Practical needs to implement advanced strategies for a proper justification of the read across/category approach

CAAT-Europe – REACH mastery

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Know the rules, play your market.

Introduction

REACH was the first Regulation accepting alternative strategies for risk assessment. This was a revolution in the area of regulatory science therefore more time is required to fully accept and implement this new opportunity.
Until now, about 14,000 registration dossiers have been submitted and published in the ECHA database (http://echa.europa.eu/information-on-chemicals/registered-substances).
Going into the details about how toxicological concern is assessed, it becomes evident that the read across approach is very much used, even though often with only weak scientific justification. Recently, ECHA (2015) has distributed a new document (Read Across Assessment Frame work, RAAF) that explains how to present read across justification. This includes the application of *in vitro* methods to elucidate mechanistic biological behavior that should demonstrate the similarity between two substances or within a category of substances.
According to personal experience, this was never the case even though acting as REACH consultant it was proposed to clients several times.

Possible reasons

Beyond the fear of a positive result that can be difficult to manage, there are other reasons why companies are generally reluctant in performing *in vitro* tests.

1. **There is still no culture on alternative methods. Most Universities are preparing professionals in toxicology in a traditional way, considering *in vivo* tests the gold standard for an endpoint-by-endpoint approach rather than in a more holistic way. Now in the EU, few scientists are aware of alternative methods even though concepts like IATA (Integrated Approach for testing and Assessment) and AOP (Adverse Outcome Pathways) are getting more and more familiar among toxicologists.**
2. **There is the misconception that regulators are not accepting alternative methods. This is false as regulators usually rejects alternative strategies only if they are not duly explained and justified.**
3. **Another practical constraint is the lack of well-equipped CROs, or better, the fact that *in vitro* and *in vivo* methods are generally available in different facilities, with all the problems related to the shipping of the sample, following the studies in different labs and combining results written into reports with different format. The final cost of a testing strategy is often higher than the cost of the correspondent *in vivo* test.**

No culture on alternative methods

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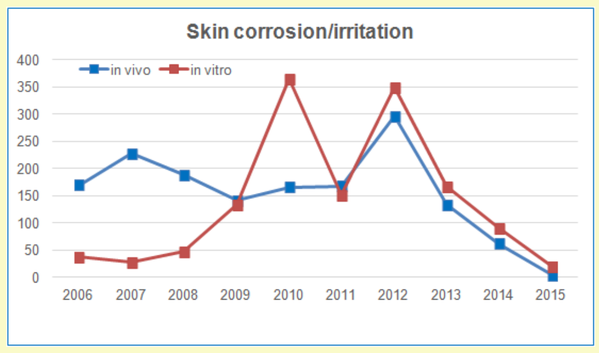
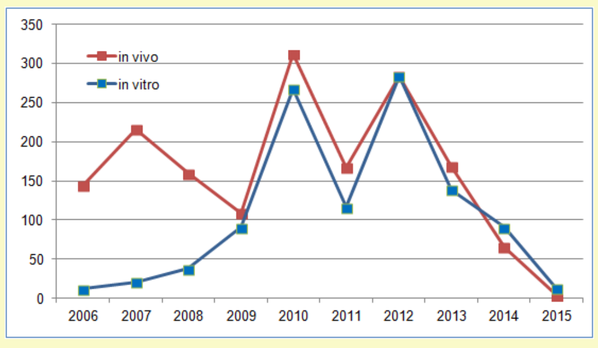
Counting alternative approaches in REACH registration dossier is impossible also because IUCLID is not designed for this purpose.
Just to scout the familiarity of registrants with *in vitro* capabilities, the number of *in vitro* and *in vivo* tests per year was counted for skin and eye irritation.





The entries of years 2012 and 2014 were manually checked to count the number of substances tested both *in vitro* and *in vivo*. Validated *in vitro* tests are available for both and therefore repetition *in vivo* is redundant (Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance)

% *in vitro* tests repeated *in vivo*		
	Skin irritation	Eye irritation
2012	12%	26%
2014	14%	17%

In 2015, a French journalist investigated on the reason why Companies still perform *in vivo* test for skin/eye irritation. Some of the answers received (forwarded by private email):
"*There are no approved methodologies recommended by ECHA for in vitro skin and eye irritations end points which will be acceptable to comply with Annex VIII requirements*"
"*When the in vitro test is negative and the tonnage is important, REACH requires the registrants to go in vivo, even if the in vitro test is validated and gives negative results*"
(The documentary is available at http://buzzmonclick.com/special-investigation-cobayes-bye-bye-replay-18-janvier-2016/)

Regulatory acceptance of alternative methods

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29.5.2007 EN Official Journal of the European Union L 136/119

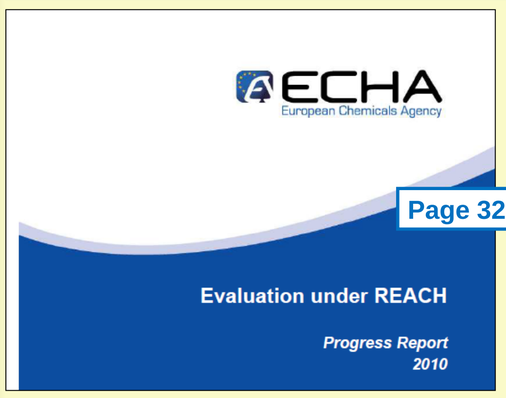
ANNEX XI

GENERAL RULES FOR ADAPTATION OF THE STANDARD TESTING REGIME SET OUT IN ANNEXES VII TO X

1.4. *In vitro* methods

…….

If the results obtained from the use of such *in vitro* methods do not indicate a certain dangerous property, the relevant test shall nevertheless be carried out at the appropriate tonnage level to confirm the negative result, unless testing is not required in accordance with Annexes VII to X or the other rules in this Annex.
Such confirmation may be waived, if the following conditions are met:
(1) results are derived from an *in vitro* method whose scientific validity has been established by a validation study, according to internationally agreed validation principles;
(2) results are adequate for the purpose of classification and labelling and/or risk assessment; and
(3) adequate and reliable documentation of the applied method is provided.



Skin irritation-corrosion

Annexes VIII-X requires an *in vivo* test to assess Skin irritation/corrosion. However, there are currently several *in vitro* methods available that can be used in a weight-of-evidence approach, to fully replace animal testing.

It is generally agreed that the EU B.46 (OECD 439) *in vitro* methods for Skin irritation represent a full replacement of the respective *in vivo* method (OECD 404) in a tiered testing strategy and in conjunction with *in vitro* skin corrosivity tests, if necessary. It should be noted that B.46 method does not address corrosivity; therefore, in case of positive result in a B46 test, a test addressing skin corrosion has to be performed.

It is recommended that the following testing strategy is followed when performing in vitro tests to assess skin-irritation and corrosion (see also *Guidance on information requirements and chemical safety assessment Chapter R.7a: Endpoint specific guidance)*

- Skin corrosion shall be tested first; in case of positive results, no further testing is necessary; the substance shall be classified accordingly.
- If the results of the skin corrosion test is negative, then a skin irritation study according to EU method B.46 shall be performed; if the result is positive, no further testing is necessary but classification of the substance.
- A negative result in the B.46 test does not need to be confirmed by additional testing.

RAAF document, Supporting evidence to read across:
"*Any scientific evidence provided to support the read-across hypothesis. Such supporting evidence may be, for example, information on the toxicokinetic properties of the substances, information from valid (Q)SARs, in vitro or in vivo experimental data addressing specific aspects of the read-across hypothesis*."

Few CROs are equipped for *in vitro* strategies

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Most CROs have implemented only official OECD guidelines, even though only few can offer also the most recent ones, based on *in vitro* testing.
In the EU, no lab can offer for example the ToxCast set of tests, which are very promising to support read across and category approach.
Other facilities are specialised in specific *in vitro* investigation, like neurotoxicity, ADME, liver toxicity, and so on, but they are not equipped for traditional OECD tests.
In these cases, the experts are focused on the drug sector and have few experience in regulatory needs for chemistry.
As REACH consultant, we face the problem of finding the most suitable lab, make it work from the proper perspective and convincing companies to spend money in one or more studies for which there is no experience.

Prosposals to ECHA for improvements

1. **Raise awareness in the Universities about the needs of the Toxicology in the 21st century**
2. **Improve IUCLID to accept alternative strategies and ask for justification to companies which performed *in vivo* test even if validated alternative test are available (skin and eye irritation, acute oral toxicity, acute fish toxicity). This will help in disseminating the culture of alternative strategies.**
3. **Foster the creation of a network of experts and labs to facilitate the implementation of alternative strategies and**

Preparing a common template for reporting the studies