

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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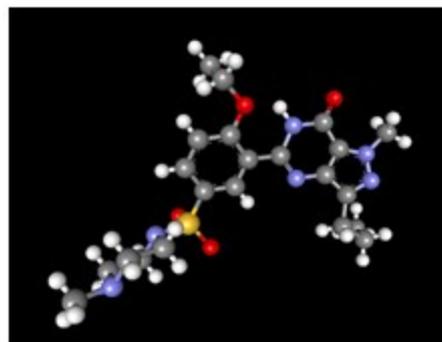
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Outline

- **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

Chemical grouping

More Formalised
in structure

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



- 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

$$\text{Log (1/EC3)} = 0.25 + 0.28 * \text{LogP} + 0.86 * \text{Rs}^*$$

- 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

QSAR TOOLBOX

Input Profiling Endpoint Category Definition Data Gap Filling Report

Profiling Profiling Schemes

Apply New View Delete

Filter endpoint tree... 1 [target]

Structure

Skin irritation/corrosion Inclusion rules by BFR (Endpoint Specific) - Profiling Scheme Browser

Advanced

Skin irritation/corrosion Inclusion rules by BFR - Category d Profile Description

Category: Acid anhydrides

This category includes the following structural alerts:

R-C(=O)-O-C(=O)-R

R-C(=O)-O-O-C(=O)-R

R = any

References:

Hulzebos E, Walker JD, Gerner I and Schlegel K, Use of structural alerts to

GPMT (1/1) M: Positive

LLNA (1/1) M: Positive

Undefined Assay M: sensitising

Considerations before testing

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

GHS classifications

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

**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 $100\ \mu\text{m}$ in diameter are inhalable and can enter the respiratory tract via nose or mouth

Thresholds of Toxicological Concern (TTC) for inhalation

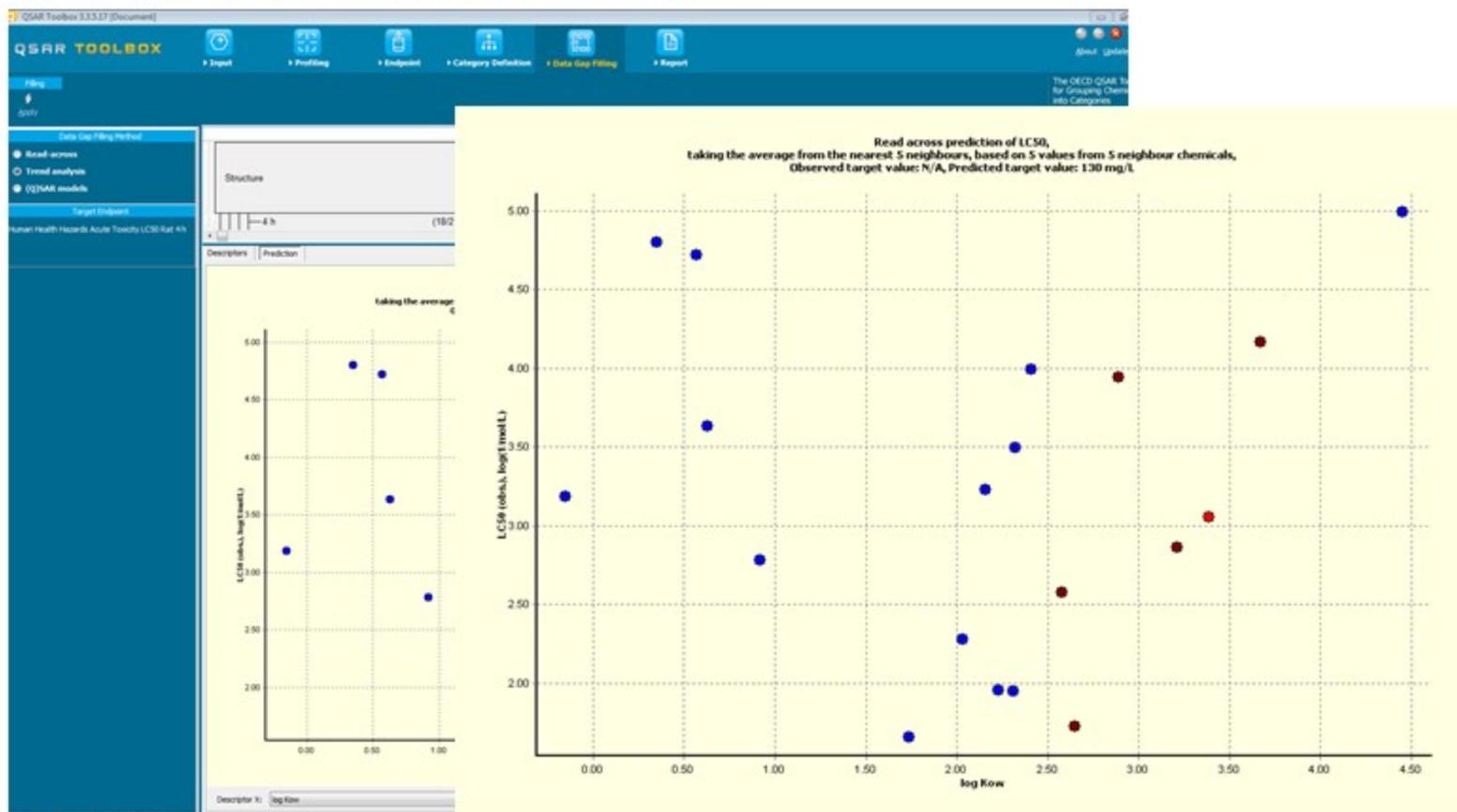
- TTC is a principle that refers to the establishment of a human exposure threshold value for (groups of) chemicals below which there would be no appreciable risk to human health
- Developed on the basis of oral data, refinements have included other routes of entry such as inhalation
 - Carthew et al (2009) see Food Chem Toxicol. 47(6):1287-95
 - Escher et al (2010) using the RepDose see Regul Toxicol Pharmacol 58(2):259-74
 - RepDose database developed by Fraunhofer Institute of Toxicology and Experimental Medicine <http://www.fraunhofer-repdose.de/>
 - Updated analysis reported in Tluczkiwicz 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
- The acute toxicity of neutral organics at 1 atm pressure
- Unspecific acceptors for reactive
- Uses the 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

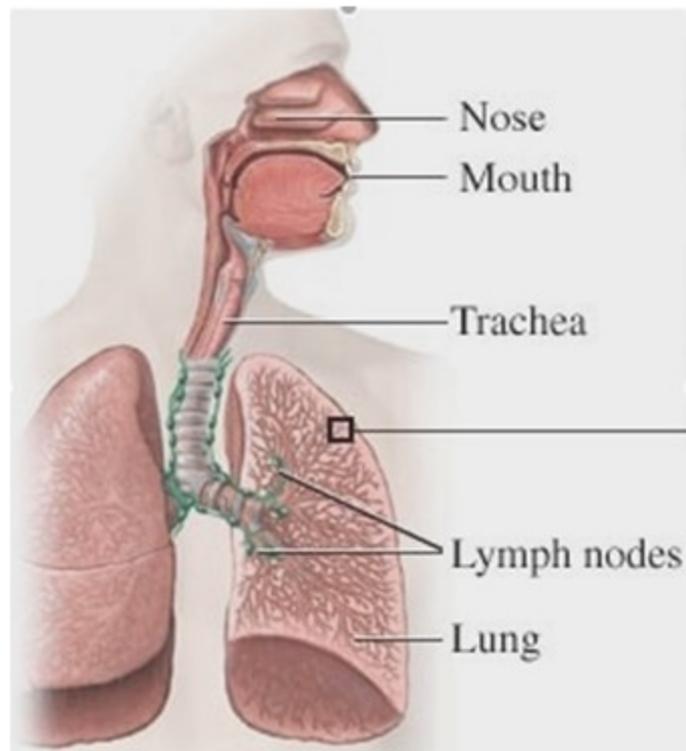
Kendall B. Wallace, Eli Petkova, Gilman D. Veith

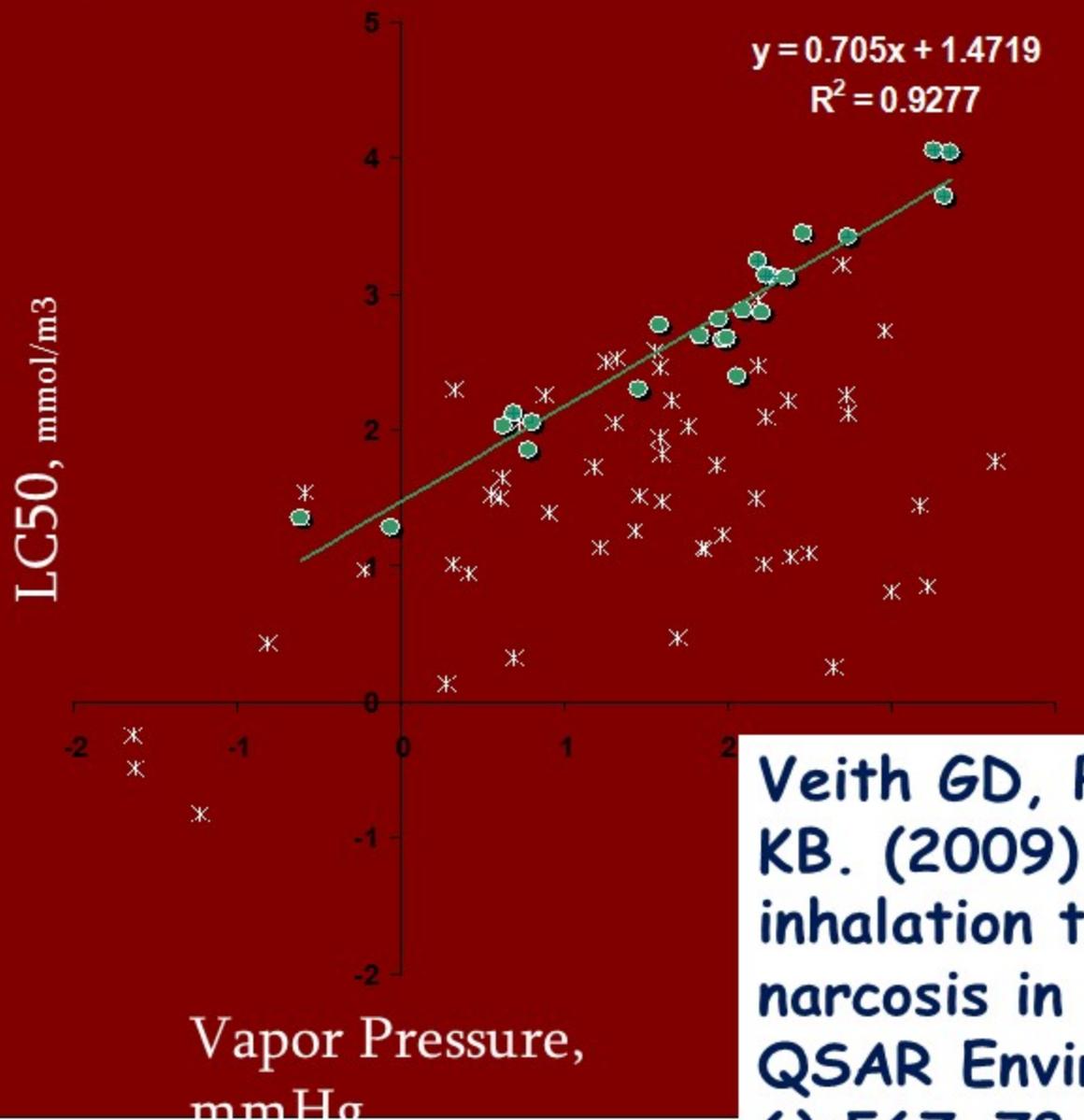
University of Minnesota – Duluth Medical School & International QSAR Foundation



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

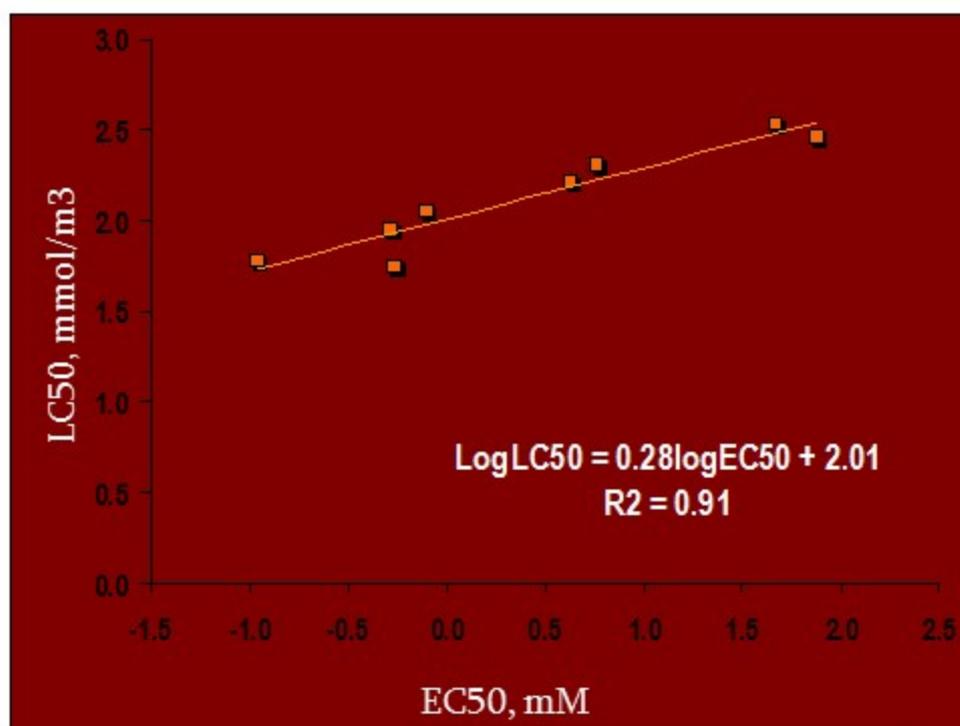
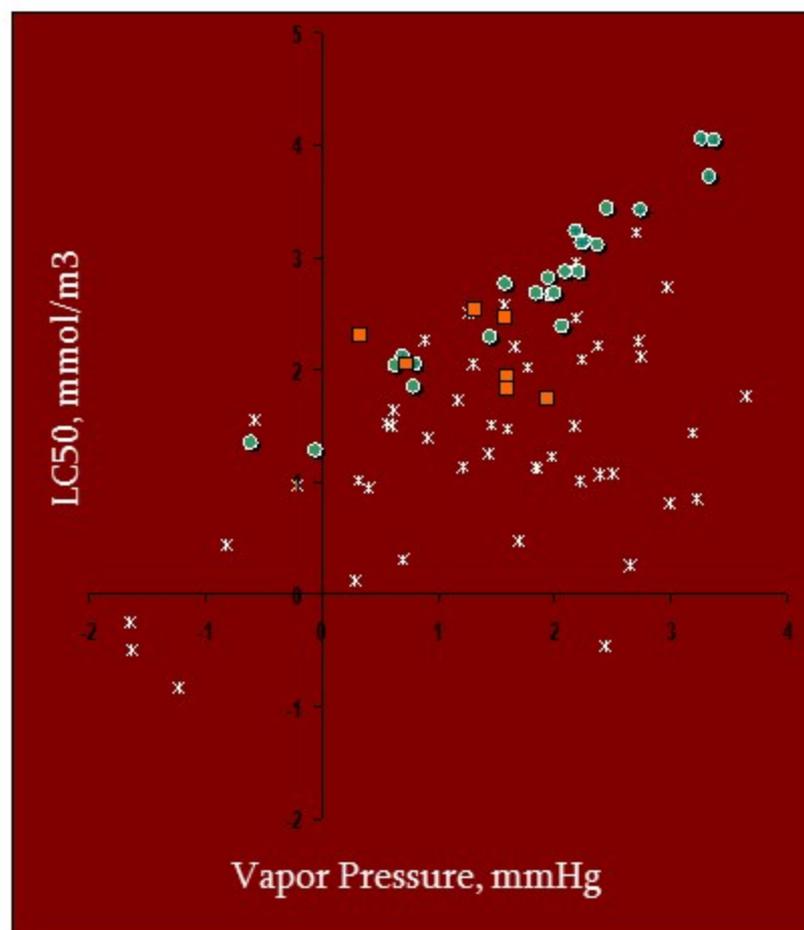




LC50 /rat/4h vs Vapor Pressure for chemicals previously classified as NON-REACTIVE

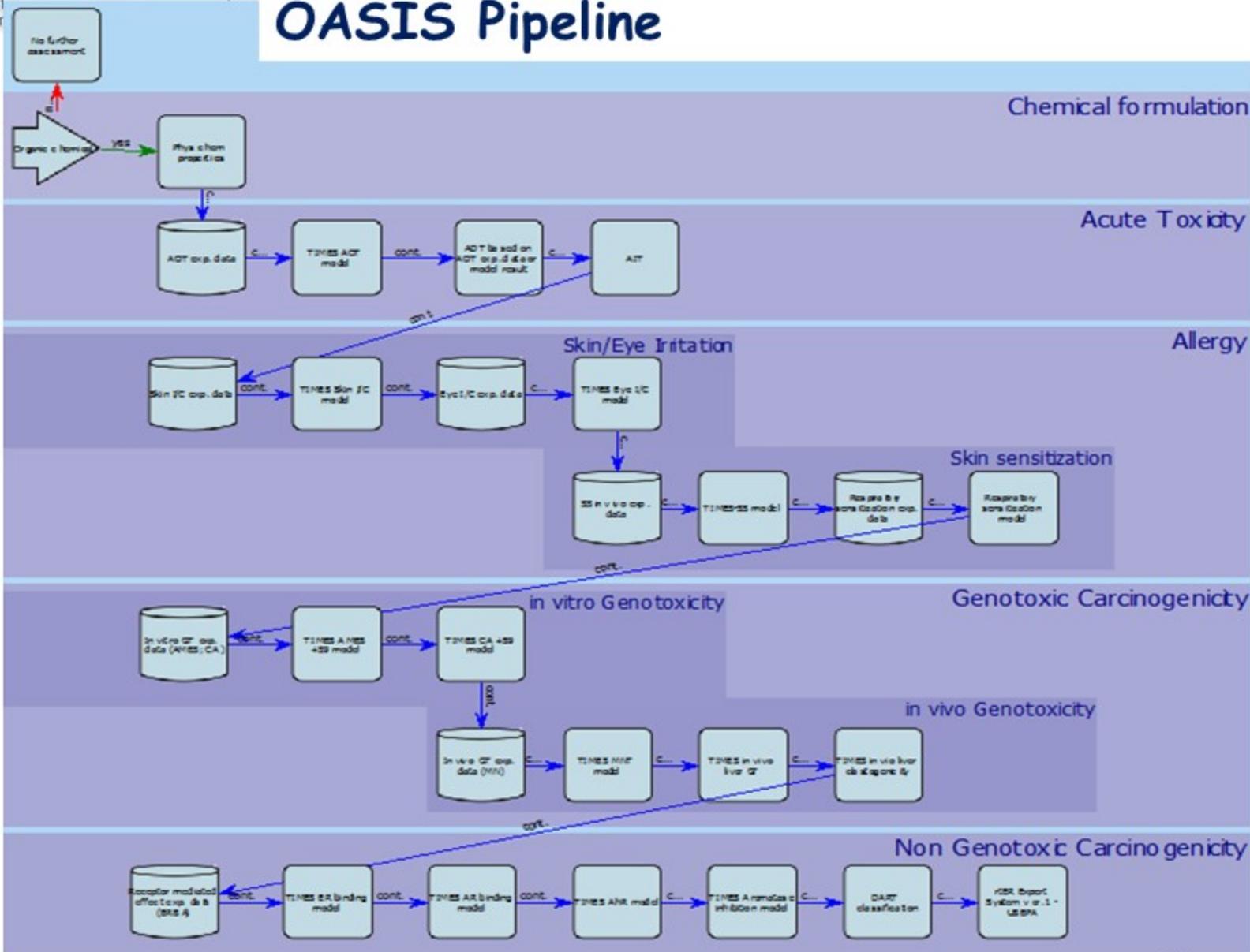
Veith GD, Petkova EP, Wallace KB. (2009) A baseline inhalation toxicity model for narcosis in mammals. SAR QSAR Environ Res. 20(5-6):567-78.

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 = predicted activity of chemical (c_i)

k = up to k nearest neighbours

$\alpha = \{chm, bio, bc\}$

Where x_j^{α} , in this case, is the *in vivo* toxicity of chemical j

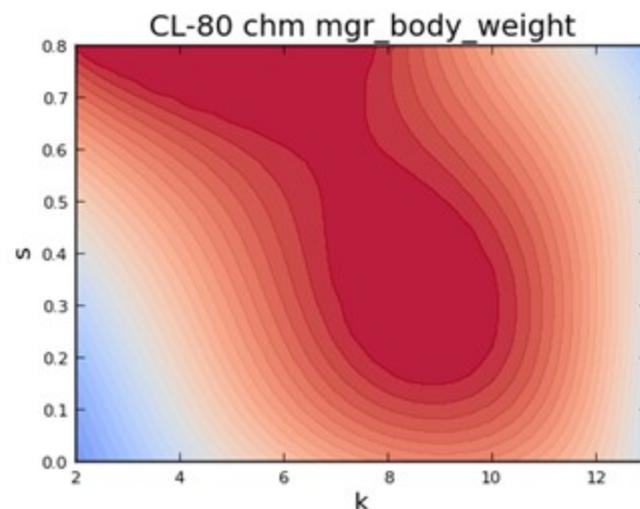
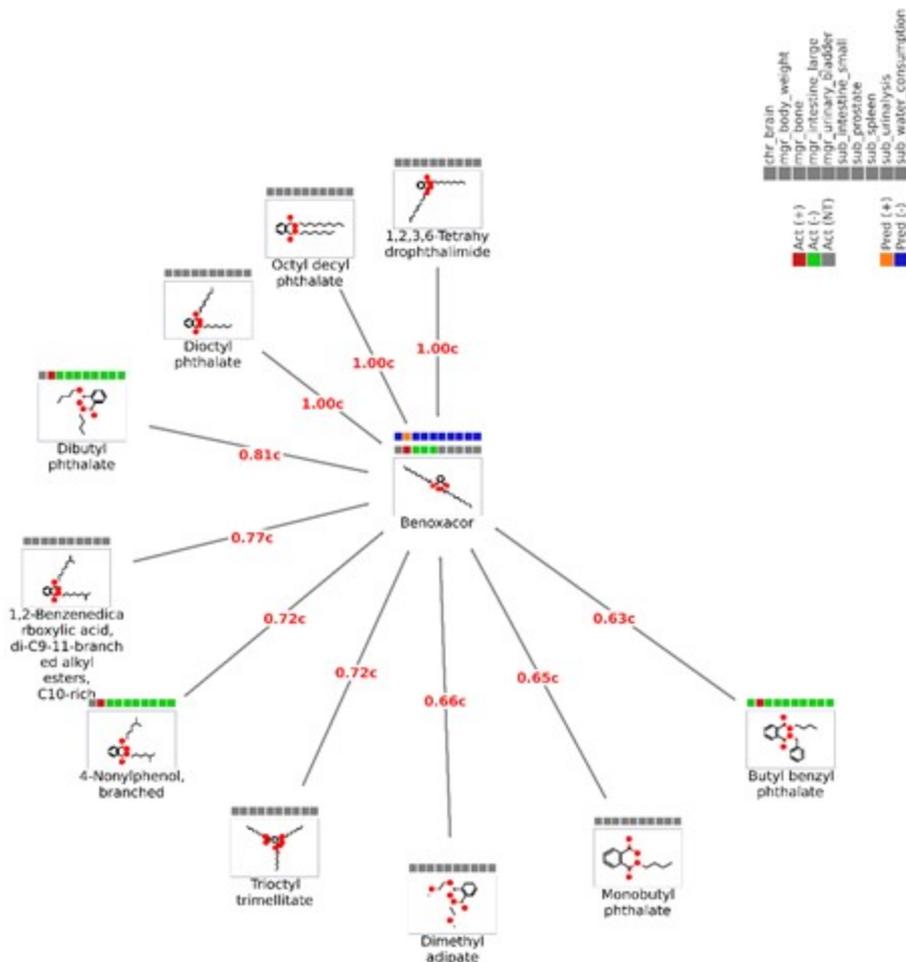
$$y_i^{tox} = \frac{\sum_j^k S_{ij}^{\alpha} x_j^{tox}}{\sum_j^k S_{ij}^{\alpha}}$$

Shah et al (2016), in press

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

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**