XCellR8
Redefining testing

In Vitro Skin Corrosion Testing

CHEMICAL HAZARDS COMMUNICATION SOCIETY (CHCS) WEBINAR

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Introduction and definitions

Definitions of skin irritation and corrosion

- Dermal irritation: the production of reversible damage of the skin following the application of a test chemical for up to 4 hours
- Dermal corrosion: the production of irreversible damage of the skin; namely, visible necrosis of the epidermis and into the dermis, following the application of a test chemical for up to 4 hours
  - Corrosive reactions are typified by ulcers, bleeding, scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions

Source: OECD TG 404, 2015

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Definitions of GHS categories for skin corrosion and irritation

DEFINED ACCORDING TO ANIMAL STUDIES USING TG 404 (Draize)

GHS category	Definition
Category 1*	Corrosive: Destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least one tested animal after exposure ≤ 4 h
Category 1A	Corrosive responses in at least one animal following exposure ≤ 3 min during an observation period ≤ 1 h
Category 1B	Corrosive responses in at least one animal following exposure > 3 min and ≤ 1 h and observations ≤ 14 days
Category 1C	Corrosive responses in at least one animal after exposures > 1 h and ≤ 4 h and observations ≤ 14 days
Category 2	A substance is irritant to skin when it produces reversible damage to the skin following its application for up to 4 hours. The major criterion for the irritation category is that at least 2 of 3 tested animals have a mean score of ≥ 2.3 and ≤ 4.0
No Category	No corrosive or irritant effects observed

*Category 1 used as default if no sub-categorisation is possible

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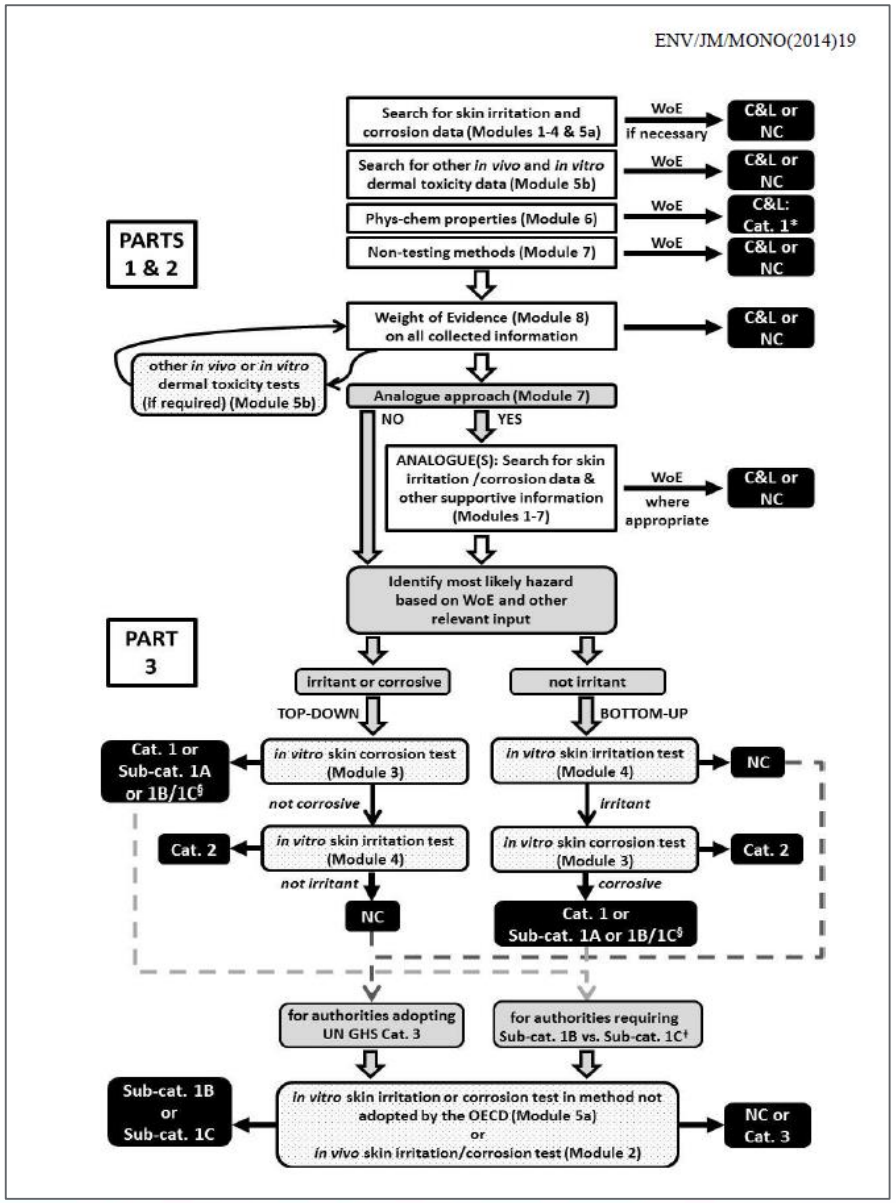
OECD guidance

GD 203: Integrated Approach on Testing and Assessment (IATA) for Skin Irritation and Corrosion (published 2014)

The IATA has 3 parts:

- Part 1: Review of existing data
- Part 2: Weight of evidence analysis
- Part 3: Additional testing

Link to IATA online



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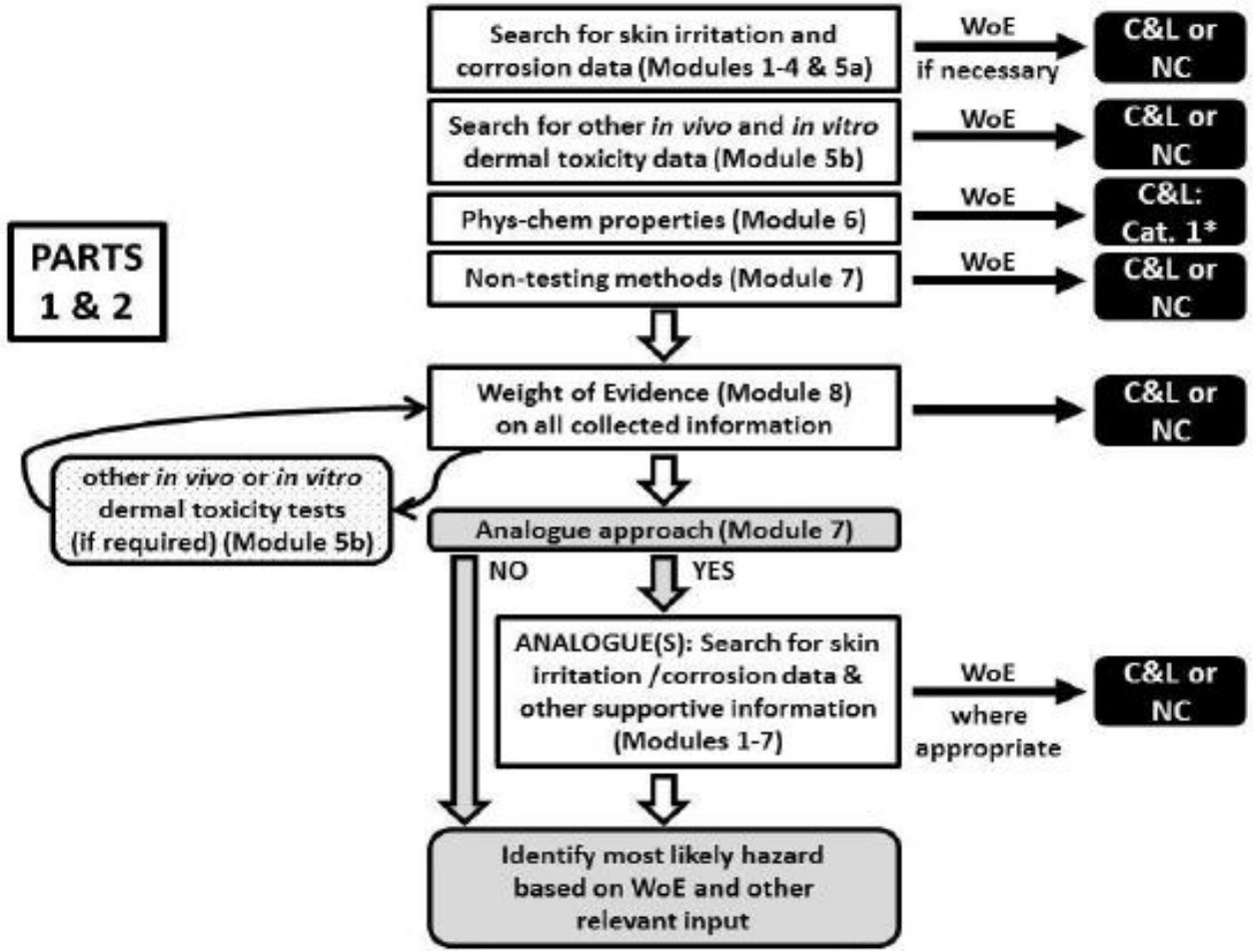
IATA on Skin Irritation and Corrosion

PARTS 1 & 2



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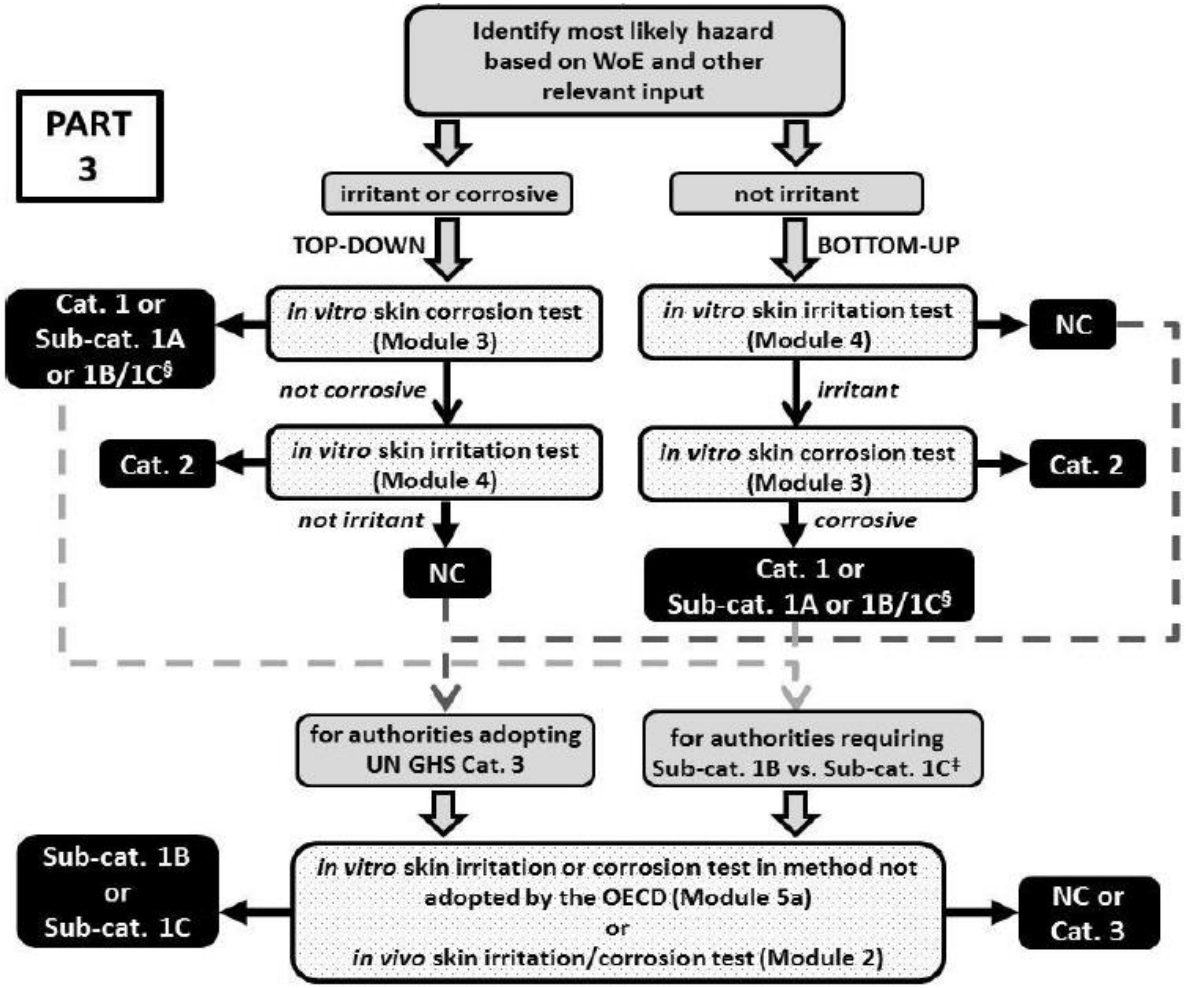
IATA on Skin Irritation and Corrosion

PART 3



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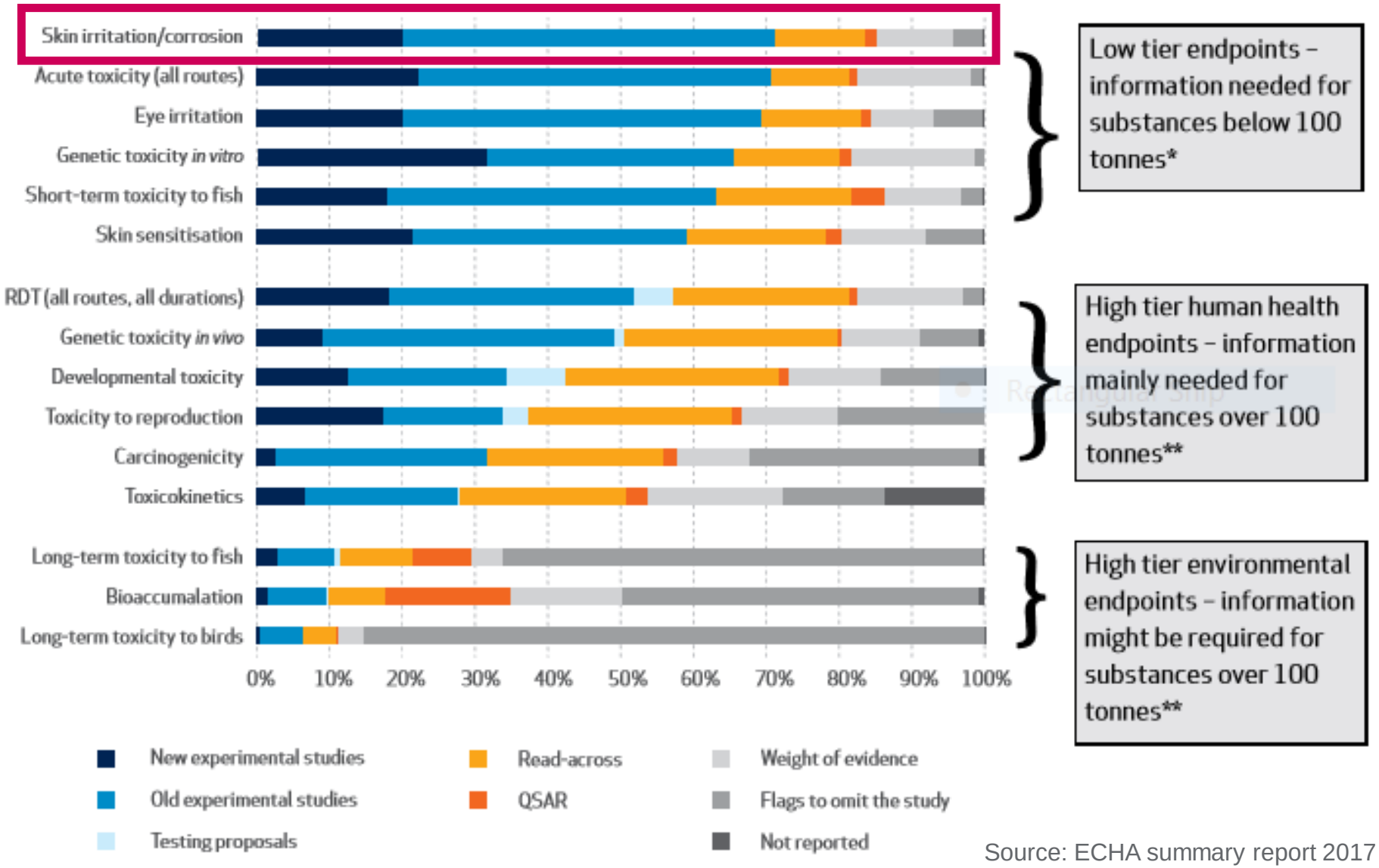
Options used by REACH registrants



New testing:
in vivo and *in vitro*

Source: ECHA summary report 2017

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In vitro skin corrosion tests described in OECD Guidance Document 203 (IATA)

In vitro skin corrosion tests described in the IATA

OECD TG 431:

Human Reconstructed Epidermis

OECD TG 435:

***In Vitro* Membrane Barrier**

OECD TG 430:

Transcutaneous Electrical Resistance (TER)

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OECD Test Guideline 431: *In Vitro* Skin Corrosion: Reconstructed Human Epidermis (RhE) Method

First adopted 13th April 2004
Latest edition published 18th June 2019

OECD TG 431: Reconstructed Human Epidermis (RhE)

- **Scientific rationale:** Corrosive chemicals cross the skin barrier by diffusion or erosion and are toxic to cells in the underlying layers
- Closest simulation of real-life human exposure
  - Closely models human skin in form and function
  - Barrier function (*stratum corneum*)
- Suitable for substances and mixtures:
  - Solids, liquids and emulsions
- Topical application for 3 mins and 1 hour (and 4 hours for EpiSkin™)
- Cell viability measured by metabolism of tetrazolium salt MTT to a blue formazan product, quantified by spectrophotometry
- Corrosive chemicals identified by a decrease in cell viability below defined threshold levels
- 4 major RhE models have been validated and are permitted for use:
  - EpiSkin™, EpiDerm™ SCT, SkinEthic™RhE and epiCS®

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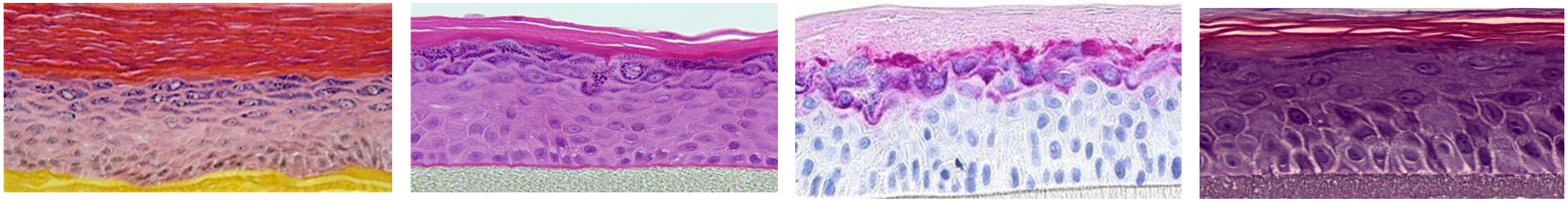
OECD TG 431: which skin model?

- Factors to consider include: predictive capacity / sub-classification needs, expected result based on WoE, historical data, accessibility (geography), price. Discuss with test lab

- Performance data for sub-categorisation by all 4 models included in Annex 3 of OECD TG 431

EpiSkin™	**EpiDerm SCT™**	**SkinEthic™ RhE**	**epiCS®**
EpiSkin France	Mattek US and Slovakia	EpiSkin France	CellSystems Germany



Cross sections through reconstructed human epidermis

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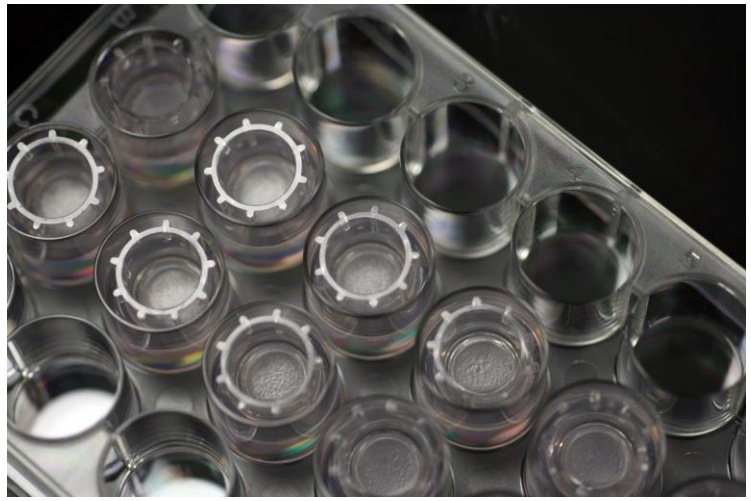
OECD TG 431: predictive capacity



Performance (compared with TG 404 Draize):

- Sensitivity >95%
- Specificity >90%
- Accuracy ≥ 82.5%

GHS categories:

- Discriminates skin corrosives (Cat.1) from non-corrosives (No Category)
- Discriminates between corrosive sub-categories 1A and 1B/C (*formal approval*)
- EpiSkin™ can discriminate between corrosive sub-categories 1B and 1C (*but without formal regulatory approval. Published studies mentioned in IATA – recommended use for weight of evidence to avoid animal studies*)

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OECD TG 431: strengths and weaknesses

STRENGTHS	WEAKNESSES
Formal validation and OECD approval	Additional steps required for substances interfering with MTT metabolism or detection of the formazan salt (eg coloured substances)
Widespread recognition by regulatory authorities	Not validated for gases and aerosols (same as other methods *in vitro* and *in vivo*)
High physiological relevance – reconstructed human tissue	Sub-categorisation has a tendency for over-classification into Cat. 1A (21-46% depending on method)
Partial sub-categorisation possible	

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OECD TG 431: limitations

- None of the methods may currently be used to distinguish 1B from 1C due the limited set of well-known *in vivo* corrosive 1C reference chemicals for validation (note: this has not been raised as an issue for TG435)

- Does not discriminate skin irritants (Cat.2) from chemicals not requiring classification from skin irritation / corrosion (No Cat)
  - However this can be addressed using OECD TG 439 (*in vitro* skin irritation – reconstructed human epidermis method)

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Proficiency testing

"*Prior to routine use of any of the validated RhE test methods... laboratories should demonstrate technical proficiency by correctly classifying the 12 Proficiency Substances*"

OECD TG 431

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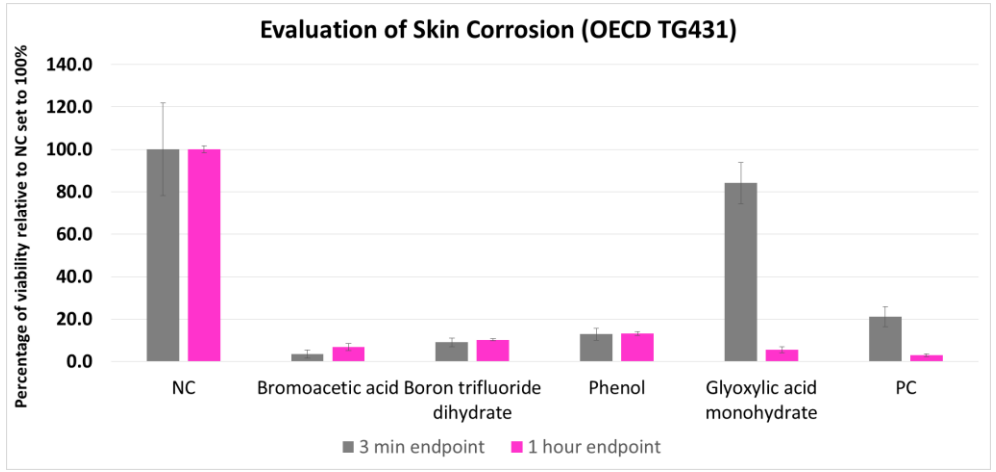
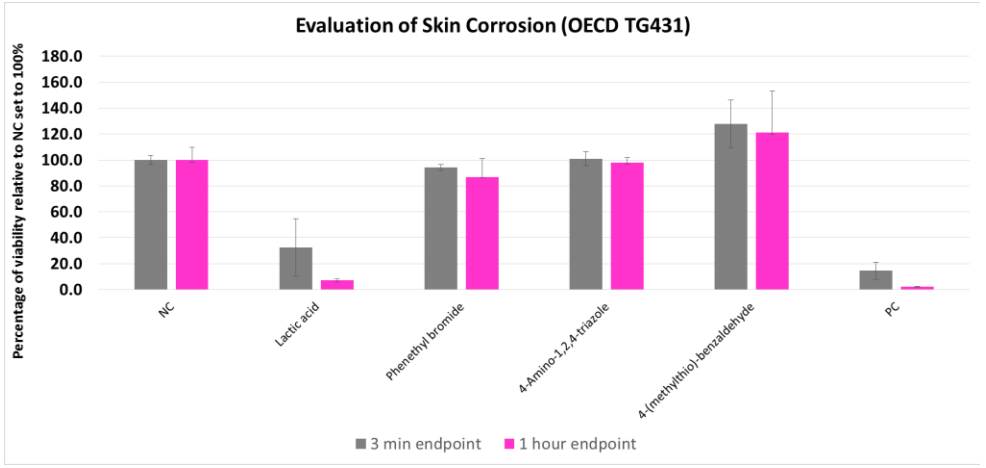
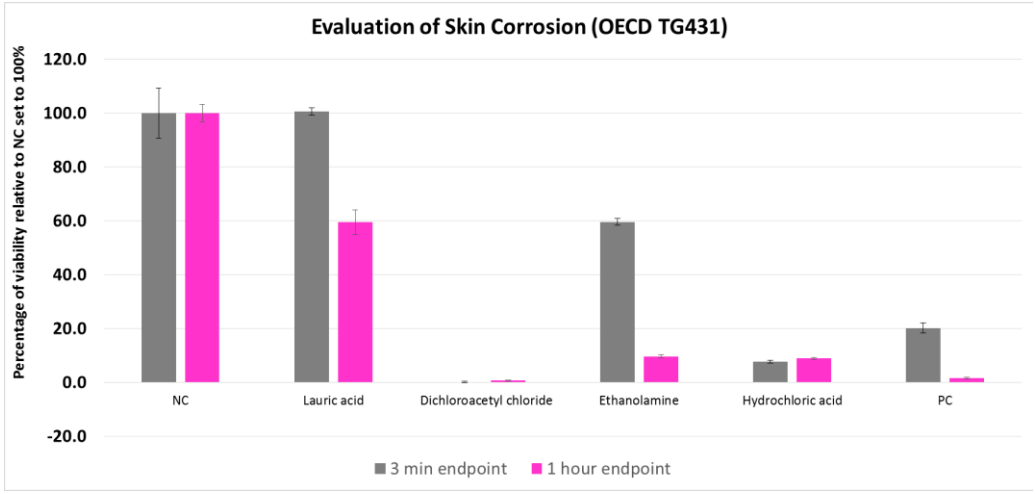
Proficiency testing

SAMPLE DATA USING EPIDERM™ SCT







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Proficiency testing

SAMPLE DATA USING EPIDERM™ SCT

Test Item name	CAS#	Test Item ID	Chemical Class	UN GHS Cat. Based on *In Vivo* results	XCellR8 classification
Bromoacetic acid	79-08-3	TA1	Organic Acid	Corrosive 1A	**Corrosive**
Boron trifluoride dihydrate	13319-75-0	TA2	Inorganic Acid	Corrosive 1A	**Corrosive**
Phenol	108-95-2	TA3	Phenol	Corrosive 1A	**Corrosive**
Glyoxylic acid monohydrate	563-96-2	TA4	Organic Acid	Corrosive 1B and 1C	**Corrosive**
Lactic acid	50-21-5	TA5	Organic Acid	Corrosive 1B and 1C	**Corrosive**
Phenethyl bromide	103-63-9	TA6	Electrophile	Non-Corrosive	**Non-Corrosive**
4-Amino-1,2,4-triazole	584-13-4	TA7	Organic base	Non-Corrosive	**Non-Corrosive**
4-(methylthio)-benzaldehyde	3446-89-7	TA8	Electrophile	Non-Corrosive	**Non-Corrosive**
Lauric acid	143-07-7	TA9	Organic Acid	Non-Corrosive	**Non-Corrosive**
Dichloroacetyl chloride	79-36-7	TA10	Electrophile	Corrosive 1A	**Corrosive**
Ethanolamine	141-43-5	TA11	Organic base	Corrosive 1B	**Corrosive**
Hydrochloric acid	7647-01-0	TA12	Inorganic Acid	Corrosive 1B and 1C	**Corrosive**

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OECD Test Guideline 435: *In Vitro* Membrane Barrier Test Method for Skin Corrosion

First adopted 19th July 2006
Latest edition published 28th July 2015

OECD TG 435: Membrane Barrier (Corrositex®)

- **Scientific rationale:** Artificial membrane acts as a surrogate for *in vivo* membrane barrier damage, assuming the same mechanism(s) of corrosion that operate on living skin
- Test system has two components:
  - Synthetic macromolecular bio-barrier
  - Chemical Detection System (CDS) (pH sensitive dyes)
- Substances breaking through the membrane barrier are measured by a change in the colour of the pH indicator dye
- Currently only one commercial test kit (Corrositex®) is validated for use
- Suitable for substances and mixtures:
  - Solids, liquids and emulsions

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OECD TG 435: predictive capacity

- **Performance (compared with TG 404 Draize)**
  - Sensitivity 86% (54/63)
  - Specificity 68% (15/22)
  - Accuracy 81% (69/85)

- **GHS categories**
  - Identifies non-corrosives and skin corrosive sub-categories 1A, 1B and 1C

- **Applicability domain**
  - Limited to specific chemical classes:
    - Organic and inorganic acids
    - Acid derivatives
    - Bases

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OECD TG 435: strengths and weaknesses

STRENGTHS	WEAKNESSES
Formal validation and OECD approval	No cellular components – not a living system
Allows full sub-categorisation into skin corrosive sub-categories 1A, 1B and 1C	In some cases colour changes may be transient and difficult to interpret (reference photos provided by the test developer)
Simple test method – quick turnaround	Not validated for gases and aerosols (same as other methods *in vitro* and *in vivo*)
	Usually not applicable to chemicals with pH 4.5-8.5 (not detected): EU validation study: 58% of the test chemicals were incompatible with the Chemical Detection System (CDS)

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OECD TG 435: limitations

- Test method valid only for limited applicability domain of acids, bases and acid derivatives

- Does not discriminate skin irritants (Cat.2) from chemicals not requiring classification from skin irritation / corrosion (No Cat)
  - However this can be addressed using OECD TG 439 (*in vitro* skin irritation – reconstructed human epidermis method)

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OECD Test Guideline 430: *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance (TER)

First adopted 13th April 2004
Latest edition published 26th July 2013

OECD TG 430: Transcutaneous Electrical Resistance (TER)

- **Scientific rationale:** Transcutaneous electrical resistance (TER) as a readout of corrosive effects on the skin and its barrier eg erosion of the *stratum corneum*
- Skin discs taken from “humanely killed” rats
- Test material applied for up to 24 hours in a 2-compartment system separated by the skin discs
- Corrosive materials identified by their ability to reduce stratum corneum integrity and barrier function, measured by reduction in TER
- Suitable for substances and mixtures:
  - Solids and liquids

Important note:
this method not performed at XCellR8

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OECD TG 430: predictive capacity

- **Performance (compared with TG 404 Draize)**
  - Sensitivity 88.1%
  - Specificity 72.4%
  - Accuracy 79.4%

- **GHS categories**
  - Discriminates skin corrosives (Cat.1) from non corrosives
  - Not accepted for identifying sub-categories 1A, 1B and 1C

Important note: this method not performed at XCellR8

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OECD TG 430: strengths and weaknesses

STRENGTHS	WEAKNESSES
Formal validation and OECD approval	Requires animal sacrifice (not a non-animal test)
Different mechanism from TG 431 and TG 435 – may complement evidence (however all 3 are stand-alone methods)	May be considered *in vivo* animal experiment in some countries due to shaving, washing and treating animals for 4-6 days prior to sacrifice
	TER cut-off value varies with age and strain of rats
	Not validated for gases and aerosols (same as other methods *in vitro* and *in vivo*)

Important note: this method not performed at XCellR8

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OECD TG 430: limitations

- Unable to sub-categorise corrosives (Cat.1A, 1B, 1C)

- Does not discriminate skin irritants (Cat.2) from chemicals not requiring classification from skin irritation / corrosion (No Cat)
  - However this can be addressed using OECD TG 439 (*in vitro* skin irritation – reconstructed human epidermis method)

Important note: this method not performed at XCellR8

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Summary

Predictive capacity of the 3 *in vitro* skin corrosion tests *vs* TG404 *in vivo* Draize test

	Sensitivity	Specificity	Accuracy
TG431: RhE	>95%	>90%	>82.5%
TG435: Memb. Barrier	86%	68%	81%
TG430: TER	88.1%	72.4%	79.4%
TG404: Draize	N/A (reference)	N/A (reference)	N/A (reference)

DEFINITIONS

- Sensitivity: ability to identify a true positive result
- Specificity: ability to identify a true negative result
- Accuracy: correctness of a single measurement (combination of sensitivity and specificity)

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In vitro skin corrosion testing might be desirable even if it's not essential

- CLP Regulation EC 1272/2008 is a key consideration for many companies commissioning skin corrosion tests
- Logistics and cost implications for transport and labelling / Packing Groups: advantages in achieving the least harmful sub-category possible

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When could an *in vivo* test (TG 404 Draize test) still be a requirement?

- Key regulations, including EU REACH, CLP and BPR allow animal testing only as a last resort
- REACH Annex VIII (>10 tonnes per year): "*An* in vivo *test should only be performed in case the in vitro studies (as required in Annex VII) are not applicable or the results of these studies are not adequate for classification.*"
- OECD GD 203 (IATA): "*In the interest of both sound science and animal welfare,* in vivo *testing should not be undertaken until all available data relevant to the potential dermal corrosivity/irritation of the test chemical have been evaluated in a weight-of-the-evidence (WoE) analysis as presented in the IATA"*
- *In vitro* tests able to meet the vast majority of technical and regulatory requirements
- In some cases, TG404 still used and the extent varies by geography / global regulations
- 2018: 123 Draize tests conducted in the UK. (2017: 112)

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Summary: recommended approach to *in vitro* skin corrosion testing

- Follow the guidance of OECD IATA on skin corrosion and irritation (Guidance Document 203, published 2014) to determine whether *in vitro* testing is required
- Depending on expected result, adopt “Top down” or “Bottom up” approach (starting from corrosion or irritation endpoints respectively)
- Selecting from the 3 approved *in vitro* tests described in Module 3 of the IATA:
  - Starting point: TG431 (RhE) has overall highest predictive capacity and a broad applicability domain. It also has formal approval to discriminate between sub-categories 1A and 1B/C. *Consider most appropriate choice of skin model*
  - If clear need for sub-categorisation between 1B and 1C, or for substances of extreme pH, consider adding TG435 (Corrositex®) – but limited applicability domain (acids, bases and acid derivatives)
  - Alternatively, consider using additional RhE ability (published studies referenced in IATA) to discriminate between 1A, 1B and 1C for weight-of-evidence purposes, or default to 1B
  - TG430 (TER) poorest predictive capacity, no sub-categorisation potential and requires *in vivo* steps – not a non-animal method

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Thank you!

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