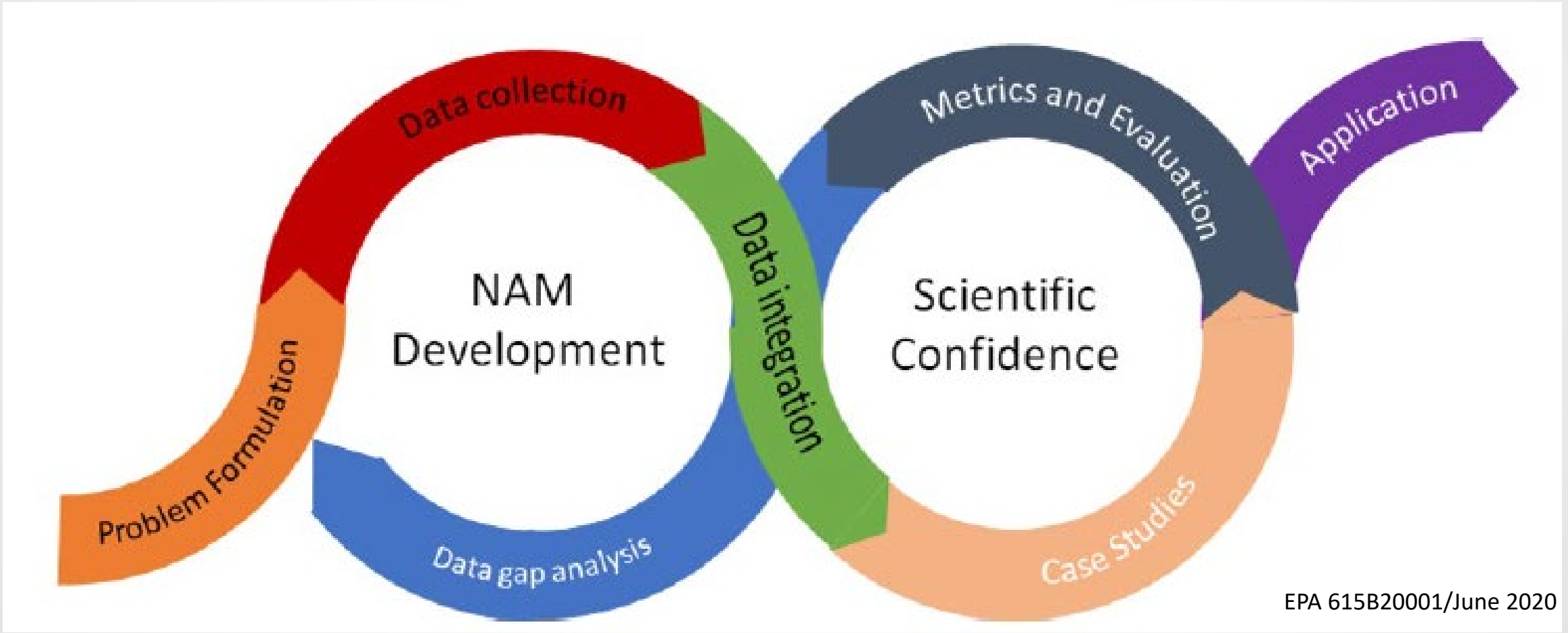
APPLYING NAMS TO INFORM HERA ASSESSMENTS

Luci Lizarraga
EPA, ORD, CPHEA
Cincinnati, OH
February 4, 2021

Note: this document may contain some elements that are not fully accessible to users with disabilities. If you need assistance accessing any information in this document, please contact ORD_Webmaster@epa.gov.

EPA NAM Work Plan: Problem-focused Research Planning and Implementation Process at EPA

HERA's role in building scientific confidence and application of NAMs



EPA 615B20001/June 2020

Applying NAMs in HERA

Output 3.1 - Advance, translate, and build confidence in the application of new approach methods (NAMs) and data in risk assessment

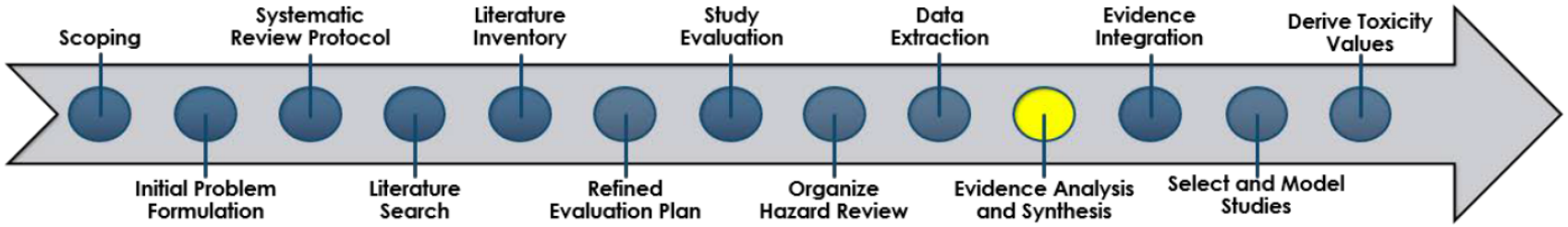
- This output encompasses the research required to use and build confidence in the application of information and data from NAMs into HERA science assessments

Overview and Strategy

- Fit-for-purpose approach to NAMs in HERA assessments:
  - Data-poor chemicals ➡ NAM serves as a driver
  - Data-rich chemicals ➡ NAM fills a data gap
- Focus on the use of NAMs to inform qualitative and quantitative hazard conclusions
- Develop case studies that demonstrate utility and increase confidence and reliability in NAMs
- Establish collaborations between scientists in HERA and CSS (or others)

Using NAMs to Inform Hazard Conclusions

ORD Staff Handbook for Developing IRIS Assessments



ANALYSIS AND SYNTHESIS OF MECHANISTIC INFORMATION

Purpose

- To consider the available mechanistic data in light of other identified hazard-specific information to inform evidence integration conclusions.

Who

- The assessment team, in consultation with appropriate disciplinary workgroup(s) and subject matter experts.

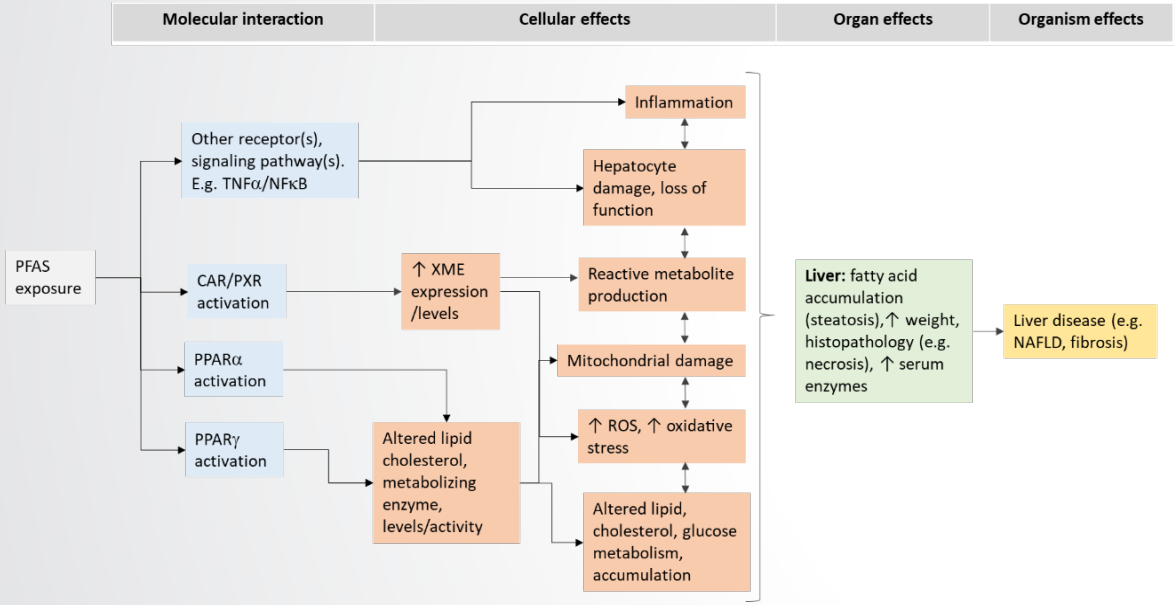
What

- Draft mechanistic synthesis sections for selected health effects describing the assessment-specific mechanistic questions or issues, as well as the interpretations drawn from the mechanistic data.

- IRIS assessments consider mechanistic information, including NAMs, to inform hazard identification and dose-response

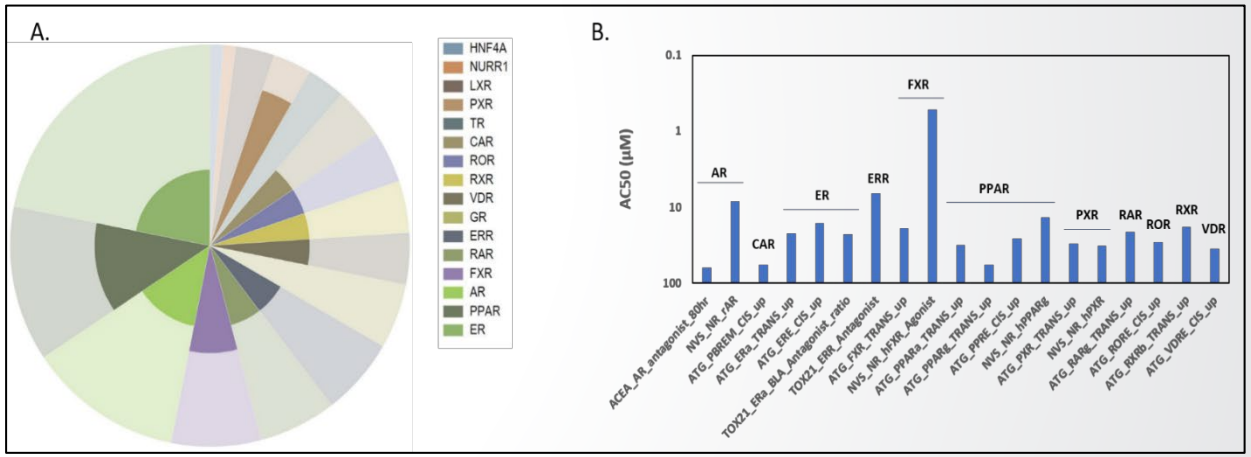
Using NAMs to Inform Hazard Conclusions

Proposed MOA/AOP Approach for evalauting PFAS-induced liver toxicity



EPA/635/R-19/049

Bioactivity data from the ToxCast/Tox21 database were synthesized as part of the mechanistic evidence

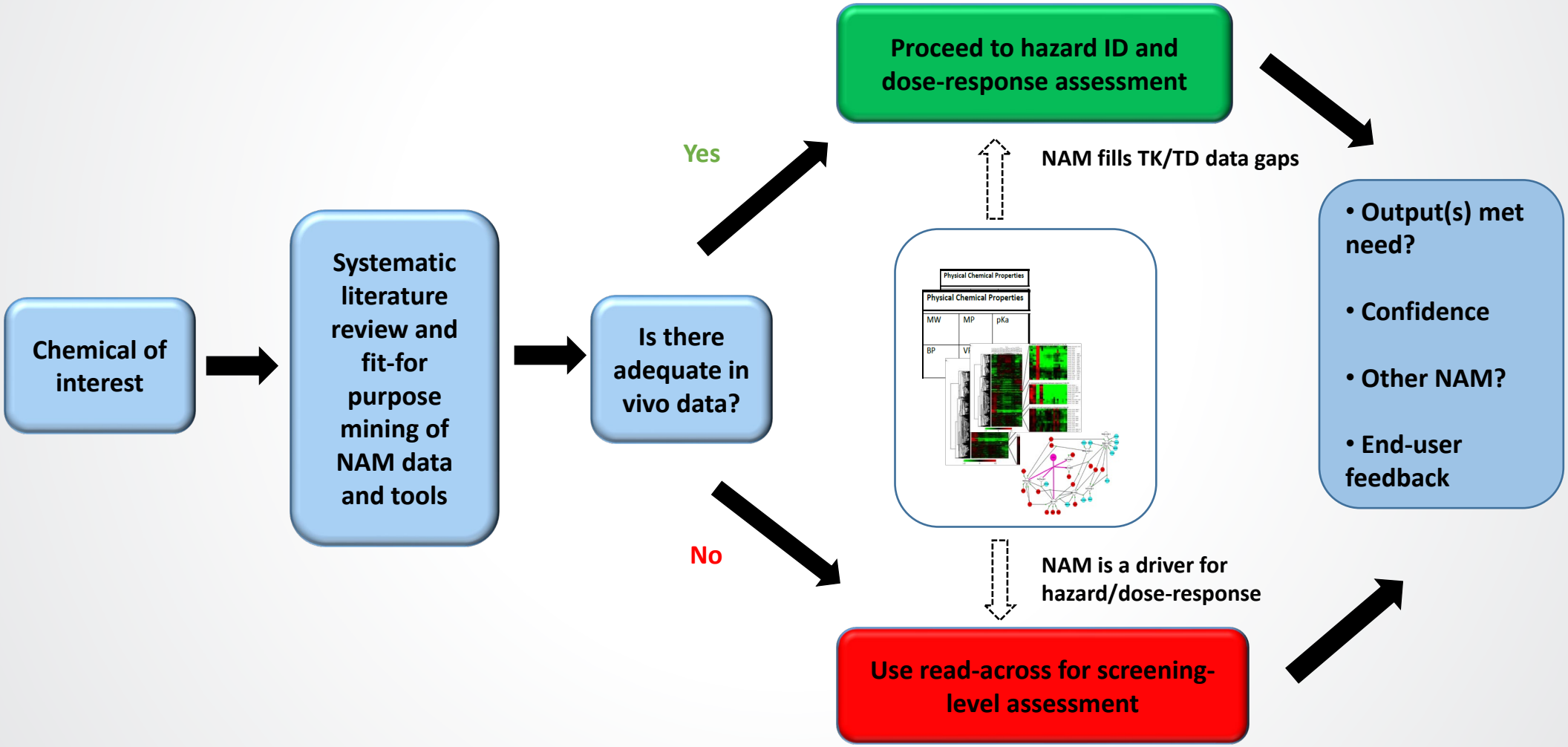


- **Evaluation of mechanistic data (including ToxCast/Tox21 assays) in support of biological plausibility and human relevance for PFAS-induced liver effects**

HERA NAM Research Portfolio

Assessment Data Gaps/Uncertainties	Proposed NAM Applications
Data-poor chemicals	Advancing read-across for screening-level assessment
Toxicokinetic data gaps	Integrated approach to evaluate metabolism
Toxicodynamic data gaps	Using gene expression data to inform MOA, hazard and dose-response
Chemical mixture assessment	AOP footprint approach for hazard grouping and dose additivity
Inhalation dosimetry	Applications to advance IVIVE, IATA and NAMs
Cumulative risk assessment	AEP and AOP integration to support source-to-outcome approaches

Integrated Approach to Human Health Assessments



Conclusions

- Output objectives and proposed products are consistent with broader NAM EPA efforts
- Research is tethered to assessment products and technical support efforts within HERA
- Coordinates with other National Research Programs and seeks partner engagement

Charge Question

As the development of NAMs advances and chemicals with little-to-no data require assessment, research is required to translate and build confidence in the application of NAMs in HERA science assessment contexts. Are the case studies and planned research appropriate to advance the integration of NAM data streams and approaches in HERA science assessments? [Research Area 3, Output 3.1]

Acknowledgments

Output leads/contributors:

Matthew Boyce
Jeffry Dean
Annie Jarabek
Jason Lambert
Luci Lizarraga
Roman Mezencev
Grace Patlewicz
Glenn Suter

HERA National Program:

Beth Owens, RAC
Samantha Jones, Director

Abbreviations

AEP – Aggregate exposure pathway
AOP – Adverse outcome pathway
CSS – Chemical safety for sustainability research program
HERA – Human and environmental risk assessment research program
IATA – Integrated approaches to testing and assessment
IVIVE – In vitro to in vivo extrapolation
NAM – New approach methods

ADVANCING READ-ACROSS APPLICATIONS IN HERA

Luci Lizarraga
EPA, ORD, CPHEA
Cincinnati, OH
February 4, 2021

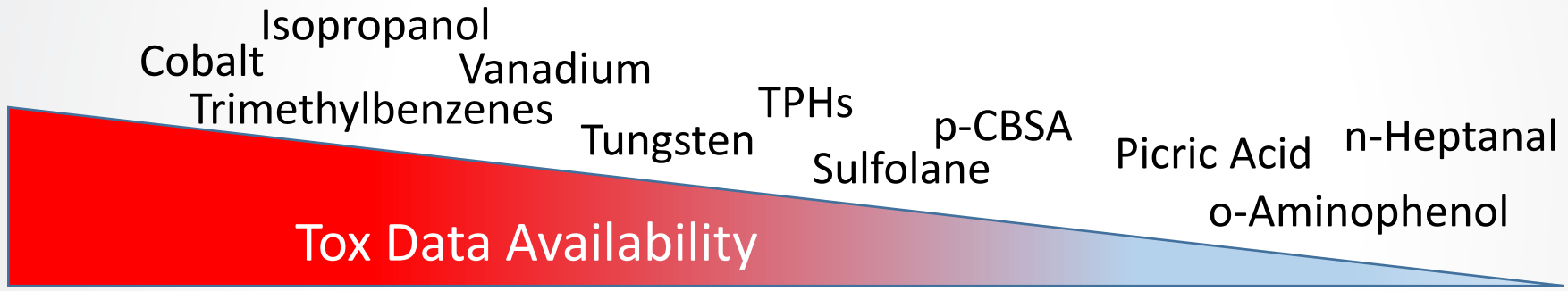
The views expressed in this presentation are solely those of the author(s) and do not necessarily reflect the views and policies of the U.S. Environmental Protection Agency (EPA)

Overview

- What are PPRTVs?
- What is read-across?
- Read-across methodology and PPRTV example
- Key lessons and revised methodology
- Expanding read-across applications
- Conclusions

Provisional Peer-Reviewed Toxicity Values (PPRTVs)

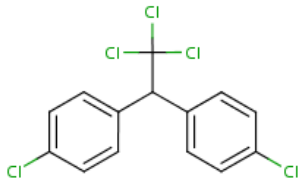
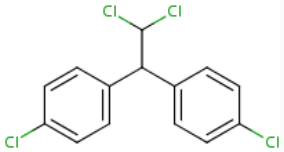
- > 400 chemicals assessed with various hazard database sizes



- Developed under the Center for Public Health and Environmental Assessment (CPHEA) and supported by the Health and Environmental Risk Assessment (HERA) program
- Source of toxicity information and cancer and noncancer toxicity values for the Superfund Program
- Apply read-across for screening-level assessment of data-poor chemicals

What is Read-across?

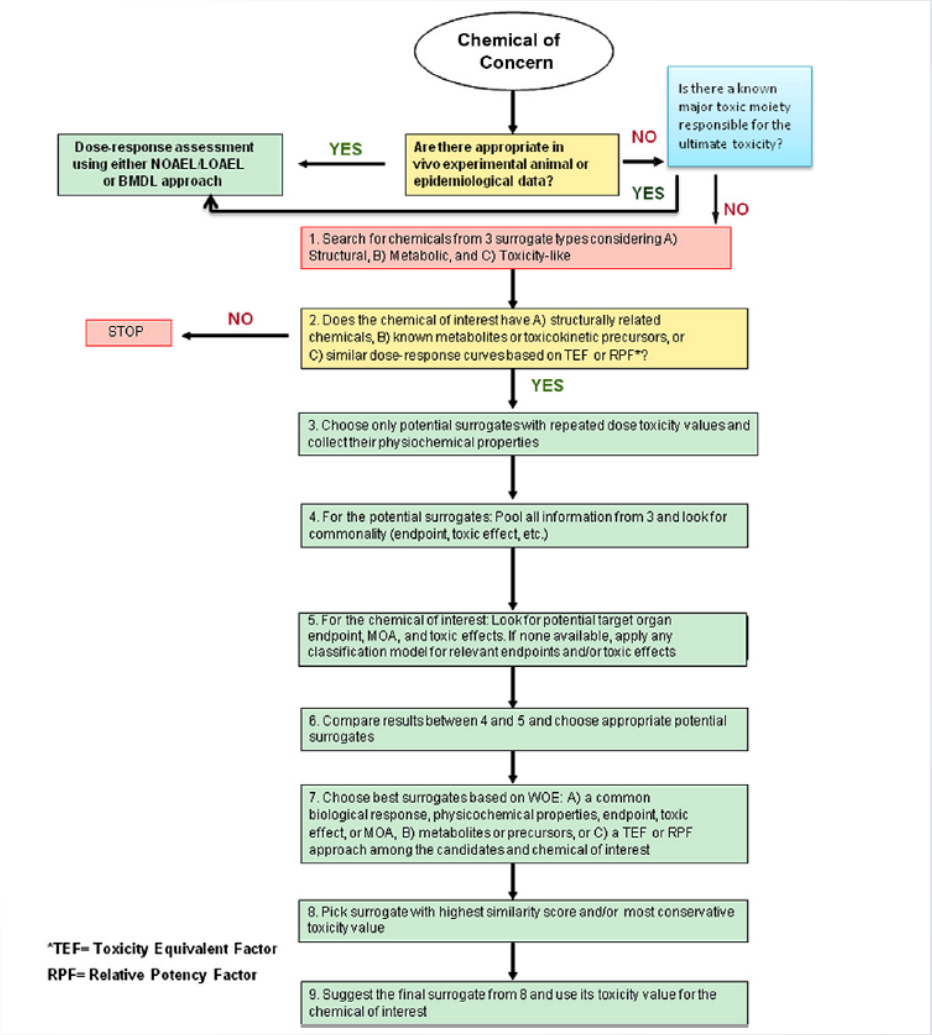
- Technique used to fill data gaps for a target chemical using information from one or more **source analogue(s)**, which are considered to be "similar" by scientific justification (OECD, 2014)

	Source Analogue	Target
		
Endpoint effect		

- Applications: EU's REACH legislation and EPA High Production Volume Challenge Programme and PPRTV program

Read-across Methodology

- Uses structural, toxicokinetic, and toxicodynamic similarities to identify and evaluate the suitability of analogues for quantitative read-across
- 35 PPRTV chemicals have been evaluated via read-across (~55 toxicity values derived)
- Methodology has evolved over time



Wang et al., 2012, Regul Toxicol Pharmacol 63:10-19

Read-Across in PPRTVs: structural similarity

EPA United States Environmental Protection Agency

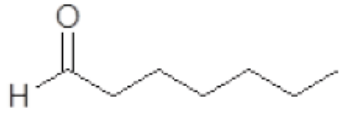
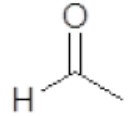
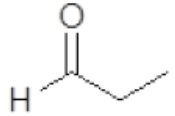
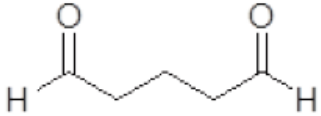
EPA/690/R-17/002
FINAL
09-13-2017

Provisional Peer-Reviewed Toxicity Values for

n-Heptanal
(CASRN 111-71-7)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Table A-1. Structural and Physicochemical Properties of *n*-Heptanal (CASRN 111-71-7) and Candidate Analogs[a]

	***n*-Heptanal (heptaldehyde)**	**Acetaldehyde (ethanal)**	**Propionaldehyde (propanal)**	**Glutaraldehyde (pentanedial)**
Structure				
CASRN	111-71-7	75-07-0	123-38-6	111-30-8
Molecular weight	114.19	44.05	58.08	100.12
DSSTox similarity score (%)[b]	100	33	47	69
OECD QSAR Toolbox similarity score (%)[c]	100	10	33	36
Melting point (°C)	−43.3	−123.37	−80	−29.86 (estimated)[a]
Boiling point (°C)	153	20.1	48	188[c]
Vapor pressure (mm Hg at 25°C)	3.52	902	317	0.6
Henry's law constant (atm-m^3/mole at 25°C)	2.7×10^{-4}	6.7×10^{-5}	7.3×10^{-5}	3.3×10^{-8}
Water solubility (mg/L)	1,250	1,000,000	306,000	167,200 (estimated)[a]
Log K_{ow}	2.8[d]	−0.34	0.59	−0.33
pKa	NV	13.57	NV	NV

[a]Data was gathered from the PHYSPROP database for each respective compound unless otherwise specified (U.S. EPA, 2012b).
[b]DSSTox (2016).
[c]OECD (2017).
[d]U.S. EPA (2015).

US EPA, 2017, Provisional Peer-Reviewed Values for n-Heptanal, Cincinnati, OH.

Read-across PPRTV Example: toxicokinetic similarity

Table A-2. Available Metabolism and Excretion Data for *n*-Heptanal (CASRN 111-71-7) and Candidate Analogs

Compound	Metabolism and Excretion	Reference
n-Heptanal	• Oxidation to heptanoic acid. • Heptanoic acid undergoes β-oxidation, yielding acetyl-CoA and propionyl-CoA. • Propionyl-CoA is converted to succinyl-CoA. • Acetyl-CoA and succinyl-CoA are used by Krebs cycle and ultimately exhaled as CO_2.	WHO (1999); RIFM (1979)
Acetaldehyde	• Oxidation to acetic acid. • Acetic acid condenses with CoA to form acetyl-CoA. • Acetyl-CoA is used by the Krebs cycle and ultimately exhaled as CO_2.	U.S. EPA (2008b); WHO (1999)
Propionaldehyde	• Oxidation to propanoic acid. • Propanoic acid condenses with CoA to form propionyl-CoA. • Propionyl-CoA is converted to succinyl-CoA. • Succinyl-CoA is an intermediate in the Krebs cycle and is ultimately exhaled as CO_2.	U.S. EPA (2008b); WHO (1999)
Glutaraldehyde	• Oxidation to glutaric acid. • Glutaric acid reacts with CoA to form glutaconyl-CoA. • Glutaconyl-CoA is decarboxylated to crotonyl-CoA, followed by hydration to β-hydroxybutyrl-CoA. • β-hydroxybutyrl-CoA condenses to form acetyl-CoA, which is used by the Krebs cycle and ultimately exhaled as CO_2.	Beauchamp et al. (1992); NTP (1999)

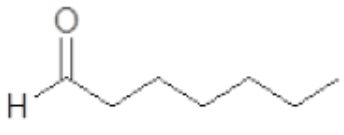
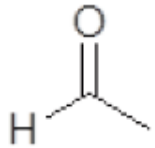
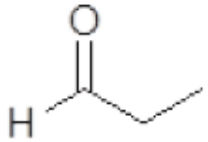
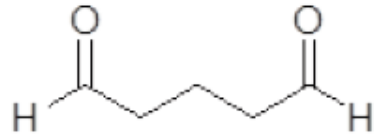
CoA = coenzyme A; CO_2 = carbon dioxide.

- Similarities in metabolism and excretions pathways
- Aldehyde dehydrogenase (ALDH) is a key enzyme in the metabolism/detoxification of aldehydes
- Similar metabolic rates for target and analogues in human liver samples (Wang et al., 2002)

US EPA, 2017, Provisional Peer-Reviewed Values for n-Heptanal, Cincinnati, OH.

Read-across PPRTV Example: toxicodynamic similarity

Table A-3. Comparison of Available Toxicity Data for *n*-Heptanal (CASRN 111−71−7) and Candidate Analogs

	***n*-Heptanal (heptaldehyde)**	**Acetaldehyde (ethanal)**	**Propionaldehyde (propanal)**	**Glutaraldehyde (pentanedial)**
Structure				
CASRN	111-71-7	75-07-0	123-38-6	111-30-8
Repeated-dose toxicity (inhalation)				
POD (mg/m^3)	NV	8.7	8	0.002
POD type	NV	NOAEL (HEC)	$BMCL_{10}$ (HEC)	BMC_{05} (HEC)
UF_C	NV	1,000	1,000	30
RfC or REL (mg/m^3)	NV	9×10^{-3}	8×10^{-3}	8×10^{-5}
Critical effects	NV	Degeneration of olfactory epithelium at concentrations ≥400 ppm.	Atrophy of the olfactory epithelium in rats at concentrations ≥150 ppm.	Squamous metaplasia of the respiratory epithelium at concentrations ≥62.5 ppb.

US EPA, 2017, Provisional Peer-Reviewed Values for n-Heptanal, Cincinnati, OH.

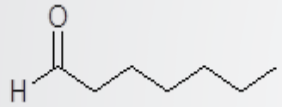
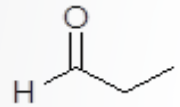
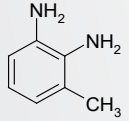
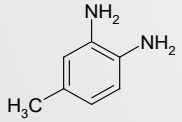
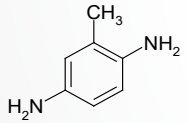
Read-across PPRTV Example: WOE conclusions and toxicity value derivation

- **Propionaldehyde** is selected as the source analogue based primarily on the shared reactive aldehyde moiety and taking into consideration the effect on carbon chain length on p-chem properties and acute toxicity

Screening Chronic p-RfC	=	Surrogate POD (HEC) ÷ UF_C
	=	$8\ mg/m^3 \div 3{,}000$
	=	$\mathbf{3 \times 10^{-3}\ mg/m^3}$

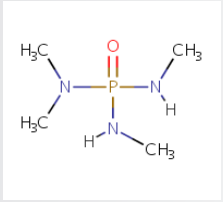
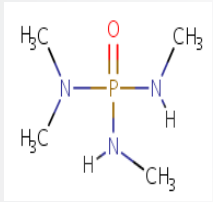
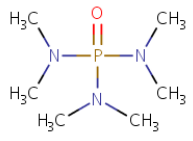
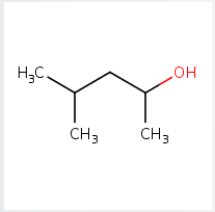
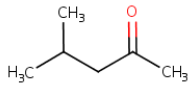
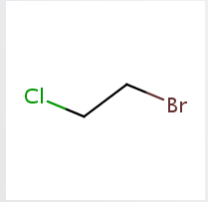
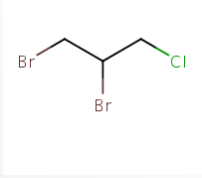
US EPA, 2017, Provisional Peer-Reviewed Values for n-Heptanal, Cincinnati, OH.

Key Lessons from Application of Read-cross to PPRTVs

Target Chemical(s) (CASRN)	Source Analogue (CASRN)	Key Lessons (general and case-specific)
 n-Heptanal (111-71-1)	 Propanal (123-38-6)	• Structural similarity metrics used to identify and rank analogues have inherent limitations • Additional software tools and expert judgement are needed during the analogue search process • The requirement for analogues to have toxicity values for inclusion in the read-across restricts the pool of candidates and chemical grouping approaches
 2,3-toluenediamine (2687-25-4)  3,4-toluenediamine (496-72-0)	 2,5- toluenediamine (95-70-5)	

Lizarraga et al., In preparation

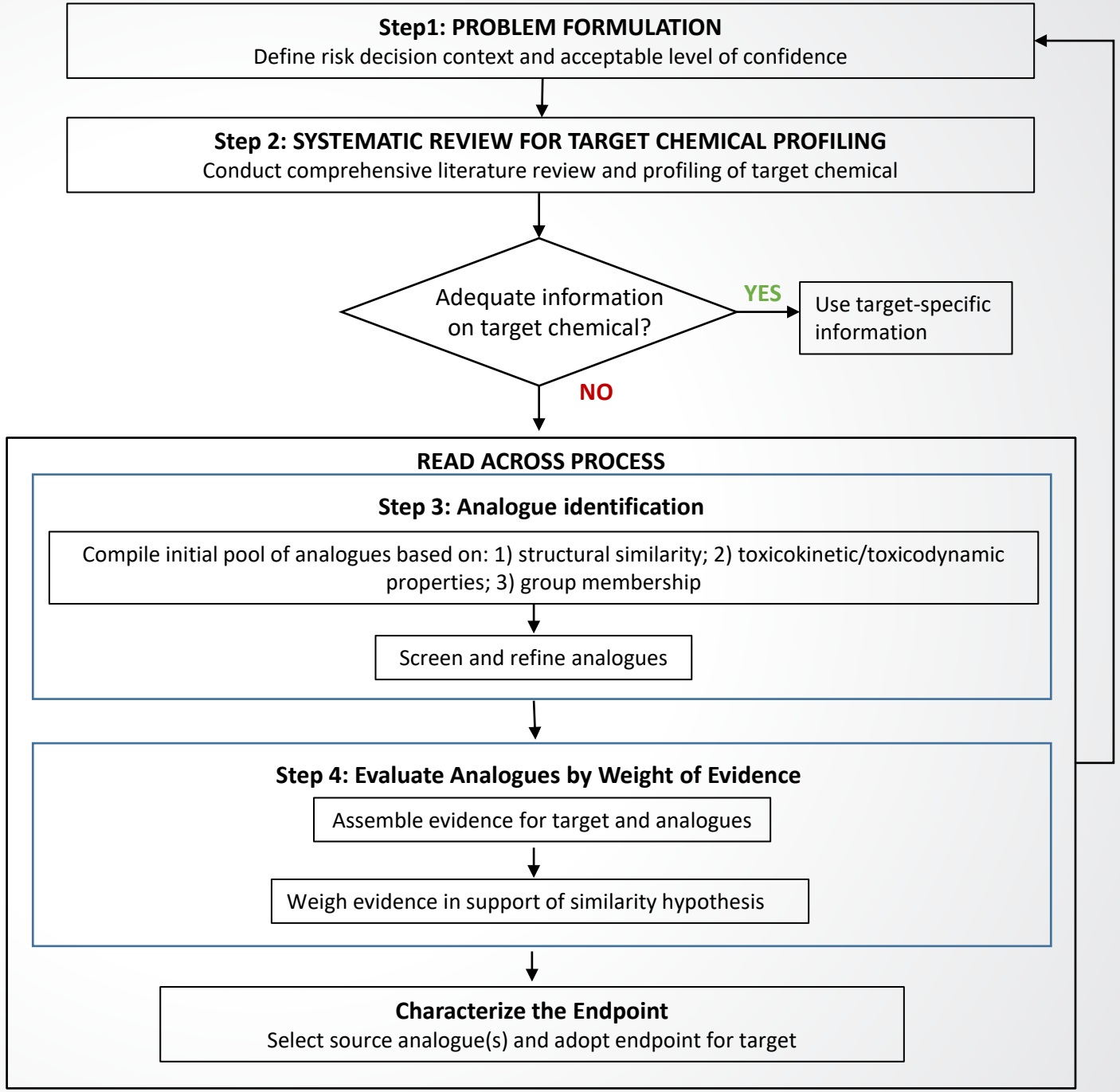
Key Lessons from Application of Read-cross to PPRTVs

Target Chemical(s) (CASRN)	Source Analogue (CASRN)	Key Lessons (general and case-specific)
 PMPA (10159-46-3)  TMPA (16853-36-4)	 HMPA (680-31-9)	• Emphasis on using biological similarity as a means for grouping chemicals for read-across • Case studies highlight the use of metabolism and mechanistic considerations to select source analogues • Test data are often limited or unavailable • NAM data streams (e.g., in vitro or in silico metabolism predictions) are necessary to fill in knowledge gaps
 MIBC (108-11-2)	 MIBK (108-10-1)	
 1-Bromo-2-chloroethane	 1,2-Dibromo-3-chloropropane	

Lizarraga et al., In preparation

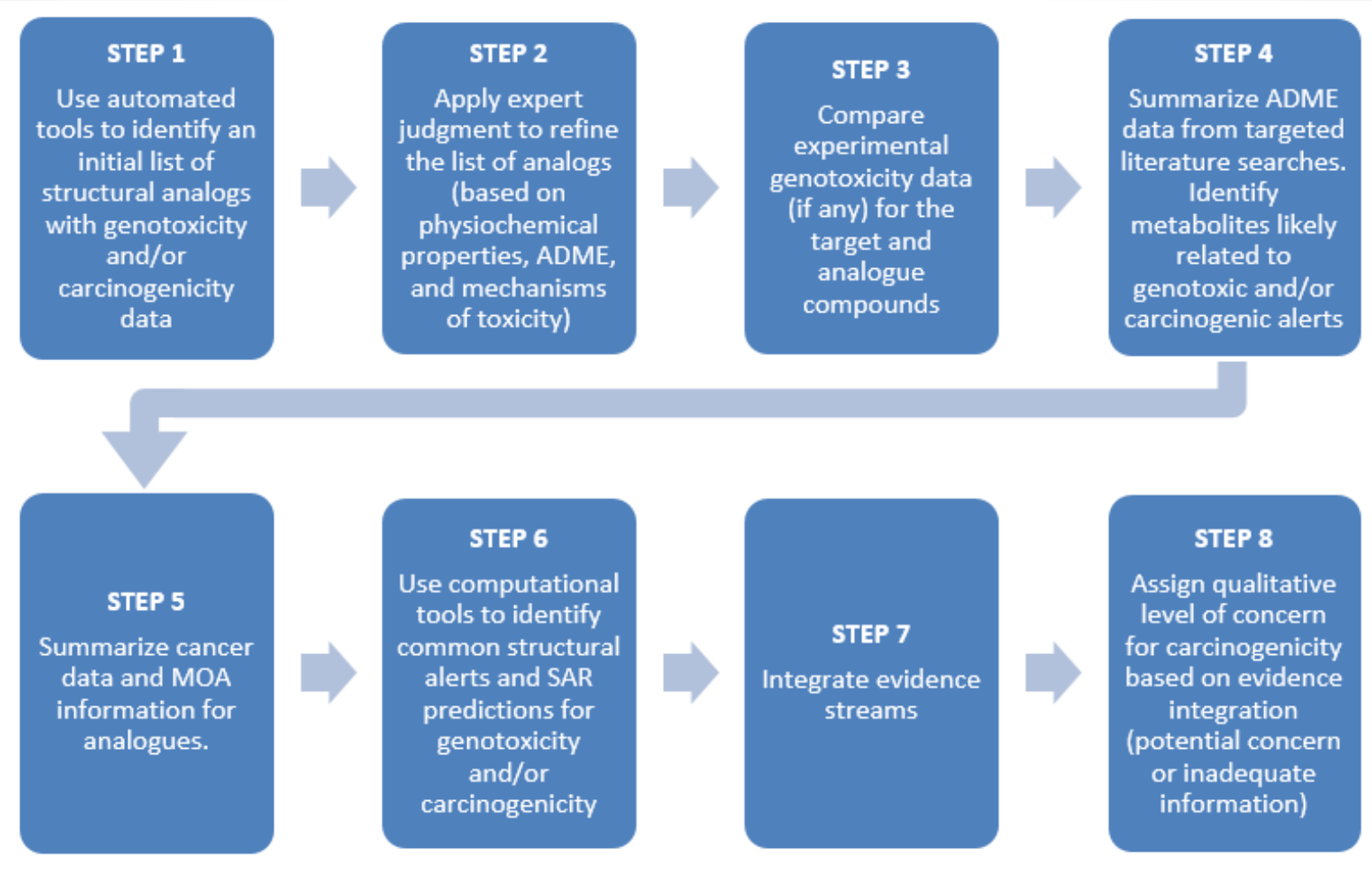
Revised Methodology

- Considerations for problem formulation, target chemical profiling, analogue identification, analogue evaluation by WoE and incorporation of NAMs



Lizarraga et al., In preparation

Expanding Read-across Applications: Qualitative Level of Concern for Carcinogenicity



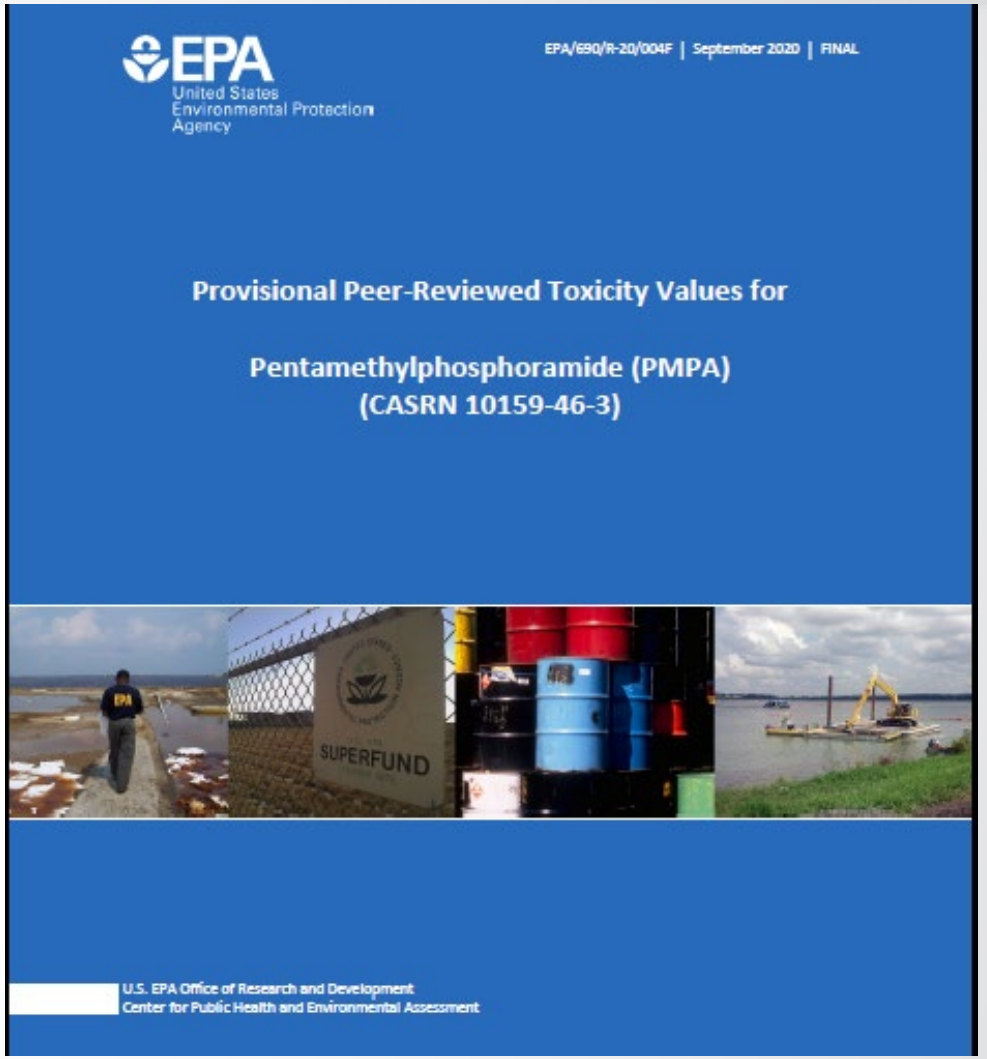
Dean et al., In preparation

Expanding Read-across Applications: Qualitative Level of Concern for Carcinogenicity

Table C-3. Qualitative Level of Concern for Carcinogenicity of PMPA (CASRN 10159-46-3)

Level of Concern	Designation	Comments
Concern for Potential Carcinogenicity	Selected	HMPA is an appropriate analogue for assessing the carcinogenicity of PMPA because it is a metabolic precursor to the target chemical, and because both compounds generate the same carcinogenic metabolite, formaldehyde. There is *"Suggestive Evidence of Carcinogenic Potential"* for HMPA by the inhalation route of exposure based on increased incidences of squamous cell carcinomas of the rat nasal cavity in both sexes. The intracellular release of formaldehyde, which has been suggested to be responsible for HMPA's carcinogenic effects, would be expected to occur for PMPA as well.
Inadequate Information for Assigning Qualitative Level of Concern	NS	NA

HMPA = hexamethylphosphoramide; NA = not applicable; NS = not selected;
PMPA = pentamethylphosphoramide.



US EPA, 2020, Provisional Peer-Reviewed Values for Pentamethylphosphoramide (PMPA), Cincinnati, OH.

Expanding Read-across Applications: IRIS Mercury Salts Assessment

EPA

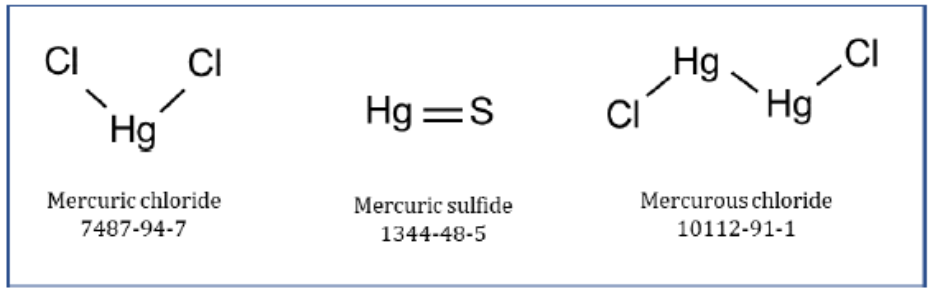
EPA/635/R-20/239
IRIS Assessment Protocol
www.epa.gov/iris

Systematic Review Protocol for the Inorganic Mercury Salts IRIS Assessment

CASRN 7487-94-7 (Mercuric Chloride)
CASRN 1344-48-5 (Mercuric Sulfide)
CASRN 10112-91-1 (Mercurous Chloride)

Integrated Risk Information System
Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

DRAFT- DO NOT CITE OR QUOTE



Mercuric chloride
7487-94-7

Mercuric sulfide
1344-48-5

Mercurous chloride
10112-91-1

10.2.2. Strategies To Identify Analogues To Inform Read-Across for Mercurous Chloride

Based on the preliminary literature search, appropriate data for conducting hazard identification and dose-response analysis are not available for mercurous chloride. Thus, an analogue-based, read-across approach will be attempted for this salt to calculate toxicity values.

The analogue approach allows for the use of data from related compounds to calculate toxicity values when data for the compound of interest are limited or unavailable. Details regarding searches and methods for surrogate analysis are presented in Wang et al., (2012). Three types of potential surrogates (structural, metabolic, and toxicity-like) will be identified to facilitate the final surrogate chemical selection. The surrogate approach might or might not be route specific or applicable to multiple routes of exposure. All information will be considered together as part of the final weight-of-evidence (WOE) approach to select a potentially suitable surrogate both toxicologically and chemically.

This WOE approach will be used to evaluate information from potential candidate surrogates, as described by Wang et al., (2012). Commonalities in structural/physicochemical properties, toxicokinetics, metabolism, toxicity, or MOA between potential surrogates and chemical(s) of concern will be identified. Emphasis will be given to toxicological or toxicokinetic similarity over structural similarity. Surrogate candidates will be excluded if they do not have commonality or demonstrate significantly different physicochemical properties, and toxicokinetic profiles that set them apart from the pool of potential surrogates and chemical(s) of concern. From the remaining potential surrogates, the most appropriate surrogate will be selected. The selection will be based on consideration of the biological and toxicological relevant analogues, structural similarities as well as sensitivity of toxicological values.

Conclusions

- Read-across is routinely used for hazard assessment and deriving toxicity values within the PPRTV program
- A revised methodology is proposed based on practical experience and advances in the field of read-across and NAMs
- Opportunity to expand the scope of read-across applications to support HERA-related products

Acknowledgments

PPRTV Read-across Team:
Glenn Suter
Jeffry Dean
Jay Zhao
Dan Petersen

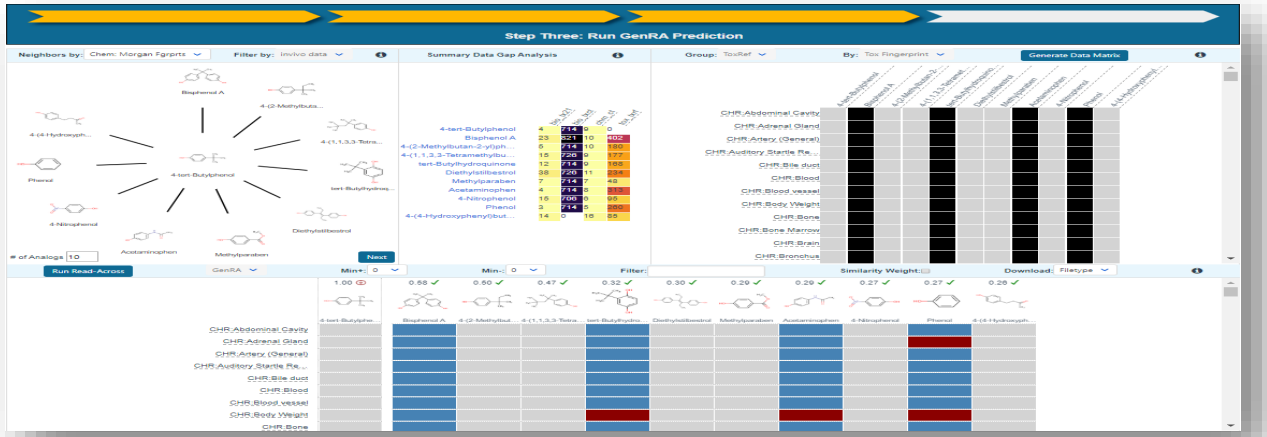
PPRTV Management:
J. Phillip Kaiser
Glenn Rice
Teresa Shannon
Kris Thayer

CCTE:
Grace Patlewicz
Jason Lambert

IRIS:
Nagu Keshava

HERA National Program:
Beth Owens, RAC
Samantha Jones, Director

Filling Metabolism Data Gaps in Read-across



Matthew Boyce, Brian Meyer, Vicente Samano, Chris Grulke, Lucina Lizarraga*, Grace Patlewicz

Center for Computational Toxicology & Exposure (CCTE), US EPA

*Center for Public Health and Exposure Assessment (CPHEA), US EPA

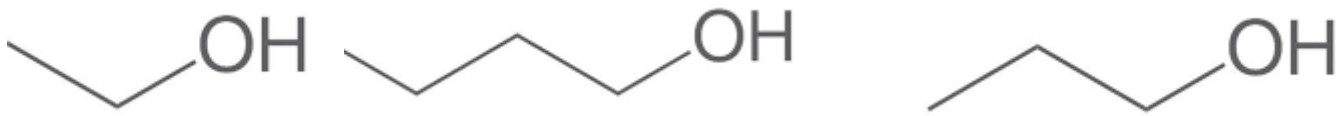
Outline

- Read-across definition
- Addressing shortcomings of current read-across approaches
- Contexts of similarity for read-across: Metabolic similarity
- Comparing and contrasting different data streams that provide metabolism information
- *In silico* metabolism: Assessing performance and coverage of tools
- Summary & Next steps

What is Read-Across?

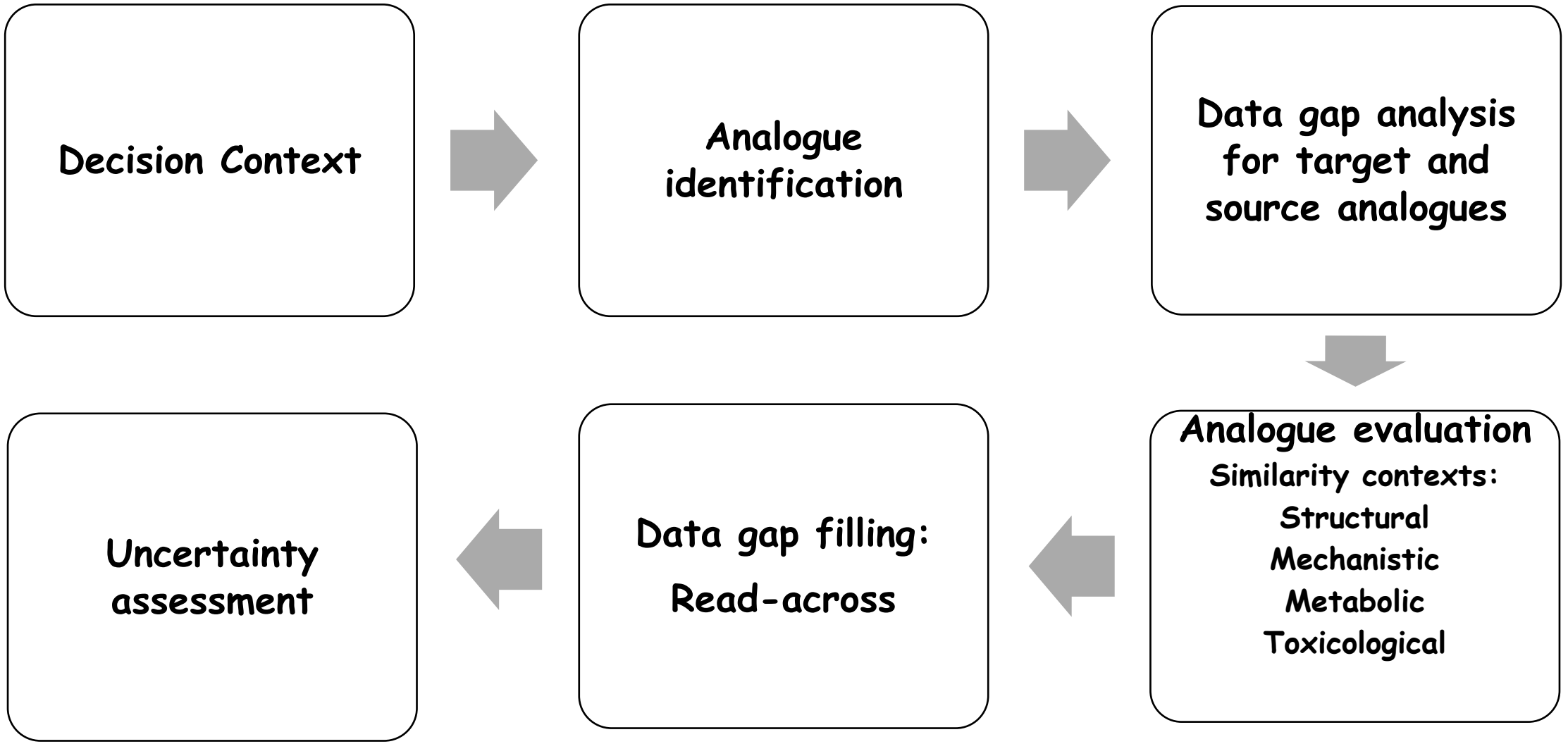
- Read-across describes the method of filling a data gap whereby a chemical with existing data values (source analogue) is used to make a prediction for a ‘similar’ chemical (target).

Source **Target**



	Ethanol	Butanol		Propanol
Property 1	●	●	→	○
Property 2	●	●		●
Property 3	●	●	→	○

Generic Read-across workflow



Ongoing issues with read-across

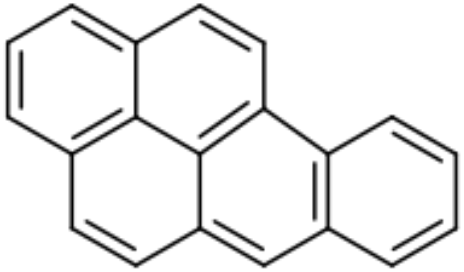
- Although there is much guidance for developing read-across assessment, acceptance remains an issue, not helped since read-across still remains a subjective, expert driven assessment.
- One issue thwarting acceptance relates to the “uncertainty of the read-across prediction”.
- As such there have been many efforts to identify the sources of uncertainty in read-across, characterize them in a consistent manner and identify practical strategies to address and reduce those uncertainties.
- Notable in these efforts have been the development of frameworks for the assessment of read-across, evaluating the utility of New Approach Methods (NAMs).
- Quantifying uncertainty and performance of read-across is still a need as are ways to better characterize different similarity contexts (metabolism, reactivity etc.)

5

Metabolism is an important similarity context

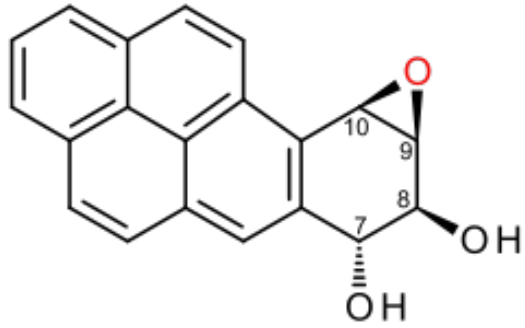
- Through metabolic activation, compounds can see a significant increase in toxicity which is not captured by the parent structure.

Example: Phase 1 metabolism of benzo[a]pyrene yields an epoxide ring that allows it to bond to DNA



Benza[a]pyrene

CYP1A1/CYP1B1 CYP1A1/CYP1B1

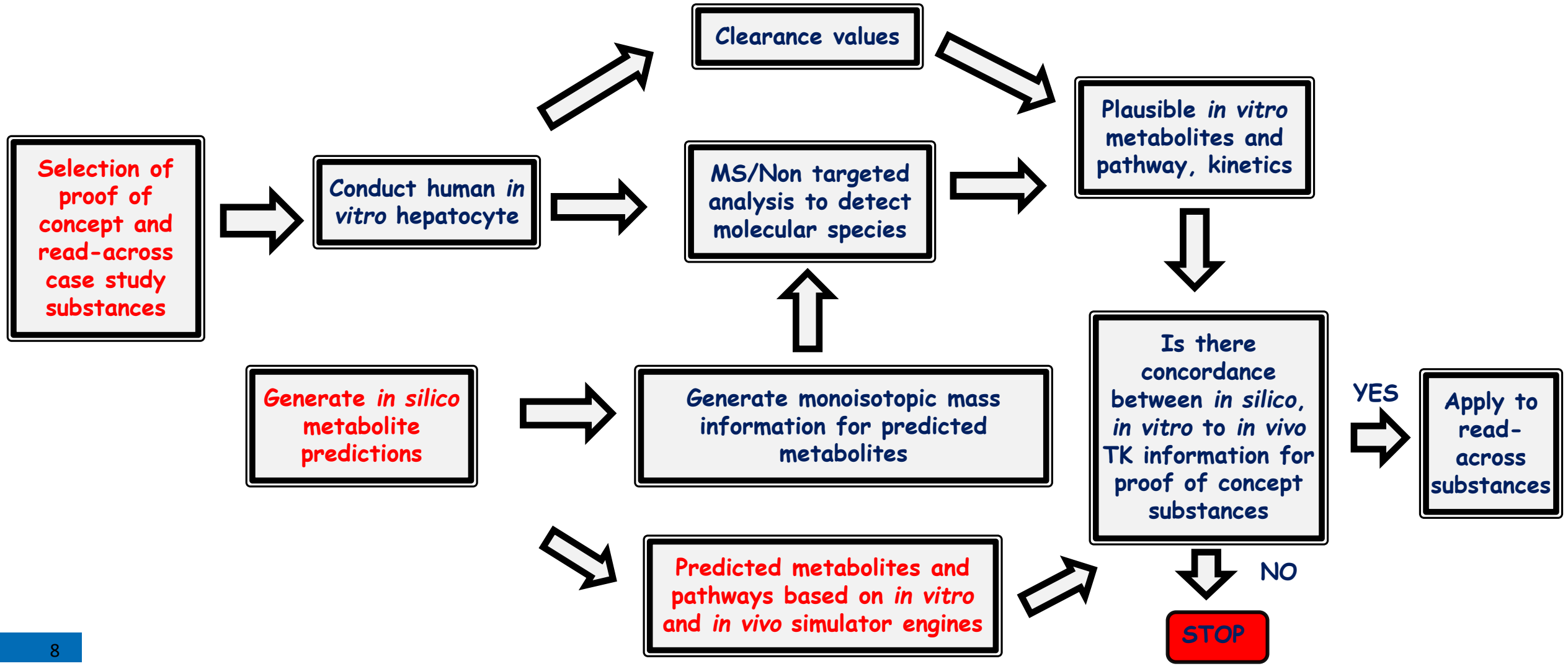


Benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide

Compare and contrast different sources of metabolism information

- Aim to investigate the concordance between *in vivo*, *in vitro* and *in silico* metabolism information and how it can be utilized to assess metabolic similarity for read-across
- Data streams
  - *In vitro*:
    - Perform *in vitro* human hepatocyte study to determine intrinsic clearance
    - Apply analytical spectroscopy (MS) for the detection of molecular species and non-targeted analysis for metabolite identification
  - *In silico*
    - Use third party expert systems for the prediction of potential metabolites and their pathways to facilitate MS analysis
  - *In vivo*
    - Extract data in the peer reviewed literature

Overall Project Workflow

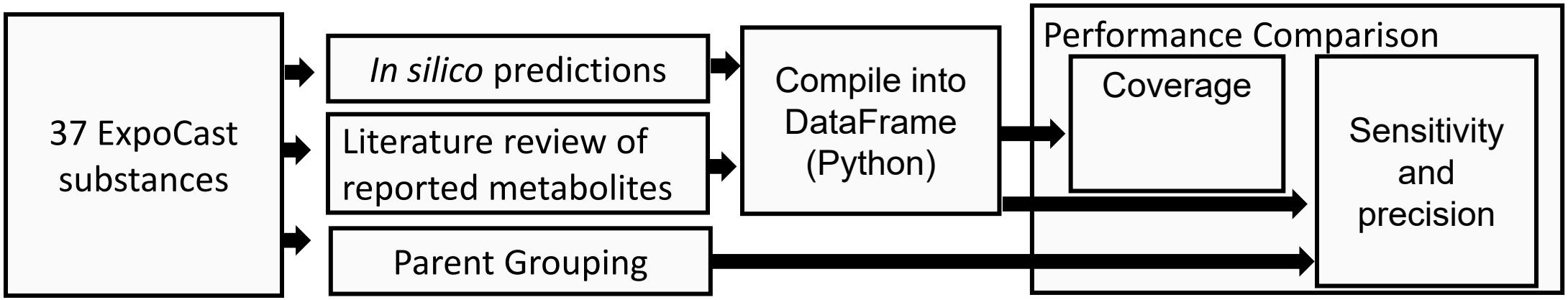


In silico tools for metabolite predictions

- *In silico* tools can provide a rapid and efficient method of predicting metabolites for compounds that lack published research.
- There are a number of metabolism prediction tools. Examples include: MetaPrint 2D, Meteor Nexus, TIMES, the simulators contained within the OECD Toolbox, Symga and Biotransformer.
- Some are freely available, others such as TIMES and Meteor are commercial.
- Few studies have been performed to directly compare the performance of these tools.

Evaluating *in silico* tools

37 proof of concept substances were selected from the ToxCast library—these compounds represented a broad spectrum of pharmaceutical, agrochemical, and industrial chemistries.



EPA United States Environmental Protection Agency

Selected *in silico* tools

	TIMES*	QSAR ToolBox	Meteor	BioTransformer	SyGMa	CTS
Developer	LMC	OECD and ECHA	Nexus	Wishart Group	Riddler & Wagener	EPA
Availability	Commercial	Public	Commercial	Public	Public	Public
Knowledge base	Expert + Statistical Score	Expert + Statistical Score	Expert + Statistical Score	Expert + ML	Expert	-
Customizable Met.	Yes	No	Yes	Yes	Yes	Limited
Interface	GUI	GUI/API	GUI‡	API/CMD Prompt/Terminal	API/Phyton	WebApp
Available Modules	Rat liver (*in vivo*), Rat liver (S9, *in vitro*), Lung (mammal), Gut (mammal), Autoxidation	Autoxidation, Hydrolysis, Rat (S9, *in vitro*), Rat (*in vivo*)	Mammals (Dog, Human, Mouse, Rat)	Human (Liver, Gut), Microbial	Human (Liver)	Human
#Predictions	211 (*vitro*), 459 (*vivo*)	312	459	827	5215	472

*Two modules were used separately for this work: rat *in vivo*, rat *in vitro*

‡ Batch mode requires command prompt or terminal

Literature review

Identification of known metabolites for all 37 ToxCast compounds:

-Extracted metabolites from 49 papers (prioritized primary articles)

-Identified 438 metabolites across all compounds

-Species were recorded, but not considered in performance comparison

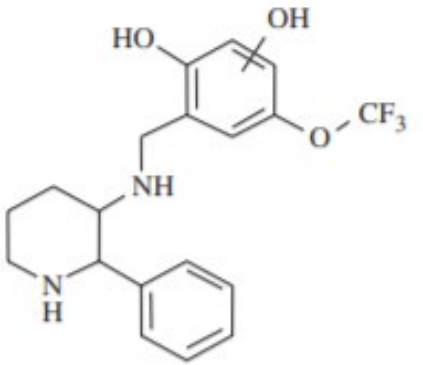
Metabolites were registered into EPA's DSSTox chemical registration system to generate specific identifiers (DTXSID/DTXCIDs) to facilitate subsequent data analysis

-Metabolism pathways captured using Proceeding/Preceding structures

-SMILES and InChI Keys were queried and retrieved for downstream analysis

Accounting for structural isomers

CP-122,721 metabolite



20 Isomers reported in literature

- Prediction software generates discrete structures, which need to be reconciled against literature for evaluation

- Requires enumeration of each potential metabolite to generate InChI Key
- Generated 585 Markush children

Quantifying performance: metrics used

Metric of model similarity

Coverage:

How well does model A match the predictions of model B?

$$\frac{Predictions\ A\ \cap Predictions\ B}{Predictions\ B}$$

Metrics of model performance

Sensitivity:

Does the model cover all reported metabolites?

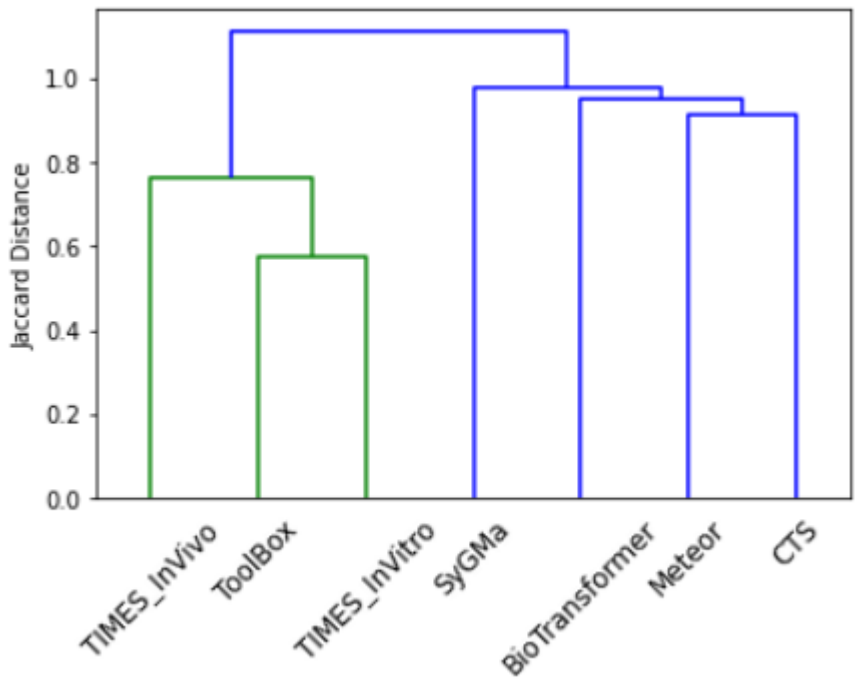
General equation: $\frac{True\ Predictions}{All\ Reported}$

Precision:

Are the predicted metabolites true?

General equation: $\frac{True\ Predictions}{All\ Predictions}$

Comparing the *in silico* tools: relative coverage

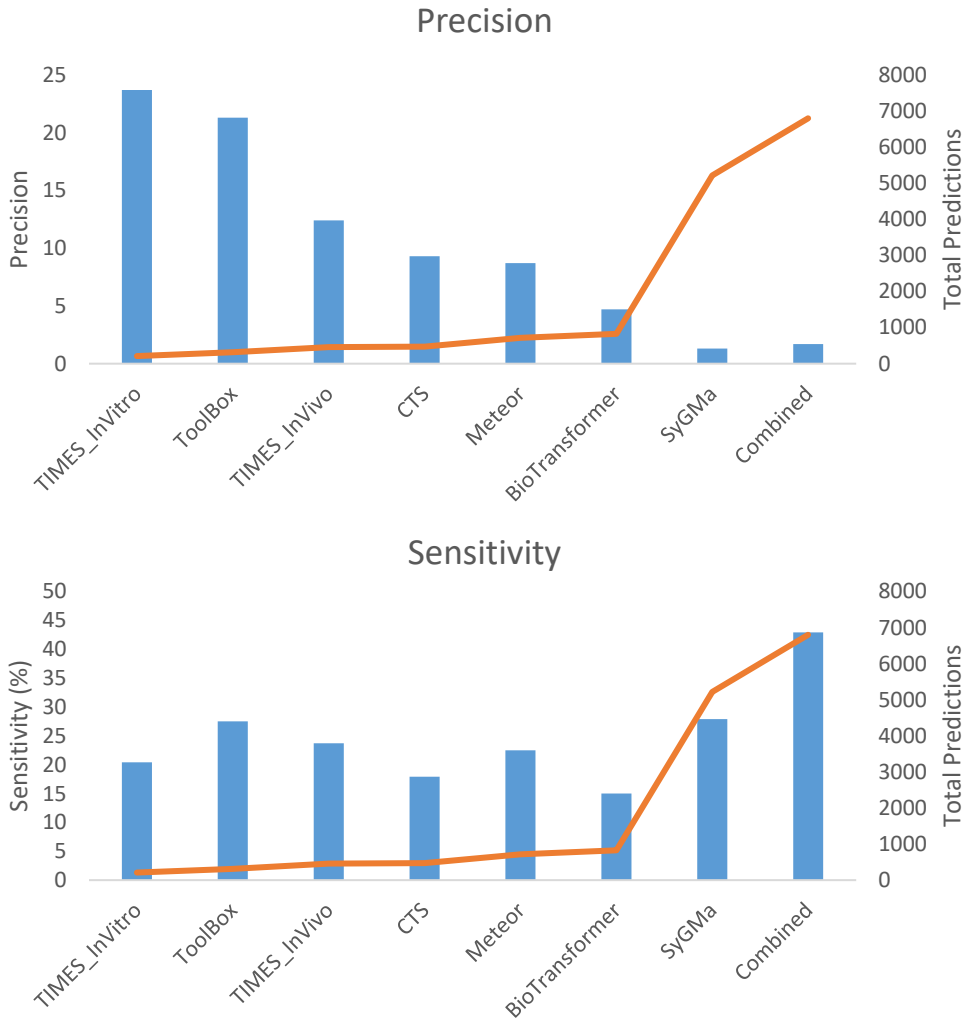


Model	%Coverage of other software							
	ToolBox	Meteor	BioTransformer	TIMES In Vivo	TIMES In Vitro	SyGMa	CTS	Avg.
ToolBox	-	15.13	7.62	40.74	74.41	2.86	27.33	28.02
Meteor	34.39	-	9.07	25.93	33.65	4.60	19.92	21.26
BioTransformer	20.06	10.50	-	10.24	23.70	4.31	17.16	14.33
TIMES In Vivo	59.55	16.67	5.68	-	61.61	3.99	14.41	26.99
TIMES In Vitro	50.00	9.94	6.05	28.32	-	2.13	16.74	18.86
SyGMa	47.45	33.61	27.21	45.32	52.61	-	32.42	39.77
CTS	41.08	13.17	9.79	14.81	37.44	2.93	-	19.87

- Significant overlap between Toolbox and TIMES models
- To ensure coverage – need a battery of different tools

Comparing *in silico* performance: Precision and Sensitivity

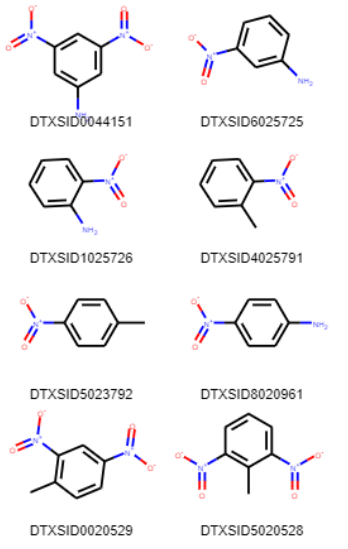
Model	Total	Unique	Precision	Sensitivity
ToolBox	314	12	21.3	27.5
Meteor	714	436	8.7	22.5
BioTransformer	827	570	4.7	15.0
TIMES_InVivo	459	122	12.4	23.7
TIMES_InVitro	211	10	23.7	20.4
SyGMa	5215	4667	1.3	27.9
CTS	472	252	9.3	17.9
Combined	6799	-	1.7	42.9



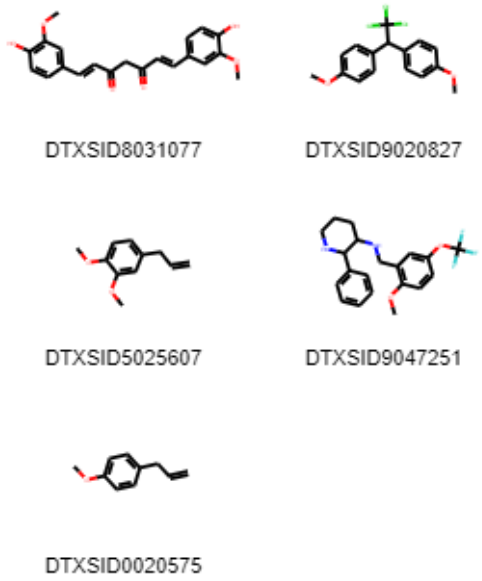
Assessing ‘local’ performance

To compare performance differences relative to the parent compound, five groupings were generating using ClassyFire classifications (a structural/functional group hierarchy) combined with clustering approaches

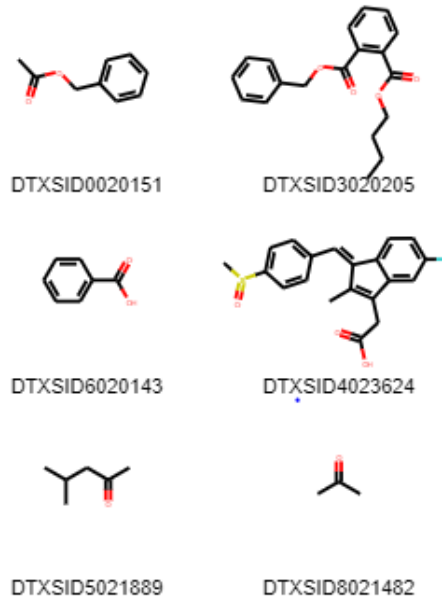
Group 1
Nitrobenzenes



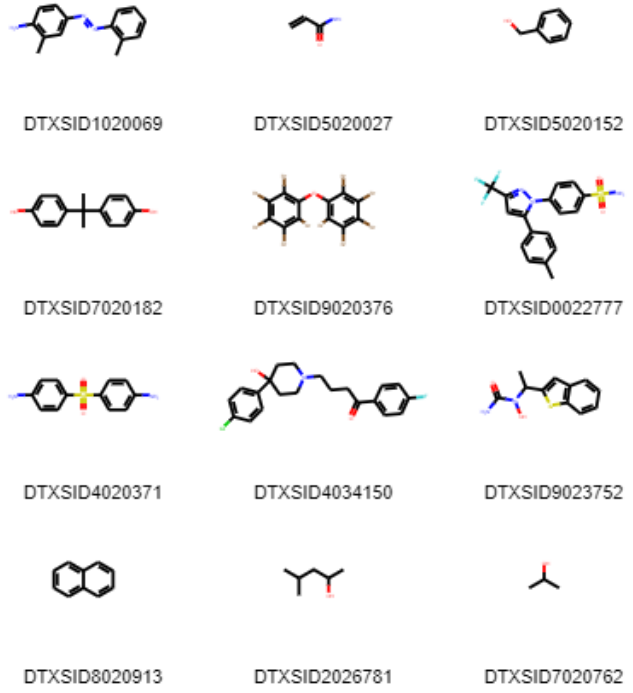
Group 2
Methoxybenzene



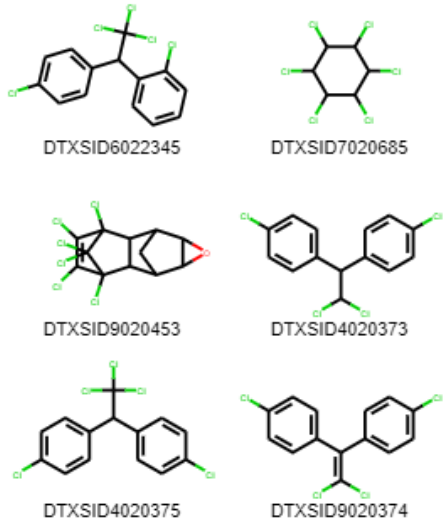
Group 3
Ketone/Benzoyl



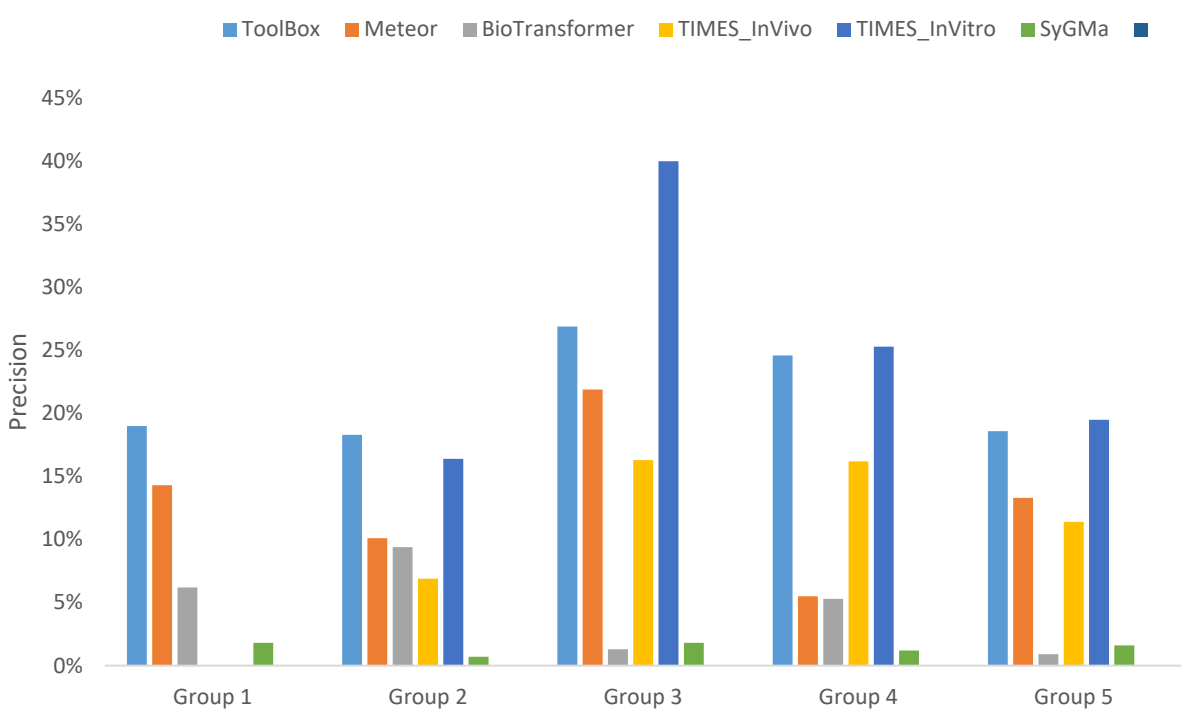
Group 4
Misc

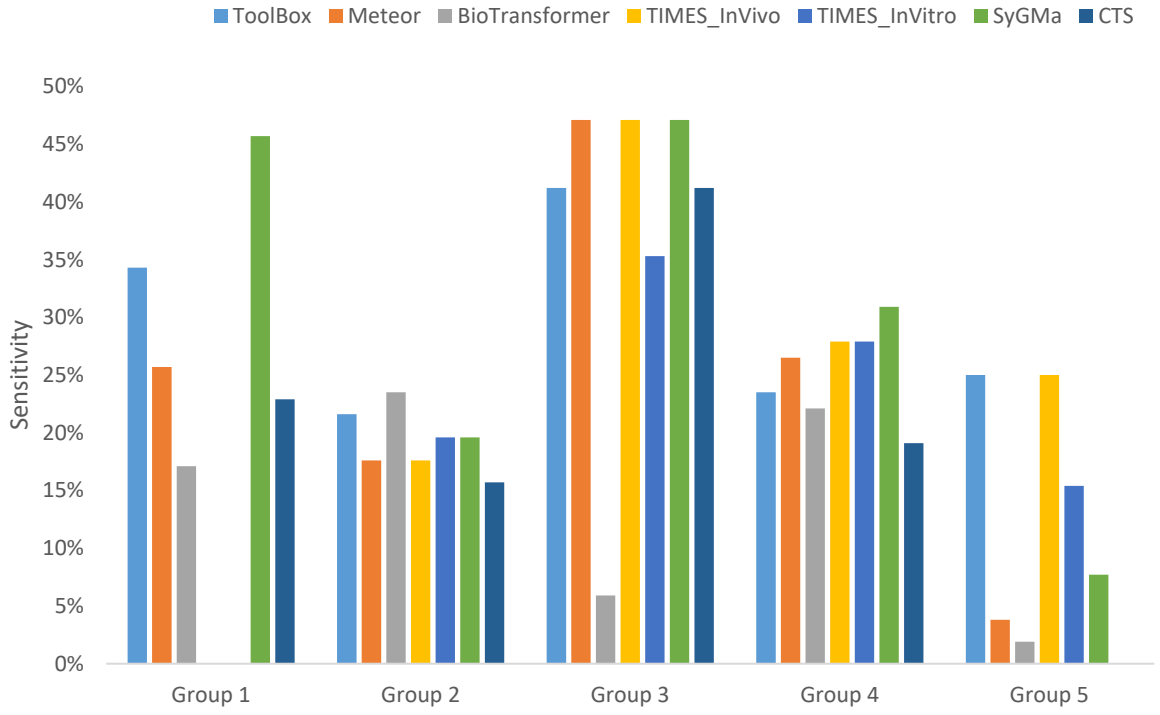


Group 5
Poly-chlorinated



‘Local’ chemical space performance





Summary

- Metabolic similarity is an important component in evaluating analogue suitability within a read-across.
- Approaches to characterize and quantify metabolic similarity is needed
- A proof of concept study is ongoing to compare and contrast different metabolism information sources and evaluate their utility for read-across amongst other purposes.
- Specific *in vitro* data has been generated and is currently being evaluated.
- Predictions have been generated using a selection of *in silico* tools.
- Experimental data has been extracted from the literature.

Summary

- The performance of *in silico* metabolite prediction tools has been evaluated.
    - Coverage was calculated to provide relative comparisons between each tool and provided a metric for prediction similarity
    - Sensitivity and precision were determined by comparing predictions against metabolites reported in literature
    - Using ClassyFire classifications, model performance could be evaluated relative to the 'local' chemical space of the starting compounds
- A manuscript to summarize the *in silico* evaluation is in preparation.
- Next steps include:
    - Evaluating the concordance of *in vitro* data generated relative to the literature data collected and the *in silico* tools
    - Generate *in silico* predictions for other substances relevant to PPRTV
    - Investigate how to codify and quantify the metabolism information from 1 or more of the *in silico* tools for the purposes of read-across

The Advent of Adverse Outcome Pathway Footprinting

Jason C. Lambert, PhD, DABT
U.S. EPA, ORD, Center for Computational Toxicology and Exposure

Board of Scientific Councilors meeting
February 4, 2021

Background

- Human environmental exposures are typically to mixtures of chemical, biological, and physical stressors
- Rarely are hazard and dose-response data available for chemical mixtures of interest (e.g., component proportions; relevant doses), and 'traditional' assay data may also be lacking for individual mixture component chemicals
- This lack of available assessment relevant information may lead to under-estimation of risk to human health and the environment due to mixture exposures
- The time and resources needed to conduct traditional chemical by chemical analyses that inform phenotypic outcomes are not conducive for informing a broad landscape of current human health assessment concerns

➢ Integration of data from New Approach Methods (NAM) may provide opportunities to evaluate hazards associated with exposure to mixtures containing data-poor component chemicals

Bold new world with NAMs…

Conceptual Approach to Integrated Testing and Assessment (IATA) of Mixtures

(A) Problem formulation
- Screening/Prioritization?
- Hazard identification/grouping?
- Mixtures dose-response assessment?

(B) 'Fit-for-purpose' toolbox
- *Data mining* - exposure and hazard data
- *Cheminformatics* – (Q)SAR/read-across
- *High-throughput exposure modeling* – ExpoCast
- High-throughput TK – IVIVE/reverse dosimetry
- *Bioactivity* – ToxCast, Tox21, REACH
- *Adverse Outcome Pathway 'Footprinting'*

(C) WOE for mixture chemicals
- AOPs available for chemicals of interest?
- Anchor chemical(s) identified?
- D-R data available for mixture chemicals?

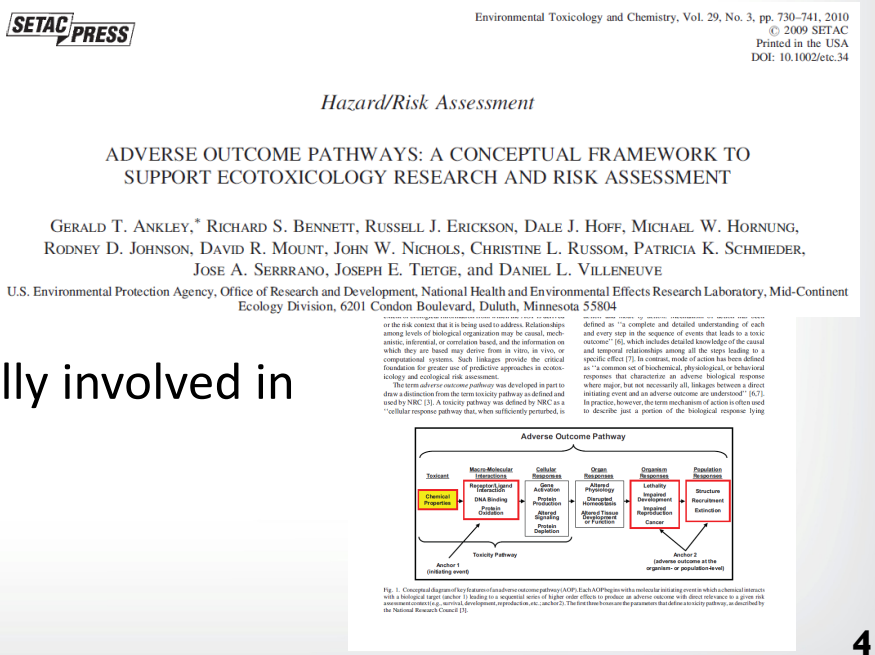
(D) Component-based Mixtures RA
- Apical and/or key event-based PODs
- D-R curves suitable for potency eval
- Multiple active AOPs/MOAs

Adverse Outcome Pathway (AOP)

- A way to organize potentially diverse streams of biological information to inform a given source to health outcome continuum
- Data used in an AOP may span different levels of biological organization relevant to human health and/or ecological assessment (e.g., molecular, cellular, tissue/organ, up to whole organismal and/or population based)

<u>Key Principles of AOPs</u>

- ❖ AOPs are chemical agnostic
- ❖ AOPs are commonly simplifications of complex biology
- ❖ AOPs are nodal/modular
- ❖ Multiple AOPs (i.e., AOP network) typically involved in phenotypic expression of bioactivity



SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 29, No. 3, pp. 730–741, 2010

Printed in the USA
DOI: 10.1002/etc.34

Hazard/Risk Assessment

ADVERSE OUTCOME PATHWAYS: A CONCEPTUAL FRAMEWORK TO SUPPORT ECOTOXICOLOGY RESEARCH AND RISK ASSESSMENT

GERALD T. ANKLEY,* RICHARD S. BENNETT, RUSSELL J. ERICKSON, DALE J. HOFF, MICHAEL W. HORNUNG, RODNEY D. JOHNSON, DAVID R. MOUNT, JOHN W. NICHOLS, CHRISTINE L. RUSSOM, PATRICIA K. SCHMIEDER, JOSE A. SERRRANO, JOSEPH E. TIETGE, and DANIEL L. VILLENEUVE

U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, 6201 Condon Boulevard, Duluth, Minnesota 55804

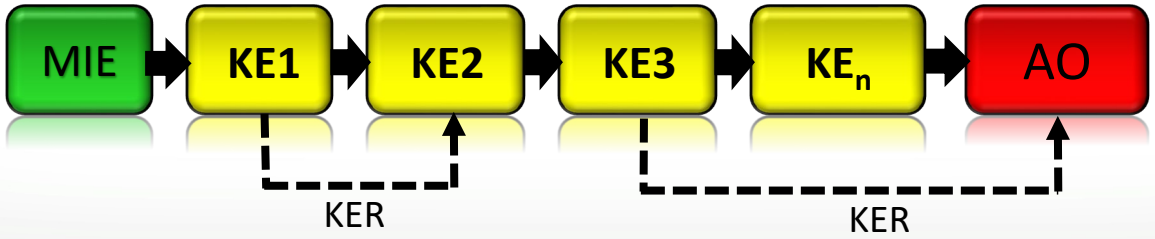
AOP Development

Key event (KE)

- *Functional qualitative unit of observation (i.e., what happened)*
- Observable Δ in biological state (measurable)
- Essential (but not necessarily sufficient to induce AO alone)

Key event Relationship (KER)

- *Functional unit of quantitative inference/extrapolation (i.e., relationship between direction and magnitude of Δ in a KE and other members of AOP)*
- State of $KE_{up/down}$ has some causal relationship to one or more other $KE_{up/down}$ and or $AO_{up/down}$
- Supported by biological plausibility and weight-of-evidence



AOP "Footprinting" Concept

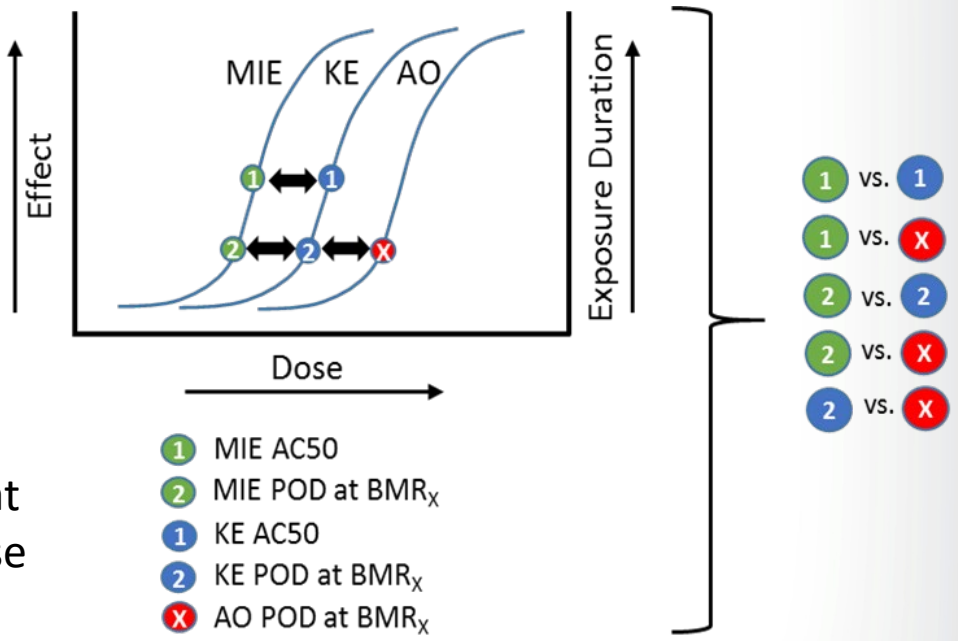
- **In contrast to AOP theory which posits a chemical agnostic description of the MIE to AO pathway, the footprinting approach first requires identification of well-characterized (hazard and dose-response) chemical(s) as the "anchor" or "index" for each toxicologically operative AOP**

- AOP footprinting is the stepwise profiling and comparison of AOPs at the level of key events moving backward from the most downstream key event to the molecular initiating event

- The goal is to identify the key event(s) within each AOP suspected of contributing to a given adverse outcome at which similarity between mixture chemicals can confidently be determined. These key events are identified as the 'footprint' for a given AOP
- Mixture chemicals are then assigned to the appropriate 'footprint' category, and the key event dose-response relationship(s) (KER) for each chemical within a category are then used to evaluate mixture additivity

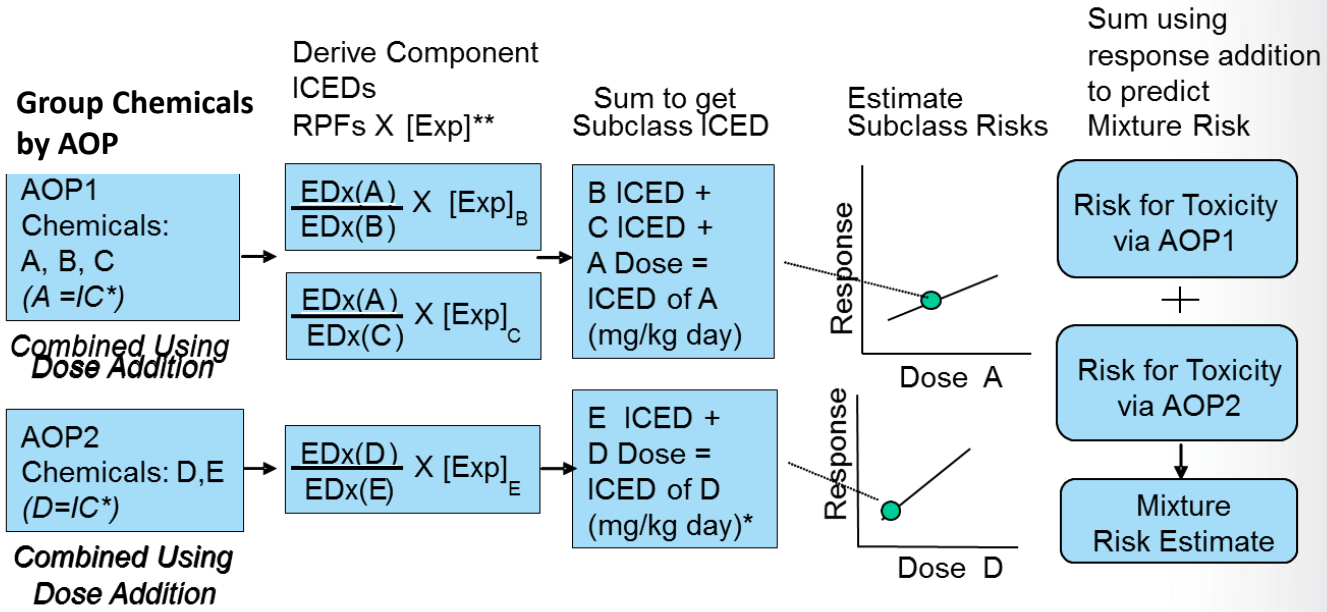
Footprint Identification and KER evaluation

- A key to identifying the 'footprint' is the WOE supporting the hazard and dose-response relationship to the AO (i.e., if the KE went away would incidence and/or severity of the AO change?)
- For most AOPs, there may be greater confidence in a 'footprint' if it is mechanistically proximal to the AO, however this will be dependent on KE data availability
- Quantitatively, benchmark doses (BMD) at biologically-informed benchmark response levels (BMR) are ideal for comparisons
- If BMD modeling is not feasible, effect level calls (e.g., LO[A]ELs) based upon biological understanding and/or statistical significance could be used



Mixtures Assessment Approach: Integrated Addition

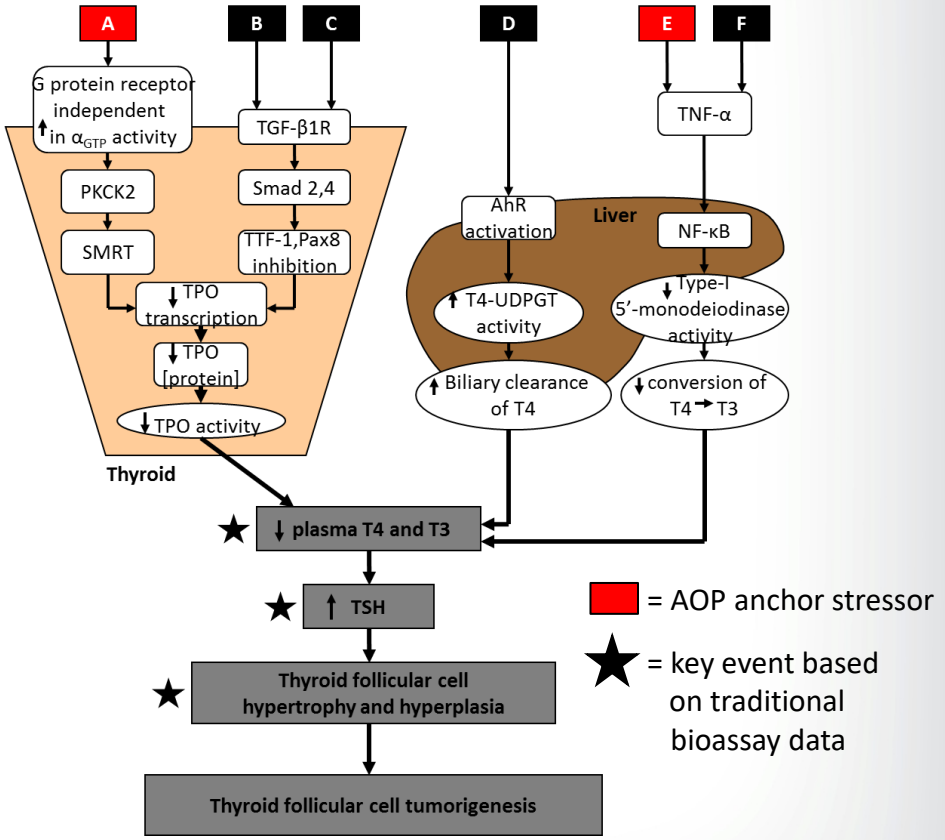
- Toxicity outcomes are rarely a single pathway phenomenon
- Environmental chemicals typically induce a messy network of perturbations and endpoints
- Integrated Addition method ideal for evaluating diversity of AOPs
- Entails integration of dose- and response-additive approaches



*IC = Index (AOP Anchor) Chemical
** [Exp]= Exposure Dose; Internal dose metric such as Total Absorbed Dose is desirable
ICED = Index Chemical Equivalent Dose

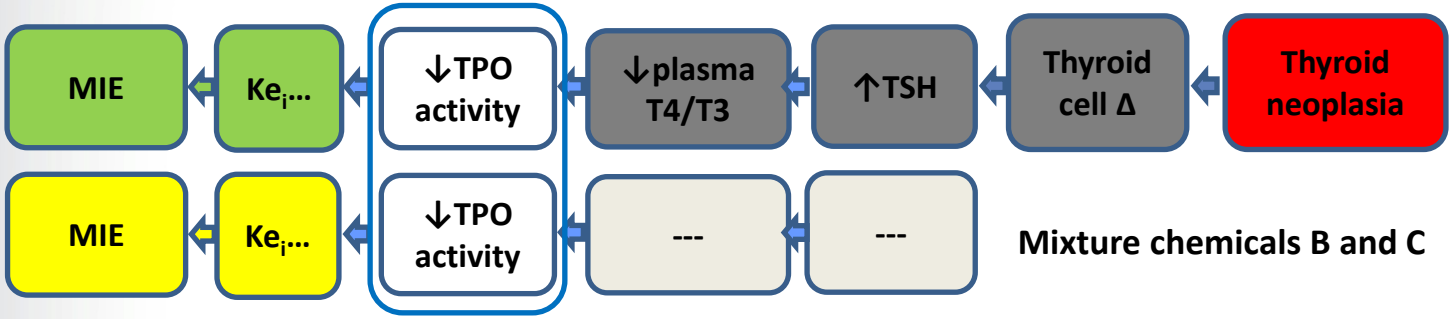
AOP Footprinting Conceptual Example

- Hypothetical mixture of six chemicals
- Two of six chemicals (e.g., A and E) have a replete AOP database including in vivo data indicating an exposure-response relationship resulting in thyroid follicular cell tumorigenesis
- Two of the other four chemicals have alternative toxicity testing data streams supporting WOE for bioactivity up to T3/T4 perturbations
- The remaining two chemicals have alternative toxicity testing data supporting WOE for perturbations in hepatocellular processes involved in thyroid hormone synthesis/metabolism

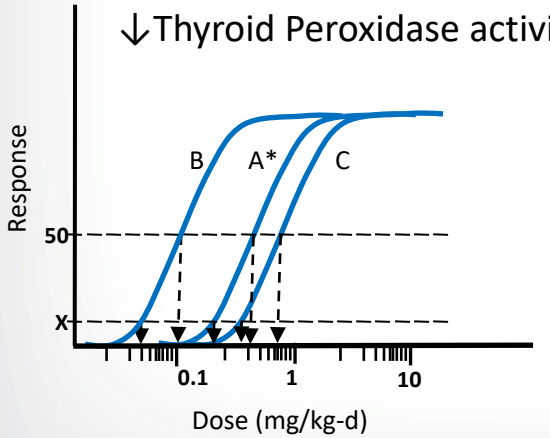


Thyroid AOP Footprint

Example Thyroid AOP footprint evaluation



Dose-response modeling of AOP footprints

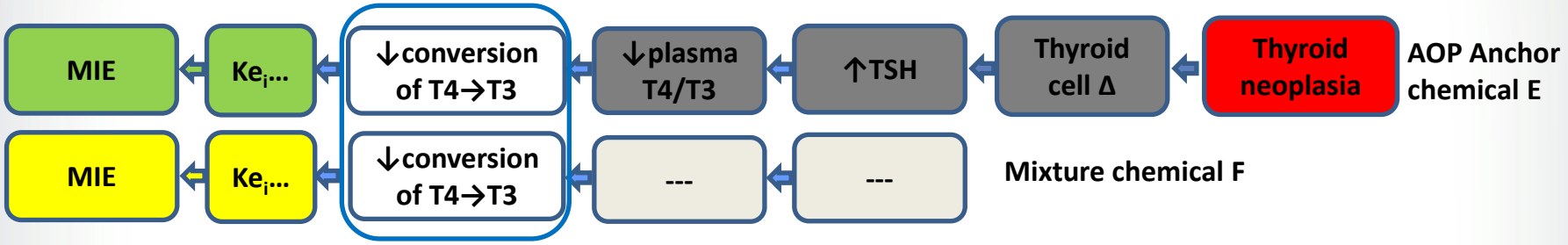


Chemical	BMD_X	BMD_{50}
A*	0.18	0.32
B	0.03	0.1
C	0.27	0.65

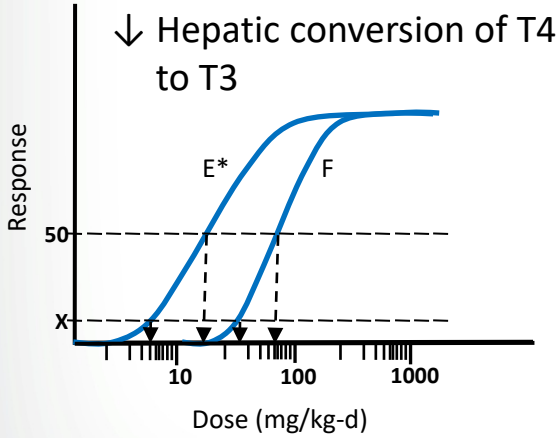
* = anchor chemical for AOP

Liver AOP Footprint

Example Liver AOP footprint evaluation



Dose-response modeling of AOP footprints

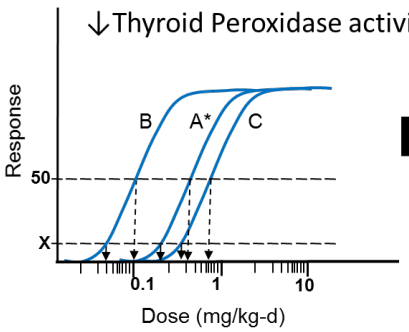


Chemical	BMD_X	BMD_{50}
E*	4	16
F	27	65

* = anchor chemical for AOP

Integrated Addition

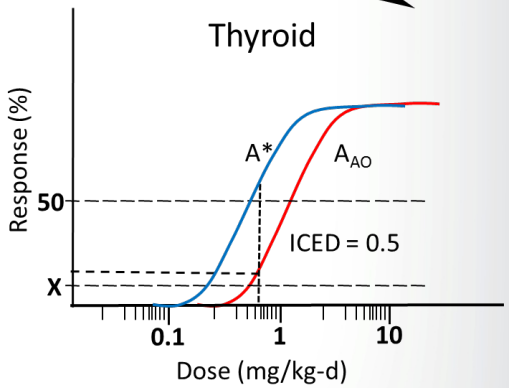
- Chemicals are evaluated based on the assumption of dose additivity within "common" footprint groupings and relative potency factors are derived
- An index chemical (i.e., AOP anchor) equivalent dose (ICED) is calculated for each chemical and summed within footprint groupings
- ICED(s) are then used to estimate AO response due to mixture exposure based off of AOP anchor dose-response function



Chemical	BMD_{50}	RPF_{50}	Exp	ICED
A*	0.32	1	0.003	0.003
B	0.1	3	0.002	0.06
C	0.65	0.5	0.8	0.4
Total ICED				0.5

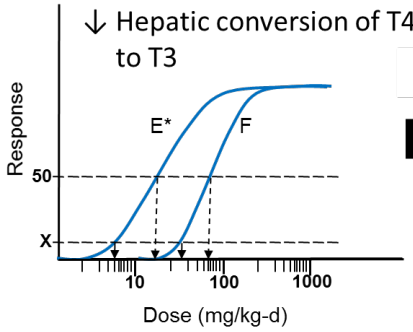
= dose-response of AOP anchor for thyroid follicular cell tumorigenesis

= dose-response of AOP anchor for ↓thyroid peroxidase (TPO) activity



Integrated Addition

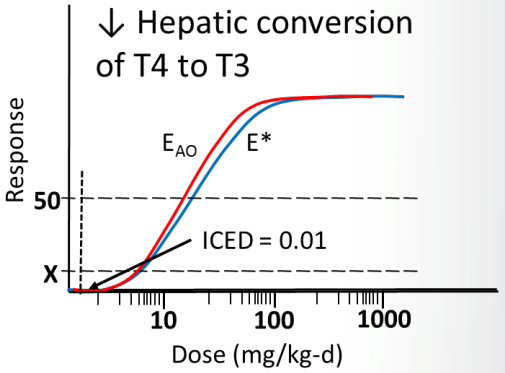
- Same RPF exercise for the liver compartment
- For mixture stressor D there is no AOP anchor
- Available information is non-apical (ends at T4-UDPGT activity in hepatocytes in vitro)
- Uncertain contribution to overall cancer mixture risk
- Until further AOP data becomes available (i.e., downstream key events), integrating stressor D into mixtures evaluation is difficult in a relative potency factor approach



Chemical	BMD_{50}	RPF_{50}	Exp	ICED
E*	16	1	0.01	0.01
F	65	0.25	0.0005	0.0001
Total ICED				0.01

= dose-response of AOP anchor for thyroid follicular cell tumorigenesis

= dose-response of AOP anchor for ↓hepatic conversion of T4 to T3



Moving Forward and Next Steps

- AOP footprinting leverages or integrates elements of AOP and MOA
- Identification of AOP anchor chemicals are key
- Uses existent EPA mixtures assessment methods for component chemicals
- Provides opportunities to integrate NAM into qualitative and quantitative assessment
- Careful about default assumptions of additivity within an AOP or across an AOP network
- Situations may arise where mixture component chemicals may not have sufficient WOE for quantitative evaluation via use of NAM data, however, decisions on AOP footprint membership can still inform potential for additivity

➢ Over the next 1-2 years: publish case study applications of AOP footprinting

  ➢ Known AOPs/networks; how to approach when AOP(s) do(es) not exist

➢ Out years: Leverage other CSS computational tools and platforms in developing algorithmic approach to AOP footprint identification, doses for RPF and ICED calculations, and mixture assessment outputs