Webinar: use of alternative methods to animal testing in your REACH registration

Using alternative methods to meet your information requirements

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Kimmo Louekari
Evaluation Unit
European Chemicals Agency

Overview

- Revision of information requirements (REACH Annexes)

- Using alternative methods and approaches to meet your information requirements

Introduction

- Animal tests traditionally used to study toxicity of chemicals

- Classification, labelling and other risk management measures often based on animal studies (*in vivo)*

Introduction

- More alternatives to animal studies now available
- *In vitro* tests can often be used as alternatives for lower tier studies:
  - skin corrosion/irritation
  - serious eye damage/eye irritation
  - skin sensitisation

- QSAR, read-across or human data can be used with proper justification → avoids animal testing and saves costs

Annex revisions

- Effective from 30 May 2016 for irritation and acute toxicity
- Foreseen in Autumn 2016 for skin sensitisation
- Information requirements affected:
  - skin corrosion/irritation
  - serious eye damage/eye irritation
  - acute toxicity: dermal route adaptations
  - skin sensitisation
- Relevant for all dossiers submitted to ECHA

Annex VII revisions: skin corrosion/irritation

'8.1. Skin corrosion/irritation	8.1. The study/ies do(es) not need to be conducted if: — the substance is a strong acid (pH ≤ 2,0) or base (pH ≥ 11,5) and the available information indicates that it should be classified as skin corrosion (Category 1), or — the substance is spontaneously flammable in air or in contact with water or moisture at room temperature, or — the substance is classified as acute toxicity by the dermal route (Category 1), or — an acute toxicity study by the dermal route does not indicate skin irritation up to the limit dose level (2 000 mg/kg body weight). If results from one of the two studies under point 8.1.1 or 8.1.2 already allow a conclusive decision on the classification of a substance or on the absence of skin irritation potential, the second study need not be conducted.
8.1.1. Skin corrosion, *in vitro*	
8.1.2. Skin irritation, *in vitro*	

Skin corrosion/irritation

No *in vivo* test needed

Relevant test methods: irritation

- If effects are observed: skin corrosion testing needed to find whether the substance is Cat 1 or Cat 2

Skin irritation		
Test method	**EU Test Methods /OECD test guideline**	**Classification according to CLP Regulation**
EpiDerm ™ SIT	B.46/TG 439	Cat. 1/Cat. 2 or NC
EpiSkin ™	B.46/TG 439	Cat. 1/Cat. 2 or NC
SkinEthic ™ RHE	B.46/TG 439	Cat. 1/Cat. 2 or NC
LabCyte EPI-MODEL24 SIT	B.46/TG 439	Cat. 1/Cat. 2 or NC

Relevant test methods: corrosion

Skin corrosion		
Test method	**EU Test Methods /OECD test guideline**	**Classification according to CLP Regulation**
TER	B.40/TG 430	Cat. 1 or non corrosive
EpiDerm ™ SCT	B.40 bis/TG 431	Cat. 1, 1A, 1B/1C or non-corrosive
EpiSkin ™	B.40 bis/TG 431	Cat. 1, 1A, 1B and 1C or non-corrosive[1]
SkinEthic ™ RHE	B.40 bis/TG 431	Cat. 1, 1A, 1B/1C or non-corrosive
epiCS®	B.40 bis/TG 431	Cat. 1, 1A, 1B/1C or non-corrosive
Corrositex (*in vitro* membrane barrier test method)	N.A./TG 435	Cat. 1, 1A, 1B and 1C or non-corrosive

Annex VII revisions: serious eye damage and eye irritation

8.2. Serious eye damage/eye irritation	8.2. The study/ies do(es) not need to be conducted if: — the substance is classified as skin corrosion, leading to classification as serious eye damage (Category 1), or — the substance is classified as skin irritation and the available information indicates that it should be classified as eye irritation (Category 2), or — the substance is a strong acid (pH ≤ 2,0) or base (pH ≥ 11,5) and the available information indicates that it should be classified as serious eye damage (Category 1), or — the substance is spontaneously flammable in air or in contact with water or moisture at room temperature.
8.2.1. Serious eye damage/eye irritation, *in vitro*	8.2.1. If results from a first *in vitro* study do not allow a conclusive decision on the classification of a substance or on the absence of eye irritation potential, (an) other *in vitro* study/ies) for this endpoint shall be considered.'

Serious eye damage/irritation

No *in vivo* test needed

Test methods for serious eye damage and eye irritation

- *In vitro* methods can **only** identify substances causing serious eye damage (Cat 1), and substances not requiring classification
- **No** *in vitro* methods are available for identification of eye irritants (Cat 2)
- Combination of multiple *in vitro* tests (or as a last resort *in vivo* tests) for the correct identification of Cat 2 substances, but still **no *in vivo* test at this tonnage**

Serious eye damage / eye irritation		
Test method	**EU Test Method /OECD test guideline**	**Classification according to CLP Regulation**
BCOP	B.47 / OECD TG 437	Cat. 1 or NC
ICE	B.48 / OECD TG 438	Cat. 1 or NC
FL	N.A. / OECD TG 460	Cat. 1
STE	N.A. / OECD TG 491	Cat. 1 or NC
RhCE	N.A. / OECD TG 492	NC

Additional test methods and their application

- Positive results of these tests can be used but not recommended for use under REACH
- Preference given to OECD/EU approved methods (see previous slide)

Serious eye damage / eye irritation		
Test method	**EU Test Method /OECD test guideline**	**Classification according to CLP Regulation**
CM	N.A. / OECD draft TG available and being considered for adoption	Cat. 1 or NC
Ocular Irritection® Assay	N.A. / N.A.	Cat. 1
IRE	N.A. / N.A.	Cat. 1
HET-CAM	N.A. / N.A.	Cat. 1

Annex VII revisions: skin sensitisation

"8.3. Skin sensitisation Information allowing - a conclusion whether the substance is a skin sensitiser and whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A), and - risk assessment, where required	The study(ies) under point 8.3.1. and 8.3.2. do not need to be conducted if: — the substance is classified as skin corrosion (Category 1), or — the substance is a strong acid (pH ≤ 2,0) or base (pH ≥ 11,5), or — the substance is spontaneously flammable in air or in contact with water or moisture at room temperature.
8.3.1. Sk… *vitro/in c…* Informati… *chemico* … recognise… article 13… each of th… events of skin sensitis… (a) Molecular interaction with skin proteins (b) Inflammatory response in keratinocytes (c) Activation of dendritic cells	…f available, or …thods are not adequate for …o point 8.3. …ing one or two …e key events in column 1 already allows classification and risk assessment according to point 8.3, studies addressing the other key event(s) need not to be conducted.

In vitro refers to non-animal tests usually in cell cultures

In chemico refers to reactivity or other physico-chemical properties of compounds. In this case peptide binding

8.3.2. Skin sensitisation, *in vivo*.
An in-vivo study shall be conducted only if *in vitro/in chemico* test methods described under point 8.3.1. are not applicable, or the results obtained from those studies are not adequate for classification and risk assessment according to point 8.3. The Murine Local Lymph Node Assay (LLNA) is the first-choice method for *in vivo* testing. Only in exceptional circumstances should another test be used. Justification for the use of another *in vivo* test shall be provided. In-vivo skin sensitisation studies that were carried out or initiated before [date of entry into force], and that meet the requirements set out in Article 13(3), first subparagraph, and Article 13(4) shall be considered appropriate to address this standard information

Test methods for skin sensitisation (1)

- 3 skin sensitisation test methods adopted by OECD, one for each key event, specified in the adverse outcome pathway
- **"Key event"**: step or phase within a toxic mechanism. A toxic mode of action can be split into key events
- **Adverse outcome pathway (AOP)**: structured representation of biological events (key events) leading to adverse effects (i.e. toxicity)

Note

In vivo test (LLNA) enables sub-categories of sensitisers to be identified

In vitro methods often do not. (New methods are likely to change this)

Test methods for skin sensitisation (2)

- **Key event 1 (KE1)**: Molecular interaction with skin proteins
- **KE2**: Inflammatory response in keratinocytes
- **KE3**: Activation of dendritic cells

Skin sensitisation		
Test method	**EU Test Methods/ OECD test guideline**	**Classification according to CLP Regulation**
DPRA (KE1)	B.59 / TG 442C	SS or NS with complementary information
KeratinoSens (KE2)	B.60 / TG 442D	SS or NS with complementary information
h-CLAT (KE3)	N/A / TG 442E	SS or NS with complementary information

Annex VIII revisions

'8.1. Skin corrosion/irritation	8.1. An *in vivo* study for skin corrosion/irritation shall be considered only if the *in vitro* studies under points 8.1.1 and 8.1.2 in Annex VII are not applicable, or the results of these studies are not adequate for classification and risk assessment. The study does not need to be conducted if: — the substance is a strong acid (pH ≤ 2,0) or base (pH ≥ 11,5), or — the substance is spontaneously flammable in air or in contact with water or moisture at room temperature, or — the substance is classified as acute toxicity by the dermal route (Category 1), or — an acute toxicity study by the dermal route does not indicate skin irritation up to the limit dose level (2 000 mg/kg body weight).
8.2. Serious eye damage/eye irritation	8.2. An *in vivo* study for eye corrosion/irritation shall be considered only if the *in vitro* study(ies) under point 8.2.1 in Annex VII are not applicable, or the results obtained from these study(ies) are not adequate for classification and risk assessment. The study does not need to be conducted if: — the substance is classified as skin corrosion, or — the substance is a strong acid (pH ≤ 2,0) or base (pH ≥ 11,5), or — the substance is spontaneously flammable in air or in contact with water or moisture at room temperature.'

Eye irritation

No suitable *in vitro* tests available

Annex VIII revision: acute dermal toxicity

8.5.3. By dermal route	8.5.3. Testing by the dermal route is appropriate if: (1) inhalation of the substance is unlikely; and (2) skin contact in production and/or use is likely; and (3) the physicochemical and toxicological properties suggest potential for a significant rate of absorption through the skin. Testing by the dermal route does not need to be conducted if: — the substance does not meet the criteria for classification as acute toxicity or STOT SE by the oral route and — no systemic effects have been observed in *in vivo* studies with dermal exposure (e.g. skin irritation, skin sensitisation) or, in the absence of an *in vivo* study by the oral route, no systemic effects after dermal exposure are predicted on the basis of non-testing approaches (e.g. read across, QSAR studies).'

Acute oral toxicity

- No annex revision
- Revised ECHA guidance describes possibility for adaptation/waiving
- Two criteria must be:
  - No effect was observed in the sub-acute oral toxicity test. Highest dose: 1 000 mg/kg
  - Weight-of-evidence approach with at least one additional piece of information provided

In vitro testing by default

- Skin corrosion/irritation, serious eye damage and skin sensitisation: if new testing is required: **must always start with *in vitro* test methods**

- *In vivo* testing only if:
  - Methods are not suitable for the substance
  - Results of the *in vitro* tests are not adequate for classification and risk assessment.

Potency estimation for skin sensitisers

- Potency estimation (strong/extreme (Cat 1A) vs moderate (Cat 1B): **now mandatory** for skin sensitisation

- But not mandatory when **existing** guideline and GLP compliant data available (performed before the new annex entered into force)
  - (e.g. results from guinea pig test (EU method B.6) may not allow a conclusion on whether a substance could be 1A due to test design)

Skin sensitising substances

Precautionary Cat 1A classification may be applied

Note

existing other information or generation of non-animal test data may be helpful in refining the potency assessment (Cat 1A vs 1B).

Tips from ECHA (1)

- Suitability and scope of relevant *in vitro* test methods addressed on ECHA's website: https://echa.europa.eu/support/oecd-eu-test-guidelines and guidance updates

- If the registered substance does not fit the scope of the *in vitro* methods or if the *in vitro* results are not adequate for classification and labelling: *in vivo* test should be performed
  - Justification for not performing *in vitro* tests must be provided in your dossier

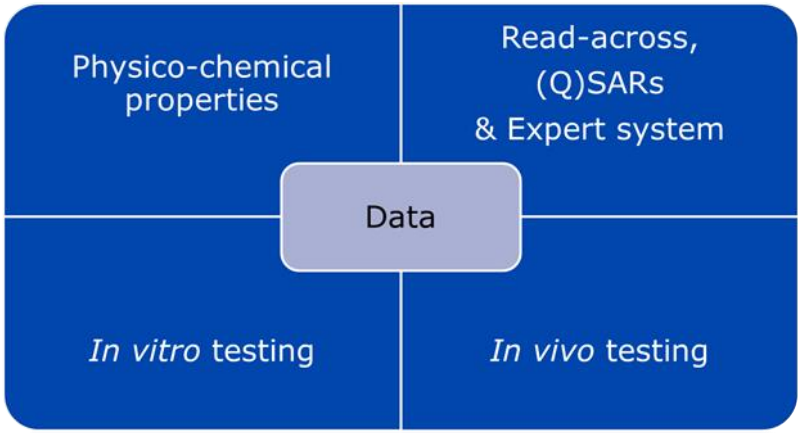
Tips from ECHA (2)

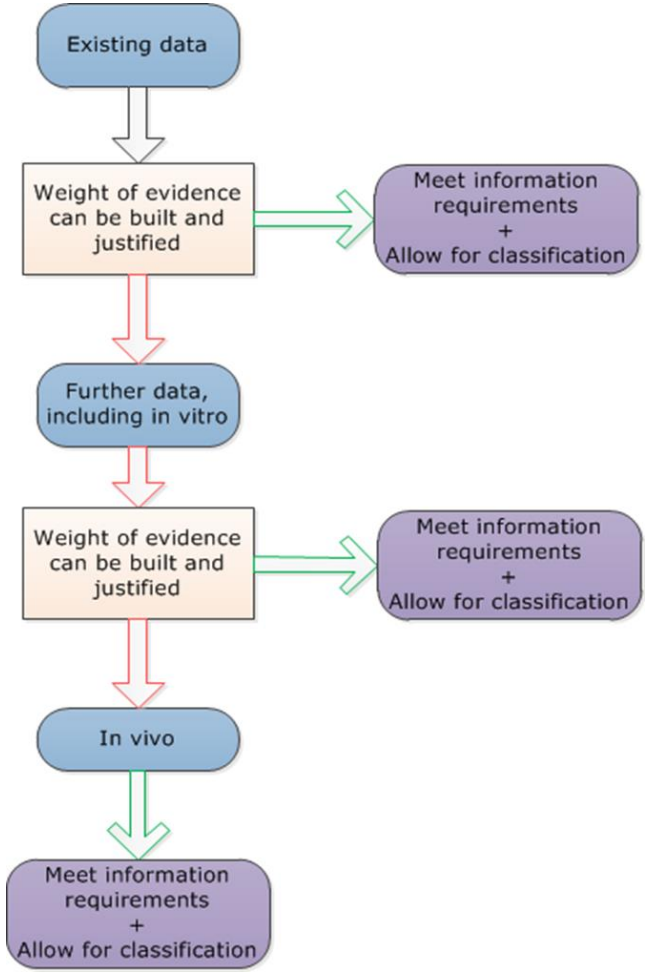
- If registrant provides more than one test result (per endpoint): separate endpoint study records need to be submitted for each test

- Conclusions drawn from all the data obtained should be given in a *weight-of-evidence* approach

Testing and assessment strategies

- Gather existing data
- Consider additional testing needs and the appropriate test to start with





Concluding remarks

- Number of *in vitro* tests have been accepted for use under REACH
- Other methods e.g. QSAR and grouping have matured and often have potential in regulatory use
- Always examine the possibility of meeting information requirements with non-animal testing or other data
- ECHA anticipates that the alternatives can be used in a significant number of cases
- Detailed guidance on non-animal methods and approaches: Practical Guide: How to use alternatives to animal testing to fulfil your information requirements for REACH registration

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kimmo.louekari@echa.europa.eu

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