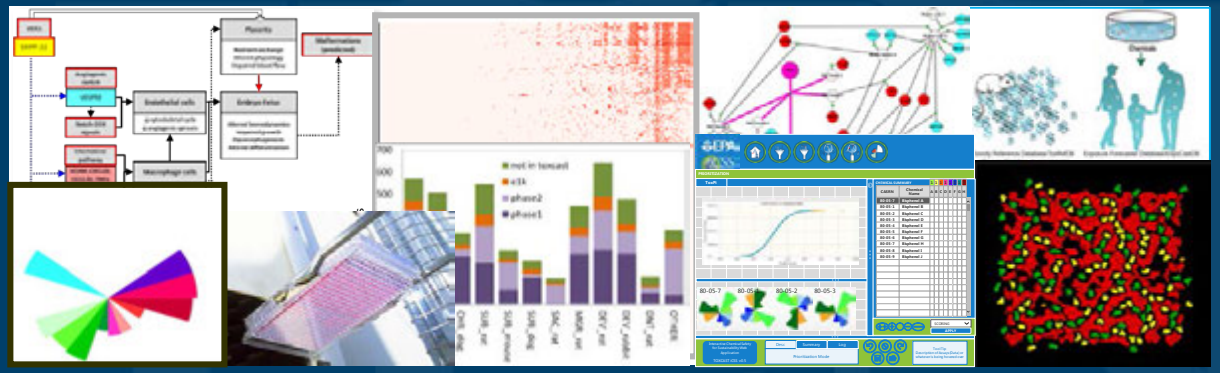
EPA
United States
Environmental Protection
Agency

Exploiting enhanced non-testing approaches to meet the needs for sustainable chemistry



250th ACS National Meeting & Exposition
16-20 August 2015

G Patlewicz, K Houck, R Judson, A Richard, I Shah, A.J. Williams

National Center for Computational Toxicology

Today's talk…

- Provide overview of current and future states of non-testing development & application
- Review application of AOPs and scientific confidence framework
- Show examples of non-testing approaches to AOP informed Integrated Approaches to Testing and Assessment (IATA)- skin sensitization and genotoxicity
- Exploiting bioactivity data for read-across – Generalized Read-Across combining chemistry and biology data

Sustainable chemistry

- Design and use of chemicals that minimize impacts to human health, ecosystems and the environment

- Chemicals need to be evaluated for:
  - their toxicity to humans and other species
  - environmental persistence
  - potential formation of toxic products as a result of biotic and abiotic transformations

- "Non-testing approaches" could be helpful

Continuum of non-testing approaches

United States Environmental Protection Agency

Activity = Function

Properties of a chemical and how it will interact with a defined system are inherent in its molecular structure

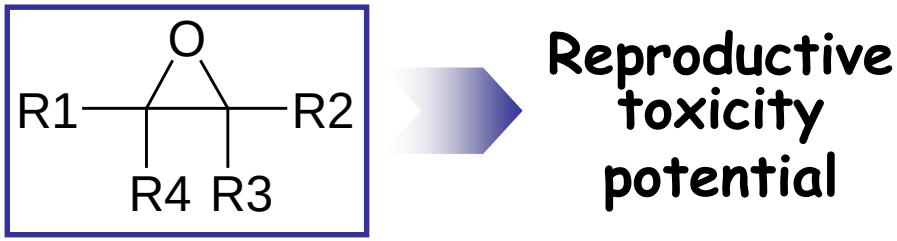
Chemical grouping → (Q)SARs

Less Formalized in structure

More Formalized in structure

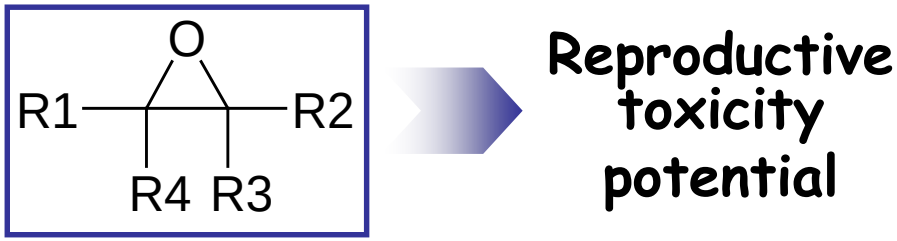
The (Q)SAR concept

- **SAR:** qualitative association between substructure(s) and the potential of a chemical to exhibit a biological effect



The (Q)SAR concept

- **SAR:** qualitative association between substructure(s) and the potential of a chemical to exhibit a biological effect

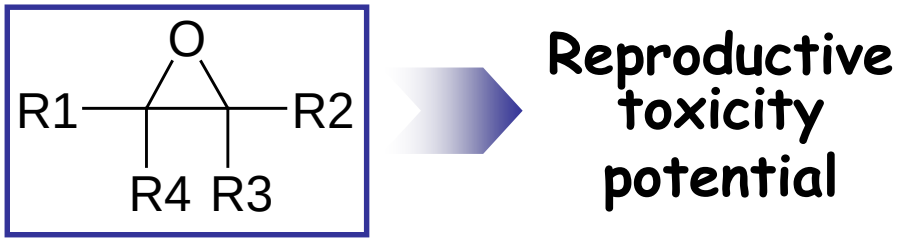


- **QSAR**: statistically established correlation relating quantitative parameter(s) derived from chemical structure or determined by experimental chemistry to a quantitative measure of biological activity

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

The (Q)SAR concept

- **SAR:** qualitative association between substructure and the potential of a chemical to exhibit a biological effect



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

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

- **Expert systems:** packaged (Q)SARs for ease of use

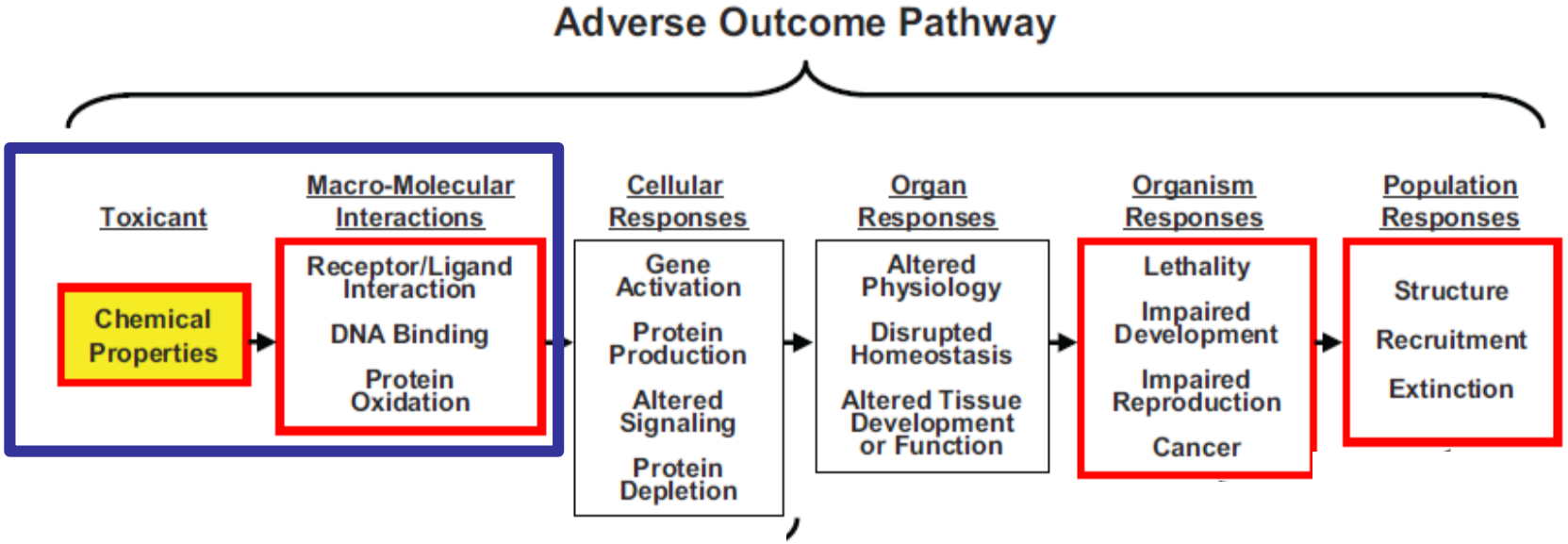
Chemical grouping approaches

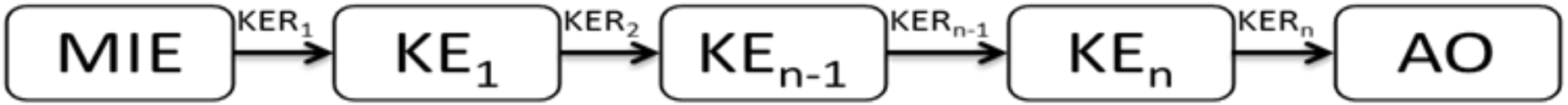
- "Analogue approach" grouping based on a very limited number of chemicals (e.g. target substance is "like" source substance). Commonly "expert-driven".
- "Category approach" grouping based on a more extensive range of analogs (e.g. larger data sets).
- "Read-across" is data gap filling using *either* an analog or category approach

Current approach for non-testing development and application

Mechanistically a Black Box: *Not transparent but fast…*

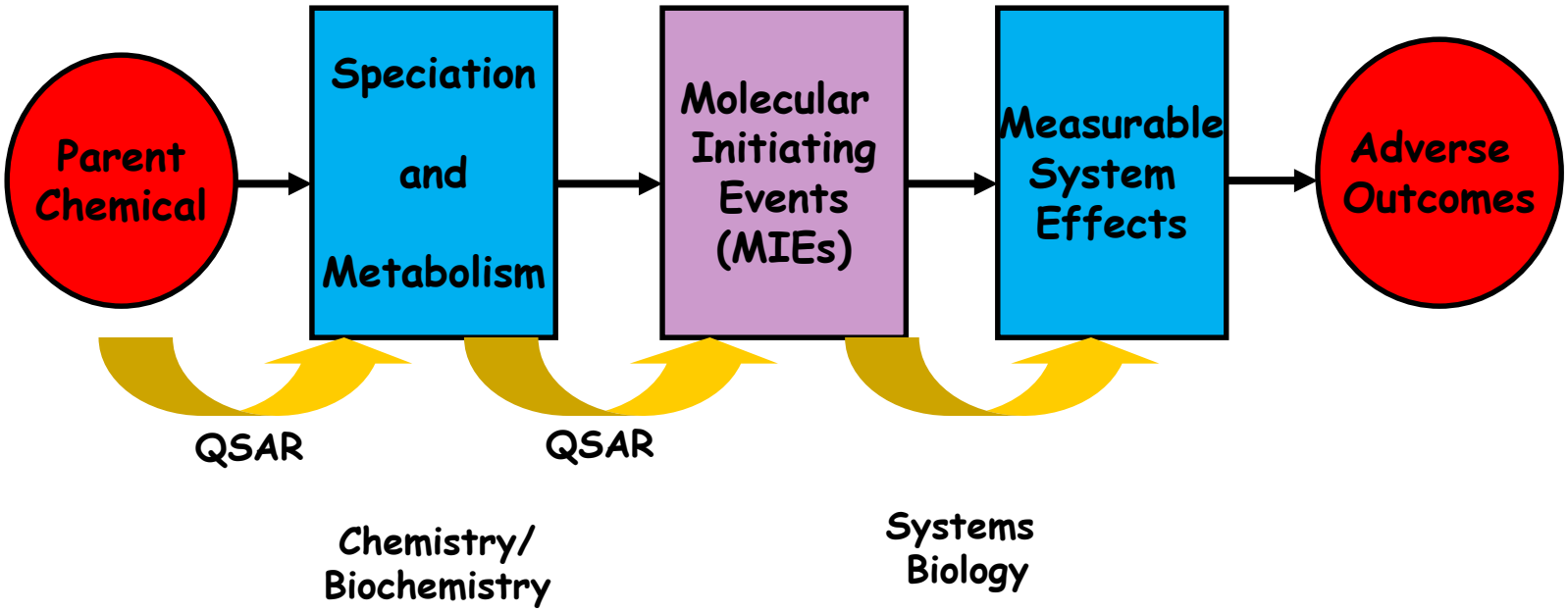
AOP – framework for developing non-testing approaches differently….





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.

Emerging conceptual approach for non-testing development and application



1. Identify Plausible MIEs
2. Explore Linkages in Pathways to Downstream Effects
3. Develop QSARs to predict MIEs from Structure or characterize other KEs as SARs

Applications of AOPs

- The three main applications of AOPs are:
  - Developing Integrated Approaches to Testing and Assessment (IATA)
  - Group chemicals into chemical categories to facilitate read-across
  - Informing test method development & refinement

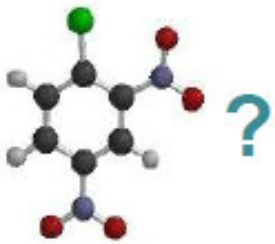
Establishing Scientific Confidence in the application of AOPs (GRACE)

1	**Develop the AOP**
2	**Develop new (or map existing) specific assays to key events within the AOP**
3	Conduct (or document) **Analytical Validation** of each assay
4	Develop new (or map existing) models that predict a specific key event from one or more pre-cursor key events. (The input data for the prediction models comes from the assays described in Steps 2 and 3 above.)
5	Conduct (or document) **Qualification** of the prediction models
6	**Utilization**: defining and documenting where there is sufficient scientific confidence to use one or more AOP-based prediction models **for a specific purpose** (e.g., priority setting, chemical category formation, integrated testing, predicting *in vivo* responses, etc.)
7	For regulatory acceptance and use, processes need to be agreed upon and utilized to ensure robust and transparent review and determination of fit-for-purpose uses of AOPs. This should include dissemination of all necessary datasets, model parameters, algorithms, etc., to enable stakeholder review and comment, fully independent verification and independent scientific peer review. Whilst these processes have yet to be defined globally, in time, these should evolve to enable credible and transparent use of AOPs with sufficient scientific confidence by all stakeholders.

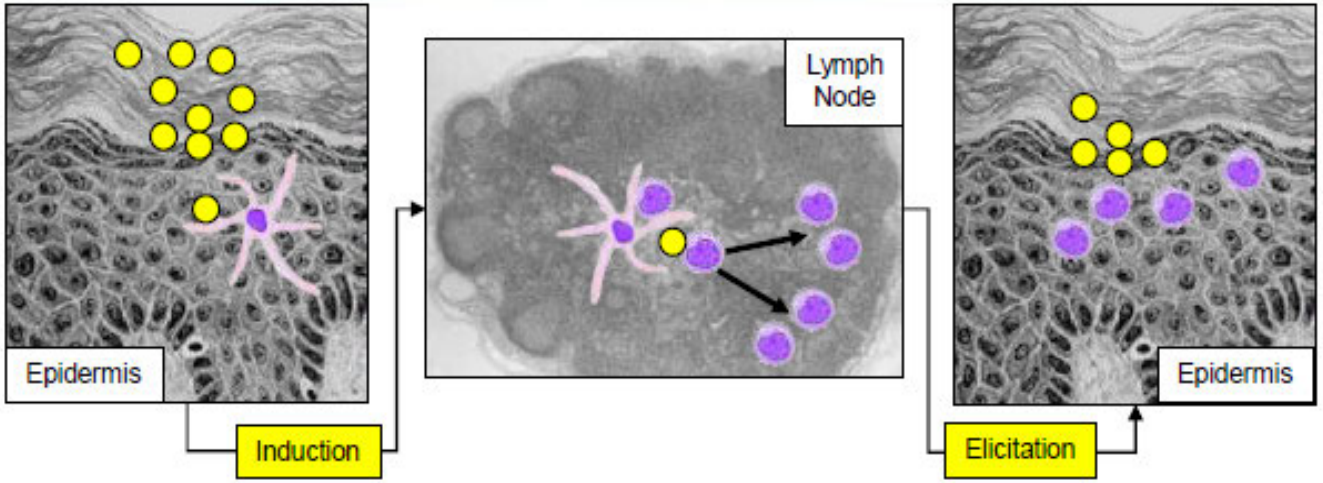
Patlewicz et al (2015) Regul Toxicol Pharmacol. 71(3):463-77.

Skin sensitization AOP (OECD, 2012)

United States Environmental Protection Agency

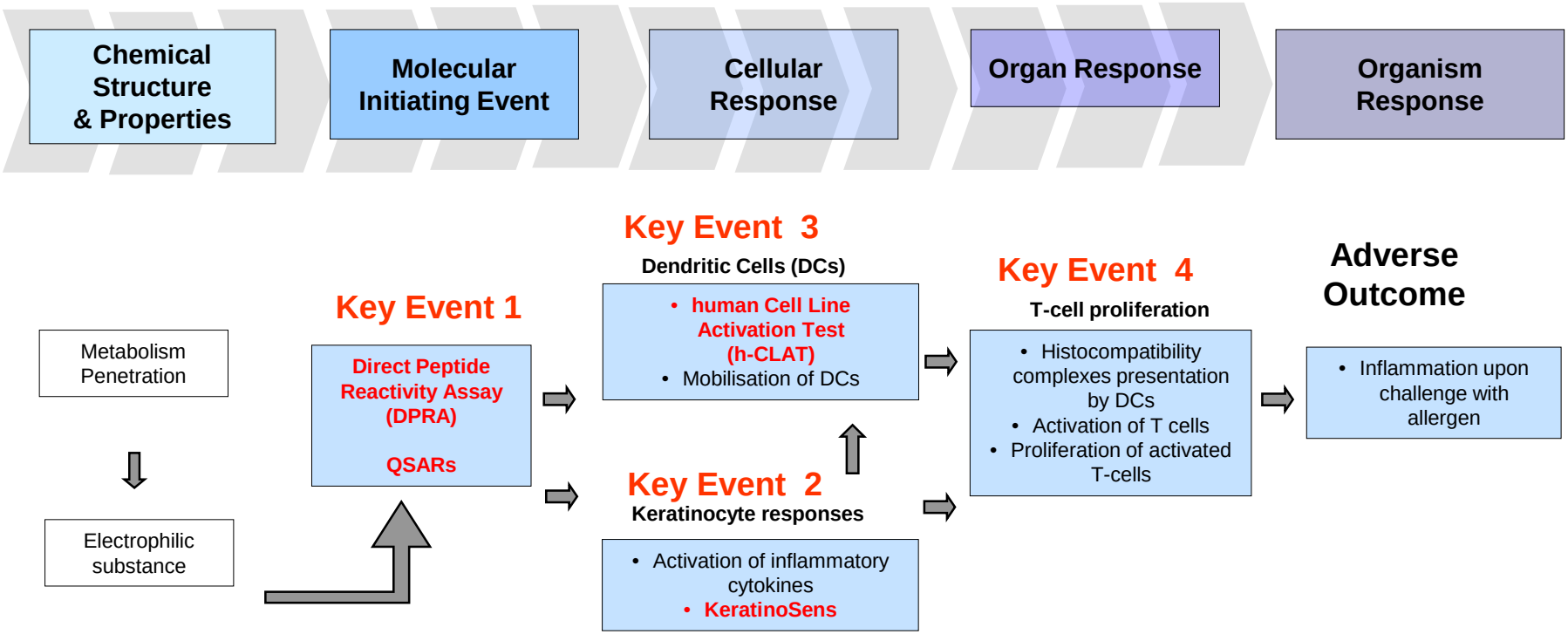


?



Chemical Structure & Properties	Molecular Initiating Event	Cellular Response	Organ Response	Organism Response
1. Skin Penetration 2. Electrophilic substance: directly or via auto-oxidation or metabolism	3-4. Haptenation: covalent modification of epidermal proteins	5-6. Activation of epidermal keratinocytes & Dendritic cells	7. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells	8-10. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death
	Key Event 1	**Key Event 2 + 3**	**Key Event 4**	**Adverse Outcome**

Mapping methods to key events



Related Work: Data Generation ToxCast HTS screening

ToxCast™

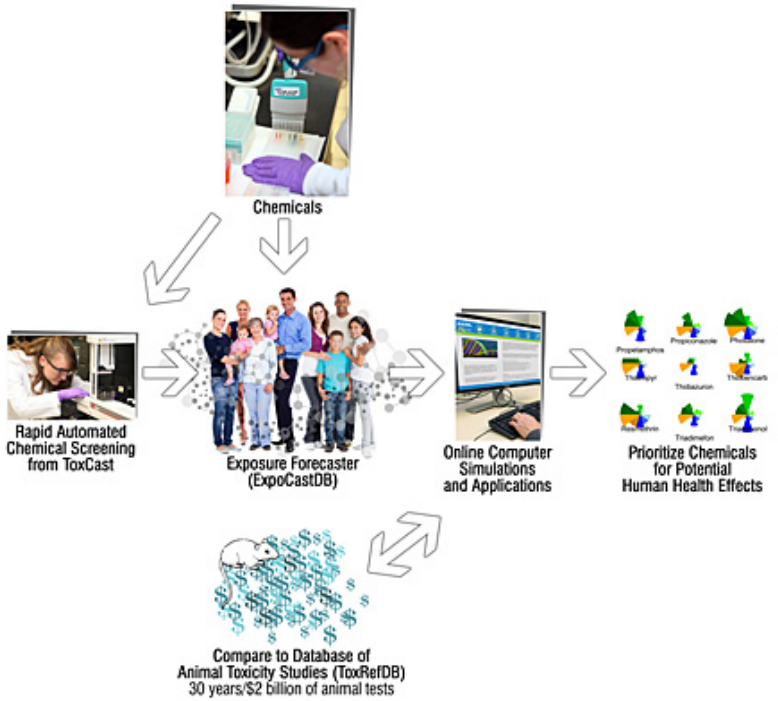
Advancing the next generation of chemical safety evaluation

A major part of EPA's CompTox research is the Toxicity Forecaster (ToxCast™). ToxCast is a multi-year effort launched in 2007 that uses automated chemical screening technologies (called "high-throughput screening assays") to expose living cells or isolated proteins to chemicals. The cells or proteins are then screened for changes in biological activity that may suggest potential toxic effects and eventually potential adverse health effects. These innovative methods have the potential to limit the number of required laboratory animal-based toxicity tests while quickly and efficiently screening large numbers of chemicals.

Chemical Prioritization

ToxCast data is starting to help inform chemical prioritization.

- EPA's Endocrine Disruption Screening Program has already started the scientific review process needed to begin using ToxCast data to prioritize the thousands of chemicals that need to be tested for potential endocrine-related activity.



How ToxCast Fits Into CompTox Research

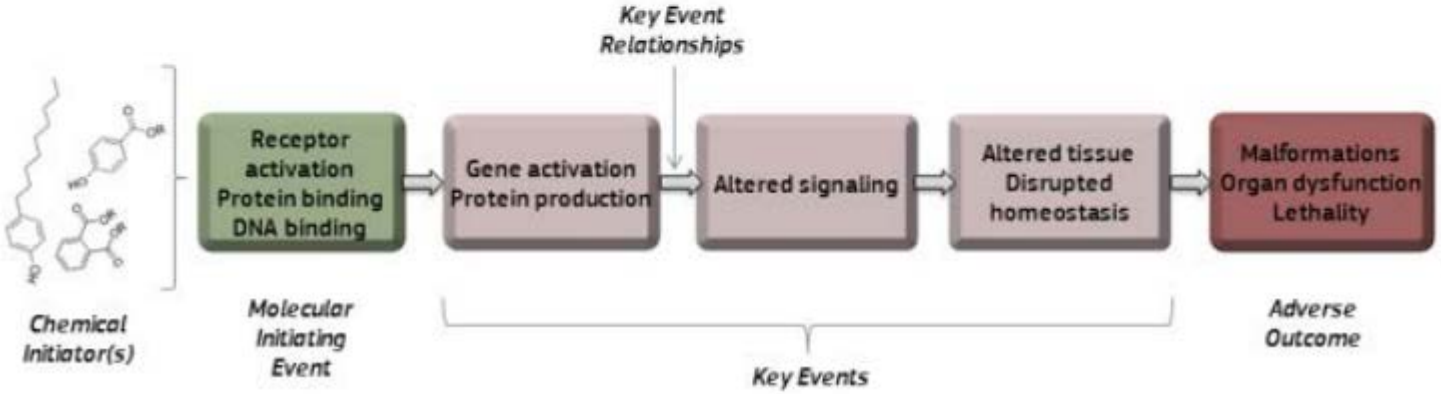
AOP Wiki
Community AOP Development

www.epa.gov/research

science in ACTION

INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE

CHEMICAL SAFETY RESEARCH: ADVERSE OUTCOME PATHWAYS

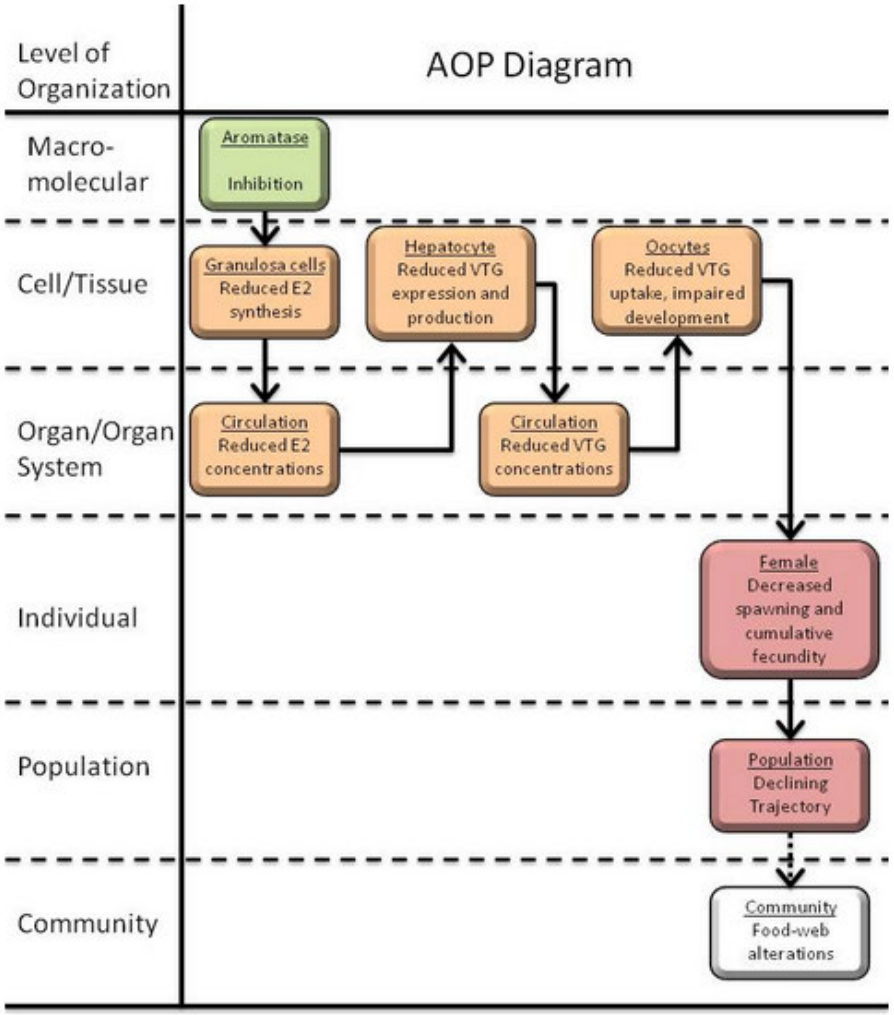


AOP Wiki
Community AOP Development

Relationships Among Key Events and the Adverse Outcome

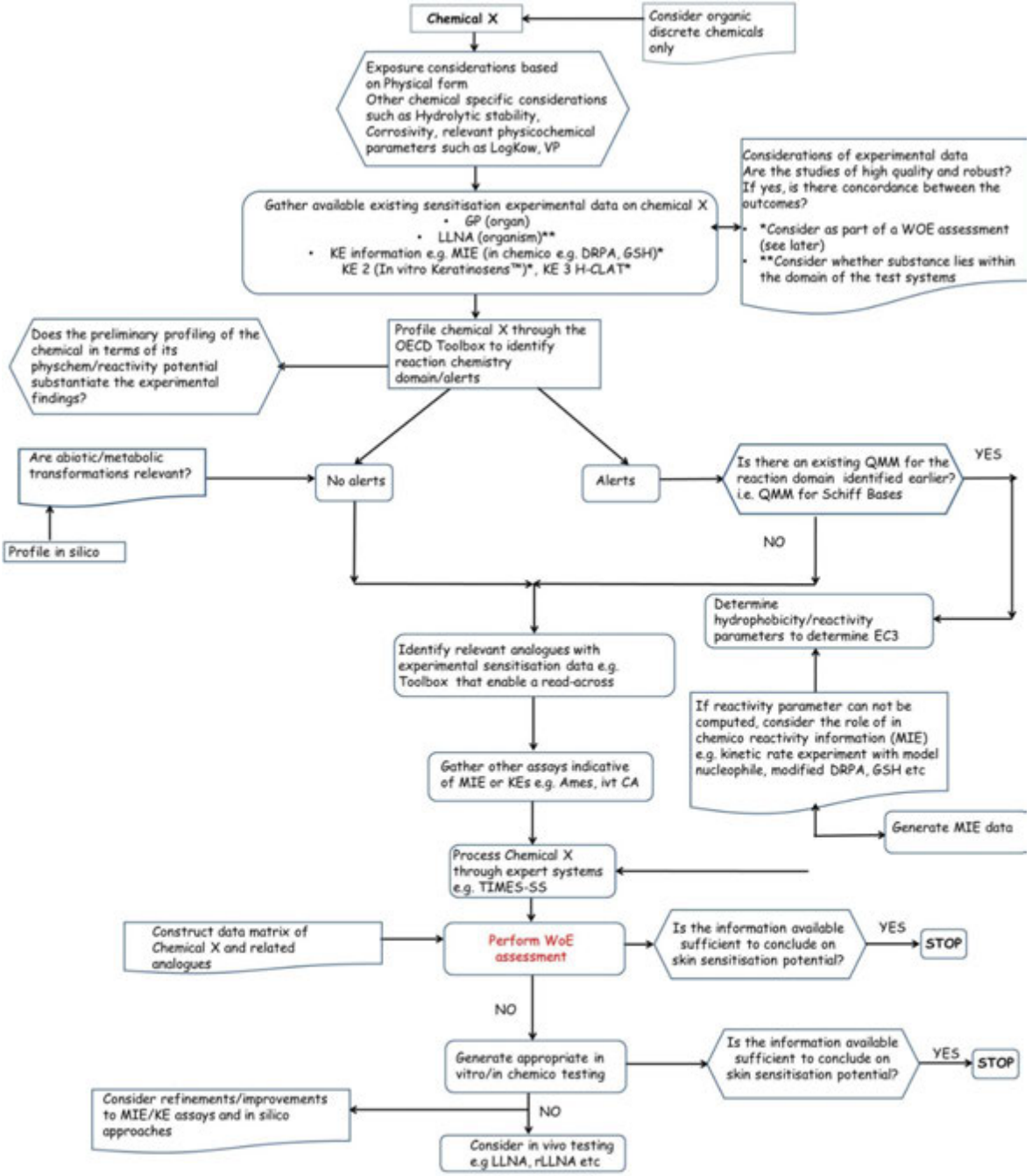
Event	Description	Triggers	Weight of Evidence	Quantitative Understanding
Aromatase, Inhibition	Directly Leads to	17beta-estradiol synthesis by ovarian granulosa cells, Reduction	Strong	Moderate
17beta-estradiol synthesis by ovarian granulosa cells, Reduction	Directly Leads to	Plasma 17beta-estradiol concentrations, Reduction	Strong	Moderate
Plasma 17beta-estradiol concentrations, Reduction	Directly Leads to	Transcription and translation of vitellogenin in liver, Reduction	Strong	Moderate
Cumulative fecundity and spawning, Reduction	Directly Leads to	Population trajectory, Decrease	Moderate	Moderate
Vitellogenin uptake into oocytes and oocyte growth/development, Reduction	Directly Leads to	Cumulative fecundity and spawning, Reduction	Moderate	Moderate
Plasma vitellogenin concentrations, Reduction	Directly Leads to	Vitellogenin uptake into oocytes and oocyte growth/development, Reduction	Moderate	Weak
Transcription and translation of vitellogenin in liver, Reduction	Directly Leads to	Plasma vitellogenin concentrations, Reduction	Strong	Moderate

AOP Wiki
Community AOP Development



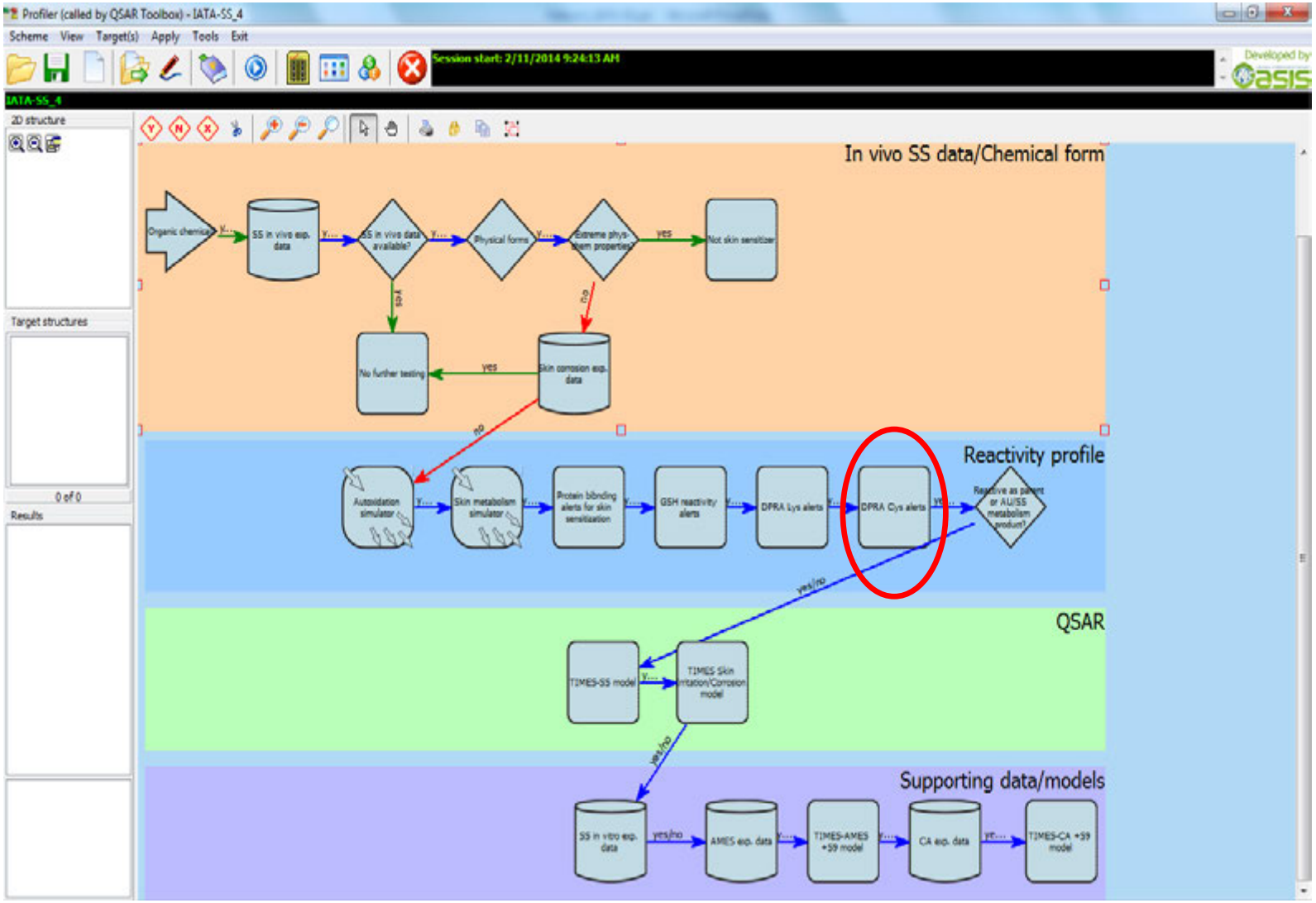
United States Environmental Protection Agency

IATA for Skin Sensitization IATA-SS

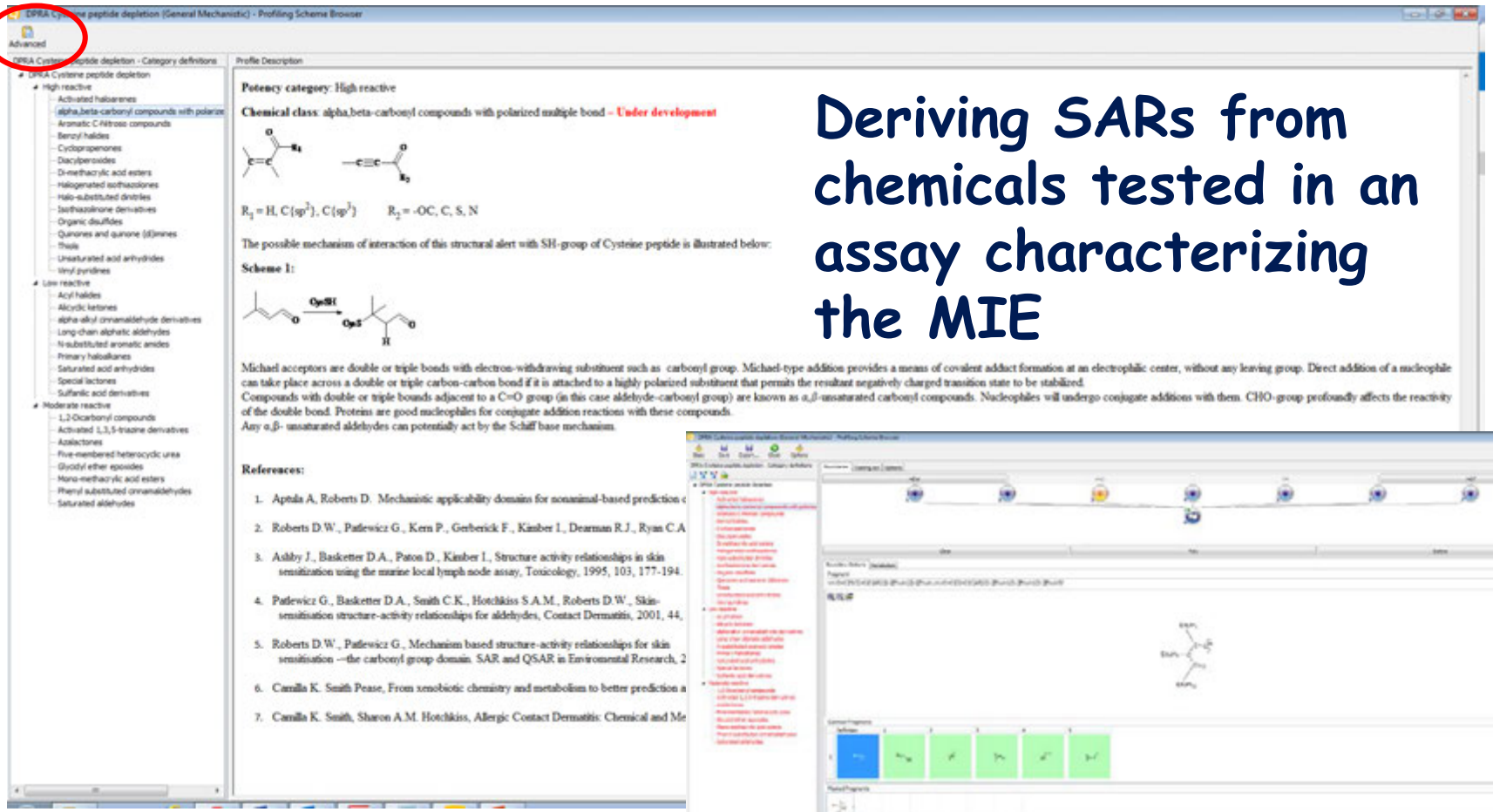


Patlewicz G, et al. 2014
Reg. Toxicol. Pharmacol.
69(3):529-45.

Implement IATA-SS in a Pipeline tool for re-use



Mechanistic basis – SAR Profiler for cysteine depletion

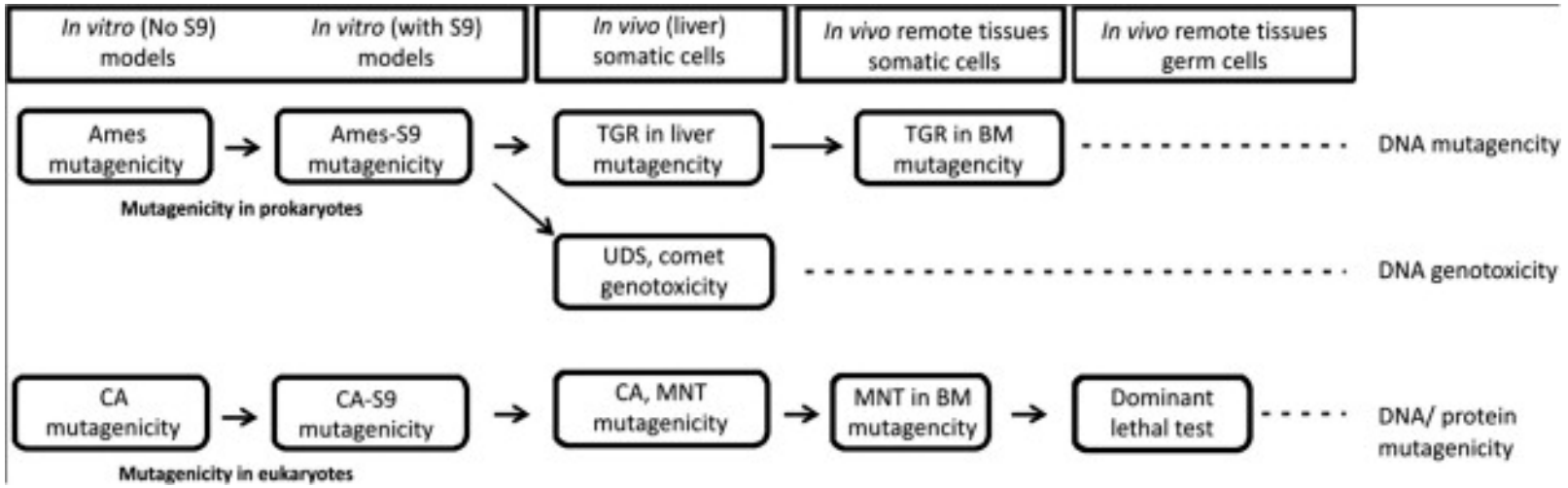


Deriving SARs from chemicals tested in an assay characterizing the MIE

IATA for genetox – work in progress

Level I A	Level I B	Level II A	Level II B	Level II C	Level III
in vitro Mutagenicity Bacterial (Ames-S9)	*in vitro* Mutagenicity Mammalian (CA, MLA)	*in vivo* Genotoxicity Liver (comet, UDS)	*in vivo* Mutagenicity Liver (TGR)	*in vivo* Mutagenicity Liver (CA, MNT)	*in vivo* Mutagenicity Bone Marrow (MNT)

Structuring a non-testing workflow taking into account the capability of each test system



Petkov et al (2015) Reg Tox Pharmacol 72(1): 17-25

Current work on read-across

- Develop a systematic approach for read-across and evaluate its performance:
  - Local validity approach – similarity weighted activity of nearest neighbors across different descriptor spaces (chemical, bioactivity and a hybrid)
  - Establish baseline performance and quantify uncertainty
  - Extend and refine to codify expert insights – as derived from e.g. SARs.

Generalized Read Across

- Generalized Read Across (GenRA) - refinement of the Chemical-Biological Read-Across (CBRA)

Integrative Chemical-Biological Read-Across Approach for Chemical Hazard Classification

Yen Low[†,‡], Alexander Sedykh[†], Denis Fourches[†], Alexander Golbraikh[†], Maurice Whelan[∥], Ivan Rusyn[‡,*], and Alexander Tropsha[†,*]

- Predict toxicity as a similarity-weighted activity of neighbors across different descriptor spaces:

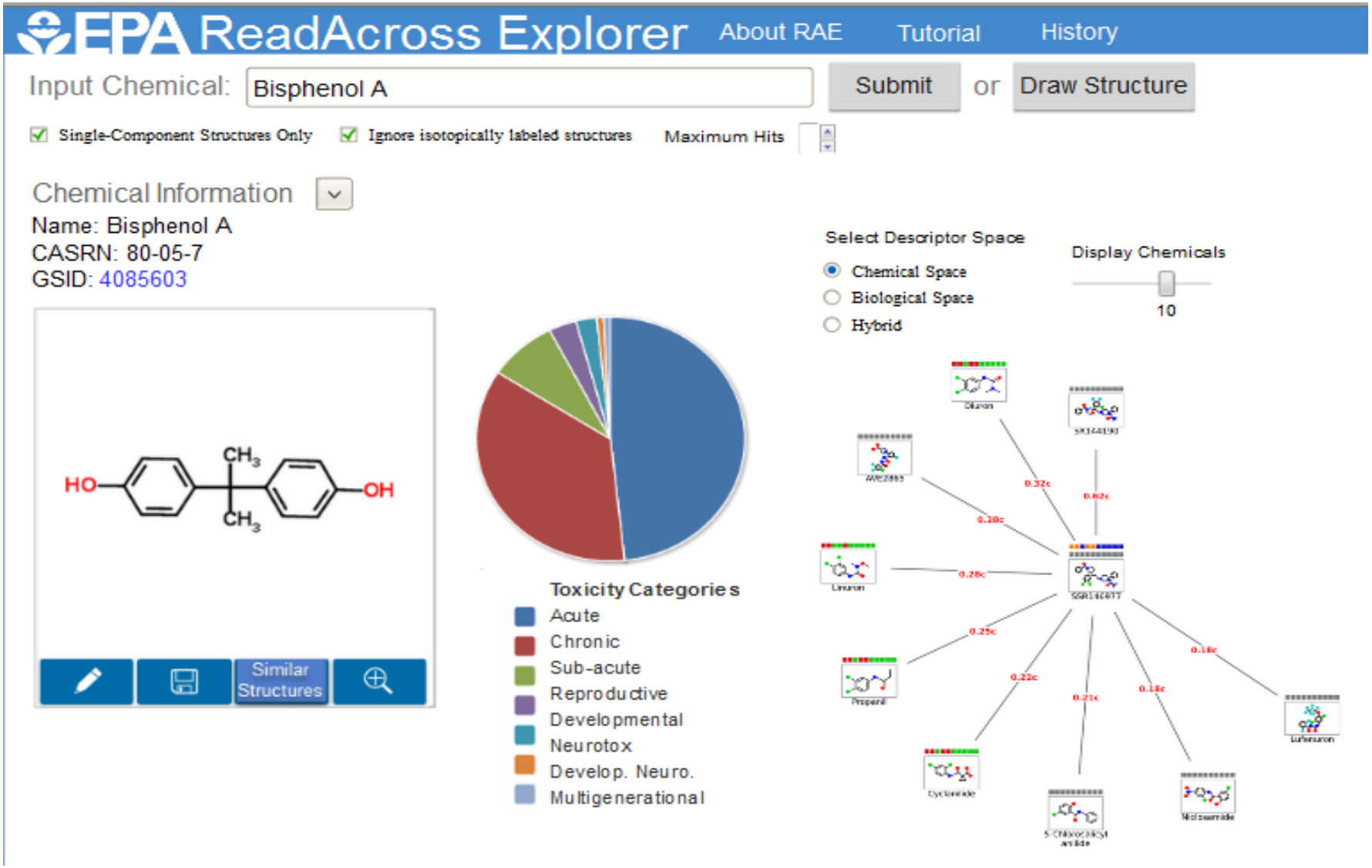
$$y_i^{tox} = \frac{\sum_j^k s_{ij}^{\alpha} x_j^{tox}}{\sum_j^k s_{ij}^{\alpha}}$$

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

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

Shah et al, in prep

Software development in progress

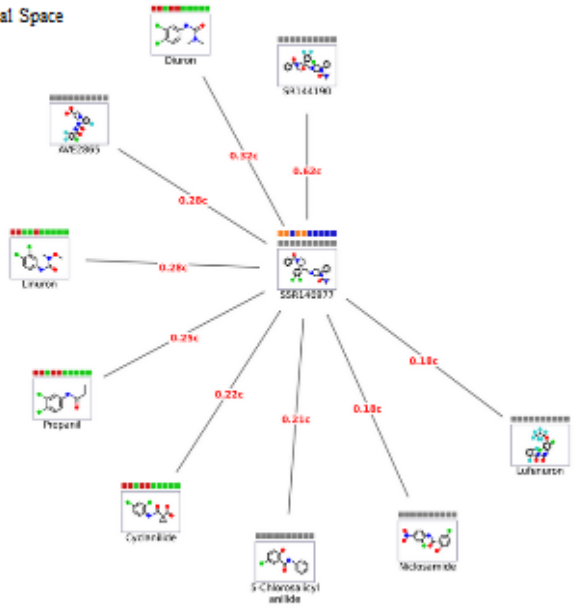


Software development in progress

EPA ReadAcross Explorer

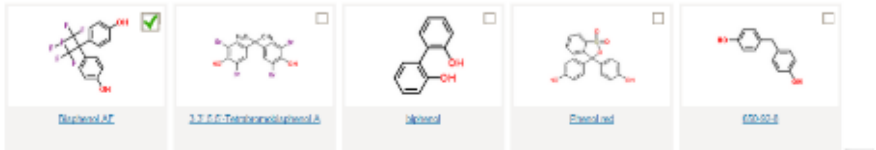
Select Descriptor Space

- (•) Chemical Space
- ( ) Biological Space
- ( ) Hybrid



Score Threshold

Nearest Neighbors



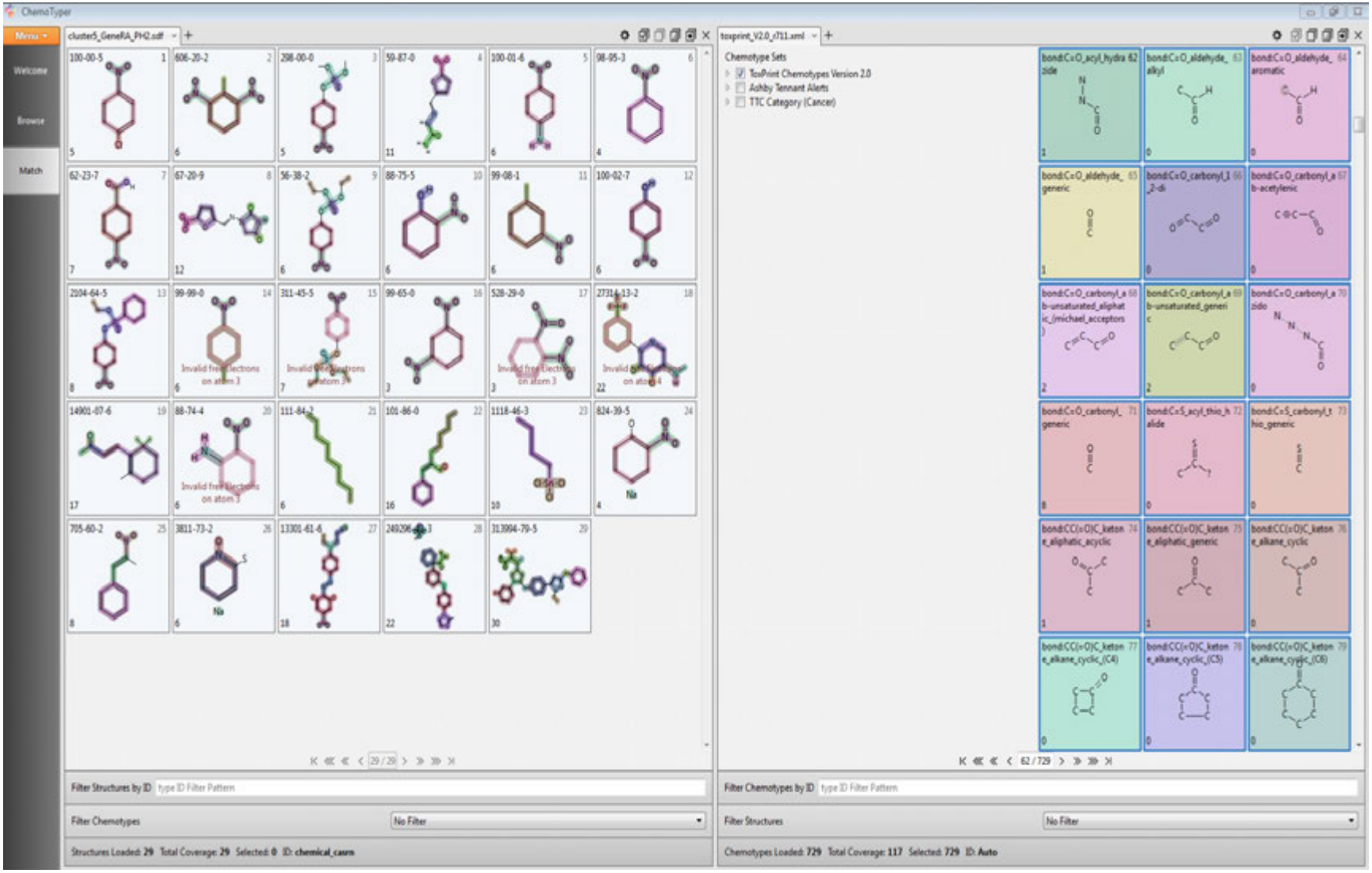
Acute		1	1	0	0
Chronic	1		1		1
Sub-acute		1	0		
Reproductive			0	0	0

Select Chemical and Predict

Predict

- ( ) CBRA
- (•) kNN
- ( ) GeneRA

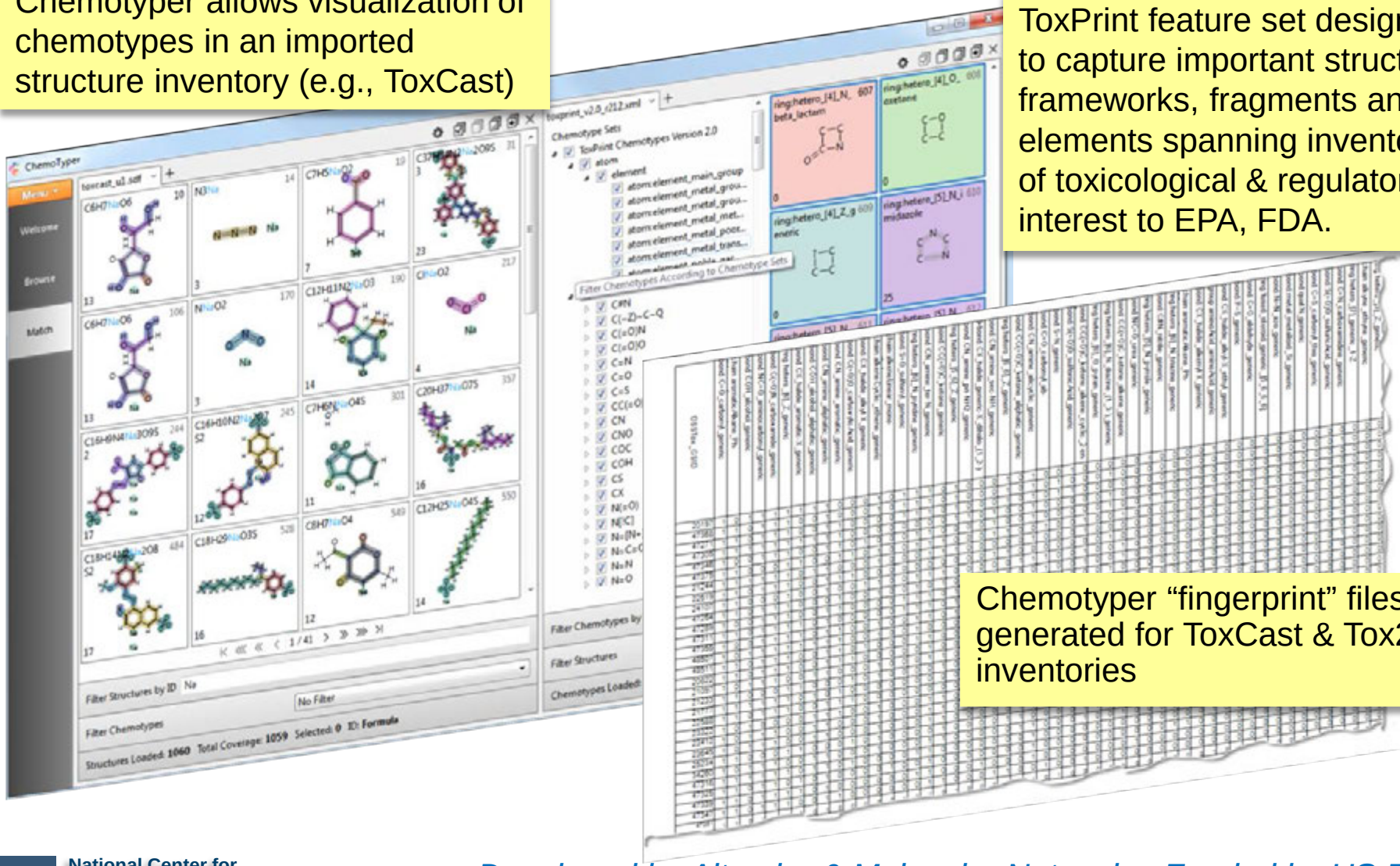
Using chemotypes for clustering



Now Investigating Public Resources: Chemotyper & ToxPrint Chemotypes

Chemotyper allows visualization of chemotypes in an imported structure inventory (e.g., ToxCast)

ToxPrint feature set designed to capture important structural frameworks, fragments and elements spanning inventories of toxicological & regulatory interest to EPA, FDA.



Chemotyper “fingerprint” files generated for ToxCast & Tox21 inventories

Developed by Altamira & Molecular Networks, Funded by US FDA

Summary

- Future non-testing approaches could be constructed into IATA underpinned by AOPs
- 2 case studies for skin sensitization and genotoxicity investigated
- We believe read-across within category and analogue approaches could be enhanced with mechanistic information – either through AOPs or from bioactivity information
- Software tools are in development and will allow for expert judgement using existing SARs or new SARs from chemotypes