	No Cat
2,4-Dicyano-1-butene	1572-52-7	No Cat	Cat 1/Cat 2	No Cat
2,4-Pentanediol	625-69-4	No Cat	No Cat	No Cat
2-Methylpentane	107-83-5	No Cat	No Cat	No Cat
Bromo-2-butane	78-76-2	No Cat	No Cat	Cat 2
Dodecane	112-40-3	No Cat	No Cat	No Cat
Glycerol	56-81-5	No Cat	No Cat	No Cat

Methyl tetraglycol	23783-42-8	No Cat	No Cat	No Cat
Pentaerythritol, propoxylated	9051-49-4	No Cat	No Cat	No Cat
Polyethylene glycol 400	25322-68-3	No Cat	No Cat	No Cat
Silan 108 (Chemical name: Trimethoxyoctylsilane)	3069-40-7	No Cat	No Cat	No Cat

添付資料 7　DAS と SkinEthic™ TTS の予測性を比較した被験物質リスト (71 物質)

Test Chemical Name	CAS RN	UN GHS 区分	DAS 結果	SkinEthic™ TTS 結果
(2R,3R)-3-((R)-1-(tert-butyldimethylsiloxy)-ethyl)-4-oxoazetidin-2-yl acetate	76855-69-1	Cat 2A	No Cat	No Cat
Sodium benzoate	532-32-1	Cat 2A	Cat 2	Cat 1
1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl) urea	101-20-2	No Cat	No Cat	No Cat
2,2'-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol)	103597-45-1	No Cat	No Cat	No Cat
Potassium tetrafluoroborate	14075-53-7	No Cat	No Cat	No Cat
4,4'-methylene bis-(2,6-di-tert-butylphenol)	118-82-1	No Cat	No Cat	No Cat
2,5,6-triamino-4-pyrimidinol sulphate	1603-02-7	No Cat	No Cat	Cat 2
1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol	72956-09-3	No Cat	No Cat	No Cat
propyl-4-hydroxybenzoate	94-13-3	No Cat	Cat 2	Cat 2
2-hydroxy-1,4-naphthoquinone	83-72-7	Cat 2B	Cat 1	Cat 2

1,4-dibutoxy benzene	104-36-9	Cat 2B	No Cat	No Cat
4-nitrobenzoic acid	62-23-7	Cat 2B	Cat 2	Cat 2
2,6-dichloro-5-fluoro-beta-oxo-3-pyridine propionate	96568-04-6	Cat 2B	Cat 2	Cat 2
sodium chloroacetate	3926-62-3	Cat 2B	Cat 2	Cat 2
3,3'-dithiopropionic acid	1119-62-6	Cat 2A	Cat 2	Cat 2
2-amino-3-hydroxy pyridine	16867-03-1	Cat 2A	Cat 2	No Cat
ammonium nitrate	6484-52-2	Cat 2A	Cat 2	Cat 2
dodecanoic acid	143-07-7	Cat 1	Cat 1	Cat 1
4,4'-(4,5,6,7-tetrabromo-3H-2,1-benzoxathiol-3-ylidene)bis[2,6-dibromophenol] S,S-dioxide	4430-25-5	Cat 1	Cat 1	Cat 2
1,2-benzisothiazol-3(2H)-one	2634-33-5	Cat 1	Cat 1	Cat 1
2-Benzyl-4-chlorophenol	120-32-1	Cat 1	Cat 1	Cat 1
2-Hydroxy iso-butyric acid	594-61-6	Cat 1	Cat 1	Cat 1
4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	Cat 1	Cat 2	Cat 2
alpha-Ketoglutaric acid	328-50-7	Cat 1	Cat 1	Cat 1
Dibenzoyl-L-tartaric acid	2743-38-6	Cat 1	Cat 1	Cat 1
1-Naphthalene acetic acid Na salt	61-31-4	Cat 1	Cat 1	Cat 1
Captan 90-concentrate	133-06-2	Cat 1	Cat 2	Cat 2
p-tert-Butylphenol (10 mg)	98-54-4	Cat 1	Cat 1	Cat 1
Sodium perborate tetrahydrate	10486-00-7	Cat 1	Cat 1	Cat 1
Sodium salicylate	54-21-7	Cat 1	Cat 1	Cat 1
Benzoic acid	65-85-0	Cat 1	Cat 1	Cat 1
Chlorhexidine	55-56-1	Cat 1	Cat 1	Cat 1

Paraformaldehyde	30525-89-4	Cat 1	Cat 2	Cat 2
N-(2-Methylphenyl)-iminodicarbonimidic diamide (1-(o-Tolyl)biguanide)	93-69-6	Cat 1	Cat 1	Cat 1
N-Acetyl-DL-methionine	1115-47-5	Cat 1	Cat 1	Cat 1
Triethanolamine orthovanadate	13476-99-8	Cat 1	Cat 2	Cat 2
4-Carboxybenzaldehyde	619-66-9	Cat 2A	Cat 1	Cat 2
Dibenzyl phosphate	1623-08-1	Cat 2A	Cat 1	Cat 1
m-Dinitrobenzene	99-65-0	Cat 2B	No Cat	No Cat
Ethylenediaminetetraacetic acid dipotassium salt	25102-12-9	No Cat	Cat 2	Cat 2
2-Mercaptopyrimidine	1450-85-7	No Cat	No Cat	No Cat
Anthracene	120-12-7	No Cat	No Cat	No Cat
Phenothiazine	92-84-2	No Cat	No Cat	No Cat
Phenylbutazone	50-33-9	No Cat	No Cat	No Cat
Silicic acid ~~(neat)~~	1343-98-2	No Cat	No Cat	No Cat
Tetrabromobisphenol A	79-94-7	No Cat	No Cat	No Cat
Theobromine	83-67-0	No Cat	No Cat	No Cat
2-Aminophenol	95-55-6	No Cat	No Cat	No Cat
4,4′ -Sulfonylbisbenezenamide	80-08-0	No Cat	No Cat	No Cat
4'-Aminoazobenzene-4-sulphonic acid	104-23-4	No Cat	No Cat	No Cat
Benzenamine, 4,4′ -[1,4-phenylenebis(1-methylethylidene)]bis-	2716-10-1	No Cat	No Cat	No Cat
Iminodibenzyl	494-19-9	No Cat	No Cat	No Cat
Magnesium carbonate	56378-72-4	No Cat	No Cat	No Cat
Phenylthiourea	103-85-5	No Cat	No Cat	No Cat

beta-Resorcyclic acid	89-86-1	Cat 1	Cat 1	Cat 1
Promethazine HCL	58-33-3	Cat 1	Cat 1	Cat 1
Aluminium hydroxide (Al(OH)3)	21645-51-2	No Cat	No Cat	No Cat
DL-Glutamic acid	19285-83-7	No Cat	Cat 2	Cat 2
Methanaminium, 1-carboxy-N,N,N-trimethyl-, hydroxide (1:1)	590-47-6	No Cat	No Cat	Cat 2
1,3-bis-(2,4-Diaminophenoxy) propane tetrachloride	74918-21-1	Cat 2A	Cat 1	Cat 1
Acid Red 92	18472-87-2	Cat 1	Cat 1	Cat 1
m-Phenylene diamine	108-45-2	Cat 1	Cat 1	Cat 1
gamma-(Aminocarbonyl)-N-methyl-N,N-bis(1-methyletheyl)-gamma-phenyl-,	71-81-8	Cat 2A	Cat 2	No Cat
4-Amino-3-nitrophenol	610-81-1	Cat 2A	Cat 1	Cat 2
1H-Indole-2,3-dione	91-56-5	No Cat	Cat 2	No Cat
3,5-Dihydroxyacetophenone	51863-60-6	No Cat	Cat 2	Cat 2
Gluconolactone	90-80-2	No Cat	Cat 2	Cat 1
Methyl p-hydroxybenzoate	99-76-3	No Cat	Cat 2	Cat 2
Sodium tripolyphosphate	7758-29-4	No Cat	Cat 2	No Cat
Theophylline sodium acetate	8002-89-9	No Cat	Cat 2	Cat 2
Xanthinol nicotinate	437-74-1	No Cat	Cat 2	No Cat

添付資料 3

Adopted:
25 June 2025

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Defined Approaches (DAs) for Serious Eye Damage and Eye Irritation

Table of contents

Tables

Figures

1 Introduction

1.1. General Introduction

1. The assessment of eye irritation/serious eye damage originally involved the use of albino rabbits according to the Draize eye test method (OECD Test Guideline 405) (1). The hazard potential of a test chemical was determined based on its effect on corneal opacity (CO), iritis (IR), conjunctival redness (CR), and conjunctival chemosis (CC). Based on the severity of effects and/or the timing of their reversibility, classifications are derived according to the serious eye damage/eye irritation classification criteria defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (2). According to the UN GHS classification system, Category 1/serious damage (Cat. 1) is defined as causing irreversible effects (not fully reversible within 21 days) on the eye/serious damage to the eye. Category 2/irritation (Cat. 2) is defined as causing reversible effects (fully reversible within 21 days) on the eye/eye irritation. This category may be divided into the optional Categories 2A (effects fully reversible within 21 days) and 2B (effects fully reversible within 7 days). When none of the Cat. 1 or Cat. 2 classification criteria are met, the test chemical does not require classification which corresponds to No Category (No Cat.).

2. In 2022, a stand-alone *in vitro* method (OECD TG 492B was adopted for the identification of test chemicals not requiring classification (UN GHS No Cat), requiring classification for eye irritation (UN GHS Cat 2) and requiring classification for serious eye damage (UN GHS Cat 1) (3). Furthermore, several Test Guidelines (TGs) on *in vitro* methods have been adopted for the identification of test chemicals inducing serious eye damage (UN GHS Cat. 1) or for the identification of test chemicals not requiring classification for eye irritation and serious eye damage hazards (UN GHS No Cat.), notably OECD TG 437, TG 438, TG 460, TG 491, TG 492, TG 494, and TG 496 (4, 5, 6, 7, 8, 9, 10). Data generated with these *in vitro* methods are proposed to be used together, as well as with information sources such as physicochemical properties, *in silico* and read-across predictions from chemical analogues, within integrated approaches to testing and assessment (IATA) or defined approaches (DAs) (11). Results from the individual information sources cannot be used in DAs if the chemicals are known to clearly fall outside the applicability domains of the methods, as may be detailed in the respective assay TGs. The prediction from a DA may be used alone or along with further information as part of an IATA (11) or according to the applicable legal criteria.

3. The major difficulty for a single *in vitro* test method to fully replace the *in vivo* rabbit eye test (TG 405) is to predict the middle category (UN GHS Cat. 2) and it is therefore recommended to make use of testing strategies (e.g., Top-Down or Bottom-Up approach) that combine the strengths of individual *in vitro* test methods to address the required ranges of irritation potential (12). The determination of the most relevant *in vivo* endpoint(s), in particular the effects on cornea, iris or conjunctiva, is important for the development of adequate *in vitro* methods as it allows to better understand the relationship between the *in vitro* and the *in vivo* data (13, 14). For this reason, it is recommended to take into consideration the most important drivers for Cat. 1 and Cat. 2 classifications as well as the

distribution of *in vivo* effects for chemicals not requiring classification when selecting reference chemicals for the development, evaluation and/or validation of alternative methods and/or strategies for serious eye damage and eye irritation testing (11) (see "*In vivo* reference data (Draize eye test)" in the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation (15)*).

4. Modes of action for eye irritation are unknowable for the majority of chemicals and do not provide additional insight in evaluation of the test methods and DAs, and thus they are not considered for the analysis of the test chemicals for the DAs in the current Guideline (see paragraph 27 of the SD for more information).

5. Results from multiple information sources can be used together in DAs to predict the eye hazard potential of test chemicals. A DA consists of a fixed data interpretation procedure (DIP) (*i.e.* a mathematical model, a rule-based approach) applied to data (*e.g in silico* predictions, *in chemico*, *in vitro* data) generated with a defined set of information sources to derive a prediction without the need for expert judgment. The DAs use method combinations intended to overcome some of the limitations of the individual, stand-alone methods in order to provide increased confidence in the overall obtained result. The DAs provide information that can be used for eye hazard identification.

6. Testing laboratories should consider all relevant available information on the test chemical prior to conducting the studies according to a DA. Such information could include, for example, the identity and chemical structure of the test chemical and its physicochemical properties. Such information should be considered in order to determine whether the individual OECD TG methods under a specific DA are applicable for the test chemical.

7. When performing a hazard evaluation based on the output from the *in vivo* Draize eye test, from an *in chemico* test, from an *in vitro* test, from an *in silico* approach, from a DA, and any combination thereof, the same principles always apply, *i.e.* all available information relevant to the chemical in question should be taken into consideration as well as toxicological data on structurally related test chemicals, if available. However, specific regulatory requirements in the applicable legislation should be applied.

8. Two rule-based DAs for non-surfactant liquids and one rule-based DA for neat solids are included in this GL, and are described with respect to their intended regulatory purpose: hazard identification, *i.e.* discrimination between three UN GHS categories i.e., Category 1 (Cat. 1) on "serious eye damage"; Category 2 (Cat. 2) on "eye irritation" and No Category (No Cat.) for chemicals "not requiring classification and labelling" for eye irritation or serious eye damage (2). The evaluation and review of the DAs are described in detail in the *Supporting Document for Evaluation and Review of TG 467 on DAs for Serious Eye Damage / Eye Irritation (15).* For the non-surfactant liquids, a dataset of at least 86 chemicals with DA predictions, data on individual information sources, highly curated Draize eye test data, and physicochemical properties, was compiled and is attached as **Annex B (spreadsheets)** to the *Supporting Document for Evaluation and Review of TG 467 on DAs for Serious Eye Damage / Eye Irritation (15)*. A list of 109 solids with DA predictions, data on individual information sources, and highly curated Draize eye test data was compiled and is attached as **Annex A (spreadsheets)** to the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15)*. The list of chemicals was used to evaluate the performance of the DAs. The set of liquids and solids covers a broad range of uses and chemicals classes, with a wide range of organic functional groups (79 different OFGs for the liquids and 111 different OFG for the solids) defined according to OECD QSAR Toolbox analysis (version 3.2; https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

9. The dataset is chemically diverse as shown by the physicochemical properties covered by these chemicals: it contains small and large molecules, as well as hydrophobic

and hydrophilic substances. Further details on the chemical characterization of the reference database are available in **Section 5.1.2.** of the *Supporting Document for Evaluation and Review of TG 467 on DAs for Serious Eye Damage / Eye Irritation (15)*.

10. Other DAs may be included in this GL following future review and approval.

1.2. DAs and Use Scenarios included in the Guideline

11. The DAs currently described in this GL are:

- **Part I** - Defined Approaches 1 for Eye hazard identification based on physicochemical properties and *in vitro* data (16), for neat non-surfactant liquids (DAL-1).
- **Part II** - Defined Approaches 2 for Eye hazard identification based on *in vitro* data (17) for non-surfactant neat liquids, liquids and solids dissolved in water (DAL-2).
- **Part III** - Defined Approach (DAS) for Eye hazard identification based on *in vitro* data for neat solids (DAS).

12. The DAL-1 described in this GL is based on the use of a combination of test methods described in OECD TG 437 and TG 492 as well as the physicochemical properties (PCP) of the test chemical. DAL-2 in contrast, is based on the use of a combination of test methods described in the OECD TG 437 and TG 491. The methods used in DAL-1 and DAL-2 encompass the following validated test methods: the Bovine Corneal Opacity and Permeability (BCOP) using the laser light-based opacitometer (LLBO)[1] according to the OECD TG 437, the Reconstructed human Cornea-like Epithelium (RhCE) (EpiOcular™ Eye Irritation Test or SkinEthic™ Human Corneal Epithelium (HCE) EIT) according to the OECD TG 492[2] and the Short Time Exposure in vitro (STE) according to the OECD TG 491. The DALs are hereafter referred to as DAL-1 PCP/EpiOcular/LLBO, DAL-1 PCP/SkinEthic/LLBO, and DAL-2 STE/LLBO. The DAS is based on the use of combination of test methods described in the OECD TG 437 and TG 492. The methods used in DAS encompass the following validated test methods: the BCOP LLBO[3] according to the OECD TG 437 and the SkinEthic™ HCE EIT according to the OECD TG 492[4] and is hereafter referred to as DAS SkinEthic/LLBO. Transferability, within- and between-laboratory reproducibility of these individual test methods have been assessed during their respective validation studies (18, 19, 20, 21, 22).

13. The DAs described in this GL can each be used to address countries' requirements for identifying chemicals causing serious eye damage (*i.e.* UN GHS Category 1), eye irritation (*i.e.* UN GHS Category 2), and test chemicals not requiring classification (*i.e.* UN GHS No Category), though they do so with different performance (detailed in the respective descriptions of each DA).

[1] DIP with the BCOP OP-KIT test method did not meet the acceptance criteria for the current GL and thus is not used for DAL-1 or DAL-2 (see Annex A of the supporting document).

[2] Other similar methods from OECD TG 492 were not used for the DAL-1 analysis due to insufficient availability in data for those methods.

[3] DIP with the BCOP OP-KIT test method did not meet the acceptance criteria for the current GL and thus is not used for DAS.

[4] DIP with the EpiOcular™ EIT test method did not meet the acceptance criteria for the current GL and thus is not used for DAS.

14. The DAs described in this GL are not designed to distinguish between Categories 2A and 2B.

15. DAL-1 and DAL-2 are applicable to liquids (i.e., pipettable test substances) and DAS is applicable to solids (i.e., not pipettable test substances). For additional details see **Section 2** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15)*.

16. The performance of DAL-1 PCP/EpiOcular/LLBO described in this GL for discriminating between the three UN GHS categories was evaluated using 94 non-surfactant liquids (17 Cat. 1, 22 Cat. 2, and 55 No Cat.) for which physicochemical properties, EpiOcular™ EIT predictions, BCOP LLBO predictions (available for all *in vivo* classified results but missing for 14/55 *in vivo* No Cat. substances), and classifications based on Draize Eye test data are available (for additional details see **Section 2** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15))*.

17. The performance of DAL-1 PCP/SkinEthic/LLBO described in this GL for discriminating between the three UN GHS categories was evaluated using 86 non-surfactant liquids (17 Cat. 1, 23 Cat. 2, and 46 No Cat.) for which physicochemical properties, SkinEthic™ HCE EIT predictions, BCOP LLBO predictions (available for all *in vivo* classified results but missing for 11/46 *in vivo* No Cat. substances), and classifications based on Draize Eye test data are available (for additional details see **Section 2** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15))*.

18. The performance of the DAL-2 STE/LLBO described in this GL for discriminating between the three UN GHS categories was evaluated using 164 non-surfactant liquids (17 Cat. 1, 24 Cat. 2, and 123 No Cat.) for which STE predictions, BCOP LLBO predictions (available for all *in vivo* classified results but missing for 67/123 *in vivo* No Cat. substances), and classifications based on Draize Eye test data are available (for additional details see **Section 2** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15)*.

19. The performance of the DAS SkinEthic/LLBO described in this GL for discriminating between the three UN GHS categories was evaluated using 109 solids (31 Cat. 1, 18 Cat. 2, and 60 No Cat.) for which SkinEthic™ HCE EIT predictions, BCOP LLBO predictions (available for all *in vivo* classified results but missing for 5/60 *in vivo* No Cat. substances), and classifications based on Draize Eye test data are available (for additional details see Section 2 and Annex B.3 of the *Supporting Document to the GL on DAs for Serious Eye Damage/Eye Irritation (15).*

1.3. Performance and Applicability

1.3.1. Performance of the DAs

20. Table 1.1. Summary of the DAs included in this Guideline – Eye hazard identification**.1** provides an overview of the DAs included in this Guideline (GL), their information sources used, and summarises their performance against the Draize Eye reference data. More details are provided in Part I, Part II, and Part III of this GL, as well as in the *Supporting documents to the GL on DAs for Serious Eye Damage and Eye Irritation (15)*.

21. The performance of the DAs for UN GHS classification (Cat. 1, Cat. 2, and No Cat) when compared to the Draize Eye test reference data yielded balanced accuracies of 69.2% (DAL-1 PCP/EpiOcular/LLBO), 75.2% (DAL-1 PCP/SkinEthic/LLBO), 74.3% (DAL-

2 STE/LLBO), and 66.7% (DAS SkinEthic/LLBO). Note that there is a raised degree of uncertainty relating to the derived Cat. 1 and Cat. 2 accuracies (correct predictions), as compared with the No Cat. accuracies due to the lower number of reference chemicals within these categories. It was however not possible to increase the number of chemicals because of the limited number of available Draize Eye test results with a Cat. 1 or Cat. 2 classification. Detailed performance statistics are reported in Part I, Part II, and Part III and in **Section 5** of the *Supporting documents to the GL on DAs for Serious Eye Damage and Eye Irritation (15).*

Table 1.1. Summary of the DAs included in this Guideline – Eye hazard identification

DA	DAL-1 PCP/EpiOcular/LLBO (N=94)	DAL-1 PCP/SkinEthic/LLBO(N=86)	DAL-2 STE/LLBO (N=164)	DAS Skinethic/LLBO (N=109)
Information Sources	Physicochemical properties, EpiOcular™ EITL (TG 492), BCOP LLBO (TG 437)	Physicochemical properties, SkinEthic™ HCE EITL (TG 492), BCOP LLBO[a] (TG 437)	STE (TG 491), BCOP LLBO (TG 437)	SkinEthic™ HCE EITS (TG 492), BCOP LLBO[b] (TG 437)
Applicable	Non-surfactant neat liquids	Non-surfactant neat liquids	Non-surfactant neat liquids, liquids and solids dissolved in water	Non-surfactant neat solids
Performance vs. Draize Eye test (Correct classification)	70.5% No Cat. (N=55) 59.1% Cat. 2 (N=22) 76.5% Cat. 1 (N=17)	79.7% No Cat. (N=46) 68.7% Cat. 2 (N=23) 76.5% Cat. 1 (N=17)	85.3% No Cat. (N=123) 56.3% Cat. 2 (N=24) 81.2% Cat. 1 (N=17)	70.0% No Cat. (N=60) 52.3% Cat. 2 (N=18) 77.4% Cat. 1 (N=31)

[a] BCOP LLBO based on opacity only; [b] BCOP LLBO based on opacity and/or permeability
Note: For performance, accuracy reflects correct classification rate within each UN GHS category.
EITL: Eye Irritation Test protocol for liquids and EITS protocol for solids.
Solid: non-pippetable neat substance.

1.3.2. Applicability domain of the DAs and of the individual components of the DAs

22. DAL-1 PCP/EpiOcular/LLBO and DAL-1 PCP/SkinEthic/LLBO are not applicable for surfactants and solids. Both DAs are applicable to neat liquids, excluding mixtures, UVCBs and multi-constituent substances. For impurities with concentration > 5% and < 20%, the physicochemical properties of the impurities also need to be determined, and only when all components meet the exclusion criteria, the liquid is predicted No Cat., in all other cases, proceed with an RhCE test method.

23. DAL-2 STE/LLBO is not applicable for surfactants and solids dispersed in water. The DAL-2 STE/LLBO is applicable to non-surfactant neat liquids, liquids and solids dissolved in water.

24. DAS SkinEthic/LLBO is not applicable to liquids. The DAS SkinEthic/LLBO is applicable to non-surfactant neat solids (i.e., not pipettable test substances).

25. Users should refer to the limitations of the individual *in vitro* test methods as specified in their respective TGs, which are revised as new data become available and should be consulted regularly. The most up-to-date published version of the respective TGs should always be used. Users should also refer to the limitations of the individual methods for measuring the physicochemical properties as specified in their respective GLs.

1.3.3. Uncertainty of DAs

26. Details on accepting the results of individual information sources to determine confidence in DA predictions are provided in **Sections 2.1.4**, **3.1.4**, and **4.1.4** and in the respective TGs (TG 437; TG 491; TG 492) (4, 7, 8).

1.4. References

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(10) OECD (2023), OECD Guidelines for the Testing of Chemicals No. 496: In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/970e5cd9-en.

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2 PART I - Defined Approaches 1 (DAL-1), based on physicochemical properties and *in vitro* data, for neat non-surfactant[5] liquids

27. Part I of this GL applies to DAL-1 that is intended for hazard identification, *i.e.* distinguishing between serious eye damage and eye irritation potential of test chemicals (or the absence thereof), specifically for neat non-surfactant liquids based on physicochemical properties and *in vitro* data. A summary of the DAL-1 for hazard identification is provided below; additional detailed information can be found in the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation.*

2.1. DAL-1

2.1.1. Summary

28. The DAL-1 is intended for the identification of the eye irritation hazard of a test chemical without the use of animal testing, *i.e.* UN GHS Cat. 1 vs. UN GHS Cat. 2 vs. UN GHS No Cat. The data interpretation procedure (DIP) is not designed to provide information on sub-categorisation of Cat. 2 into 2A and 2B.

29. The DAL-1 presented in this GL describes the combination of one and/or three physicochemical properties with the results of two *in vitro* test methods (RhCE and BCOP LLBO) for the identification of the eye hazard potential of non-surfactant liquid substances primarily for the purposes of classification and labelling without the use of animal testing (1). The physicochemical properties can be retrieved from publicly available databases, can be determined by new experimental studies, or may be predicted using computational methods (e.g. Quantitative Structure-Activity Relationships ((Q)SAR)). The RhCE models that are part of DAL-1 are the EpiOcular™ EITL and the SkinEthic™ HCE EITL (OECD TG 492) (2). Furthermore, the Bovine Corneal Opacity and Permeability (BCOP) test method with the laser light-based opacitometer (LLBO) is used (OECD TG 437) (3).

30. The DAL-1 PCP/EpiOcular/LLBO was compared to 94 chemicals with curated Draize Eye test reference data and demonstrated a balanced accuracy of 68.7% (see Table 2.1). The DAL-1 PCP/SkinEthic/LLBO was compared to 86 chemicals with curated

[5] Surfactant, also called surface-active agent, this is a substance, such as a detergent, that can reduce the surface tension of a water and thus allow it to foam or penetrate solids; it is also known as a wetting agent.

Draize Eye test reference data and demonstrated a balanced accuracy of 75.0% (see Table 2.2).

2.1.2. Data interpretation procedure

31. The data interpretation procedure (DIP) applied uses the readout of the prediction models of each of the individual test method as defined by the TGs and/or information on the physicochemical properties. A scheme of DAL-1 is presented in Figure 2.1. Physicochemical property exclusion rules based on water solubility (WS) or a combination of octanol-water partition coefficient (LogP), vapour pressure (VP) and surface tension (ST) of the neat liquid are used in a first step to identify liquid chemicals with no serious eye damage or eye irritation potential (details are provided in section 5.1.2. of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation*). Liquids that are not identified as No Cat. according to the physicochemical property-based exclusion rules, are then evaluated based on a RhCE test method (EpiOcular™ EIT or SkinEthic™ HCE EIT) in Step 2. Liquids that result in a tissue viability > 60% are classified No Cat. Liquids that result in a tissue viability ≤ 60% are then evaluated based on the BCOP LLBO test method in a third step. Liquids that result in an opacity > 145 are predicted Cat. 1 and the remaining liquids are assigned Cat. 2. Note that it is also possible to start with a RhCE method, followed by the physicochemical property exclusion rules in case the tissue viability measured with EpiOcular™ EIT or SkinEthic™ HCE EIT > 60% (Figure 2.2). Furthermore, when a RhCE method is used as a first step and if the tissue viability > 60%, the prediction is based on the stand-alone method.

Figure 2.1. Scheme of the DAL-1 option 1; step 1 physicochemical exclusion rules (WS: water solubility in mg/mL; or LogP: octanol-water partition coefficient / VP: vapour pressure in mm Hg / ST: surface tension of the neat liquid in dyne/cm) to identify No Cat., step 2 RhCE EITL test method used to identify No Cat., and step 3 BCOP LLBO used to identify Cat. 1

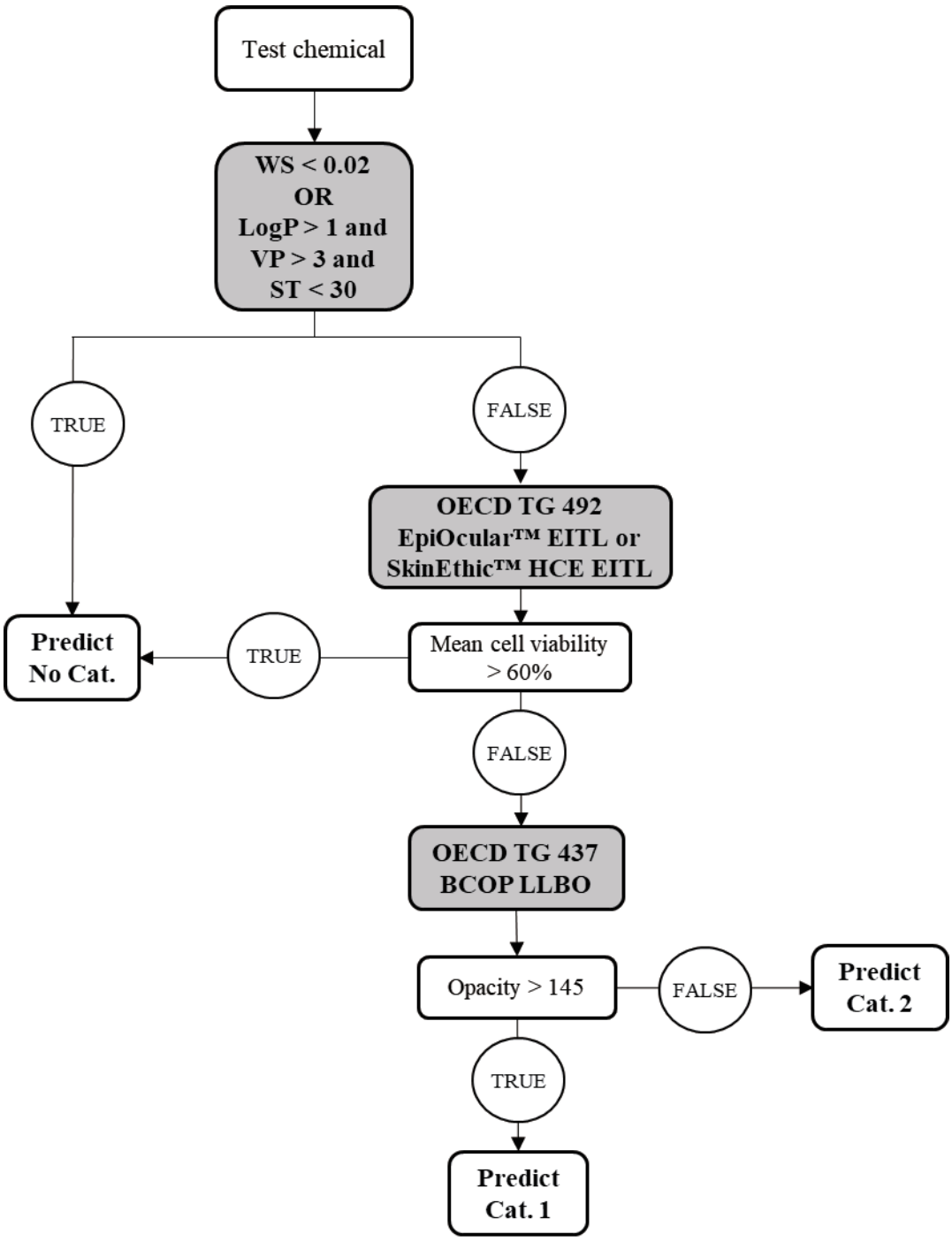
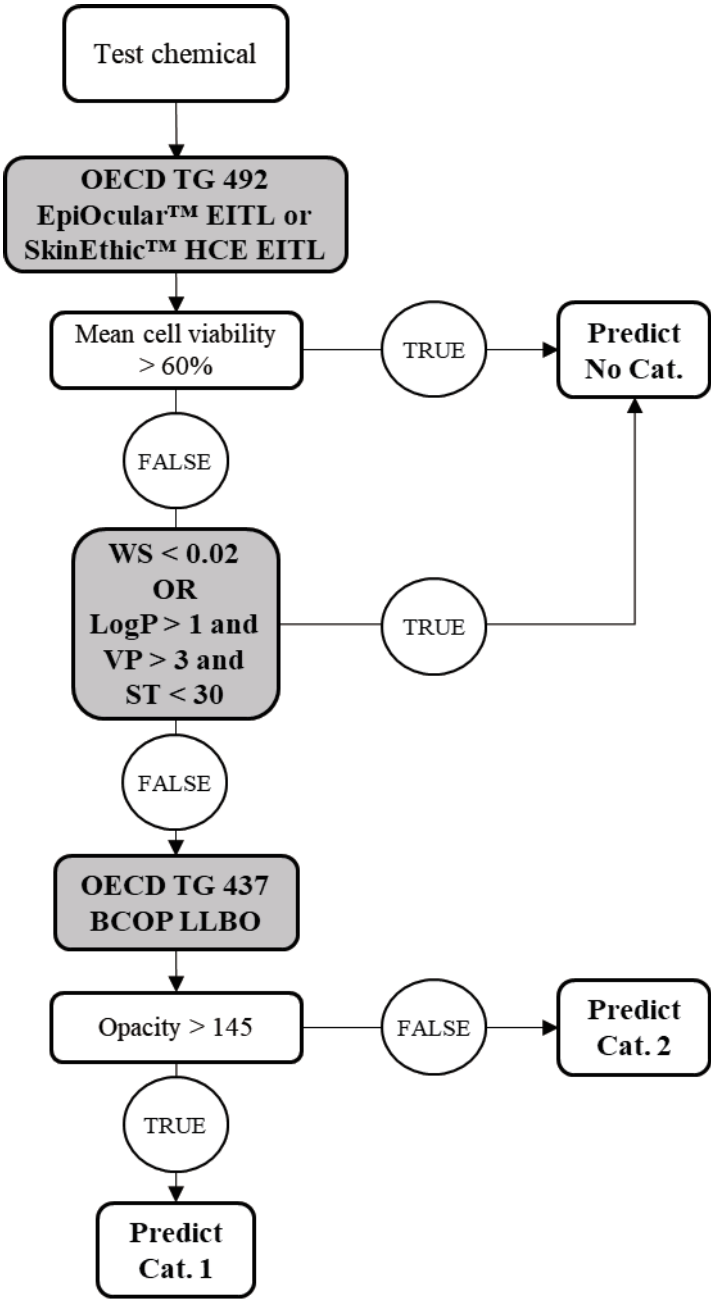


Figure 2.2. Scheme of the DAL-1 option 2; step 1 RhCE EITL test method used to identify No Cat., step 2: physicochemical exclusion rules (WS: water solubility in mg/mL; or LogP: octanol-water partition coefficient / VP: vapour pressure in mm Hg / ST: surface tension of the neat liquid in dyne/cm) to identify No Cat., and step 3 BCOP LLBO used to identify Cat. 1



2.1.3. Description and limitations of the individual information sources

32. The individual information sources in the DA are the physicochemical properties and test methods included in OECD TG for serious eye damage/eye irritation or the absence thereof (OECD TG 437, 492) (2, 3), and the protocols are detailed therein.

33. The following *in vitro* test methods from those TGs have been characterised and included in the DAL-1.

- The RhCE EITL test methods: the methods measure the ability to induce cytotoxicity. In case borderline results are obtained, additional testing should be conducted, as specified in OECD TG 492 (2).
- BCOP LLBO test method: the eye hazard potential of a test chemical is measured by its ability to induce opacity and permeability in an isolated bovine cornea. Note that only opacity measurement is considered in the DAs. In case borderline results are obtained for opacity measurements, additional testing should be conducted, as specified in OECD TG 437 (3).

34. Any restriction regarding the applicability domain identified in the respective test method TGs (TG 437, TG 492) and analytical methods for measuring the physicochemical properties (GL 104, GL 105, GL 107, GL 115, GL 117, GL 123) is applicable to this GL (2, 3).

35. Measurements of physicochemical properties should be performed according to the OECD Guidelines (GL) and test reports are required corresponding with the information requested on data and reporting in each specific OECD GL (see Annex E). Prediction of physicochemical properties should use models that are based on the 5 OECD principles for QSAR models and that have a QMRF (QSAR Model Reporting Format).

2.1.4. Procedure for dealing with borderline result in test guidelines relevant to DAL-1

36. The first decision on whether each information element can be used is dictated by the applicability domain as described in the TGs of the respective *in vitro* methods (TG 437, TG 492) (2, 3). Even for within-domain substances, test results are inherently subject to variation and these variations increase the uncertainty of a test result, especially when close to a (classification) cut-off threshold, i.e. in the borderline range. The following procedures are in place to control the degree of uncertainty are described within the TGs of the respective information sources.

- TG 492 (RhCE EITL): A single test composed of at least two tissue replicates should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent tissue viability equal to 60±5% a second test should be considered, as well as a third one in case of discordant results between the first two tests.
- TG 437 (BCOP LLBO): UN GHS Cat. 1 prediction based on opacity (Lux/7, mean opacity > 145), but 1 of 3 corneas with opacity (Lux/7) < 130; in cases of borderline results in the first testing run, a second testing run should be considered, as well as a third one in case of discordant predictions between the first two testing runs.

2.1.5. Predictive capacity of the DAL-1 PCP/EpiOcular/LLBO vs. the Draize Eye test

37. The predictive capacity of DAL-1 PCP/EpiOcular/LLBO is reported based on data generated by the Draize eye test (see **Table 2.1**) (see **Section 2.1** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation)*.

Performance statistics are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 5** and **Annex B.2** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation for liquids (5).*

Table 2.1. Performance of DAL-1 PCP/EpiOcular/LLBO in comparison to Draize Eye reference data

UN GHS	Prediction using DAL-1 PCP/EpiOcular/LLBO[b]		
	Cat 1	Cat 2	No Cat
Cat. 1 (N='17),' %[a] (n/N)	**76.5%** **(13.0/17.0)**	23.5% (4.0/17.0)	0.0% (0.0/17.0)
Cat. 2 (N='22)' , %[a] (n/N)	27.3% (6.0/22.0)	**59.1%** **(13.0/22.0)**	13.6% (3.0/22.0)
No Cat. (N='55)' , %[a] (n/N)	5.5% (3.0/55.0)	24.0% (13.2/55.0)	**70.5%** **(38.8/55.0)**
68.7% balanced accuracy overall			

[a] The proportion given is based on a weighted calculation which takes into account (where they exist) multiple results from an individual information source for a given chemical, and applying a correction factor so that all chemicals have a weight of 1. To improve the readability of the numbers in the table, the numbers n/N have been rounded, so they may deviate slightly from the percentage corresponding to the weighted calculation.
[b] EpiOcular™ EITL protocol for liquids.
Note 1: The performance is the same for the two versions of the DIP (Fig. 2.1 and Fig 2.2).

2.1.6. Predictive capacity of the DAL-1 PCP/SkinEthic/LLBO vs. the Draize Eye test

38. The predictive capacity of DAL-1 PCP/SkinEthic/LLBO is reported based on data generated by the Draize eye test (see **Table 2.2**) (see **Section 2.1** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation)*. Performance statistics are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 5** and **Annex B.2** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation for liquids (5).*

Table 2.2. Performance of DAL-1 PCP/SkinEthic/LLBO in comparison to Draize Eye reference data

UN GHS	Prediction using DAL-1 PCP/SkinEthic/LLBO [b]		
	Cat 1	Cat 2	No Cat
Cat. 1 (N='17),' %[a] (n/N)	**76.5%** **(13.0/17.0)**	23.5% (4.0/17.0)	0.0% (0.0/17.0)
Cat. 2 (N='23),' %[a] (n/N)	30.4% (7.0/23.0)	**68.7%** **(15.8/23.0)**	0.9% (0.2/23.0)
No Cat. (N='46),' %[a] (n/N)	3.1% (1.4/46.0)	17.2% (7.9/46.0)	**79.7%** **(36.7/46.0)**
75.0% balanced accuracy overall			

[a] The proportion given is based on a weighted calculation which takes into account (where they exist) multiple results from an individual information source for a given chemical, and applying a correction factor so that all chemicals have a weight of 1. To improve the readability of the numbers in the table, the numbers n/N have been rounded, so they may deviate slightly from the percentage corresponding to the weighted calculation.
[b] SkinEthic™ HCE EITL protocol for liquids
Note 1: The performance is the same for the two versions of the DIP (Fig. 2.1 and Fig 2.2).

2.1.7. Demonstration of Proficiency

39. The DAL-1 relies on a simple, rule-based data interpretation procedure and requires no expert judgment. Proficiency chemicals for the individual information sources are defined in the respective TGs (2, 3). Proficiency for the individual information sources demonstrates proficiency for the DA.

2.1.8. Reporting of the DA

40. The reporting of the DA application should include at a minimum the following elements:

- Test chemical identification (*e.g.,* chemical name, structural formula, composition, isomers, purity, chemical identity of impurities including their quantities as available, CAS number, batch and lot number, and other relevant identifiers).
- The DAL-1 option used, and the RhCE method used.
- Individual test reports performed per corresponding TGs (OECD TG 437, TG 492). Note that the chemical identity for each test report should match that above.
- Individual test reports on physicochemical properties corresponding with the information requested on data and reporting in each specific OECD GL (Annex E).
- Discussions on any uncertainties in the data with the *in vitro* methods and physicochemical properties applied in the DA that was used.
- Outcome of the DA application, including discussion of any uncertainties in the applied DA, as well as their predicted impact (e.g., over- or under-classification).
- Any deviation from or adaptation of the DA.
- Conclusion

2.2. References

(1) Alépée N, Adriaens E, Abo T, Bagley D, Desprez B, Hibatallah J, Mewes KR, Pfannenbecker U, Sala A, Van Rompay AR, Verstraelen S, McNamee P. (2019). Development of a defined approach for eye irritation or serious eye damage for neat liquids based on Cosmetics Europe Analysis of in vitro RhCE and BCOP test methods. Toxicology In Vitro 59, 100-114. https://doi: 10.1016/j.tiv.2019.04.011.

(2) OECD (2023), OECD Guidelines for the Testing of Chemicals No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264242548-en.

(3) OECD (2023), OECD Guidelines for the Testing of Chemicals No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264203846-en.

(4) OECD (2014), Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models, OECD Series on Testing and Assessment, No. 69, OECD Publishing, Paris, https://doi.org/10.1787/9789264085442-en.

(5) OECD (2022), Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage and Eye Irritation. Series on Testing and Assessment No. 354. Organisation for Economic Cooperation and Development, Paris. Available at: [https://www.oecd.org/chemicalsafety/testing /series-testing-assessment-publications-number.htm]

3 PART II – Defined Approaches 2 (DAL-2), based on *in vitro* data, for non-surfactant[6] neat liquids, liquids and solids dissolved in water

41. Part II of this GL applies to DAL-2 STE/LLBO that is intended for hazard identification, *i.e.* distinguishing between serious eye damage and eye irritation potential of test chemicals (or the absence thereof), specifically for non-surfactant neat liquids, liquids and solids dissolved in water based on *in vitro* data. A summary of the DAL-2 for hazard identification is provided below; additional detailed information can be found in the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation.*

3.1. DAL-2

3.1.1. Summary

42. The DAL-2 STE/LLBO is intended for the identification of the eye irritation hazard of a test chemical without the use of animal testing, *i.e.* UN GHS Cat. 1 vs. UN GHS Cat. 2 vs. UN GHS No Cat. The data interpretation procedure (DIP) is not designed to provide information on sub-categorisation of Cat. 2 into 2A and 2B.

43. The DAL-2 STE/LLBO presented in this GL describes the combination of two *in vitro* test methods (STE: OECD TG 491 and BCOP LLBO: OECD TG 437) for the identification of the eye hazard potential of non-surfactant neat liquids, liquids and solids dissolved in water primarily for the purposes of classification and labelling without the use of animal testing (1, 2, 3).

44. The DAL-2 STE/LLBO was compared to 164 chemicals with curated Draize Eye test reference data and demonstrated a balanced accuracy of 74.3% (see **Table 3.1**).

3.1.2. Data interpretation procedure

45. The DIP applied uses the readout of the prediction models of each of the individual test methods as defined by the TGs (OECD 437, OECD 491) (1, 2). A scheme of DAL-2 STE/LLBO is presented in Figure 3.1. Scheme of the DAL-2 STE/LLBO option 1: start with

[6] Surfactant, also called surface-active agent, this is a substance, such as a detergent, that can reduce the surface tension of a water and thus allow it to foam or penetrate solids; it is also known as a wetting agent.

the STE test method followed by the BCOP LLBO test method. The STE test method is used to identify liquid chemicals with no serious eye damage or eye irritation potential (No Cat.: liquids that result in a mean cell viability > 70% at a 5% and 0.05% concentration) or to identify liquids that cause serious eye damage/eye irritation (Cat. 1: liquids that result in a mean cell viability ≤ 70% at a 5% and 0.05% concentration). For liquids that result in a mean cell viability ≤ 70% at 5% concentration but > 70% at 0.05%, the BCOP LLBO is needed. Liquids that result in an opacity > 145 are predicted as Cat. 1 and the remaining liquids are assigned to Cat. 2. Note that it is also possible to start with the BCOP LLBO followed by the STE test method, this scheme of DAL-2 is presented in Figure 3.2.

Figure 3.1. Scheme of the DAL-2 STE/LLBO option 1: start with the STE test method followed by the BCOP LLBO test method

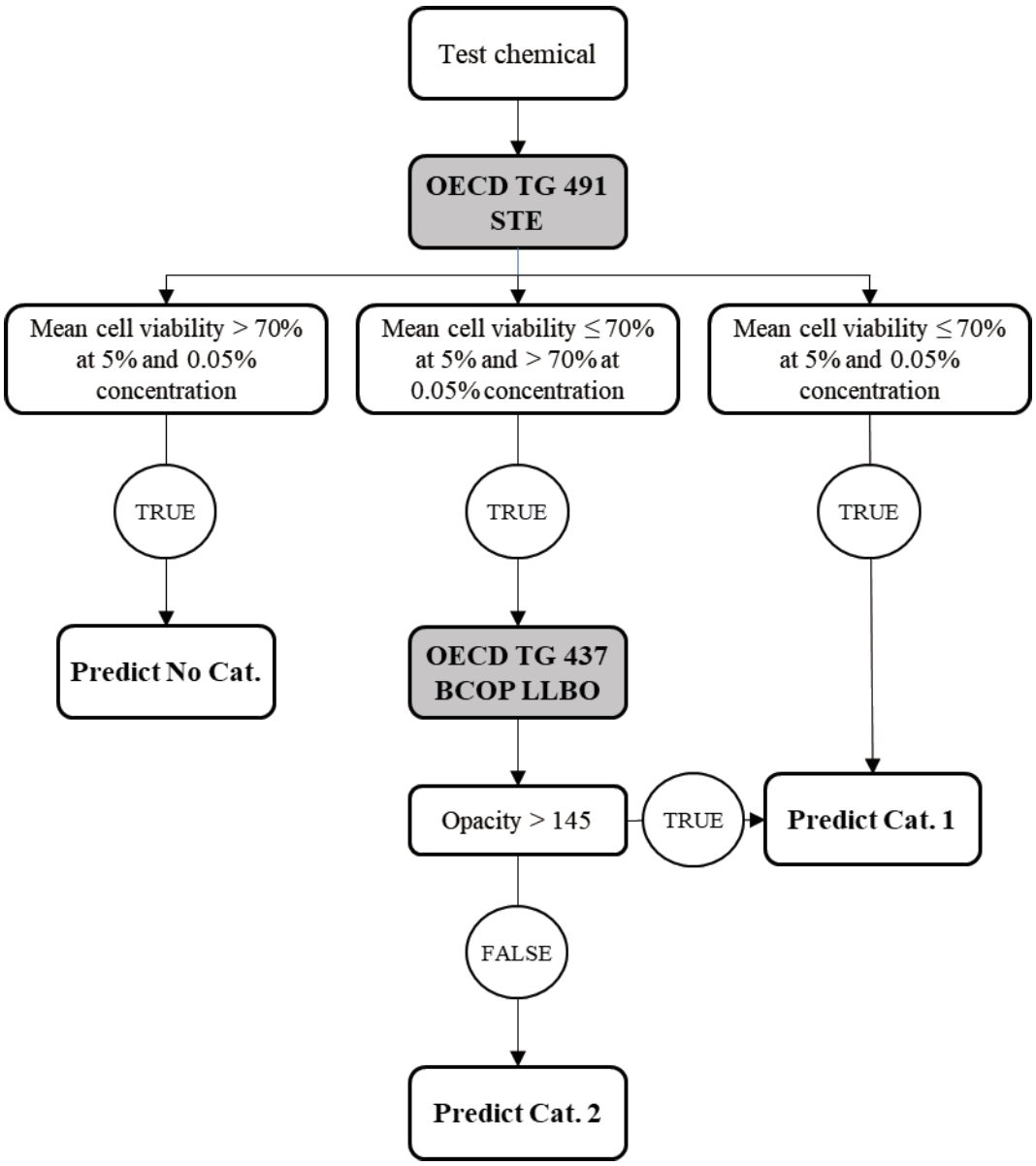
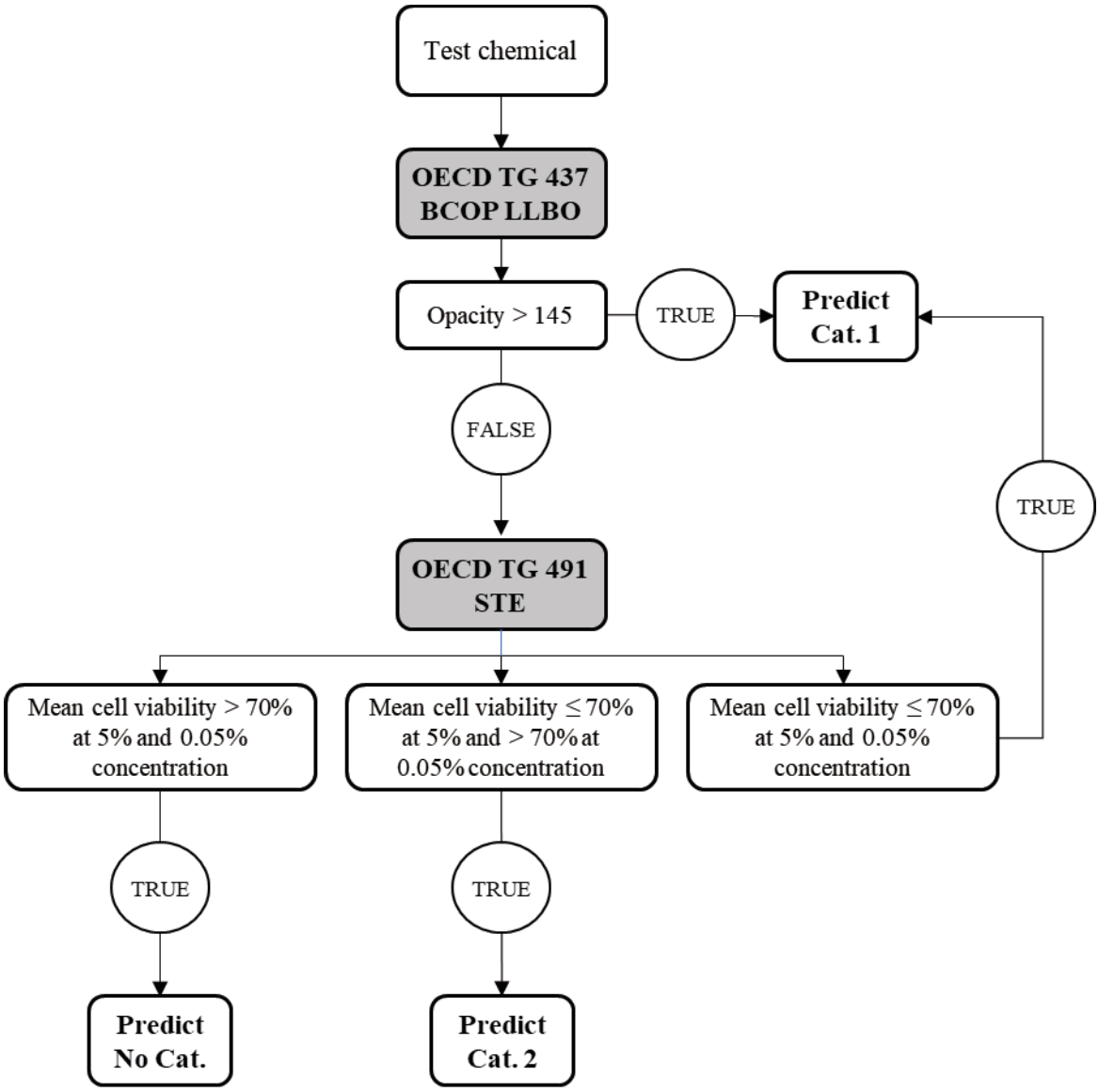


Figure 3.2. Scheme of the DAL-2 STE/LLBO option 2: start with the BCOP LLBO test method followed by the STE test method.



3.1.3. Description and limitations of the individual information sources

46. The individual information sources in the DA are test methods included in OECD TGs (OECD TG 437, 491) for serious eye damage/eye irritation or the absence thereof (1, 2), and the protocols are detailed therein.

47. The following test methods from those TGs have been characterised and included in the DAL-2 STE/LLBO.

- BCOP LLBO test method: the eye hazard potential of a test chemical is measured by its ability to induce opacity and permeability in an isolated bovine cornea. Note that only opacity measurement is considered in the DAL-2. In case borderline results are obtained for opacity measurements, additional testing should be conducted, as specified in OECD TG 437 (1).
- STE test method: the eye hazard potential of a test chemical is assessed based on its ability to induce cytotoxicity on a confluent monolayer of Statens Seruminstitut Rabbit Cornea (SIRC) cells.

48. Any restrictions regarding the applicability domain identified in the respective TGs (TG 437, TG 491) are applicable to this GL (1, 2).

3.1.4. Procedures for dealing with borderline results in the test guidelines relevant to DAL-2 STE/LLBO predictions

49. The first decision on whether each information element can be used is dictated by the practical limitations as described in the TGs of the respective *in vitro* methods (TG 437, TG 491) (1, 2). Even for within-domain substances, test results are inherently subject to variation and these variations increase the uncertainty of a test result, especially when close to a (classification) cut-off threshold, i.e. in the borderline range. The following procedures to control the degree of uncertainty are described with the TG of the respective information sources.

- TG 437 (BCOP LLBO): UN GHS Cat. 1 prediction based on opacity (Lux/7, mean opacity > 145), but 1 of 3 corneas with opacity (Lux/7) < 130; in cases of borderline results in the first testing run, a second testing run should be considered, as well as a third one in case of discordant predictions between the first two testing runs.
- TG 491 (STE): Standard deviation of the final cell viability derived from three independent repetitions should be less than 15% for both 5% and 0.05% concentrations of the test chemical. If the standard deviation is greater than or equal to 15%, the results should not be used and three more repetitions should be performed.

3.1.5. Predictive capacity of the DAL-2 STE/LLBO vs. the Draize Eye test

50. The predictive capacity of DAL-2 STE/LLBO is reported based on data generated by the Draize eye test (see Table 3.1) (see **Section 2.1** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation)*. Performance statistics are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 5** and **Annex B.2** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation.*

Table 3.1. Performance of DAL-2 STE/LLBO in comparison to Draize Eye reference data

UN GHS	Prediction using DAL-2 STE/LLBO		
	Cat 1	Cat 2	No Cat
Cat. 1 (N=17), % [a] (n/N)	**81.2%** **(13.8/17.0)**	17.6% (3.0/17.0)	1.2% (0.2/17.0)
Cat. 2 (N=24), % [a] (n/N)	30.2% (7.2/24.0)	**56.3%** **(13.5/24.0)**	13.5% (3.2/24.0)
No Cat. (N=123), % [a] (n/N)	4.1% (5.1/123.0)	10.6% (13.0/123.0)	**85.3%** **(104.9/123.0)**
74.3% balanced accuracy overall			

[a] The proportion given is based on a weighted calculation which takes into account (where they exist) multiple results from an individual information source for a given chemical, and applying a correction factor so that all chemicals have a weight of 1. To improve the readability of the numbers in the table, the numbers n/N have been rounded, so they may deviate slightly from the percentage corresponding to the weighted calculation.
Note 1: The performance was obtained using the version of the DIP provided in Fig 3.1.

3.1.6. Proficiency chemicals

51. The DAL-2 STE/LLBO relies on a simple, rule-based data interpretation procedure and requires no expert judgment. Proficiency chemicals for the individual information sources are defined in the respective TGs (1, 2). Proficiency for the individual information sources demonstrates proficiency for the DAL-2 STE/LLBO.

3.1.7. Reporting of the DA

52. The reporting of the DA application should include at a minimum the following elements:

- Test chemical identification (*e.g.,* chemical name, structural formula, composition, isomers, purity, chemical identity of impurities including their quantities as available, CAS number, batch and lot number, and other relevant identifiers).
- Describe the DAL-2 option used.
- Individual test reports performed per corresponding TGs (OECD TG 437, TG 491). Note that the chemical identity for each test report should match that above.
- Discussions on any uncertainties in the data with the *in vitro* methods applied in the DA that was used.
- Outcome of the DA application, including discussion of any uncertainties in the applied DA, as well as their predicted impact (e.g., over- or under-classification)
- Any deviation from or adaptation of the DA.
- Conclusion.

3.2. References

(1) OECD (2023), OECD Guidelines for the Testing of Chemicals No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264203846-en.

(2) OECD (2023), OECD Guidelines for the Testing of Chemicals No. 491: Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264242432-en.

(3) Alépée N, Adriaens E, Abo T, Bagley D, Desprez B, Hibatallah J, Mewes KR, Pfannenbecker U, Sala A, Van Rompay AR, Verstraelen S, McNamee P. (2019). Development of a defined approach for eye irritation or serious eye damage for liquids, neat and in dilution, based on cosmetics Europe analysis of *in vitro* STE and BCOP test methods. Toxicology In Vitro 57, 154-163. doi: 10.1016/j.tiv.2019.02.019.

(4) OECD (2022). Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage and Eye Irritation. Series on Testing and Assessment No. 354. Organisation for Economic Cooperation and Development, Paris. Available at: [https://www.oecd.org/chemicalsafety/testing/ series-testing-assessment-publications-number.htm]

4 PART III – Defined Approaches (DAS), based on *in vitro* data, for neat solids[7]

54. Part III of this GL applies to DAS SkinEthic/LLBO that is intended for hazard identification, i.e., distinguishing between serious eye damage and eye irritation potential of test chemicals (or the absence thereof), specifically for neat solids based on *in vitro* data. A summary of the DAS for hazard identification is provided below; additional detailed information can be found in the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (3)*.

4.1. DAS SkinEthic/LLBO

4.1.1. Summary

55. The DAS SkinEthic/LLBO is intended for the identification of the eye irritation hazard of a test chemical without the use of animal testing, i.e., UN GHS Cat. 1 vs. UN GHS Cat. 2 vs. UN GHS No Cat. The data interpretation procedure (DIP) is not designed to provide information on sub-categorisation of Cat. 2 into 2A and 2B.

56. The DAS SkinEthic/LLBO presented in this GL describes the combination of two *in vitro* test methods (SkinEthic™ HCE EITS: OECD TG 492 and BCOP LLBO: OECD TG 437) for the identification of the eye hazard potential of neat solids primarily for the purposes of classification and labelling without the use of animal testing (1, 2).

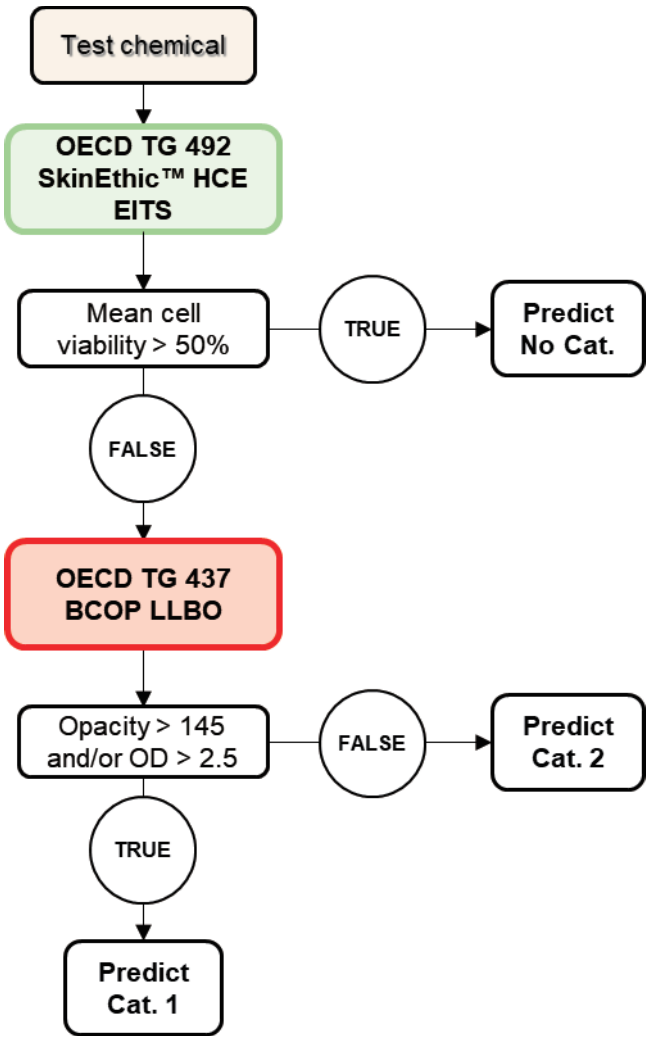
57. The DAS SkinEthic/LLBO was compared to 109 chemicals with curated Draize Eye test reference data and demonstrated a balanced accuracy of 66.7% (see Table 4.1. Performance of DAS SkinEthic/LLBO in comparison to Draize Eye reference data).

4.1.2. Data interpretation procedure

58. The DIP uses the readout of the prediction models of each of the individual test methods as defined by the TGs (OECD 437, OECD 492) (1, 2). A scheme of DAS SkinEthic/LLBO is presented in Figure 4.1. The SkinEthic™ HCE EITS test method is used to identify solid chemicals with no serious eye damage or eye irritation potential (No Cat.: solids that result in a mean tissue viability > 50%). For solids that result in a mean tissue viability ≤ 50%, the BCOP LLBO is needed. Solids that result in an opacity > 145 or OD > 2.5, or both opacity > 145 and OD > 2.5 are predicted as Cat. 1 and the remaining solids are assigned to Cat. 2.

[7] A solid is a non-pipettable test substance.

Figure 4.1. Scheme of the DAS SkinEthic/LLBO: start with the SkinEthic™ HCE EITS test method followed by the BCOP LLBO test



4.1.3. Description and limitations of the individual information sources

59. The individual information sources in the DA are test methods included in OECD TGs (OECD TG 492, 437) for serious eye damage/eye irritation or the absence thereof (1, 2), and the protocols are detailed therein.

60. The following test methods from those TGs have been characterised and included in the DAS SkinEthic/LLBO.

- SkinEthic™ HCE EITS test method: the method measures the ability to induce cytotoxicity. In case borderline results are obtained, additional testing should be conducted, as specified in OECD TG 492 (1).
- BCOP LLBO test method: the eye hazard potential of a test chemical is measured by its ability to induce opacity and permeability in an isolated bovine cornea. In case borderline results are obtained for opacity or permeability measurements, additional testing should be conducted, as specified in OECD TG 437 (2).

61. Any restrictions regarding the applicability domain identified in the respective TGs (TG 437, TG 492) are applicable to this GL (1, 2).

4.1.4. Procedures for dealing with borderline results in the test guidelines relevant to DAS SkinEthic/LLBO predictions

62. The first decision on whether each information element can be used is dictated by the practical limitations as described in the TGs of the respective *in vitro* methods (TG 437, TG 492) (1, 2). Even for within-domain substances, test results are inherently subject to variation and these variations increase the uncertainty of a test result, especially when close to a (classification) cut-off threshold, i.e., in the borderline range. The following procedures to control the degree of uncertainty are described within the respective TG.

- TG 492 (SkinEthic™ HCE EITS): A single test composed of at least two tissue replicates should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent tissue viability equal to 50±5% a second test should be considered, as well as a third one in case of discordant results between the first two tests.
- TG 437 (BCOP LLBO): UN GHS Cat. 1 prediction based on (i) opacity (Lux/7, mean opacity > 145), but 1 of 3 corneas with opacity (Lux/7) < 130 or (ii) OD (mean OD > 2.5), but 1 of 3 corneas with OD < 2.0; in cases of borderline results in the first testing run, a second testing run should be considered, as well as a third one in case of discordant predictions between the first two testing runs.

4.1.5. Predictive capacity of the DAS SkinEthic/LLBO vs. the Draize Eye test

63. The predictive capacity of DAS SkinEthic/LLBO is reported based on data generated by the Draize eye test (see Table 4.1. Performance of DAS SkinEthic/LLBO in comparison to Draize Eye reference data) (see **Section 5.1** and **Annex A.2** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation for neat solids (3)*). Performance statistics are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 7** and **Annex A.2** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation for neat solids (3).*

Table 4.1. Performance of DAS SkinEthic/LLBO in comparison to Draize Eye reference data

UN GHS	Prediction using DAS SkinEthic/LLBO		
	Cat 1	Cat 2	No Cat
Cat. 1 (N=31), % [a] (n/N)	**77.4%** **(24.0/31.0)**	22.6% (7.0/31.0)	0.0% (0.0/31.0)
Cat. 2 (N=18), % [a] (n/N)	29.5% (5.3/18.0)	**52.3%** **(9.4/18.0)**	18.2% (3.3/18.0)
No Cat. (N=60), % [a] (n/N)	1.7% (1.0/60.0)	28.3 (17.0/60.0)	**70.0%** **(42.0/60.0)**
66.7% balanced accuracy overall			

[a] The proportion given is based on a weighted calculation which takes into account (where they exist) multiple results from an individual information source for a given chemical, and applying a correction factor so that all chemicals have a weight of 1. To improve the readability of the numbers in the table, the numbers n/N have been rounded, so they may deviate slightly from the percentage corresponding to the weighted calculation.

4.1.6. Proficiency chemicals

64. The DAS SkinEthic/LLBO relies on a simple, rule-based data interpretation procedure and requires no expert judgment. Proficiency chemicals for the individual information sources are defined in the respective TGs (1, 2). Proficiency for the individual information sources demonstrates proficiency for the DAS.

4.1.7. Reporting of the DA

65. The reporting of the DA application should include at a minimum the following elements:

- Test chemical identification (*e.g.,* chemical name, structural formula, composition, isomers, purity, chemical identity of impurities including their quantities as available, CAS number, batch and lot number, and other relevant identifiers).
- Individual test reports performed per corresponding TGs (OECD TG 437, TG 492). Note that the chemical identity for each test report should match that above.
- Discussions on any uncertainties in the data with the *in vitro* methods applied in the DA that was used.
- Outcome of the DA application, including discussion of any uncertainties in the applied DA, as well as their predicted impact (e.g., over- or under-classification).
- Any deviation from or adaptation of the DA.
- Conclusion.

4.2. References

(1) OECD (2023), OECD Guidelines for the Testing of Chemicals No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264242548-en.

(2) OECD (2023), OECD Guidelines for the Testing of Chemicals No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264203846-en.

(3) OECD (2024), Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage and Eye Irritation(Second Edition). Series on Testing and Assessment No. 354. Organisation for Economic Cooperation and Development, Paris. Available at: [https://www.oecd.org/chemicalsafety/testing /series-testing-assessment-publications-number.htm]

5 PART IV – Defined Approach (DA), based on in vitro data, for surfactants (DASF)

67. Part IV of this GL applies to DASF that is intended for hazard identification, *i.e.* distinguishing between serious eye damage and eye irritation potential of test chemicals (or the absence thereof), for surfactants[8] (neat and in dilution in deionized water) based on *in vitro* data. A summary of the DASF for hazard identification is provided below; additional detailed information can be found in the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation.*

5.1. DASF

5.1.1. Summary

68. The DASF, which was develop specifically for surfactants, is intended for the identification of the eye irritation hazard of a test chemical without the use of animal testing, *i.e.* UN GHS Cat. 1 vs. UN GHS Cat. 2 vs. UN GHS No Cat. The data interpretation procedure (DIP) is not designed to provide information on sub-categorisation of Cat. 2 into 2A and 2B. DASF fills an important gap in this Test Guideline, however, due to the currently small dataset for Cat.2 (n=10), if a surfactant is predicted as Cat.2, additional information may be required, taking into account that the prospective use of animals is only to be used as a last resort.

69. The DASF presented in this GL describes the combination of two *in vitro* test methods (EpiOcular™ Eye Irritation Test (EIT) or SkinEthic™ HCE EIT: OECD TG 492 and STE$^{0.5}$ for surfactants, as part of OECD TG 491) for the identification of the eye hazard potential of surfactants (liquids and solids) primarily for the purposes of classification and labelling without the use of animal testing (1, 2).

70. The performance of the DASF was compared to 50 surfactants (45 liquids and 5 solids) with curated Draize eye test reference data and demonstrated a balanced accuracy of 79.1% (see Table 5.1. Performance of DASF in comparison to Draize Eye reference data). No *in vivo* data on neat Cat. 2 surfactants were found to assess the performance of the DASF, therefore when the DASF predicts a Cat.2 for a neat surfactant, additional information may be required,-taking into account that the prospective use of animals is only

[8] Surfactant means any substance and/or mixture, which has surface-active properties and which consists of one or more hydrophilic and one or more hydrophobic groups of such a nature and size that it is capable of reducing the surface tension of water, and of forming spreading or adsorption monolayers at the water-air interface, and of forming emulsions and/or microemulsions and/ or micelles, and of adsorption at water-solid interfaces.

to be used as a last resort. Note that caution should be exercised when interpreting the DASF results for anionic surfactants as this class has a tendency to overpredict.

5.1.2. Data interpretation procedure

71. The DIP applied uses the readout of the prediction models of each of the individual test methods as defined by the OECD TG 492 and $STE^{0.5}$ for surfactants (1, 2). A schematic representation of DASF is provided in Figure 5.1. Bottom up approach of the DASF; step 1 EpiOcular™ EITL (Liquids) / EITS (Solids) or SkinEthic™ HCE EITL / EITS test method used to identify No Cat., and step 2 $STE^{0.5}$ for surfactants used to identify Cat. 1.

(bottom-up approach) and Figure 5.2. Top-down approach of the DASF; step 1 $STE^{0.5}$ for surfactants used to identify Cat. 1, and step 2 EpiOcular™ EITL / EITS or SkinEthic™ HCE EITL / EITS test method used to identify No Cat.

72. (top-down approach). Depending on the available data, the most relevant approach will be selected: if the test substance is most likely classified as UN GHS Cat. 1, or if there is high certainty that no classification is needed (UN GHS No Cat), either a Top-Down or Bottom-Up approach will be considered, respectively (3). The EpiOcular™ EIT or SkinEthic™ HCE EIT test method are used to identify surfactants with no serious eye damage or eye irritation potential. Surfactants predicted as No Cat. are those that result in a mean tissue viability > 60% for EpiOcular™ EIT (liquid or solid) or > 60% (liquid) or > 50% (solid) for SkinEthic™ HCE EIT, respectively. For surfactants that result in a mean tissue viability below the threshold value, the $STE^{0.5}$ for surfactants is needed. Surfactants (liquid or solid) that result in a mean cell viability ≤ 20% at a 0.5% test concentration are predicted as Cat. 1 and the remaining surfactants are assigned to Cat. 2.

Figure 5.1. Bottom up approach of the DASF; step 1 EpiOcular™ EITL (Liquids) / EITS (Solids) or SkinEthic™ HCE EITL / EITS test method used to identify No Cat., and step 2 STE$^{0.5}$ for surfactants used to identify Cat. 1.

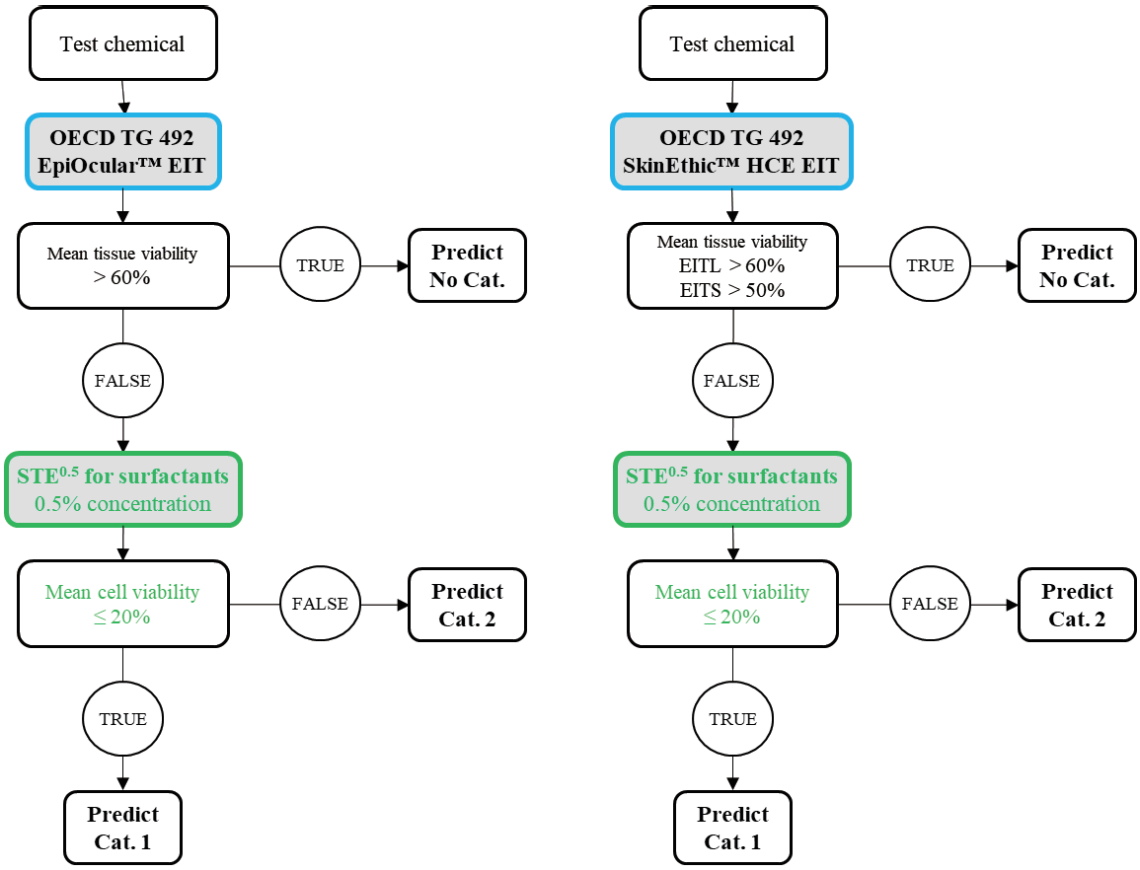
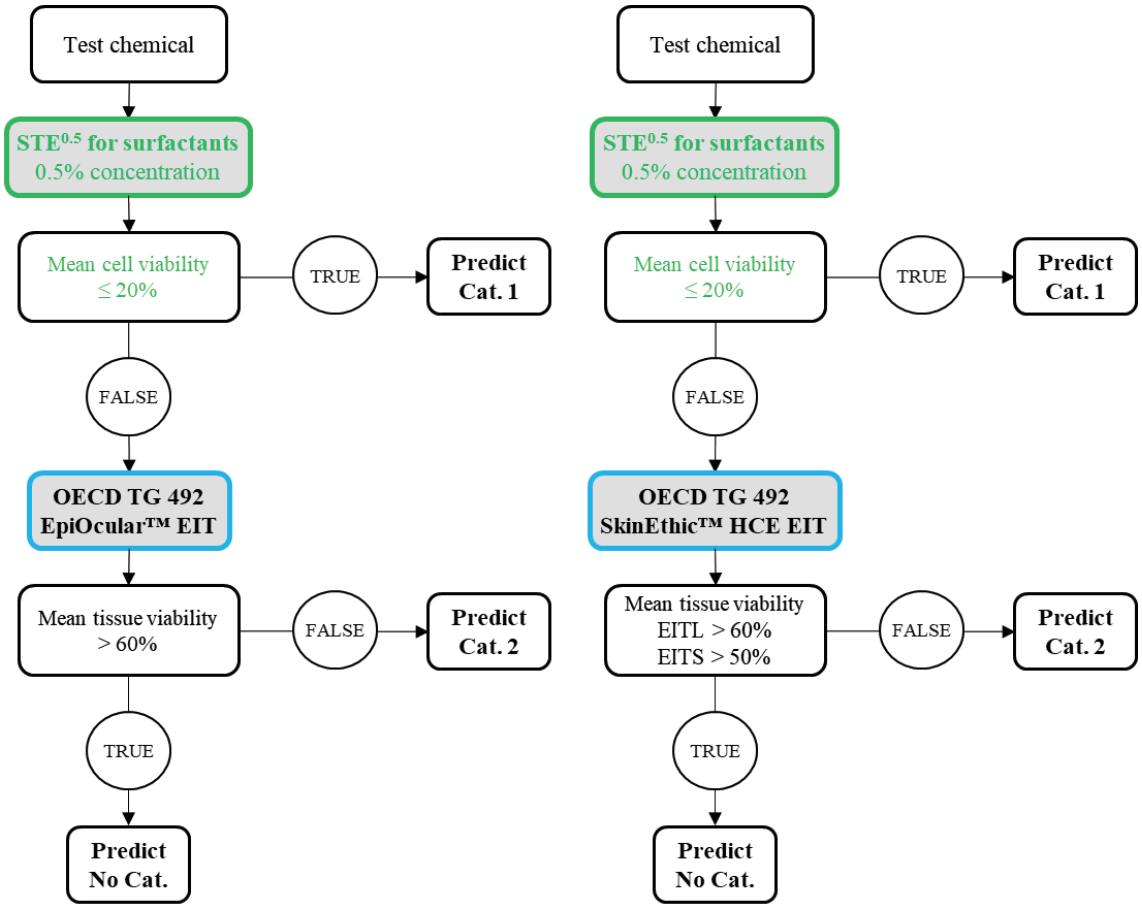


Figure 5.2. Top-down approach of the DASF; step 1 STE$^{0.5}$ for surfactants used to identify Cat. 1, and step 2 EpiOcular™ EITL / EITS or SkinEthic™ HCE EITL / EITS test method used to identify No Cat.



5.1.3. Description and limitations of the individual information sources

73. The individual information sources in the DA are test methods included in OECD TGs (OECD TG 492, 491) for serious eye damage/eye irritation or the absence thereof (1, 4), and the protocols are detailed therein.

74. The following test methods from those TGs have been characterised and included in the DASF.

- The RhCE (EpiOcular™ EIT and SkinEthic™ HCE EIT) test methods: the methods measures the ability to induce cytotoxicity. In case borderline results are obtained, additional testing should be conducted, as specified in OECD TG 492 (1).
- STE test method: the eye hazard potential of a test chemical is assessed based on its ability to induce cytotoxicity on a confluent monolayer of Statens Seruminstitut Rabbit Cornea (SIRC) cells. The STE$^{0.5}$ for surfactants differs from the procedure described in OECD TG 491 in that cell viability in SIRC cells is measured after a 5 minutes exposure to a 0.5% concentration and using a different cut-off value of 20% cell viability for classification (2, 4), instead of measuring cell viability after a 5

minutes exposure to a 5% and 0.05% concentration and using a cut-off value of 70% cell viability for classification (4).

75. Any restrictions regarding the applicability domain identified in the respective TGs (TG 492, TG 491) are applicable to this GL (1, 4) as well.

76. No *in vivo* data was found to assess the performance of DASF for neat Cat. 2 surfactants. When the DASF predicts a Cat.2 for a neat surfactant, additional information may be required, taking into account that the prospective use of animals is only to be used as a last resort. Furthermore, caution should be exercised when interpreting the DASF results for anionic surfactants as this class has a tendency to overpredict (see **Section 7.4** and **Table 7-10** of the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation)*.

5.1.4. Procedures for dealing with borderline results in the test guidelines relevant to DASF prediction

77. The first decision on whether each information element can be used is dictated by the practical limitations as described in the TGs of the respective *in vitro* methods (TG 492, TG 491) (1, 4). Even for within-domain substances, test results are inherently subject to variation and these variations increase the uncertainty of a test result, especially when close to a (classification) cut-off threshold, i.e. in the borderline range. The following procedures to control the degree of uncertainty are described with the TG of the respective information sources.

- TG 492 (EpiOcular™ EIT): A single test composed of at least two tissue replicates should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent tissue viability equal to 60±5% (EITL and EITS) a second test should be considered, as well as a third one in case of discordant results between the first two tests.
- TG 492 (SkinEthic™ HCE EIT): A single test composed of at least two tissue replicates should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent tissue viability equal to 60±5% (EITL) or to 50±5% (EITS) a second test should be considered, as well as a third one in case of discordant results between the first two tests.
- TG 491 ($STE^{0.5}$ for surfactants): A single test composed of at least three replicate wells should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent viability equal to 20±5%, a second test should be considered, as well as a third one in case of discordant results between the first two tests.

5.1.5. Predictive capacity of the DASF vs. the Draize Eye test

78. The predictive capacity of DASF is reported based on data generated by the Draize eye test (see Table 5.1. Performance of DASF in comparison to Draize Eye reference data) (see **Section 2.1** and **Annex B.2** of the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation)*. Performance statistics

are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 5** and **Annex A.B** of the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation.*

Table 5.1. Performance of DASF in comparison to Draize Eye reference data

UN GHS	Prediction using DASF		
	Cat 1	Cat 2	No Cat
Cat. 1 (N=23), % [a] (n/N)	**91.3% (21.0/23.0)**	8.7% (2.0/23.0)	0.0% (0.0/23.0)
Cat. 2 (N=10), % [a] (n/N)	30.0% (3.0/10.0)	**70.0% (7.0/10.0)**	0.0% (0.0/10.0)
No Cat. (N=17), % [a] (n/N)	5.9% (1.0/17.0)	18.1 (3.1/17.0)	**76.0% (12.9/17.0)**
	79.1% balanced accuracy overall		

[a] The proportion given is based on a weighted calculation which takes into account (where they exist) multiple results from an individual information source for a given chemical, and applying a correction factor so that all chemicals have a weight of 1 (see Annex D of OECD SD 354). To improve the readability of the numbers in the table, the numbers n/N have been rounded, so they may deviate slightly from the percentage corresponding to the weighted calculation.
Note that no neat UN GHS Cat. 2 reference surfactant could be identified, which prevents establishing confidence in the DASF for this category. As a result, when the DASF predicts a Cat. 2 for a neat surfactant, additional information may be required, taking into account that the prospective use of animals is only to be used as a last resort.. Furthermore, caution should be exercised when interpreting the DASF results for anionic surfactants as this class has a tendency to overpredict.

5.1.6. Demonstration of Proficiency

79. The DASF relies on a simple, rule-based data interpretation procedure and requires no expert judgement. Proficiency chemicals for the individual information sources are defined in the respective TGs (1,4). Proficiency for the individual information sources demonstrates proficiency for the DAs (e.g., para 39 for DAL-1).

5.1.7. Reporting of the DA

80. The reporting of the DA application should include at a minimum the following elements:

- Test chemical identification (*e.g.* chemical name, structural formula, composition, isomers, purity, chemical identity of impurities including their quantities as available, CAS number, batch and lot number, and other relevant identifiers)
- Individual test reports performed per corresponding TGs (OECD TG 492, TG 491), including the elements described therein. Note that the chemical identity for each test report should match that above.
- Discussions on any uncertainties in the data on *in vitro* methods applied in the DA used.
- Outcome of the DA application, including discussion of any uncertainties in the applied DA, as well as their predicted impact (e.g., over- or under-classification)

- Any deviation from or adaptation of the DA
- Conclusion

5.2. References

1. OECD (2019), OECD Guidelines for the Testing of Chemicals No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264242548-en.
2. Alépée N, Adriaens E, Abo T, Magby J, Mewes KR, Giusti A (2023). Development of a Defined Approach for Eye hazard identification of chemicals having surfactant properties according to the three UN GHS categories. Toxicol In Vitro, 89, 105576. https://doi.org/10.1016/j.tiv.2023.105576
3. OECD (2023), OECD Guidelines for the Testing of Chemicals No. 491: Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264242432-en.
4. Alépée N, Mewes KR, Abo T, Cavarzan A, O'Driscoll A, Adriaens E (2024). Performance of the DASF compared to other combinations of OECD NAMs for eye hazard identification of surfactants. ALTEX. 2024 Aug 28. doi: 10.14573/altex.2406031. Epub ahead of print. PMID: 39228327.