CAAT Europe
Inclusion of animal testing alternatives into QRA for skin sensitisation

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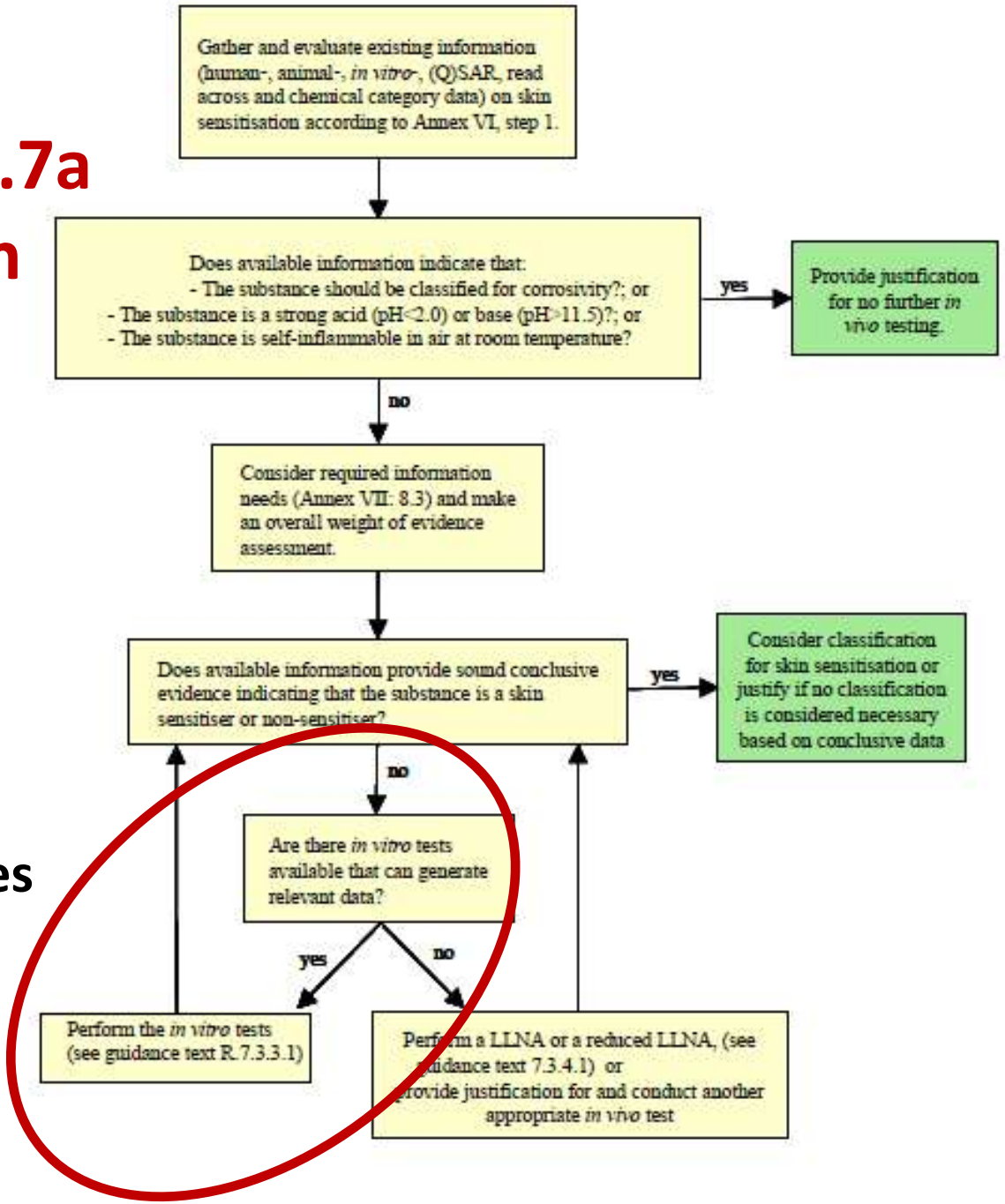
REACH Guidance R.7a skin sensitisation

First Edition

Publication: January 2008

"*vitro*" mentioned 36 times



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Coming-soon guideline

- "vitro" mentioned 141 times (4 times more)
- New paragraphs about:
  - Read Across
  - Mechanisms of skin sensitisation
  - *In chemico/in vitro data*
  - Predictive capacity of the existing *in vivo and non-animal tests when compared to human data*
  - How to deal with the lack of or limited metabolic capacity of the non-animal test methods?
  - Use of non-animal data (e.g. *in vitro methods) to support a category approach*
  - How to perform and report a *Weight-of-Evidence analysis*

Existing data on physico-chemical properties

1 **Is the substance a strong acid (pH≤ 2.0) or base (pH≥ 11.5), corrosive to the skin or (spontaneously) flammable in air at room temperature?**

Existing human data

2 **Are there adequate existing human data, which provide evidence that the substance is a skin sensitiser?**

Existing animal data from sensitisation studies

3 **Are there data from existing studies on skin sensitisation in laboratory animals (LLNA, GPMT, or Buehler test, OECD TGs 429, 442A, 442B and 406), which provide sound conclusive evidence that the substance is a sensitiser, or non-sensitiser?**

Existing (Q)SAR data and read-across

4 **Do "read-across" from structurally and mechanistically related substances and do suitable (Q)SAR predictions reliably indicate**

ARE toxicity pathway in an EU/OECD adopted *in vitro* test (e.g. OECD TG 442d)? (*Key event 2 of the AOP*)

***In vitro* test methods that have been validated and are considered scientifically valid but are not yet adopted by the EU and/or OECD may also be used if the provisions defined in Annex XI to the REACH Regulation are met.**

7c Does the substance demonstrate induction of the cell surface markers (CD54 and/or CD86) on monocytic cells in an validated *in vitro* test (e.g. h CLAT)? (*Key event 3 of the AOP*)

***In vitro* test methods that have been validated and are considered scientifically valid but are not yet adopted by the EU and/or OECD may also be used if the provisions defined in Annex XI to the REACH Regulation are met.**

7d Is any additional testing/generation of data considered necessary in order to conclude on classification, or e.g. to explain the inconsistent data obtained in previous elements or to address the *Key event 4 of the AOP* (T cell proliferation) with an *in vitro* test? d

Weight-of-Evidence analysis

8 **The "elements" described above may be arranged as appropriate. Taking all existing and relevant data (elements 1-7) into account, is there sufficient information to meet the respective information requirement of Section 8.3 of Annex VII and to make a decision on whether classification and labelling are warranted?**
For specific guidance on *Weight of Evidence see below*.

Generation of new in vivo data for sensitisation as a last resort (Annex VII to the REACH Regulation)

9 **Does the substance demonstrate sensitising properties in an EU/OECD adopted *in vivo* test, the LLNA (EU B.42/OECD TG 429, EU B.50/442A or EU B.51/442Be)? →**

ATP 2: COMMISSION REGULATION (EU) No 286/2011

Table 3.4.3

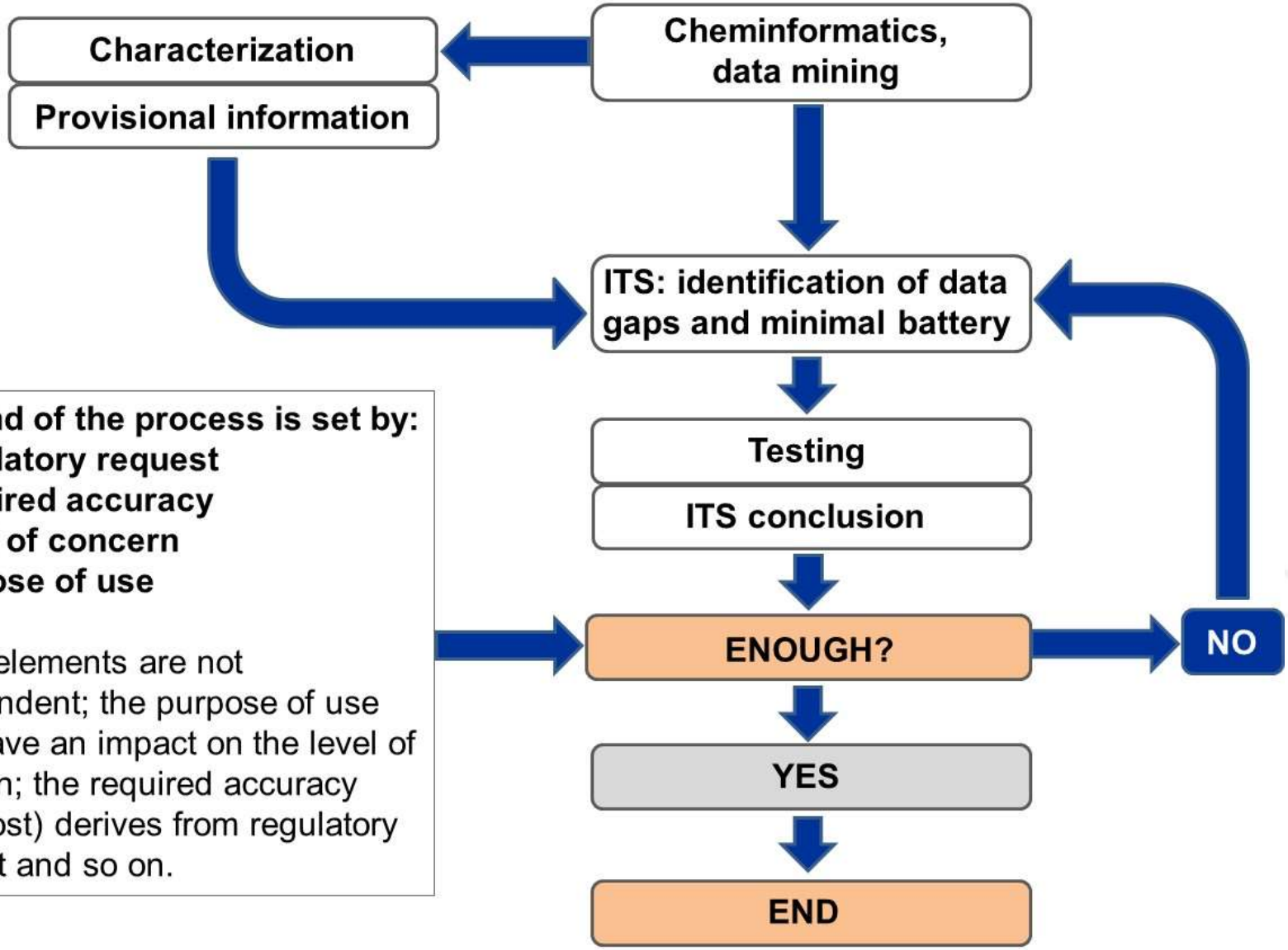
Animal test results for sub-category 1A

Assay	Criteria
Local lymph node assay	EC3 value ≤ 2 %
Guinea pig maximisation test	≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose
Buehler assay	≥ 15 % responding at ≤ 0,2 % topical induction dose or ≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose

Table 3.4.4

Animal test results for sub-category 1B

Assay	Criteria
Local lymph node assay	EC3 value > 2 %
Guinea pig maximisation test	≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
Buehler assay	≥ 15 % to < 60 % responding at > 0,2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose



The end of the process is set by:

- **Regulatory request**
- **Required accuracy**
- **Level of concern**
- **Purpose of use**
- **Cost**

Some elements are not independent; the purpose of use may have an impact on the level of concern; the required accuracy (and cost) derives from regulatory request and so on.

ALTEX. 2015;32(1):25-40

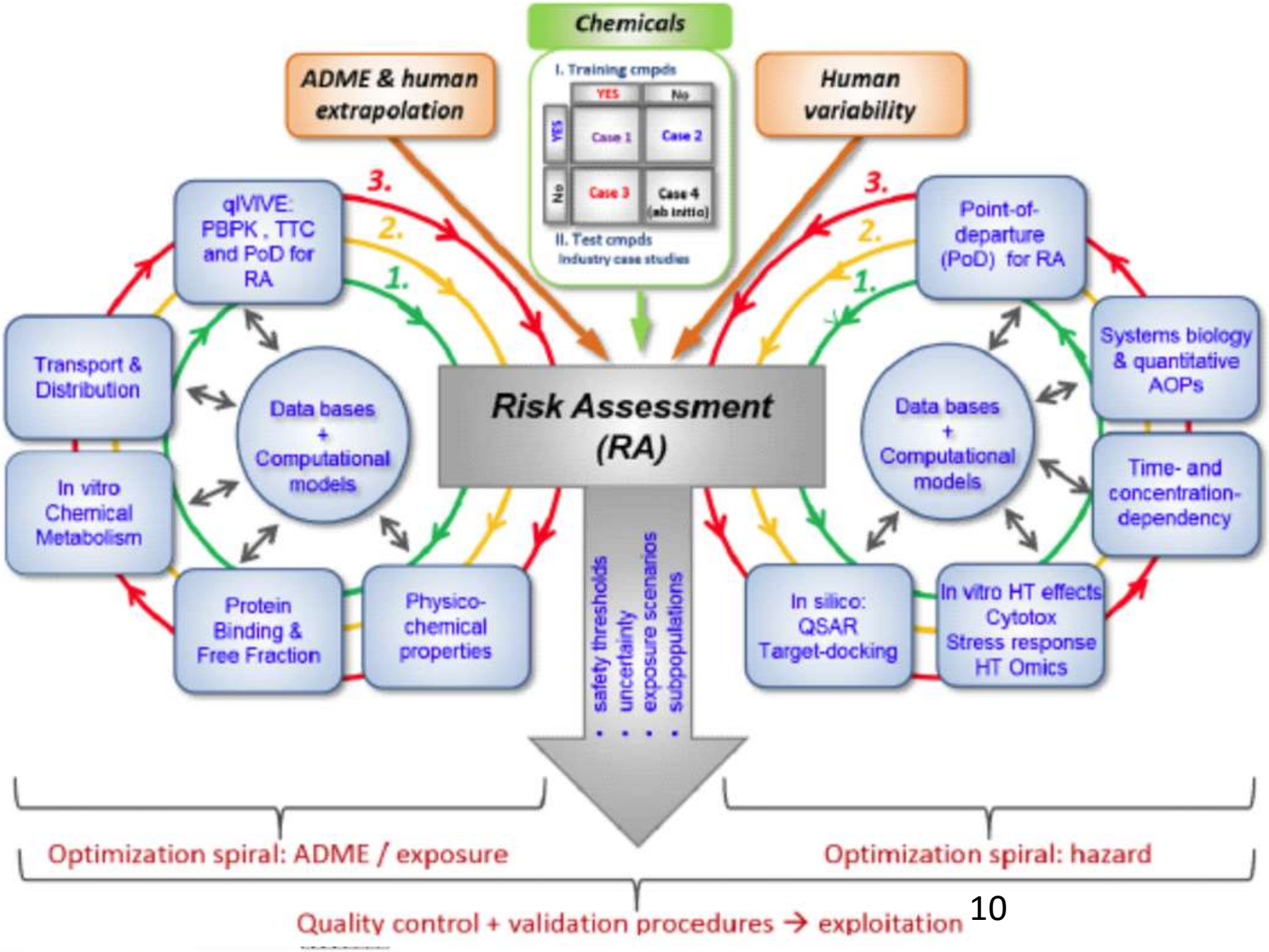
EU-ToxRisk

An Integrated European 'Flagship' Program Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st Century

12 October 2015

www.eu-toxrisk.eu

Two pillar tiered strategy of EuToxRisk



Research article

Journal of Applied Toxicology

Received: 9 February 2015, Revised: 6 April 2015, Accepted: 13 April 2015 Published online in Wiley Online Library: 5 June 2015

(wileyonlinelibrary.com) DOI 10.1002/jat.3172

Probabilistic hazard assessment for skin sensitization potency by dose–response modeling using feature elimination instead of quantitative structure–activity relationships

Thomas Luechtefeld[a†], Alexandra Maertens[a†], James M. McKim[b], Thomas Hartung[a,c*], Andre Kleensang[a] and Vanessa Sá-Rocha[a,d]

Table 2. Sensitization class to dose specific binary class transformation

Class	Low dose	Medium dose	High dose
Non-sensitizer	Negative	Negative	Negative
Moderate sensitizer	Negative	Negative	Positive
Strong sensitizer	Negative	Positive	Positive
Extreme sensitizer	Positive	Positive	Positive

Table 3. Example chemical 1-bromobutane - LLNA reference classification: non-sensitizer

LLNA	Low dose	Medium dose	High dose
Transformed LLNA classification	Negative	Negative	Negative
Possible problematic supervised model prediction	Negative	Positive	Negative

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Feature selection and variable importance

- Skin sensitization difficult to predict from chemoinformatic methods alone
- More informed ranking of in vitro assays: using all available data does not improve accuracy
- Account for dermal penetration data
- Applicability domain and prediction model!

Local Lymph Node Assay: How Testing Laboratories Apply OECD TG 429 for REACH Purposes

- Positive reference standard
- Applicability Domain
- Species
- Vehicle
- Selection of testing dose
- Housing conditions
- Other?

Thank you for your attention!