통합시험평가접근법 국내 및 글로벌 활용 사례 소개

안자극에 대한 통합시험평가접근법 활용 사례

Amorepacific R&D Center
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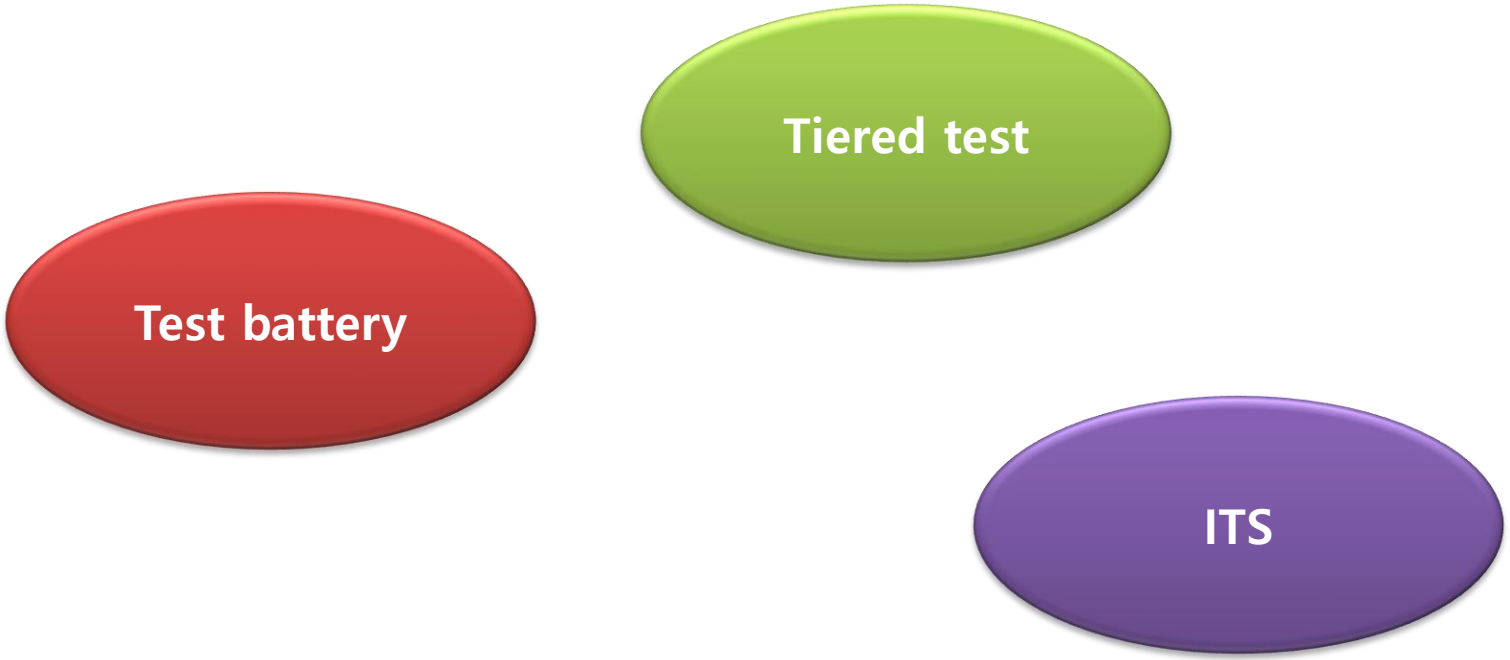
2020. 10. 22


Tiered test
Test battery
ITS

Test battery	•a group of assays conducted together to predict a toxicity endpoint •the results of each individual assay could be equally weighted •or a statistical weight could be used an attempt to better model the in vivo response
Tiered test scheme	•based on sequential assessments, where a result at one tier is used to determine the next step •usually a decision-tree type of assessment
Integrated testing strategy	•integrate different types of data and information into the decision-making process •may incorporate approaches such as weight-of-evidence and exposure/population data into the final risk assessment

- http://alttox.org/mapp/emerging-technologies/integrated-testing-strategies-risk-assessment/

Purpose of eye irritation test

- 국제 규제 대응
  GHS category : Classification & Labeling
  Substance & Mixture

- 소비자 사용 환경에서의 안전성 확보
- 안전한 원료의 선별
- 사용 가능한 제품 유형 결정
- 사용 가능한 농도 범위의 결정
- 제품 평가 (mixture)

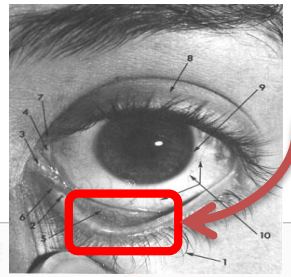
Definition of eye irritation

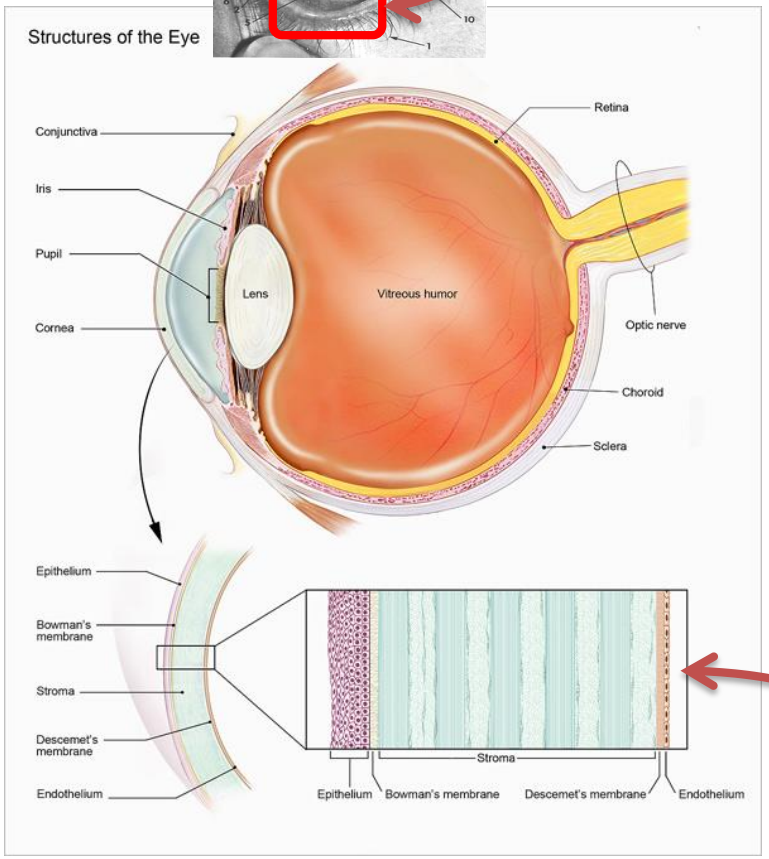
- Eye corrosion is the production of irreversible tissue damage in the eye following application of a test substance to the anterior surface of the eye.

- Eye irritation is the production of reversible changes in the eye following the application of a test substance to the anterior surface of the eye.

Structure of eyes and animal alternative test

For Conjunctiva Response
- **Vascular response**
- **HET-CAM : hemorrhage, lysis, Coagulation**





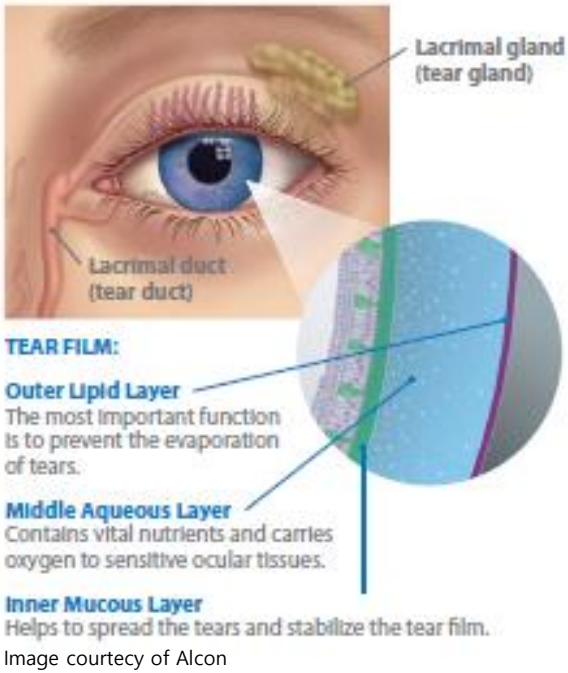


Image courtesy of Alcon

For Cornea Response
- **3-5 layers of flattened/ columnar epithelia**
- **Avascular response**
- **STE, RhCE EIT : cell viability**
- **BCOP, ICE : opacity, etc**
- **FL test : permeability**

https://nei.nih.gov/health/cornealdisease

Initial considerations

(1) Strongly acidic or alkaline substances, for example, with a demonstrated pH of 2 or less or 11.5 or greater, need not be tested owing to their predictable corrosive properties. Buffer capacity should also be taken into account.

(2) Materials which have demonstrated definite corrosion or severe irritation in a dermal study need not be further tested for eye irritation. It may be presumed that such substances will produce similarly severe effects in the eyes.

(3) Results from well validated and accepted *in vitro* test systems may serve to identify corrosives or irritants such that the test material need not be tested *in vivo*.

OPPTS 870.2400 Acute Eye Irritation

안점막 자극 평가등급
< 의약품등의 독성시험기준 >

안자극 구분표

Rating	Evaluation value		
	A.O.I.[a]	M.O.I.[b]	Day 7- I.O.I.[c]
Non irritant	0 ~ 5	0 (after 48 hrs)	
Minimally irritant	5 ~ 15	≤5 (after 48 hrs)	
Mildly irritant	15 ~ 30	≤5 (after 4 days)	
Moderately irritant	30 ~ 60	≤20 (after 7 days)	≤30(tested animals all)
Severely irritant	60 ~ 80	≤40 (after 7 days)	≤60(tested animals all)
Extremely irritant	80 ~ 100		

a) A.O.I. (Acute ocular irritation index): max. among M.O.I.
b) M.O.I. (Mean ocular irritation index): total score/tested animal No. in each observation time
c) Day-7 I.O.I. (Individual ocular irritation index): score of each animal on Day-7

(1) 각 막
A) 혼탁: 안구의 농후한 정도 (가장 농후한 지점을 관찰함)
· 화농이나 혼탁이 없음 ---------- 0
· 혼탁이 분산 혹은 밀집되어 있음 (정상적인 투명성이 약간 둔화된 것과는 다름), 홍채의 말단이 명확히 관찰됨 ---------- 1
· 반투명한 부분이 쉽게 관측됨. 홍채의 말단이 약간 불명확함. ---------- 2
· 진주 색깔을 나타냄. 홍채의 말단이 관찰 안됨, 동공의 크기가 가까스로 관측됨. ---------- 3
· 각막이 불투명. 혼탁 때문에 홍채가 관찰 안됨. ---------- 4

B) 혼탁된 각막의 범위
· 정상 (0) ---------- 0
· 1/4 이하 (그러나 0은 아님) ---------- 1
· 1/4 이상 1/2 미만 ---------- 2
· 1/2 이상 3/4 미만 ---------- 3
· 3/4 이상 1 까지 ---------- 4
A × B × 5 최대치 = 80

(2) 홍 채
A) 반응치
· 정상 ---------- 0
· 현저한 주름의 형성, 충혈, 종창, 각막 주위에 중정도의 충혈, 이상과 같은 단독 혹은 혼합, 홍채는 빛에 대해 반응함. (둔한 반응은 양성)---------- 1
· 빛에 대해 반응 없음, 출혈, 대부분 파괴. (이상과 같은 증상의 일부 혹은 전부) ---------- 2
A x 5 최대치 = 10

(3) 결 막
A) 발적 (안검 결막, 안구 결막에 한함. 각막, 홍채 제외)
· 혈관은 정상 ---------- 0
· 몇몇 혈관은 명확히 충혈 ---------- 1
· 넓은 심홍색 색조, 각각의 혈관은 쉽게 관찰 안됨 ---------- 2
· 엷은 선홍색 ---------- 3

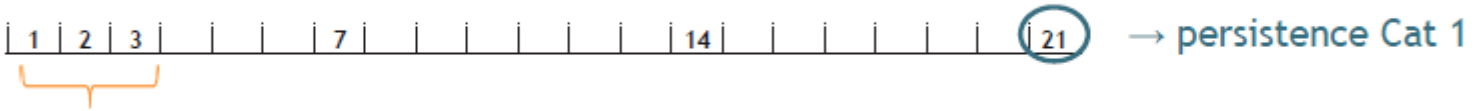
B) 결막 부종
· 부풀지 않음 ---------- 0
· 정상보다 약간 종창 (순막 포함) ---------- 1
· 안검의 부분적 외전을 동반한 현저한 종창 ---------- 2
· 눈이 반쯤 감길 정도의 안검의 종창 ---------- 3
· 눈이 반 이상 감길 정도의 안검의 종창 ---------- 4

C) 배출물
· 배출물 없음 ---------- 0
· 약간의 배출물(정상동물의 내부눈꼬리에서 관찰되는 작은양 제외) ---------- 1
· 속눈썹과 눈꺼풀을 적시는 배출물 ---------- 2
· 눈 주위의 상당 부위와 속눈섭과 눈꺼풀을 적시는 배출물 ---------- 3
(A + B + C) × 2 최대치 = 20

8

Draize rabbit eye test

Observe tissues



Calculate for each rabbit mean CO, mean IR, mean CR, and mean CC values over day 1 - 3

Tab. 1: Description of Draize Scoring Rules

Endpoint	Description	Range
Cornea	degree opacity and ulcerations	0-4
Iris	swelling, hyperaemia	0-2
Conjunctivae	redness, vessel discernibility	0-3
Chemosis	swelling, lids closed/open	0-4

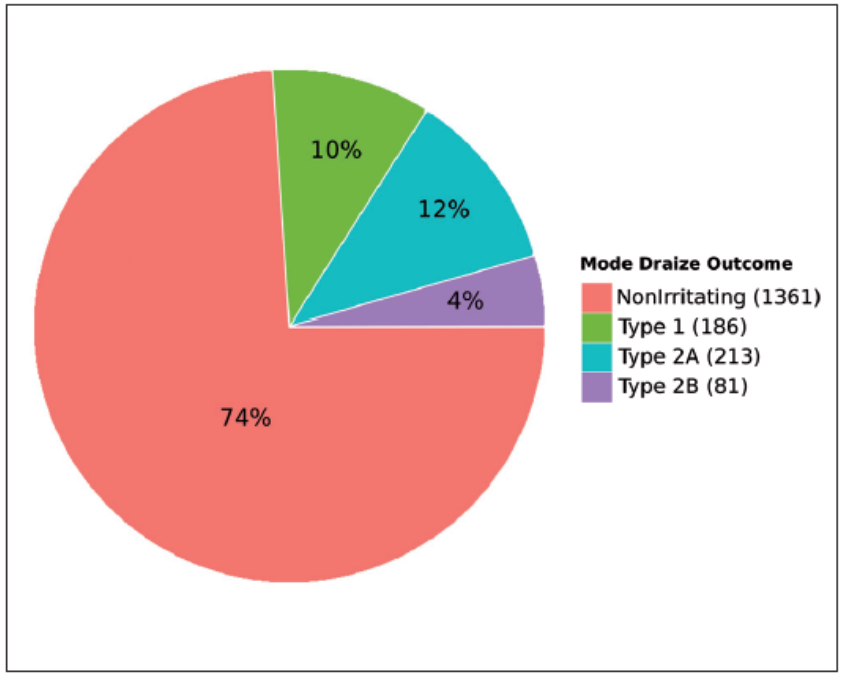


Fig. 1: Prevalence of outcomes for substances tested with OECD TG 405 (Draize rabbit eye test) in REACH registrations 2008-2014

ALTEX 33(2) 2016

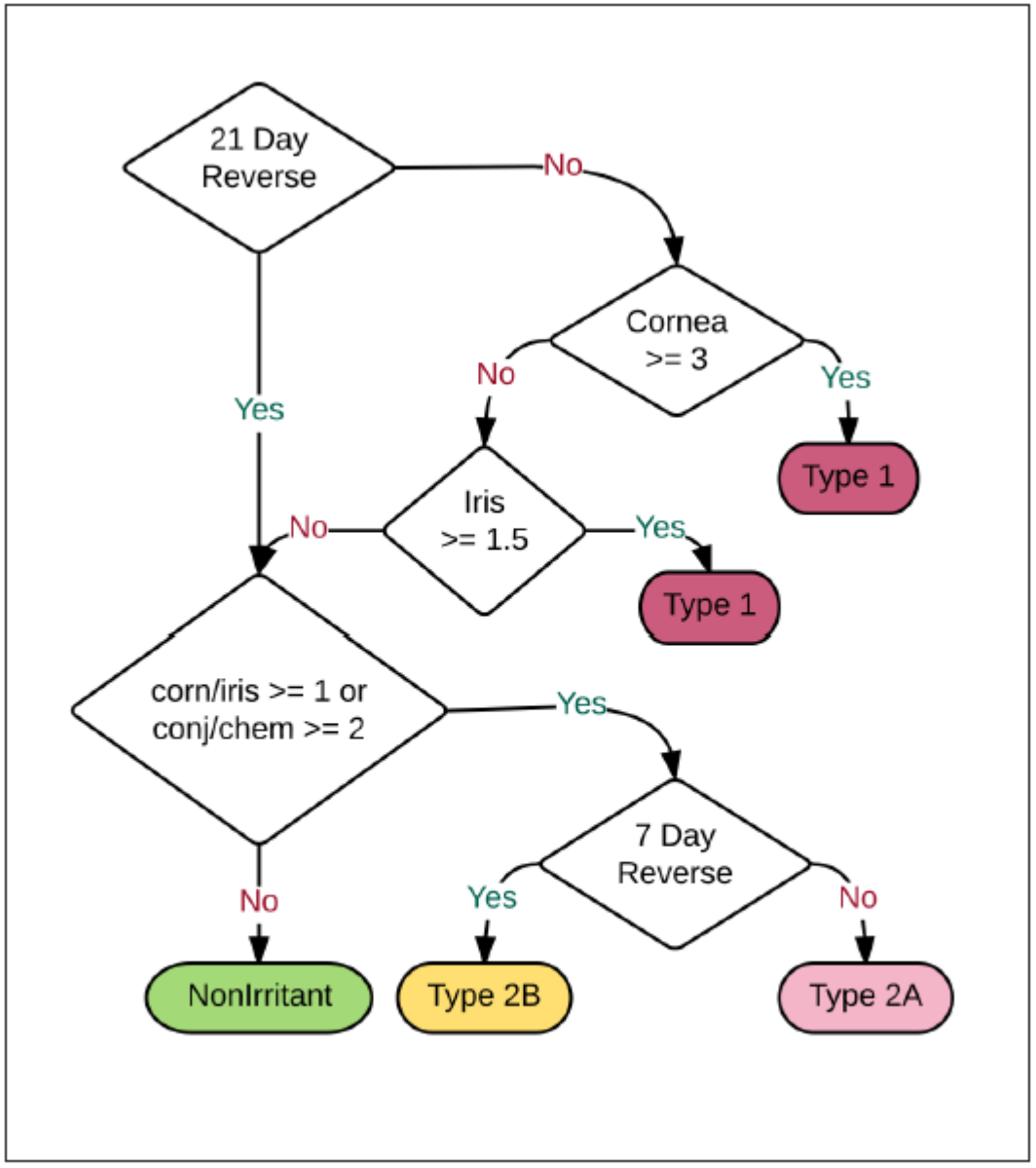


Fig. 4: Draize endpoint classification strategy as represented by IDRI

ALTEX 33(2) 2016

Table 3.3.1: Irreversible eye effects categories[a]

An eye irritant Category 1 (irreversible effects on the eye) is a test material that produces:

(a) at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(b) at least in 2 of 3 tested animals, a positive response of:

(i) corneal opacity ≥ 3; and/or
(ii) iritis > 1.5;

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

ST-SG-AC10-30-Rev4e (Unece)

Table 3.3.2: Reversible eye effects categories

An eye irritant Category 2A (irritating to eyes) is a test material that produces:

(a) at least in 2 of 3 tested animals a positive response of:

(i) corneal opacity ≥ 1; and/or

(ii) iritis ≥ 1; and/or

(iii) conjunctival redness ≥ 2; and/or

(iv) conjunctival oedema (chemosis) ≥ 2

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of normally 21 days.

Within this category an eye irritant is considered **mildly irritating to eyes (Category 2B)** when the effects listed above are fully reversible within 7 days of observation.

ST-SG-AC10-30-Rev4e (Unece)

Classification of mixture

Table 3.3.3: Concentration of ingredients of a mixture classified as skin Category 1 and/or eye Category 1 or 2 that would trigger classification of the mixtures as hazardous to the eye (Category 1 or 2)

Sum of ingredients classified as	Concentration triggering classification of a mixture as	
	Irreversible eye effects	Reversible eye effects
	Category 1	Category 2
Eye or skin Category 1	≥ 3%	≥ 1% but < 3%
Eye Category 2/2A		≥ 10%
(10 × eye Category 1) + eye Category 2/2A		≥ 10%
Skin Category 1 + eye Category 1	≥ 3%	≥ 1% but < 3%
10 × (skin Category 1 + eye Category 1) + eye Category 2A/2B		≥ 10%

Table 3.3.4: Concentration of ingredients of a mixture for which the additivity approach does not apply, that would trigger classification of the mixture as hazardous to the eye

Ingredient	Concentration	Mixture classified as: Eye
Acid with pH ≤ 2	≥ 1%	Category 1
Base with pH ≥ 11.5	≥ 1%	Category 1
Other corrosive (Category 1) ingredients for which additivity does not apply	≥ 1%	Category 1
Other irritant (Category 2) ingredients for which additivity does not apply, including acids and bases	≥ 3%	Category 2

ST-SG-AC10-30-Rev4e (Unece)

Alternative methods currently available to the Draize rabbit eye test

1. Isolated organs

- 1.1. Bovine Corneal Opacity and Permeability (BCOP) Test
- 1.2. Isolated Rabbit Eye (IRE)
- 1.3. Chicken Enucleated Eye Test (CEET)

2. Organotypic Methods - Chorio-allantoic membrane methods

- 2.1 Hen`s egg test on the chorio-allantoic membrane (HET-CAM Assay)
- 2.2. Chorioallantoic membrane vascular assay (CAMVA)
- 2.3. Chorioallantoic membrane - trypan blue staining (CAM-TB)

3. Human corneal epithelium models

- 3.1.The EpiOcularTM assay (ET50-based assay)
- 3.2. The SkinEthic in vitro reconstituted human corneal epithelium (HCE model)
- 3.3. HCE-T Tissue Construct (Gillette)

4. Cell based cytotoxicity methods

- 4.1. Neutral Red Uptake
- 4.2. Neutral Red Release Assay
- 4.3. Red blood cell (RBC) haemolysis test

5. Cell function based assays

- 5.1. Fluorescein leakage (FL)
- 5.2. Silicon Microphysiometer (SM) or Cytosensor Microphysiometer

6. Other assays

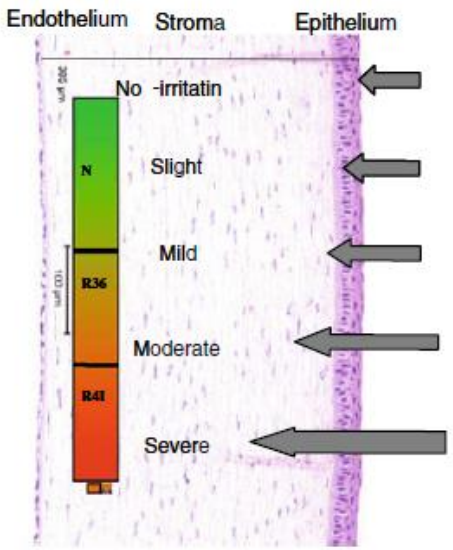
- 6.1. Mucosal irritation model: using slugs (e.g. Arion lusitanicus)
- 6.2. The IRRITECTION® assay
- 6.3. The Pollen Tube Growth (PTG) assay

http://ec.europa.eu/consumers/sectors/cosmetics/files/doc/antest/(5)_chapter_3/3_eye_irritation_en.pdf

OECD test guidelines for eye irritation

Title	Date	**Existing animal test method**
TG 437 Bovine Corneal Opacity and Permeability (BCOP) Test	29 Jun 2020	TG 405 Acute Eye Irritation/Corrosion
TG 438 Isolated Chicken Eye (ICE) Test Method	27 Jun 2018	
TG 460 Fluorescein Leakage (FL) Test Method	09 Oct 2017	
TG 491 Short Time Exposure (STE) in vitro test method	29 Jun 2020	
TG 492 Reconstructed Human Cornea-like Epithelium (RhCE) test	18 Jun 2019	
TG 494 Vitrigel-Eye Irritancy Test Method	18 Jun 2019	
TG 496 In vitro Macromolecular Test Method	22 Nov 2019	

Depth of Injury to the Cornea



Scottet et al. 2010, Tox. In vitro 24: 1-9

Non irritants vs others

Neutral Red Release
Fluorescein Leakage
Cytosensor
Irritection
Human Corneal Epithelium
EpiOcular
HET-CAM
Red Blood Cell
Slug Mucosal Irritation
Organ Based Methods (below)

Severe irritants vs others

Chicken Enucleated EyeTest
Bovine Corneal Opacity Test
Porcine Corneal Opacity Test
Rabbit Enucleated Eye Test
Rabbit Ex-Vivo
CAM-TBS

IATA for eye irritation (Parts and Modules)

Part (*)	Modules
Part 1: Existing information, physico-chemical properties and non-testing methods	1. Existing human data on serious eye damage and eye irritation
	2. Existing *in vivo* animal data according to OECD TG 405 on serious eye damage and eye irritation
	3. Existing *in vitro* data from OECD adopted test methods on serious eye damage and eye irritation a) OECD TG 437 on the BCOP test method b) OECD TG 438 on the ICE test method c) OECD TG 491 on the STE test method d) OECD TG 492 on the RhCE test methods e) OECD TG 460 on the FL test method
	4. Other existing animal data from non-OECD adopted test methods on serious eye damage and eye irritation
	5. Other data from non-OECD adopted alternative test methods on serious eye damage and eye irritation
	6. Existing data on skin corrosion (human, animal and *in vitro*)
	7. Physicochemical properties (existing, measured or estimated) such as pH and acid/alkaline reserve
	8. Non-testing data on serious eye damage and eye irritation a) Substances: (Q)SAR, expert systems, grouping and read-across b) Mixtures: bridging principles and theory of additivity
Part 2: WoE analysis	9. Phases and elements of WoE approaches
Part 3: New testing	4.Testing onOECD adopted *in vitro* test methods for serious eye damage and eye irritation **OECD TG491 STE**
	6. Testing on other non-OECD adopted alternative test methods for serious eye damage and eye irritation **HET-CAM**
	3.As a last resort, testing on *in vivo* animal test method according to OECD TG 405 for serious eye damage and eye irritation

** While the three Parts are considered as a sequence, the order of Modules 1 to 8 of Part 1 (here shown in decreasing order of complexity) might be arranged as appropriate. Furthermore, if sufficient and adequate data exist, each module may lead on its own to a classification decision or the absence of classification where relevant, as described in the figure.*

OECD GD 263 on IATA for Serious eye damage and eye irritation (2019)

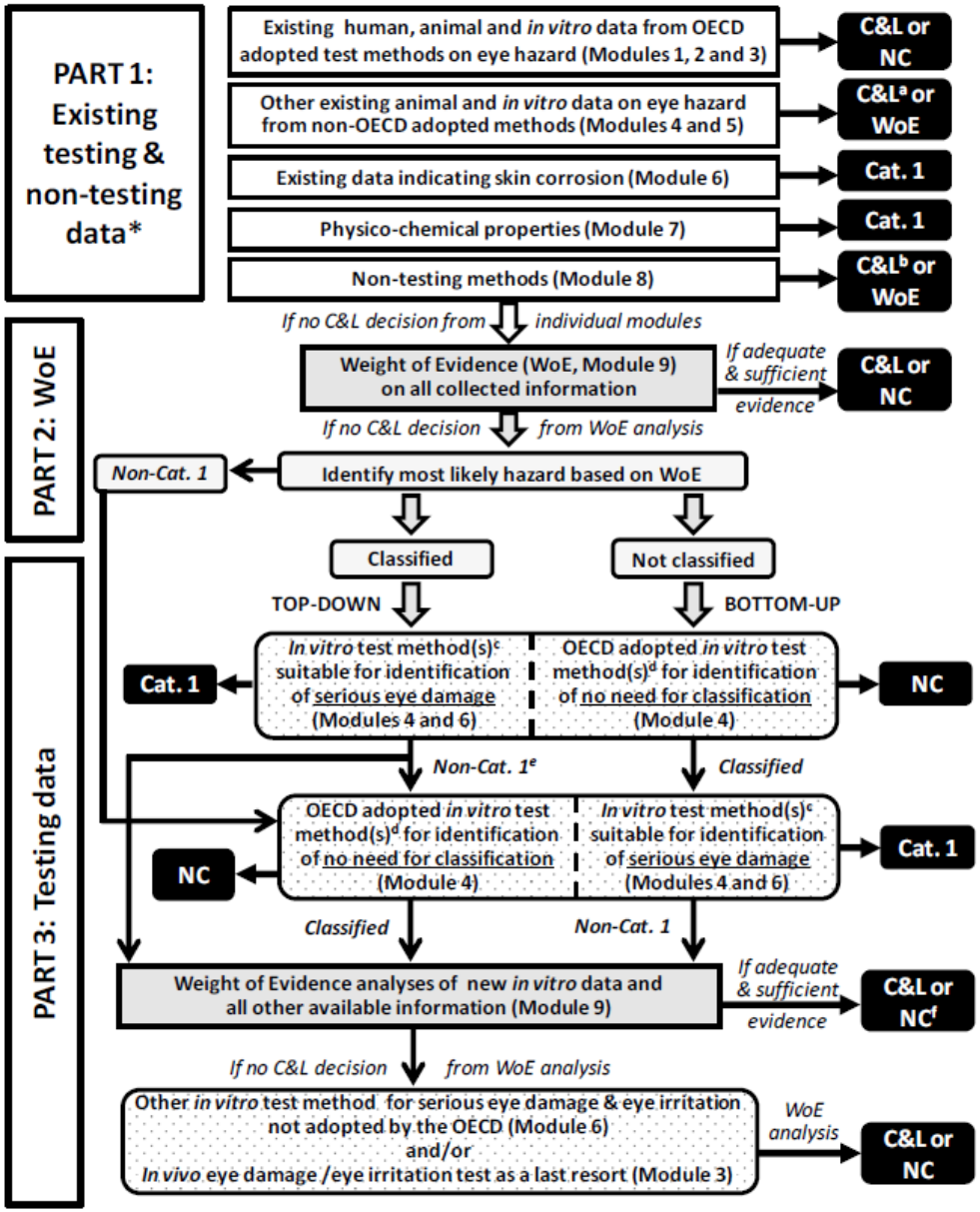


Figure 2.1. Detailed IATA for serious eye damage and eye irritation. C&L: Classification and labelling (i.e., UN GHS Cat. 1 or Cat. 2); NC: UN GHS No Category.

** While the three Parts are considered as a sequence, the order of Modules 1 to 8 of Part 1 (here shown in decreasing order of complexity) might be arranged as appropriate. Furthermore, if sufficient and adequate data exist, each module may lead on its own to a classification decision or the absence of classification where relevant, as described in the figure.*

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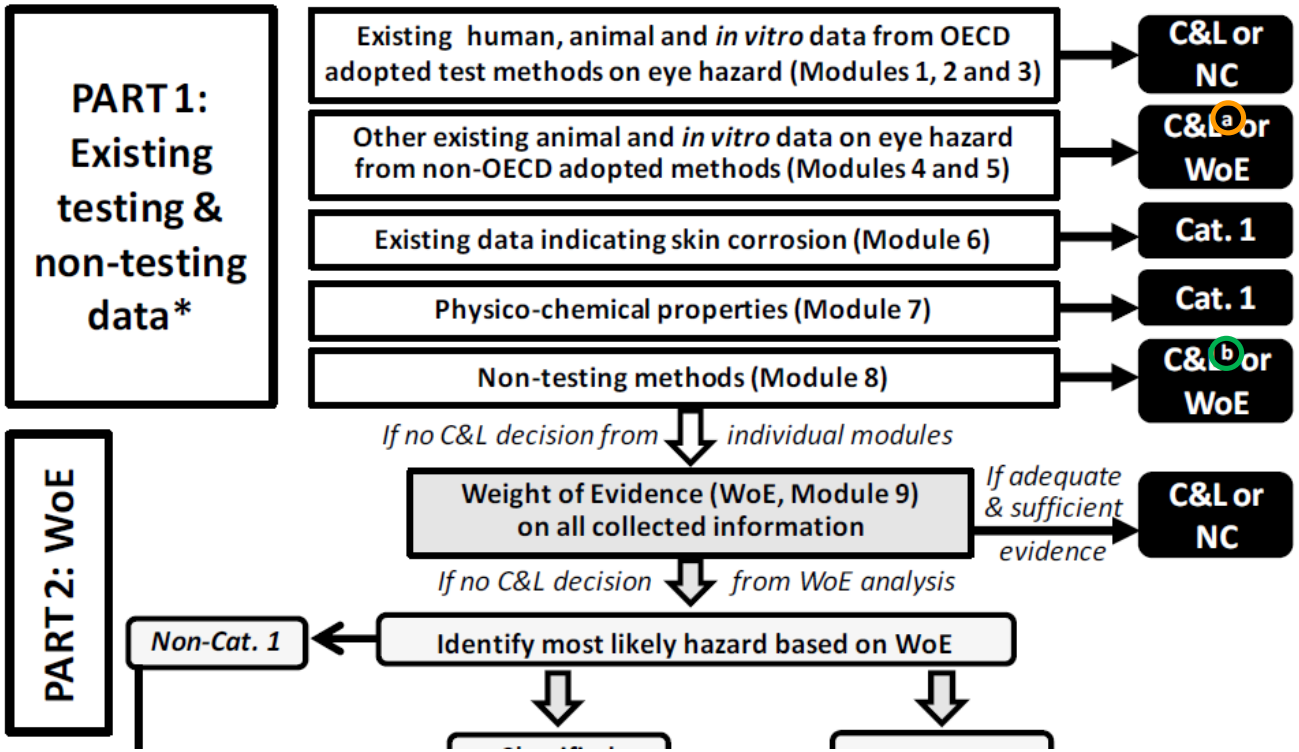
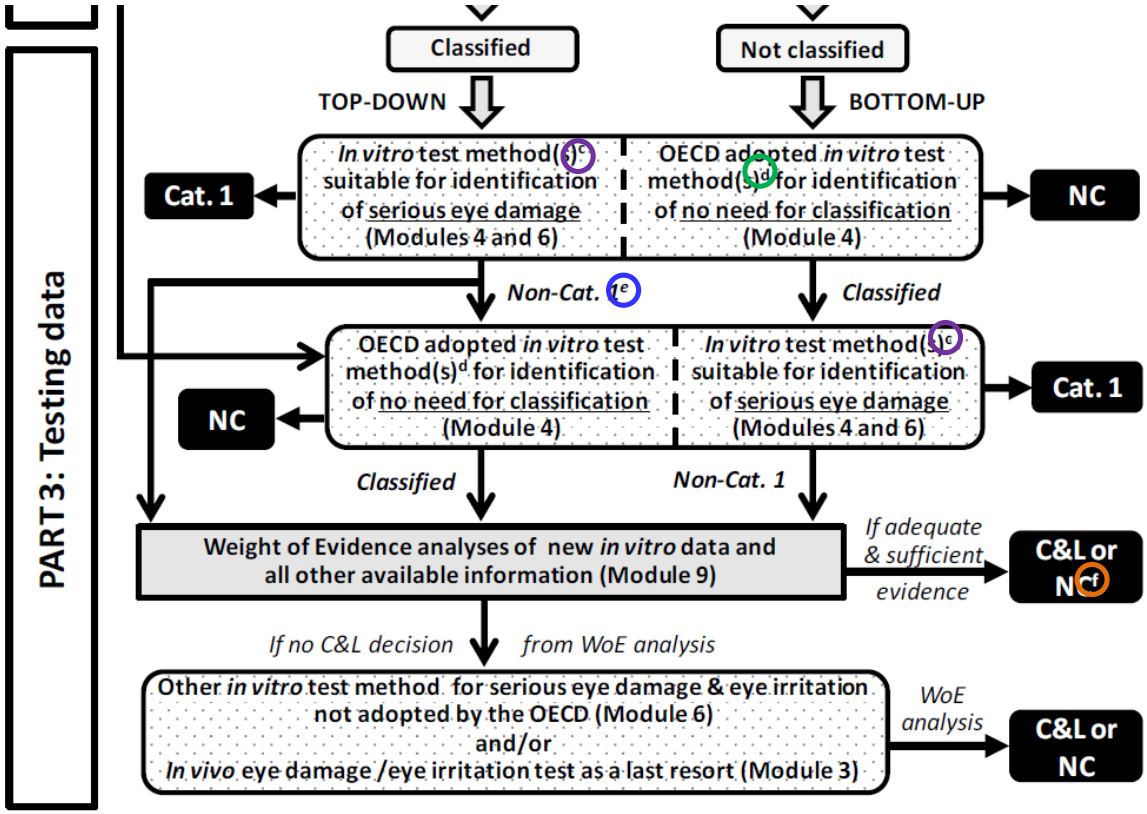


- *the order of Modules 1 to 8 of Part 1 (here shown in decreasing order of complexity) might be arranged as appropriate.*
- *Furthermore, if sufficient and adequate data exist, each module may lead on its own to a classification decision or the absence of classification where relevant, as described in the figure.*

a For example results obtained with other existing in vivo test methods (e.g., the FHSA method 16CFR 1500,42 (CPSC. 2003)) might be used to derive a final classification, which might include also identification of UN GHS No Category. Furthermore, results obtained with optimized non-OECD adopted test methods (e.g., Isolated Rabbit Eye Test) might be used to identify UN GHS Cat. 1 test chemicals. Finally, **negative results obtained with optimized non-OECD adopted test methods might be used in a WoE approach.**

b For example, **the application of bridging principles might be used to derive a classification of the tested mixture**, which might include also identification of UN GHS No Category. In contrast, results obtained from **(Q)SARs might be used in a WoE approach.**



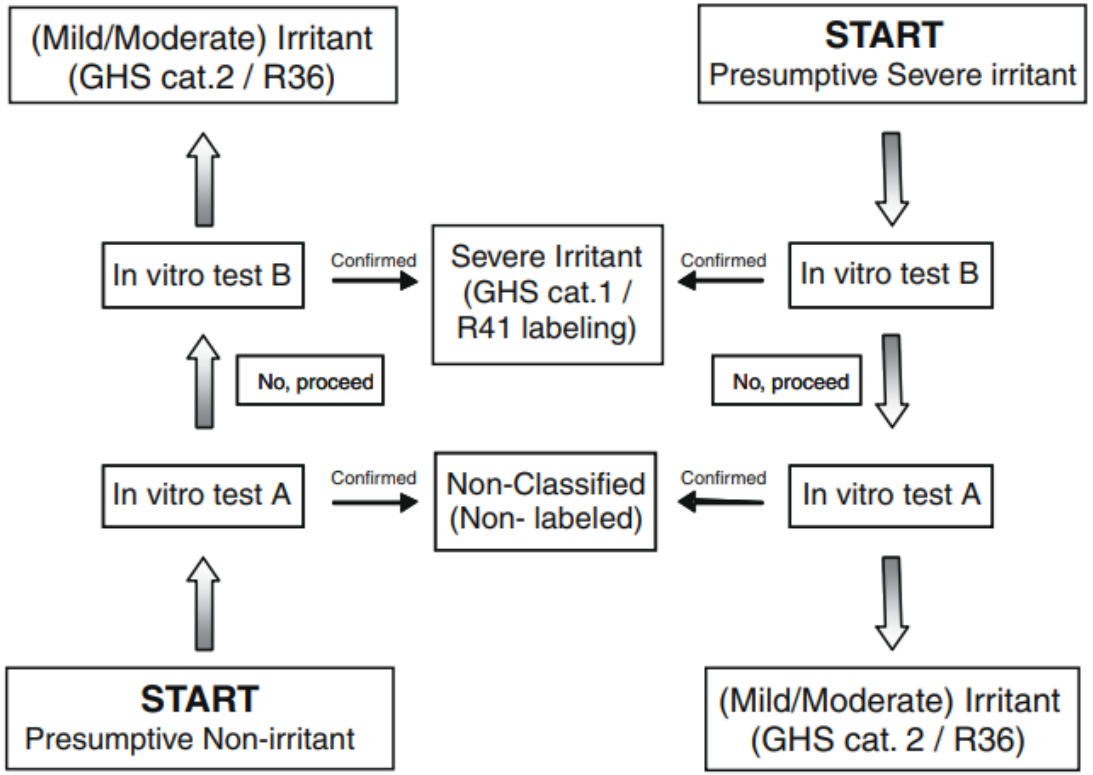
c The use of additional in vitro test methods suitable for identifying UN GHS Cat. 1, based if possible on different mechanisms of action, may be considered in case a negative result is obtained with a first in vitro test method used for this purpose. This is due to the fact that a single in vitro test method aiming at the identification of UN GHS Cat. 1 may not cover all mechanisms of action resulting in serious eye damage (e.g. persistence of effects) and may therefore produce a certain amount of false negatives (see chapters 3 and 4.3).

d The use of additional OECD adopted in vitro test methods for identifying UN GHS No Cat. may be considered in case a positive result is obtained with a first in vitro test method used for this purpose. This is due to the fact that the currently OECD adopted in vitro test method aiming at the identification of UN GHS No Cat. produce a significant amount of false positives (see chapters 3 and 4.3).

e In cases where the WoE evaluation in Part 2 indicates that a classification is warranted with a high degree of certainty, testing with an in vitro test method for identification of UN GHS No Cat. may be waived, and the next steps in the strategy should be undertaken.

f UN GHS Cat. 2 classification is to be considered **only in cases** where the WoE evaluation indicates that **the test chemical is not UN GHS Cat. 1 with a high degree of certainty.**

- **A Top-Down approach**, starting with in vitro test methods that can identify test chemicals causing serious and/or irreversible eye damage (UN GHS Cat. 1) with low false positive predictions and the highest possible accuracy.



Scottet et al. 2010, Tox. In vitro 24: 1-9

- **A Bottom-Up approach**, starting with in vitro test methods that can identify test chemicals not requiring classification for eye hazard (UN GHS No Cat.) with low false negative predictions and the highest possible accuracy.

Comparison of various methods

	HET-CAM	STE	BCOP	RhCE
장점	결막 반응 모사 유정란의 수급과 보관 용이 적은 비용 물질 처치 용이 (용해도의 영향 작음)	각막 반응 모사 시험 용이 적은 비용 저자극 물질에 대한 민감도 정량적 수치 평가	각막 반응 모사 물질 처치 용이 정량적 수치 평가	각막 반응 모사 물질 처치 용이 정량적 수치 평가
단점	동물실험으로 오인 폐기 비용 위양성 (일부 polyol 등) 육안 평가 (주관적 요소) 물리적 자극, 눈물 영향 변별 못함	용해도의 영향 물리적 자극, 눈물 영향 변별 못함	재료 수급 어려움 폐기비용 조직의 시간 내 소비 물리적 자극, 눈물 영향 변별 못함	고비용 시험모델 의존성 물리적 자극, 눈물 영향 변별 못함

- 한가지 안자극 대체법이 복잡한 눈의 구조와 작용에 의한 결과를 반영하는데 한계가 있음. => HET-CAM + STE
- 물리적 자극이나, 실제 눈 환경에서의 눈물에 의한 희석, 버퍼 작용, 반대로 눈물을 유발하는 현상에 대해 평가할 수 없음.
- 눈의 감각적 현상 (눈시림)을 반영할 수 없음
- 미약한 안자극을 변별할 수 없음
- 휘발성이 높은 물질에 의한 위양성/위음성 반응 가능성 -> 휘발환경 모사 시험

Two tiered approaches combining alternative test methods and minimizing the use of reconstructed human cornea-like epithelium tests

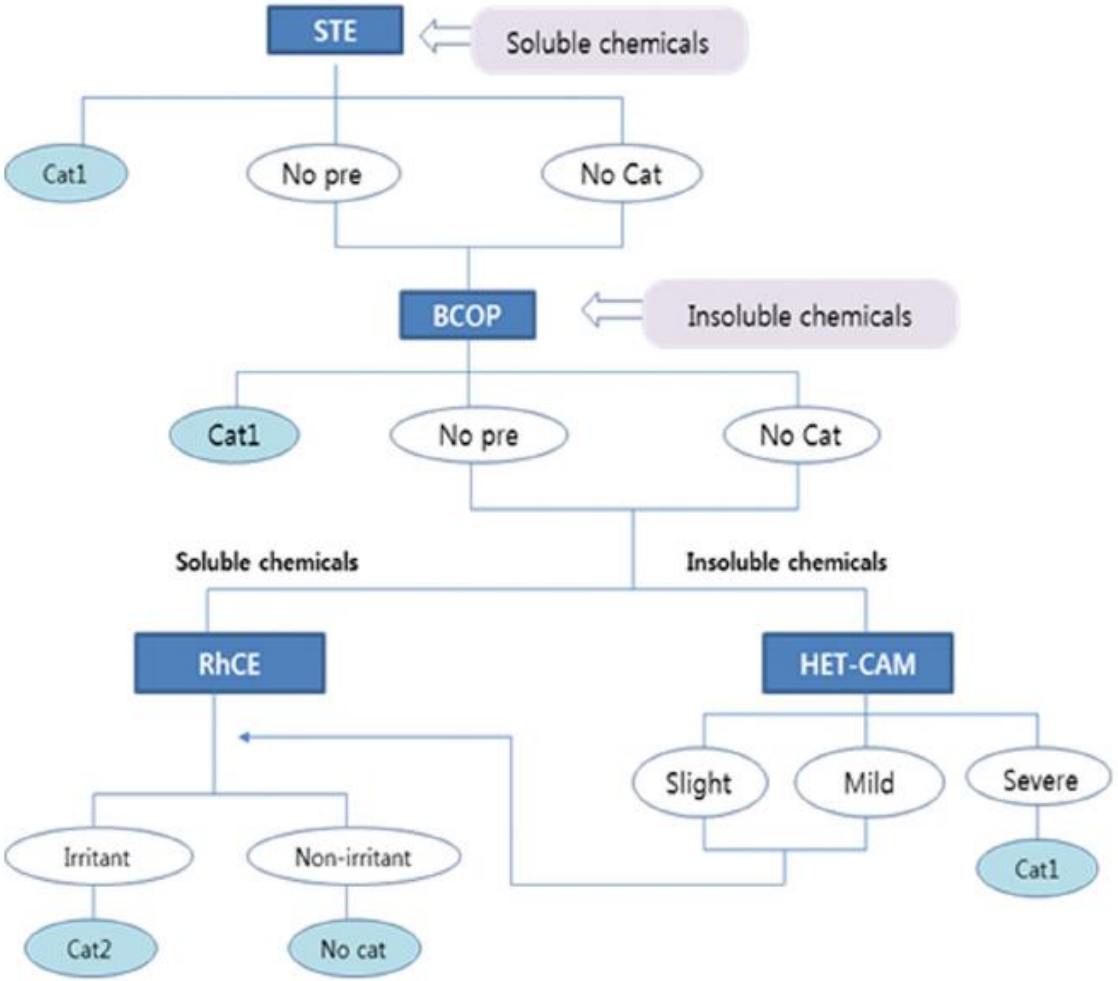


Fig. 1. The tiered approach A proposed from the present study.

Kyung Yuk Ko, et al. 2020, Tox. In vitro 63, 104675

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