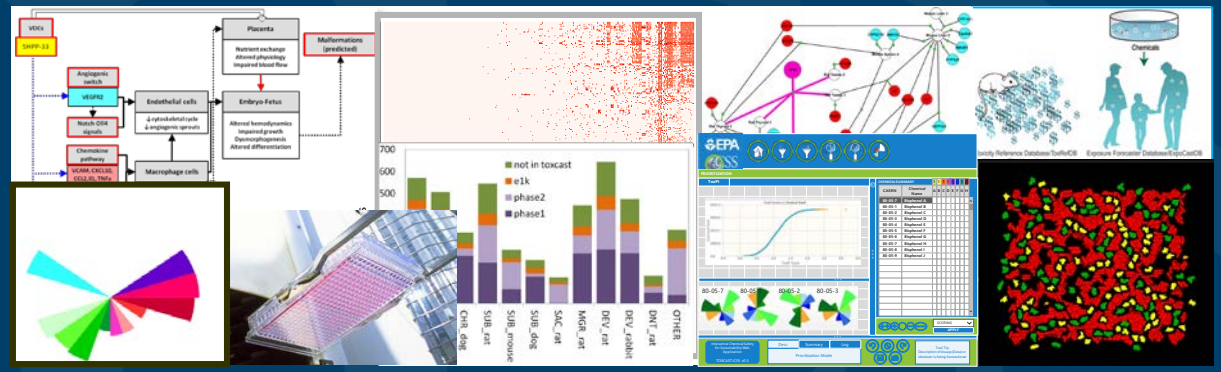
EPA United States Environmental Protection Agency

Toxicity Testing in the 21st Century and NexGen Risk Assessments



Grace Patlewicz
National Center for Computational Toxicology (NCCT), US EPA

Outline

- Regulatory Drivers
- Integrated Approaches to Testing and Assessment (IATA) – definitions and Adverse Outcome Pathway (AOP) informed
- Decision contexts and their impact on the approaches applied
- Practical workflow – where and what approaches can be used
  - Using the US EPA Chemistry Dashboard
- Summary remarks

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 New Approach Methodologies (NAM) to reduce the extent of experimental testing in animals.

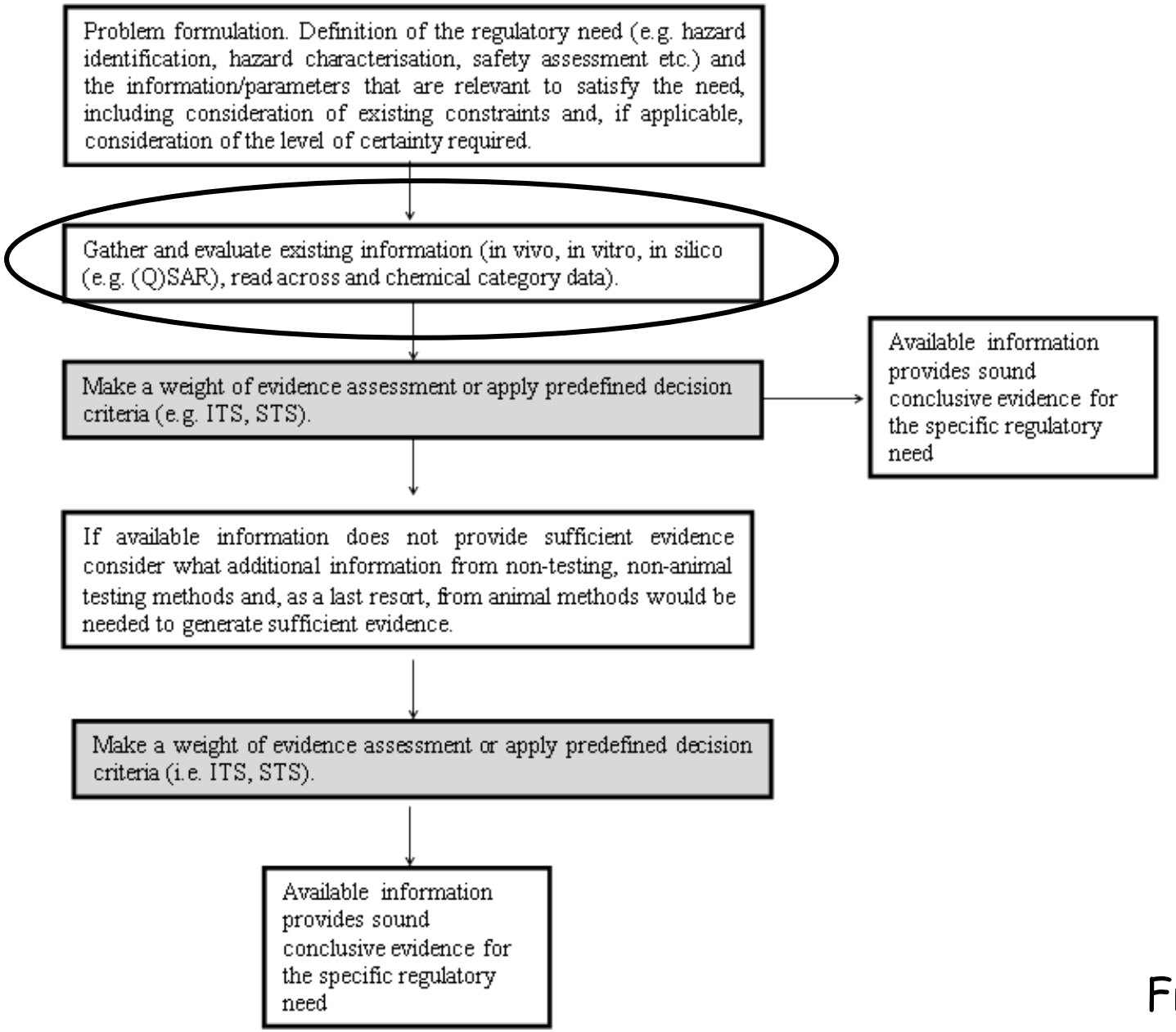
Regulatory drivers

- 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 prioritisation is also an important aspect of regulatory frameworks in Canada (the Domestics Substance List), Australia and the EU.
- NAM offer a means of facilitating the regulatory challenges in chemical safety assessment

Integrated Approaches to Testing and Assessment (IATA)

- A means of integrating existing data and non-testing data together, determining what new information needs to be generated in order to make a decision with sufficient confidence for the purpose in mind
- IATA can be likened to workflows depicting the steps of gathering information for a substance and evaluate its fitness for purpose for the decision required
- Some IATA are more complex than others but the generic building blocks of considering existing data, NAM (i.e. *in vitro* methods, non-testing approaches) BEFORE instigating new *in vivo* testing are the same
- NAM fit within the context of these IATA schemes and should not necessarily be considered *in vacuo*

General framework of an IATA



From OECD

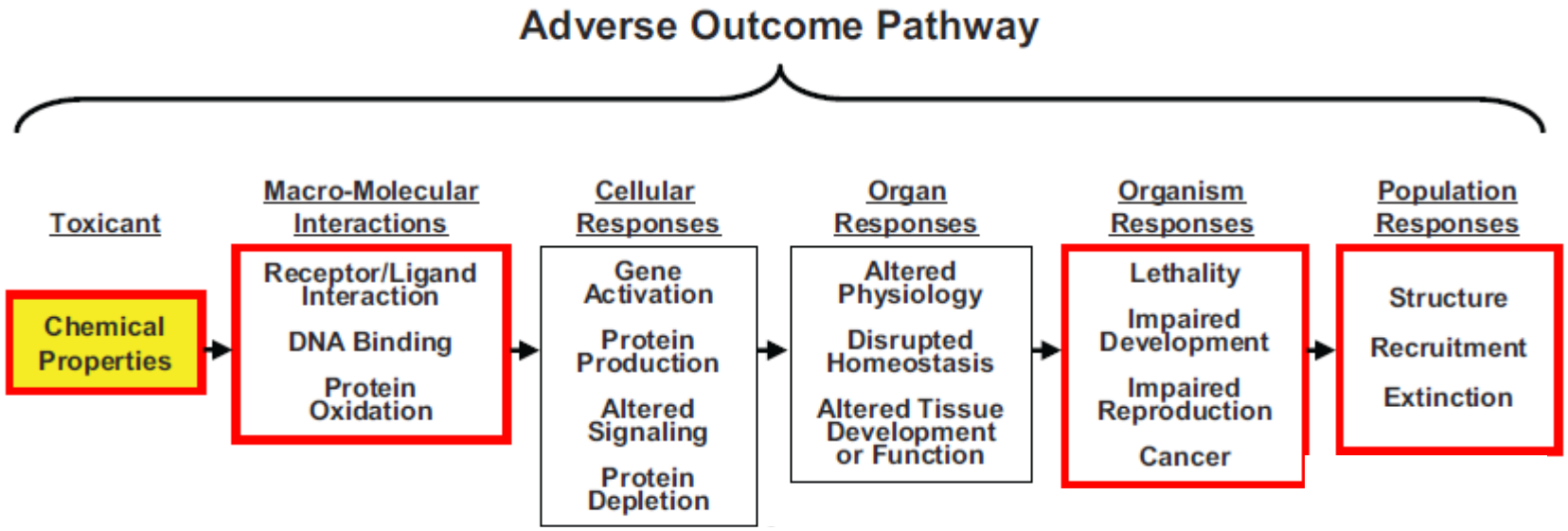
Typical Information within an IATA

- 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

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

AOPs



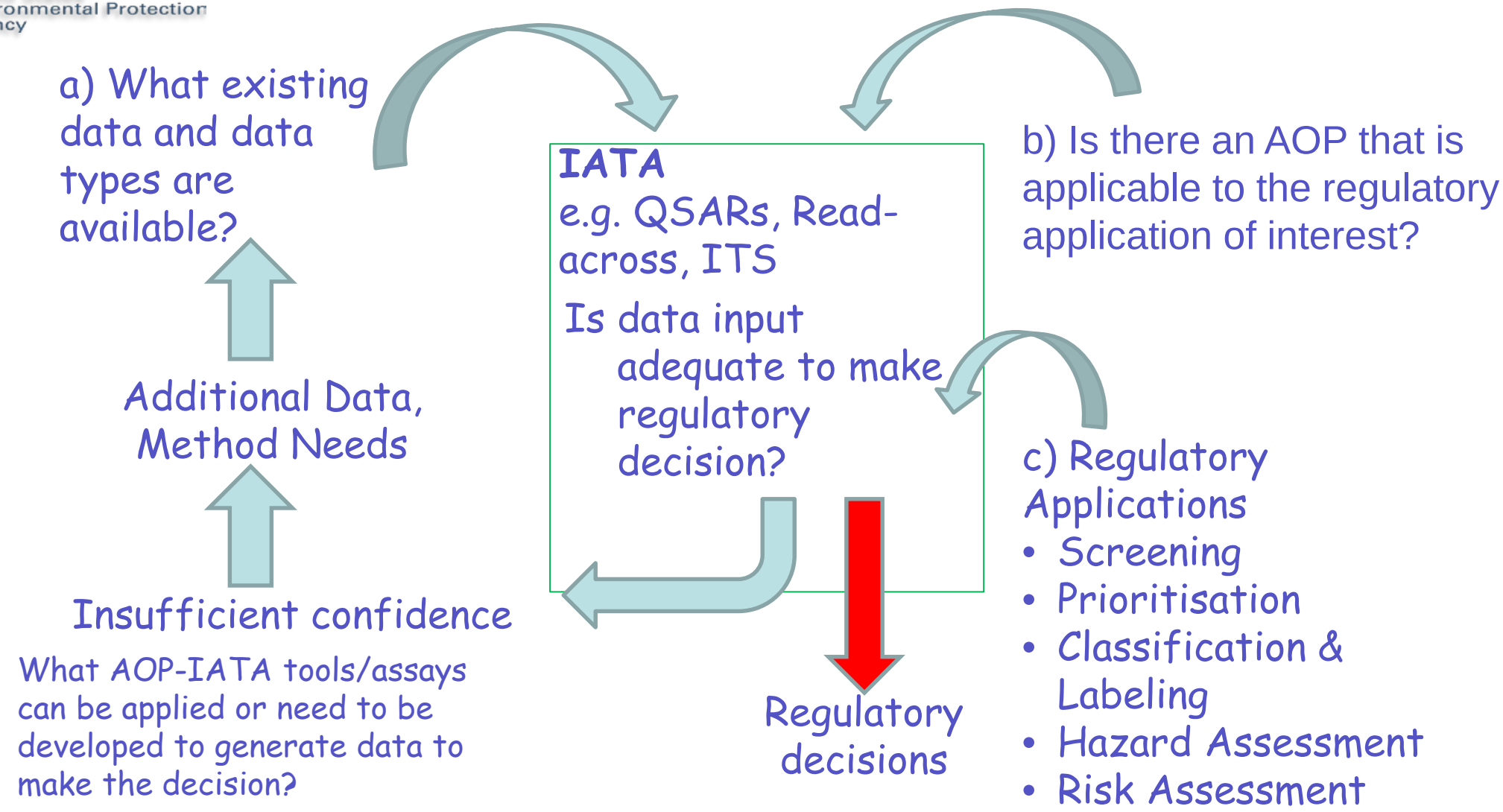
An AOP represents existing knowledge concerning the sequence of events and causal linkages between initial molecular events, ensuing key events and an adverse outcome at the individual or population level.

Establishing Scientific Confidence in the application of AOPs for IATA

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.

AOP-informed IATA



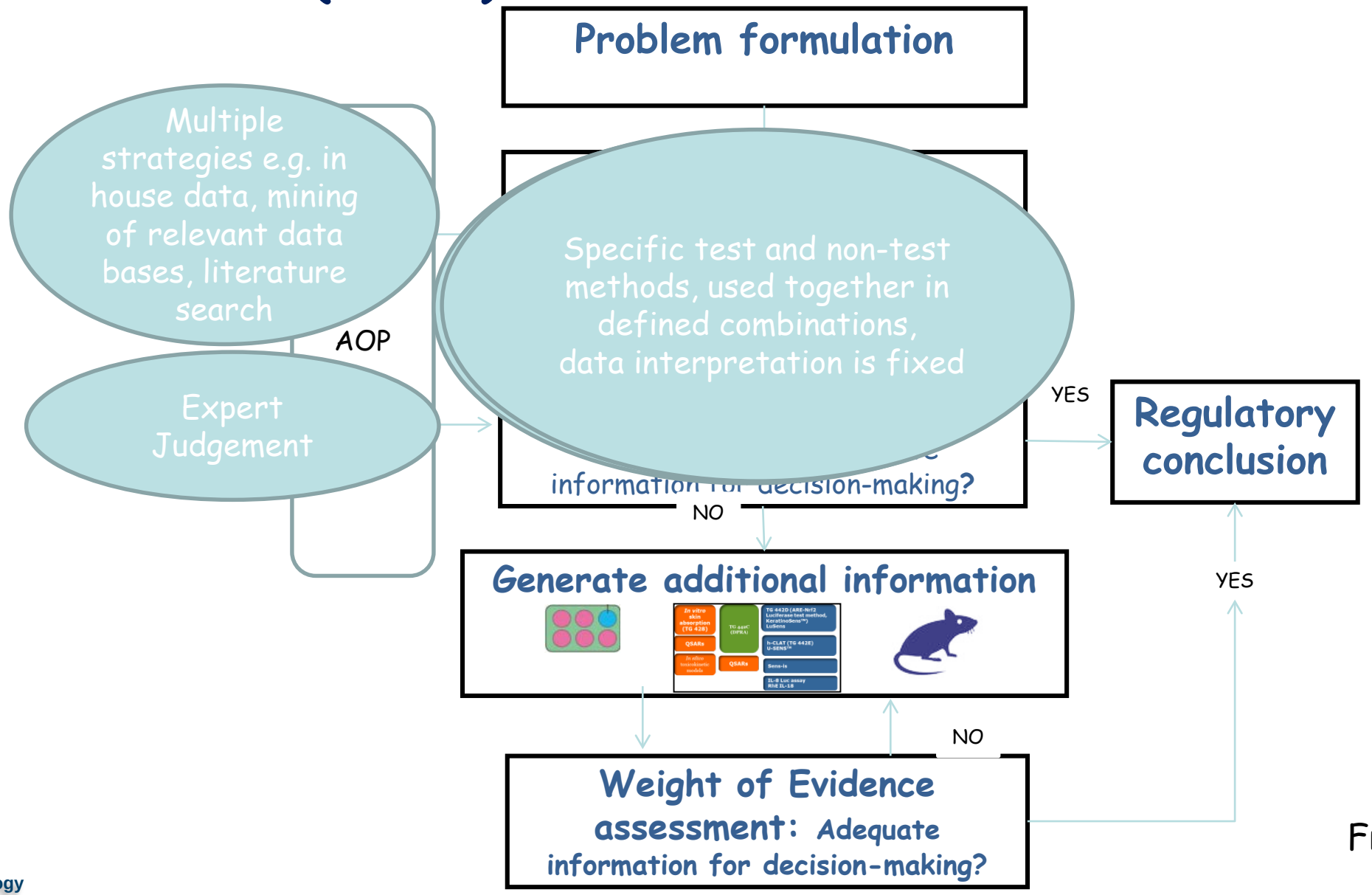
Tollefsen et al, 2014

Scientific confidence considerations for IATA

- Proposed validation principles:
  - define the endpoint being assessed;
  - define the purpose/application for which the IATA is proposed;
  - describe the rationale underlying the construction of the IATA;
  - describe how the individual information sources constituting the IATA are integrated to derive the final prediction/assessment and,
  - describe the predictive capacity of the approach, the limitations in the application of the approach and the known uncertainties associated with the IATA application.

Worth and Patlewicz 2016

General workflow in Integrated Approaches to Testing and Assessment (IATA)



From OECD

Defined approaches within IATA

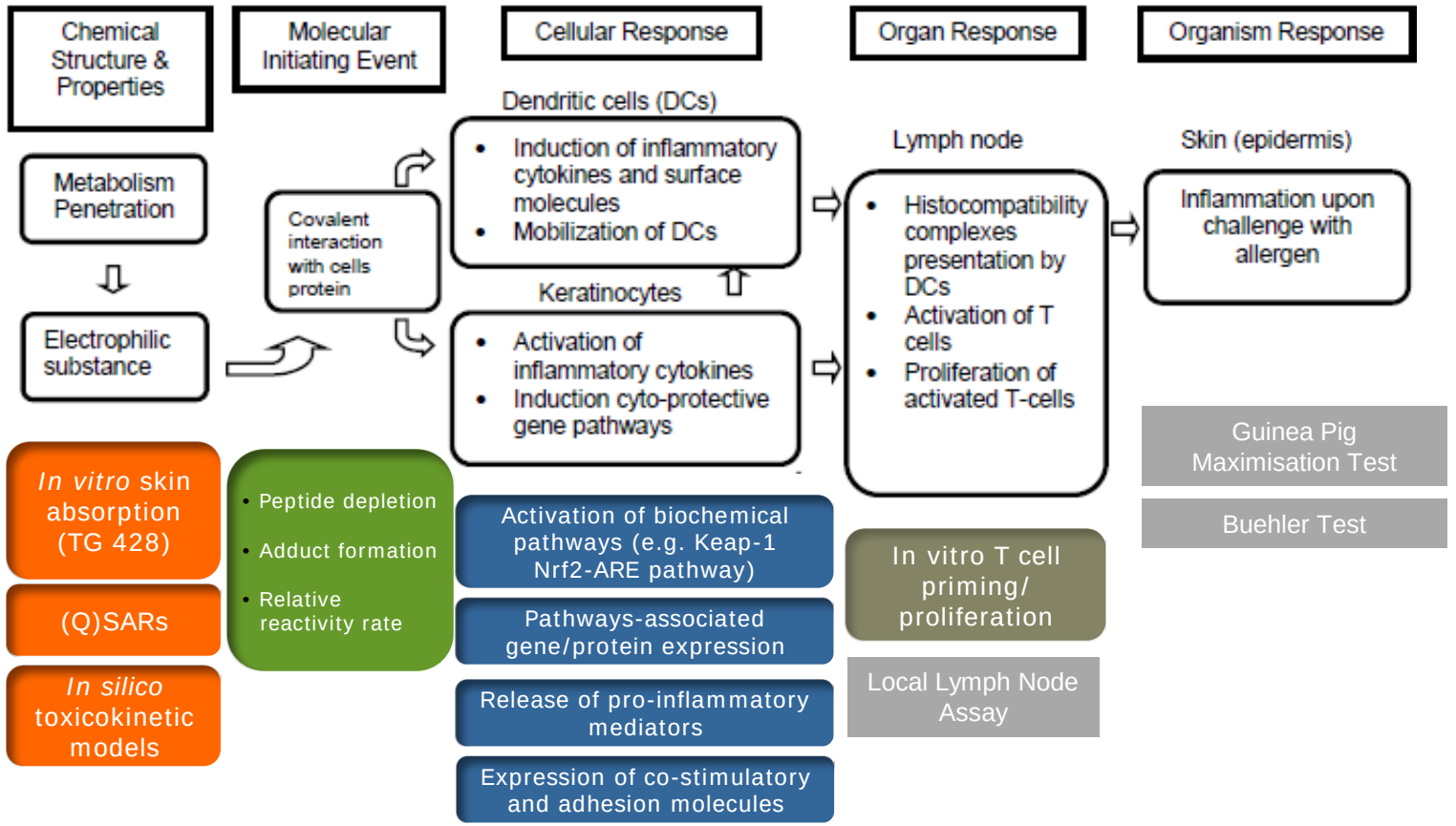
- A defined approach to testing and assessment consists of a fixed data interpretation procedure (DIP) used to interpret data generated with a defined set of information sources, that can either be used alone or together with other information sources, to satisfy a specific regulatory need.
  - Guidance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

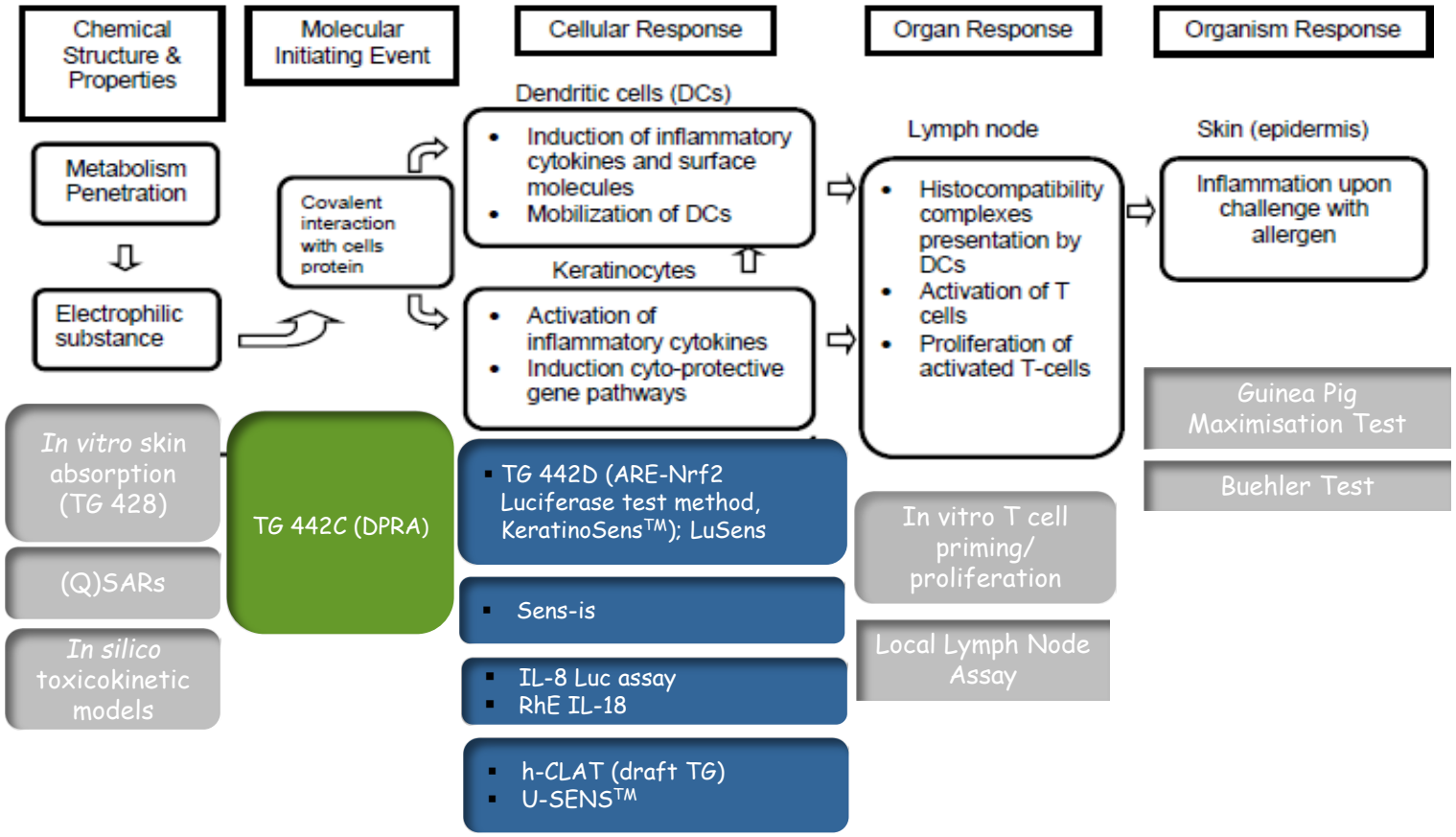
Defined approaches within IATA: Skin sensitisation

AOP and available toolbox of non-animal methods



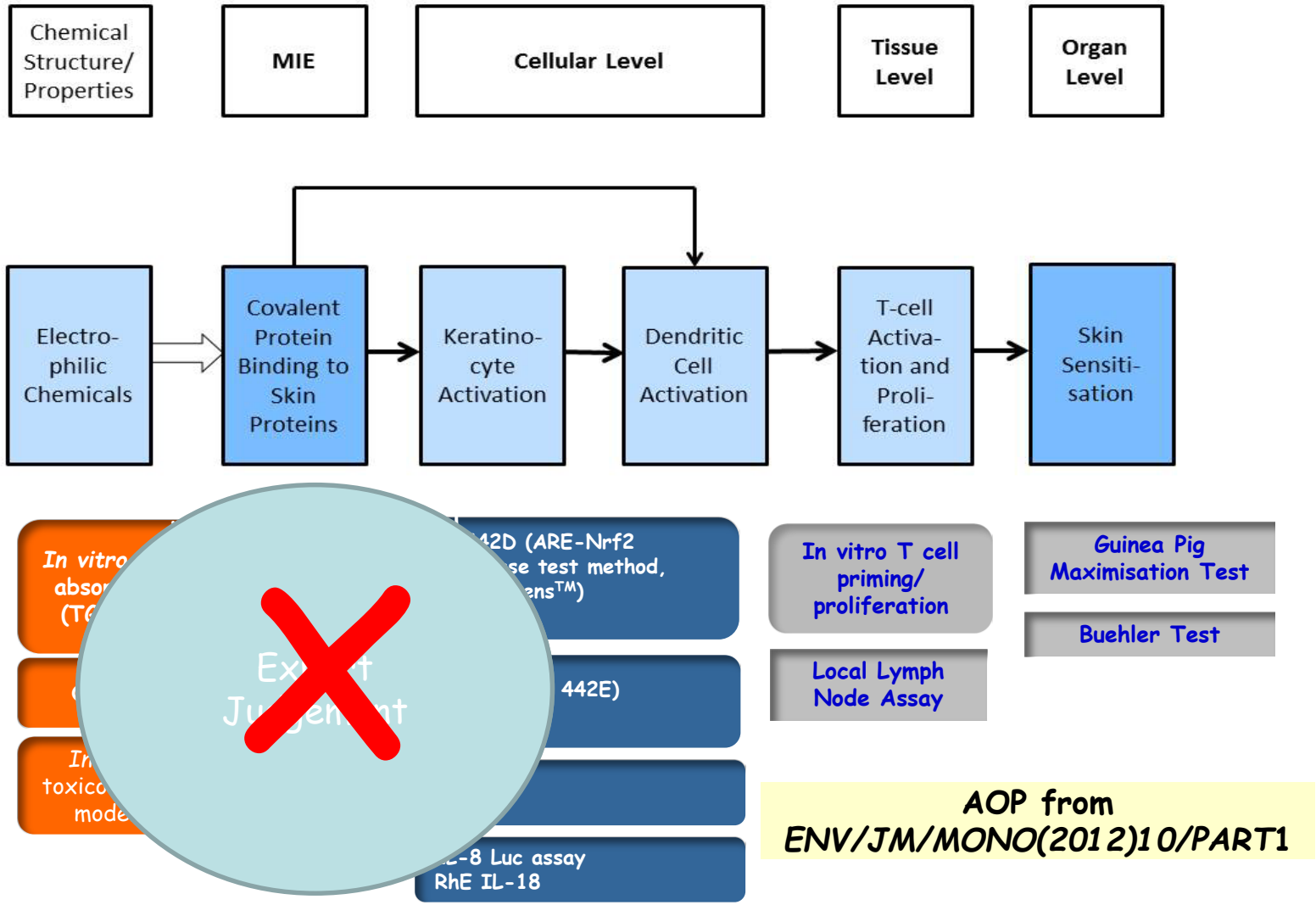
Presented by S Casati, JRC

Defined approaches within IATA: Skin sensitisation (SS)



Presented by S Casati, JRC

Defined approaches within IATA-SS



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https://aopwiki.org/wiki/index.php/Aop:40

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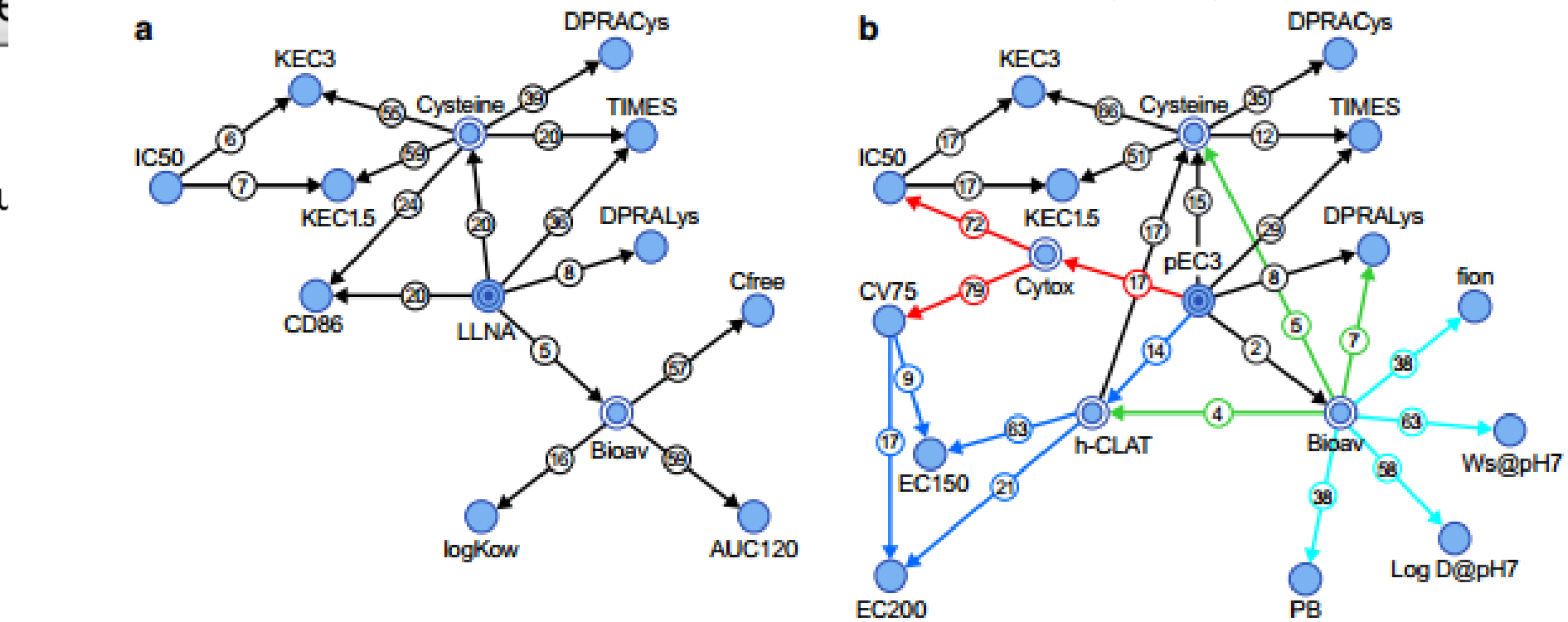
Defined approaches for skin sensitisation

Bayesian Networks for
Skin sensitization
Jaworska et al (2015)



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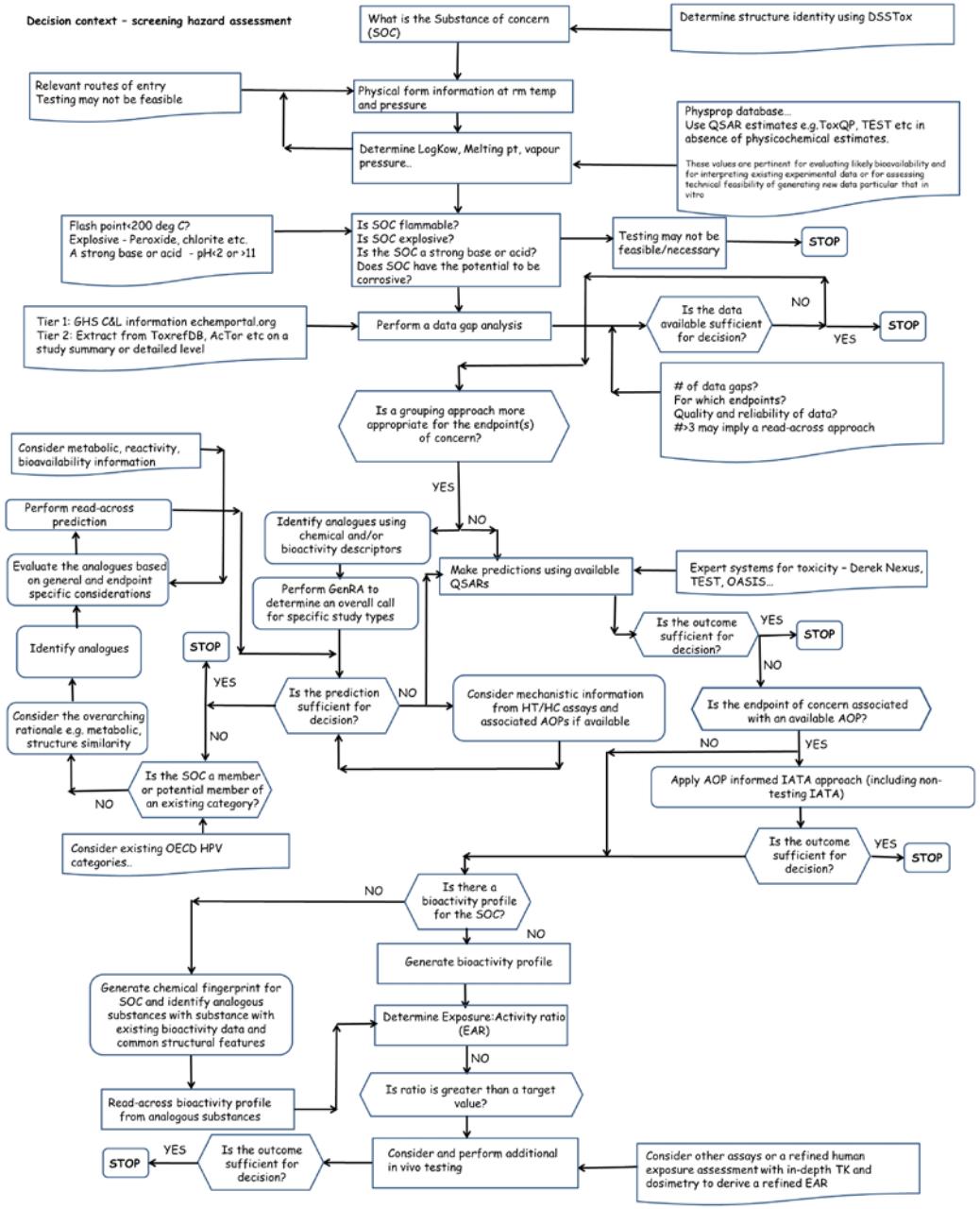
IATA in practice

- What is the Substance of concern (SOC)?
- What is already known about the SOC?
- What is the Decision context?

An Assessment workflow



Patlewicz and Fitzpatrick 2016

An Assessment workflow

Data gap analysis

Strategies for filling the data gaps using read-across

Strategies for filling data using QSARs, AOP-informed DA etc.

Strategies for filling data using bioactivity information

Patlewicz and Fitzpatrick 2016

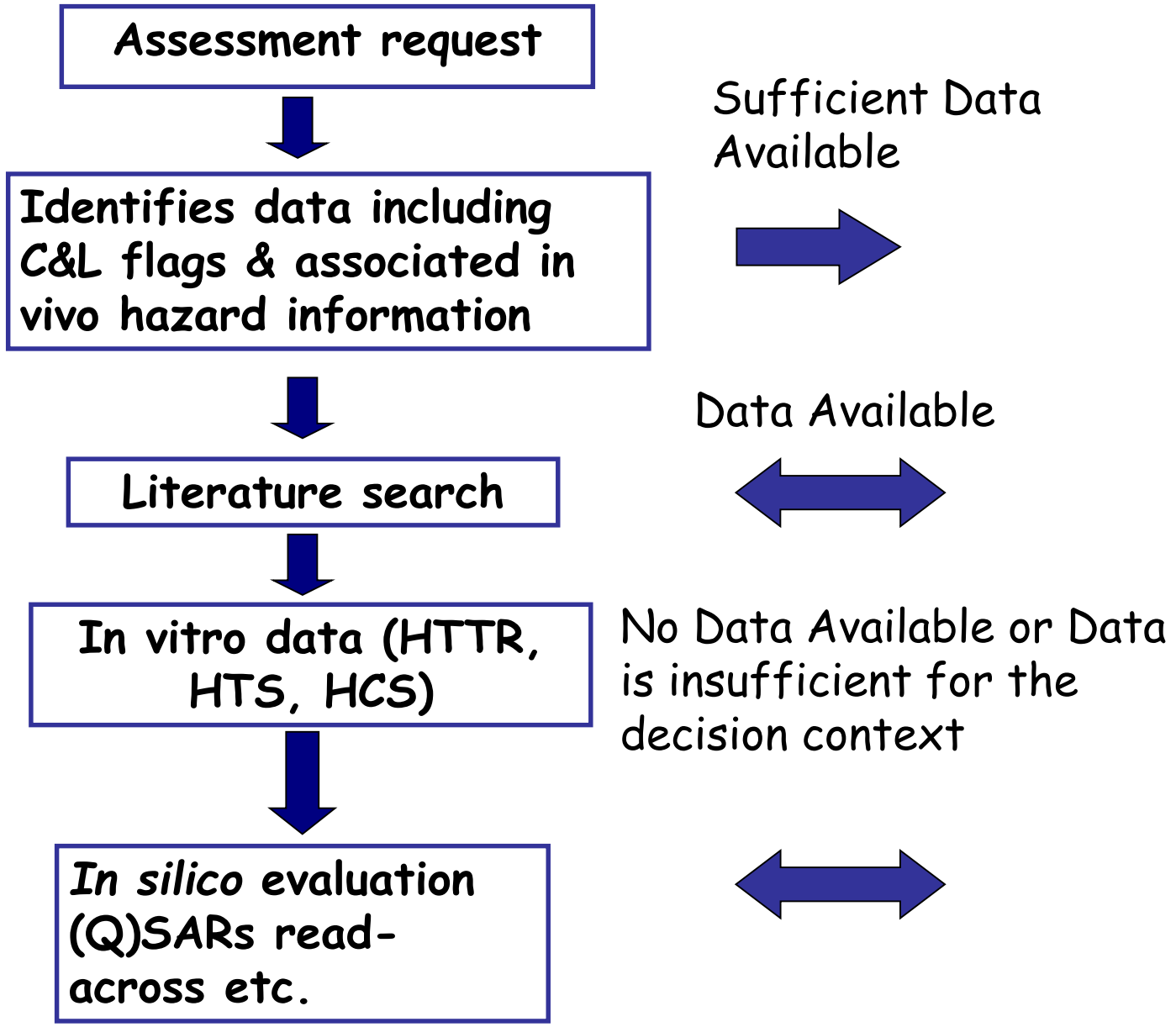
Decision contexts

- Prioritisa
- Screening
- Risk Asse

What do we know about our substance of interest..

A Data gap analysis is typically the first step

Data gap analysis



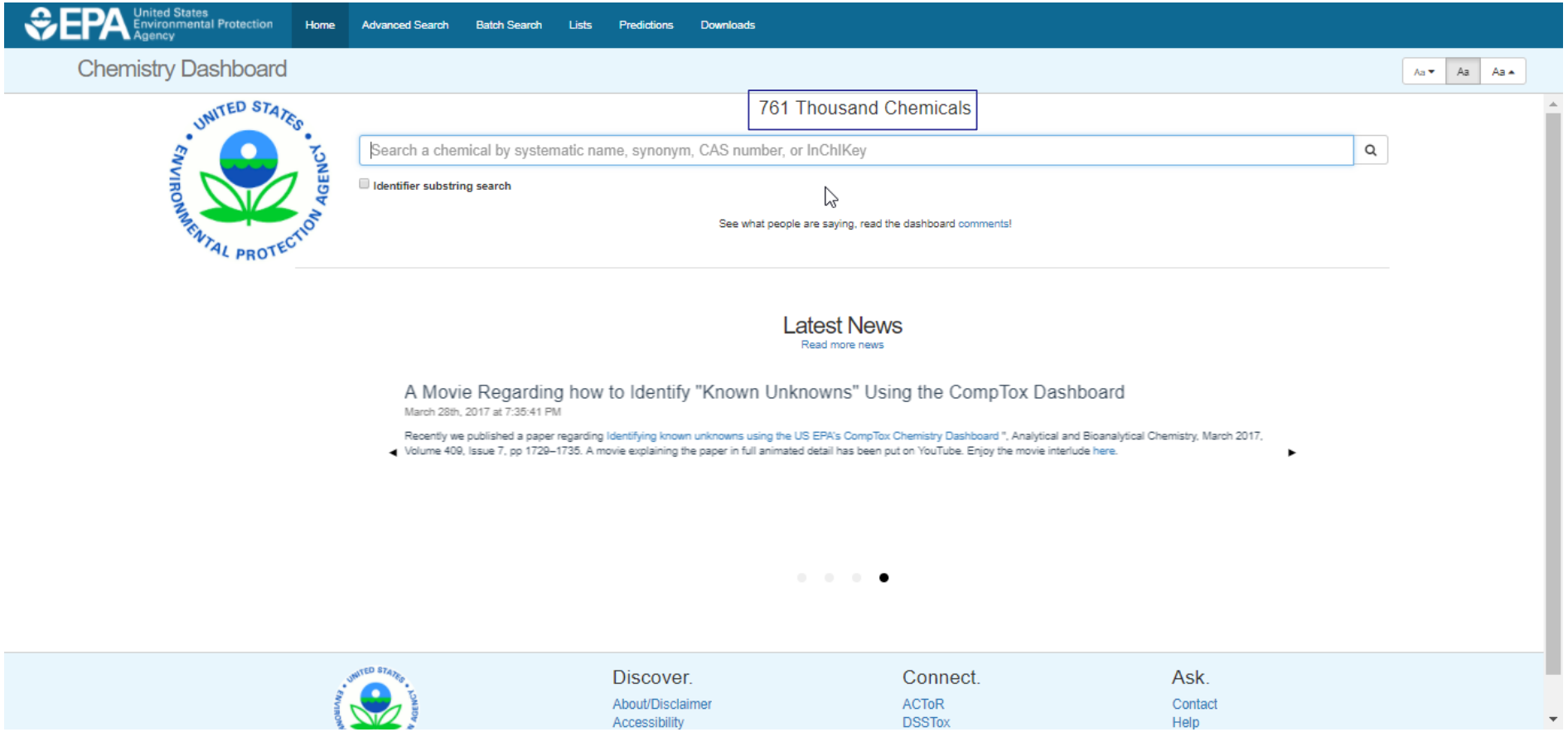
United States Environmental Protection Agency

The CompTox Chemistry Dashboard

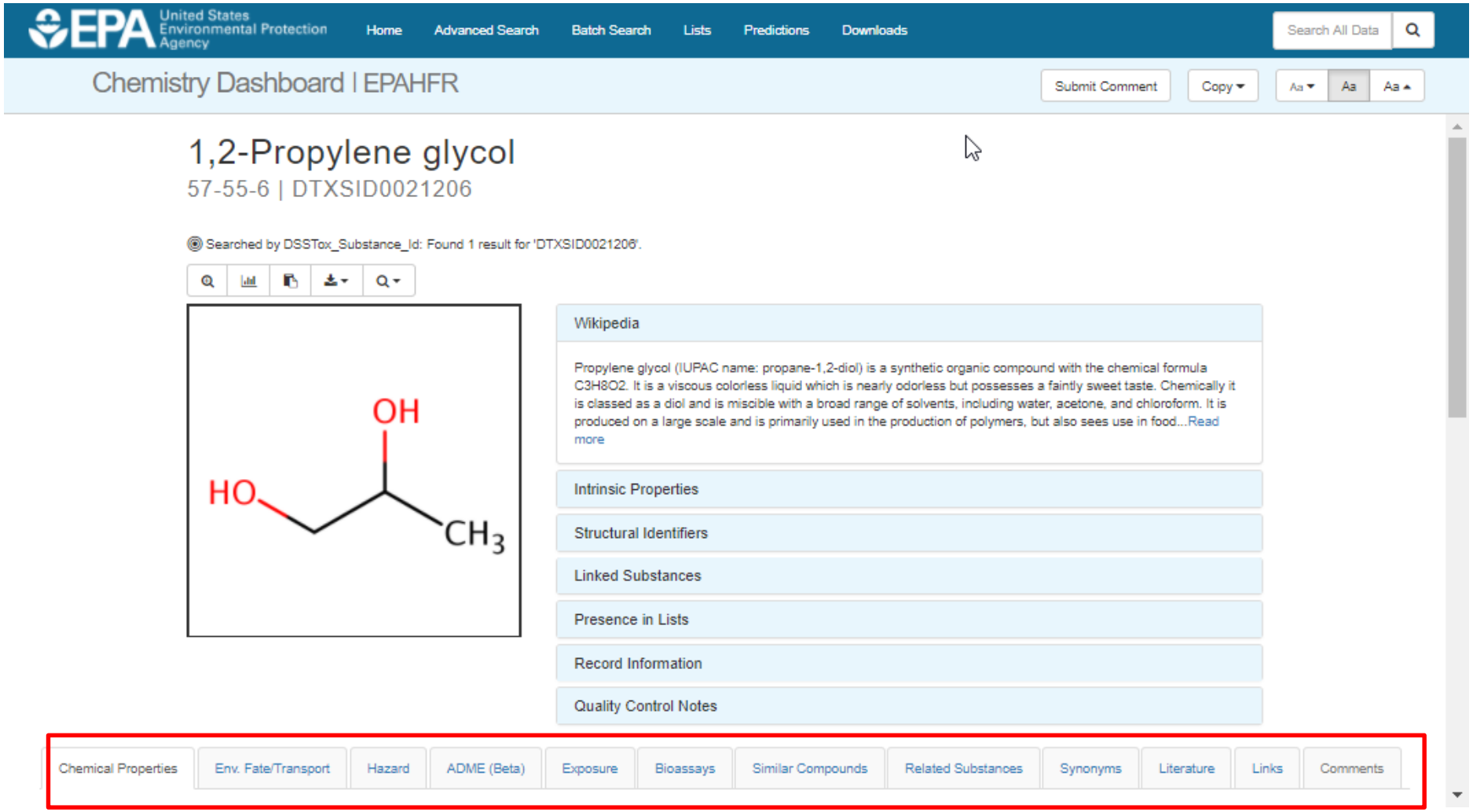
- A publicly accessible website delivering access:
  - ~760,000 chemicals with related property data
  - Experimental and predicted physicochemical property data
  - Integration to "biological assay data" for 1000s of chemicals
  - Information regarding consumer products containing chemicals
  - Links to other agency websites and public data resources
  - "Literature" searches for chemicals using public resources
  - "Batch searching" for thousands of chemicals
  - DOWNLOADABLE Open Data for reuse and repurposing

CompTox Chemistry Dashboard

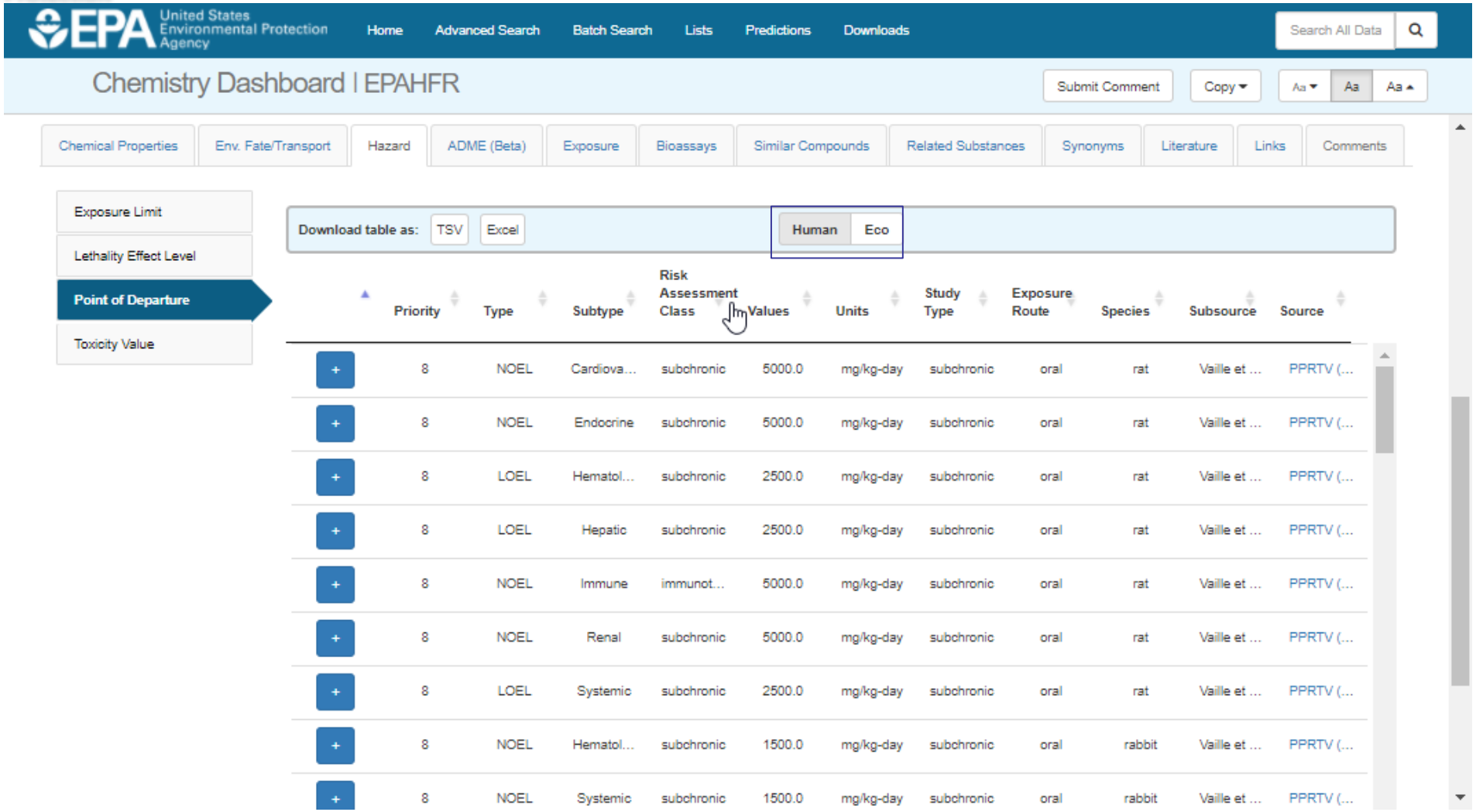
https://comptox.epa.gov/dashboard



Detailed Chemical Pages



Access to Chemical Hazard Data

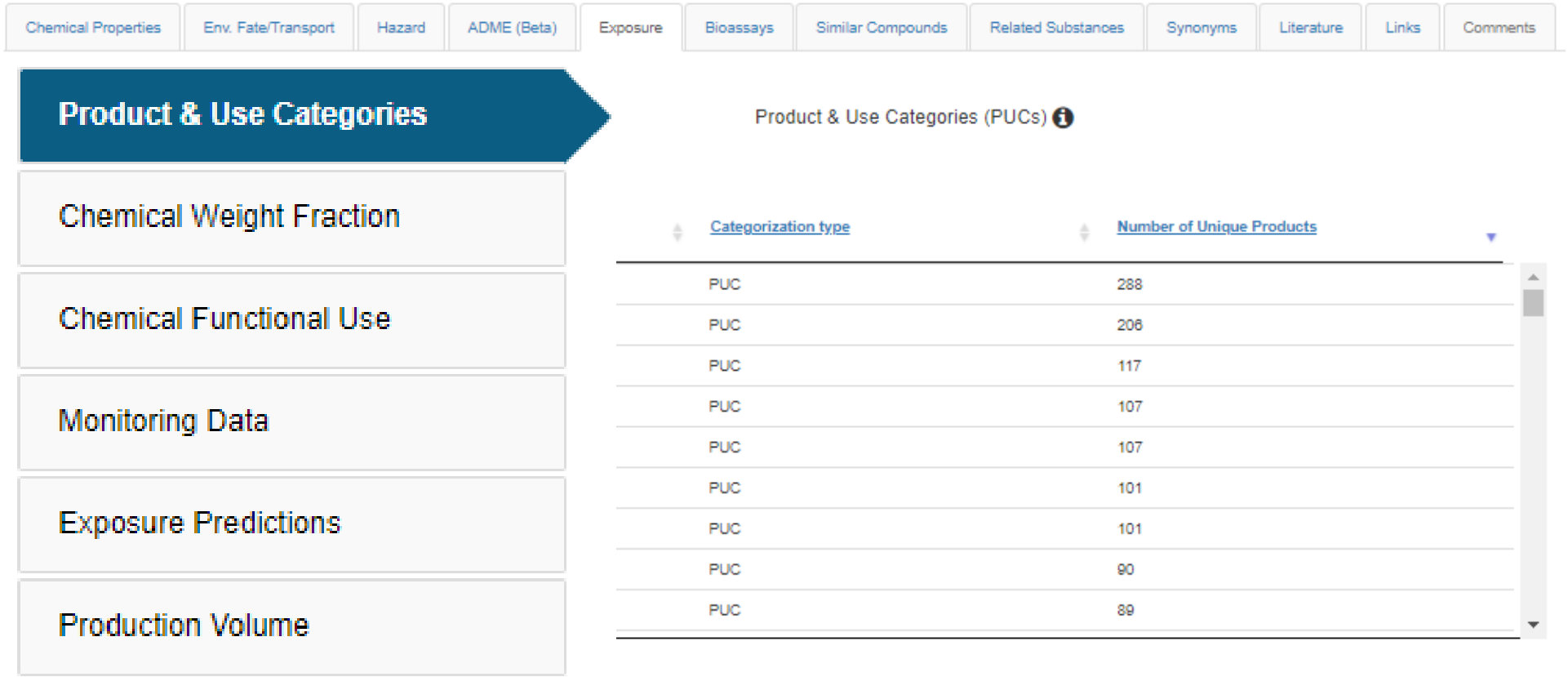


In Vitro Bioassay Screening

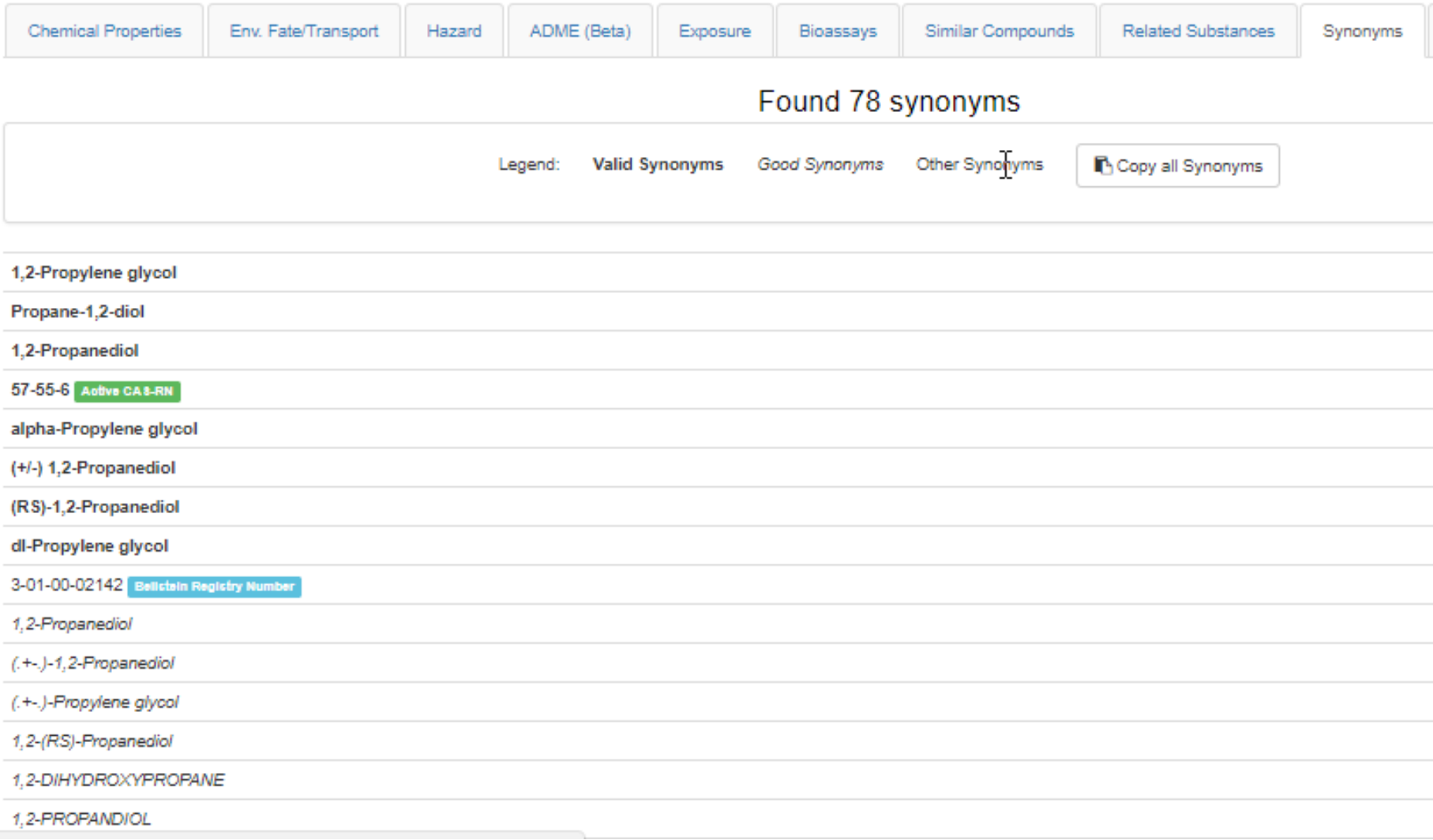
ToxCast and Tox21

Sources of Exposure to Chemicals

United States Environmental Protection Agency

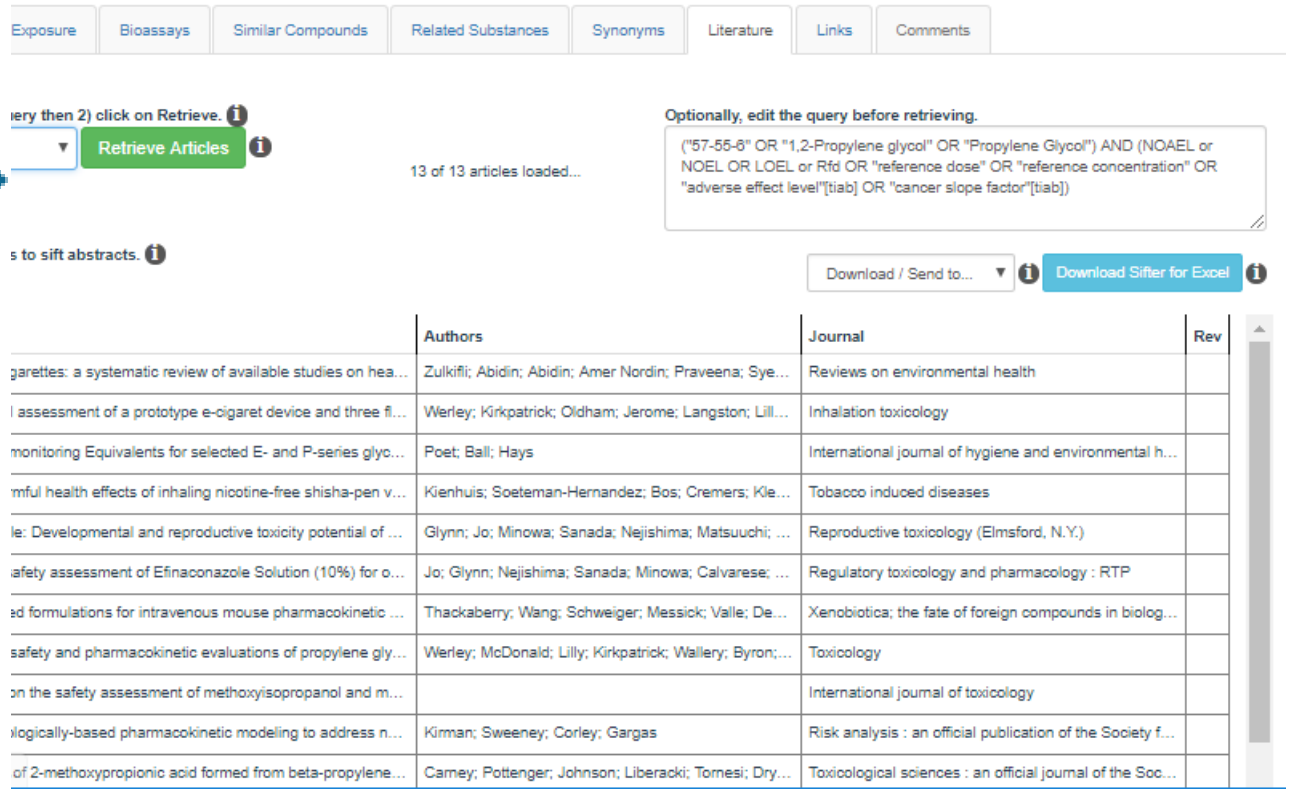


Identifiers to Support Searches

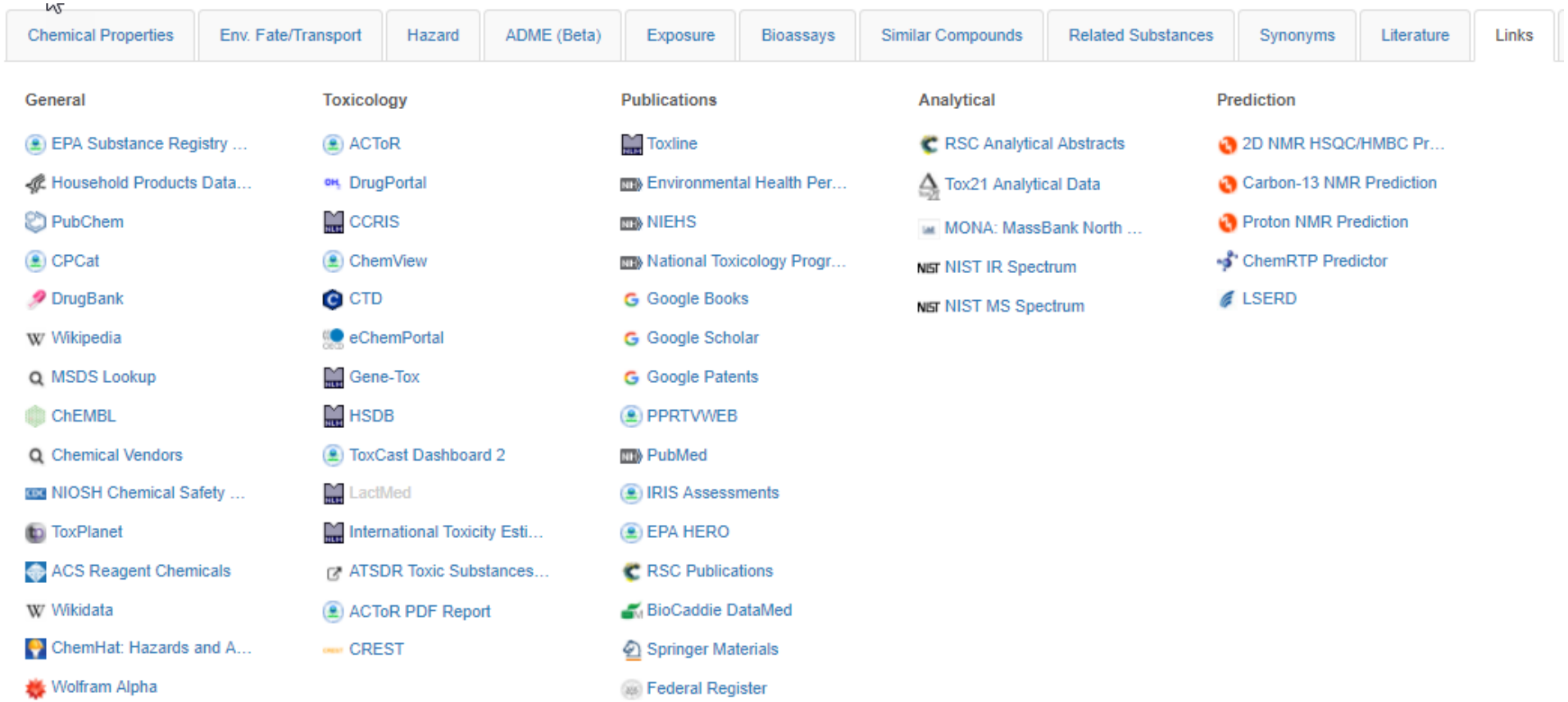


Literature Searches and Links

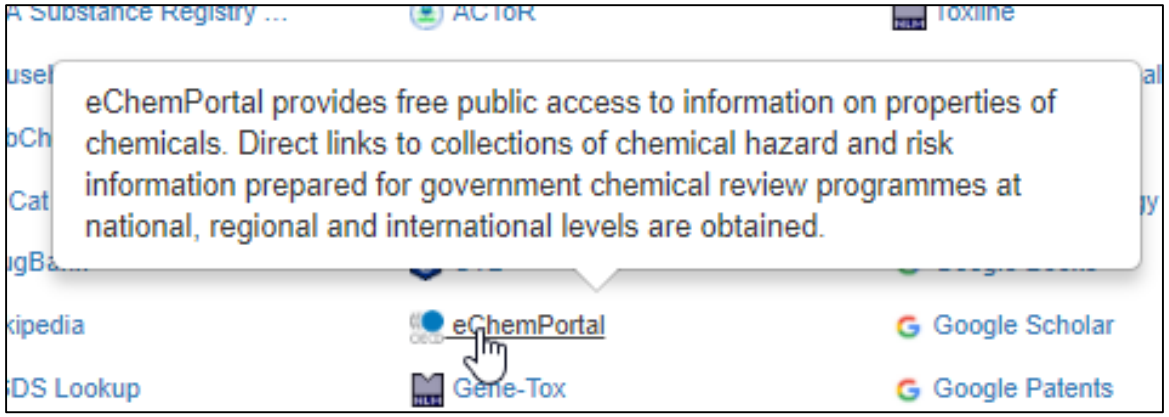
- Google Scholar
- **PubMed Abstract Sifter**
- PubChem Articles
- PubChem Patents
- PPRTV
- IRIS

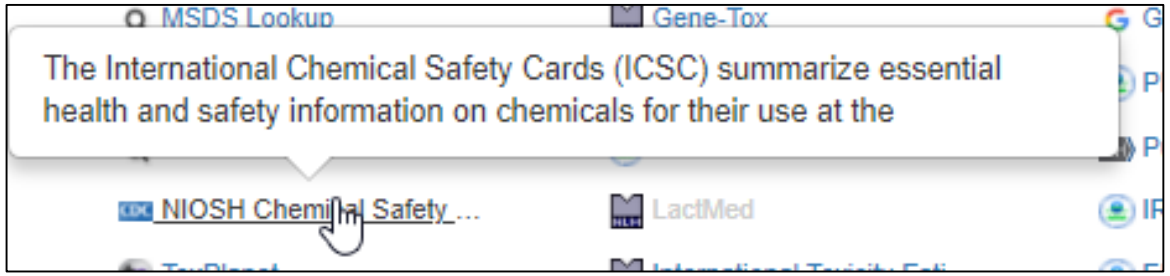


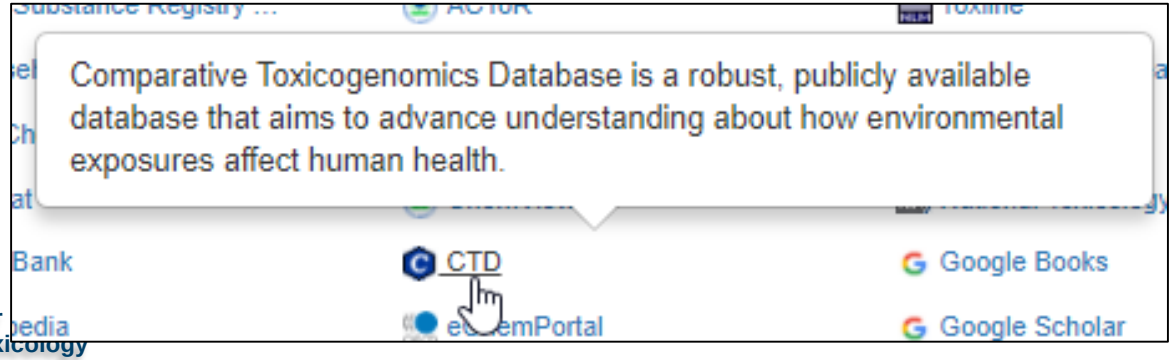
External Links to Data and Services



Integrated Linkouts







Integrated Linkouts

Comparative Toxicogenomics DB

ctd *Illuminating how chemicals affect human health.*

Comparative Toxicogenomics Database

Your queries | Contact us

Chemicals ▼ Name, CAS RN, ID Search

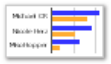
Home ▼ | Search ▼ | Analyze ▼ | Download ▼ | Help ▼

Propylene Glycol

Basics | Gene Interactions | Genes | Diseases | Phenotypes | Comps | Pathways | GO | Exposure Studies | Exposure Details | References

These diseases are associated with *Propylene Glycol* or its descendants. Each association is *curated* (M *marker/mechanism* and/or T *therapeutic*) and/or *inferred* (via a curated gene interaction).

Disease categories [Show chart]



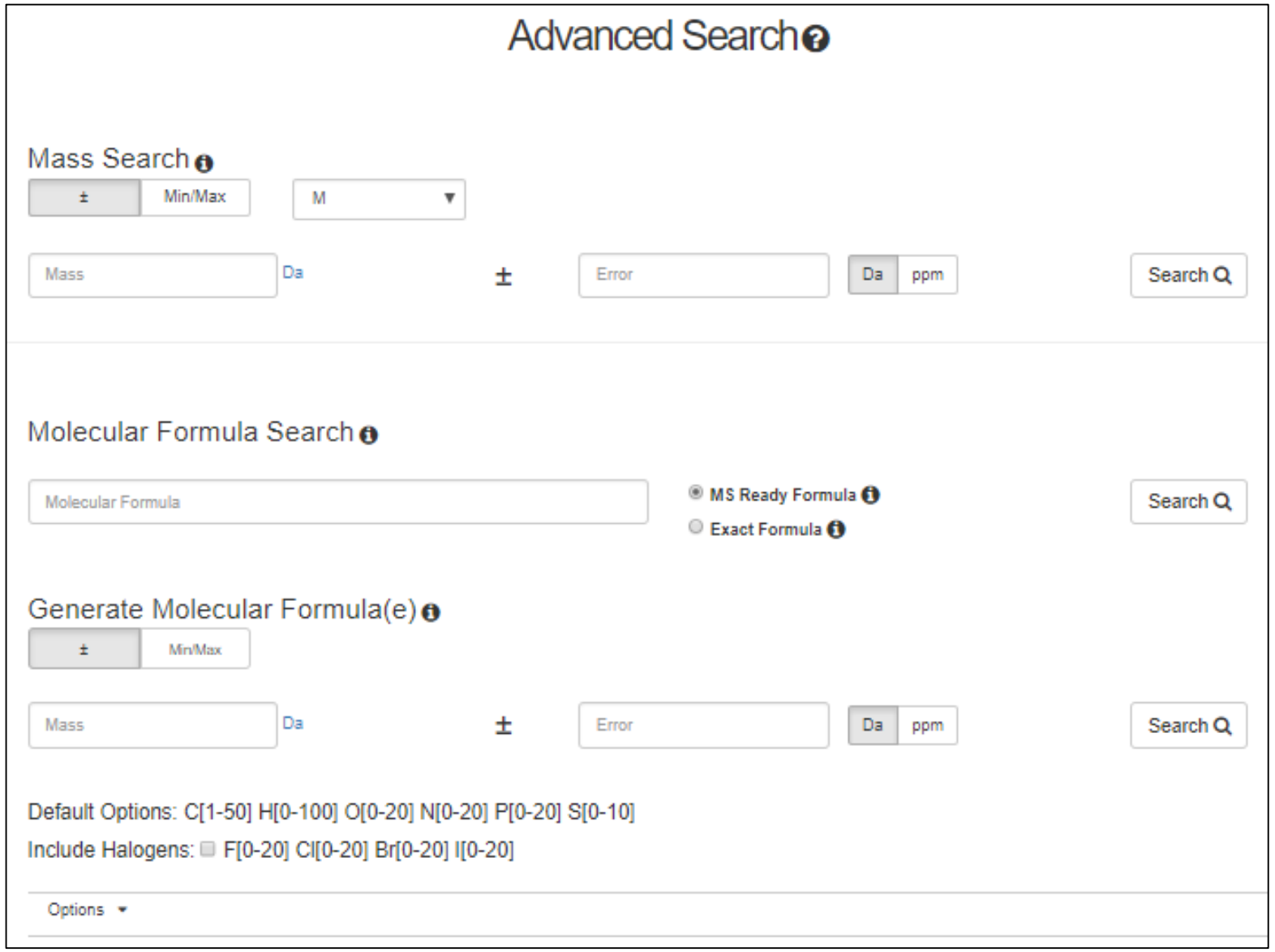
Filter by | Disease category: ALL | Association type: ALL | Filter

1–50 of 240 results.

First | Previous | 1 | 2 | 3 | 4 | 5 | Next | Last

	Chemical	Disease	Direct Evidence	Enrichment Analysis	Inference Network	Inference Score	References
1.	Propylene Glycol	Drug-Related Side Effects and Adverse Reactions	M		*2 genes:* ABCC2 | ABCC4	4.09	5
2.	Propylene Glycol	Acute Kidney Injury	M		*2 genes:* IL6 | TGFB1	3.78	3
3.	Propylene Glycol	Chemical and Drug Induced Liver Injury	M		*2 genes:* ABCC2 | IL6	2.82	5
4.	Propylene Glycol	Kidney Diseases	M		*1 gene:* TGFB1	2.54	4

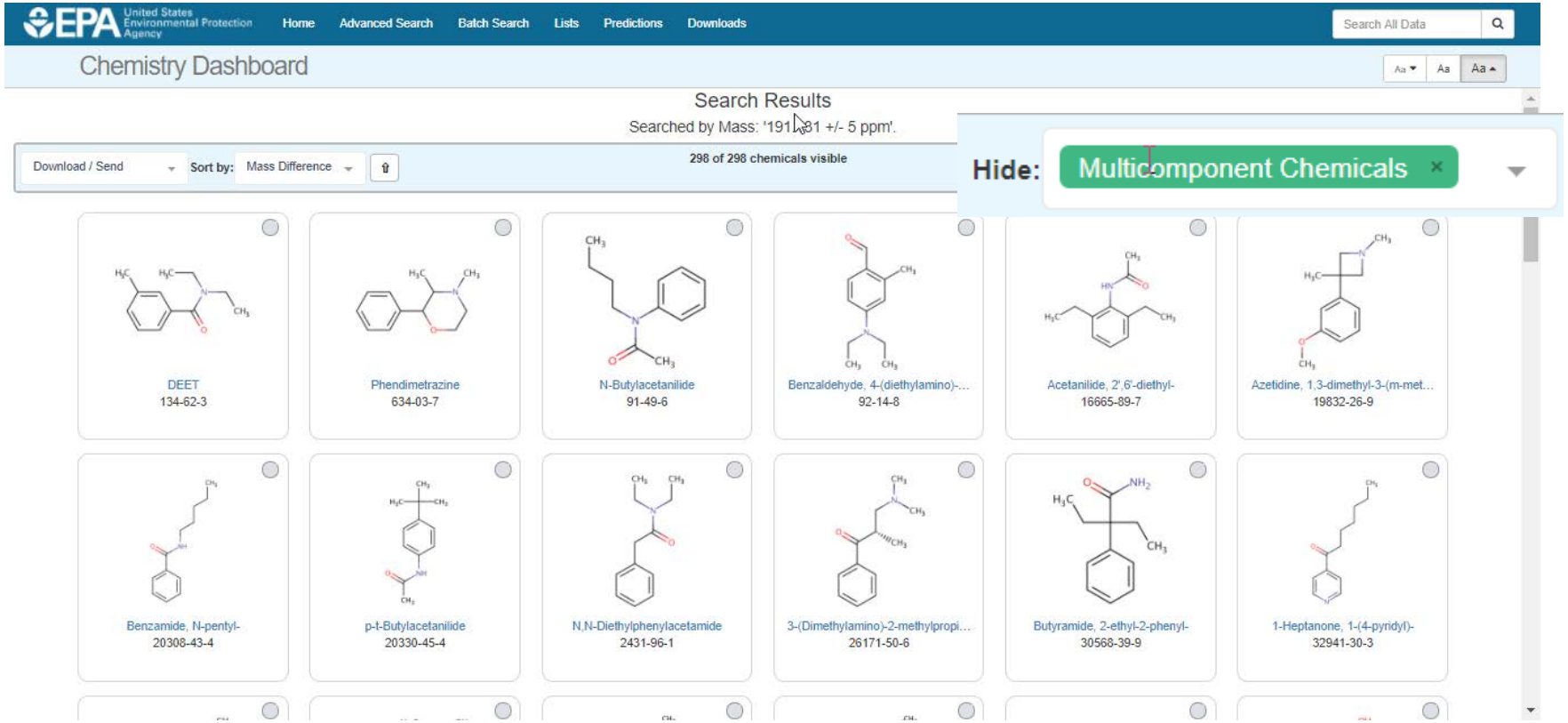
Advanced Searches



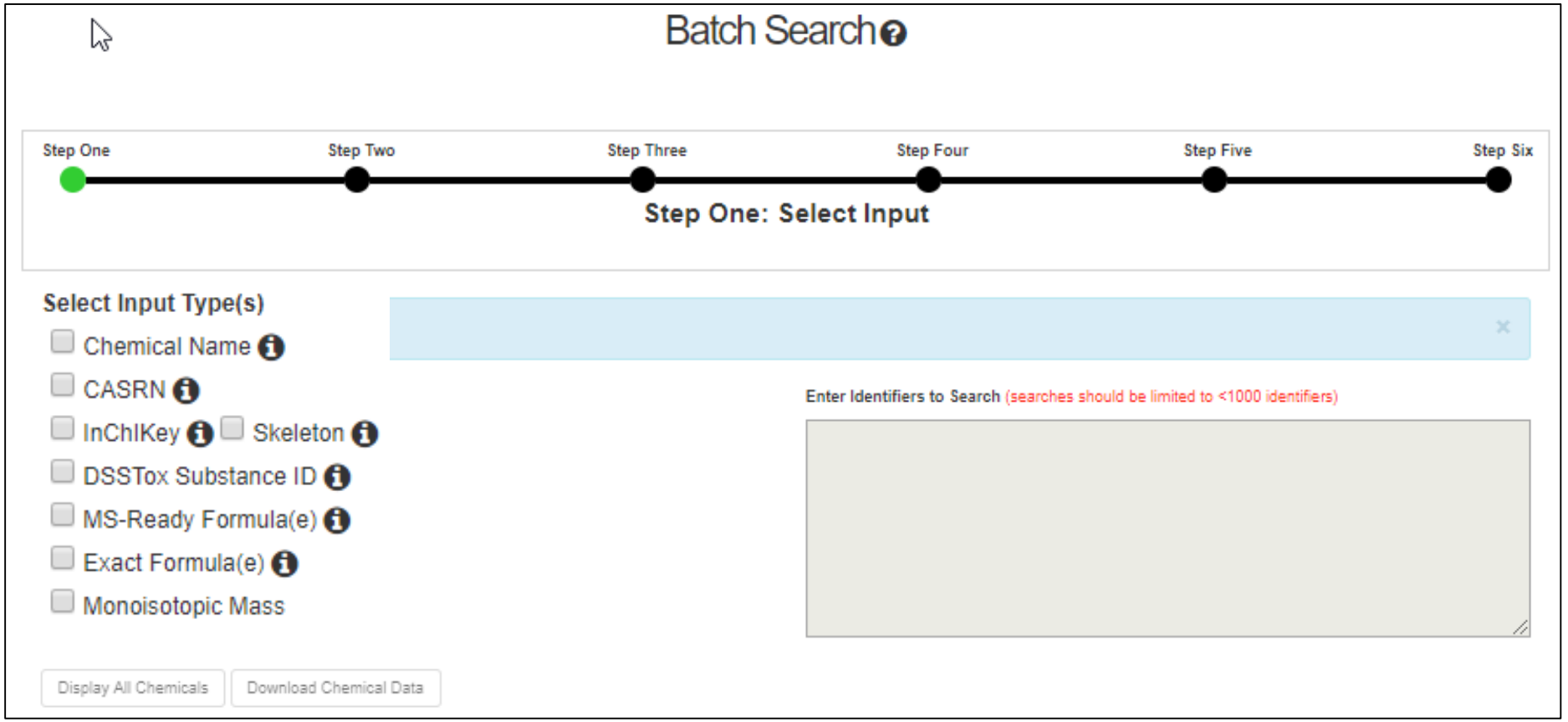
United States Environmental Protection Agency

Advanced Searches
Mass Based Search

Advanced Searches



Batch Searches



Batch Search

United States Environmental Protection Agency

Select Input Type(s)

- [ ] Chemical Name
- [ ] CASRN
- [ ] InChIKey [ ] Skeleton
- [ ] DSSTox Substance ID
- [x] MS-Ready Formula(e)
- [ ] Exact Formula(e)
- [ ] Monoisotopic Mass

Enter Identifiers to Search (searches should be limited to <1000 identifiers)

```
C6H12O3
C7H7N3
C8H11NO
C7H5NOS
C9H15NO
C11H12O
C9H8O3
C6H12O5
C9H15NO2
```

Metadata

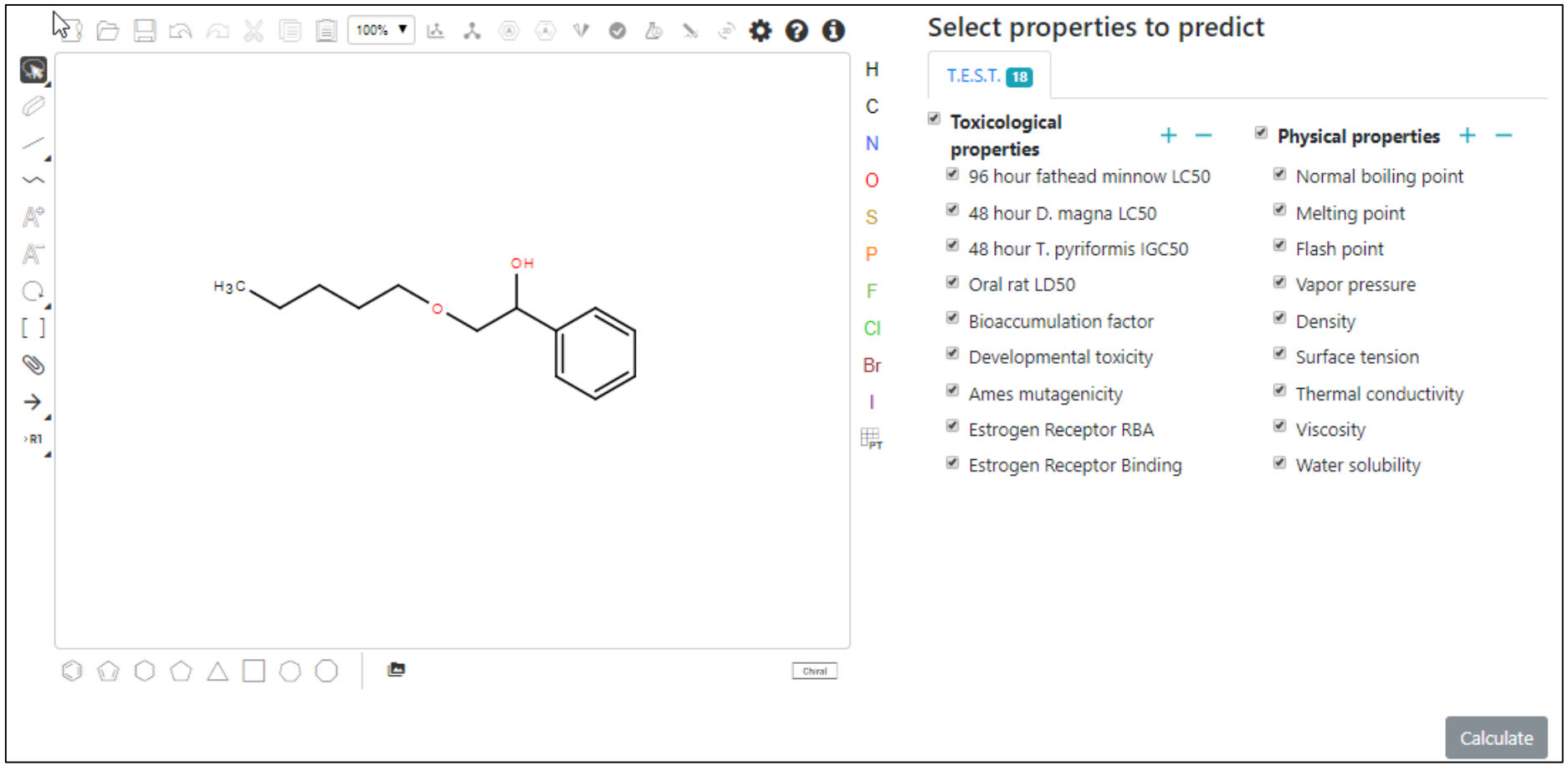
- [ ] Curation Level Details
- [x] Data Sources
- [x] Assay Hit Count
- [ ] Include links to ACToR reports - SLOW! (BETA)
- [x] NHANES/Predicted Exposure
- [x] Include ToxVal Data Availability
- [x] Number of PubMed Articles
- [ ] Abstract Sifter Input File (Beta)
- [ ] MetFrag Input File(Beta)
- [x] IRIS
- [x] PPRTV
- [x] PubChem Data Sources
- [ ] ToxPrint fingerprints

- [ ] NIOSH IDLH Values
- [ ] NIOSH International Chemical Safety Cards
- [ ] NIOSH Pocket Guide to Chemical Hazards
- [ ] NIOSH Skin Notation Profiles
- [ ] NORMAN Collaborative Trial 2015 Targets and Suspects
- [ ] Norman Network PFAS (KEMI Report)
- [ ] NORMAN Network Priority List
- [ ] NormaNEWS: Norman Early Warning System
- [ ] PFAS list provided by X.Trier et al
- [ ] Pharmaceutical List with EU, Swiss and US Consumption Data
- [ ] Provisional Peer Reviewed Toxicity Values
- [ ] Stockholm Convention on Organic Pollutants
- [x] STOFF-IDENT Database of Water-Relevant Substances
- [ ] Superfund Chemical Data Matrix
- [x] Surfactant List Screened in Swiss Wastewater (2014)

Excel Output

INPUT	FOUND_BY	DTXCID_IN	DATA_SOU	TOXVAL_D	TOXCAST_	TOXCAST_	NUMBER_C	PUBCHEM_	STOFFIDEI
C6H12O3	MS Ready I	DTXCID701	91	Y	0.36	2/562	24	83	Y
C6H12O3	MS Ready I	DTXCID003	67	Y	0.36	1/276	376	80	Y
C6H12O3	MS Ready I	DTXCID106	65	Y	4.42	5/113	6	77	Y
C6H12O3	MS Ready I	DTXCID105	45	Y	0.0	0/163	3	94	-
C6H12O3	MS Ready I	DTXCID901	38	Y	-	-	14	110	Y
C6H12O3	MS Ready I	DTXCID402	34	Y	0.0	0/113	-	53	Y
C6H12O3	MS Ready I	DTXCID202	31	Y	-	-	-	36	Y
C6H12O3	MS Ready I	DTXCID202	30	-	2.54	7/276	-	54	-
C6H12O3	MS Ready I	DTXCID109	26	Y	-	-	-	46	-
C6H12O3	MS Ready I	DTXCID202	24	Y	0.0	0/113	-	47	-
C6H12O3	MS Ready I	DTXCID303	22	Y	-	-	-	89	-
C6H12O3	MS Ready I	DTXCID302	20	Y	-	-	2	25	Y
C6H12O3	MS Ready I	DTXCID407	19	Y	-	-	12	62	-
C6H12O3	MS Ready I	DTXCID704	17	Y	-	-	-	64	-
C6H12O3	MS Ready I	DTXCID704	16	Y	-	-	3	49	-

Real-Time Predictions



Real-Time Predictions

Property	Experimental Value	Prediction				
		Consensus	Hierarchical clustering	Single model	Group contribution	Nearest neighbor
96 hour fathead minnow LC50		4.477 -Log10(mol/L) 6.954 mg/L	4.195 -Log10(mol/L) 13.288 mg/L	3.994 -Log10(mol/L) 21.110 mg/L	3.478 -Log10(mol/L) 69.224 mg/L	6.238 -Log10(mol/L) 0.120 mg/L
48 hour D. magna LC50		4.398 -Log10(mol/L) 8.328 mg/L	3.877 -Log10(mol/L) 27.677 mg/L	4.039 -Log10(mol/L) 19.026 mg/L	4.084 -Log10(mol/L) 17.173 mg/L	5.593 -Log10(mol/L) 0.532 mg/L
48 hour T. pyriformis IGC50		4.063 -Log10(mol/L) 18.039 mg/L	3.731 -Log10(mol/L) 38.668 mg/L		3.386 -Log10(mol/L) 85.610 mg/L	5.070 -Log10(mol/L) 1.773 mg/L
Oral rat LD50		1.758 -Log10(mol/kg) 3640.950 mg/kg	1.982 -Log10(mol/kg) 2172.756 mg/kg			1.533 -Log10(mol/kg) 6101.245 mg/kg
Bioaccumulation factor		1.797 Log10 62.700	2.202 Log10 159.310	1.287 Log10 19.346	1.181 Log10 15.157	2.520 Log10 330.834
Developmental toxicity		false	false	false		true
Ames mutagenicity		false	false			false
Estrogen Receptor RBA		-3.075 Log10 $8.418*10^{-4}$	-3.078 Log10 $8.356*10^{-4}$	-3.720 Log10 $1.907*10^{-4}$		-2.427 Log10 0.004
Estrogen Receptor Binding		true	true	true	false	true

Real-Time Predictions

United States Environmental Protection Agency

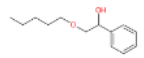
Predicted Water solubility at 25°C for OC(C=1C=CC=CC1)COCCCCC from Consensus method

Prediction results

Endpoint	Experimental value	Predicted value
Water solubility at 25°C -Log10(mol/L)	N/A	2.46
Water solubility at 25°C mg/L	N/A	723.26

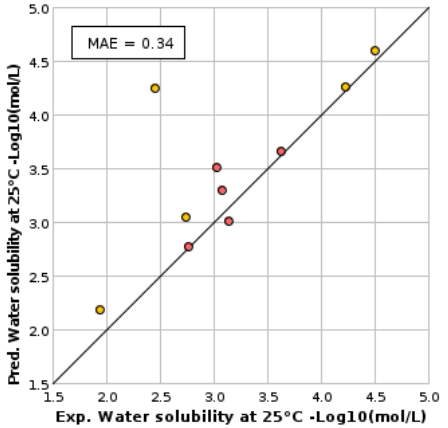
Individual Predictions

Method	Predicted value -Log10(mol/L)
Hierarchical clustering	2.42
Group contribution	2.32
Nearest neighbor	2.64



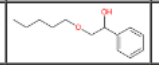
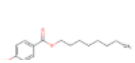
Predictions for the test chemical and for the most similar chemicals

Prediction results (colors defined in table below)

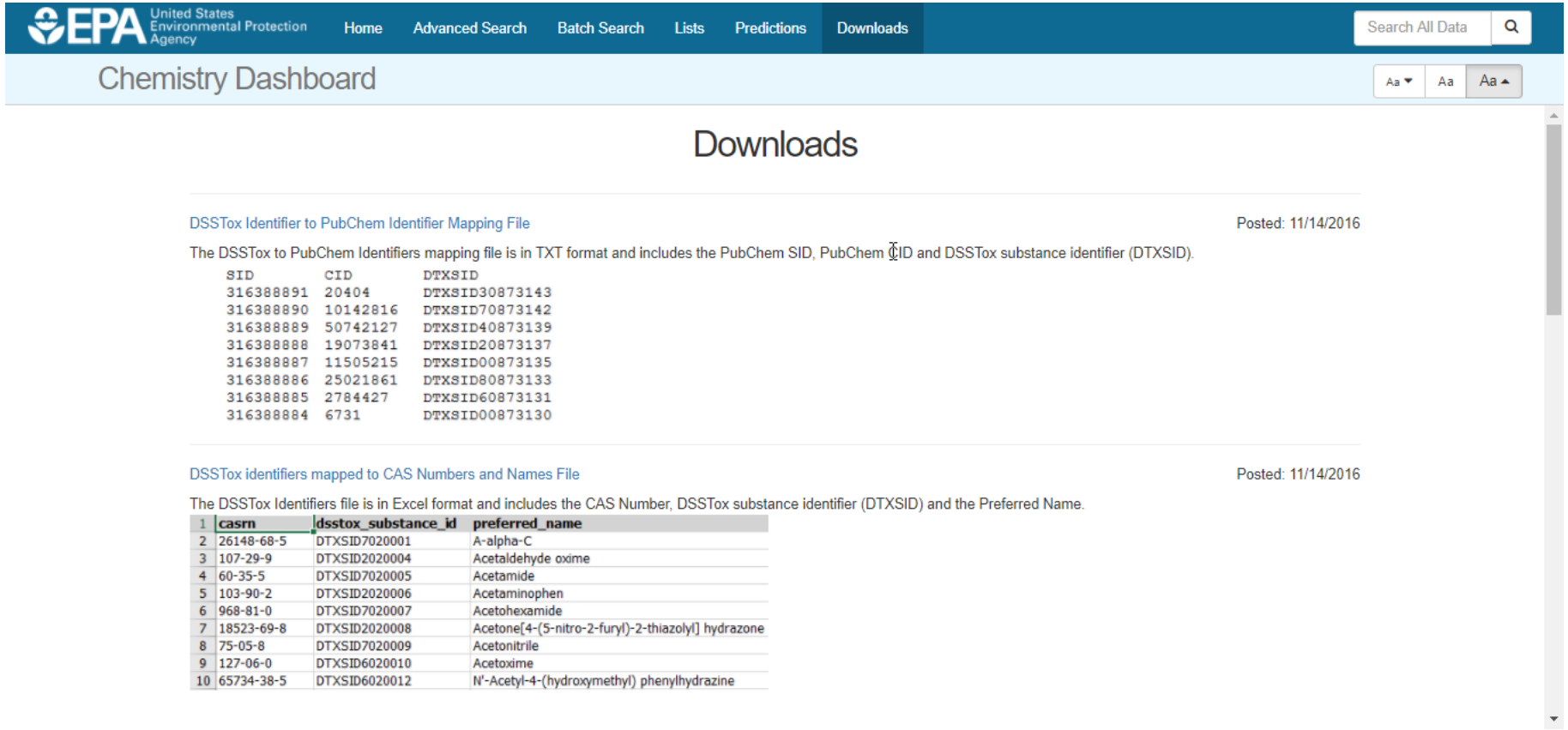


Chemicals	MAE*
Entire set	0.58
Similarity coefficient ≥ 0.5	0.34

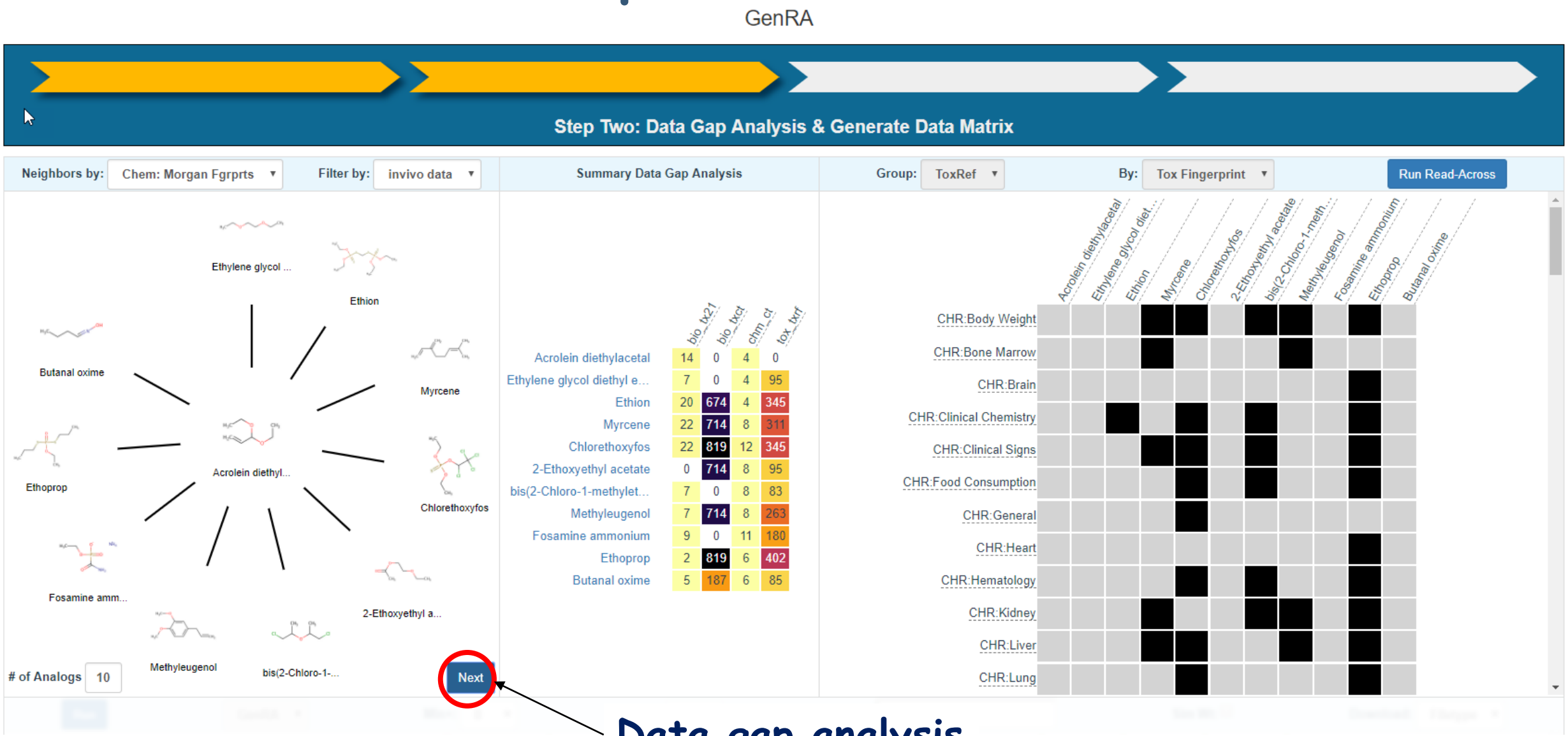
*Mean absolute error in -Log10(mol/L)

CAS	Structure	Similarity Coefficient	Experimental value -Log10(mol/L)	Predicted value -Log10(mol/L)
OC(C=1C=CC=CC1)COCCCCC (test chemical)			N/A	2.46
104-40-5		0.68	4.50	4.60
1219-38-1		0.67	4.22	4.26

Downloadable Data

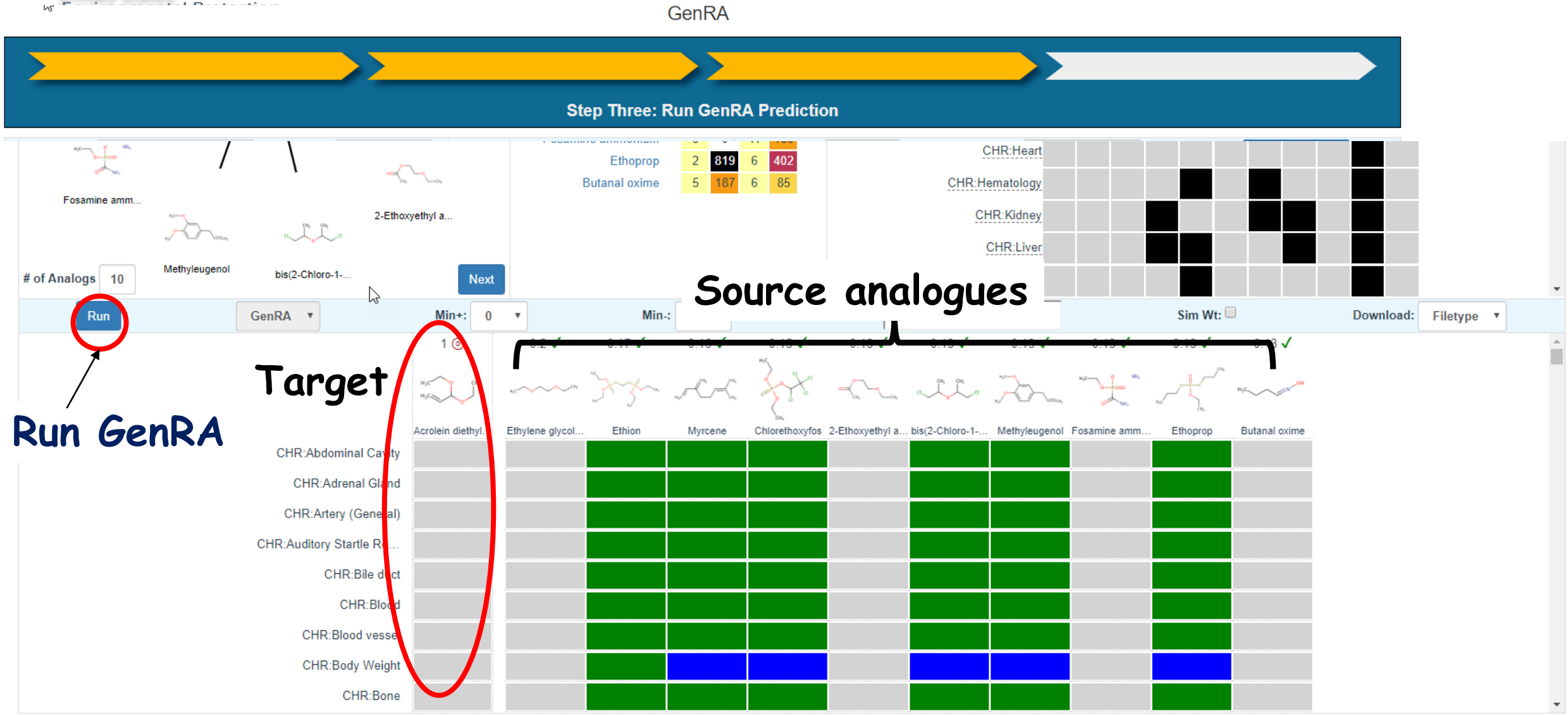


Future Development: GenRA-Read-across tool



Data gap analysis

Future Development: GenRA-Read-across tool



Future Development
Real Time OPERA Predictions

Mansouri *et al. J Cheminform (2018) 10:10*
https://doi.org/10.1186/s13321-018-0263-1

Journal of Cheminformatics

RESEARCH ARTICLE Open Access

OPERA models for predicting physicochemical properties and environmental fate endpoints

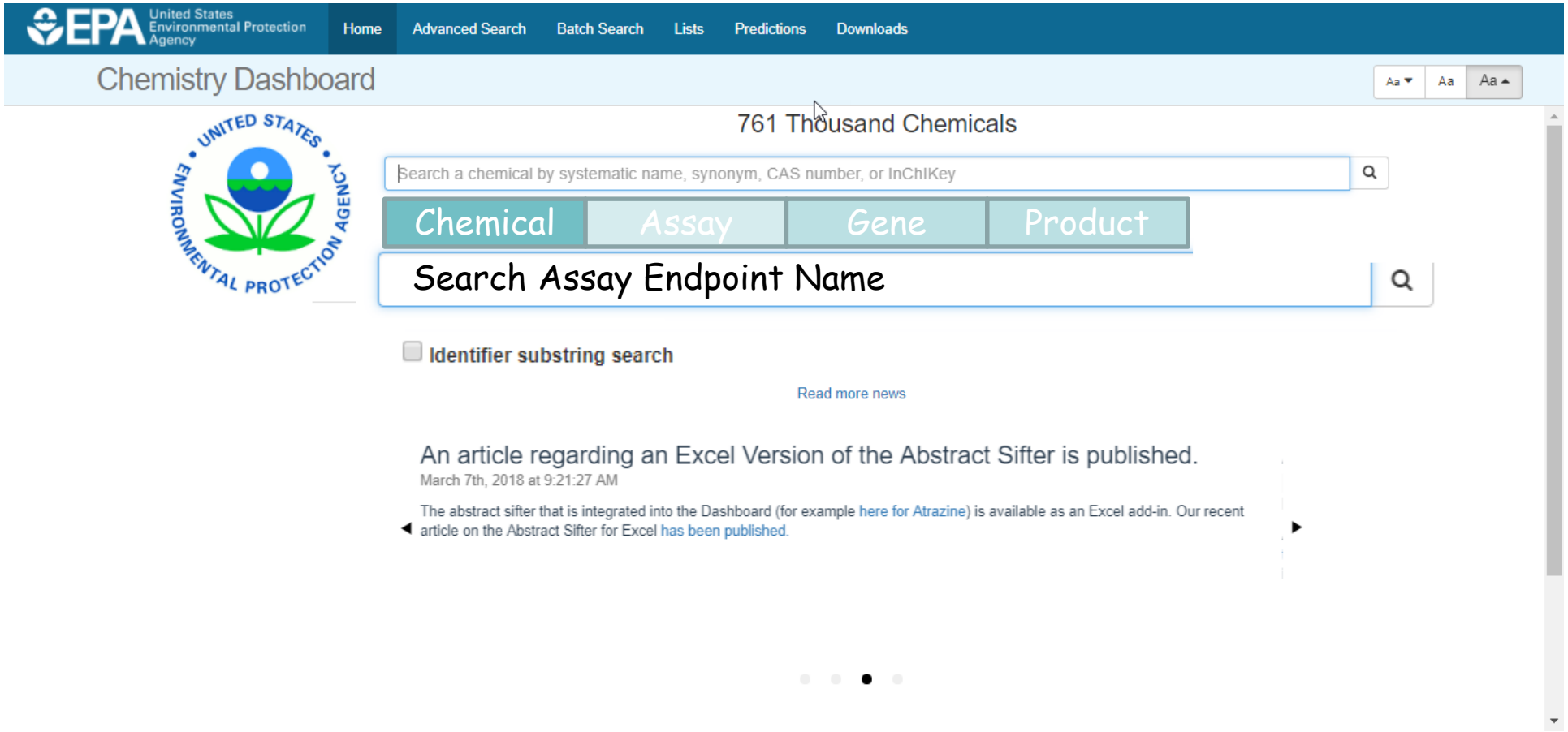
Kamel Mansouri[1,2,3*], Chris M. Grulke[1], Richard S. Judson[1] and Antony J. Williams[1]

Select properties to predict

T.E.S.T. 18 | OPERA | Search

- Toxicological properties + −
  - 96 hour fathead minnow LC50
- Physical properties + −
  - Normal boiling point

Future Search Possibilities



Summary remarks on the Dashboard

- The CompTox Chemistry Dashboard provides access to data for ~760,000 chemicals
- High quality data from ongoing curation efforts
- An integration hub for multiple "modules"
  - Experimental and predicted properties
  - Human and Ecological Hazard data
  - Exposure data – products, data in the environment
  - *In vitro* bioassay data – ToxCast/Tox21
  - Literature searching – Google Scholar and PubMed
  - Specialized searches – mass/formula for analytical support
  - Batch searching and Real Time Predictions
- The primary architecture for NCCT data

Take home messages

- Outlined Regulatory Drivers
- What Integrated Approaches to Testing and Assessment (IATA) are and how they have evolved taking into account Adverse Outcome Pathways (AOPs)
- How different decision contexts impact the types of NAMs applied
- Practical workflow – where and what approaches (including NAMs) can be used with reference to the US EPA Chemistry Dashboard

Acknowledgments

- The NCCT CompTox Chemistry Dashboard Development Team
- Ann Richard, Chris Grulke
- NERL scientists (Jon Sobus, Elin Ulrich) – Mass Spectrometry
- Kamel Mansouri – OPERA models
- Todd Martin – TEST predictions
- Nancy Baker – Abstract Sifter
- George Helman, Imran Shah, Grace Patlewicz - GenRA

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