# Effectiveness of toxicology-based webinars to promote nonstandard methods for REACH

Jeffrey Brown[1], João Barroso[2], Grace Patlewicz[3,4*], Richard Butterworth[5], Amy Clippinger[1], Gilly Stoddart[1]

[1]PETA International Science Consortium Ltd.; [2]EURL ECVAM / Systems Toxicology Unit, IHCP, European Commission Joint Research Centre; [3]DuPont Haskell Global Centers; [4]US EPA; [5]CW Research Ltd

## Introduction

The REACH regulation is designed to protect human health and the environment whilst promoting the use of alternatives to animal testing.

The regulation explicitly states that tests using vertebrates must be undertaken only as a last resort and includes mechanisms that allow use of non-animal approaches in lieu of the standard animal testing regime.

**Here we report on the impact of a free webinar series designed to increase uptake of non-animal testing strategies for REACH, alongside a case study illustrating how non-animal methods can be used to predict skin sensitisation in a WoE assessment.**

## Webinar content and assessment

Led by experts in relevant fields, and made available to an international audience through a collaboration between the PETA International Science Consortium Ltd. and online news service Chemical Watch, each webinar explored use of validated non-animal approaches to meet REACH Annex VII and VIII endpoints. Following the live sessions, recordings were permanently archived online and made freely available at PISCLtd.org.uk, chemicalwatch.com, and eurl-ecvam.jrc.ec.europa.eu.

At the end of each webinar, participants were emailed an optional 12 question web-based survey. Responses to key survey questions for the first four sessions are presented at right.

**Figure 1:** Summary of webinars to date.

| Topic | Date | Webinar Registrants |
|---|---|---|
| OECD QSAR Toolbox and Read-Across (QSAR/RA) | October 22, 2014 | 835 |
| Skin Irritation and Skin Corrosion (SI/SC) | November 11, 2014 | 736 |
| Serious Eye Damage and Eye Irritation (SED/EI) | December 4, 2014 | 493 |
| Skin Sensitisation (SS) | January 28, 2014 | 950 |
| Alternative Approaches to Mammalian Acute Systemic Toxicity Testing | March 5, 2015 | 547 |

## Webinar impact

**Figure 2:** In which country are you mainly based?

**Figure 3:** As a result of attending this webinar, are you more or less likely to use non-testing/non-animal methods for REACH 2018?

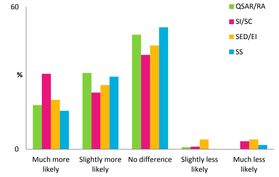



**Figure 4:** I gained some useful guidance on how to incorporate non-testing/non-animal methods into a testing strategy

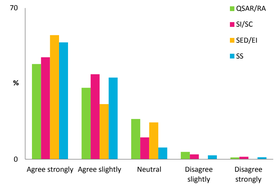



**Figure 5:** Regarding the complexity of information, was it:

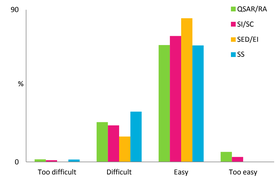



**Figure 6:** What do you think are the main barriers to the uptake of non-testing methods (e.g. QSARs, read-across) for REACH?

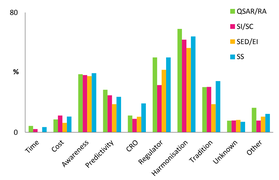
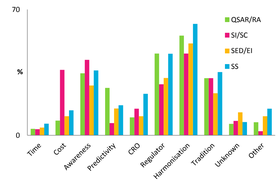


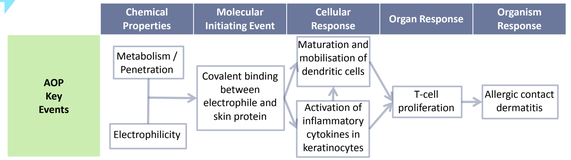

**Figure 7:** What do you think are the main barriers to the uptake of non-animal testing methods for REACH?




## Using a weight of evidence approach to replace animal testing

Webinar participants routinely request demonstrations of potential WoE approaches applied to registered substances. Here we exemplify the use of non-testing/non-animal methods for two substances for the skin sensitisation endpoint retrieved from the REACH registration database, using the AOP construct as a framework for organising the available data on the target substance and/or related analogues.

**1 Simplified AOP for Skin Sensitisation**




**2 Identify Test Methods for Evaluating Key Events**

| Testing and Non-testing Approaches for Assessing AOP Key Events† | QSARs<br>Read-across | DPRA[1,2,3]<br>Read-across | ARE-Nrf2 Luciferase[4,5,6,7]<br>h-CLAT[8]<br>MUSST[9]<br>Read-across | LLNA[10]<br>QMM<br>Read-across | GPMT[11]<br>HRIPT<br>Read-across |
|---|---|---|---|---|---|

**3 Assess Existing Data on Target Substances**

**Example: Icosyl Acrylate (CAS 48076-38-6)**

| | | | | | |
|---|---|---|---|---|---|
| **Target chemical data for AOP key events** | OECD QSAR Toolbox profiles target as a Michael acceptor<br><br>Hydrophobicity will make DRPA execution technically difficult<br><br>Minimal activity in DRPA using Behenylacrylate (C18-C22) (CAS 85085-17-2) as source analogue | No data | MUSST positive for Behenyl-acrylate (CAS 85085-17-2) as source analogue | Source analogues: 2-ethylhexyl-acrylate EC3 9.7% and 2-propyl-heptylacrylate 26%<br><br>But large $LogK_{ow}$ difference suggests icosyl acrylate will be non-sensitising | No data |

**Predicted to be non-sensitising**

**Example: Castor oil, Dehydrated (CAS 64147-40-6)**

| | | | | | |
|---|---|---|---|---|---|
| **Target chemical data for AOP key events** | No alerts identified<br>Minimal activity in DRPA using target | Positive | MUSST negative for target | No data | Coconut oil as source analogue negative in GPMT |

**Predicted to be non-sensitising**

## Discussion

Evidence suggests that registrants may not be fully implementing non-animal methods[12,13] to meet REACH data requirements as required by law. Furthermore, there is a view that ECHA is not fully applying its authority to ensure registrants minimise animal experiments.[14] Separate and apart from regulatory oversight, measures must be taken to ensure that registrants understand how to use non-animal methods that can be used to meet REACH requirements. Feedback from participants of these sessions suggests that:

> Webinars can provide comprehensible information on strategies incorporating non-standard approaches (Fig. 4, 5)

> This information increases participant intention to use non-testing/non-animal methods (Fig. 3)

> Lack of clear regulatory acceptance of non-standard approaches is perceived as the most likely barrier to their successful use in place of animal methods for REACH registration (Fig. 6, 7)

Additional webinars are scheduled that will cover the (Zebra)Fish Embryo Acute Toxicity Test to Predict Short Term Toxicity to Fish (April 2015) and ECHA perspectives on the use of non-animal methods (date TBA), but survey results indicate that perceived regulatory barriers may not be overcome by registrant education alone. Regulatory authorities must address these concerns.

## References and acronyms

Full reference list available at piscltd.org.uk/reaching-alternatives-animal-testing or via the QR code below.

| | | | |
|---|---|---|---|
| **AOP** | Adverse Outcome Pathway | **IATA** | Integrated Approach on Testing and Assessment |
| **ARE-Nrf2** | Antioxidant/electrophile responsive element, Nrf2 reporter gene assay | **LLNA** | Local Lymph Node Assay |
| **CRO** | Contract Research Organisation | **$LogK_{ow}$** | Octanol-Water Partition Coefficient (Logarithmic) |
| **DPRA** | Direct Peptide Reactivity Assay | **MUSST** | Myeloid U937 Skin Sensitisation Test |
| **ECHA** | European Chemicals Agency | **OECD** | Organisation for Economic Co-operation and Development |
| **GPMT** | Guinea Pig Maximisation Test | **QMM** | Quantitative Mechanistic Modeling |
| **h-CLAT** | Human Cell Line Activation Test | **QSAR** | Quantitative structure activity relationship |
| **HRIPT** | Human Repeat Insult Patch Testing | **REACH** | Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals |
| | | **TBA** | To Be Announced |

† The test method examples given are not exhaustive of what could potentially be used to address key events. Other examples include, but are not limited to, GSH, PPRA, SENS-IS, SenCeeTox, RhE IL-18, IL-8 Luc, SensiDerm, GARD, VITOSENS, PBMDC, hTCPA.

* Present address.