REACH Webinar Series
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Questions and Answers from REACH webinar 1: OECD QSAR Toolbox and Read-Across
Dr. Grace Patlewicz, DuPont's Haskell Global Centers for Health and Environmental Sciences
Dr. Mark Cronin, Liverpool John Moores University, England.

Where to start?

Is there a recommendation for external help to get an evaluation by means of the OECD QSAR Toolbox?
Users can start to become familiar with the Toolbox either from:
dedicated training, for example REACH Monitor holds regular training seminars
(http://www.reachmonitor.com);
by working through the OECD tutorials designed to help users understand the Toolbox
(http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm#Guidance_Documents_and_Training_Materials_for_Using_the_Toolbox); or
by posing questions to the OECD Discussion Forum
(https://community.oecd.org/community/toolbox_forum).

What kind of expertise is required to use OECD QSAR Toolbox?
A mix of chemistry and toxicology, and the basics of chemoinformatics.

How can we find out whether our chemical of interest was used to develop the read-across algorithm?
There appears to be some confusion between read-across and QSAR. The substance of interest will be the target in the category formation and if that substance has associated data it might well be used to derive the estimate by qualitative/quantitative read-across. In the associated report generated, one can determine whether the substance of interest's data were used in the estimate.

If the substance is not in the existing category in OECD QSAR Toolbox, what should I do?
The OECD QSAR Toolbox has profilers to help identify whether a substance falls within the scope of an existing category, such as an OECD High Production Volume (HPV) category. The OECD HPV categories are documented in the OECD existing chemicals website (see http://webnet.oecd.org/hpv/ui/Default.aspx), where a record of the original category description and associated data can be found. It is up to the user to review the information and make a determination of whether the category data and supporting information can be supplemented further.
Alternatively, the profiling outcome might merely provide an indication of whether the substance of interest could be a member of that category. At that point, it is up to the user to make an evaluation of whether a justification can be made to substantiate membership.
If the profiler(s) do not flag membership or potential membership, then the tactical strategy starts from exploring what information is available for the substance of interest to help formulate an initial grouping hypothesis and determination of what data gaps need to be addressed for the information requirements for the tonnage band of interest.

A critical step in doing QSAR/SAR/read-across is to find at least one molecule with known toxicities which is structurally similar to the target molecule. How similar is similar enough? This is a somehow subjective issue. Could you give two examples to describe their experience, one with a positive outcome, and the other with a negative result – that is no similar molecule was identified out of several candidates.

It is a subjective issue since similarity is a relative, not absolute, concept. From a practical standpoint, one could start by gathering what information you can for your substance of interest as that will help inform the evaluation of any "similar" analogues identified following a search for structurally-related substances. How similar is then characterised by reference to what you know about your substance of interest and how those preliminary related analogues compare in terms of their physicochemical characteristics, such as their likely bioavailability, reactivity, and metabolic profiles.

Many times, whilst similar analogues can be identified, finding analogues with relevant good quality data to address the data gaps of interest becomes the rate-limiting step.

Of course, structural similarity might not be the starting rationale all the time – take the case of a substance that is rapidly hydrolysed – in such a case, information on the metabolite would be used to read-across the data gaps for the target of interest.

As an example, there are a number of publications on saturated long-chain aliphatic alcohols where categories can be formed as there is "low" toxicity and the variation in effects with chain length is predictable (see for instance: Sanderson H et al (2009) *Ecotoxicol. Environ. Saf.* 72: 973-979; Belanger SE et al (2009) *Ecotoxicol. Environ. Saf. 72:* 1006-1015). However, what may be considered as structurally-similar molecules may show large variations in activity. For instance, addition of a double bond in the aliphatic chain adjacent to the hydroxyl group can lead to a large change in toxicity due to the compound becoming potentially reactive. This is referred to as an activity cliff, that is, a minor change in structure can result in a large difference in toxicity. Thus, the user of read-across must be careful to have a well explained and defined domain for a group, with expert knowledge of the group's boundaries.

Regulatory acceptance

Under what circumstances in the regulatory world might you need to provide a category reporting format document?

When constructing a category and justifying the read-across for different endpoints, the category reporting format can be used as a template. A category reporting format document would be used for any regulatory submission of a read-across prediction, and it provides a useful framework for anyone doing a prediction to try and ensure good practice.

Is there somewhere where specific cases of in silico predictions accepted, not just received, by ECHA for REACH have been documented?

There is little evidence at this time. One could refer to ECHA's forthcoming Read Across Assessment Framework (RAAF) or their published case studies.

An example of the complexity in using the read-across approach for regulatory submissions is provided by Ball et al. (2014). "The challenge of using read-across within the EU REACH regulatory framework; how much uncertainty is too much? Dipropylene glycol methyl ether acetate, an exemplary case study" *Reg Tox and Pharmacol.* 68: 212–221.

For the endpoint of mutagenicity, what is the experience regarding the acceptance by authorities to endpoint specific QSARs? Is there a difference in Europe versus the US with regard to the acceptance?

It is difficult to talk about acceptance by different agencies when the decision contexts can be so different. Given the way in which a percentage of dossiers are evaluated by ECHA for their technical compliance, it is difficult to judge whether a QSAR approach or read-across has been accepted or whether the dossier simply has not been selected for evaluation. That said we have on occasion elected to use a QSAR approach for certain human health endpoints such as Ames mutagenicity where QSARs are available. We attempt to characterise the QSAR model of interest, justify the prediction in accordance with the OECD Validation principles (http://www.oecd.org/chemicalsafety/risk-assessment/validationofqsarmodels.htm), use the templates provided (QMRF, QPRF) and favour a weight-of-evidence approach that is consistent with the relevant integrated testing strategies described in the technical guidance (see technical regulatory guidance links below).

For the endpoints of irritation and corrosion, in case the result based on having met/not met the German Federal Institute for Risk Assessment (BfR) inclusion and exclusion rules is contradicting, how do you determine whether a substance is predicted to be or not to be irritant or corrosive?

The BfR rulebase comprises inclusion rules which are based on structural alerts – substances that flag particular features would be indicative of irritation or corrosion effects. The exclusion rules are based on physicochemical properties that might render substances unlikely to be irritant or corrosive.

The original documentation on the structural alerts was described by Gerner and colleagues (Gerner et al. (2005) Assessment of the eye irritating properties of chemicals by applying alternatives to the Draize rabbit eye test: The use of QSARs and in vitro tests for the classification of eye irritation. *ATLA*, 33:215-237). It may be easier for the purpose of predicting irritation /corrosion to use the ToxTree implementation. It should also be noted that the OECD QSAR Toolbox was not specifically designed to make direct predictions of toxicity, but to assist in the formation of groups of categories from which read-across may be inferred.

For the endpoint of reprotoxicity, can you expand on how QSAR could be used as part of weight of evidence or read across?

It depends on the substance of interest – but a SAR to help group chemicals in conjunction with predictions from a QSAR and other information could start to form the basis of a weight of evidence. More information can be found in:

- Piersma et al. (2013). Evaluation of an alternative in vitro test battery for detecting reproductive toxicants. *Reproductive Toxicology*, 38: 53– 64.
- OECD (2014). New Guidance Document on an Integrated Approach on Testing and Assessment (IATA) for Skin Corrosion and Irritation. Series on Testing and Assessment No. 203.
- Patlewicz et al. (2014). Towards AOP application--implementation of an integrated approach to testing and assessment (IATA) into a pipeline tool for skin sensitization. *Regul Toxicol Pharmacol*. 69(3):529-45.
- Patlewicz et al. (2014). Read-across approaches – misconceptions, promises and challenges ahead. *ALTEX*. 31 (4/14): 387-396.
- Piparo and Worth. Review of QSAR Models and Software Tools for predicting Developmental and Reproductive Toxicity. https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/doc/EUR_24522_EN.pdf

Other questions

Are these QSAR models freely available?
Some QSAR models such as ECOSAR or TEST are freely available. Others such as Derek Nexus, TOPKAT, and TIMES require payment for licences. Additionally, the Antares EU Project website (http://www.antares-life.eu/index.php?sec=modellist) provides a useful list of models and suppliers and the QSAR-DB from the University of Tartu a database of QSARs. (https://qsardb.org/repository/)

Can you provide links to toxicology databases?
Under the endpoint tab in the OECD QSAR Toolbox, each data source can be probed in turn. If you right-click on the database name and click on "About", you will obtain more information. It is essential for users to understand that it is their responsibility to confirm the quality of these databases and the information contained within them.

Is the Laboratory of Mathematical Chemistry (LMC) Domain Manager (http://oasis-lmc.org/) part of the OECD QSAR Toolbox?
The algorithms underpinning LMC Domain Manager are part of the OECD QSAR Toolbox; the standalone package referenced in the presentation is not.

Could you explain the use of non testing approaches with an example of how it is applied for testing a herbal supplement?
The major and/or active ingredients would need to be identified. Due to the nature of herbal supplements, QSAR and read-across approaches are not currently suitable for an herbal supplement.

How to approach UVCB substances? Can QSAR be used?
Exploring predictions for representative structures might be an approach to help evaluate UVCBs, but this depends on the type of UVCB. Further research may take place in this area in the future.

Dr. Mark Cronin is a Professor of Predictive Toxicology at the School of Pharmacy and Chemistry at Liverpool John Moores University, England. At the time of the webinar, Dr Grace Patlewicz was employed as a computational toxicologist at DuPont's Haskell Global Centers for Health and Environmental Sciences.

Disclaimer: The views expressed are personal opinions of the authors and are not reflections or endorsements by DuPont or Liverpool John Moores University nor those of the European Chemicals Agency.

Additional Information
Please visit www.piscltd.org.uk for more information on non-animal testing methods and for a recording of Dr Patlewicz and Cronin's webinar.

Useful Links
Domain tools
- http://ambit.sourceforge.net/download_ambitdiscovery.html
- http://oasis-lmc.org/

Technical regulatory guidance
- https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology
- http://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf

- http://echa.europa.eu/support/grouping-of-substances-and-read-across
- http://echa.europa.eu/practical-guides

OECD Toolbox
- http://www.qsartoolbox.org/

Industry guidance and experiences
- ECETOC TR116 Category approaches, read-across, (Q)SAR
- Blackburn, K., and Stuard, S. B. A framework to facilitate consistent characterization of read across uncertainty. *RegToxicol Pharmacol 2014*, 68, 353-362.

Publications
- Ball N, Bartels MJ, Budinsky, RA, Klapacz J, Hayes SM, Kirman CR, Patlewicz GY. (2014). The challenge of using Read-Across within the EU REACH regulatory framework; how much uncertainty is too much? Dipropylene Glycol Methyl Ether Acetate, an exemplary case study. *Reg. Toxicol. Pharmacol.*, 68: 212-221.
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- Patlewicz G, Roberts DW, Aptula A, Blackburn K, Hubesch B. (2013). Workshop: Use of 'read-across' for chemical safety assessment under REACH. *Reg. Toxicol. Pharmacol.*, 65(2): 226-228.
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- Cronin MTD et al. (2013). Chemical Toxicity Prediction: Category Formation and Read-Across. Royal Society of Chemistry.
- Cronin MTD and Madden JC. (2010). In Silico Toxicology. Principles and Applications. Royal Society of Chemistry.
- Tollefsen KE, Scholz S, Cronin MTD et al. (2014). Applying Adverse Outcome Pathways (AOPs) to support Integrated Approaches to Testing and Assessment (IATA). *Reg. Toxicol. Pharmacol.*, pii: S0273-2300(14)00214-1.