Perspectives on the Development, Evaluation, and Application of *in Silico* Approaches for Predicting Toxicity

9 January 2018
This webinar will cover

- Part 1
  - (Q)SARs
  - Grouping approaches, chemical categories, read-across

- Part 2
  - Integrated Approaches to Testing and Assessment (IATA)
  - General framework and where non-testing approaches fit
  - Adverse Outcome Pathways (AOPs) and AOP-informed IATA
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Please contact the PETA International Science Consortium Ltd., for assistance in avoiding animal testing
Today’s speakers

• Dr. Grace Patlewicz, US EPA
  Dr. Grace Patlewicz is currently a research chemist at the National Center for Computational Toxicology within the US Environmental Protection Agency. She started her career at Unilever UK before moving to the European Commission Joint Research Centre in Italy and then to DuPont in the US. Her research has focused on the development and application of QSARs and read-across for regulatory purposes. A chemist and toxicologist by training, she has also authored over 100 journal articles and book chapters, chaired a number of industry groups, and contributed to the development of technical guidance for QSARs, chemical categories, and adverse outcome pathways under various OECD work programmes.

• Prof. Mark Cronin, Liverpool John Moores University
  Dr. Mark Cronin is professor of predictive toxicology at the School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, UK. He has more than 30 years' experience in the application of in silico approaches to predicting the toxicity and fate of chemicals as well as in the development of integrated testing strategies for identifying alternatives to whole-animal toxicity testing. His current research includes the application of chemical grouping and read-across to assessing human health and environmental endpoints, particularly the linking of adverse outcome pathways to category formation.
Perspectives on the Development, Evaluation, and Application of \textit{in Silico} Approaches for Predicting Toxicity

Grace Patlewicz, NCCT, U.S. EPA, RTP, NC, USA  
Mark Cronin, Liverpool John Moores University, England

9 January 2018

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA or Liverpool John Moores University
Regulatory drivers

• Societal demands for safer and sustainable chemical products are stimulating changes in toxicity testing and assessment frameworks

• Chemical safety assessments are expected to be conducted faster and with fewer animals, yet the number of chemicals that require assessment is also rising with the number of different regulatory programmes worldwide.

• In the EU, the use of alternatives to animal testing is promoted.

• Animal testing is prohibited in some sectors e.g. cosmetics

• The European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) legislation lays out specific information requirements, based on tonnage level triggers. However, the regulation explicitly expresses the need to use non-testing approaches to reduce the extent of experimental testing in animals.
Regulatory drivers

- REACH-like schemes also have been established in China, South Korea, and Turkey.
- In the US, the new Frank Lautenberg Chemical Safety for the 21st Century Act (LCSA) requires that a risk based prioritisation is conducted for all substances in commerce, some 80,000, many of which are lacking sufficient publicly available toxicity information.
- The LCSA also suggests developing alternative methods to reduce/refine animal testing.
- Risk based prioritization is also an important aspect of regulatory frameworks in Canada (the Domestics Substance List), Australia and the EU.
- Non-testing approaches offer a means of facilitating the regulatory challenges in chemical safety assessment
Aims of this webinar

• To review current practices in the development and assessment of non-testing approaches; focussing on (Q)SAR and read-across

• To provide an overview of integrated approaches to testing and assessment (IATA) and where non-testing approaches fit within such a framework

• To highlight advances in the Tox21 field that are shaping how Adverse Outcome Pathways (AOPs) are informing IATA development and application with particular emphasis on read-across
Outline – Part 1

- Non-testing approaches
- Definitions
- (Q)SARs
- Grouping approaches, chemical categories, read-across
- Frameworks for development and assessment of read-across
- Read-across tools
- Challenges in read-across and research directions
Outline – Part 2

- Integrated Approaches to Testing and Assessment (IATA)
- General framework and where non-testing approaches fit
- Adverse Outcome Pathways (AOPs) and AOP-informed IATA
- Defined approaches (DA) for skin sensitisation in the context of AOP-informed IATA
Part 1
Computational (In Silico) Toxicology

- Databases of existing information
- Category formation (grouping) read-across
- Structure-Activity Relationships (SAR)
- Quantitative Structure-Activity Relationships (QSAR)
- Expert Systems
  - Bioinformatics
  - Chemoinformatics
  - Biokinetics (PBPK)
Computational *(In Silico)* Toxicology

- Databases of existing information
- Category formation (grouping read-across)
- Structure-Activity Relationships (SAR)
- Quantitative Structure-Activity Relationships (QSAR)
- Expert Systems
- Bioinformatics
- Chemoinformatics
- Biokinetics (PBPK)
A SAR is a (qualitative) association between a chemical substructure and the potential of a chemical containing the substructure to exhibit a certain biological effect.

Quantitative Structure-Activity Relationships (QSARs)

- A (Q)SAR attempts to relate (statistically or otherwise) the activity of one or more molecules to their physico-chemical properties or structural descriptors.

- QSAR can be used to predict:
  - Quantitative endpoints
    - e.g. potency
  - Qualitative endpoints
    - e.g. active / inactive
Collections of (Q)SARs

• An Expert System is a formalised system, usually computerised that enables an end-user to make rational predictions of toxicity based on structure alone

• Expert systems are typically categorised by whether they are underpinned by:
  • empirically based algorithms such as QSARs e.g. TOPKAT, Leadscope
  • knowledge bases such as SARs e.g. Derek Nexus, Toxtree
  • or a hybrid of the two e.g. TIMES, ChemTunes
Regulatory Applications of (Q)SARs

- “Packaged mature knowledge for systematic reuse”

- For data gap filling – to provide an estimate for a given (eco)toxicity/e-fate/phys chem endpoint in lieu of testing (replacement or supporting information)

- To rationalise spurious results in experimental data – since the (Q)SAR is based on a larger body of data, provides a more compelling Weight of Evidence (WoE) to rationalise the validity of a potential outlier

- Essential for category development and associated read-across justification - to provide a context of endpoint mechanistic similarity

- To add another line of evidence as part of a WoE within the context of an IATA
Current Experiences of (Q)SAR Approaches

• As replacements - (Q)SARs are most promising for physicochemical, ecotoxicity and environmental fate properties e.g. Log Kow, acute fish toxicity, ready biodegradability.

• (Q)SARs can also be used as “supporting information” in category/analogue approaches or as additional information as part of a Weight of Evidence assessment (WoE) – most progress has been made with (Q)SARs for endpoints such as skin/eye irritation, or genotoxicity endpoints.

• (Q)SARs for repeated dose toxicity endpoints are not sufficiently evolved to be used as replacements but can play an useful role in supporting read-across within category/analogue approaches.
Regulatory Use of (Q)SARs

• For regulatory purposes, there is an expectation that an assessment of the QSAR model and associated prediction are undertaken

• Under REACH, it is stated that “Results obtained from valid qualitative or quantitative structure-activity relationship models may be used instead of testing when the following conditions are met to indicate the presence or absence of a certain dangerous property”.

Figure taken from ECHA guidance on QSARs and read-across approaches, 2008
Scientific Validity: OECD Principles for (Q)SAR Validation

- A (Q)SAR should be associated with the following information:
  - a defined endpoint
  - an unambiguous algorithm
  - a defined applicability domain
  - appropriate measures of goodness-of-fit, robustness and predictivity
  - a mechanistic interpretation, if possible

- Principles were agreed by OECD in 2004 and associated guidance was published in 2007
**Scientific Validity: OECD Principles for (Q)SAR Validation**

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Assessing Applicability Domain to Determine if the Model is Valid for Use for a Specific Substance

- Applicability domain may be characterised using:
  - Descriptors
  - Structural features e.g. fragments, fingerprints
  - Metabolic transformations
  - Mechanistic information
- Tools exist to assess applicability domains
  - e.g. LMC Domain Manager, AMBIT Discovery etc.
Documenting the Model: QSAR Model Reporting Format (QMRF)

- QSAR Model Reporting Format (QMRF) is a harmonised template for summarising and reporting key information on (Q)SAR models, including the results of any validation studies.

- The information is structured according to the OECD (Q)SAR validation principles.

- A freely available editor is available:
The QSAR Prediction Reporting Format (QPRF) is a harmonised template for summarising and reporting substance-specific predictions generated by (Q)SAR models.

QPRF requires information on:

- The substance
- General information (e.g. date and author)
- Description of QSAR according to OECD Principles and how it relates to target substance
- Adequacy (optional)

(Q)SAR related resources

- Since (Q)SARs have become a viable approach to address regulatory purposes, there have been a plethora of tools and resources developed to help facilitate their application.

- The JRC QSAR Model inventory - provides a resource to identify well documented (Q)SARs.

- QSARDB - is a smart repository for (Q)SAR/QSPR models and datasets, ready for discovery, exploring, citing and predicting (https://qsardb.org/).

- Ochem – is a resource for developing new (Q)SARs based on uploaded publicly accessible datasets, or for applying available (Q)SARs (https://ochem.eu/home/show.do)

- US EPA Chemistry Dashboard – is a platform to search for substances within the DSSTox inventory, find associated ToxCast/Tox21 data, toxicity/physical property information, QSAR model predictions, literature resources as well as other related links (https://comptox.epa.gov/dashboard/)
US EPA Chemistry Dashboard

- Available at [https://comptox.epa.gov/dashboard/](https://comptox.epa.gov/dashboard/)
- For substances within the DSSTox inventory (~750,000 substances), model predictions are available for a range of physchem, ecotox and toxicity endpoints
- For some of these endpoints e.g. OPERA physchem models – QMRFs are available and prediction reports for specific chemicals are available for download
Category Formation (Grouping) for Read-across

• “Analogue approach” refers to grouping based on a very limited number of chemicals (e.g. target substance + source substance)

• “Category approach” is used when grouping is based on a more extensive range of analogues (e.g. 3 or more members) and there may be an apparent trend in property

• Read-across describes one of the methods for filling data gaps in either the analogue or category approaches i.e. not to be confused with the “analogue approach”

• OECD definition: “A chemical category is a group of chemicals whose physico-chemical and human heath and/or environmental toxicological and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristics)”. 
# Uses of Read-across

<table>
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<tr>
<th></th>
<th>Chemical 1</th>
<th>Chemical 2</th>
<th>Chemical 3</th>
<th>Chemical 4</th>
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<tbody>
<tr>
<td>Property 1</td>
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<td>Property 4</td>
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<td>Activity 2</td>
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<td>Activity 3</td>
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<tr>
<td>Activity 4</td>
<td></td>
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</tr>
</tbody>
</table>

- **read-across**
- **interpolation**
- **extrapolation**

- 🆙 reliable data point
- 🆔 missing data point

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Trend analysis or QSAR
Uses of Read-across

- Read-across application has been more extensive than (Q)SAR for regulatory purposes – it probably wasn’t recognised and categorised as a “read-across” in each case!
- Examples where “read-across” approaches are applied include:
  - US EPA Provisional Peer Reviewed Toxicity Values (PPRTVs) where data is lacking for a specific substance of interest
  - EPA Test Rules – Industry registrants providing information to satisfy a test rule
  - EPA Pre Manufacture Notifications (PMN) – QSARs such as those in Epiwin and ECOSAR are routinely used for e-fate and ecotox predictions but read-across is relied upon for non cancer endpoints
  - ASTDR Emergency response values – an accidental spill that requires an immediate assessment of acute toxicity for first responders
  - REACH registrations – addressing information requirements
Problem formulation/Decision context in read-across

- Decision context is even more important in read-across as the practical approaches can be markedly different.
Considerations Before Embarking on a “Read-across”

• Decision context – what level of scientific confidence is needed and how does this impact the level of effort and resources that should be applied

• How many data gaps? And for which endpoints?

• Legitimate access to sufficient, reliable data?

• Plausible hypothesis for grouping substances and ease and cost of substantiating that hypothesis?

• Accurate and credible assessment of the hazards for the substance in question? Is the scientific confidence sufficient for the purpose required?

• Consequence and cost of the read-across approach not being accepted?
Types of Groupings

Structural Analogues

Mechanistic Analogues

Mode of Action Analogues
Types of Groupings - 2

- Substances that are **metabolised** to a common molecule
- Substances that are **degraded** rapidly to common products
- The rationale underpinning the category/analogue approach might be based on 1 or more of these rationales
Developing a read-across assessment

- Existing guidance and resources that can be helpful in developing a read-across assessment:
  - Technical regulatory guidance has been published by OECD and ECHA
  - OECD guidance from 2007 was updated in 2014
  - ECHA Chapter 6 QSARs and Grouping of Chemicals as well as practical guides
- However, many papers have been published that complement and augment the regulatory guidance for development of read-across
Developing a read-across assessment

- Selected literature include:
  - ECETOC TR116 category approaches, Read-across, (Q)SAR
  - Patlewicz et al (2013) Use of category approaches, read-across and (Q)SAR general considerations
  - Ball et al (2016) Towards Good Read-across Practice
Frameworks for the development of category/analogue approaches

OECD (2014)

Wu et al, 2010

Patlewicz et al, 2015
### Frameworks for the development of read-across

<table>
<thead>
<tr>
<th>Framework</th>
<th>ECHA</th>
<th>OECD</th>
<th>Wu et al</th>
<th>Wang et al</th>
<th>Patlewicz et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Context</td>
<td>REACH</td>
<td>International regulatory purposes</td>
<td>Product Stewardship</td>
<td>Quantitative risk assessment</td>
<td>Regulatory purposes/Product stewardship</td>
</tr>
<tr>
<td>Approach</td>
<td>Analogue/Category – aim is to fill an endpoint specific study. Focused on structural similarity as a starting point</td>
<td>Analogue/Category – a generalisation of the ECHA approach</td>
<td>Analogue Systematic stepwise evaluation of analogue suitability based on structure, reactivity, p&lt;sub&gt;chem&lt;/sub&gt; and metabolism</td>
<td>Analogue Approach is based on a WOE assessment from structure, ADME and toxicity considerations</td>
<td>Analogue Stepwise approach considering general (p&lt;sub&gt;chem&lt;/sub&gt;, reactivity, metabolism) and endpoint specific considerations</td>
</tr>
<tr>
<td>Terms of reference</td>
<td></td>
<td>Analogue/Category</td>
<td></td>
<td></td>
<td>Analogue/Category</td>
</tr>
<tr>
<td>Scope</td>
<td>Endpoint specific</td>
<td>Endpoint specific</td>
<td>Systematic stepwise evaluation of analogue suitability based on structure, reactivity, p&lt;sub&gt;chem&lt;/sub&gt; and metabolism</td>
<td>Most sensitive/relevant endpoint – focused on repeated dose toxicity endpoints; quantitative risk assessment</td>
<td>Approach is aimed to identify source analogues that can be used to address as many endpoints as appropriate, even though the read-across prediction itself is justified on an endpoint per endpoint basis and some source analogues might be excluded from the prediction itself if they are not appropriate for specific endpoints of interest</td>
</tr>
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</table>

Lots of commonality between these frameworks!
Ongoing issues with read-across

• Although there is much guidance for developing read-across assessment, acceptance still remains an issue, especially for regulatory purposes.

• A key issue thwarting acceptance relates to the “uncertainty of the read-across”

• As such there have been many efforts to identify the sources of uncertainty in read-across, characterise them in a consistent manner and identify practical strategies to address and reduce those uncertainties.

• Notable in these efforts have been the development of frameworks for the assessment of read-across. These allow for a structured assessment of the read-across justification.
Sources of uncertainty in read-across

- Analogue or category approach? (no. of analogues)
- Completeness of the data matrix – no. of data gaps
- Data quality for the underlying analogues for the target and source analogues
- Consistency of data across the data matrix – concordance of effects and potency across analogues
- Overarching hypothesis/similarity rationale – how to identify similar analogues and justify their similarity for the endpoint of interest
- Address the dissimilarities and whether these are significant from a toxicological standpoint e.g. ToxDelta
- Presence vs. absence of toxicity
- Toxicokinetics
Frameworks for the assessment of read-across

- Blackburn & Stuard (2014)
- These aim to identify, document and address the uncertainties associated with read-across inferences/predictions
Frameworks for the assessment of read-across

**READ ACROSS UNCERTAINTY EVALUATION QUESTIONNAIRE FOR:**

**Target chemical (SOI) = [list CAS #]**

**INSTRUCTIONS**

Complete the Questionnaire. Answer the questions for each endpoint where SAR was conducted, and follow instructions listed in each section below. (In general, NO responses indicate potential areas of uncertainty in the proposed read across.)

<table>
<thead>
<tr>
<th>Questions</th>
<th>Responses by Endpoint</th>
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<tr>
<td></td>
<td>Repeat Dose Toxicity</td>
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</tbody>
</table>

**Section 1: Chemical similarity between source (analogue) and target (SOI)**

1. For each endpoint, list the CAS#s of the source (analogs) contributing theoretical study for the read across for:

   - CAS#

2. What is the ‘suitability rating’ of the analogue?

   - Suitable
   - Not suitable
   - (skip to next question)

   - Suitable
   - (skip to next question)

3. Are any differences in functional groups and associated properties better suited?

   - Yes
   - No
   - Unknown
   - No differences

**Suitability of Analogs contributing data**

- Are all features of SOI covered or differences in conservative direction

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<th>NOTES, if any:</th>
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**Blackburn & Stuard (2014)**

**Table 2: Scientific confidence considerations in Read-across evaluation.**

<table>
<thead>
<tr>
<th>Data issues</th>
<th>Similarity rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analogue/category approach</td>
<td>Similarity rationale/hypothesis that underpins the analogue/category approach</td>
</tr>
<tr>
<td>Complete ment of data matrix – No of data gaps e.g. source analogue(s) have many data points to address, target substance has a handful of data gaps.</td>
<td>- Metabolic transformation</td>
</tr>
<tr>
<td>Quality of data for source analogues – e.g. Klimisch scores of 1 or 2</td>
<td>- Structural similarity</td>
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<tr>
<td>Concordance of any available anchor data</td>
<td>- Analogue validity</td>
</tr>
<tr>
<td>Concordance of any available anchor data</td>
<td>- Analogue similarity with respect to general and endpoint specific considerations</td>
</tr>
<tr>
<td>Concordance of any available anchor data</td>
<td>- Rationalization of why structural differences do not impact the toxicity</td>
</tr>
</tbody>
</table>

**Patlewicz et al (2015)**
Frameworks for the assessment of read-across

- Outlined a strategy for structuring and reporting a read-across
- Defined different read-across scenarios
- Two main aspects tackled:
  - an assessment of the similarity of the source analogues
  - an assessment of the mechanistic relevance and completeness of the read-across (number of analogues, absence/presence of toxicity, quality of underlying data, temporal and dose response relationship between mechanistically relevant endpoints)
- Three scale grading of the overall read-across confidence Low, Medium, High
Frameworks for the assessment of read-across: RAAF

HYPOTHESIS

- Biotransformation to common compound(s)
  - Scenario 1: Effect(s) of the target substance predicted to be quantitatively equal to those of the source substance or prediction based on worst case.
  - Scenario 2: Effect(s) of the target substance predicted to be quantitatively equal to those of the source substance or prediction based on worst case.

- Different compounds have the same type of effect(s)
  - Scenario 3: Variations in the strength of effect(s) observed among source substances. Prediction based on a regular pattern or on a worst case approach.
  - Scenario 4: Variations in the strength of effect(s) observed among source substances. Prediction based on a regular pattern or on a worst case approach.

- No variations among the category members
  - Scenario 5: No relevant variations in the strength observed among source substances and the same strength predicted for the target substance.
  - Scenario 6: No relevant variations in the strength observed among source substances and the same strength predicted for the target substance.

Frameworks for the assessment of read-across: RAAF

- Six scenarios identified
- For each scenario there will be a number of scientific considerations
- Each is associated with an “assessment element” (AE)
- Each AE is scored from 1-5 where 5 is “acceptable with high confidence” to 1 is not acceptable
- These scores are termed Assessment Options (AO)
- A minimum score of 3 is needed for a read-across to be taken up and used to inform decision making
- There are common assessment elements e.g. reliability of the underlying data and there are scenario specific elements e.g. common underlying mechanism for scenario 2
### Frameworks for the assessment of read-across

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<tbody>
<tr>
<td>Context</td>
<td>REACH</td>
<td>Product Stewardship</td>
<td>Regulatory purposes &amp; Product stewardship</td>
<td>Regulatory purposes &amp; Product stewardship</td>
</tr>
<tr>
<td>Scope</td>
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</table>

#### Lots of commonality between these frameworks!

- **ECHA RAAF (2017)**
  - Scenarios addressing analogue (2) and category (4) approaches as described above.
  - Each scenario is associated with a number of assessment elements (AE) (both common and scenario specific).
  - 3 aspects: analogue suitability (covered in Wu et al, 2010); data quality of the analogues; consistency of the data across the analogues and relative to the target.

- **Blackburn and Stuard (2014)**
  - Identifies the sources of uncertainty in relationship to the data and similarity context.
  - Different scenarios are articulated to frame up to 11 different similarity criteria.

- **Patlewicz et al (2015)**
  - 8 factors proposed to evaluate mechanistic relevance and completeness of the read-across.

- **Schultz et al (2015)**
  - Lots of commonality between these frameworks!
Ongoing issues with read-across

- These frameworks allow for a structured assessment of the read-across justification.
- The next step is how those uncertainties can be addressed
- One approach per Blackburn and Stuard (2014) is to use assessment factors
- Alternatively the RAAF and the work by Schultz et al (2015) advocate the use of New Approach Methods (NAM) (e.g. High Throughput Screening (HTS) data) to enhance the scientific confidence of a read-across
- Examples have been published by Schultz (2017) and colleagues
- These examples rely on the qualitative use of NAM data and preferably in the context of an organising framework such as an AOP to ensure the appropriate biological context for interpretation (see Part 2)
- Others such as Shah et al (2016) have explored quantifying the uncertainties of read-across and using NAM data in conjunction with chemical structure information in a ‘QSAR-like’ read-across (Generalised Read-Across [GenRA])
- Some of these efforts have been implemented into read-across tools
## Selected read-across tools

<table>
<thead>
<tr>
<th>Tool</th>
<th>AIM</th>
<th>ToxMatch</th>
<th>AMBIT</th>
<th>OECD Toolbox</th>
<th>CBRA</th>
<th>ToxRead</th>
<th>GenRA</th>
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</thead>
<tbody>
<tr>
<td>Analogue identification</td>
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<td>X</td>
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<td>X</td>
<td>X For Ames &amp; BCF</td>
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<td>User driven</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Uncertainty assessment</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>X</td>
<td>NA</td>
<td>NA</td>
<td>X</td>
</tr>
<tr>
<td>Availability</td>
<td>Free</td>
<td>Free</td>
<td>Free</td>
<td>Free</td>
<td>Free</td>
<td>Free</td>
<td>Beta for Internal testing</td>
</tr>
</tbody>
</table>

- **AIM**: Free
- **ToxMatch**: Free
- **AMBIT**: Free
- **OECD Toolbox**: Free
- **CBRA**: Free
- **ToxRead**: Free
- **GenRA**: Beta for Internal testing
Selected read-across tools
OECD QSAR Toolbox

• A software tool which facilitates the development, evaluation, justification and documentation of chemical categories for read-across

• Software workflow mimics that described in the OECD and REACH guidance on categories

• Contains regulatory inventories and data plus “profilers” which encode SAR type information which represent molecular initiating events (MIEs) within Adverse Outcome Pathways (AOPs)

• Profilers include those for “DNA Binding”, “Protein Binding”, “Aquatic toxicity MOAs” etc. – hence works best for skin sensitisation, mutagenicity and aquatic toxicity endpoints

• Ongoing development is focusing on how to implement new MIEs and AOPs into the Toolbox to facilitate read-across for repeated dose toxicity endpoints

• First AOP implemented into the OECD Toolbox - skin sensitisation
Selected read-across tools: OECD QSAR Toolbox

Target

Source substances

Endpoint specific
Similarity rationale

Data gap
Selected read-across tools: Toxmatch

Source analogues

Similarity matrix for all source analogues as characterised by fingerprints
Similarity index = Tanimoto distance

Target

Selected read-across tools: ToxRead

http://www.toxgate.eu/

Neighbouring source analogues, colour coded by activity (positive = red) and by similarity index
Selected read-across tools: CBRA
Generalised Read-Across (GenRA)

- GenRA (Generalised Read-Across) is a “local validity” approach
- Predicts toxicity (toxicity binary outcomes observed from different study types) as a similarity-weighted activity of nearest neighbors based on chemistry and/or bioactivity (HTS) descriptors
- Generalised version of Chemical-Biological Read-Across (CBRA) developed by Low et al (2013)
- Systematically evaluates read-across performance and uncertainty using available data
Generalised Read-Across (GenRA)

I. Data
1,778 Chemicals
3,239 Structure descriptors (chm)
820 Bioactivity assays (bio)
ToxCast
574 Apical outcomes (tox)
ToxRefDB

II. Define Local neighborhoods
Use K-means analysis to group chemicals by similarity
Use cluster stability analysis
~ 100 local neighborhoods

III. GenRA
Use GenRA to predict apical outcomes in local neighborhoods
Evaluate impact descriptors (chm, bio, bc) on prediction
Quantify uncertainty

Use GenRA to predict the similarity weighted toxicity scores
for each:
Toxicity type ($\beta$)
Descriptor =\{chm,bio,bc\} ($\alpha$)
No. of nearest neighbors ($k$)
Similarity score threshold ($s_{ij}^{\alpha}$)

Calculate performance by comparing predicted $y_{i}^{tox}$ and true $x_{i}^{tox}$ for all chemicals using area under ROC curve (AUC)

\[
y_{i}^{\beta,a} = \frac{\sum_{j=1}^{k} s_{ij}^{\alpha} x_{j}^{\beta}}{\sum_{j=1}^{k} s_{ij}^{\alpha}}
\]
**Selected read-across tools: GenRA**

<table>
<thead>
<tr>
<th>GenRA (Beta)</th>
<th>Chemical Properties</th>
<th>Synonyms</th>
<th>External Links</th>
<th>Env. Fate/Transport</th>
<th>Toxicity Values (Beta)</th>
<th>Bioassays</th>
<th>Exposure</th>
<th>Literature</th>
<th>Similar Molecules (Beta)</th>
<th>Comments</th>
</tr>
</thead>
</table>

**Summary:**
- **NN By:** chm_mrgn
- **K:** 10
- **Sel by:** tox_trif
- **Grip:** tox_trif
- **By:** study

**Threylene glycol**

**Run** GenRA | Min: | 0 | Min: | 0 | Filter by: | Enter text | Sim wt | Export |

**CHR:**
- Adrenal Gland
- Body Weight
- Bone Marrow
- Clinical Signs
- Histology
- Kidney
- Liver
- Lung
- Mortality
- Nose

**Compounds:**
- 2-Methoxyethanol
- Ethylene glycol
- Threylene glycol
- 2-Hydroxyacetophenone
- Isopropyl acetate
- 2-Biodyne-1,4-di
- Ethylene glycol
- 2-Allyl-1-prop
- N,N-Diethyletha
- Diethanolamine
Integrated Approaches to Testing and Assessment (IATA)

• “IATA is a means of organising and analysing all the available relevant data on a given substance or group of substances coupled with mechanistic, exposure, and dosimetry information where possible, to focus testing when needed and facilitate an assessment conclusion” – OECD definition

• “Integrated Testing Strategies (ITS) are …. approaches that integrate different types of data and information into the decision-making process. In addition to the information from individual assays, test batteries, and/or tiered test schemes, integrated testing strategies may incorporate approaches such as weight-of-evidence and exposure/population data into the final risk assessment for a substance”

• http://www.alttox.org/ttrc/emerging-technologies/its/
Integrated Approaches to Testing and Assessment (IATA)

• In practice:
  
  • “A means of integrating existing data and non-testing data, determining what new information needs to be generated in order to make a decision”

• Some IATA are more complex than others but the generic building blocks of considering existing data, *in vitro* methods, non-testing approaches BEFORE instigating new *in vivo* testing are the same

• Non-testing approaches fit within the context of these IATA schemes and should not be considered *in vacuo*
Typical Information within an IATA

- Historical information on the chemical of interest
- Non-standard *in vivo* tests
- Information from “similar” chemicals
- Predictions from other non-testing approaches such as (Q)SAR
- *In chemico* tests
- *In vitro* tests
- Molecular biology, -omics
- Exposure, (bio-)kinetics
General framework of an IATA

From OECD
Mechanistic based and AOP-informed IATA

• As noted earlier, there is a shift towards non animal alternatives as a response to regulatory drivers

• Integration of different non-animal approaches requires an organising framework to ensure that the different information sources are being interpreted in their appropriate context. This is particularly relevant for New Approach Methodologies (NAMs).

• AOPs serve to provide this organisational framework and hence play an important role in developing and applying IATA for different purposes as well as provide a roadmap for future QSAR development

• AOPs provide the linkage from chemistry, through the Molecular Initiating Event (MIE) to Adverse Effect

• Data from key events provides support to, and will enhance, read-across especially for regulatory acceptance as well as supports definition of domains for MIEs
An AOP represents existing knowledge concerning the sequence of events and causal linkages between initial molecular events, ensuing key events and an adverse outcome at the individual or population level.
AOP-informed IATA

a) What existing data and data types are available?

Additional Data, Method Needs

Insufficient confidence

What AOP-IATA tools/assays can be applied or need to be developed to generate data to make the decision?

IATA
e.g. QSARs, Read-across, ITS

Is data input adequate to make regulatory decision?

b) Is there an AOP that is applicable to the regulatory application of interest?

Additional Data, Method Needs

Insufficient confidence

What AOP-IATA tools/assays can be applied or need to be developed to generate data to make the decision?

Regulatory decisions

c) Regulatory Applications
   • Screening
   • Prioritisation
   • Classification & Labeling
   • Hazard Assessment
   • Risk Assessment

Tollefsen et al, 2014
General workflow in Integrated Approaches to Testing and Assessment (IATA)

Multiple strategies e.g. in house data, mining of relevant data bases, literature search

Expert Judgement

Problem formulation

Gather existing information

Weight of Evidence Assessment: Adequate information for decision-making?

Generate additional information

Weight of Evidence assessment: Adequate information for decision-making?

Regulatory conclusion

From OECD
Defined approaches within IATA

• A defined approach to testing and assessment consists of a fixed data interpretation procedure (DIP) used to interpret data generated with a defined set of information sources, that can either be used alone or together with other information sources, to satisfy a specific regulatory need.

• Guidance Document on the Reporting of Defined Approaches to be Used within Integrated Approaches to Testing and Assessment ENV/JM/MONO(2016)28

• Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation ENV/JM/MONO(2016)
Defined approaches within IATA

- Work currently underway within the OECD is aiming to establish Performance-based Defined Approaches for skin sensitisation

- Aims to substitute the need for animal testing for skin sensitisation based on a combination of methods which predict key endpoint responses in the AOP

- DA will be evaluated based on their performance using the same data sets/reference chemicals for the endpoint of interest
Defined approaches within IATA: Skin sensitisation

AOP and available toolbox of non-animal methods

- **Chemical Structure & Properties**
- **Molecular Initiating Event**
- **Cellular Response**
- **Organ Response**
- **Organism Response**

- **Metabolism Penetration**
  - Covalent interaction with cells
  - Peptide depletion
  - Adduct formation
  - Relative reactivity rate
  - Activation of biochemical pathways (e.g. Keap-1 Nrf2-ARE pathway)

- **Electrophilic substance**
  - Activation of co-stimulatory and adhesion molecules
  - Release of pro-inflammatory mediators
  - Pathways-associated gene/protein expression

- **In vitro skin absorption (TG 428)**
- **(Q)SARs**
- **In silico toxicokinetic models**

- **Guinea Pig Maximisation Test**
- **Buehler Test**
- **Local Lymph Node Assay**

- **In vitro T cell priming/proliferation**

Presented by S Casati, JRC
Defined approaches within IATA: Skin sensitisation

AOP and *some* of the more advanced non-animal methods (i.e. OECD adopted, evaluated or under evaluation in ring trials)

- **Chemical Structure & Properties**
  - Metabolism Penetration
  - Electrophilic substance

- **Molecular Initiating Event**
  - Covalent interaction with cells protein

- **Cellular Response**
  - Activation of inflammatory cytokines
  - Induction of anti-inflammatory cytokines and surface molecules
  - Mobilization of DCs
  - Keratinocytes

- **Organic Response**
  - Lymph node
    - Histocompatibility complexes presentation by DCs
    - Activation of T cells
    - Proliferation of activated T-cells

- **Organism Response**
  - Skin (epidermis)
    - Inflammation upon challenge with allergen

- **In vitro skin absorption (TG 428)**
  - TG 442C (DPRA)
  - (Q)SARs
  - In silico toxicokinetic models

- **In vitro T cell priming/proliferation**
  - TG 442D (ARE-Nrf2 Luciferase test method, KeratinoSens™); LuSens
  - Sens-is
  - IL-8 Luc assay
  - RhE IL-18
  - h-CLAT (draft TG)
  - U-SENS™

- **Local Lymph Node Assay**
- **Guinea Pig Maximisation Test**
- **Buehler Test**

Presented by S Casati, JRC
Defined approaches within IATA: Skin sensitisation

- Defined approaches within IATA: Skin sensitisation
- AOP from ENV/JM/MONO(2012)10/PART1
- Expert Judgement
- In vitro T cell priming/proliferation
- Guinea Pig Maximisation Test
- Buehler Test
- Local Lymph Node Assay
- AOP from ENV/JM/MONO(2012)10/PART1

https://aopwiki.org/wiki/index.php/Aop:40
Defined approaches for skin sensitisation examples

Method 1 (KE a)  Method 2 (KE b)

Results concordar

Defined Approach (BASF) ‘2 out of 3 approach’

Bayesian Networks for Skin sensitization Jaworska et al (2015)
Take Home Messages - 1

• QSARs are most effectively used for ecotox, efate and physchem endpoints as replacement values and as supporting information for “simpler” mammalian endpoints within an IATA.

• The OECD principles provide a framework to assess a QSAR model and its prediction and document both.

• Many QSAR resources exist to identify QSARs, make/extract predictions, or develop new models.

• Read-across tends to be more routinely relied upon for “more complex” endpoints such as repeated dose 28 day or developmental toxicity screening tests – an analogue/category approach is likely to be more effective – an overarching hypothesis and evidence to support the read-across is essential – (Q)SARs can be helpful in providing some of this evidence.

• There is much guidance for read-across, and many frameworks exist that guide how to develop a read-across. Many of these frameworks are very complementary to each other.
Take Home Messages - 2

• Despite these development frameworks, acceptance of read-across remains a challenge. The main reason thought to be thwarting acceptance is characterising and addressing the uncertainties of the read-across prediction.

• Many frameworks exist that provide a structure for how to characterise these uncertainties. Research has been undertaken to explore to what extent NAM can be used to enhance the scientific confidence in read-across. Most approaches have been limited to a qualitative application of NAM. Other researchers have attempted to quantify the uncertainties in order to explore the performance of read-across and how and to what extent NAM is impactful in improving that performance.

• There are many tools that can be used in the development and assessment of read-across. A selection have been highlighted from those tools that are publicly available.
Take Home Messages - 3

- (Q)SARs and read-across are categorised as non-testing approaches and ordinarily form components of an IATA.

- There are different ways in which IATA can be constructed but there is a lot of commonality in the main steps.

- Increasingly IATA are being underpinned by mechanistic information such as captured within AOPs.

- For the skin sensitisation endpoint, an AOP is available and efforts have been made to explore to what extent more formalised prediction models can be developed that integrate different KE information. These sorts of prediction models are termed defined approaches (DA).

- OECD is undertaking work to explore to what extent performance based standards can be established for defined approaches to obviate formalised & lengthy validation exercises of specific DA.

- Examples of DA developed for skin sensitisation are highlighted to demonstrate the range of complexity that a DA might encompass.
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  • The European Community’s 7th Framework Programme Innovative Medicines Initiative Joint Undertaking (IMI-JU) eTox Project (grant agreement n° 115002).
  • European Chemicals Agency (EChA) Service Contract No. ECHA/2008/20 /ECA/203.
Useful Links – (Q)SARs

QSAR resources (Models, Formats etc.)

- US EPA Chemistry Dashboard comp.tox.epa.gov/dashboard/
- QSARDB - https://qsardb.org/
- Ochem https://ochem.eu/home/show.do
- Applicability Domain software tools
- http://oasis-lmc.org/
Useful Links – (Q)SARs and Read-across

Technical regulatory guidance

- ECHA. 2017. RAAF ECHA-17-R-01-EN
- ECHA. 2017. RAAF - considerations on multi-constituent substances and UVCBs ECHA-17-R-04-EN
Useful Links – Read-across

Read-Across tools

- AMBIT - http://cefic-iri.org/toolbox/ambit/
- OECD QSAR Toolbox - http://www.qsartoolbox.org/
- CBRA - https://www.fourches-laboratory.com/software

Useful Links – Read-across

Read-Across literature

Useful Links – Read-across

Read-Across literature


Useful Links – Read-across

Read-Across literature


Useful Links – Read-across

AOPs, IATA & DA

Useful Links – Read-across

AOPs, IATA & DA

- OECD 2016a Guidance Document for the Use of Adverse Outcome Pathways in Developing IATA. STA No. 260, ENV/JM/MONO(2016)67

- OECD 2016b. OECD Guidance Document on the Reporting of Defined Approaches (DAs) to Be Used within IATA. STA No. 255, ENV/JM/MONO(2016)28

- OECD 2017 Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA) Series on Testing and Assessment No. 260

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Prof. Mark Cronin, Liverpool John Moores University |
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25 January 2018, 4–5 pm GMT | Dr. Gertrude-Emilia Costin, Institute for In Vitro Sciences  
Dr. Costanza Rovida, TEAM Mastery and CAAT-Europe |
| Skin Sensitisation  
1 February 2018, 4–5 pm GMT | Dr. Susanne Kolle, BASF SE  
Dr. Silvia Casati, EURL ECVAM |
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