State-of-the-science and Practical Application of In Silico Methods

A practical perspective of Non-testing approaches for acute inhalation toxicity – past, present and future

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The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA
Outline

- Definitions
  - Non-testing approaches
    - (Q)SARs, TTC
- Considerations before testing
- TTC
- In silico tools
  - Local models
  - Expert systems
  - Mechanistic hybrid approaches
The continuum of non-testing approaches

Activity = Function

The properties of a chemical with respect to how it will interact with a defined system are inherent in its molecular structure

(Q)SARs

More Formalised in structure

Chemical grouping

Less Formalised in structure
The (Q)SAR concept

- 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.

![Chemical structure diagram]

- A **QSAR** is a statistically established correlation relating (a) quantitative parameter(s) derived from chemical structure or determined by experimental chemistry to a quantitative measure of biological activity.

\[
\log(1/EC3) = 0.25 + 0.28 \times \log P + 0.86 \times R_s
\]

- **Expert systems** are compilations of (Q)SARs packaged for ease of use.
Considerations before testing

- Substance is highly corrosive to skin
- Positive classification from acute toxicity derived by another route of entry
- Consideration of physical form – is the substance a liquid, vapour or solid?
Considerations before testing

- Substance is highly corrosive to skin
- Relevant to know whether local irritation or corrosion to the respiratory tract following single exposure might occur
- Evidence could include experimental data such as an in vitro skin corrosion test, in vitro or in vivo data from (a) related substance(s) [read-across], SARs such as those encoded in Expert systems and other in silico tools
Example in silico tools to assess skin corrosion
Considerations before testing

- Positive classification from acute toxicity derived by another route of entry
## GHS classifications

<table>
<thead>
<tr>
<th>Class</th>
<th>Oral LD50 (mg/kg)</th>
<th>Inhalation LC50 Gas (ppm)</th>
<th>Inhalation LC50 Vapor (mg/L)</th>
<th>Inhalation LC50 Aerosol (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>≤ 5</td>
<td>≤ 100</td>
<td>≤ 0.5</td>
<td>≤ 0.05</td>
</tr>
<tr>
<td>II</td>
<td>5-50</td>
<td>100-500</td>
<td>0.5 - 2</td>
<td>0.05 - 0.5</td>
</tr>
<tr>
<td>III</td>
<td>50-300</td>
<td>500-2500</td>
<td>2 - 10</td>
<td>0.5 - 1</td>
</tr>
<tr>
<td>IV</td>
<td>300-2000</td>
<td>2500-5000</td>
<td>10 - 20</td>
<td>1 - 5</td>
</tr>
<tr>
<td>V</td>
<td>2000-5000</td>
<td>Indication of significant effects in humans; Any mortality at class 4; Indications from other studies.</td>
<td></td>
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</tr>
</tbody>
</table>

Substances that are GHS class I or II by the oral route have tended to be of equal or stricter GHS classification by the inhalation route (Dow results – D Wilson to present)
Considerations before testing

- Consideration of physical form - is the substance a liquid, vapour or solid?
- Melting point, Boiling point and Vapour pressure measurements or estimates are helpful to make this type of determination
Considerations before testing

- Physicochemical properties may be important in determining the technical feasibility of testing
- Vapour pressure, aerodynamic particle size considerations (for substances in powder form as derived from granulometry testing e.g. MMAD - will be helpful to assess respirable and inhalable fractions)
  - Particles smaller than 100 μm in diameter are inhalable and can enter the respiratory tract via nose or mouth
Thresholds of Toxicological Concern (TTC) for inhalation

- TTC is a principle that refers to the establishment of a human exposure threshold value for (groups of) chemicals below which there would be no appreciable risk to human health.

- Developed on the basis of oral data, refinements have included other routes of entry such as inhalation.
  - RepDose database developed by Fraunhofer Institute of Toxicology and Experimental Medicine [http://www.fraunhofer-repdose.de/](http://www.fraunhofer-repdose.de/)
  - Updated analysis reported in Tluczkiewicz et al (2016) in Regul Toxicol Pharmacol 78:8-23
Local QSAR models on inhalation toxicity

- Few if any and tend to be focused on inhalational toxicity of volatile substances.
- Examples include acute (non-lethal) neurotoxicity data for the neurotropic effects of common solvents in rats and mice where LogKow, Boiling Point and molecular connectivity indices were found to be relevant chemical descriptors.
Data gap filling within the OECD Toolbox

- Identify related analogues with LC50 data to perform an endpoint specific read-across within the OECD Toolbox
Expert systems

- Only one known expert system - TOxicity Prediction by Komputer Assisted Technology (TOPKAT) which contains a rat LC50 model
- Contains five submodels related to different chemical classes
Mechanistic hybrid approaches

- Work by Gil Veith and Kendall Wallace
- Presented at the EPA conference
- The acute aquatic fish toxicity established by e.g. Verhaar et al to subcategorise chemicals (baseline narcotics, polar narcotics, unspecific reactivity, specific mechanisms)

QSAR-based Prediction of Inhalation Toxicity

Incorporating elements of dosimetry and reactivity to predict biological response

Kendall B. Wallace, Eli Petkova, Gilman D. Veith

University of Minnesota – Duluth Medical School & International QSAR Foundation
Modeling Assumptions

- Obstructive disorders
  - Low vapor pressure
  - High water solubility
  - High chemical reactivity

- Restrictive disorders
  - Low vapor pressure
  - Low water solubility
  - High chemical reactivity
  - MoA - specific disease

- Non-specific, narcotic-like effects
  - Low vapor pressure
  - Low water solubility
  - Low chemical reactivity

Slide presented at McKim 2006
LC50 /rat/4h vs Vapor Pressure for chemicals previously classified as NON-REACTIVE

For **ACRYLATES & METHACRYLATES** there is no relationship with Vapor Pressure but significant correlation with GSH reactivity.

**LC50 vs GSH reactivity for acrylates and methacrylates**
Baseline model incorporated into the OASIS Pipeline
Future approaches I

- Incorporation of bioactivity information in addition to chemical structure information for local neighbourhoods of chemicals to develop systematic read-across predictions e.g. GenRA
Systematic read-across

- **GenRA (Generalised Read-Across)** is a “local validity” approach
- Predicting toxicity as a similarity-weighted activity of nearest neighbours based on chemistry and bioactivity descriptors

\[ y_i = \text{predicted activity of chemical } (c_i) \]
\[ k = \text{up to } k \text{ nearest neighbours} \]
\[ \alpha = \{chm, bio, bc\} \]

Where \( x_j^{\text{tox}} \), in this case, is the in vivo toxicity of chemical \( j \)

\[ y_i^{\text{tox}} = \frac{\sum_{j}^{k} S_{ij} \alpha \cdot x_j^{\text{tox}}}{\sum_{j}^{k} S_{ij}} \]

- Developed using repeated dose toxicity endpoints, could be adapted for other endpoints such as acute inhalation toxicity

Shah et al (2016), in press
GenRA: Nominal cluster

Explore performance as a function of number of nearest neighbours or similarity index
Future approaches II

- Categorising chemicals by likely mode of action (MoA) into local neighbourhoods for read-across and QSAR development but going beyond the work of Wallace and Veith who exploited the Verhaar MoA
- For more information see next talk by Dan Wilson