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

Case studies: what you need to do

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Overview

- Case 1: extreme pH
- Case 2: existing *in vivo* studies
- Case 3: skin corrosion/irritation
- Case 4: eye irritation
- Cases 5a to 5d: skin sensitisation
- Case 6: acute toxicity

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Case 1: extreme pH

Skin sensitisation
In chemico/in vitro testing can be performed at suitable concentrations, if considered necessary to assess skin sensitisation potential in sub-corrosive concentrations.

This is not a formal information requirement

Starting point:

- Substance has a pH of 12

What to do:

- Column 2 adaptations can be used for:
  - skin corrosion/irritation
  - serious eye damage/eye irritation
  - skin sensitisation
  - acute dermal and inhalation toxicity

If the substance has been classified for skin corrosion (Category 1)
→ no testing needed

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Case 2: existing *in vivo* studies

Starting point:

- Existing *in vivo* data → what is needed to pass the technical completeness check

What to do:

- An adaptation for not submitting *in vitro* data needs to be submitted in the dossier → separate endpoint study record
  - An adaptation can be e.g. that existing good quality *in vivo* data is available

Skin corrosion/irritation & serious eye damage/eye irritation

in vitro testing mandatory information requirement in Annex VII since REACH entered into force in 2008

Skin sensitisation

in vivo study needs to be performed or initiated before the new annex requirement enters into force

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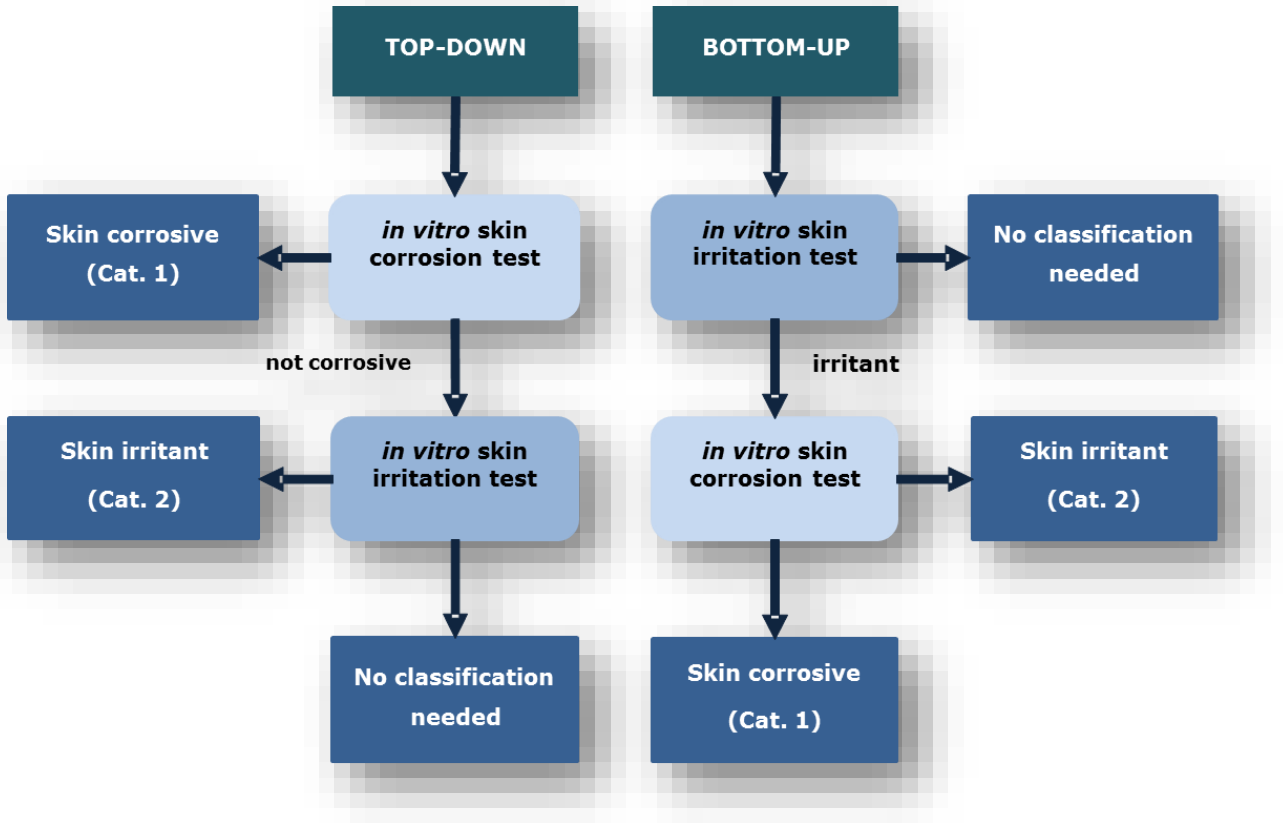
Case 3: skin corrosion/irritation



Consider top-down or bottom-up approaches based on presumed properties

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Case 4: eye irritation

- Study performed according to OECD test guideline 437: Bovine Corneal Opacity and Permeability (BCOP)
  - Outcome *in vitro* irritation score (IVIS) ≤ 3: **no classification.** No further testing needed for eye irritation
  - Outcome IVIS > 55: classify for **serious eye damage** (Cat 1). No further testing needed
  - Outcome 3 < IVIS ≤ 55: inconclusive results. **Further testing needed** (*in vitro*, or as a last resort *in vivo*)

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Case 5: skin sensitisation

Note
Data obtained from an analogue substance e.g. EC3-value from LLNA, may provide useful information on assessing skin sensitisation potency together with additional information

Starting point:

- No existing data available

What to do:

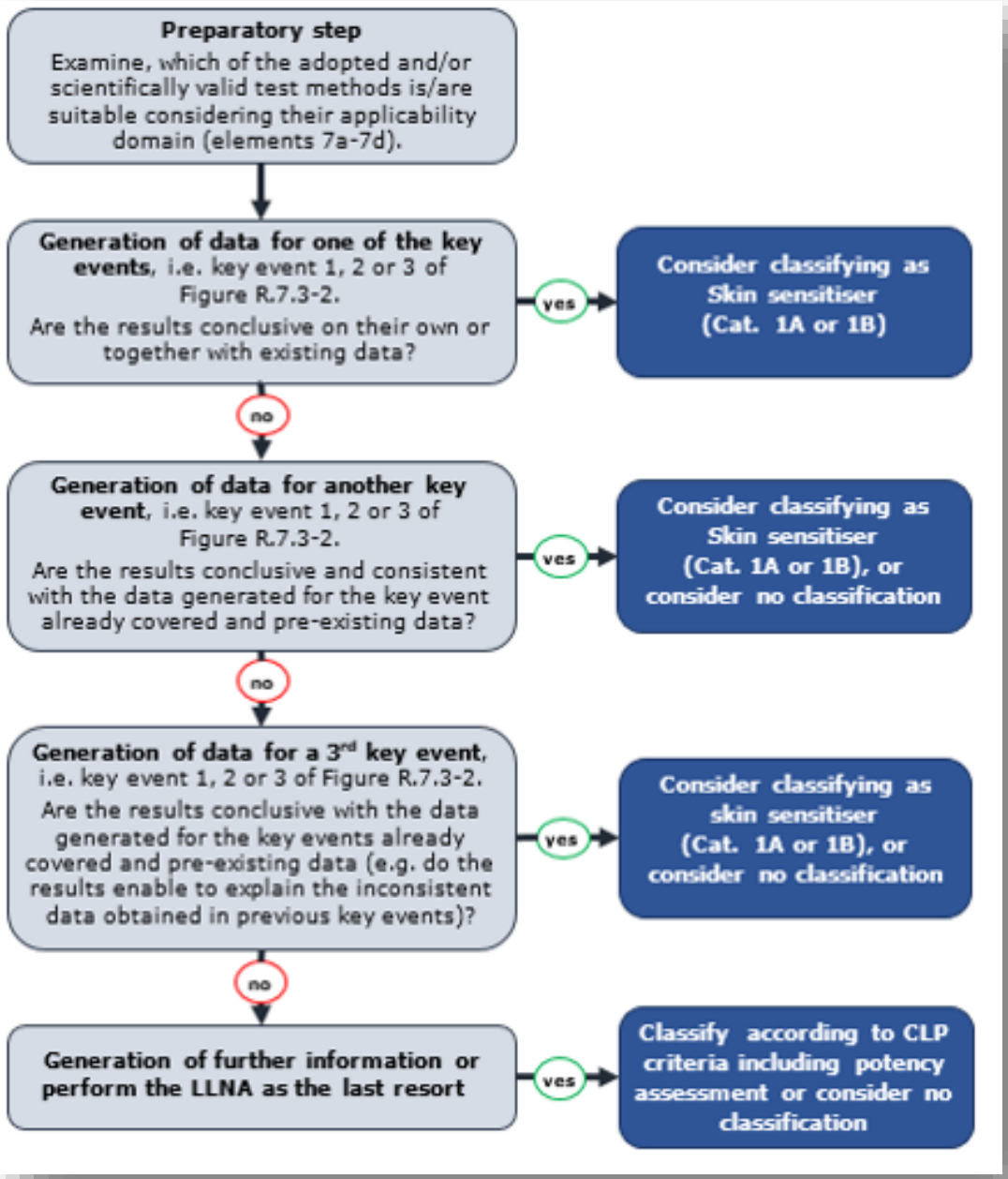
- Use of e.g. QSAR Toolbox recommended
  - Identification of existing *in chemico*, *in vitro* and *in vivo* data
  - Identification of skin sensitisation specific alerts
  - Prediction and characterisation of metabolic and abiotic transformation
  - Identification of potentially suitable analogues with existing data, i.e. read-across

Case 5: Generation of new data (1)

- Testing must start with *in chemico/in vitro* methods
- Sufficient number of key events need to be covered

Note
If *in chemico/in vitro* methods are not suitable for the substance or results are not suitable for classification (Cat. 1A vs 1B) and risk assessment, *in vivo* testing has to be conducted. Justification for performing the study is needed

Case 5: Generation of new data (2)



Case 5a: skin sensitisation *in vitro* (1)

Starting point:

- No analogues found in QSAR toolbox → new data needed
- Negative results obtained from 3 *in vitro* tests but test methods lack or only have limited metabolic capacity

Test method	Result
DPRA (KE1)	Neg
KeratinoSens (KE2)	Neg
h-CLAT (KE3)	Neg
TIMESS-SS	No alerts

Case 5a: skin sensitisation *in vitro* (2)

Challenge:

- Might the substance have a metabolite that could have skin sensitising properties?
- TIMES-SS* used for the substance → identified metabolites did not show concern for skin sensitisation

What to do:

- *Weight-of–evidence* approach based on the data obtained that adequately and consistently addressed the lack of skin sensitisation potential → no further testing (*in vitro* or *in vivo*) nor classification needed

*TImes MEtabolism Simulator platform for predicting Skin Sensitisation (TIMES-SS) is a hybrid expert system

Case 5b: Skin sensitisation *in vitro* (1)

Starting point:

- Three *in vitro* tests performed showing positive results

Test method	Result
DPRA (KE1)	Pos
KeratinoSens (KE2)	Pos
h-CLAT (KE3)	Pos

Challenge:

- How to consider skin sensitisation potency (Cat 1A vs 1B)?
  - DPRA: positive with low reactivity
  - KeratinoSens: > 1.5 fold luciferase induction noted at high concentration (non-cytotoxic substance)
  - h-CLAT: induction of CD86 and CD54 noted at high concentration (non-cytotoxic substance)

Case 5b: Skin sensitisation *in vitro* (2)

Challenge:

- Search in QSAR toolbox resulted in one analogue substance having LLNA data with EC3 value of 30 % (cut-off in CLP for 1B is EC3 > 2 %)

Note
Adequacy of the read-across needs to be provided

What to do:

- As all data indicates moderate skin sensitisation potency i.e. Cat 1B, substance is classified accordingly and no further testing is needed

Case 5c: Skin sensitisation *in vivo* (1)

Starting point:

- No existing data noted in QSAR Toolbox, inconsistent results from other expert systems on skin sensitisation alerts

Test method	Result
DPRA (KE1)	Not applicable
KeratinoSens (KE2)	Not applicable
h-CLAT (KE3)	Applicable?

Challenge:

- Suitability of the substance for the *in vitro* test methods
  - Substance is a UVCB → not suitable for DPRA.
  - Substance has a logKow of >4 → suitability for h-CLAT questionable (negative outcome not acceptable)
  - Substance is not soluble in water or DMSO → not suitable for KeratinoSens

Case 5c: Skin sensitisation *in vivo* (2)

Conclusion/what to do:

- *In vivo* testing (LLNA) needs to be performed. However a justification needs to be provided why *in vitro* testing was not performed in the registration dossier

Case 5d: existing *in vivo* study (1)

Starting point:

- Existing guinea pig test data (Guinea pig maximisation test or Buehler test) with positive result (induction values and incidence suggest Cat. 1B according to CLP criteria)
- Can be used according to Annex VII, column 2 of 8.3.2

Case 5d – existing *in vivo* study (2)

Challenge:

- Consideration of correct classification → although the criteria in the CLP for classification to Cat. 1B are fulfilled, the classification for Cat. 1A **may** not be excluded, due to high incidences at high doses (CLP Guidance)

What to do:

- Use of QSAR Toolbox or other information sources to confirm the **Cat. 1B** classification

Note
If no other supporting information can be obtained, Cat. 1 should be used for classification according to the CLP Regulation

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Starting point:

- No effect was observed in the sub-acute oral toxicity test. Other information exists, providing evidence of low toxicity

What to do:

- *Weight-of-evidence* adaptation can be made. *In vivo* acute oral toxicity test can be waived
- Acute oral toxicity has been covered → no need for acute dermal toxicity test, independent of the tonnage band

Links

- Testing methods and alternatives
- Practical guide: How to use alternatives to animal testing to fulfil your information requirements for REACH registration
- Practical guide for SME managers and REACH coordinators - How to fulfil your information requirements at tonnages 1-10 and 10-100 tonnes per year
- Endpoint specific Guidance R.7a (updates available in October 2016)

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