

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



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National Center for Computational Toxicology

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Today's talk...

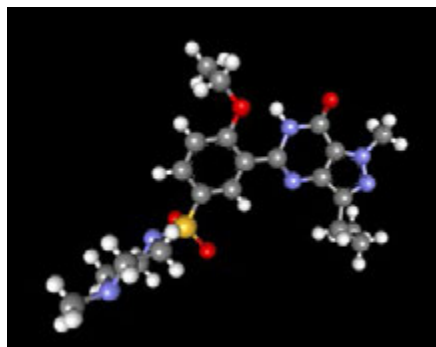
- Provide overview of current and future states of non-testing development & application
- Review application of AOPs and scientific confidence framework
- Show examples of non-testing approaches to AOP informed **Integrated Approaches to Testing and Assessment (IATA)**- skin sensitization and genotoxicity
- 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

Activity = Function



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

Chemical grouping

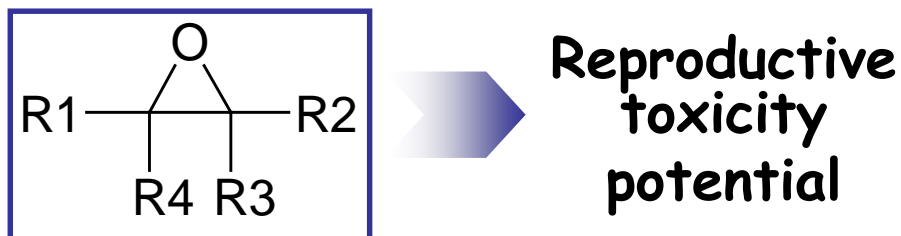
(Q)SARs

Less Formalized
in structure

More Formalized
in structure

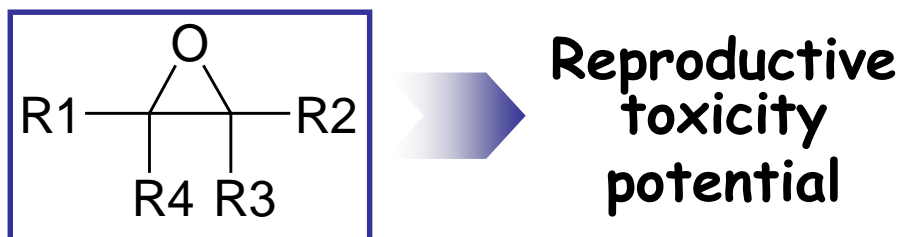
The (Q)SAR concept

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



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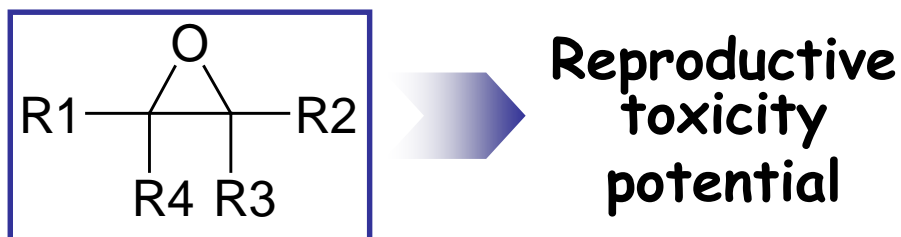


- **QSAR:** statistically established correlation relating quantitative parameter(s) derived from chemical structure or determined by experimental chemistry to a quantitative measure of biological activity

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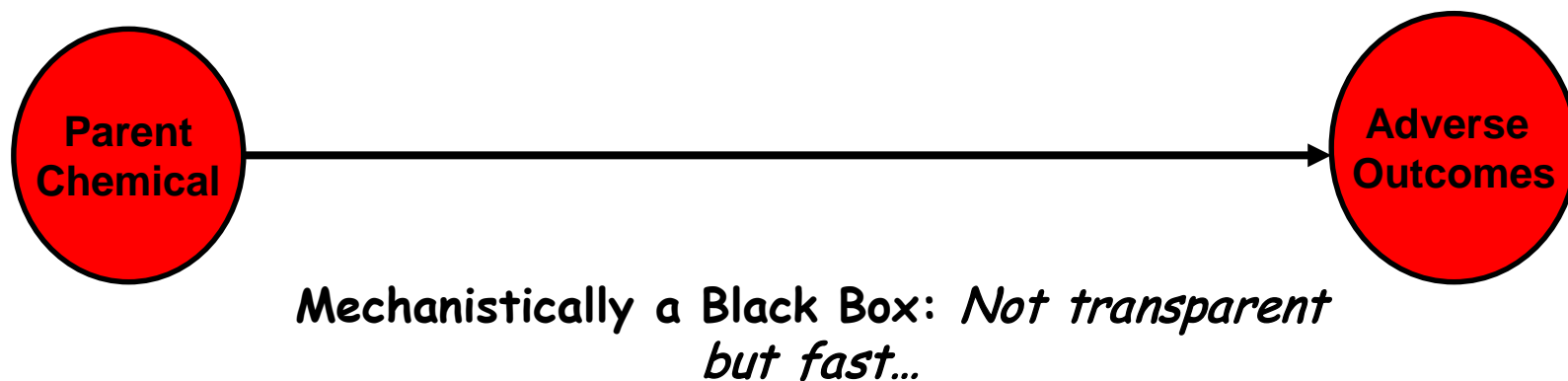
$$\text{Log (1/EC3)} = 0.25 + 0.28 * \text{LogP} + 0.86 * R_s$$

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

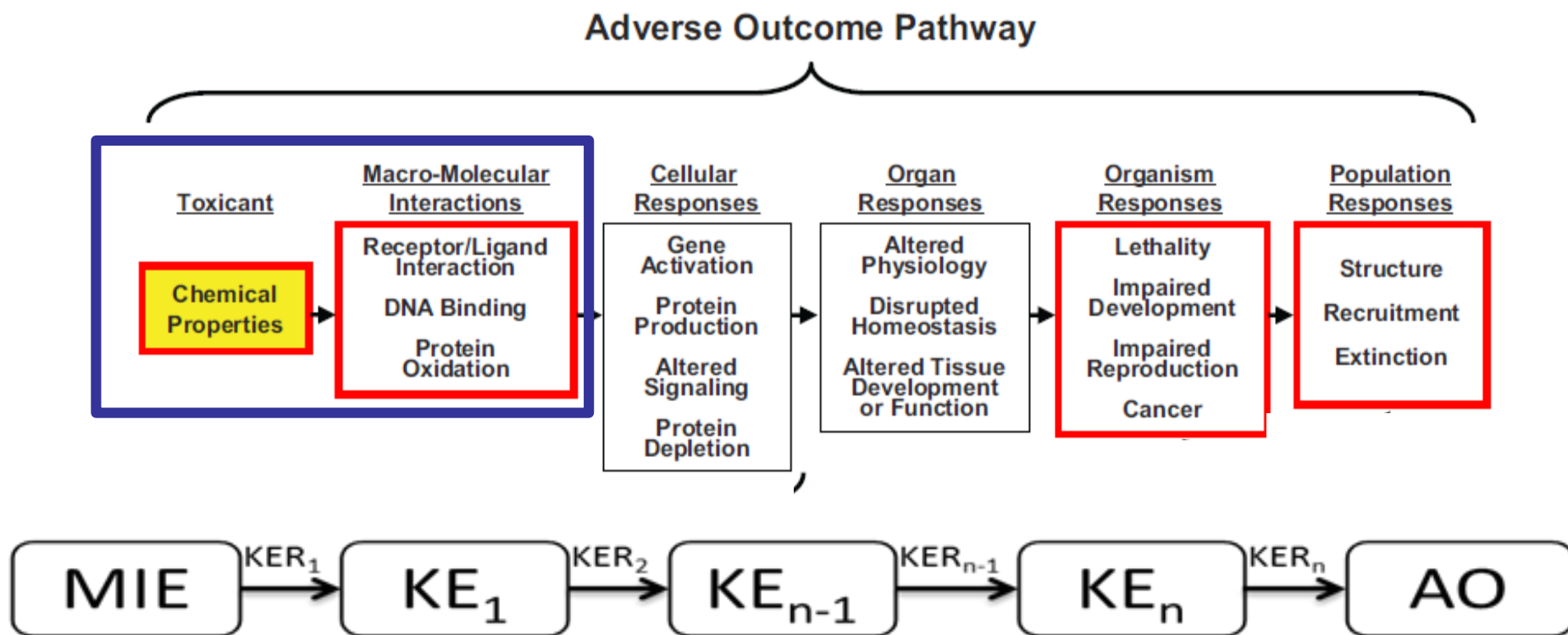
Chemical grouping approaches

- “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

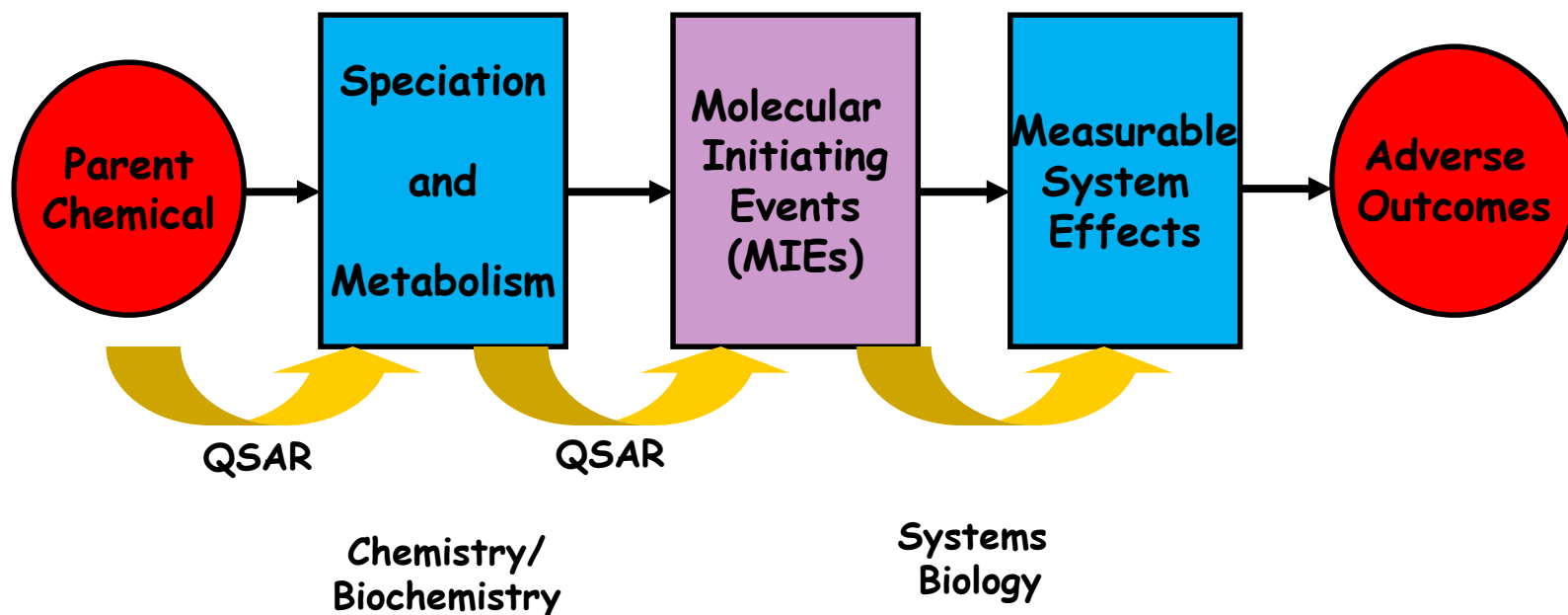


AOP - framework for developing non-testing approaches differently....



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

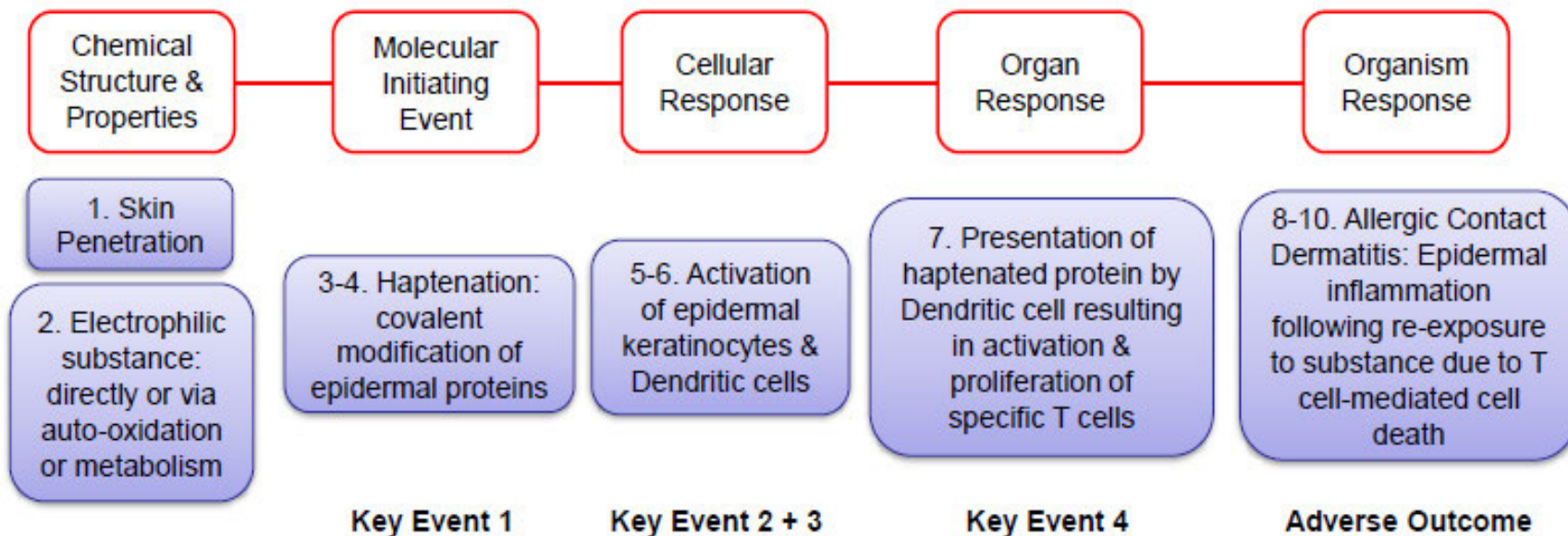
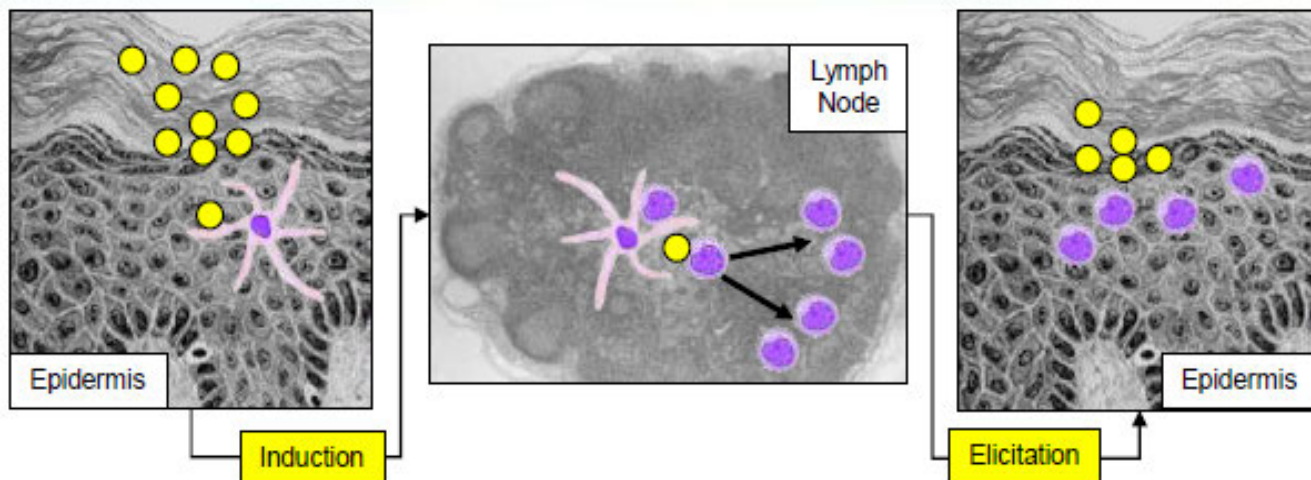
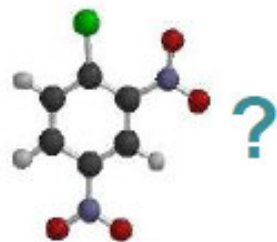
Applications of AOPs

- 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

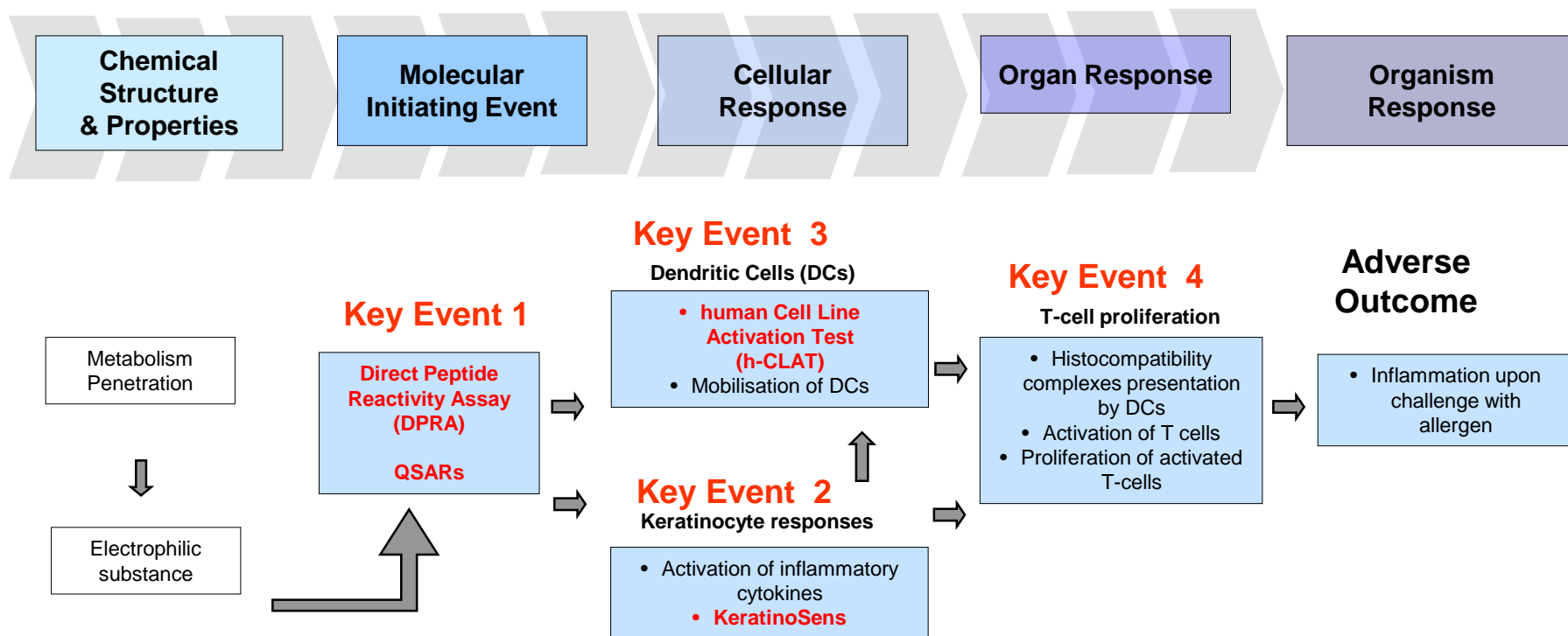
Establishing Scientific Confidence in the application of AOPs (GRACE)

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, <i>chemical category formation, integrated testing</i> , predicting <i>in vivo</i> 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.

Skin sensitization AOP (OECD, 2012)



Mapping methods to key events



Related Work: Data Generation

ToxCast HTS screening

ToxCast™

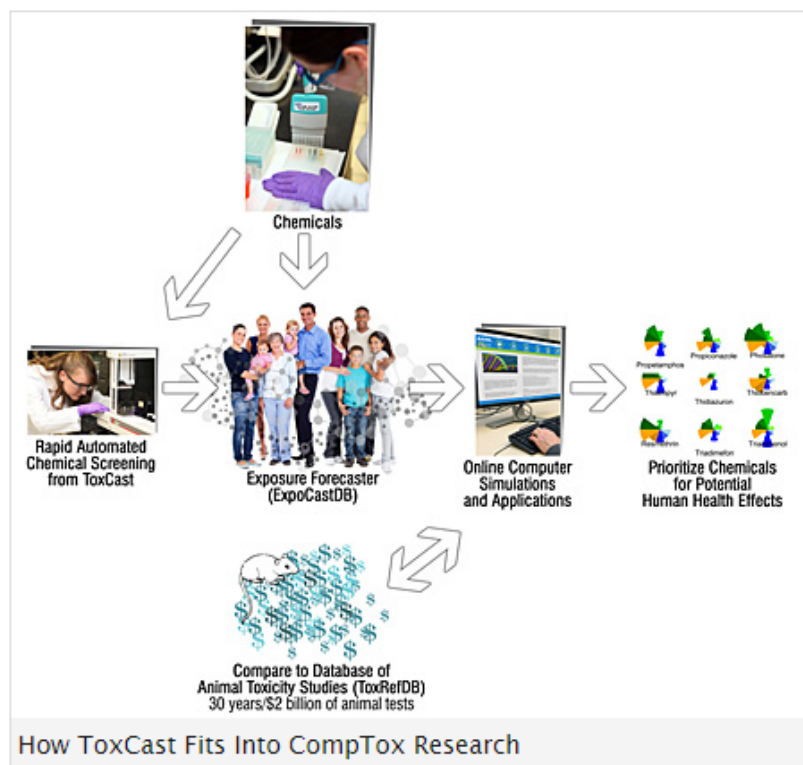
Advancing the next generation of chemical safety evaluation

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.

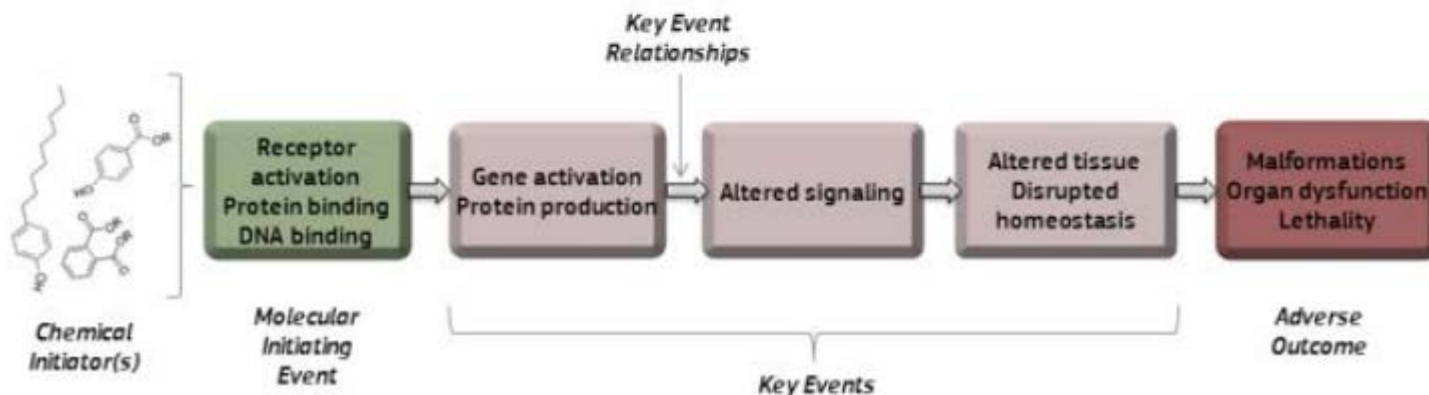


AOP Wiki

Community AOP Development



CHEMICAL SAFETY RESEARCH: ADVERSE OUTCOME PATHWAYS



AOP Wiki

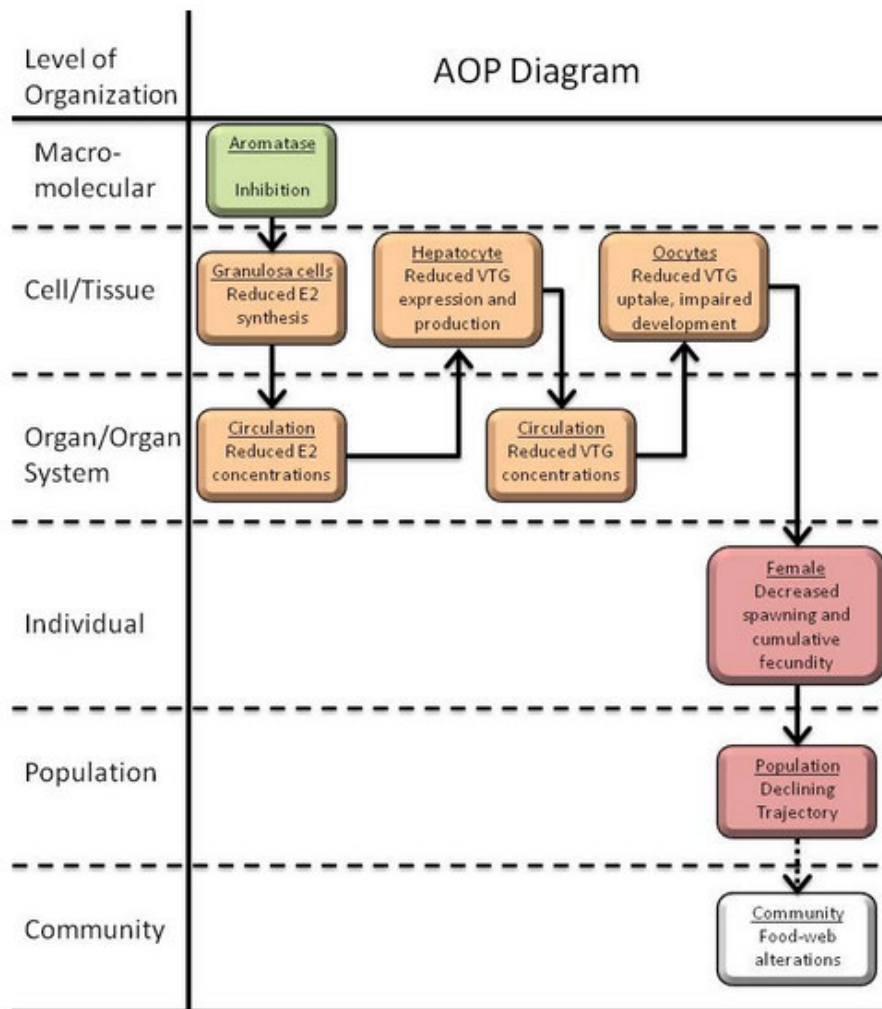
Community AOP Development

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

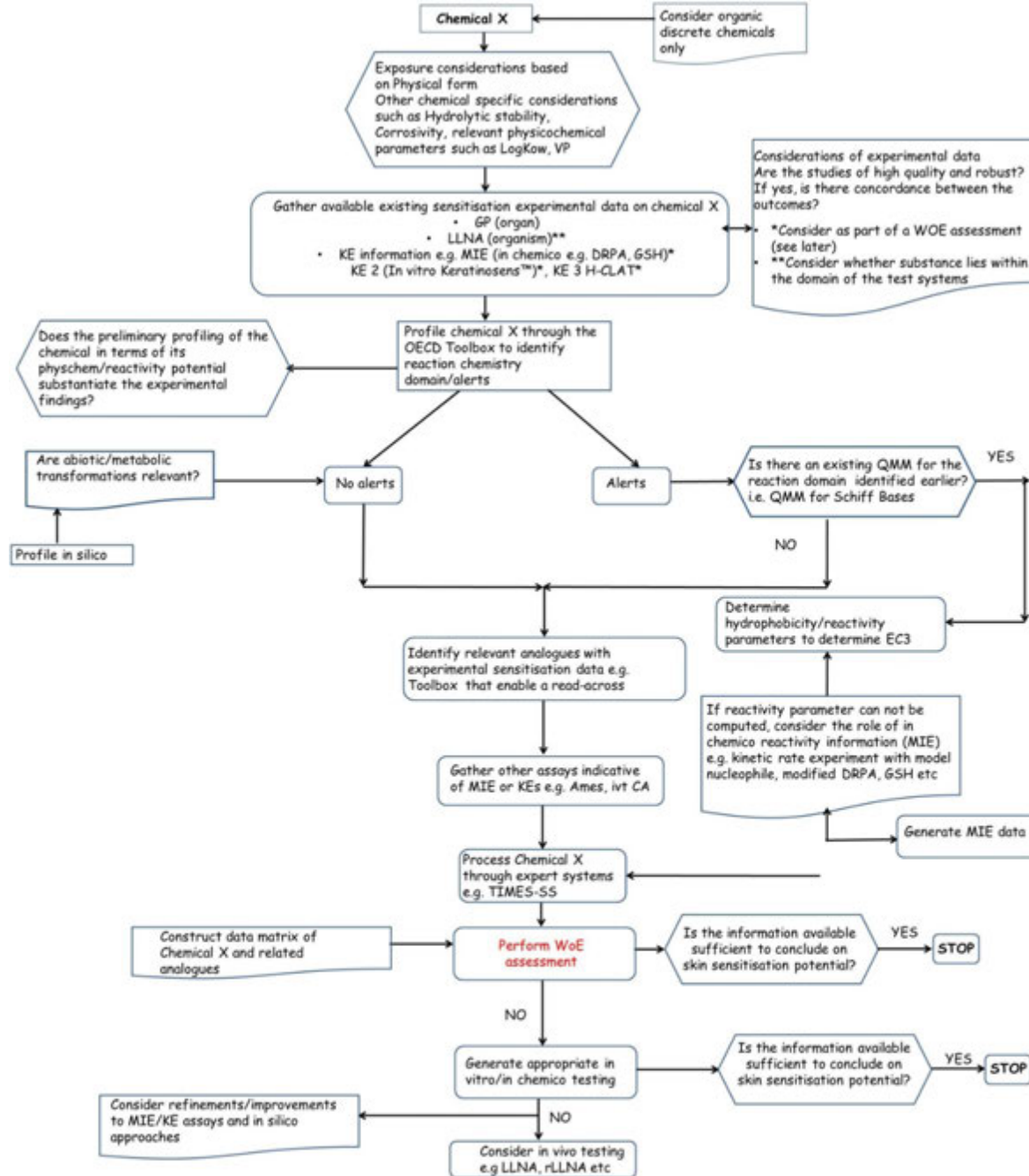
AOP Wiki

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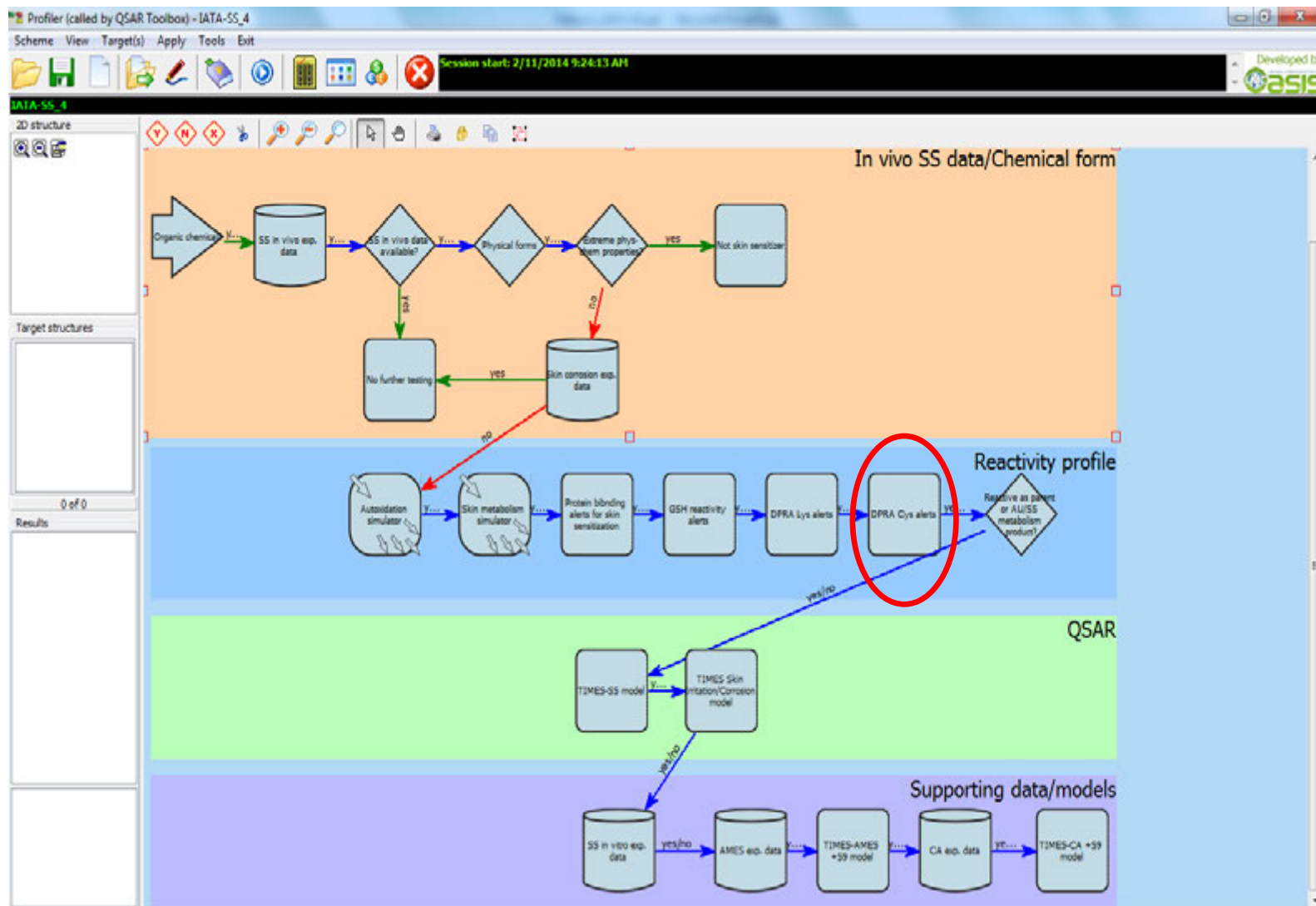


IATA for Skin Sensitization IATA-SS

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

EPA Cysteine peptide depletion (General Mechanistic) - Profiling Scheme Browser

Advanced


EPA Cysteine peptide depletion - Category definitions

- High reactive**
 - Activated haloarenes
 - alpha,beta-carbonyl compounds with polarized multiple bond
 - Aromatic C-H bonds compounds
 - Benzyl halides
 - Cyclopropanones
 - Dialkylperoxides
 - Di-methacrylic acid esters
 - Halogenated isothiazolones
 - Halo-substituted dinitriles
 - Sulfonamide derivatives
 - Organic disulfides
 - Quinones and quinone (di)imines
 - Thiols
 - Unsaturated acid anhydrides
 - Vinyl pyridines
- Low reactive**
 - Acyl halides
 - Alcyclic ketones
 - alpha-alkyl cinnamaldehyde derivatives
 - Long-chain aliphatic aldehydes
 - N-substituted aromatic amides
 - Primary haloalkanes
 - Saturated acid anhydrides
 - Special lactones
 - Sulfonic acid derivatives
- Moderate reactive**
 - 1,2-Dicarbonyl compounds
 - Activated 1,3,5-triazine derivatives
 - Asialactones
 - Five-membered heterocyclic urea
 - Glycidyl ether epoxides
 - Mono-methacrylic acid esters
 - Phenyl substituted cinnamaldehydes
 - Saturated aldehydes

Profile Description

Potency category: High reactive

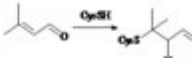
Chemical class: alpha,beta-carbonyl compounds with polarized multiple bond - **Under development**



$R_1 = H, C(sp^2), C(sp^3) \quad R_2 = -OC, C, S, N$

The possible mechanism of interaction of this structural alert with SH-group of Cysteine peptide is illustrated below:

Scheme 1:



Michael acceptors are double or triple bonds with electron-withdrawing substituent such as carbonyl group. Michael-type addition provides a means of covalent adduct formation at an electrophilic center, without any leaving group. Direct addition of a nucleophile can take place across a double or triple carbon-carbon bond if it is attached to a highly polarized substituent that permits the resultant negatively charged transition state to be stabilized. Compounds with double or triple bonds adjacent to a C=O group (in this case aldehyde-carbonyl group) are known as α,β -unsaturated carbonyl compounds. Nucleophiles will undergo conjugate additions with them. CHO-group profoundly affects the reactivity of the double bond. Proteins are good nucleophiles for conjugate addition reactions with these compounds. Any α,β -unsaturated aldehydes can potentially act by the Schiff base mechanism.

References:

1. Aptula A, Roberts D. Mechanistic applicability domains for nonanimal-based prediction of skin sensitization.
2. Roberts D.W., Patlewicz G., Kern P., Gerberick F., Kimber I., Dearman R.J., Ryan C.A.
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6. Canillo K. Smith Pease, From xenobiotic chemistry and metabolism to better prediction a
7. Canillo K. Smith, Sharon A.M. Hotchkiss, Allergic Contact Dermatitis: Chemical and Me

Deriving SARs from chemicals tested in an assay characterizing the MIE


EPA Cysteine peptide depletion (General Mechanistic) - Profiling Scheme Browser

Advanced

EPA Cysteine peptide depletion - Category definitions

High reactive


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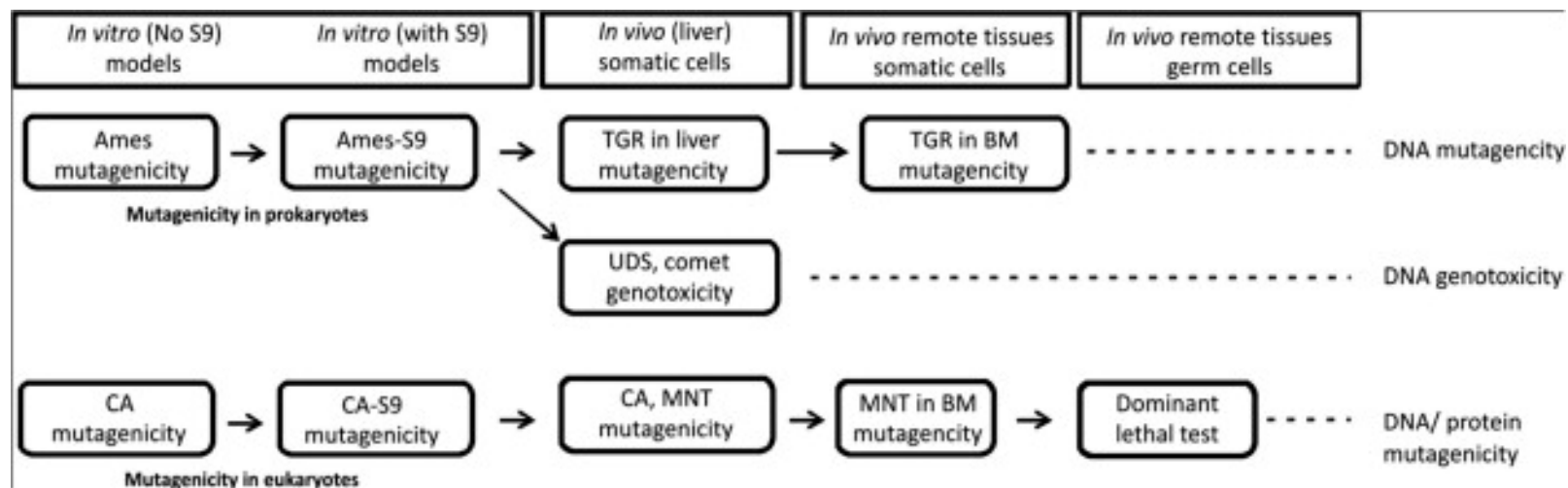
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National Center for
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IATA for genetox - work in progress

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

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



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:


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

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

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

Shah et al, in prep

Software development in progress


EPA ReadAcross Explorer

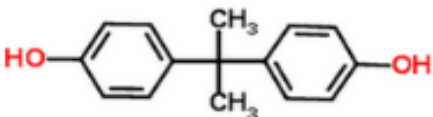
[About RAE](#)
[Tutorial](#)
[History](#)


Input Chemical:
 or

☒ Single-Component Structures Only
 ☒ Ignore isotopically labeled structures
 Maximum Hits

Chemical Information

Name: Bisphenol A
 CASRN: 80-05-7
 GSID: [4085603](#)





Toxicity Categories

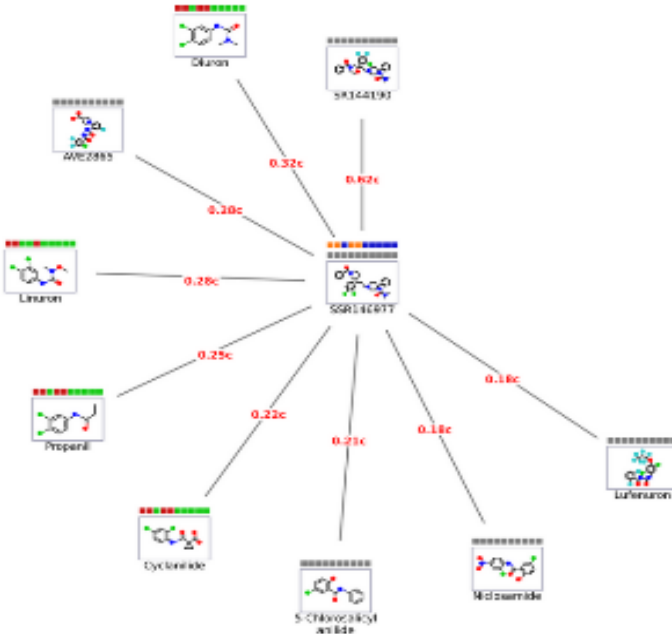
- Acute
- Chronic
- Sub-acute
- Reproductive
- Developmental
- Neurotox
- Develop. Neuro.
- Multigenerational

Select Descriptor Space

☒ Chemical Space
☐ Biological Space
☐ Hybrid

Display Chemicals

10

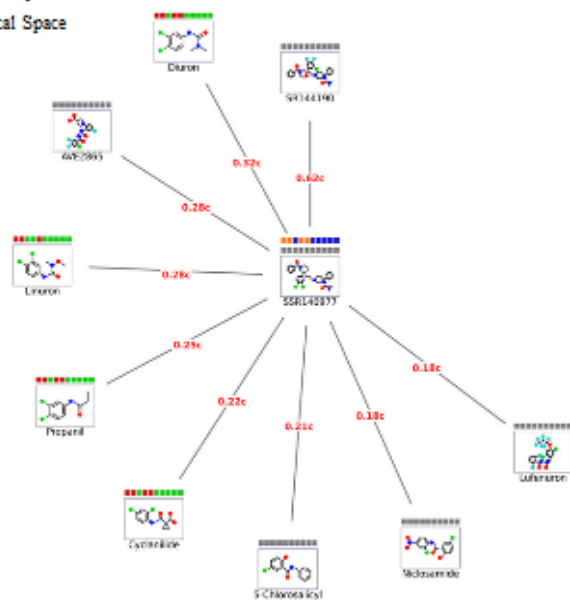


Software development in progress

EPA ReadAcross Explorer

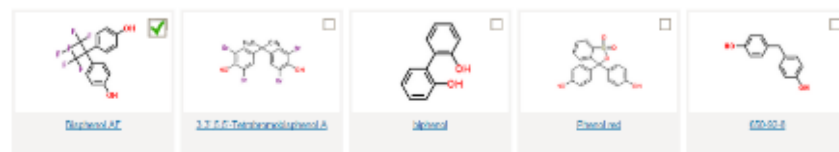
Select Descriptor Space

- ☒ 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

ChemoTyper

cluster5_GeneRA_P102.sdf

Chemotype Sets

- ☒ ToxPrint Chemotypes Version 2.0
- ☐ Ashby Tennant Alerts
- ☐ TTC Category (Cancer)

Chemotype Sets

- bondC=O_acyl_hydra 62
- bondC=O_aldehyde_63
- bondC=O_aldehyde_64
- bondC=O_aldehyde_65
- bondC=O_aldehyde_66
- bondC=O_aldehyde_67
- bondC=O_aldehyde_68
- bondC=O_aldehyde_69
- bondC=O_aldehyde_70
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- bondC=O_aldehyde_97
- bondC=O_aldehyde_98
- bondC=O_aldehyde_99
- bondC=O_aldehyde_100

Filter Structures by ID type ID Filter Pattern

Filter Chemotypes

Structures Loaded: 29 Total Coverage: 29 Selected: 0 ID: chemical_casrn

Filter Chemotypes by ID type ID Filter Pattern

Filter Structures

Chemotypes Loaded: 729 Total Coverage: 117 Selected: 729 ID: Auto

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.

The screenshot displays the Chemotyper application window. On the left, a sidebar contains 'Menu', 'Welcome', 'Browse', and 'Match' buttons. The main area is a grid of chemical structures, each with a label and a count. For example, 'C6H7N1O6' appears 13 times, 'N1O2' appears 3 times, and 'C12H11N2O3' appears 190 times. Below the grid, there are filters for 'Filter Structures by ID' (set to 'Na') and 'Filter Chemotypes'. At the bottom, it shows 'Structures Loaded: 1660' and 'Total Coverage: 1059'. On the right, a 'Chemotype Sets' panel lists various structural features like 'atom', 'element', and 'ring_hetero'. Below this, a 'Filter Chemotypes According to Chemotype Sets' section shows a list of features with checkboxes, many of which are checked. At the bottom right, a 'Chemotypes Loaded' section shows a list of chemotypes with their counts.

Chemotyper "fingerprint" files generated for ToxCast & Tox21 inventories

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