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# Advancing New Alternative Methods (NAMs) for Tobacco Harm Reduction

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## INTRODUCTION

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October 19, 2021



# 2021 NAM Symposium: Agenda

## Topics and Rosters

1. Nicole Kleinstreuer, US NIEHS

US Federal Efforts to Develop and Implement Alternatives to Animal Testing

2. Alicia Paini, EU JRC

Application of Biokinetic Modeling for IVIVE in Chemical Risk Assessment

3. Rick Corley, GCTC LLC

Inhalation Exposure Modeling for Assessing Health Risks of Toxic Aerosols and Vapors

### 10-Min Break

4. Andy O Stucki, PETA

Assessing Respiratory Toxicity of Chemicals in Two Human Bronchial in vitro Systems

5. Luis Valerio Jr., US FDA/CTP

In Silico Toxicology as a New Approach Methodology in Tobacco Regulatory Science

6. Annie Jarabek, US EPA

Application of Mechanistic Data in Risk Assessment: Exposure Alignment and Evidence Integration

### 20-Min Discussion

**DISCLAIMER:** The presentations reflect personal opinions of speakers and do not represent the views of their affiliated organizations.

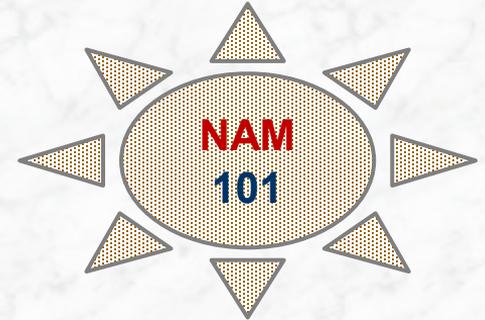
# Introduction TOC

## ❖ Background

- Setting the stage
- New Alternative Methods...for Toxicology
- Key Concepts

## ❖ Why

- Tobacco Harm Reduction
- Non-Combustible Alternatives



! Key Points !

SYMPOSIUM

Advancing New Alternative Methods (NAMs) for Tobacco Harm Reduction



# What is a NAM?

## ❖ NAMs ≈ New Alternative (or Approach) Methods

“A New Alternative Method (NAM) is **any technology, methodology, approach, or combination thereof** that can be used to provide information on **chemical hazard and risk assessment** that **avoids the use of intact animals**” (EPA, 2018)

➤ <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce>

## ❖ Other related terms ≈ “Alternative to animal testing”

➤ ([https://en.wikipedia.org/wiki/Alternatives\\_to\\_animal\\_testing](https://en.wikipedia.org/wiki/Alternatives_to_animal_testing))

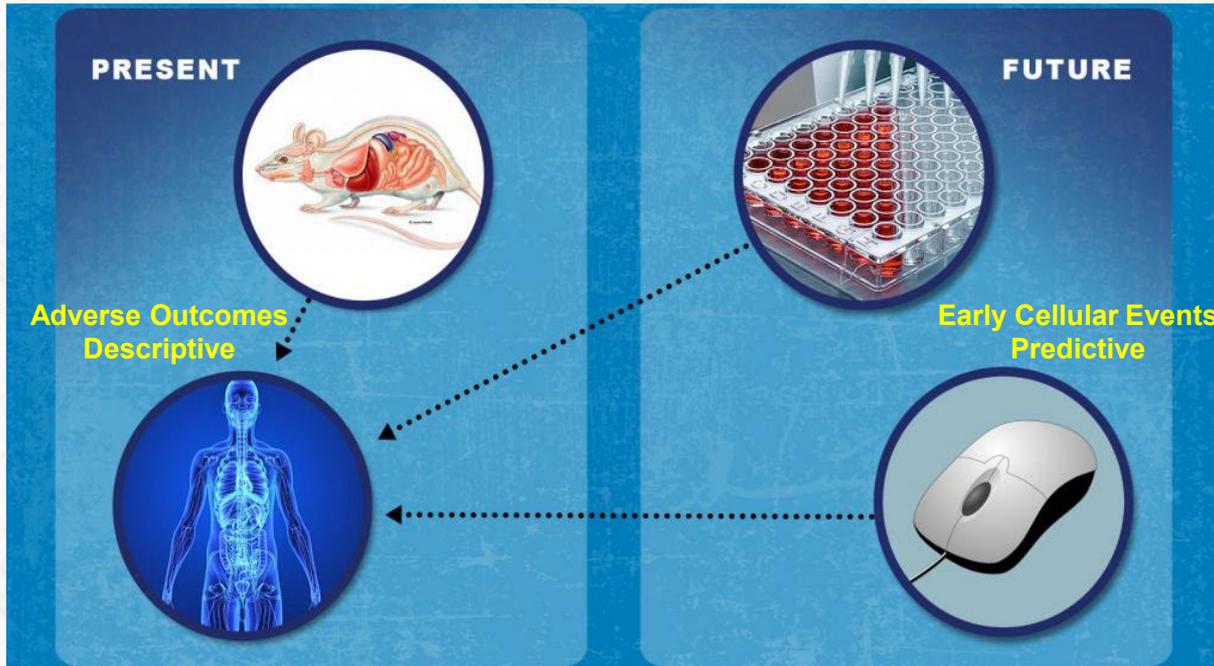
## ❖ NAMs are more than avoiding in vivo animal studies

- NAMs are not seeking a 1-to-1 replacement
- NAMs pursue a better way we do toxicology



# NAM is the 21<sup>st</sup> Century Toxicology

## ❖ NAMs are to modernize toxicology



<https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-FDA>

### NAMs can...

- ✓ Be clinically relevant - human cell-based in vitro assays
  - ✓ Be Predictive - connecting based on Mode-of-Action & early events
  - ✓ Leverage in silico - structure-based chemical evaluation; computational tools
- Drive the 3Rs (Reduce/Refine/Replace) animal-based testing

### Opportunities...

- ! Awareness vs. Application
- ! Supporting vs. Replacing what in vivo
- ! Uncertainty & Context of use

There are successful case examples

# Our World - Tobacco Harm Reduction (THR)

## Interest & Need for Non-Combustible Alternatives



Inhalable

Complete Switching

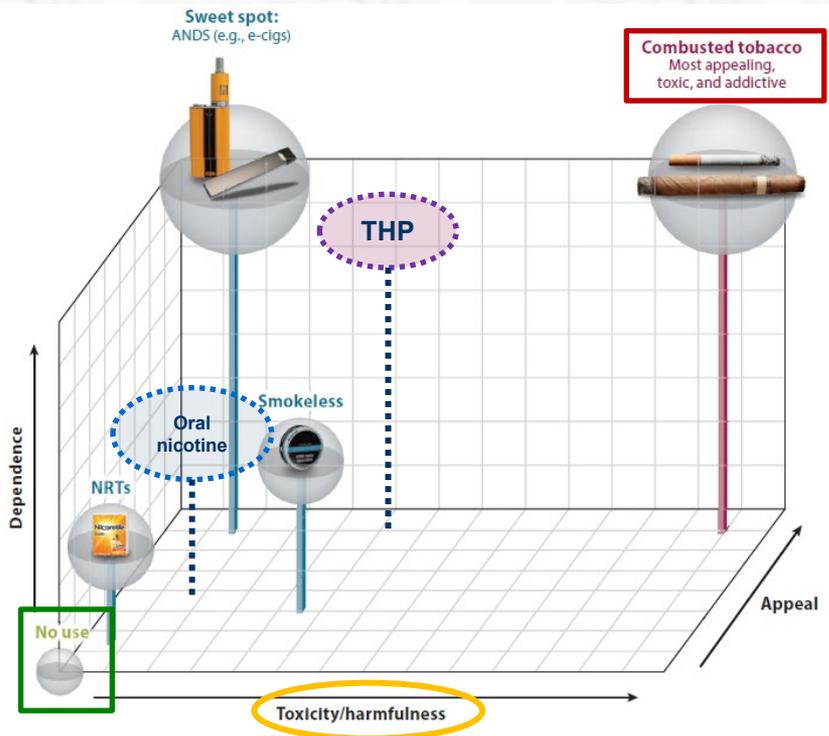
~~Tobacco combustion~~



<https://www.clivebates.com/vaping-tobacco-harm-reduction-nicotine-science-and-policy-4-2/>

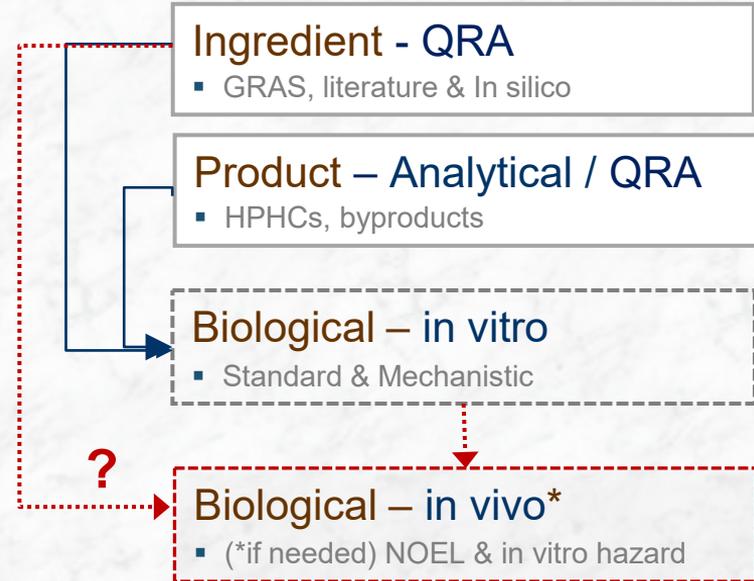
Potential Reduced-Risk Products (RRPs) – Examples (shaded, not considered RRFs)

# Evaluating Health Risk of RRP's



Abrams et al. 2018 <https://pubmed.ncbi.nlm.nih.gov/29323611/> (marked for illustration)

## WoE Toxicological Assessment



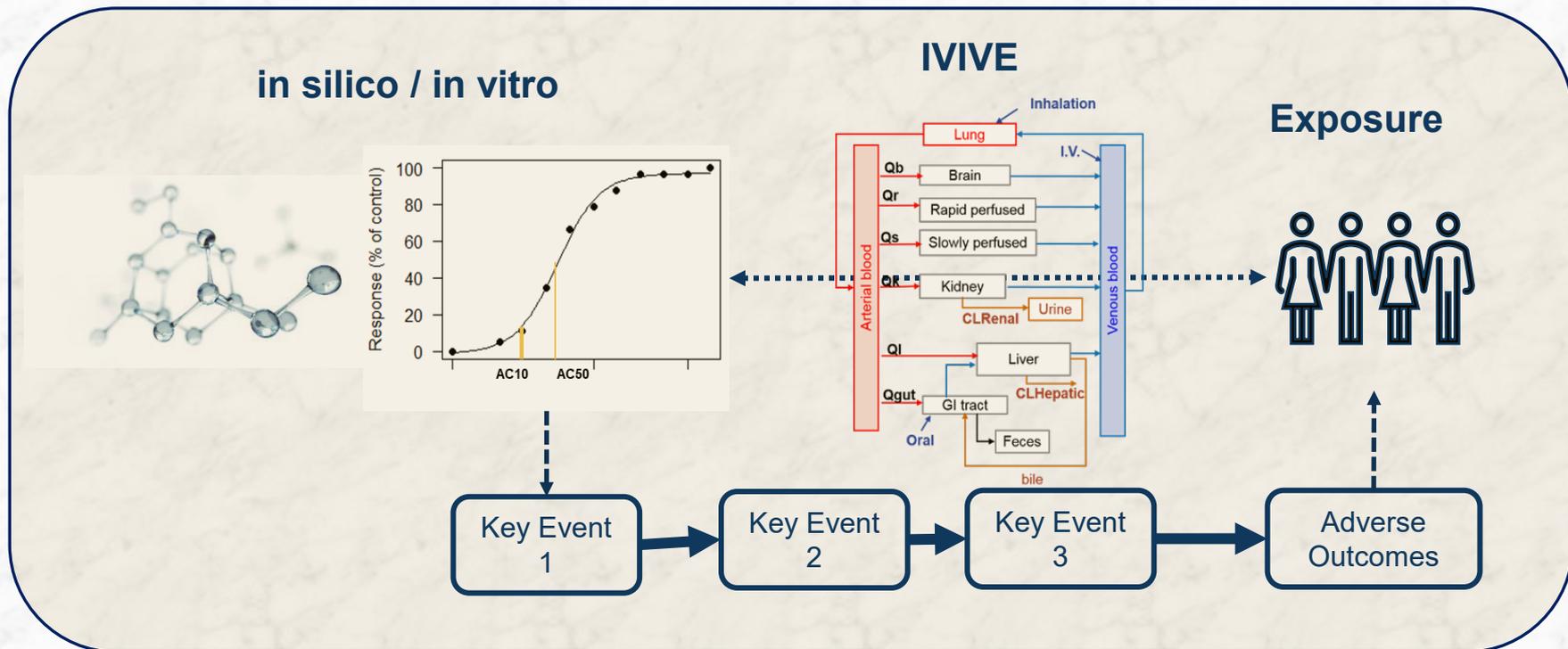
*Should we change the way we pose questions?*

WoE= Weight-of-Evidence; GRAS=Generally regarded as safe; HPHCs=Harmful and potentially harmful chemicals; QRA=Quantitative risk assessment; NOEL=No-observable-effect-level



# New Alternative Methods (NAMs)

offer a different way of “connecting the dots” for tox assessment



IVIVE ((in vitro to in vivo extrapolation) – Quantitative relationship, using kinetic modeling, between in vitro bioactivity and the in vivo exposure expected to result in adverse outcomes



## ❖ This symposium will provide:

- A high-level overview – new terminologies, some are technical
- Introducing publicly available NAM tools and resources
- Case examples with success stories, on-going efforts

## ❖ Participants are encouraged to:

- Contrast to what we currently do & context of use
- Share thoughts on potential barriers and limitations
- Consider common areas of NAMs for collective opportunity

## ❖ Comment & ask questions!

- *Don't forget the Panel Discussion at the end*

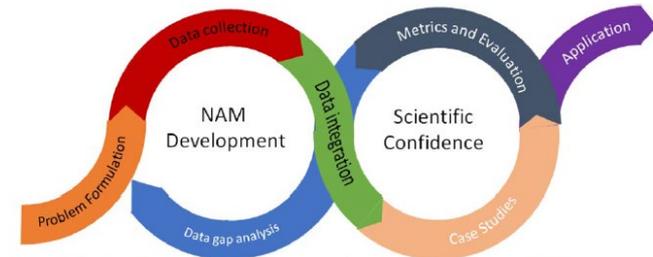
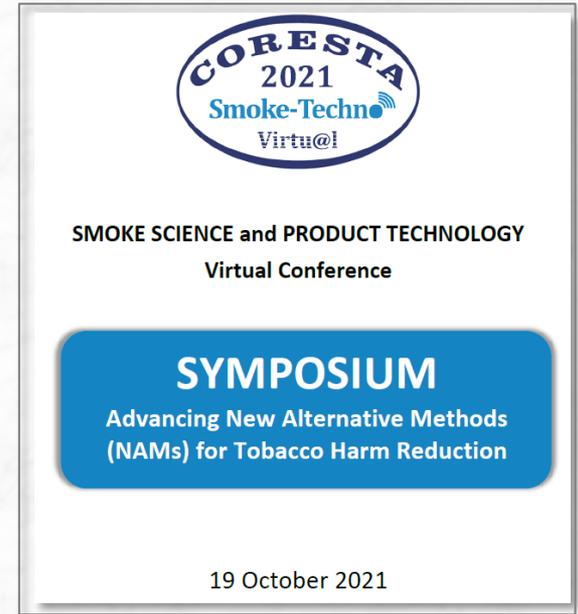


Figure 2. Problem-focused research planning and implementation process at EPA.



# THANK YOU

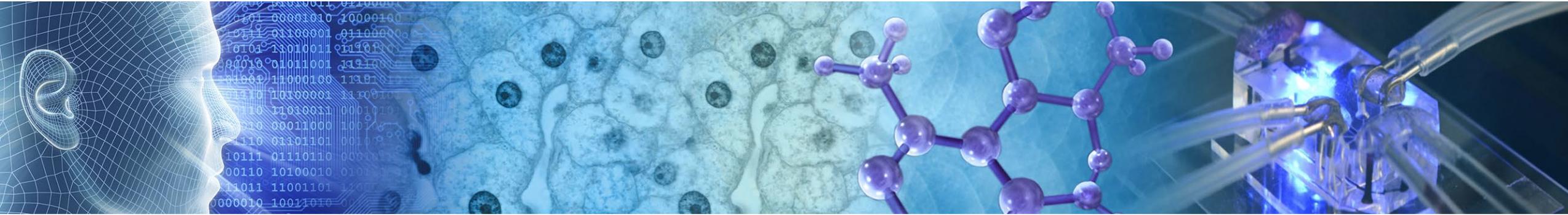
- ❖ CORESTA Scientific Commission
  - Next Generation Tox (NGT) Task Force
  - In Vitro Tox (IVT) Subgroup
  - Biomarker (BMK) Subgroup
- ❖ Invited Speakers
- ❖ Altria Client Services, LLC
- ❖ Integrated Laboratory Systems
- ❖ Participants



# Selected References

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- ❖ ICCVAM <https://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam/index.html>
- ❖ Tox21 <https://tox21.gov/overview/>
- ❖ Abrams et al. 2018 <https://pubmed.ncbi.nlm.nih.gov/29323611/>
- ❖ Avila et al. 2020 <https://pubmed.ncbi.nlm.nih.gov/32325112/>
- ❖ Parish et al. 2020 <https://pubmed.ncbi.nlm.nih.gov/32017962/>
- ❖ The Counterfactual 2020 <https://www.clivebates.com/vaping-tobacco-harm-reduction-nicotine-science-and-policy-q-a/>
- ❖ OECD TG331 2021 [https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-CBC-MONO\(2021\)1%20&doclanguage=en](https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-CBC-MONO(2021)1%20&doclanguage=en)
- ❖ New Approach Methods Work Plan, EPA 2020 [https://www.epa.gov/sites/default/files/2020-06/documents/epa\\_nam\\_work\\_plan.pdf](https://www.epa.gov/sites/default/files/2020-06/documents/epa_nam_work_plan.pdf)



# US federal efforts to develop and implement alternatives to animal testing

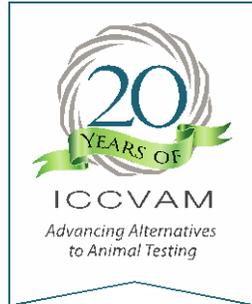
CORESTA NAMs Symposium, 2021  
19 October, 2021

Nicole Kleinstreuer  
Acting NICEATM Director



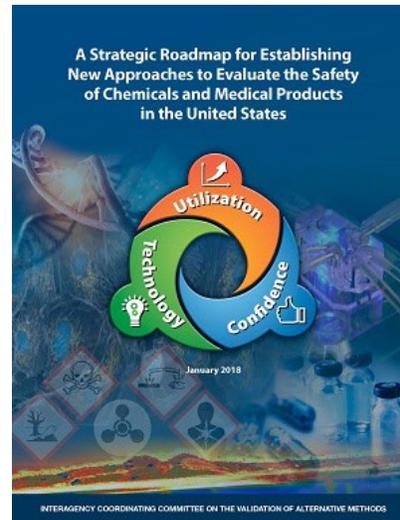


- National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (**NICEATM**), supporting the Interagency Coordinating Committee for the Validation of Alternative Methods (**ICCVAM**)
- ICCVAM Authorization Act of 2000: To establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new and revised toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing (**3Rs**) animal tests and ensuring human safety and product effectiveness.



## 10 Research Agencies

Agency for Toxic Substances and Disease Registry  
National Institute for Occupational Safety and Health  
National Cancer Institute  
National Institute of Environmental Health Sciences  
National Library of Medicine  
National Institutes of Health  
Department of Defense  
Department of Energy  
National Institute of Standards and Technology  
Veterans Affairs Office of Research and Development



## 7 Regulatory Agencies

Consumer Product Safety Commission  
Department of Agriculture  
Department of the Interior  
Department of Transportation  
Environmental Protection Agency  
Food and Drug Administration  
Occupational Safety and Health Administration

\*Other participants include: NCATS, Tox21 Representatives

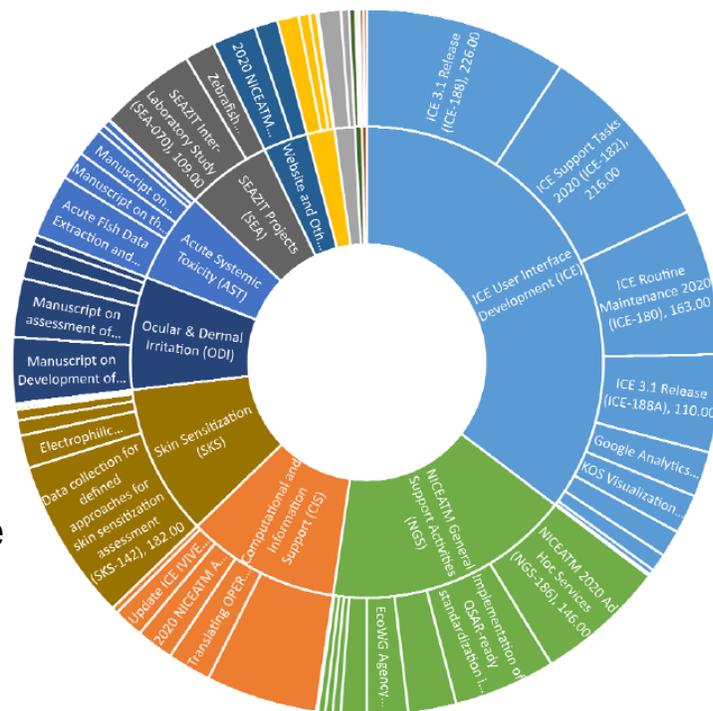
More information: <https://ntp.niehs.nih.gov/go/iccvam>





# Ongoing NICEATM and ICCVAM Projects

- Integrated Chemical Environment
- OPERA (QSAR/QSPR)
- Quantitative IVIVE
- Reference data curation
- Variability of in vivo data
- Acute Systemic Toxicity
- Dermal absorption
- Eye and skin irritation
- Skin sensitization
- ZF models (SEAZIT)
- Acute Fish Retrospective
- Carcinogenesis
- Cardiovascular toxicity
- Developmental Toxicity
- Animal-free affinity reagents
- Microphysiological Systems
- Evolving Process of Validation



- Summarizes US agency activities to promote alternatives or reduce animal use
  - Contributions from every ICCVAM member agency
- 2018-2019 report published in July 2020, available online at:  
<https://ntp.niehs.nih.gov/go/2019iccvamreport>
- [Subscribe to NICEATMNews email list](#)  
<https://ntp.niehs.nih.gov/go/niceatm>





# Acute 6-Pack Alternatives

## Dermal lethality

- US EPA Waiver guidance available

## Oral lethality

- In silico (CATMoS) for single chemicals; additivity for formulations under consideration

## Inhalation lethality

- 3D models being evaluated; LC50 database for in silico model development being built

## Eye irritation

- NAMs for Cat I and/or Cat IV (TG 437, 438, 460, 491, 492, 494); Prospective testing ongoing

## Skin irritation

- NAMs for Cat I or Cat IV (TG 430, 431, 435, 439); Prospective testing ongoing

## Skin sensitization

- EPA science policy, draft risk assessment, and OECD international DASS guideline





## CATMoS implementation in OPERA

OPERA suite of models:

- Free, open-source, and open-data
- Command line and GUI
- Single chemical and batch mode
- Windows OS and Linux
- Embeddable wrapper libraries in Java, C, C++, and Python

```

OPERA,CL
-----
OPERA models for physchem, environmental fate and tox properties.
Version 2.5 (January 2020)

OPERA is a command line application developed in Matlab providing QSAR
models predictions as well as applicability domain and accuracy assessment.

Developed by:
Kamel Mansouri
mansourikamel@gmail.com
kamel.mansouri@nih.gov

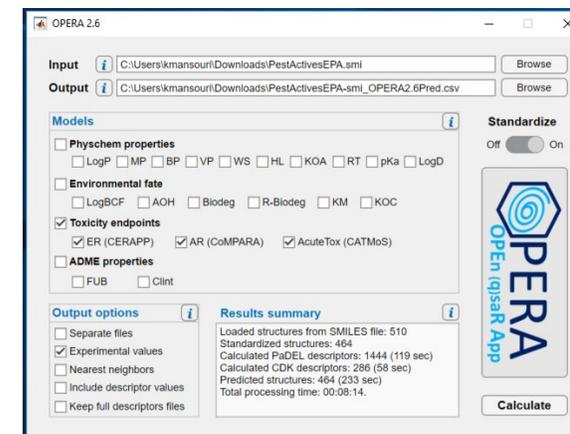
Usage: OPERA <argument_list>

Examples:
OPERA -s Sample_50.sdf -o predictions.csv -a -x -v 2
opera -d Sample_50.csv -o predictions.txt -e logP BCF -n -v 1

Type OPERA -h or OPERA --help for more info.

C:\Users\k Mansouri>

```



## Collaboration with ATWG partners and ICCVAM agencies

Agency	No. Substances	Agency	No. Substances
Air Force	421	EPA OPP	36
Army Public Health Command	18	EPA OPPT	8
Army Edgewood Chemical Biological Center	42	EPA NCCT	4815
CPSC	110	EPA EFED	160
DOT	3671	FDA CFSAN	22

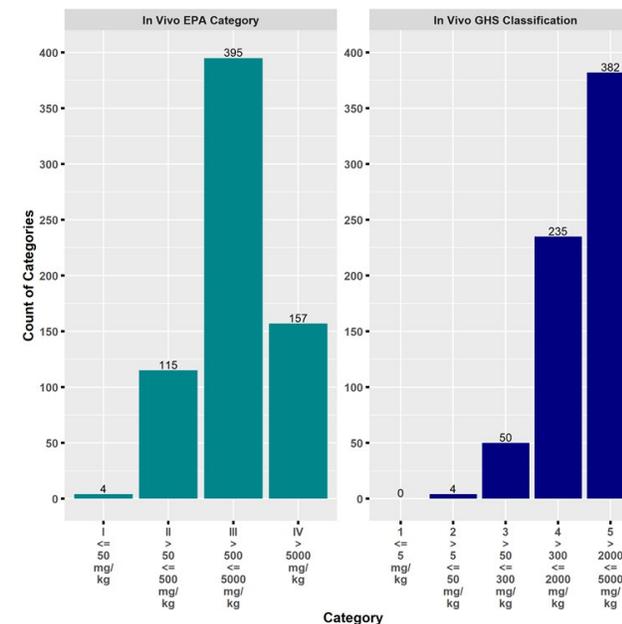
## Progress made with EPA EFED

- Compare CATMoS predictions to acute oral toxicity data on 160 pesticides registered in the last 25 years.
- Determine impact on risk assessments, leading to additional curation and characterizing confidence in predictions.



# Acute Toxicity Mixtures Equation Analyses

- GHS Mixtures Equation - mathematical approach to calculating toxicity of mixtures based on components
- Compare LD50s predicted for formulations based on the GHS Mixtures Equation to those from in vivo results with the complete formulation.
- Data set consisted of 671 formulations produced by eight companies:
  - 51 antimicrobial cleaning products (AMCPs), 620 agrochemical formulations
- Analysis based on PPE requirements demonstrated 82% concordance overall.



Within-class concordance for less toxic substances was consistently over 85% regardless of classification system (EPA, GHS).

<i>In vivo</i> LD <sub>50</sub>	Additivity LD <sub>50</sub> Prediction (mg/kg)			Within-class Concordance
	≤50	>50 to ≤500	>500	
≤50	3	1	0	75%
>50 to ≤500	4	30	81	26%
>500	1	35	514	93%
<b>Total</b>	8	66	595	82%



# Human-relevant approaches for eye corrosion/irritation potential

Clippinger et al. 2021 Cut Ocu Tox

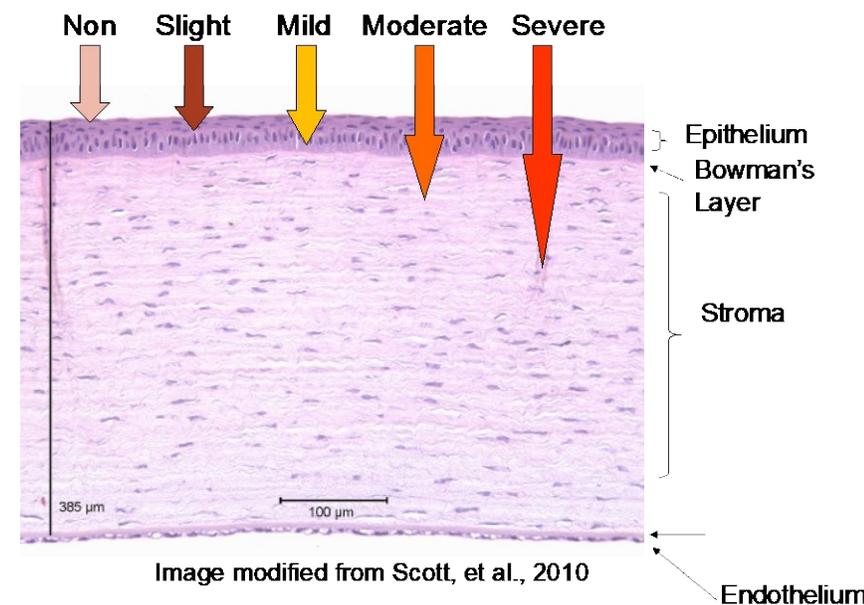
Prior GHS category	1	2A	2B	NC
1 (serious eye damage)	73%	16%	0%	10%
2A (irritant)	4%	33%	4%	59%
2B (mild irritant)	0%	4%	16%	80%
NC (non-irritant)	1%	4%	2%	94%

Adapted from Luechtefeld et al., ALTEX 33(2), 2016.

Consider strengths and limitations of all available methods with respect to:

- their relevance to human ocular anatomy
- the mechanisms of eye irritation/corrosion in humans

- The rabbit test should not be used as a reference method to demonstrate the validity of *in vitro/ex vivo* assays
- *In vitro/ex vivo* methods are as or more reliable and relevant than the rabbit test





# Skin Irritation Variability

EPA	Category I	Category II	Category III	Category IV
PDII	Corrosive	>5.0	2.1-5.0	0-2.0
Signal Word	DANGER	WARNING	CAUTION	CAUTION
PPE Required	Coveralls worn over long-sleeved shirt and long pants	Coveralls worn over short-sleeved shirt and short pants	Long-sleeved shirt and long pants	Long-sleeved shirt and long pants
	socks	socks	socks	socks
	Chemical-resistant footwear	Chemical-resistant footwear	Shoes	Shoes
	Waterproof or chemical resistant gloves	Waterproof or chemical resistant gloves	Waterproof or chemical resistant gloves	No minimum
Irritant		Non-irritant		

## Curated Dataset with Binary Approach

Prior Result	Irritant (Cat I or II)	Non-irritant (Cat III or IV)
Irritant (Cat I or II)	75.6%	24.4%
Non-irritant (Cat III or IV)	3.9%	96.1%

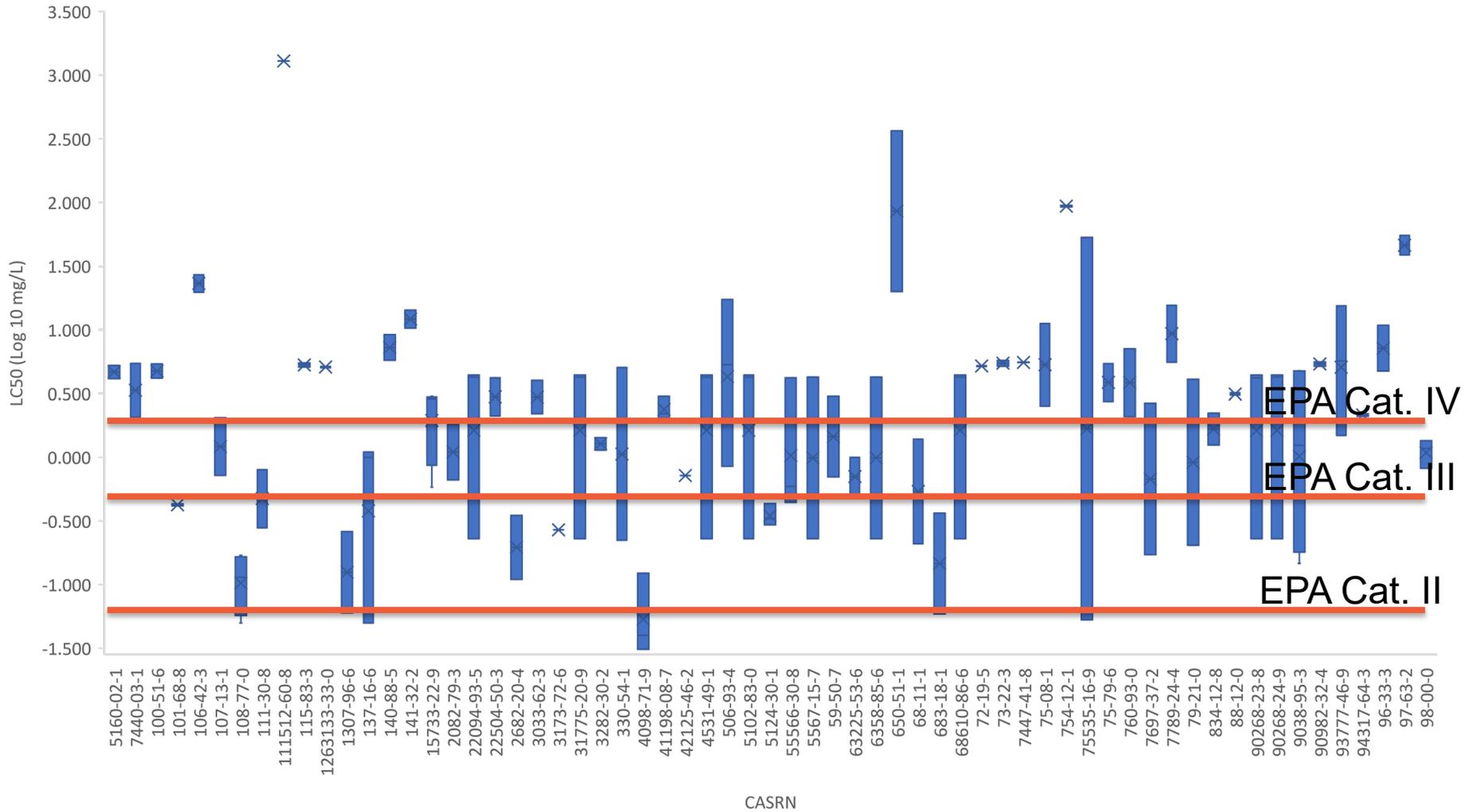
## Curated Dataset

Prior result	COR	II	III	IV
COR	86.3%	4.2%	7.1%	2.5%
II	14.1%	44.9%	20.5%	20.5%
III	6.9%	5.2%	53.6%	34.3%
IV	0.9%	2.0%	9.1%	88.0%





# Inhalation Tox Variability: LC50 Ranges Over EPA Categories



\*unpublished data

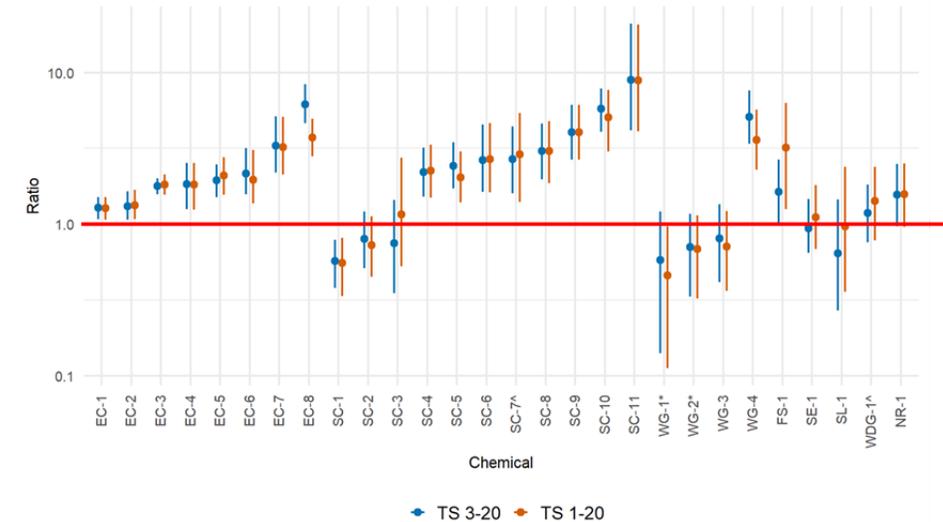




# Dermal Absorption Analyses

Allen et al. 2021 ALTEX

- Absorption through in vitro human skin was found to be similar to, or less than, that observed in rat skin (in vitro and in vivo) for all formulations.
- The human in vitro assay provided a similar or higher estimate of dermal absorption than the triple pack
- For human health risk assessment, in vitro assays using human skin would be preferable. Such tests would be directly relevant to the species of interest (humans) and avoid any overestimation of dermal absorption using rat models.
- However, rat in vitro studies would still have utility if human in vitro data were not available.
- In vitro rat data provide estimates of dermal absorption that are at least as protective as in vivo rat data, and thus could also be considered adequate for use in establishing dermal absorption factors.



$$\text{triple pack DAF} = \text{rat in vivo} \times (\text{human in vitro} \div \text{rat in vitro})$$





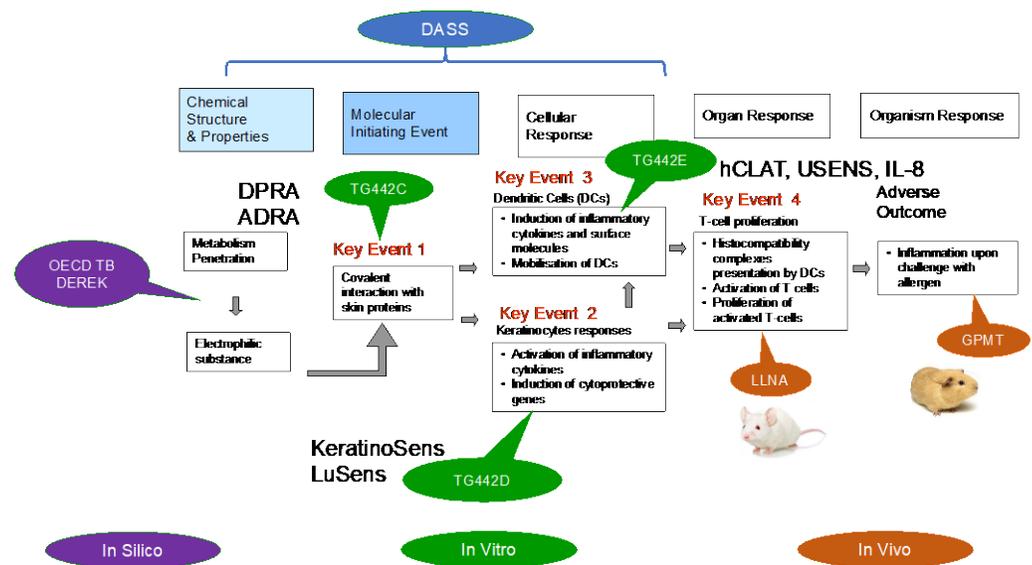
# Defined Approaches for Skin Sensitization

Section 4  
Health effects

**Guideline No. 497**  
Guideline on Defined Approaches for Skin Sensitisation

14 June 2021

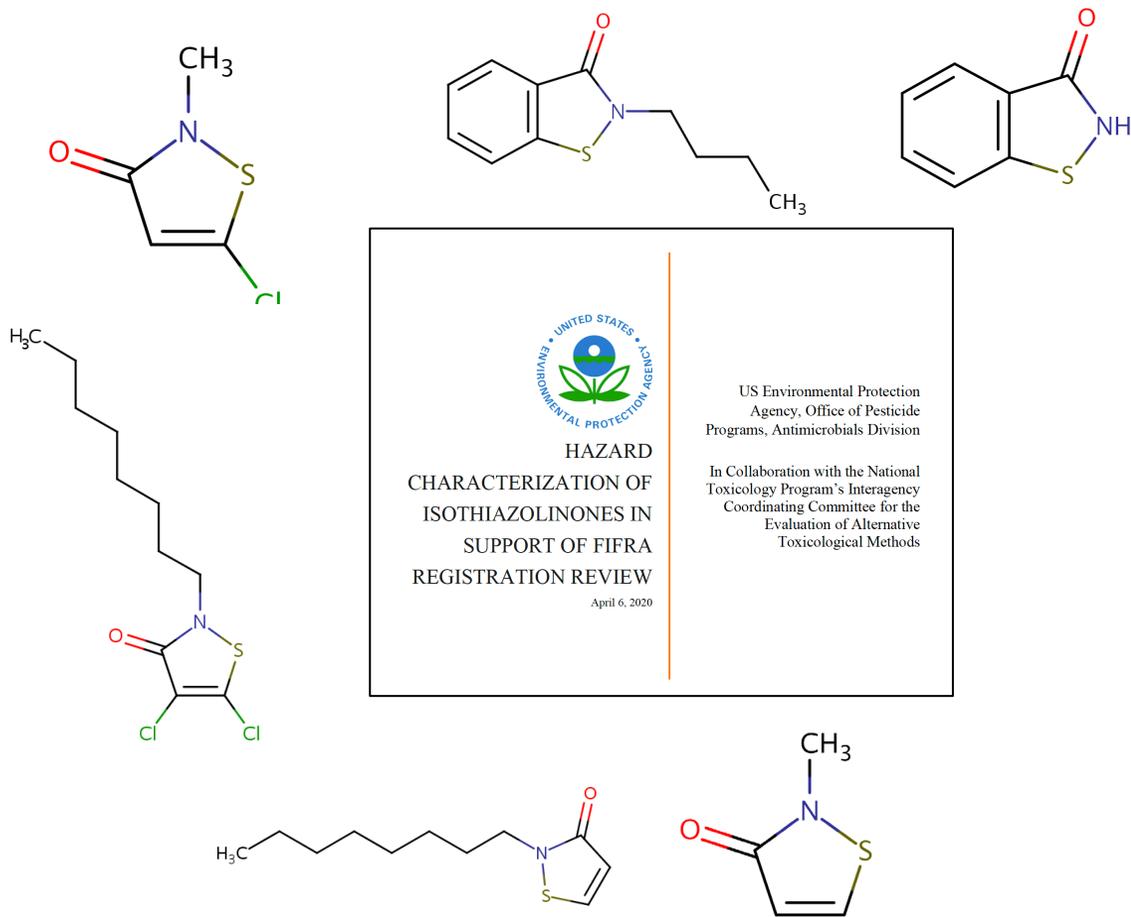
OECD Guidelines for the Testing of Chemicals



DA/Method	Information Sources	Capability (Hazard and/or Potency)	Hazard Performance vs. LLNA N~168	Hazard Performance vs. Human N~63	GHS Potency Performance vs. LLNA (Accuracy)	GHS Potency Performance vs. Human (Accuracy)
203 DA	DPRA, KeratinoSens™, h-CLAT	Hazard	84% BA, 82% Sens, 85% Spec	88% BA, 89% Sens, 88% Spec	-	-
IITSv1 DA	DPRA, h-CLAT, DEREK Nexus v6.1.0	Hazard, Potency (GHS)	81% BA, 92% Sens, 70% Spec	69% BA, 93% Sens, 44% Spec	70% NC, 71% 1B, 74% 1A	44% NC, 77% 1B, 65% 1A
IITSv2 DA	DPRA, h-CLAT, OECD QSAR Toolbox v4.5	Hazard, Potency (GHS)	80% BA, 93% Sens, 67% Spec	69% BA, 94% Sens, 44% Spec	67% NC, 72% 1B, 72% 1A	44% NC, 80% 1B, 67% 1A
LLNA (provided for comparison)	<i>in vivo</i>	Hazard, Potency	-	58% BA, 94% Sens, 22% Spec	-	25% NC, 74% 1B, 56% 1A



# Regulatory Risk Assessment

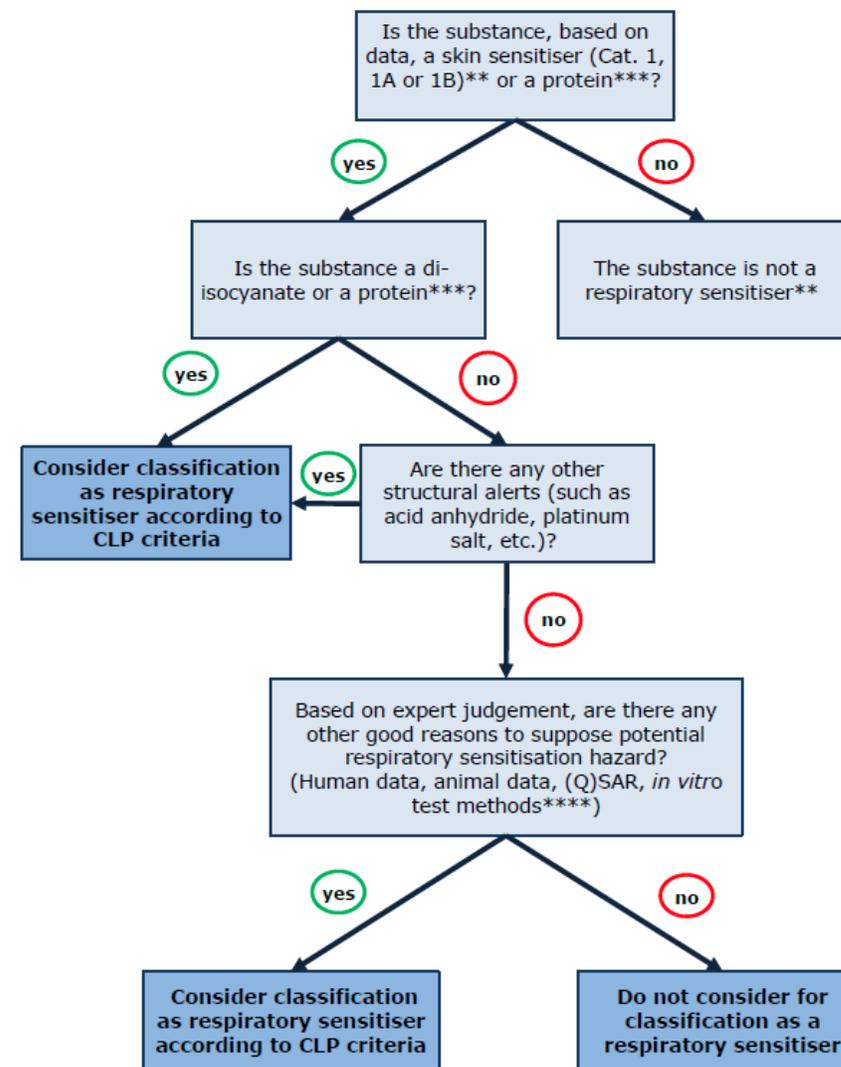


UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
HAZARD CHARACTERIZATION OF ISOTHIAZOLINONES IN SUPPORT OF FIFRA REGISTRATION REVIEW  
April 6, 2020

US Environmental Protection Agency, Office of Pesticide Programs, Antimicrobials Division  
In Collaboration with the National Toxicology Program's Interagency Coordinating Committee for the Evaluation of Alternative Toxicological Methods

DASS for Isothiazolinone biocides: material preservatives to prevent the growth of microbial organisms and are used in industrial processes and consumer products

<https://www.federalregister.gov/documents/2020/05/14/2020-10376/pesticide-registration-review-draft-human-health-and-ecological-risk-assessments-for-several>

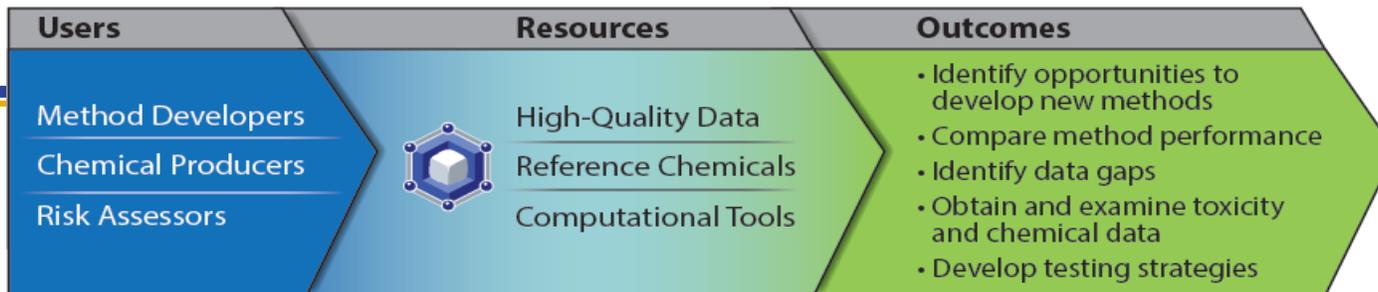


Decision tree for respiratory sensitization

Fabrice Broeckaert & Laura Rossi, ECHA



# Integrated Chemical Environment ICEv3.4



## News & Events

### ICE v3.4 Release

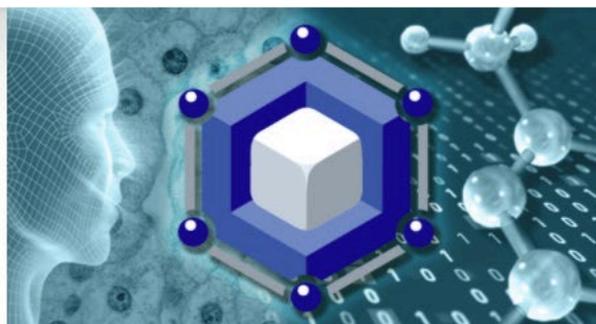
#### ICE updates include:

##### New tools and expanded capabilities:

- Chemical Quest (Beta)
- Drawing of 2D structures
- Query by multiple chemical identifiers
- Send Assays to other ICE tools

#### Learn about ICE updates

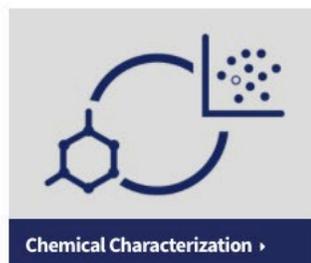
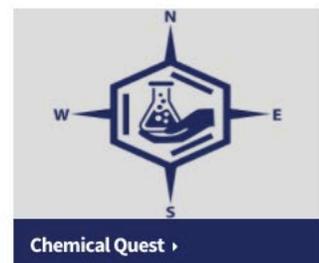
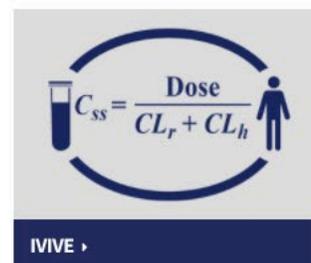
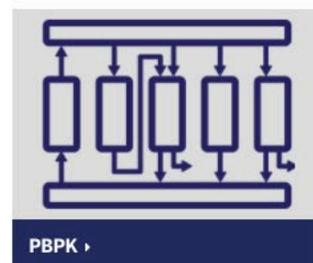
UPDATES



ICE provides data to support development of new approaches for chemical safety testing.

[Click here to learn more about ICE!](#)

PAUSE



<https://ice.ntp.niehs.nih.gov/>



# Open Structure-Activity/Property Relationship App

- OPERA is a free and open-source quantitative structure-activity relationship (QSAR) tool with a suite of AI/ML models.
- OPERA predictions include:
  - Physchem properties
    - General structural properties
    - Environmental fate
  - ADME properties
  - Tissue partition coefficient inputs
  - Models for Toxicity Endpoints
    - CERAPP: Collaborative Estrogen Receptor Activity Prediction Project
    - CoMPARA: Collaborative Modeling Project for Androgen Receptor Activity
    - CATMoS: Collaborative Acute Toxicity Modeling Suite



<https://github.com/NIEHS/OPERA>





## Curation to assist meaningful assay selection and model building

**Select Assays** ⓘ

**cHTS** | **Mode of Action**

Assay	Info	Category
<input type="checkbox"/>	ⓘ	▼ cHTS
<input type="checkbox"/>	ⓘ	Abnormal Growth and Differentiation
<input type="checkbox"/>	ⓘ	Angiogenic Process
<input type="checkbox"/>	ⓘ	> Cellular Processes
<input type="checkbox"/>	ⓘ	> Cellular Stress Response
<input type="checkbox"/>	ⓘ	▼ Endocrine-Related Processes
<input type="checkbox"/>	ⓘ	> Steroid Hormone Metabolism
<input type="checkbox"/>	ⓘ	> Thyroid Hormone Metabolic Process
<input type="checkbox"/>	ⓘ	Energy Metabolism Process
<input type="checkbox"/>	ⓘ	> Epigenetic Process
<input type="checkbox"/>	ⓘ	> Gene Expression

**Select Assays** ⓘ

**cHTS** | **Mode of Action**

Assay	Info	Category
<input type="checkbox"/>	ⓘ	▼ Mode of Action
<input type="checkbox"/>	ⓘ	> Acute Lethality MOAs
<input type="checkbox"/>	ⓘ	▼ Endocrine MOAs
<input type="checkbox"/>	ⓘ	Androgen Metabolic Process
<input type="checkbox"/>	ⓘ	Estrogen Metabolic Process
<input type="checkbox"/>	ⓘ	Gene Expression
<input type="checkbox"/>	ⓘ	Steroidogenesis
<input type="checkbox"/>	ⓘ	Steroid Hormone Metabolism
<input type="checkbox"/>	ⓘ	Thyroid Hormone Metabolic Process
<input type="checkbox"/>	ⓘ	Glucocorticoid Metabolic Process
<input type="checkbox"/>	ⓘ	Progesterone Metabolic Process
<input type="checkbox"/>	ⓘ	> Cancer MOAs
<input type="checkbox"/>	ⓘ	> DART MOAs

- Curated high-throughput screening data (cHTS) starts with EPA invitrodb and incorporates chemical QC information and technology-specific flags
- Assays are grouped by biological process, mechanistic target, and MoA, and linked to ontologies



The Curve Surfer tool allows you to view and interact with concentration response curves from cHTS.

**Curve Surfer** is an interactive concentration response visualization tool for cHTS data

- Select/filter assays based on Mechanistic Target
- View specific assays/chemicals
- Filter on activity call, AC50

Select Page: 1 of 21

Order By: Chemical Name Asc

Only showing curves for 200 chemicals. Please reduce your query to view all chemicals.

Select Mechanistic Target To View Curves: All

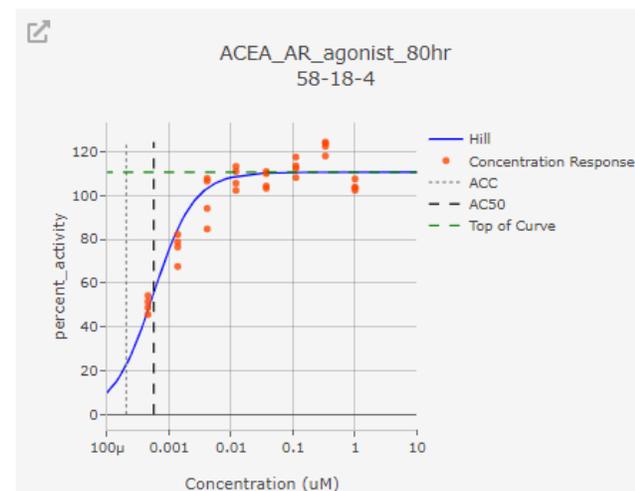
Assay Text Fil...: 0 values

Select Assay(s): 0 values

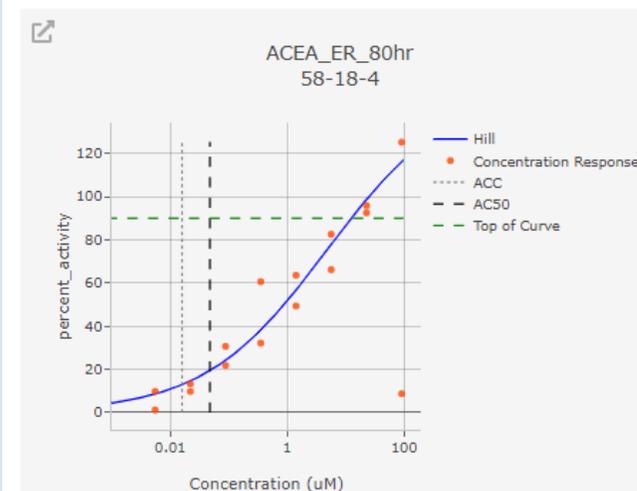
Select CASRN(s): 0 values

Select Call(s): Active Choose...

**Assay:** ACEA\_AR\_agonist\_80hr  
**Mechanistic Target:** Androgen Metabolic Process  
**CASRN:** 58-18-4  
**DTXSID:** DTXSID1033664  
**Chemical Name:** 17-Methyltestosterone  
**Winning Curve-Fit Model:** Hill  
**AC50:** 5.7E-4  
**ACC:** 2.0E-4  
**Top of Curve:** 110.66  
**Call:** Active  
[View EPA curve \(testing purposes only\)](#)



**Assay:** ACEA\_ER\_80hr  
**Mechanistic Target:** Estrogen Metabolic Process  
**CASRN:** 58-18-4  
**DTXSID:** DTXSID1033664  
**Chemical Name:** 17-Methyltestosterone  
**Winning Curve-Fit Model:** Hill  
**AC50:** 0.048  
**ACC:** 0.016  
**Top of Curve:** 89.94  
**Call:** Active  
[View EPA curve \(testing purposes only\)](#)





Curve Surfer

**PBPK**

IVIVE

Chemical Characterization

Input

Results

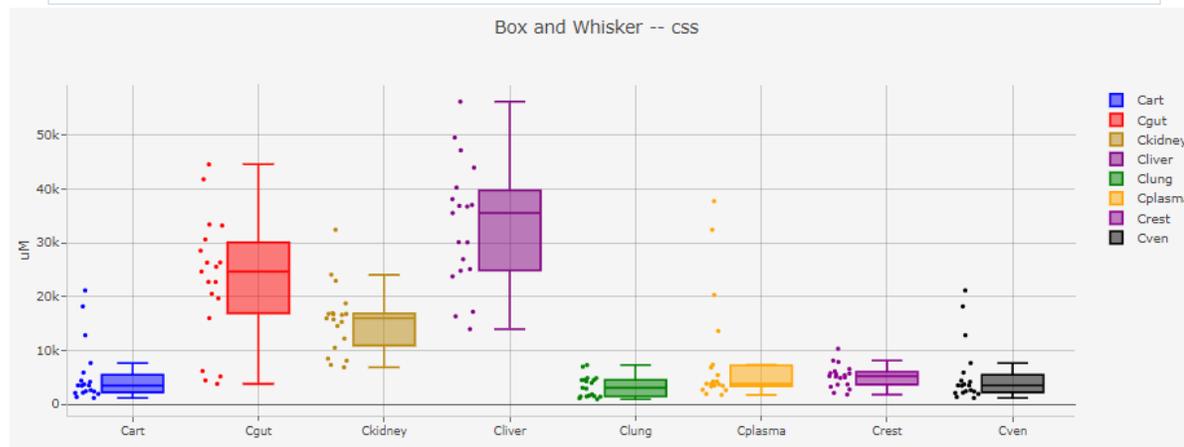
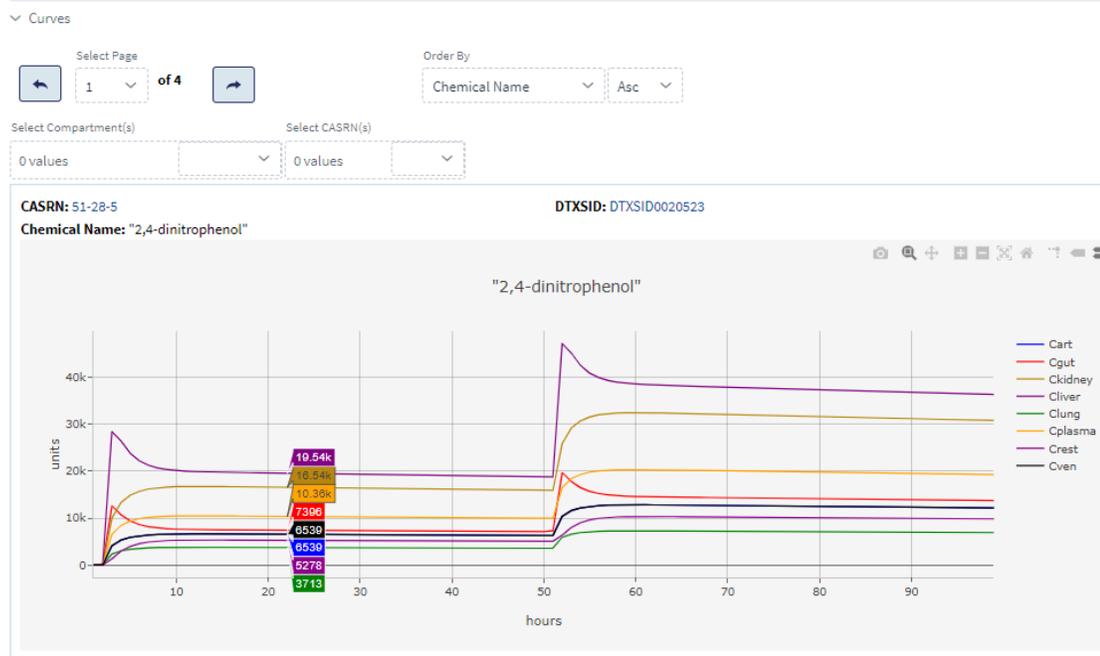
Report an Issue



PBPK tool allows you to generate predictions of tissue-specific chemical concentration profiles following a dosing event

**PBPK tool** allows users to calculate internal chemical concentrations using PBPK models from the EPA httk R package and in-house code

- Tissue level concentrations
- View individual chemical curves
- View overall distribution in different tissue compartments for all query chemicals





Curve Surfer

PBPK

IVIVE

Chemical Characterization

Input

Results

Report an Issue



The IVIVE tool uses pharmacokinetic models to predict the equivalent administered dose (EAD) from the activity concentration of selected assays.

Chemical	CASRN	DTXSID	Flag	Assay	Mode of Action	Mechanistic Targets	AC50 uM	EAD 50th Percentile (mg/kg/day)	Clint	Fraction Unbound
Testosterone	58-22-0	DTXSID8022371		TOX21_ERa_BLA_Agon...	estrog Receptor Mediated Effects	Estrogen Metabolic Process	13.0			0.39952

Annotation provided for filtering

Select EAD to visualize:

EAD 50th

Select in vivo data to display:

ion (Uterotrophic LEL)

Log Axis

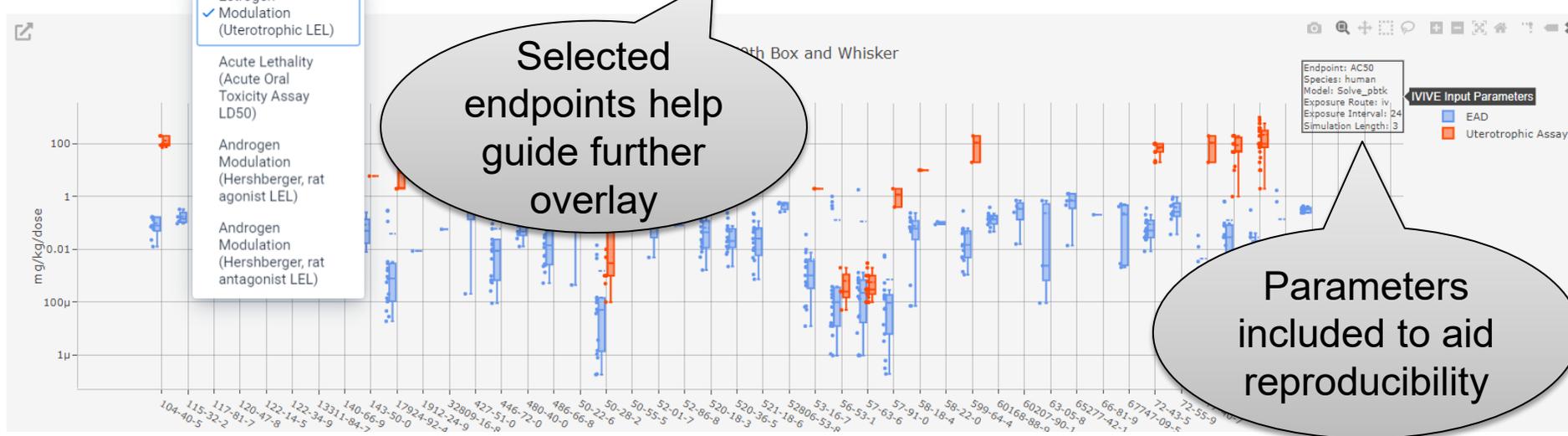
Show Name

Toxicity Endpoints represented: Endocrine

Hover over graphic for interacti

- Estrogen Modulation (Uterotrophic LEL)
- Acute Lethality (Acute Oral Toxicity Assay LD50)
- Androgen Modulation (Hershberger, rat agonist LEL)
- Androgen Modulation (Hershberger, rat antagonist LEL)

Selected endpoints help guide further overlay



Parameters included to aid reproducibility

# Transparency and annotation to help guide use and interpretation



Input

Results

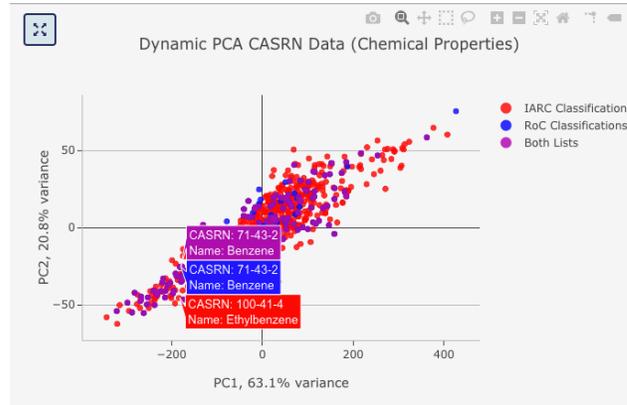
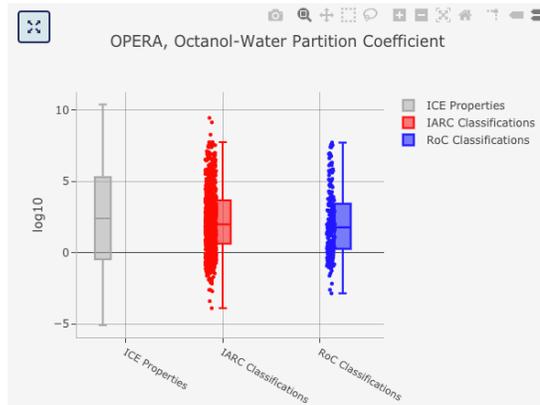
Report an Issue



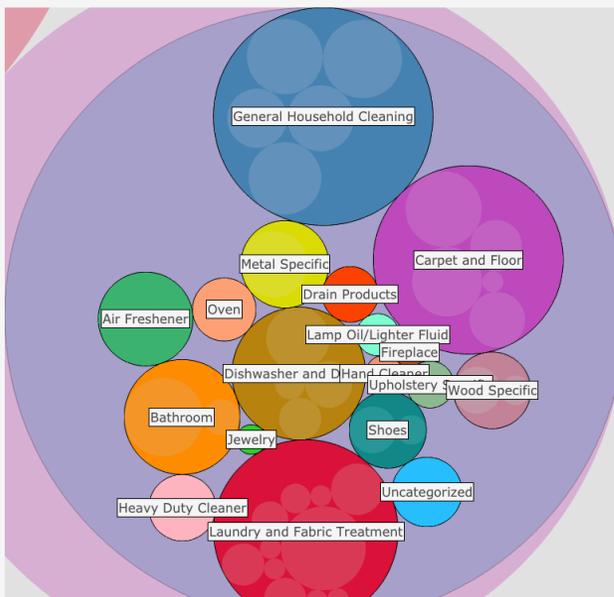
The Chemical Characterization tool allows you to view and compare one or two chemical lists based on their physicochemical properties. Comparisons are available in tabular format along with principal component analysis plots of list against subsets of the ICE chemical inventory.

**Chemical Characterization tool** allows users to explore one or two chemical lists.

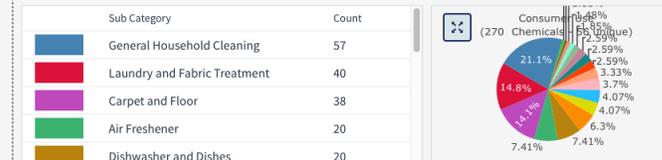
- Physicochemical property distributions
- Interactive PCA plots of chemical space coverage
- Presence in consumer products (EPA CPDat)



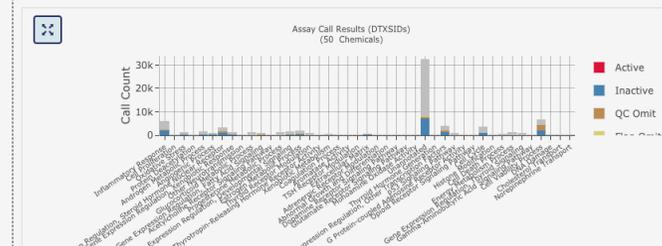
Chemical Consumer Use (1875 Chemicals - 203 unique): Cleaning Products and Household Care



Chemical Consumer Use Details: Cleaning Products and Household Care (270 Chemicals - 56 unique)



DTXSID (Dashboard)	Substance Name	CASRN (CEBS Link)	Sub Categories	Count
DTXSID7020762	Isopropanol	67-63-0		27
DTXSID9020584	Ethanol	64-17-5		27
DTXSID1024097	2-Butoxyethanol	111-76-2		24
DTXSID1020778	D-Limonene	5989-27-5		21

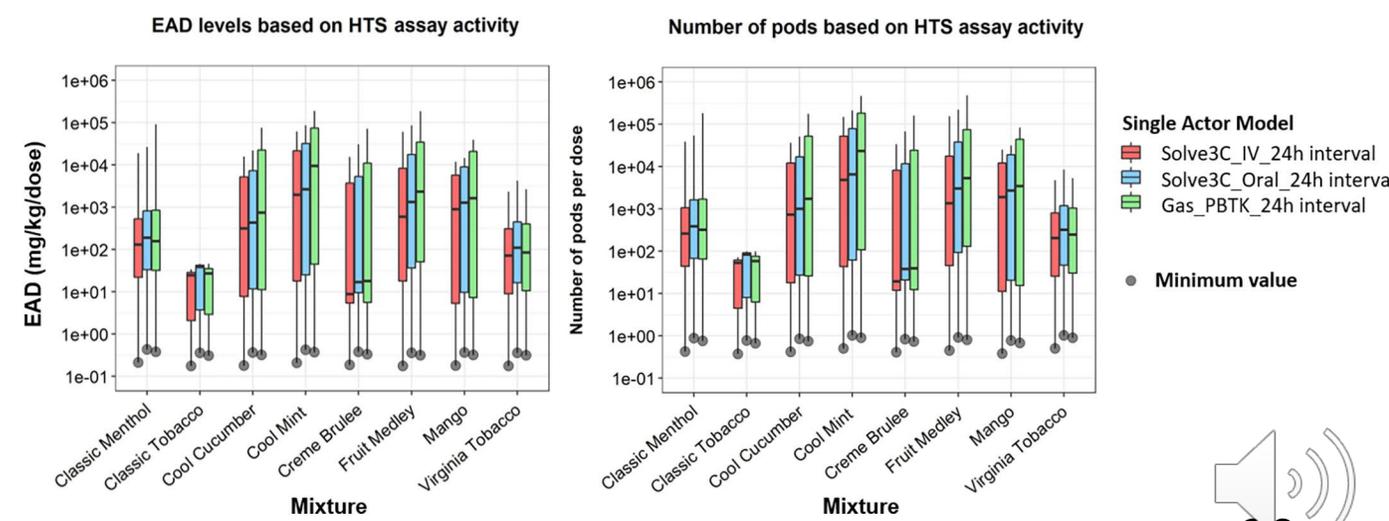
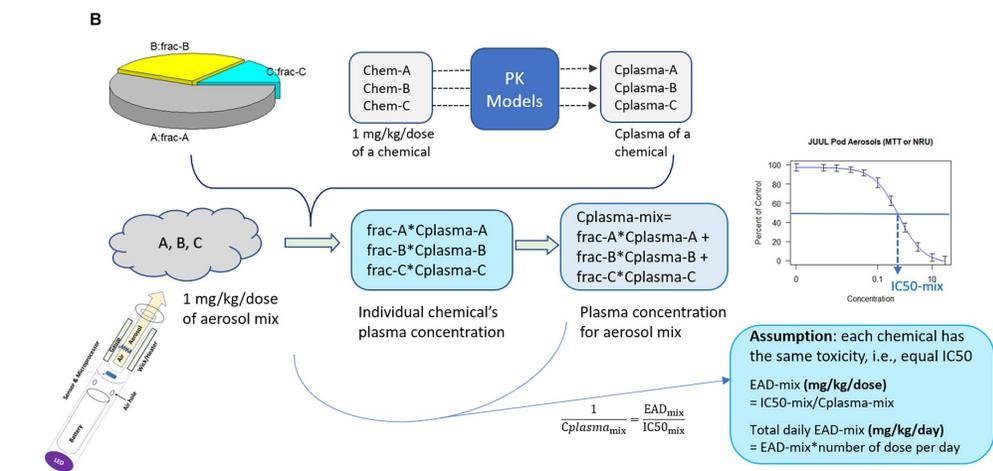
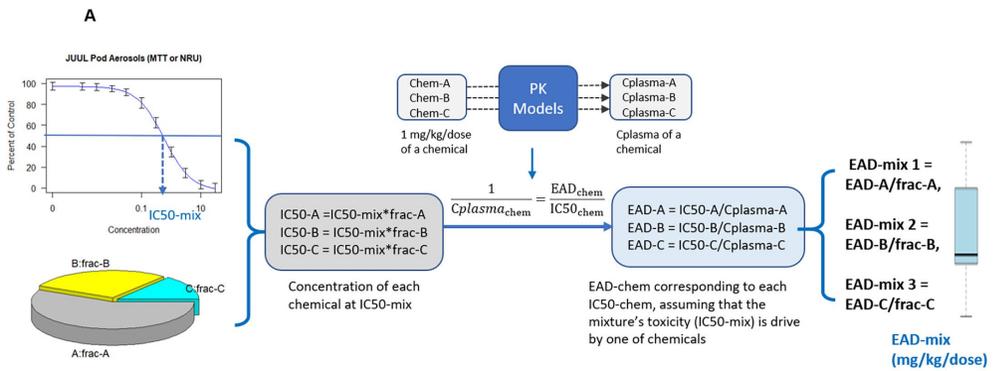




# IVIVE for exposure and health impacts of e-cigarette flavor mixtures

Chang et al. 2021 Tox In Vitro

- Explored impact of PK modeling approach, handling of chemical mixtures for IVIVE modeling, and selection of *in vitro* assays.
- Considered results of cytotoxicity assays and *in vitro* assays that have more diverse and specific mechanistic (sub toxic) targets – stronger relevance to human health risk.





- Many computational toxicology tools are available for generation and interpretation of NAM data.
- Flavor ingredients and other components can be effectively tested in in vitro assays.
- In vivo inhalation data, like many in vivo toxicology study designs, suffer from lack of reproducibility.
- NAMs are accepted alternatives for many acute toxicity endpoints.
- Combining kinetic modeling and in vitro concentration response data can serve as an effective toxicity screening approach.
- Engaging regulatory authorities early in the process is critical for effective NAM implementation.



# Acknowledgments: The NICEATM Group

Speaker View Exit Full Screen

Judy Strickland Nicole Kleinstreuer Jaleh Abedini Dave Allen John Rooney

Pei-Li Yao Amber Daniel Bethany Cook Xiaoqing Chang Agnes Karmaus

Patricia Ceger Alex Borrel Jon Hamm Cathy Sprankle Lauren Browning

Arpit Tandon Eric McAfee Jason Phillips Shannon Bell Steven Morefield

David Hines Matt Stout Kamel Mansouri Ruchir Shah Neepa Choksi

Mute Stop Video Security Participants 25 Chat Share Screen Polling Record Reactions End



# Acknowledgments: The NICEATM Group

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David Hines	Matt Stout	Kamel Mansouri	Ruchir Shah	Neepa Choksi

Mute Stop Video Security Participants 25 Chat Share Screen Polling Record Reactions End





# TK to Connect Metabolism and Variability in Humans

Toxicology Letters 312 (2019) 173-180

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: [www.elsevier.com/locate/toxlet](http://www.elsevier.com/locate/toxlet)

Metabolism of triflumuron in the human liver: Contribution of cytochrome P450 isoforms and esterases

Rim Timoumi<sup>a,b</sup>, Franca M. Buratti<sup>c,d</sup>, Salwa Abid-Essefi<sup>e</sup>, Jean-Lou C.M. Dorne<sup>f</sup>, Emanuela Testai<sup>g</sup>

Toxicology Letters

journal homepage: [www.elsevier.com/locate/toxlet](http://www.elsevier.com/locate/toxlet)

Inter-phenotypic differences in CYP2C9 and CYP2C19 metabolism: Bayesian meta-regression of human population variability in kinetics and application in chemical risk assessment

Nadia Quignot<sup>a,b,\*</sup>, Witold Wiecek<sup>b,h,i,\*</sup>, Leonie Lautz<sup>c</sup>, Jean-Lou Dorne<sup>d</sup>, Billy Amzal<sup>a</sup>

Computational Toxicology

journal homepage: [www.elsevier.com/locate/comtox](http://www.elsevier.com/locate/comtox)

Inter-ethnic differences in CYP3A4 metabolism: A Bayesian meta-analysis for the refinement of uncertainty factors in chemical risk assessment

Keyvin Darney<sup>a</sup>, Emanuela Testai<sup>b</sup>, Franca M. Buratti<sup>c</sup>, Emma Di Consiglio<sup>b</sup>, Emma E.J. Kasteel<sup>d</sup>, Nynke Kramer<sup>e</sup>, Laura Turco<sup>f</sup>, Susanna Vichi<sup>g</sup>, Alain-Claude Roudot<sup>h</sup>, Jean-Lou Dorne<sup>i</sup>, Camille Béchaux<sup>h</sup>

Environment International

journal homepage: [www.elsevier.com/locate/envint](http://www.elsevier.com/locate/envint)

Bayesian meta-analysis of inter-phenotypic differences in human serum paraoxonase-1 activity for chemical risk assessment

K. Darney<sup>a</sup>, E.E.J. Kasteel<sup>b</sup>, F.M. Buratti<sup>c</sup>, L. Turco<sup>d</sup>, S. Vichi<sup>e</sup>, C. Béchaux<sup>f</sup>, A.C. Roudot<sup>g</sup>, N.I. Kramer<sup>h</sup>, E. Testai<sup>i</sup>, J.L.C.M. Dorne<sup>j</sup>, E. Di Consiglio<sup>k</sup>, L.S. Lautz<sup>l</sup>

TOXICOKINETICS AND METABOLISM

Human variability in isoform-specific UDP-glucuronosyltransferases: markers of acute and chronic exposure, polymorphisms and uncertainty factors

E. E. J. Kasteel<sup>a</sup>, K. Darney<sup>b</sup>, N. I. Kramer<sup>c</sup>, J. L. C. M. Dorne<sup>d</sup>, L. S. Lautz<sup>e</sup>

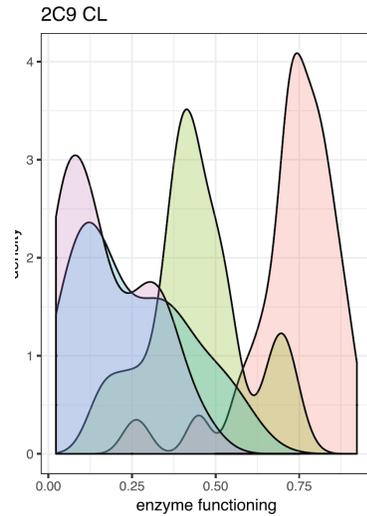
Computational Toxicology

journal homepage: [www.elsevier.com/locate/comtox](http://www.elsevier.com/locate/comtox)

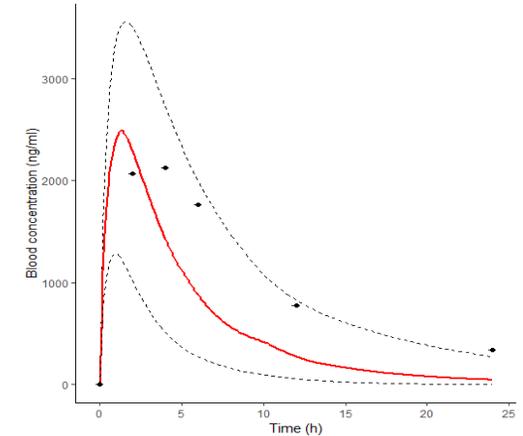
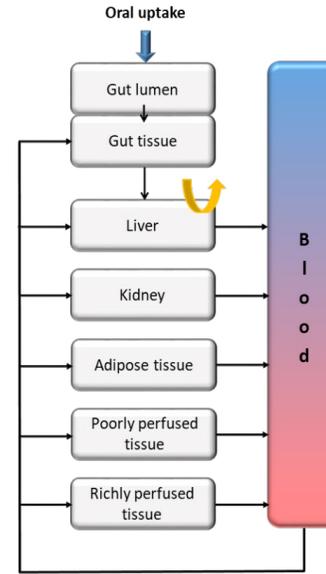
A generic Bayesian hierarchical model for the meta-analysis of human population variability in kinetics and its applications in chemical risk assessment

Witold Wiecek<sup>a,b</sup>, Jean-Lou Dorne<sup>b</sup>, Nadia Quignot<sup>c</sup>, Camille Béchaux<sup>d</sup>, Billy Amzal<sup>a</sup>

Covering Phase I  
CYP450 and Phase II  
UGTs enzymes

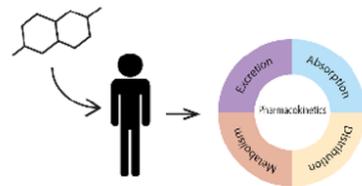


## PBPK models + virtual population

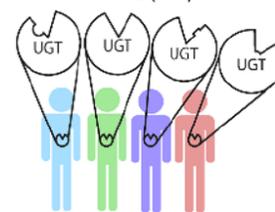
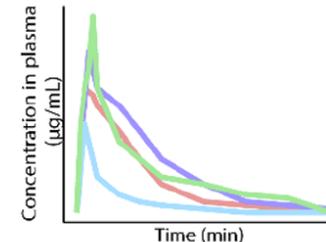


<https://ice.ntp.niehs.nih.gov/>

Pharmacokinetic data on compounds metabolised by UGT isoforms is collected and summarised in a database



Interindividual differences in kinetics and polymorphisms



UGT-related uncertainty factors

Data on polymorphism frequencies in different populations is collected and summarised



Courtesy of Jean-Lou Dorne European Food Safety Authority

# Application of biokinetic modelling for in vitro-in vivo extrapolation (IVIVE) in chemical risk assessment

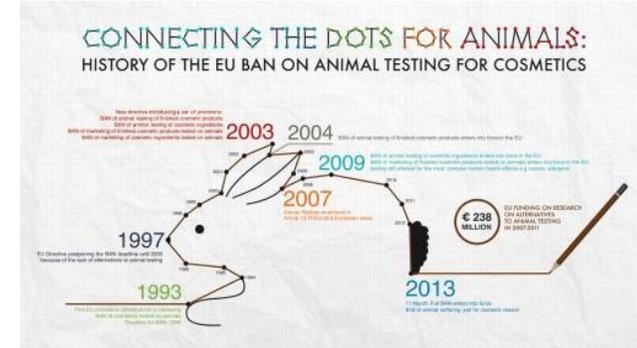
Alicia Paini & Andrew Worth

European Commission Joint Research Centre (JRC), Ispra, Italy



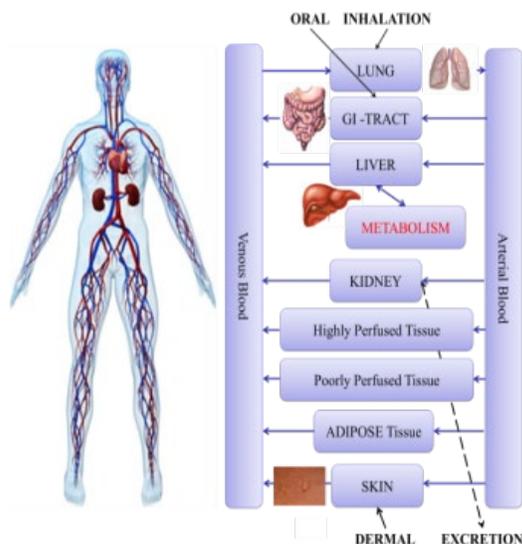
# Premise

- Chemical Risk Assessment can and should be based on non-animal data
- This implies the need to use alternatives such as *in vitro* and *in silico* methods
- Especially to interpret and use *in vitro* toxicity data in combination with biokinetic data
- Biokinetic (ADME) data can be generated by *in silico* and *in vitro* models
- Mathematical modelling is the way to accurately integrate and use *in vitro* data for the design of experiments and extrapolate *in vitro* to *in vivo* for safety assessment
- Robust and reliable mathematical models are available



# What kinds of models are in scope?

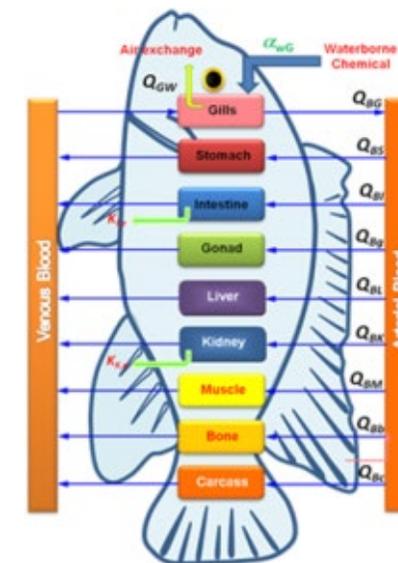
## Physiologically based kinetic (PBK) model



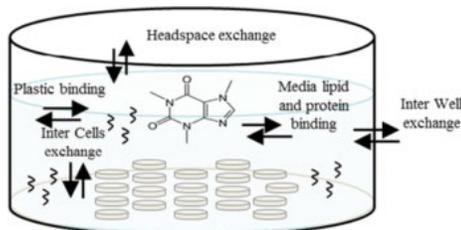
Mathematical description of the body, simulating the xenobiotic distribution into the different organs.

Throughout this presentation the more general term PBK will be used. Noting that PBK, PBPK, PBBK and PBTk are synonyms.

Physiologically based pharmacokinetic (PBPK) is the most widely used term for kinetic models describing the absorption, distribution, metabolism and excretion of a drug within the body. Although widely used in the pharmaceutical sector, the "PBPK" term is not strictly correct in the area of chemical risk assessment. An alternative is "PBTk" with the TK representing toxicokinetic, but this is not appropriate either (Clewel & Clewell, 2008). **More general terms, such as physiologically based biokinetic (PBBK) or physiologically based kinetic (PBK), are thus more appropriate.**



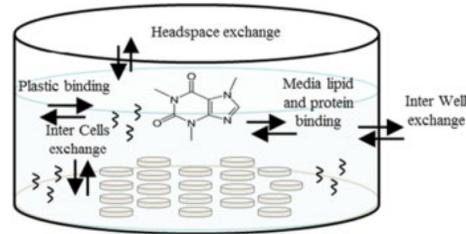
## Fate and Distribution model



Mathematical description of the well, simulating the xenobiotic distribution into the different in vitro set up compartments.



# In vitro to in vivo extrapolation



## Stream 1

Scale up of parameters

PBK model parametrisation

Scale-up of in vitro data to in vivo is performed by analyzing the correlation between in vitro and in vivo data or applying physiological correction factors.

*in vitro* data provides the parameter values for developing a model.<sup>1</sup>



## Stream 2

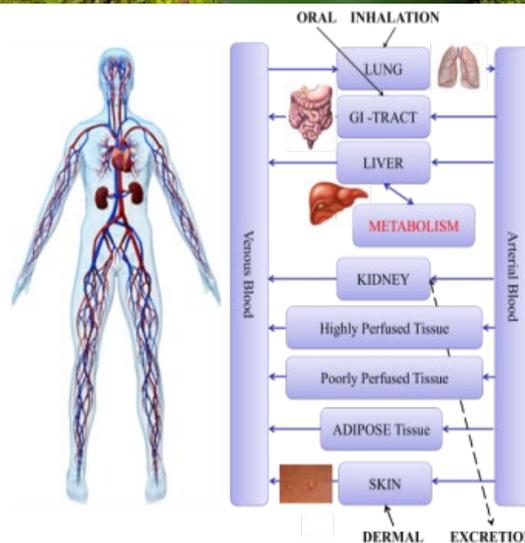
Reverse dosimetry

PBK model extrapolation

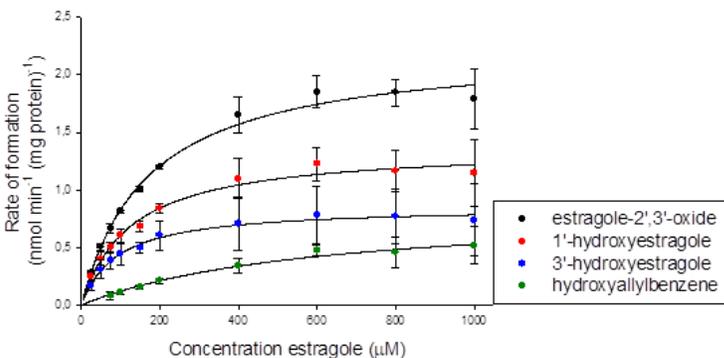
Translation of in vitro concentration effect curves into in vivo dose response curves.

Obtain an oral equivalent dose or a PoD.

Extrapolating adverse effects observed in vitro to an in vivo exposure.



# Stream 1: Scale up of parameters



In vitro incubation rate of metabolism or clearance

nmol min<sup>-1</sup> (mg protein)<sup>-1</sup>  
 nmol min<sup>-1</sup> (mg S9 protein)<sup>-1</sup>  
 (measuring rate of formation)

$$V = V_{max} (S) / K_m + (S)$$

$V_{max}$  → Needs to be scaled from in vivo to in vitro  
 $K_m$  → assumed to be the same as the in vivo  $K_m$  (uM)

Using scaling factors (from literature); hepatocellularity values or microsomal recovery factors, non specific binding and liver weights.

- Cyps abundance
- S9 abundance
- protein abundance (HLM)

$V_{max}$ , in vivo  $\mu\text{mol hr}^{-1}$   
 $Cl_{int,H}$ , in vivo  $\mu\text{L}/\text{min}/\text{g Liver}$

In vitro to in vivo extrapolation of parameters

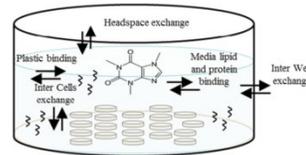
Incorporate of the scaled in vivo parameters in the PBK model  
 Liver model: Well stirred, parallel tube, dispersion

# Stream 2: Reverse dosimetry

## Difference in exposure

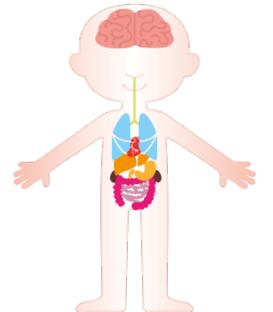
### *Effect measured in vitro*

- 5 or 10% serum
- Single cells
- High concentration
- Non bioaccumulation
- Plastics/Evaporation
- Short exposure
- Batch and experimental set up variability



### *Effect measured in vivo*

- 100% serum
- Connected complex cell system
- Low concentration
- Bioaccumulation
- No plastic/No evaporation
- Long exposure
- Inter-individual variability

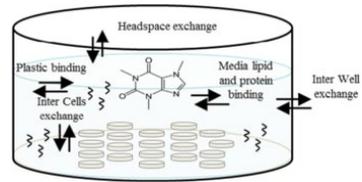


## Difference in dose metrics

Maybe best dose metric: internal concentration



# Stream 2: Reverse dosimetry - Steps



Nominal Concentrations

Experimental dilutions

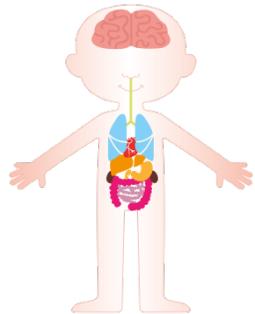
- Armitage model (2014)
- Kramer model (2010)
- Zaldivar model (2016)
- Proenca et al (2021)

Distribution math models

Free Concentrations

Free conc

\*Extrapolation Translation



Exposure dose

mg/kg BW

PBK models

- Httk
- PKSim
- SymCip
- Simulation plus
- IndusChemFate
- MeGEN
- Berkley Madonna/Matlab/R
- Among others

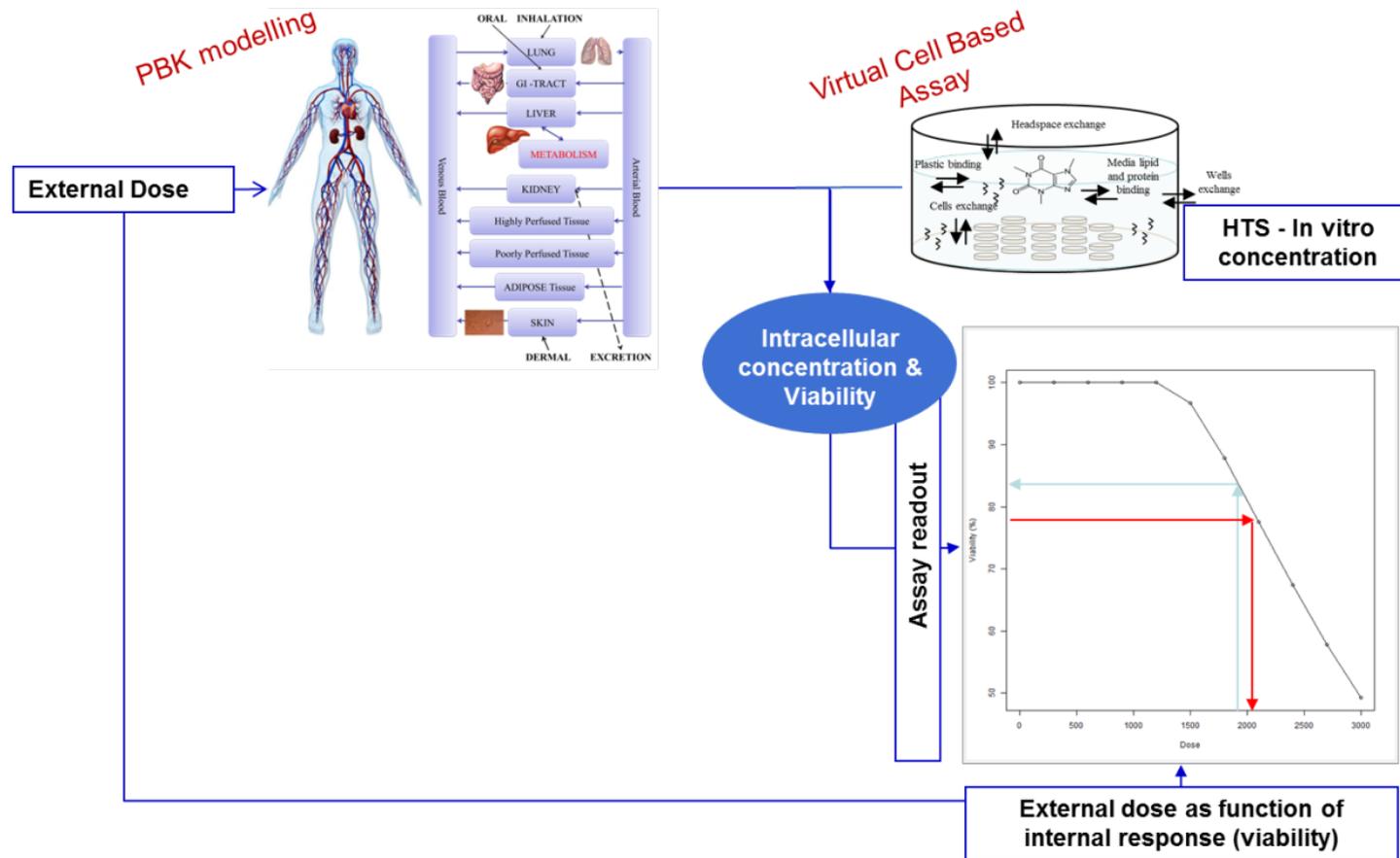
Organ concentration

Cmax & AUC

\*Assumption that the free concentration in the assay and the organ concentration can be considered =  
 Painsi et al., 2017, Tox in vitro – OECD IATA 2020 – under review Systemic Toxicity of Phenoxyethanol – Pistollato et al., 2021 Rep.Tox



# Stream 2: Reverse dosimetry – endpoint



This strategy has been applied to a number of **toxicological endpoints** including **developmental toxicity, genotoxicity, acute toxicity and hepatotoxicity, nephrotoxicity, neurotoxicity, and, more recently, endocrine disruption.**



# How to accurately integrate in vitro data



Stream 1  
Scale up

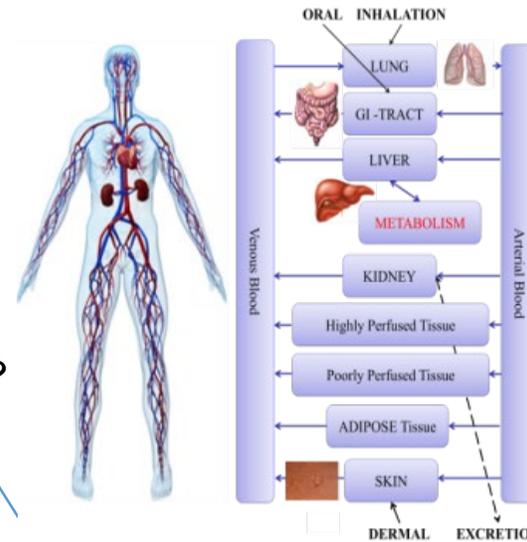
Stream 2  
Reverse dosimetry

Several PBK models  
available in the literature

PBK model parametrisation  
IVIVE

PBK model extrapolation  
QIVIVE

**How to gain  
confidence???**



Connected Streams  
PB(P)K modelling



# Connected Streams

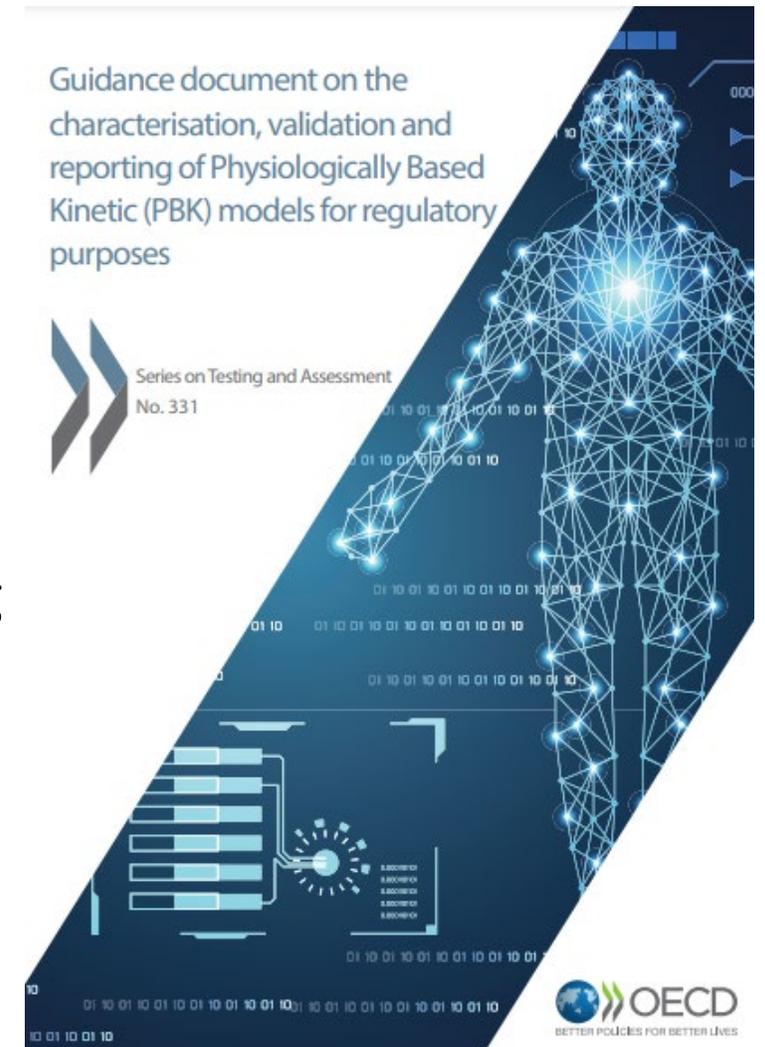
In vitro input parameters → OECD TG & GD (OHTs) or GIVIMP

In silico input parameters → OECD QSAR GD - QMRF

Evaluation/qualification/validation PBK model → OECD PBK model GD

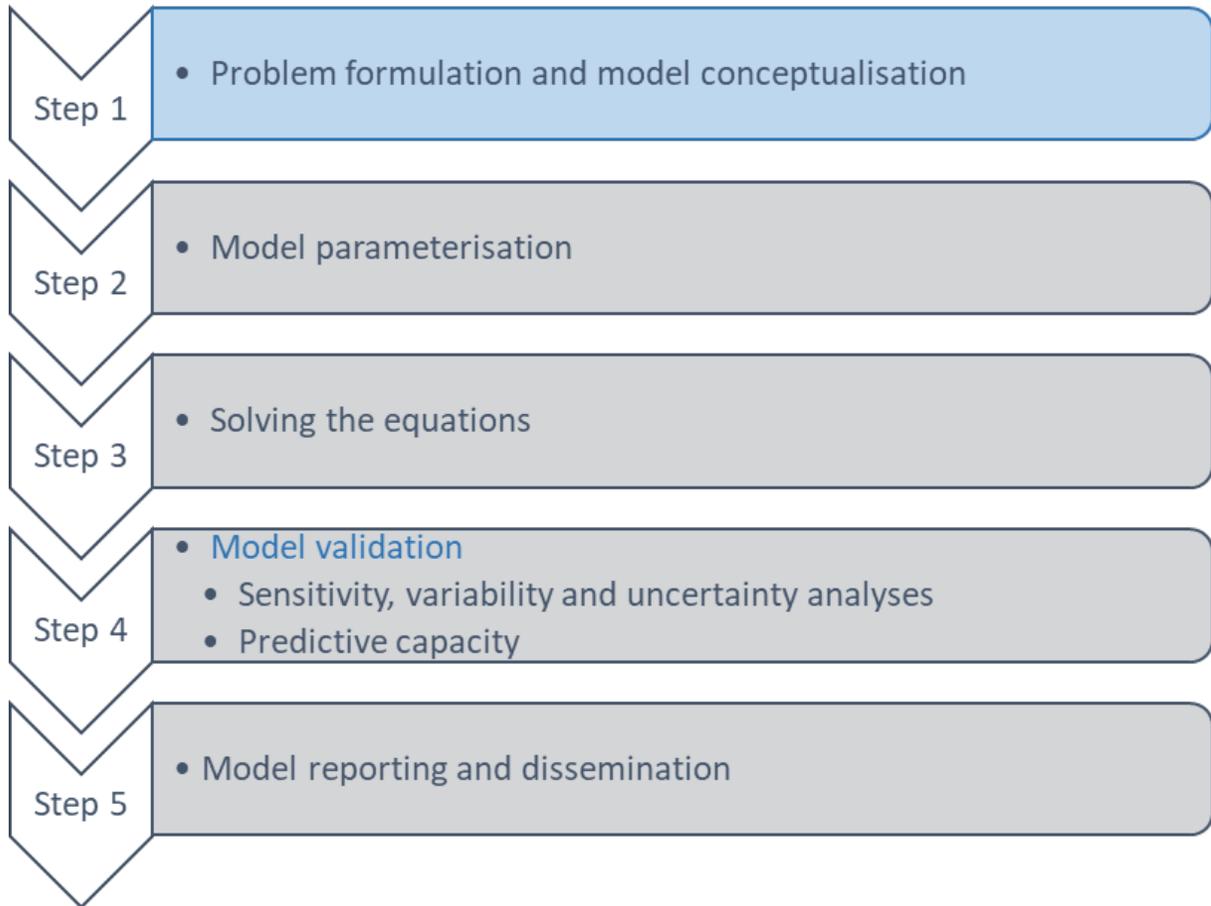
## OECD PBK model GD Purpose and scope

- Provide guidance on characterising, reporting, and evaluating PBK models used in regulatory assessment of chemicals
- Address challenges associated with developing and evaluating PBK models for chemicals without *in vivo* kinetic data
- Promote the use of PBK models in regulatory risk assessment and facilitate dialogue between model developers and users



# Contents of OECD Guidance Document

## 1. PBK Model workflow

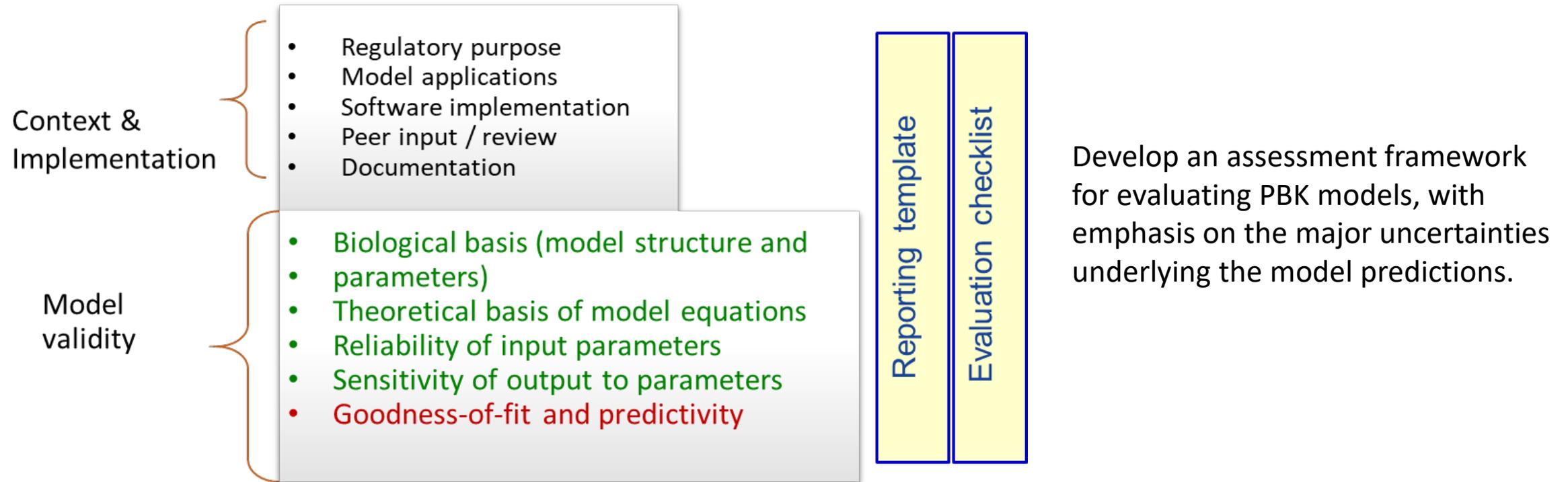


Scientific workflow for characterising and validating PBK models, with emphasis on the use of in vitro and in silico data for absorption, distribution, metabolism and excretion (ADME) parameters, and in scenarios where in vivo kinetic data are limited or unavailable to parameterise model parameters



# Contents of OECD Guidance Document

## 2. Regulatory assessment framework of PBK models

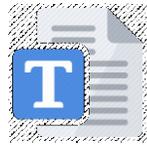


# Contents of OECD Guidance Document



## 3. PBK model Evaluation tool box

### 1. Model Reporting Template



### 2. Evaluation Checklist



### 3. Overall Evaluation Matrix (adapted from WHO 2010)

		Uncertainty in variability of the input parameter estimates		
		High	Medium	Low
SENSITIVITY	High		Parameter 1	Parameter 3 Parameter 4 Parameter 7
	Medium		Parameter 2	Parameter 10
	Low			Parameter 12 Parameter 13

		LEVEL OF CONFIDENCE		
		NONE		HIGH
Biological basis	Biological basis	The model parameters, structure or assumptions are consistent with neither the biology nor the current state of knowledge regarding the kinetics of the chemical.	The biological basis of some model parameters, structural elements or assumptions is questionable.	The model parameters and structure have reasonable biological basis and are consistent with available kinetic data in several experiments using a single set of input parameters.
	Model simulations of data	Model is unable to reproduce the shape (i.e. bumps, valleys) of the kinetic time course curves, neither for the chemical of interest nor for a suitable analogue.	Model reproduces the shape of part but not all of the kinetic time course curves, either for the chemical of interest or suitable analogue.	Model reproduces consistently all kinetic data, including the shape of time course profiles for chemical of interest.
	Uncertainty in input parameters and model output; Sensitivity of model output to input	No uncertainty and sensitivity analyses were performed	Local Sensitivity Analysis supports the robustness of the model.	Global Sensitivity Analysis supports the robustness of the model.



## Thirteen case studies (listed in Annex 4)

**Case Study I:** Generic PBK model for farm animal species: Cattle (*Bos taurus*), Swine (*Sus scrofa*), Sheep (*Ovis aries*) and Chicken (*Gallus gallus domesticus*)

Lautz et al. (2019 a,b; 2020 a,b)

**Case Study II:** Generic PBK models for four fish species

Grech et al. (2017, 2018 a,b; 2019)

**Case Study XIII:** Generic Human one compartment and QIVIVE PB-K models

Wiecek et al. (2019 a,b)

### **Case Study VIII**

PBK model application in species and route to route extrapolation

Bessems et al., 2017

### **Case XI**

Using high-throughput pharmacokinetic simulation and in silico property predictions to predict herbicide absorption and bioavailability

Clark Robert D

### **Case study IX**

Caffeine PBBK model to predict MoIE for risk assessment

IATA caffeine CS

### **Case study X**

IVIVE-PBPK model for phenyl-1,4-dihydropyridine calcium channel antagonists

Gardner et al.

### **Case Study XII**

Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products

Moxon et al. 2020

<https://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm>

### **Case Study III**

In vitro-to In vivo extrapolation (IVIVE) by PBTK modelling

Fabian et al. 2019

### **Case Study IV**

PBK model predictions using data from analogues

Paini et al., 2021

### **Case Study V**

Physiologically based pharmacokinetic (PBK) model for acrylonitrile in humans

Takano et al 2010

### **Case Study VI**

PBK model predictions for monoisononyl phthalate

Miura et al.,2019

### **Case Study VII**

Quantitative Proteomics-based Bottom-up PBK Modeling to Predict Chemical Exposure in Humans

Chan et al. 2019



# Sources

## OECD PBK model GD webinar



Welcome to the webinar, we will start in a couple of minutes.

**Gaining acceptance in next generation PBK modelling approaches for regulatory assessments**

**WHEN: 10 May 2021**  
13:20 - 15:30 (CEST) / 07:20 - 09:30 (EDT)

 **OECD**  
BETTER POLICIES FOR BETTER LIVES

The banner features a central graphic of a human figure composed of a glowing blue wireframe mesh, set against a dark blue background with various data visualization elements like bar charts and line graphs.

<https://www.oecd.org/chemicalsafety/testing/webinars-on-testing-and-assessment-methodologies.htm>

**OECD PBK model GD (n 331)**

**Case Studies to illustrate (ANNEX IV)**

<https://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm>



# Take Home

- Characterising in vitro and in vivo biokinetics is going to be critical for determining the relevance and context of your results → IVIVE!
- Connected Streams → Integration!
- As the risk assessment community increase its dependence on in vitro systems and NAMs, more PBK models are being developed without the use of in vivo data → Confidence!



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# Inhalation Exposure Modeling for Assessing Health Risks of Toxic Aerosols and Vapors

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**CORESTA 2021**  
**NAM Symposium**  
19 October 2021



# Modeling Examples for Inhaled Aerosols & Vapors

## State-of-the-art inhalation modeling approaches for cross-species and *in vitro* to *in vivo* comparisons to assess human health risks

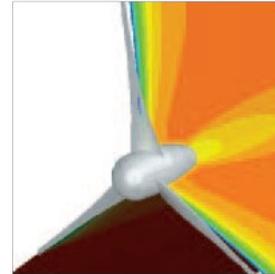
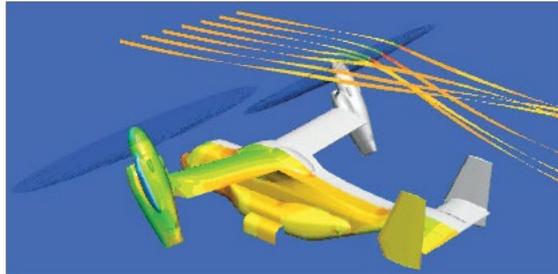
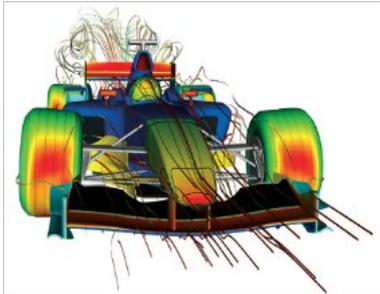
- 3D Imaging-based computational fluid dynamics (CFD) models of the respiratory system
- Incorporate species-specific 3D anatomy, physiology and clearance processes and realistic breathing and exposure scenarios for **site-specific dosimetry**
  
- **Ex 1: Ranking relative hazards of tobacco smoke constituents under a harm reduction strategy using existing animal toxicity and measured human exposure data**
  - **CFD/PBPK modeling for cell- or tissue-specific internal dose**
  - Corley et al., *Toxicol. Sci.* 146(2015)65-88
  
- **Ex 2: Reducing/replacing animal toxicity studies for pesticide re-registration with *in vitro* toxicity studies with human cells for occupational and residential exposures**
  - **CFD/Aerosol/Mucociliary clearance modeling for region-specific retained dose**
  - Corley et al., *Toxicol. Sci.* 182(2021)243-259



# What is Computational Fluid Dynamics or CFD?

## In a nutshell...

- **Numerical method for describing fluid flows**
  - Navier-Stokes Equations that describe the flow of a viscous fluid
  - Solved using a 3D computational mesh with appropriate boundary conditions (e.g. shape, mechanical properties, fluid characteristics, pressure, etc.)
  - The solution is a flow velocity field over space and time
  - Complexities added as needed (equations/mesh refinements) depending upon applications (e.g. physics of heat transfer, turbulence, material transport within fluids, material interactions, etc.)
- **Methods widely used in aerospace, automotive, energy, building HVAC, etc. industries to improve design, trouble-shooting, and decrease costs in product development**



Source: Fluent News, 2005



# What is Computational Fluid Dynamics or CFD?

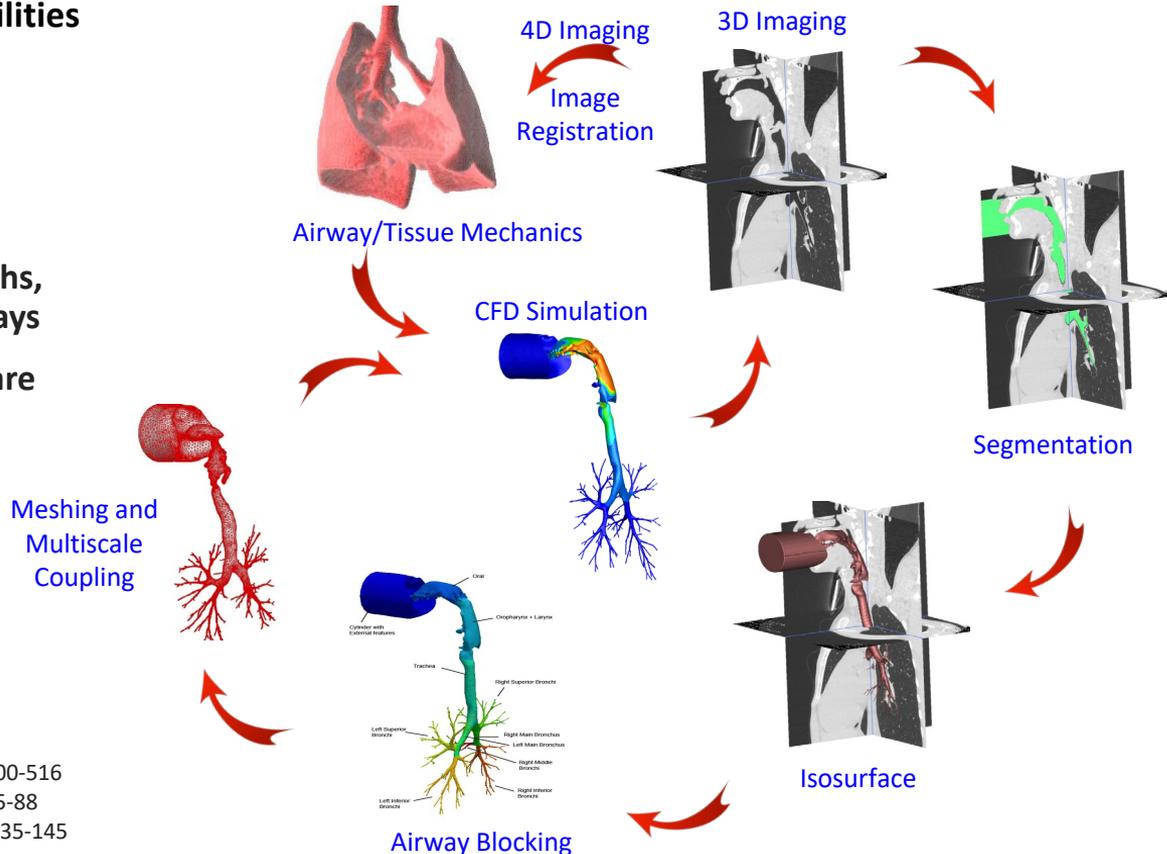
- Biological applications are a rapidly growing area with the advent of new imaging, image analysis, and computational capabilities

- **3D/4D MRI and CT**

- Mod-High resolution
- Dynamic
- Structure & Function

- **What once took months, can now be done in days**

- **Personalized models are possible**



Corley et al. Toxicol. Sci. 128(2012)500-516  
Corley et al. Toxicol. Sci. 146(2015)65-88  
Jacob et al. Exp. Lung Res. 41(2014)135-145



# Ex 1: Multi-Scale CFD/PBPK for Reactive Aldehydes

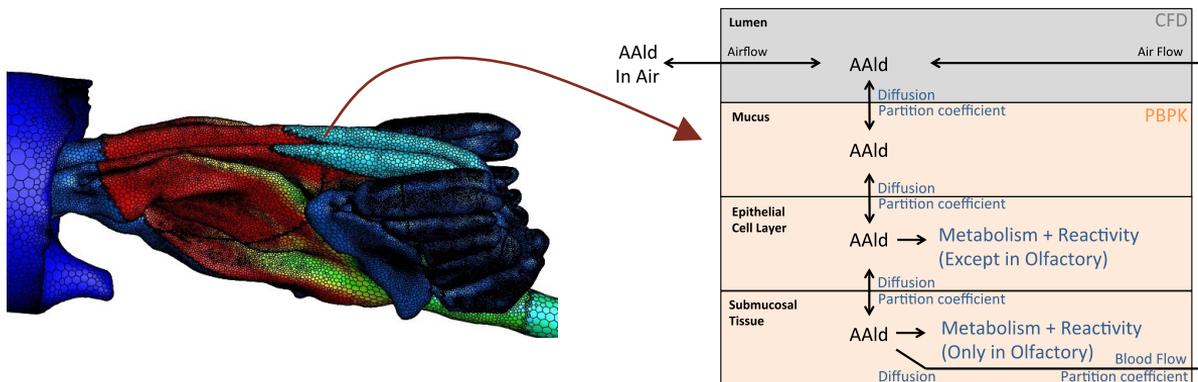


- **Highly reactive, water-soluble vapors**
- **Important industrial chemical intermediates as well as by-products of combustion including smoking of tobacco products**
  - Difficult to directly measure in tissues, endogenously produced and have dietary sources of exposure
- **Cytotoxicity and tumors in specific sites within nasal and upper respiratory tissues of rodents drive many human health risk assessments**
- **Site-specificity of lesions and species differences in anatomy, physiology and tissue clearance rates warranted a combined CFD/PBPK approach**
  - Previous constituent risk comparisons often lacked species-, site-, or exposure-specific dosimetry considerations
- **Took advantage of existing CFD and PBPK models and realistic exposures to create a combined approach**



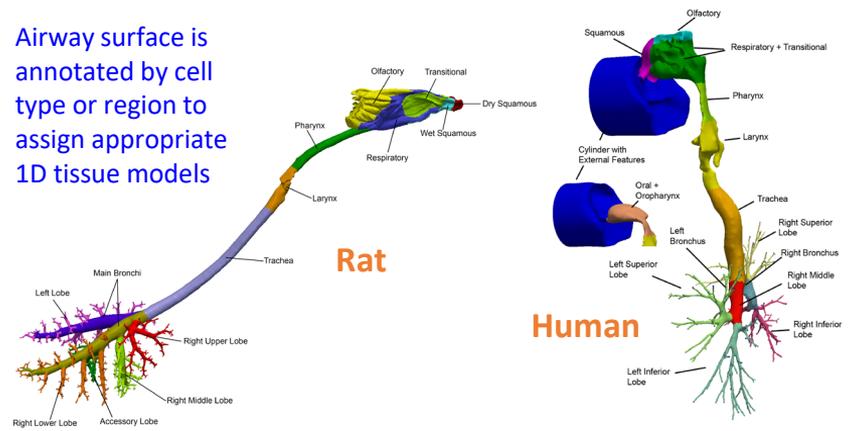
# Ex 1: CFD/PBPK for Reactive Aldehydes

## Model Structure

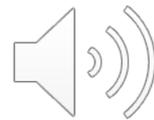


Each surface facet has its own 2-way coupled PBPK tissue model

Airway surface is annotated by cell type or region to assign appropriate 1D tissue models



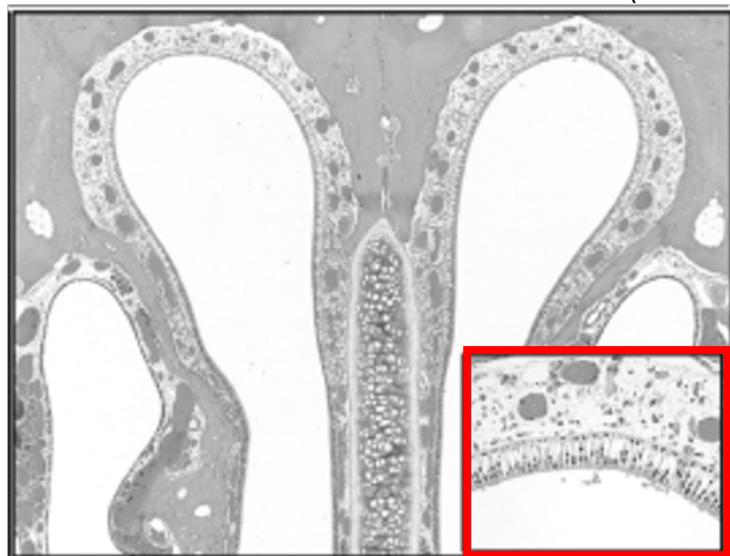
Corley et al. Toxicol. Sci. 128(2012)500-516  
 Corley et al. Toxicol. Sci. 146(2015)65-88



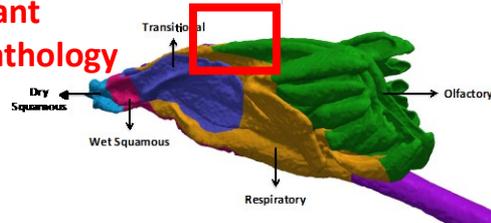
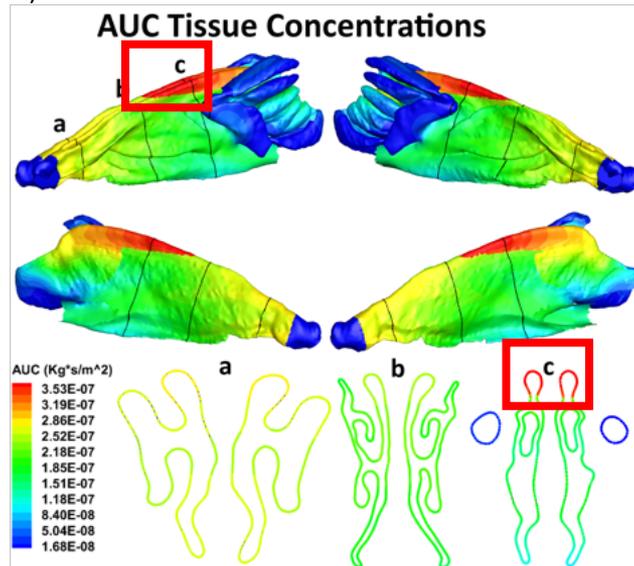
# Ex 1: CFD/PBPK for Reactive Aldehydes

## AUC Tissue Concentration “Hot Spots” vs. Lesions

Acetaldehyde  
(Rat NOAEL = 50 ppm)

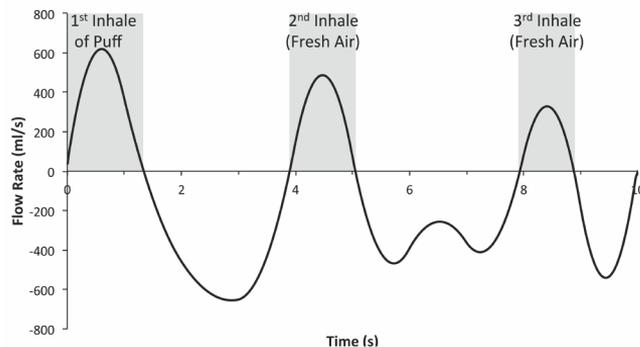


**Enzyme Location is Key Determinant**  
**→ AUC dosimetry maps to histopathology**

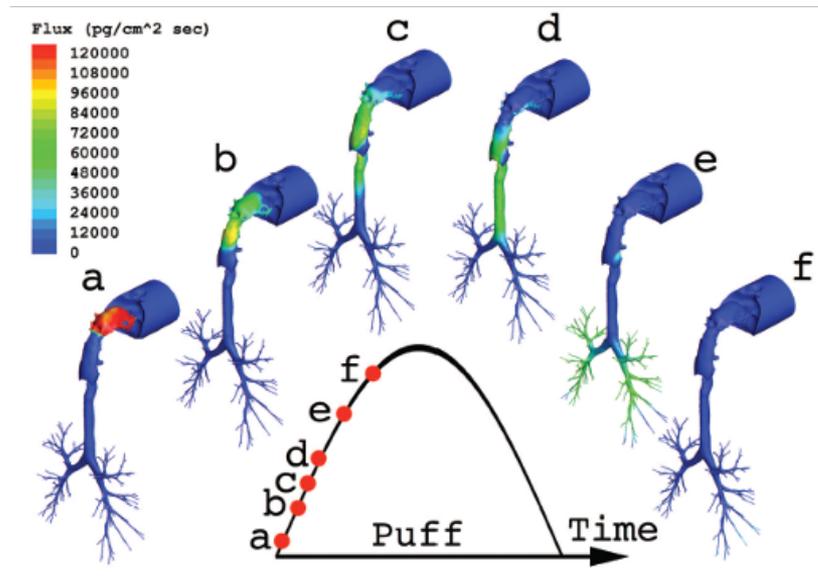


# Ex 1: CFD/PBPK for Reactive Aldehydes Human Exposure via Cigarette Smoking

## Human Smoking Profile



- Measured human puff profile
  - St. Charles et al. *Inhal. Toxicol.* 21(2009)712-718
- Measured smoke compositions for representative puff concentrations
  - (Counts et al. *Reg. Toxicol. Pharmacol.* 41(2005)185-227)
  - Acetaldehyde – 1028 ppm (857  $\mu\text{g}/\text{cig}$ )
  - Acrolein – 94 ppm (100  $\mu\text{g}/\text{cig}$ )
  - Formaldehyde – 108 ppm (61  $\mu\text{g}/\text{cig}$ )

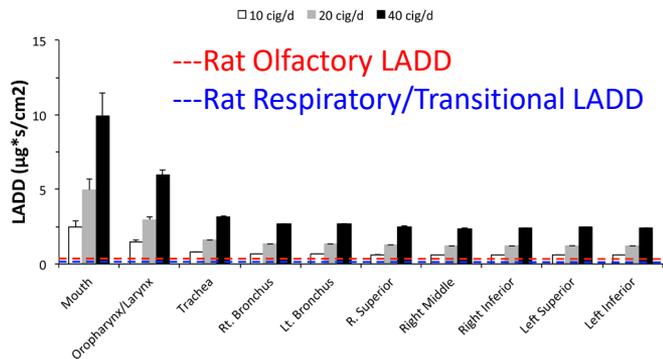


# Ex 1: CFD/PBPK for Reactive Aldehydes

## Comparative Dose Cigarette Smoke Constituents

### Acrolein

(Rat NOAEL = 0.2 ppm; Puff = 94 ppm)



### Rat - Human comparisons based upon 'Hot Spot' AUCs and Exposure-Duration/#cigs per day Adjustments

LADD Rat:  $\text{NOAEL AUC}_{2.5\%}/\text{breath} * \text{bpm} * 360 \text{ min/d} * 5 \text{ d/7 d}$

LADD Human:  $\text{AUC}_{2.5\%}/\text{puff} * 11 \text{ puff/cig} * \text{no. cigs/d}$

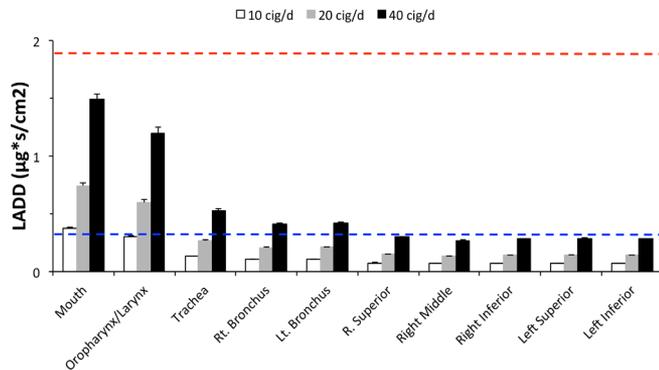
### Rank order:

Acrolein > Formaldehyde > Acetaldehyde

No significant differences when simulated as a mixture with competitive metabolism

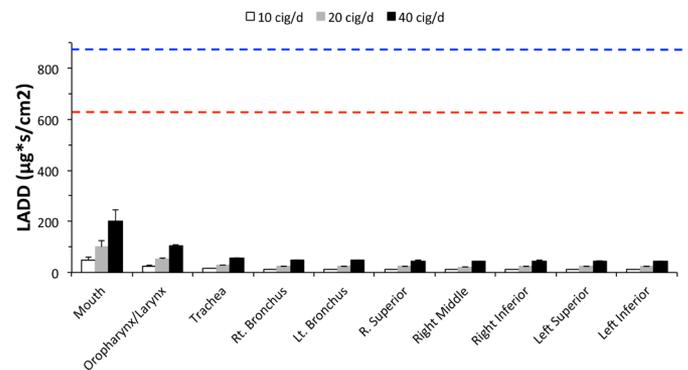
### Formaldehyde

(Rat NOAEL = 1 ppm; Puff = 108 ppm)



### Acetaldehyde

(Rat NOAEL = 50 ppm; Puff = 1024 ppm)



# Ex 2: Syngenta's Pesticide Re-Registration

## Chlorothalonil

- **A widely-used fungicide since 1966**
  - Labeled for >65 crops
  - Also used as a wood protectant, anti-mold and anti-mildew agent, bacteriocide, microbiocide, algaecide and insecticide
- **Contact irritant by all routes of exposure**
- **Extremely low volatility and water solubility**
  - Formulated as a solid or liquid suspension
  - Applications typically water-diluted spray
- **Aerosol inhalation studies in rats with formulation (acute through 2-week)**
  - Epithelial degeneration/necrosis primarily in nose and larynx; minimal effects in trachea and lung
  - Squamous cell metaplasia in nose and larynx
  - Lesions resolved or reduced following 2-wk recovery

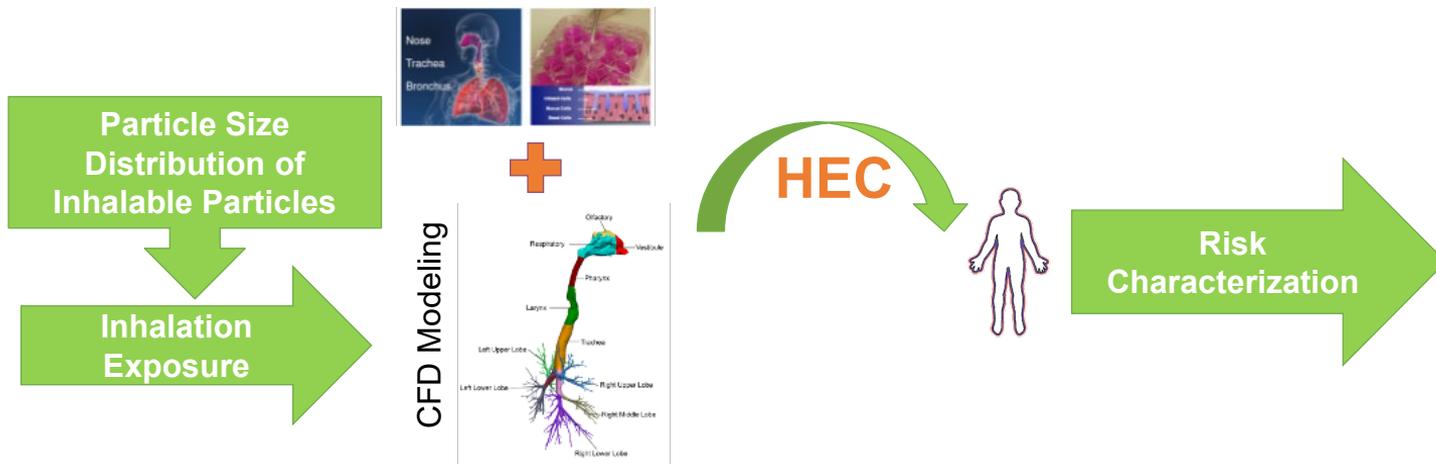


## Ex 2: Syngenta's Pesticide Re-Registration Inhalation Risk Assessment

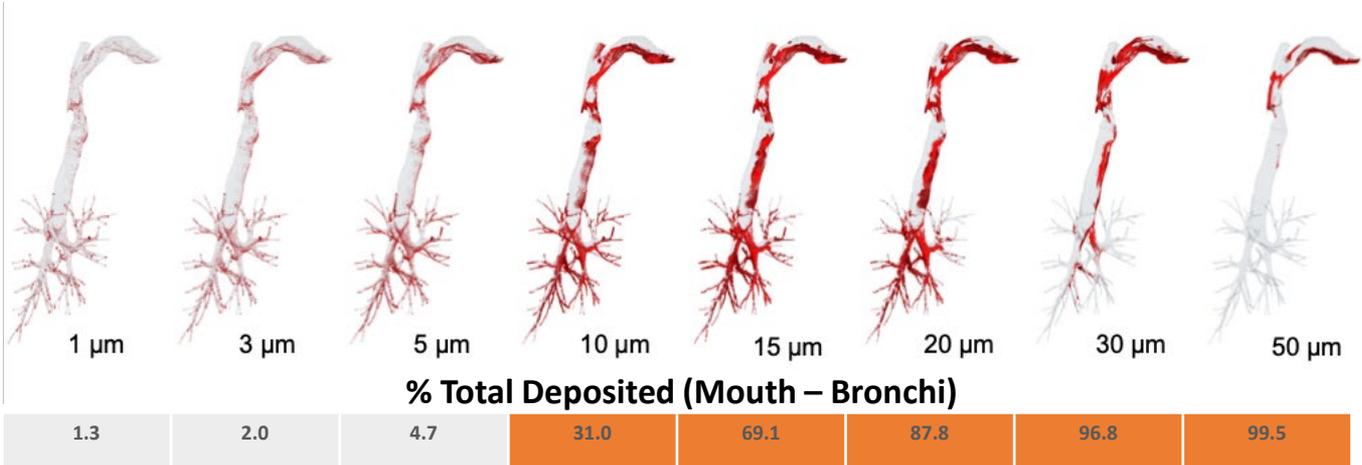
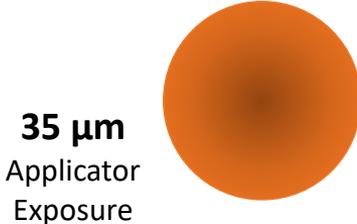
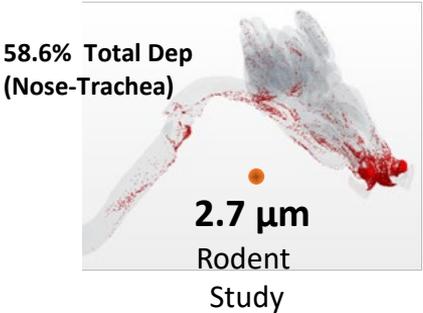
- Replace requirement for 90-day rat inhalation toxicity study with ***in vitro* studies in human cells** coupled to enhanced **characterization of exposure and target dose** relevant to risk characterization



*In vitro* Testing Based Point of Departure using MucilAir™ from Epithelix

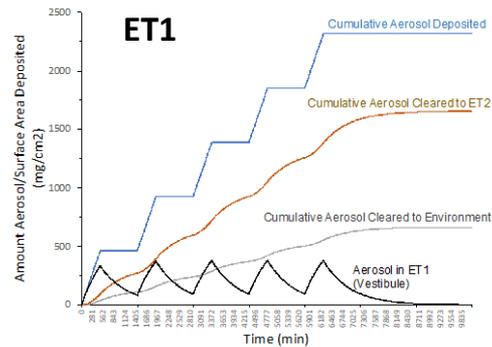
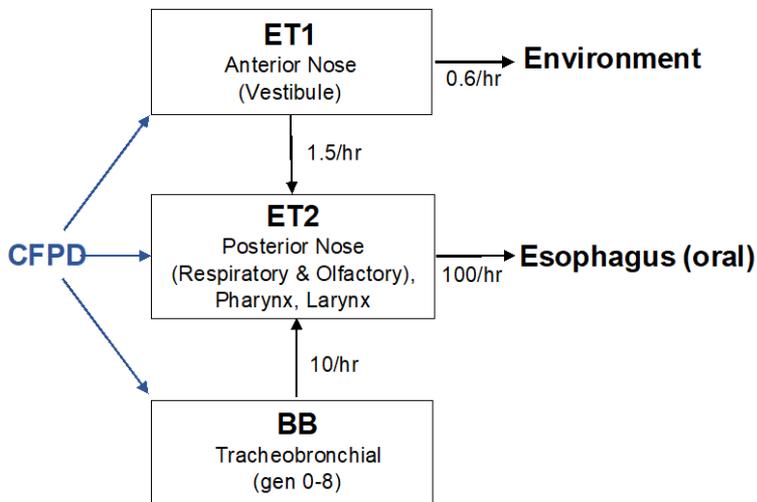


# Ex 2: CFD/Particle Dosimetry for Cross-Species and IVIVE Oral Breathing

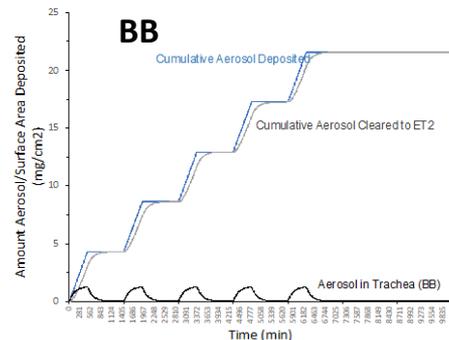
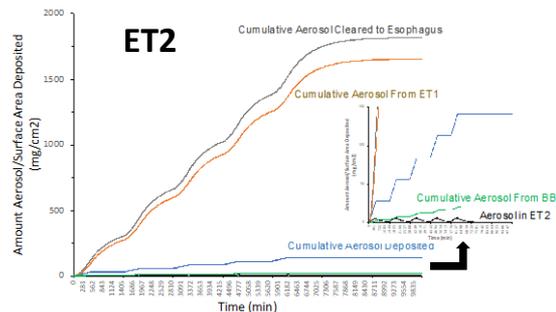


# Ex 2: Clearance Model

Abbreviated ICRP (2015)

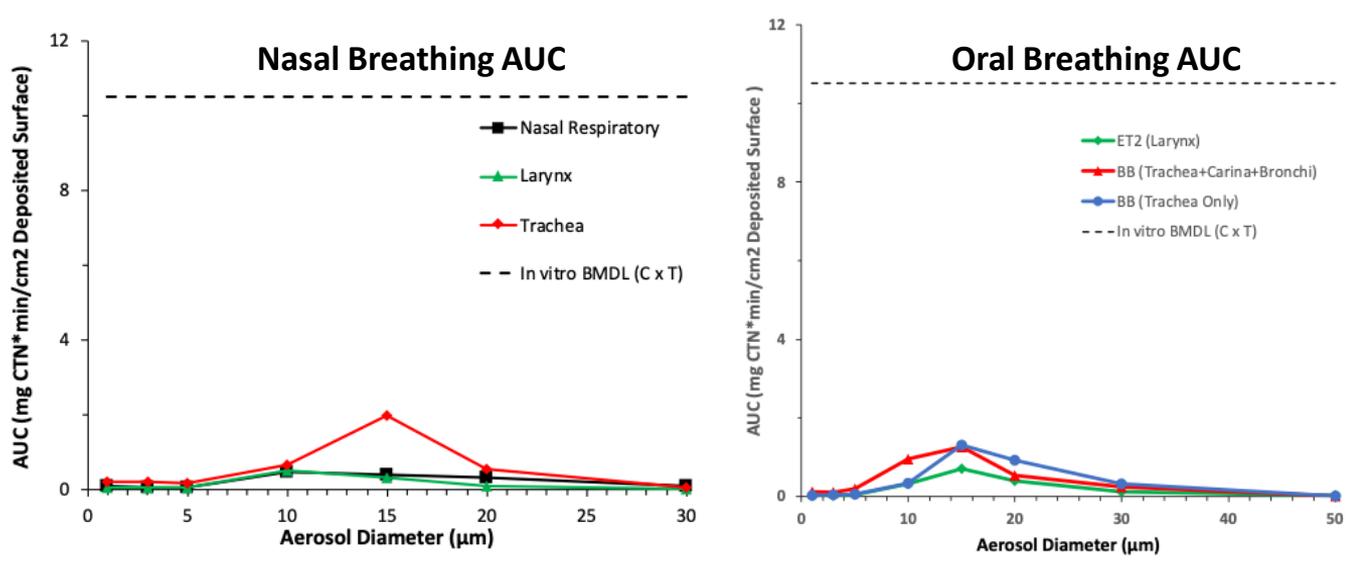


Ex: 10  $\mu\text{m}$  Aerosols  
8 hr/d, 5 d/wk



# Ex 2: CFPD/CL Human Simulations at Rat LOAEL

## AUC Retained Dose vs. In vitro BMDL



**Exposures for 8-hr/day, 5 consecutive days followed by 2 days no exposure**

- Day-to-day steady-state retention profile achieved in 2-3 days
- AUC retained doses determined for final exposure day
- AUC compared to BMDL\*24 hr (CxT)
- $HEC = (BMDL/AUC) * Aerosol\ Conc * Active\ Ingredient\ Conc$



# Ex 2: Revised Human Risk Assessment for Inhalation Exposures

- EPA determined **the NAM using human *in vitro* data and CFPD dosimetry was appropriate** for evaluating potential risk for inhalation exposure to direct contact irritants
  - **Waved requirement for additional 90-d rat inhalation studies** (EPA, 2021).
  - Human equivalent concentrations (**HEC**) and human equivalent doses (**HED**) calculated for 2, 8 and 24-hr exposures based upon human *in vitro* BMDL's for multiple polydisperse aerosol scenarios
  - **Interspecies UF reduced to 1X** (both dosimetry and toxicity determined in human)
  - **Intraspecies UF reduced to 3X** (ADME not likely an impact for direct contact irritant/cytotoxicant)
- Revised draft assessment and supporting documents open for comment until Sept. 20, 2021, at: <https://www.regulations.gov/docket/EPA-HQ-OPP-2011-0840>
  - Manuscripts for the human *in vitro* toxicity study (accepted) and human health risk assessment (in review) have also been submitted



# Bottom Lines

- **CFD-based models are well-suited for calculating HEC's from *in vitro* and *in vivo* target tissue doses when site-specificity is important for inhalation toxicity (typically upper conducting airways)**
  - A valuable part of an overall toolkit for modeling inhalation exposures
- **These approaches have been used to refine human risk assessments as well as reduce or even replace animal studies by regulatory agencies**
- **Topics not covered but still important include:**
  - Model evaluation and verification/validation were key components to both examples
    - See references included at the end of this presentation including those used in the case studies
  - Models can be templated or adjusted to fit new materials or exposure scenarios (no need to start from scratch)
    - Airway geometries available for multiple humans and animal models (see Selected References)
    - Existing CFPD simulations are being used to predict site-specific doses for other aerosols that have similar properties
  - CFD models are ideal for site-specificity in upper conducting airways (nose/mouth to generation 5-10) but do not describe the deep lung due to limitations in imaging and the computational challenges
    - However, the **Multiple Path Particle Dosimetry (MPPD) model is ideal for predicting regional dosimetry in the deep lung** and is now being adopted by the U.S.EPA to replace its RDDR model
      - MPPD is available (free) at: <https://www.ara.com/mppd/>
    - CFPD models have also been linked with the MPPD model to provide full respiratory system coverage (Kuprat et al., *J. Aerosol Sci.* 151(2021)105647) and take advantage of, and compensate for, the strengths and weaknesses of each model
      - Ongoing work: disease influences on tissue mechanics are now being incorporated into the CFPD/MPPD model and validated against experimental data in humans and rats





Questions?

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# Glossary

- **AUC = Area under the curve, typically of a concentration vs. time curve**
- **CFD = Computational fluid dynamics**
- **CFPD = Computational fluid-particle dynamics**
- **Cmax = Maximum concentration, typically of a concentration vs. time curve**
- **CT = X-ray computed tomography**
- **EPA = U.S. Environmental Protection Agency**
- **FIFRA SAP = Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel to EPA**
- **HEC = Human equivalent concentration (typically mg/L or mg/m<sup>3</sup>)**
- **HED = Human equivalent dose (typically mg/kg/d)**
- **HVAC = Heating, ventilation, air conditioning**
- **ICRP = International Commission on Radiological Protection**
- **MMAD = Mass median aerodynamic diameter**
- **MPPD = Multiple path particle dosimetry model**
- **MRI = Magnetic resonance imaging**
- **NCRP = National Council on Radiation Protection**
- **PBPK = Physiologically based pharmacokinetic model**

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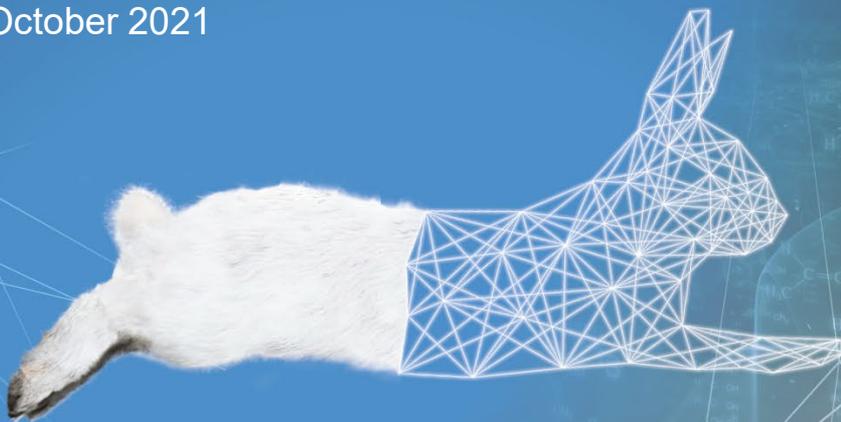
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- **NAM Links:**
  - EPA: <https://www.epa.gov/research/epa-new-approach-methods-efforts-reduce-use-animals-chemical-testing>
  - FDA: <https://www.fda.gov/news-events/fda-brief/fda-brief-fda-publishes-report-advancing-alternative-methods>



# Assessing Respiratory Toxicity of Chemicals in Two Human *in vitro* Systems

Andreas Stucki, PhD  
PETA Science Consortium International e.V.

CORESTA SSPT – NAM Symposium  
19 October 2021



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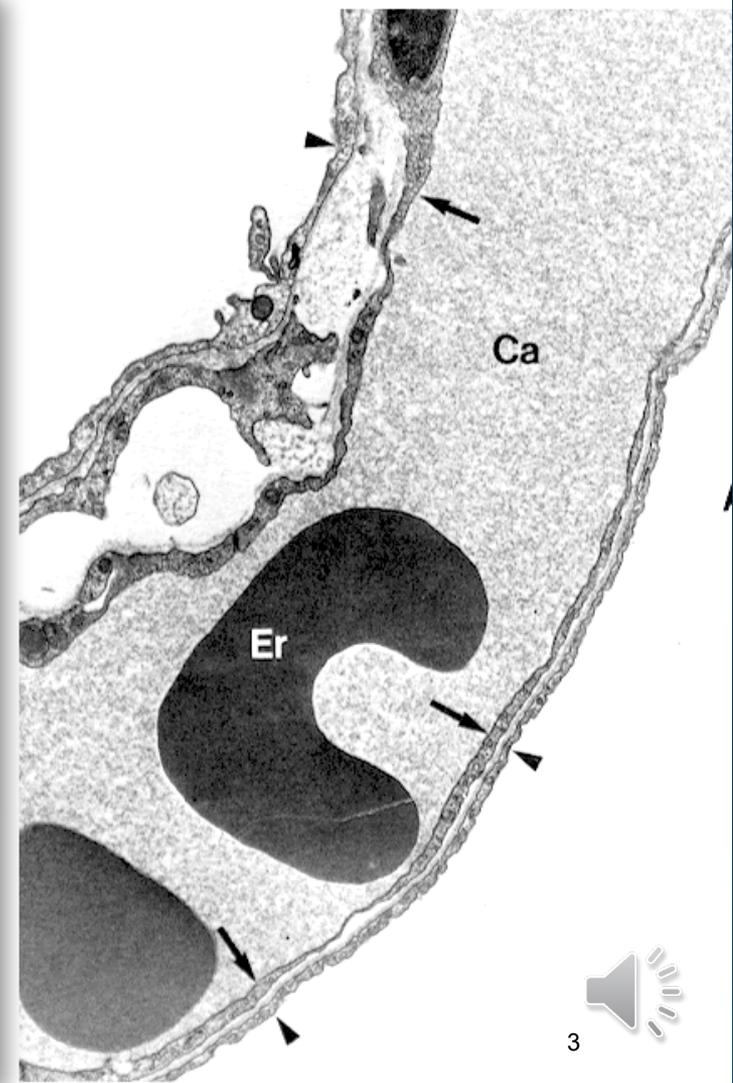


RETROSPECTIVE  
REVIEWS

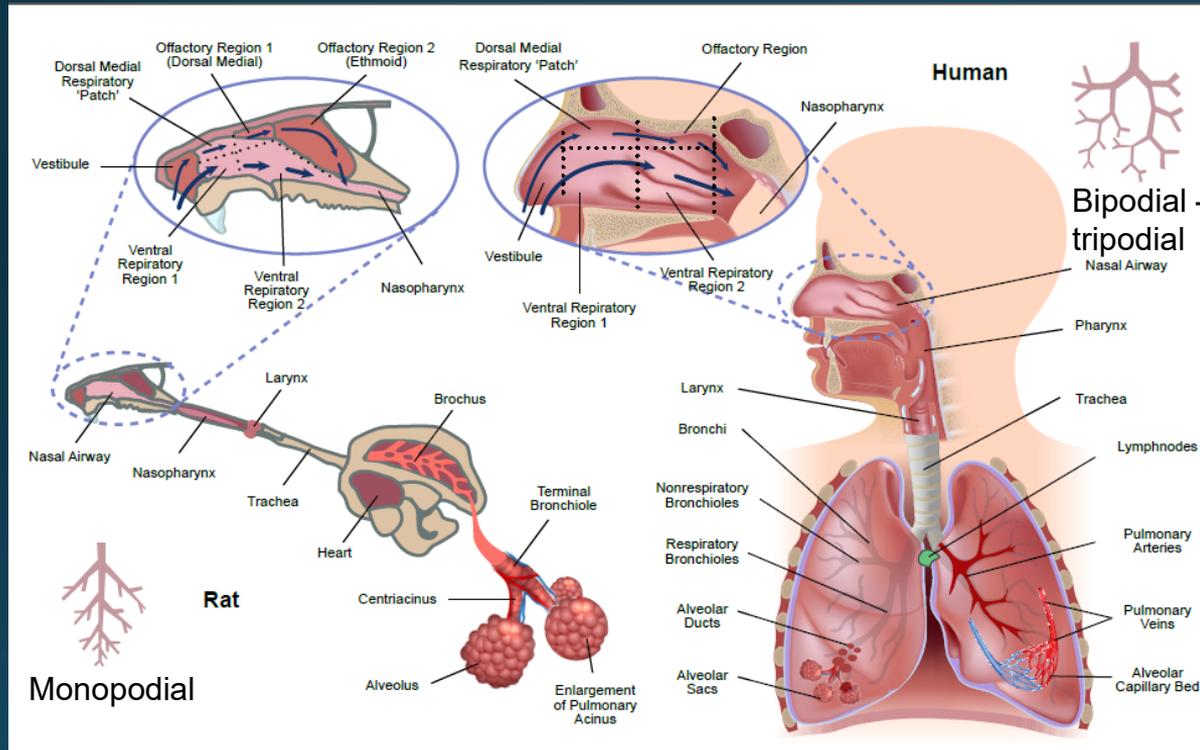


# The human lungs

- 100-150 m<sup>2</sup> surface area
- <1 μm air-blood barrier thickness
- 7-10,000 km of blood vessels
- 17,000 breaths per day
- 7,000 L of air per day
- 40+ different cell types



# Anatomical and physiological differences



Ventilation rates and breathing mode

Airway architecture and branching pattern

Cell type distribution and mucous composition

Metabolic activity

Illustration modified from Dr. Jack R. Harkema,  
Professor of Comparative Pathology, Michigan State University



# The INSPIRE Initiative: IN vitro System to Predict REspiratory toxicity



Toxicology in Vitro 52 (2018) 131–145

Contents lists available at ScienceDirect

**Toxicology in Vitro**

journal homepage: [www.elsevier.com/locate/toxinvit](http://www.elsevier.com/locate/toxinvit)



Review

## Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity

Amy J. Clippinger<sup>a,\*</sup>, David Allen<sup>b</sup>, Holger Behring<sup>c</sup>, Kelly A. Bérubé<sup>d</sup>, Michael B. Bolger<sup>e</sup>, Warren Casey<sup>f</sup>, Michael DeLorme<sup>g</sup>, Marianna Gaça<sup>h</sup>, Sean C. Gehen<sup>i</sup>, Kyle Glover<sup>j</sup>, Patrick Hayden<sup>k</sup>, Paul Hinderliter<sup>l</sup>, Jon A. Hotchkiss<sup>m</sup>, Anita Iskandar<sup>n</sup>, Brian Kevser<sup>o</sup>, Karsta Luettich<sup>o</sup>, Lawrence Milch<sup>o</sup>, Hans Raabe<sup>c</sup>, Erwin Thone<sup>o</sup>, Peter S. Thorne<sup>o</sup>

Contents lists available at ScienceDirect

**Toxicology in Vitro**

journal homepage: [www.elsevier.com/locate/toxinvit](http://www.elsevier.com/locate/toxinvit)



## Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: An international workshop report

Amy J. Clippinger<sup>a,\*</sup>, David Allen<sup>b</sup>, Annie M. Jarabek<sup>c</sup>, Marco Corvaro<sup>d</sup>, Marianna Gaça<sup>e</sup>, Sean Gehen<sup>f</sup>, Jon A. Hotchkiss<sup>g</sup>, Grace Patlewicz<sup>h</sup>, Jodie Melbourne<sup>g</sup>, Paul Hinderliter<sup>i</sup>, Miyoung Yoon<sup>j</sup>, Dongeun Huh<sup>k</sup>, Anna Lowit<sup>l</sup>, Barbara Buckley<sup>c</sup>, Michael Bartels<sup>m</sup>, Kelly Bérubé<sup>n</sup>, Daniel M. Wilson<sup>o</sup>, Ian Indans<sup>o</sup>, Mathieu Vincken<sup>o</sup>

## Goals of the INSPIRE initiative

- Present a case study on how *in vitro* approaches may be used for acute toxicity testing
- Compare a 2D cell line with 3D human reconstructed lung tissues
- Derive *in vitro* point of departure (POD)
- Strengthen scientific confidence in *in vitro* models *en lieu* of animal testing



# Initial considerations for human *in vitro* respiratory toxicity testing



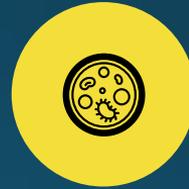
CHEMICAL OR  
SUBSTANCE TO  
TEST?



WHAT  
EXPOSURE  
SYSTEM?



WHICH IN  
VITRO/EX VIVO  
SYSTEM?



WHAT KIND OF  
CELLS?



WHAT  
ENDPOINTS/  
READOUTS?



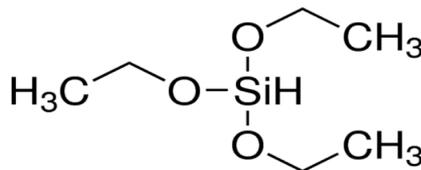


# Picking a chemical or substance to test?

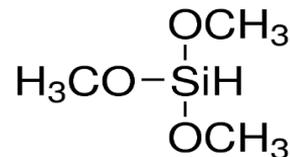
- Know your substance:
  - Physicochemical properties of the substance?
  - Locally metabolized?
  - *in vitro* or *in vivo* data available?
  - Known Adverse Outcome Pathways?

## Silanes

- Highly reactive
- Triethoxysilane more stable



Triethoxysilane  
(GHS 2, CAS # 998-30-1)



Trimethoxysilane  
(GHS 1, CAS# 2487-90-3)





# What exposure system to use?

## Pipetting

Easier to calculate exposure dose

No special equipment needed

May disturb surface lining fluid

Limited to (particles in) liquids

+

-

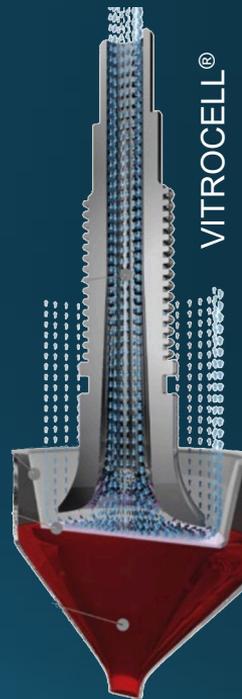
## ALI exposure

Physiologically relevant

Final formulation can be used

Special equipment needed

Monitoring exposure dose more challenging

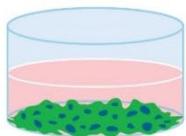




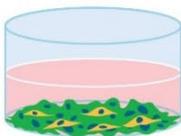
# Which *in vivo* / *ex vivo* system to use?

Do not allow for exposures at ALI

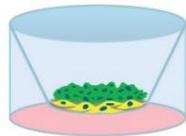
Allow for exposures at ALI



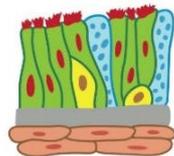
Submerged mono-cultures



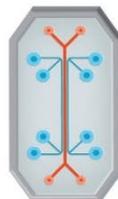
Submerged co-cultures



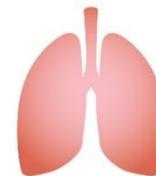
Co-cultures grown at the ALI



3D reconstructed human tissues grown at the ALI



Microfluidic human lung-on-a-chip



Human PCLS



Human *in vivo*

PHYSIOLOGICAL RELEVANCE

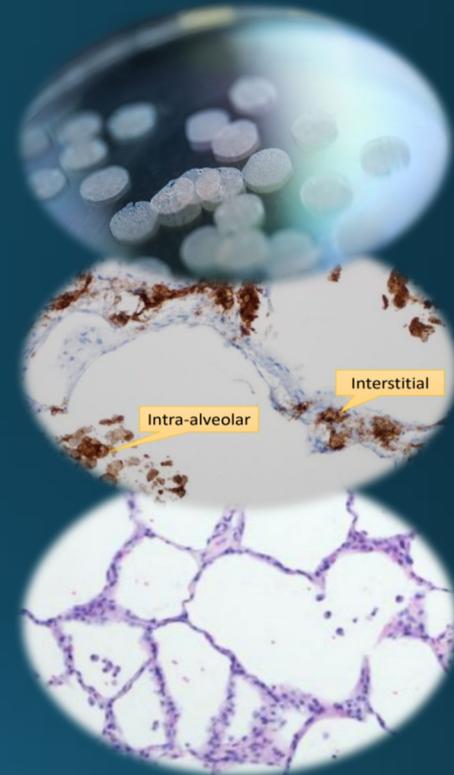
COMPLEXITY





# Human precision-cut lung slices

- PCLS from healthy and diseased donors
- All relevant cells and structures present
- Culture for 28+ days & cryopreservation possible
- Cross-section
- Multiple cell types may make readout more challenging
- Obtainment of (suitable) donor tissues





# Microphysiological Systems / Organs-on-chip

- Influencing microenvironment of tissues/cells
- Mechanical stretch or „blood“ flow possible
- Allow combination of different tissues (e.g., Lung-Liver)
- Various materials used → may influence dose (e.g. absorption)
- Choice of cell culture medium for multiple “organs”
- Standardisation and comparability difficult

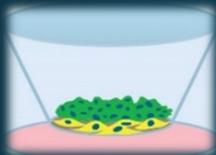


[www.thepsci.eu/chips](http://www.thepsci.eu/chips)





# 2D monocultures vs reconstructed tissues



## 2D monocultures

Simpler &  
less expensive

Higher throughput

Cell lines and their limitations

Often short-term ALI cultures

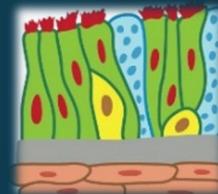


## 3D reconstructed tissues

Primary cells differentiated  
to *in situ*-like epithelium

ALI cultures for months

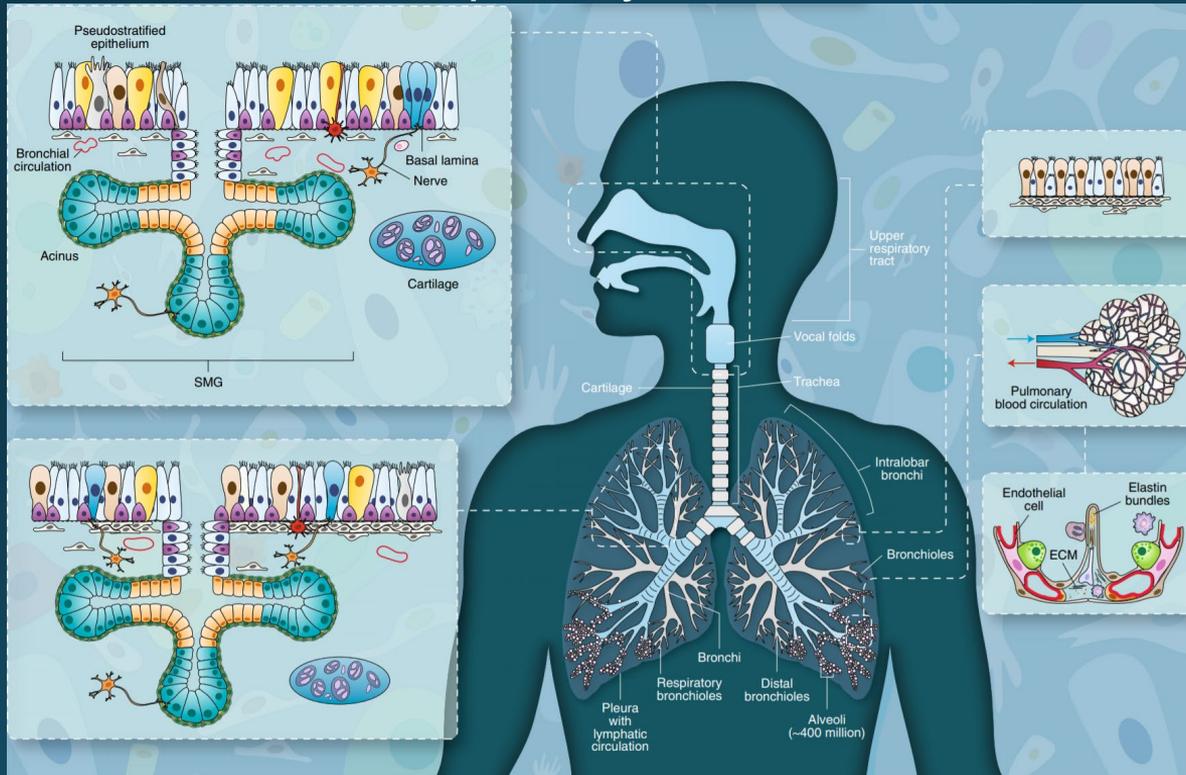
Generally more expensive





# What kind of cells to use?

## Respiratory tract



## Cell types

### Airways



**Basal cells:** TP63, KRT5, NGFR  
Function as multipotent stem cells



**Club cells:** SCGB1A1, SCGB3A2 (data from mice)  
Immunomodulatory functions



**Goblet cells:** MUC5AC, FOXA3, SPDEF  
Secrete mucins



**Ciliated cells:** FOXJ1,  $\beta$ -tubulin IV  
Remove mucus from the lung



**NE cells:** ASCL1, CALCA  
Act as sensory cells;  
communicate with neurons



**Rare cells**  
**Ionocytes:** FOXI1, CFTR high  
**Tuft (brush) cells:** TRPM5, GNG13

### Alveoli



**AEC2s:** SFTPC, DC-LAMP  
Stem cells; produce surfactant



**AEC1s:** PDPN, AGER  
Large surface area; facilitate  
gas exchange

### Immune cells



**Dendritic cells**



**Alveolar resident macrophages,  
interstitial macrophages**



**Basophils, eosinophils**



**Lymphoid cells (T and B cells)**

<https://www.nature.com/articles/s41556-019-0357-7.pdf>





# What endpoints/readouts to measure?

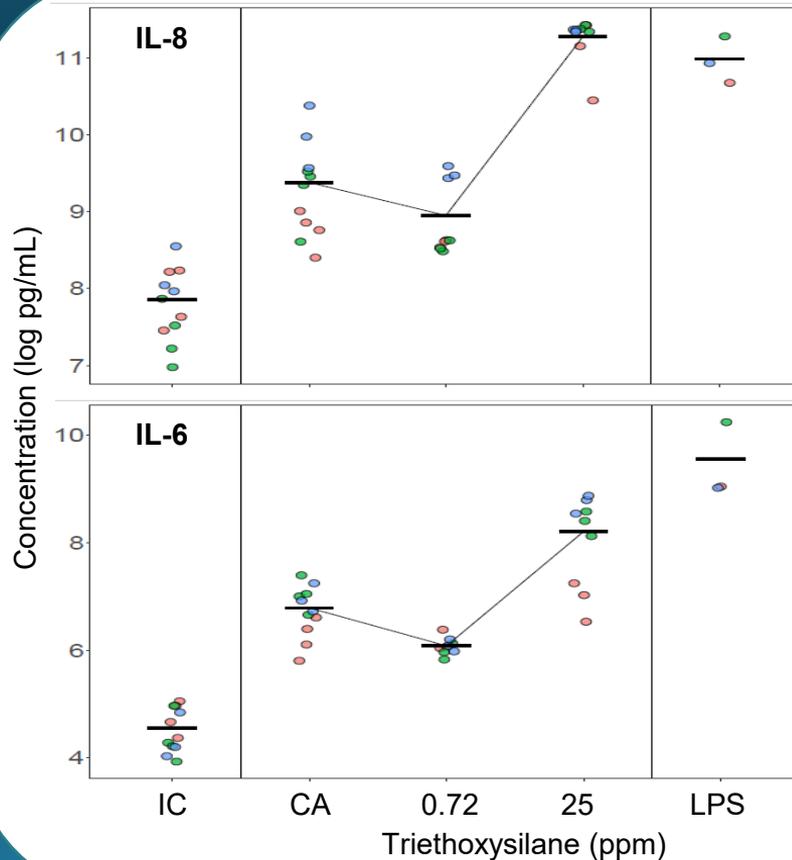
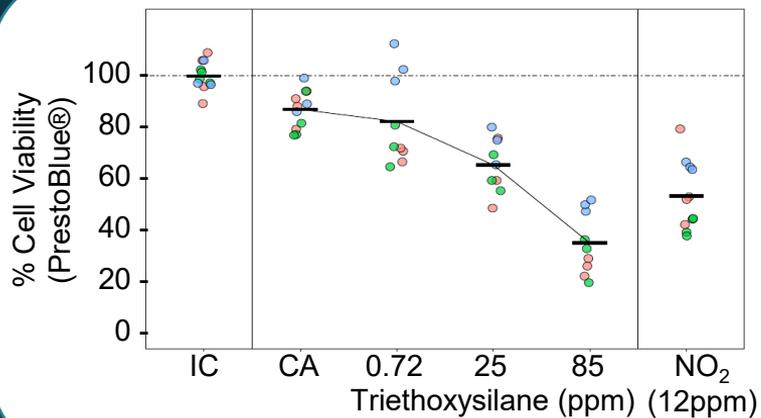
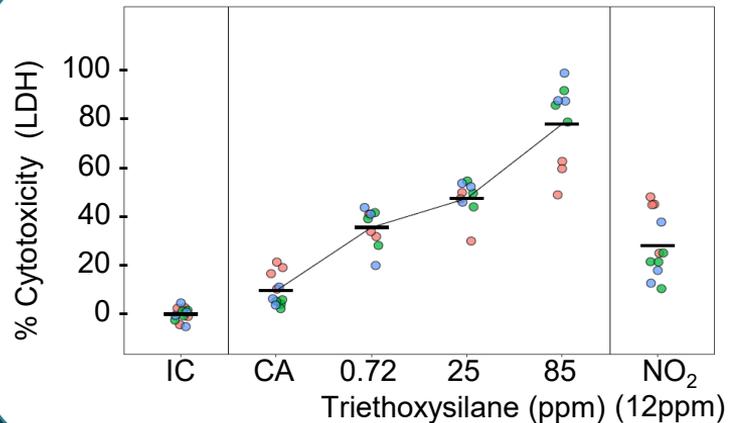


- Si content in cells and basal medium

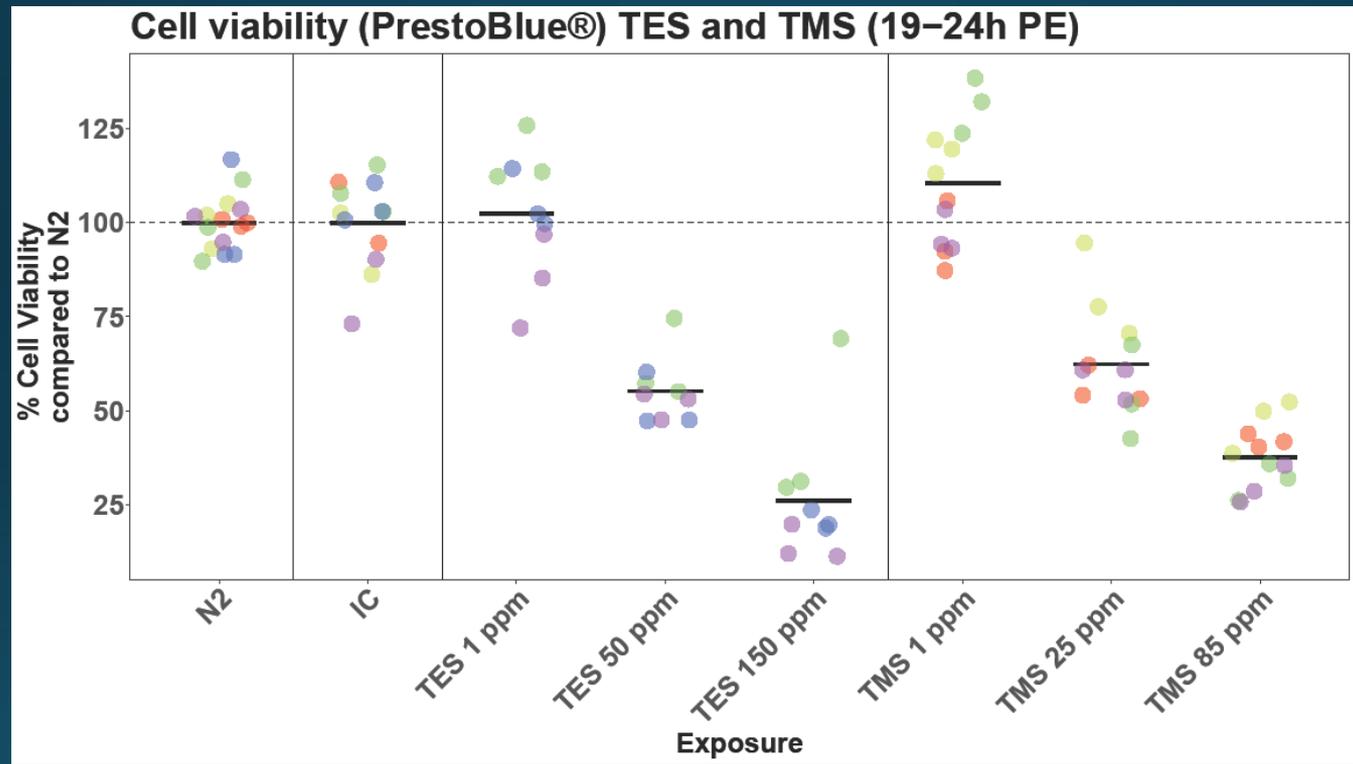
- Evident toxicity (cytotoxicity, cell viability, histology)
- Sub-toxic effects (inflammatory cytokines, cilia beating frequency)
- Trans-epithelial electrical resistance (TEER)



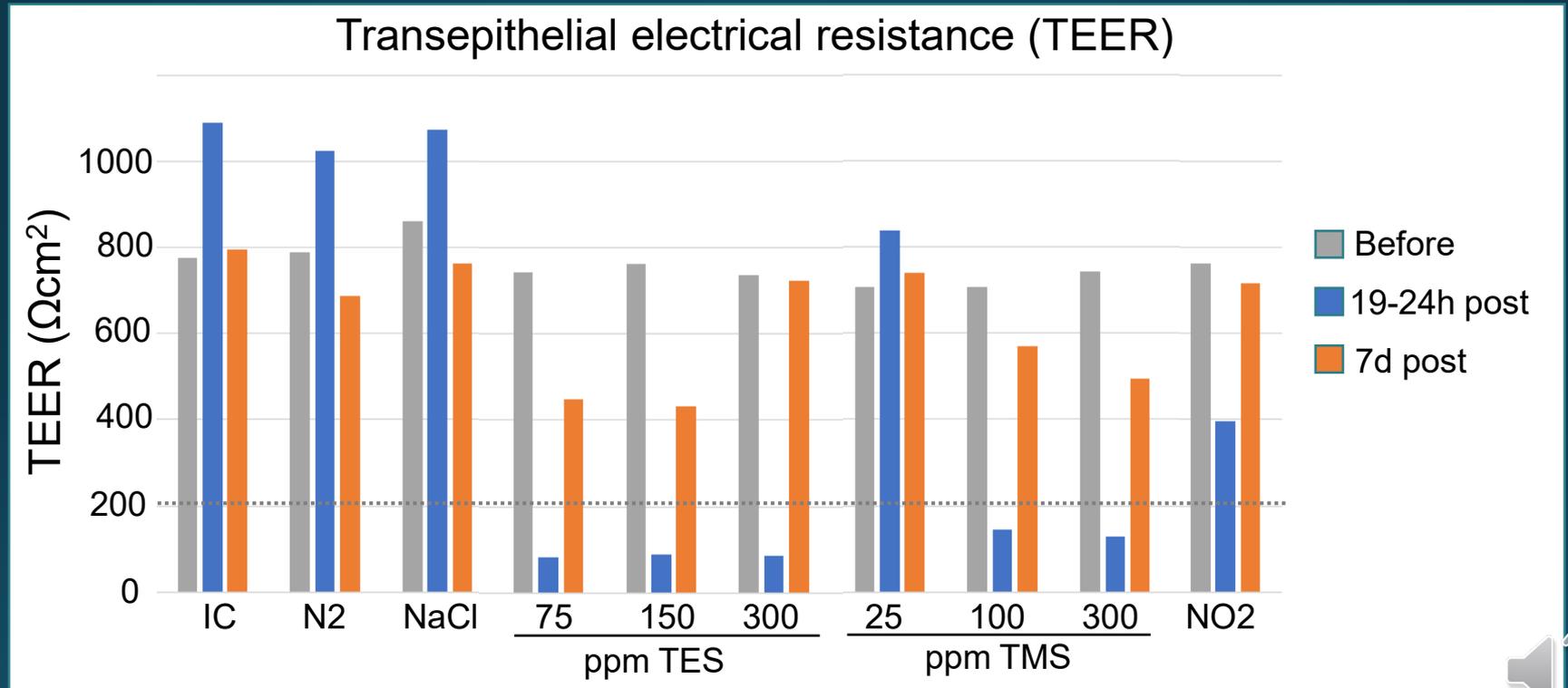
# Cell response to triethoxysilane exposure (60 min)



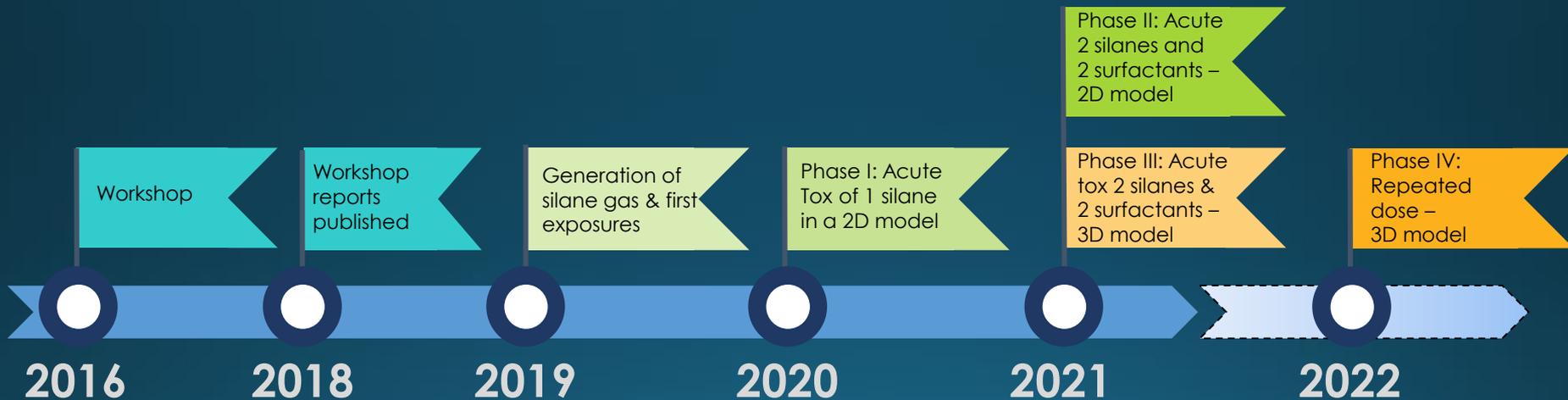
# 2D cell model response to silane exposure (30 min)



# Silane toxicity to the epithelial barrier in 3D model



# INSPIRE Initiative – Next steps



# Take home

- Developments in recent years allow for human-relevant exposures of lung cells or tissues
- No size fits all: depending on the substance to test – *in vitro* methods may need adaptation (e.g., addition of endpoints)
- Rather than only having one *in vitro* assay, a battery of assays may be needed to answer a specific question (e.g., OECD TG 497 – Defined approaches on skin sensitisation)
- Especially in combination with *in silico* models, *in vitro* models have the potential to replace inhalation testing in animals
  - Chlorothalonil human health draft risk assessment - 90-day subchronic rat inhalation study waived by EPA OPP based on *in silico* and *in vitro* methods using MucilAir® tissue model <https://www.regulations.gov/document/EPA-HQ-OPP-2011-0840-0080>
- Talk to regulators **early** in the development process to discuss your NAM strategy and to find out whether animal testing is even necessary



# Other inhalation related PSCI projects

## Generating an Alternative System to Predict Pulmonary Fibrosis (GASPP)

- MatTek EpiAlveolar commercially available
- Used in EU-Horizon 2020 project PATROLS

## Development of adverse outcome pathways (AOP)

- AOP 173: Substance interaction with the lung resident cell membrane components leading to lung fibrosis
- AOP 411: Oxidative stress leading to decreased lung function

## FBS and Animal-Component free Testing (FACT)

- A549 cells successfully transitioned to animal-free medium

## Precision-cut lung slices (PCLS)

- Study on cryopreservation ongoing

## Awards

- 3D Tissues with MatTek and Epithelix
- CellTox Sampler with MedTec Bio
- VITROCELL inhalation exposure systems
- Travel Grants

## Webinars and Workshops

- Several inhalation related webinars in 2016, 2018, 2020 and 2021
- <https://www.theptsci.eu/inhalation-webinars/>

Please visit [www.theptsci.eu/our-work/inhalation/](https://www.theptsci.eu/our-work/inhalation/)



# Selected resources for respiratory *in vitro* methods

- <https://www.thepsci.eu/inhalation-publications/>
- Petersen EJ, Sharma M, Clippinger AJ, Gordon J, Katz A, Laux P, Leibrock LB, Luch A, Matheson J, Stucki AO, Tentschert J, Bierkandt FS. Use of Cause-and-Effect Analysis to Optimize the Reliability of In Vitro Inhalation Toxicity Measurements Using an Air-Liquid Interface. *Chem Res Toxicol.* 2021;34:1370–1385
- Welch J, Wallace J, Lansley AB, Roper C. Evaluation of the toxicity of sodium dodecyl sulphate (SDS) in the MucilAir™ human airway model *in vitro*. *Regul Toxicol Pharmacol.* 2021. Epub ahead of print.
- Hargrove MM, Dobrzanski BP, Li L, Constant S, Wallace J, Hinderliter P, Wolf DC, Charlton A. Use of the MucilAir Airway Assay, a New Approach Methodology, for Evaluating the Safety and Inhalation Risk of Agrochemicals. *Appl In Vitro Toxicol.* 2021;7(2):50-60.
- Marescotti D, Serchi T, Luettich K, Xiang Y, Moschini E, Talikka M, Martin F, Baumer K, Dulize R, Peric D, Bornand D, Guedj E, Sewer A, Cambier S, Contal S, Chary A, Gutleb AC, Frentzel S, Ivanov NV, Peitsch MC, Hoeng J. How complex should an *in vitro* model be? Evaluation of complex 3D alveolar model with transcriptomic data and computational biological network models. *ALTEX.* 2019; 36(3):388-402.
- Behrsing H, Hill E, Raabe H, Tice R, Fitzpatrick S, Devlin R, Pinkerton K, Oberdörster G, Wright C, Wieczorek R, Aufderheide M, Steiner S, Krebs T, Asgharian B, Corley R, Oldham M, Adamson J, Li X, Rahman I, Grego S, Chu PH, McCullough S, Curren R. *In vitro* exposure systems and dosimetry assessment tools for inhaled tobacco products: Workshop proceedings, conclusions and paths forward for *in vitro* model use. *Altern Lab Anim.* 2017;45(3):117-158.
- Behrsing H, Raabe H, Manuppello J, Bombick B, Curren R, Sullivan K, Sethi S, Phipps R, Tesfaigzi Y, Yan S, D’Ruiz C, Tarran R, Constant S, Phillips G, Gaça M, Hayden P, Cao X, Mathis C, Hoeng J, Braun A, Hill E. Assessment of *in vitro* COPD models for tobacco regulatory science: Workshop proceedings, conclusions and paths forward for *in vitro* model use. *Altern Lab Anim.* 2016;44(2):129-166.
- Clippinger AJ, Allen D, Behrsing H, BéruBé KA, Bolger MB, Casey W, DeLorme M, Gaça M, Gehen SC, Glover K, Hayden P, Hinderliter P, Hotchkiss JA, Iskandar A, Keyser B, Luettich K, Ma-Hock L, Maione A, Makena P, Melbourne J, Milchak L, Ng S, Paini A, Page K, Patlewicz G, Prieto P, Raabe H, Reinke E, Roper C, Rose J, Sharma M, Spoo W, Thorne PA, Wilson DM, Jarabek AM. Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity. *Toxicol In Vitro.* 2018;52:131-145.



## Selected resources for *in vitro* methods in general

- PETA Science International Consortium <https://www.thepsci.eu/>
- NICEATM <https://ntp.niehs.nih.gov/whatwestudy/niceatm/>
- EPA's List of NAMs: <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce>
- EURL ECVAM <https://ec.europa.eu/jrc/en/eurl/ecvam>
- Tracking System for Alternative methods towards Regulatory acceptance (TSAR) <https://tsar.jrc.ec.europa.eu/>
- Frontiers in *in vitro* Toxicology Research Topic on Chemical Testing Using NAMs <https://www.frontiersin.org/research-topics/19075/>
- Non-Animal Technologies (NAT) Database: <https://nat-database.org/>
- AOP wiki: <https://aopwiki.org>



# Thank you!

Andreas Stucki, Ph.D.  
AndreasS@thepsci.eu

PETA Science Consortium International e.V.  
[www.thePSCI.eu](http://www.thePSCI.eu)

 @thePSCI



# IN SILICO TOXICOLOGY AS A NEW APPROACH METHODOLOGY IN TOBACCO REGULATORY SCIENCE

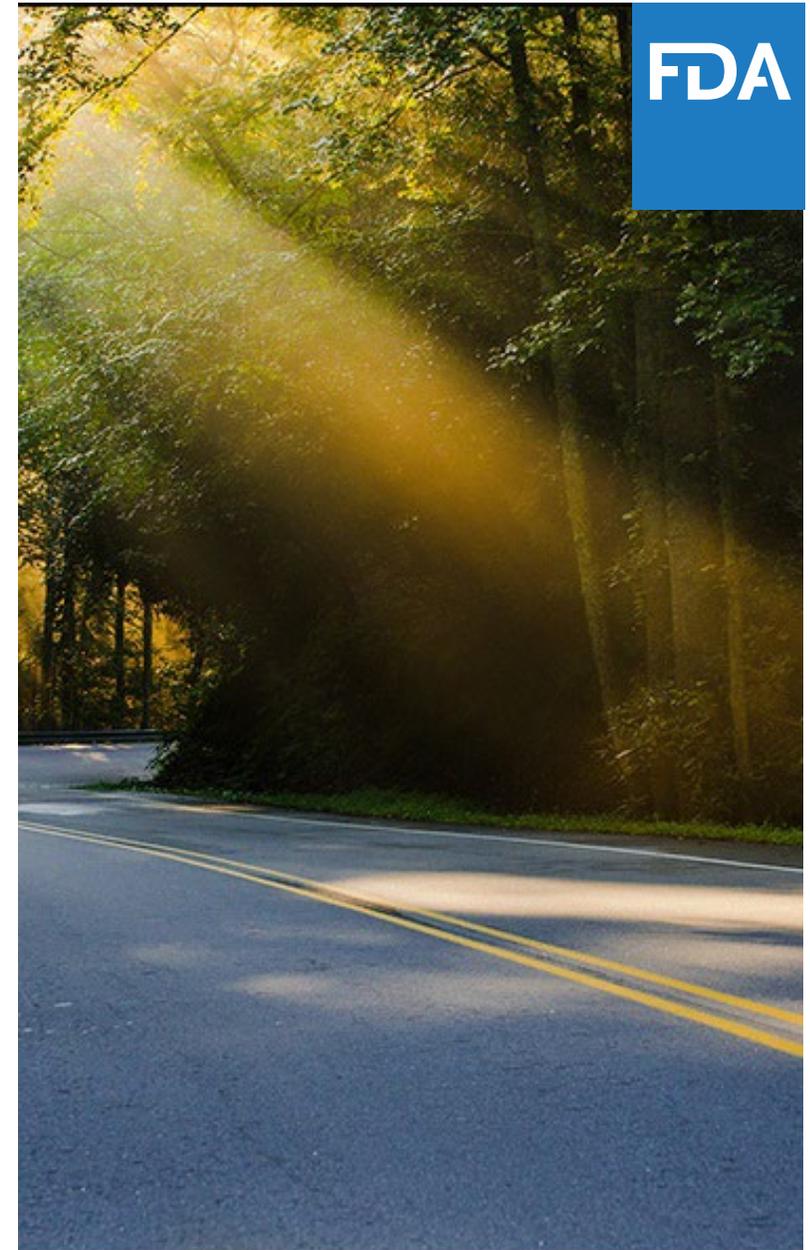
*Luis G. Valerio, Jr., Ph.D., ATS  
Associate Director  
Division of Nonclinical Science  
Office of Science | Center for Tobacco Products*



*Disclaimer: This is not a formal dissemination of information by FDA and does not represent Agency position or policy.*

# AGENDA

- In silico toxicology
  - Capabilities
  - Challenges
- Proof of concept applied regulatory research
  - Hazard identification
    - Screening for toxicity
    - Structural alerts
    - Validation testing of models
    - Chemical similarity analysis



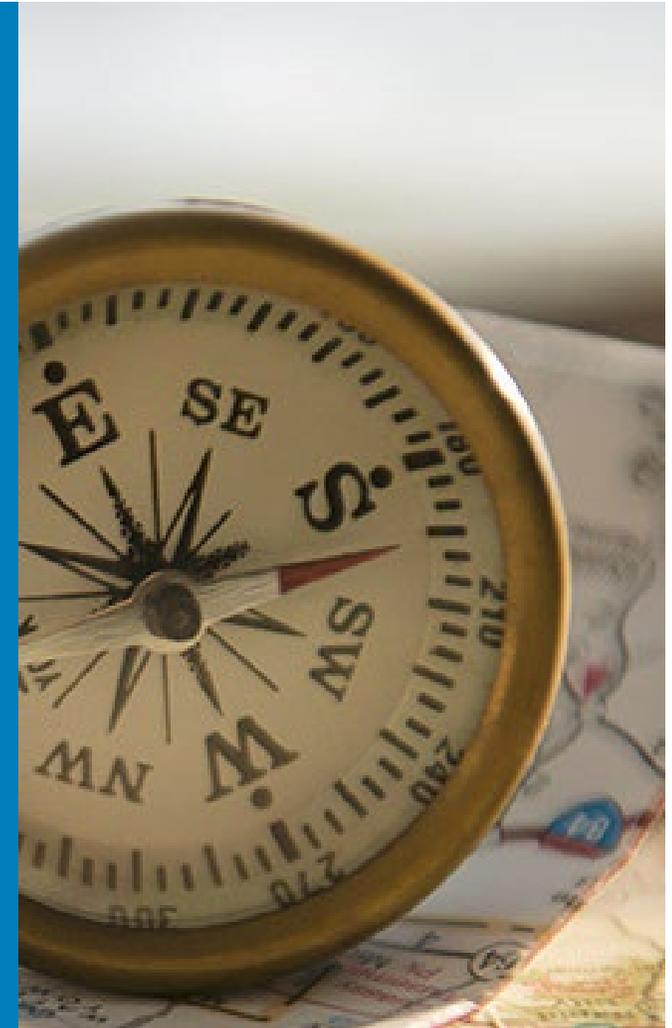
# COMPUTATIONAL SCIENCE



- Predictions for health and safety
- Ensemble of models - consensus
- Data mining for continuous updating

# ADVANTAGES OF IN SILICO TOXICOLOGY

- Rapid and cost-effective
- Maximize resources
- 3Rs principals –reduction, refinement and replacement
  - Ethical and humane
- May strengthen or serve as complementary evidence
- Analyze for non-traditional toxicity alerts
- Enabler for consensus approaches
- Strategy to support prioritization for follow-up
- Pattern recognition
- Does not require synthesis of compound
- Predict hazard and provide mechanistic insight
- Data visualization tool

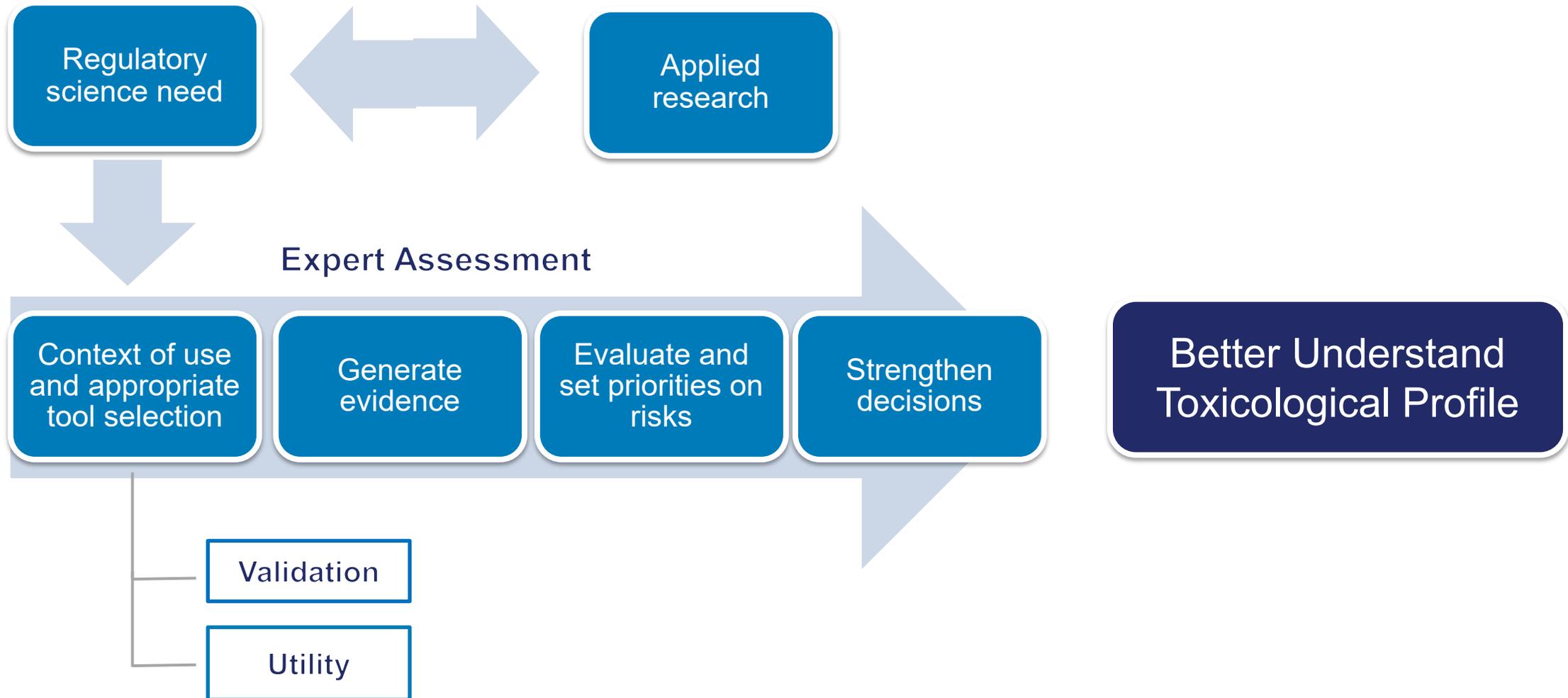


# CHALLENGES OF IN SILICO TOXICOLOGY

- Interpretation by expert assessment is important
- Explain predictions
- Selection of technique appropriate for intended use
- Appropriate use of predictive modeling data – in/out of context
- Model selection, updating, and domain space
- Data quality
- Validation and performance
  - method sensitivity
  - prospective validation rare
  - retrospective validation common but what is considered ‘good’ performance

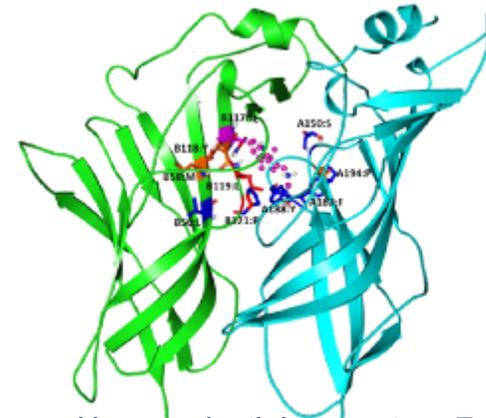


# CONCEPT OF COMPUTATIONAL TOXICOLOGY IN TOBACCO REGULATORY SCIENCE

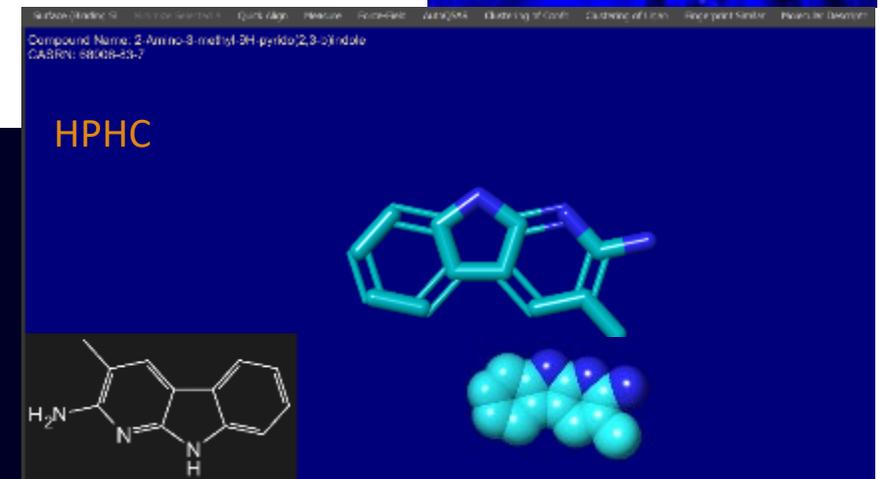
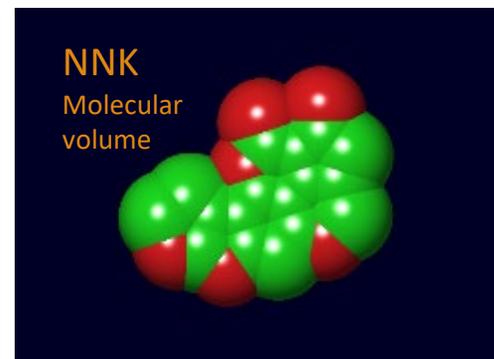
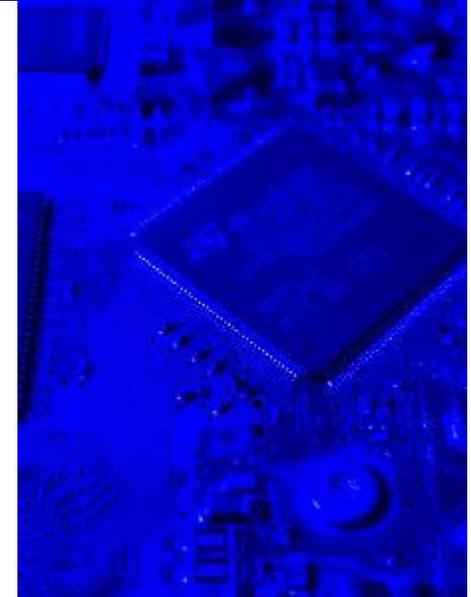


# IN SILICO TOXICOLOGY METHODS ARE VERSATILE

- Screen for hazard identification
- Similarity analysis
- Support read-across/bridging
- Rapid detection of promiscuous compounds
  - molecular filtering
- Predict toxicities based on computational models
  - organ toxicity, DNA damage, endpoints unethical to test in humans
- Toxicokinetics
- Physical chemical properties
- Uncover structural alerts
- Mechanistic information
- Mine for toxicity data



Human nicotinic receptor  $\alpha 7$



# IN SILICO TOXICOLOGY METHODS ARE RAPID

- > 3D Minimization
- Physicochemical Descriptors
- Topological Descriptors
- Ligfilter Descriptors
- QikProp Descriptors
- Feature Selection
- ▼ **Binary Fingerprints (1 new)**
  - fp\_radial\_1 Incorporated
  - fp\_dendritic\_2 Finished**
- 3D Pharmacophore Fingerprints
- Similarity/Distance Screen
- Shape Screen
- Diversity-Based Selection
- Hole-Filling and Library Optimization
- Library Comparison
- Hierarchical Clustering
- Leader Follower Clustering
- K-Means Clustering
- Self-Organizing Map (Properties)
- Self-Organizing Map (Fingerprints)
- Principal Components Analysis
- > Maximum Common Substructure
- Bayes Classification
- Multiple Linear Regression
- Partial Least-Squares Regression
- Kernel-Based PLS Regression
- Principal Components Regression
- Neural Networks
- Recursive Partitioning
- ▼ Scaffold Decomposition
  - scaffold\_01 Finished
- > Views
- Partitions
- Structure Filters
- Property Filters
- > Substructure Queries

CASRN: 706-14-9

Row	In	Title
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138	<input type="radio"/>	Ethyl lactate
139	<input type="radio"/>	Isomyl isovalerate
140	<input type="radio"/>	2,5-Dimethylpyrazine
141	<input type="radio"/>	Chromic acid methyl est.
142	<input type="radio"/>	?-Pinene
143	<input type="radio"/>	?-Terpinene
144	<input type="radio"/>	Ethyl caprylate
145	<input type="radio"/>	Ethyl phenylacetate

STRUCTURE HIERARCHY

Current Selection

- gamma-Decalactone

Entries: 153 total, 1 selected, 1 included

3000 compound data set

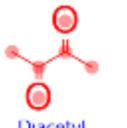
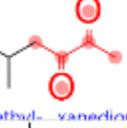
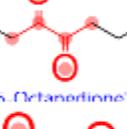
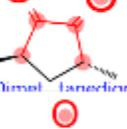
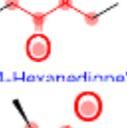
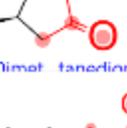
CPU time = 0.09 sec

canvasFPCombine successfully completed.

Driver script for parent canvasFPGen job fp\_dendritic\_2 finished  
Current time: Wed Sep 1 08:29:28 2021  
Elapsed time = 00:00:13

SELECTED 0 atoms 0 residues ATOMS 12 CHAINS 0 ENTRIES 1  
DISPLAYED 12 of 12 1 of 1 RESIDUES 1 MOLS 1 CHARGE 0

# IN SILICO TOXICOLOGY DATA VISUALIZATION

	Structure	NAME
1		Diacetyl
2		"5-Methyl-2,3-hexanedione"
3		"4-Methyl-2,3-pentanedione"
4		"4,5-Octanedione"
5		"3,5-Dimethyl-1,2-cyclopentanedione"
6		"3,4-Hexanedione"
7		"3,4-Dimethyl-1,2-cyclopentanedione"
8		"2,3-Undecanedione"

← **Molecular filtering**  
screen toxic substructures

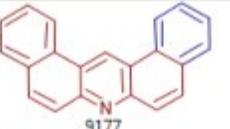
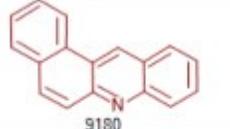
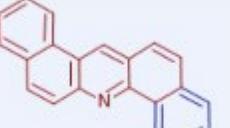
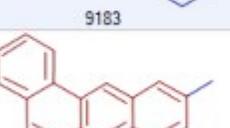
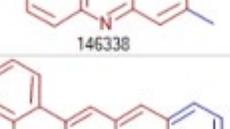
**Scaffold generation** →  
decipher alerts

Show All Filter: (none)

Scaffold Tree 2847

- Scaffold Tree\_001 514
  - Scaffold Tree\_001\_01 95
    - Scaffold Tree\_001\_01\_1 22
      - Scaffold Tree\_001\_01\_1\_13 13
        - Scaffold Tree\_001\_01\_1\_1\_2 6
          - Orphans(Scaffold Tree\_001\_01\_1)

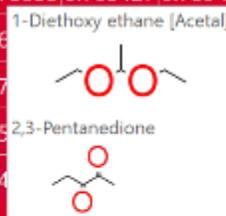
Data Table 1

	Structure	canvasUID
1		834
2		836
3		838
4		2076
5		2803
6		2856

# IN SILICO TOXICOLOGY STRUCTURE READ-ACROSS

## Similarity

1-...]	1	0.875537	0.818778	0.755556	0.809524	0.510101	0.643312	0.775533	0.765427	0.789431
1,...ol	0.875537	1	0.912711	0.749155	0.860696	0.534392	0.682432	0.765427	0.789431	0.8226
(3...ol	0.818778	0.912711	1	0.804455	0.853716	0.604079	0.647436	0.775533	0.765427	0.822581
(R...al	0.755556	0.749155	0.804455	1	0.810502	0.661465	0.68775	0.75297	0.742839	0.784341
1,...ol	0.809524	0.860696	0.853716	0.810502	1	0.485577	0.60479	0.742839	0.783772	0.84715
2...e	0.510101	0.534392	0.604079	0.661465	0.485577	1	0.693574	0.783772	0.736628	0.727496
2,...ne	0.643312	0.682432	0.647436	0.68775	0.60479	0.693574	1	0.736628	0.590643	0.749155
2,...ne	0.775533	0.765235	0.778962	0.75297	0.742839	0.783772	0.736628	1	0.764045	0.881154
2,...al	0.765427	0.796569	0.893217	0.801567	0.829047	0.622182	0.590643	0.764045	1	0.874072
2...e	0.789431	0.8226	0.822581	0.784341	0.84715	0.727496	0.749155	0.881154	0.874072	1

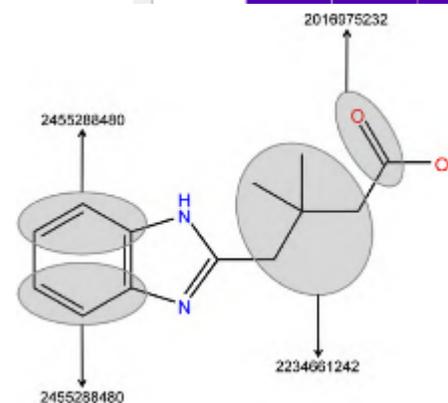


## Distance

1-...]	0	2.23607	2.64575	3.31662	3	3.87298	2.82843	3	3.31662	2.82843
1,...ol	2.23607	0	2.44949	3.16228	2.82843	3.74166	2.64575	2.82843	3.16228	2.64575
(3...ol	2.64575	2.44949	0	3.4641	3.16228	4	3	3.16228	3.16228	3
(R...al	3.3	3.16228	3.4641	0	3.4641	4.47214	3.60555	3.74166	4	3.60555
1,...ol	3	2.82843	3.4641	0	4.24264	3.31662	3.4641	3.4641	3	3
2...e	3.8	3.74166	4.47214	4.24264	0	4.12311	4.24264	4.47214	4.12311	4.12311
2,...ne	2.8	2.64575	3.60555	3.31662	4.12311	0	3.31662	3.60555	3.16228	3.16228
2,...ne	3	2.82843	3.16228	3.74166	3.4641	4.24264	3.31662	0	3.74166	3.31662
2,...al	3.31662	3.16228	3.16228	4	3.4641	4.47214	3.60555	3.74166	0	3
2...e	2.82843	2.64575	3	3.60555	3	4.12311	3.16228	3.31662	3	0

## Dendritic fingerprint

To encode both linear and branched fragments, linear paths are augmented with intersections of linear paths, with a maximum 5 bonds per path.



Duan et al. 2010. J Mol Graphics Modeling. 29:157-170

# IN SILICO ASSESSMENT OF CHEMICAL SIMILARITY

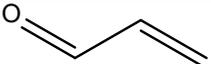
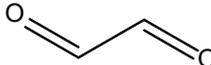
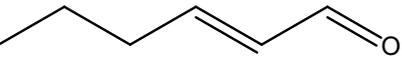
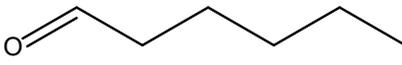
## Advantages:

- ✓ Science-based
- ✓ Transparent
- ✓ Mech. hypothesis
- ✓ Calculate PCPs
- ✓ Abundant molecular descriptors, metrics, and atom typing

Read-Across

## Similarity coefficient

### Buser metric, 2D Linear Chemical fingerprints

 Acrolein	1.0
 Glyoxal	0.9786
 Methylglyoxal	0.9120
 <i>trans</i> -2-Hexanal	0.9050
 Hexanal	0.8757

Data rich

Higher

Confidence

Lower

Buser metric:

$$(\sqrt{cd}+c)/\sqrt{cd+a+b-c}$$

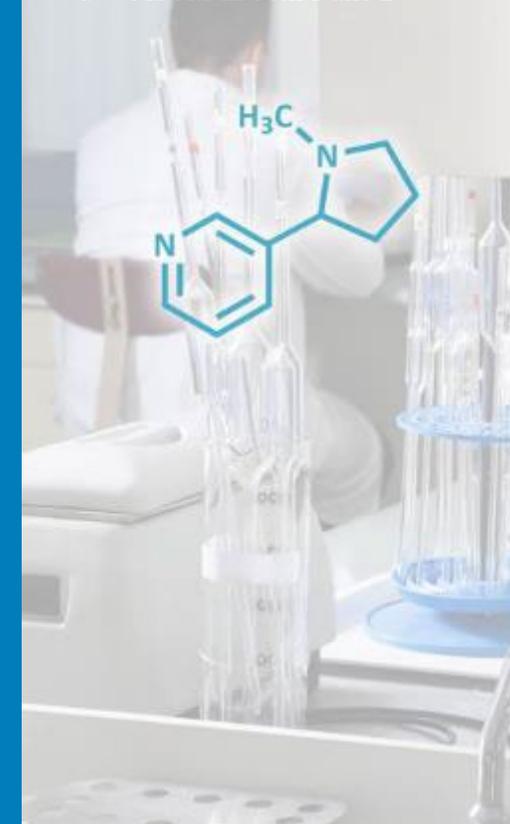
Formula key:

a = On bits in structure 1

b = On bits in structure 2

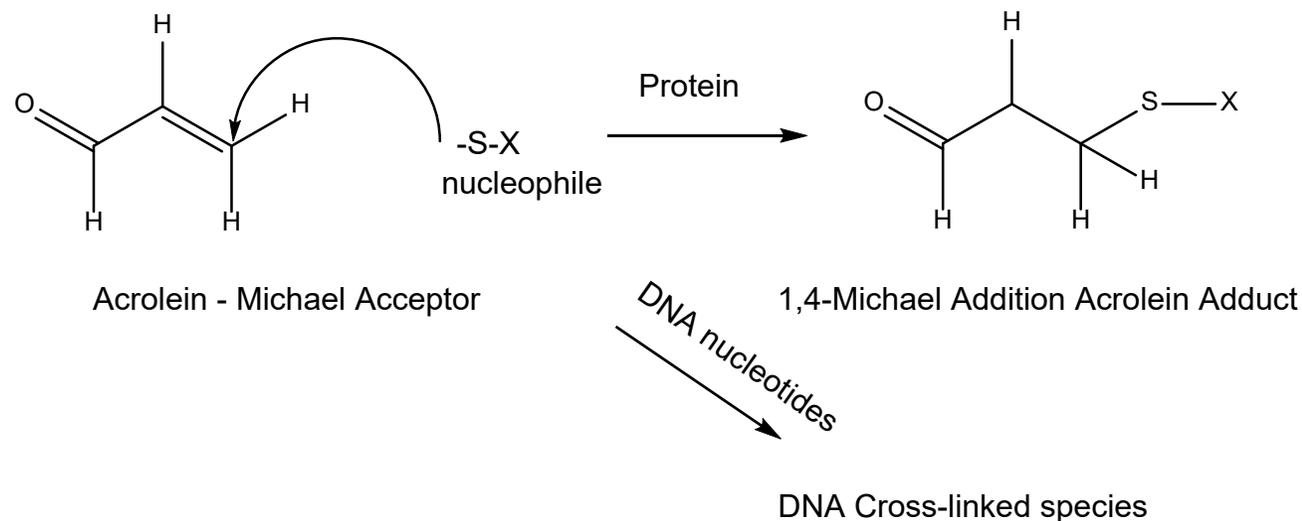
c = On bits in both 1 and 2

d = Off bits in both 1 and 2



## Michael Acceptors

“Soft” electrophiles that can alkylate nucleophilic sites on proteins or nucleic acids on DNA forming covalent adducts

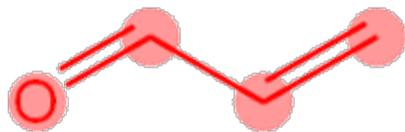


## Other Factors

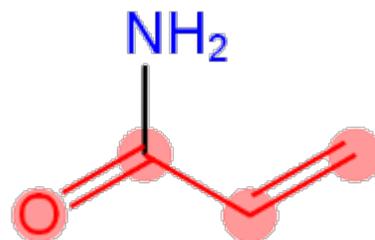
- Good leaving group
- Presence of electron withdrawing groups

Ou et al. 2020. J. Agric. Food Chem. 68(18):5039-5048.  
Cai et al. 2009. Chem. Res. Toxicol. 22(4):708-716.  
Kozekov et al. 2003. JACS. 125(1):50-61.

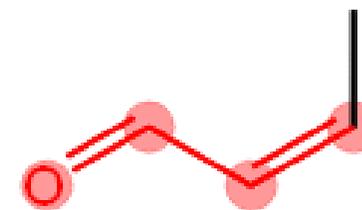
**Michael Acceptors** SMARTS: [#6]=[#6][#6,#16]=[O]



Acrolein (107-02-8)

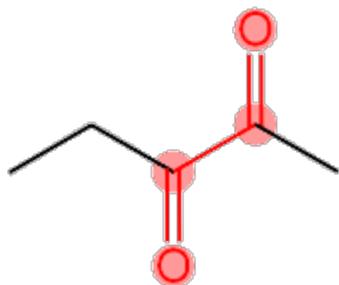


Acrylamide (79-06-1)

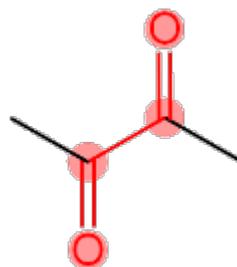


Crotonaldehyde (123-73-9)

**Dicarbonyl** SMARTS: [C;X3](=O)([C;X3](=O))



Pentanedione (600-14-6)



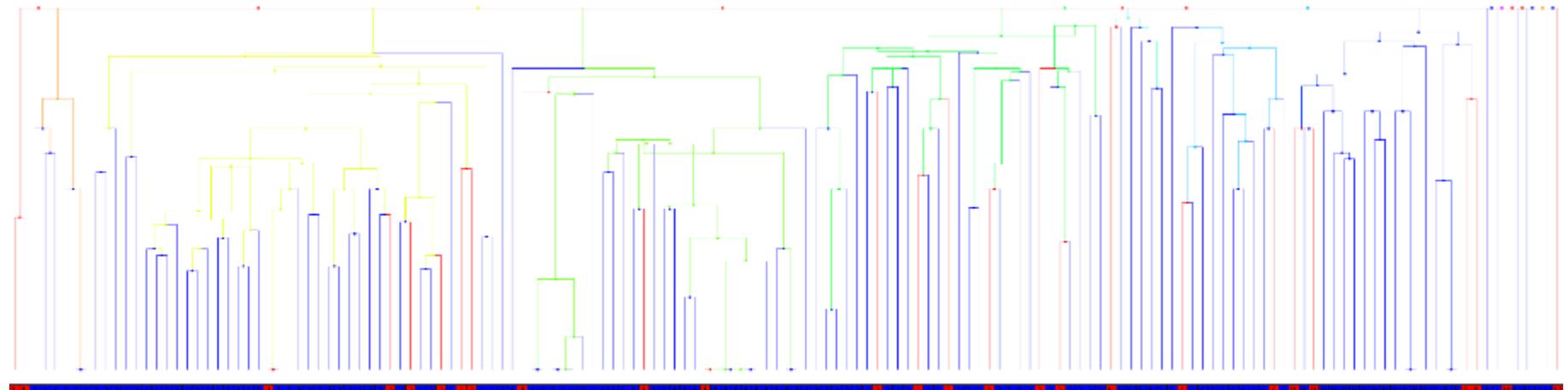
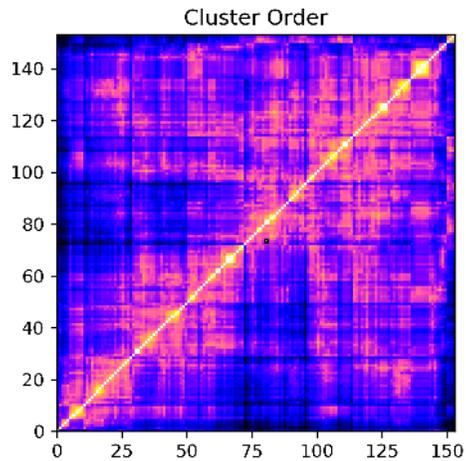
Diacetyl (431-03-8)

SMARTS: SMiles ARbitrary Target Specification

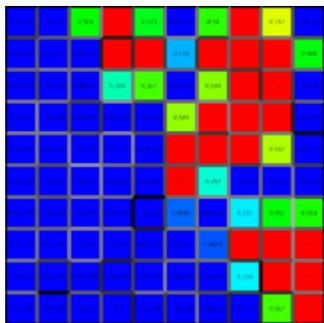


## Hierarchical Clustering Dendrogram

By property for in vitro DNA damage



Self Organizing Map



Property	Display	Min	Max
DNA Damage ...	<input checked="" type="checkbox"/>	0	1



Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: [www.elsevier.com/locate/taap](http://www.elsevier.com/locate/taap)



Received: 19 February 2020 | Revised: 12 May 2020 | Accepted: 16 May 2020  
DOI: 10.1002/jat.4020

## RESEARCH ARTICLE

Journal of  
**Applied Toxicology** WILEY

Investigating DNA adduct formation by flavor chemicals and tobacco byproducts in electronic nicotine delivery system (ENDS) using *in silico* approaches

Jueichuan (Connie) Kang<sup>a,b,\*</sup>, Luis G. Valerio Jr.<sup>a</sup>

<sup>a</sup> United States Food and Drug Administration, Center for Tobacco Products, Office of Science, Division of Nonclinical Science, 11785 Beltsville Drive, Calverton, MD 20705, USA

<sup>b</sup> US Public Health Service Commissioned Corps, Rockville, MD, USA



## In vitro and in silico genetic toxicity screening of flavor compounds and other ingredients in tobacco products with emphasis on ENDS

Pei-Hsuan Hung<sup>1</sup> | Matthew Savidge<sup>1</sup> | Mamata De<sup>1</sup> |  
Jueichuan (Connie) Kang<sup>1,2</sup> | Sheila M. Healy<sup>1</sup> | Luis G. Valerio Jr.<sup>1</sup>

**JCIM**  
JOURNAL OF  
CHEMICAL INFORMATION  
AND MODELING

[pubs.acs.org/jcim](http://pubs.acs.org/jcim)

Article

## Development of a Nicotinic Acetylcholine Receptor nAChR $\alpha 7$ Binding Activity Prediction Model

Sugunadevi Sakkiah, Carmine Leggett,\* Bohu Pan, Wenjing Guo, Luis G. Valerio, Jr., and Huixiao Hong\*

Cite This: *J. Chem. Inf. Model.* 2020, 60, 2396–2404

Read Online

TOXICOLOGY MECHANISMS AND METHODS  
<https://doi.org/10.1080/15376516.2020.1805836>

Taylor & Francis  
Taylor & Francis Group

Check for updates

## ORIGINAL ARTICLE

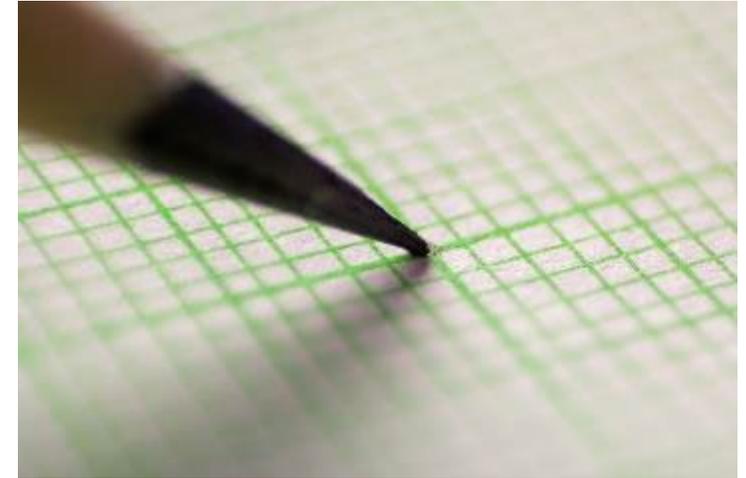
## Predicting the mutagenic potential of chemicals in tobacco products using *in silico* toxicology tools

Reema Goel and Luis G. Valerio, Jr.

United States Food and Drug Administration, Division of Nonclinical Science, Office of Science, Center for Tobacco Products, Calverton, MD, USA

# PROJECT-BASED ASSESSMENTS

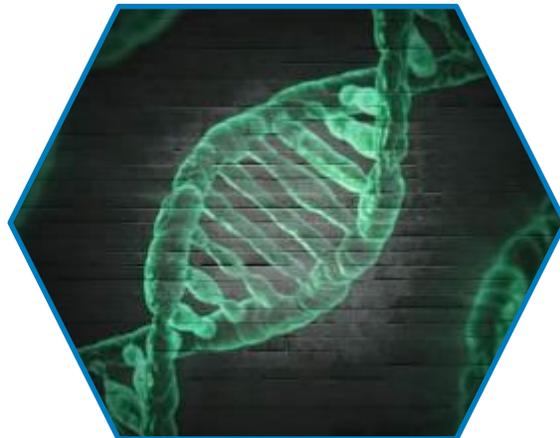
- Designed to assess utility and a proof of concept
  - Machine learning techniques
  - Endpoint
  - Performance
  - Applicability



Constituents



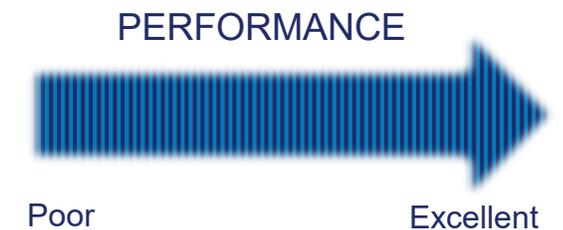
Endpoint



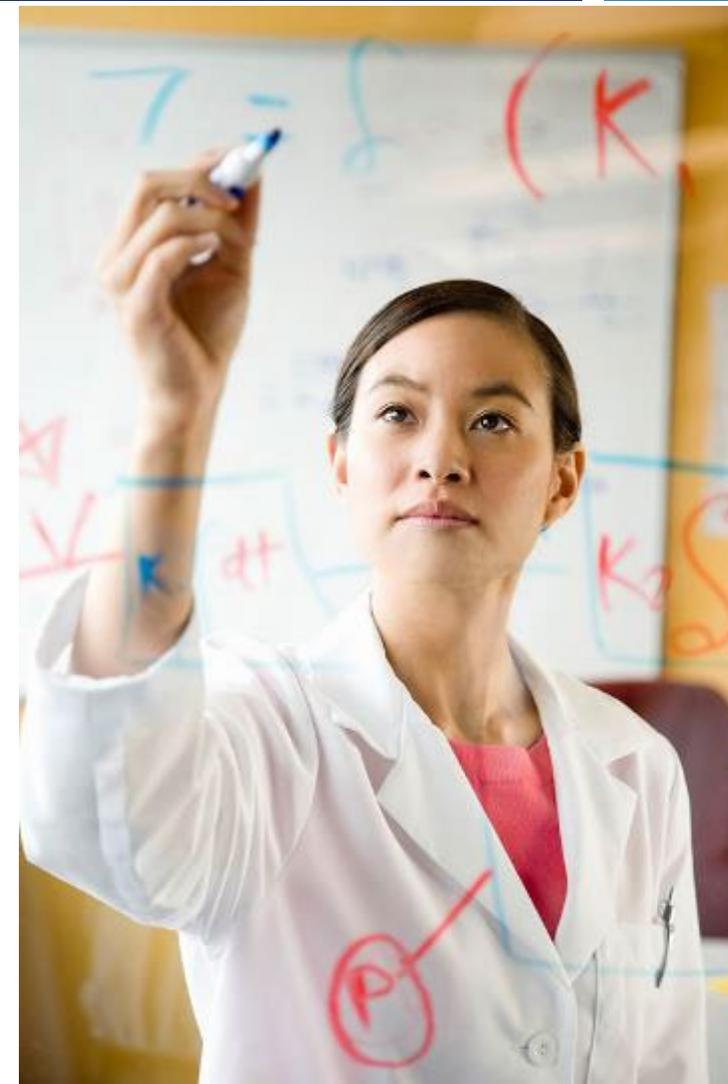
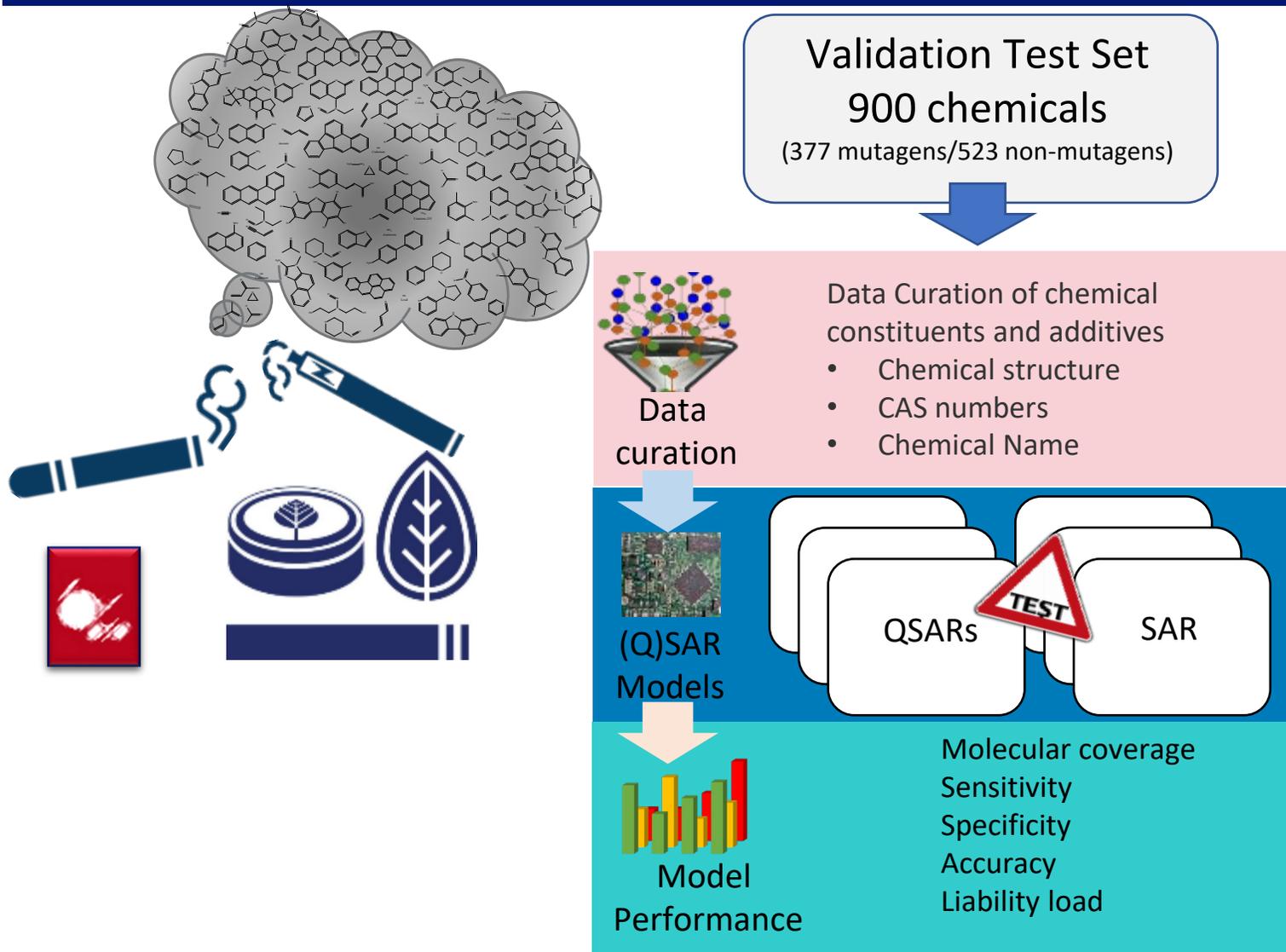
Computational



Evaluation



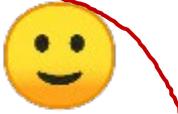
# PREDICTING MUTAGENIC POTENTIAL OF CHEMICALS FROM TOBACCO PRODUCTS



# PREDICTIVE PERFORMANCE OF THE (Q)SAR MODELS

## Ames test for mutagenicity

- ✓ Predicted tobacco chemicals
- ✓ SAR and QSAR models performed similarly
- ✓ Combination of SAR/QSAR did not enhance performance
- ✓ Average Accuracy 89%

		Predicted	
Actual		Non-Toxic	Toxic
Non-Toxic	 Specificity	 Type I Error (False +)	
Toxic	 Type II Error (False -)	 Sensitivity	

<i>In Silico</i> Model	Molecular Coverage	Sensitivity	Specificity	Accuracy	Liability Load
Model 1 (QSAR)	99.89%	77.5%	72.8%	74.7%	48.33%
Model 2 (QSAR)	98.56%	92.0%	96.9%	94.8%	41.67%
Model 3 (QSAR)	94.44%	93.8%	93.2%	93.4%	47.56%
Model 4 (SAR)	99.00%	91.8%	96.9%	94.7%	41.11%
Model 5 (QSAR+SAR)	91.44%	66.2%	92.3%	81.5%	37.67%
Model 6 (QSAR+SAR)	98.22%	92.0%	97.2%	95.0%	41.78%

TP = True Positive; FP = False Positive; TN = True Negative; FN = False Negative

# NEW APPROACH METHODOLOGIES IN PARALLEL

## Tested 150 Chemical flavors

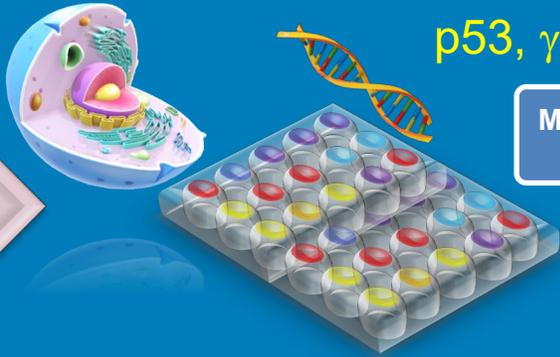
**In Vitro**

• MultiFlow<sup>®</sup> DNA Damage Assay

**In Silico**

• SAR/QSAR Computer Models

Biomarkers relevant to DNA Damage Response Pathways  
p53,  $\gamma$ H2AX, phospho-histone-H3



Machine Learning

Random Forest,  
Artificial Neural  
Networks,  
Logistic Regression

**PREDICTIONS**

**Mode  
of  
Action**

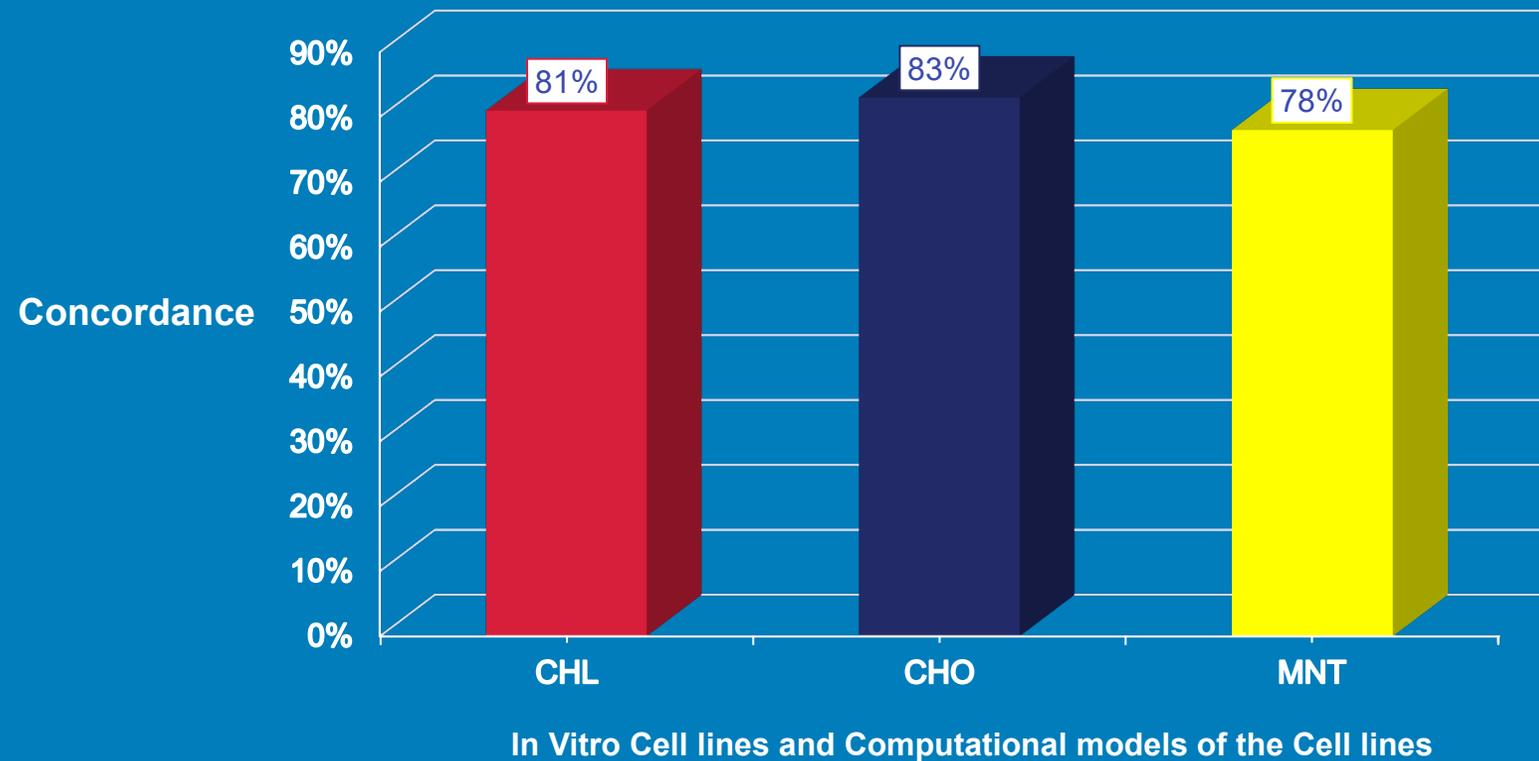
**Chemical Hazard ID  
Risk Prioritization**

Hung et al. 2020. J Appl Toxicol. 40(11):1566-1587

\*Contract: HHSF2232015100091

# AGREEMENT BETWEEN IN VITRO AND IN SILICO RESULTS

## Clastogenicity Predictions for 150 Flavors



# CONTRIBUTIONS OF THIS APPLIED RESEARCH

- First validation study using QSAR/SAR computational models for predicting mutagenic potential of tobacco chemicals
- Evaluated utility of in vitro and in silico screening technologies for assessing DNA damage by chemically defined flavors
- Assessed use of in silico molecular filters/substructure searching as a screening tool for chemically reactive moieties that portend to respiratory toxicity and DNA modification
- Evaluated use of chemical fingerprints for similarity analysis for potential application to support read-across of compound structures

# ACKNOWLEDGEMENTS



Reema Goel, PhD

Connie Kang, PhD

Hans Rosenfeldt, PhD

Todd Cecil, PhD

Office of Science

Division of Regulatory Science and Informatics

# Application of Mechanistic Data in Risk Assessment: Exposure Alignment and Evidence Integration

Annie M. Jarabek  
Senior Science Advisor

*Advancing New Approach Methods for Tobacco Harm Reduction*  
**Virtual Symposium**

**CORESTA Smoke Science and Product Technology Conference**

October 19, 2021



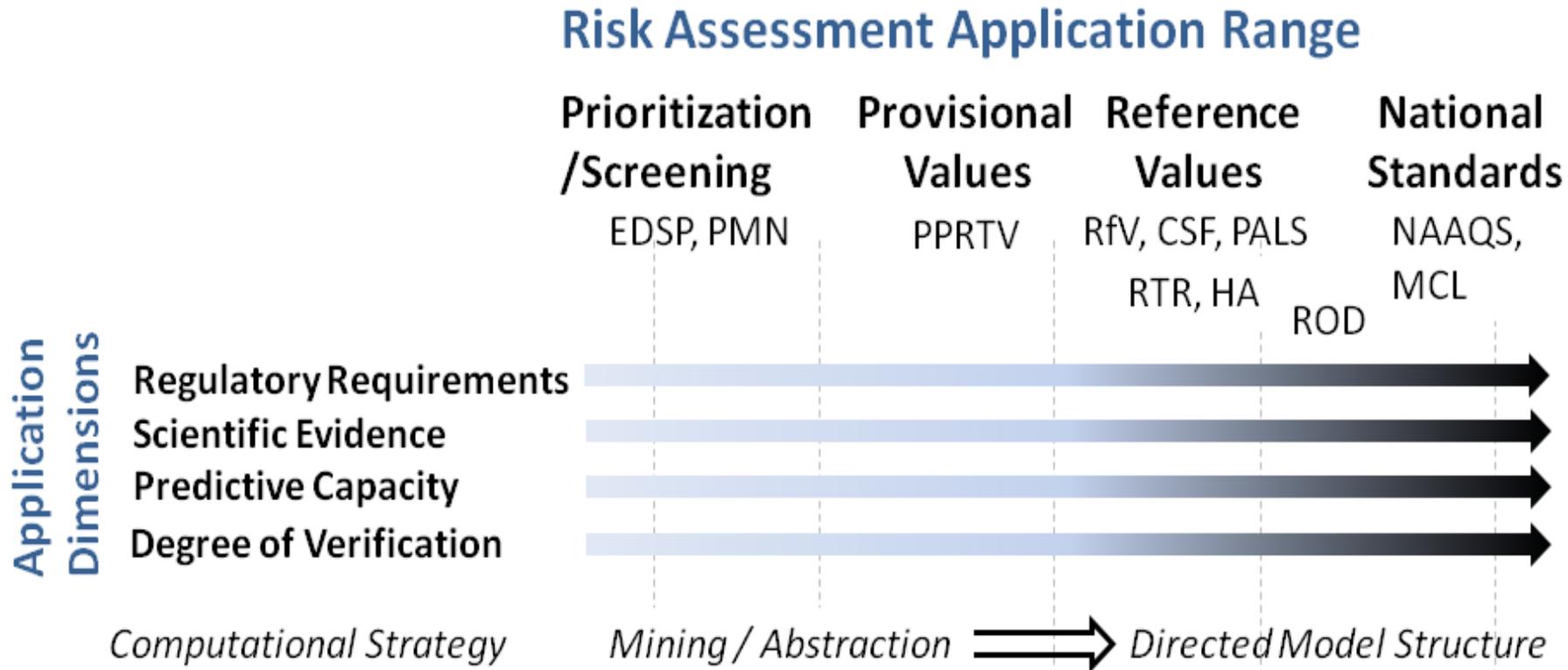
Progress for a Stronger Future

# Topics

- **Challenge:** Coherent evidence integration across large landscape of risk assessment applications
- **Building confidence:** Create context for translation based on mechanistic modeling to advance novel approach methods (NAMs)
  - AEP and AOP frameworks
  - Exposure alignment
  - Quantitative AOP and IATA
- **Case study:** Evaluation of new chemical substances under TSCA
- **Specific considerations:** Communication and characterization
  - Reporting standards
  - Uncertainty / variability and new translation factors
- **Summary**

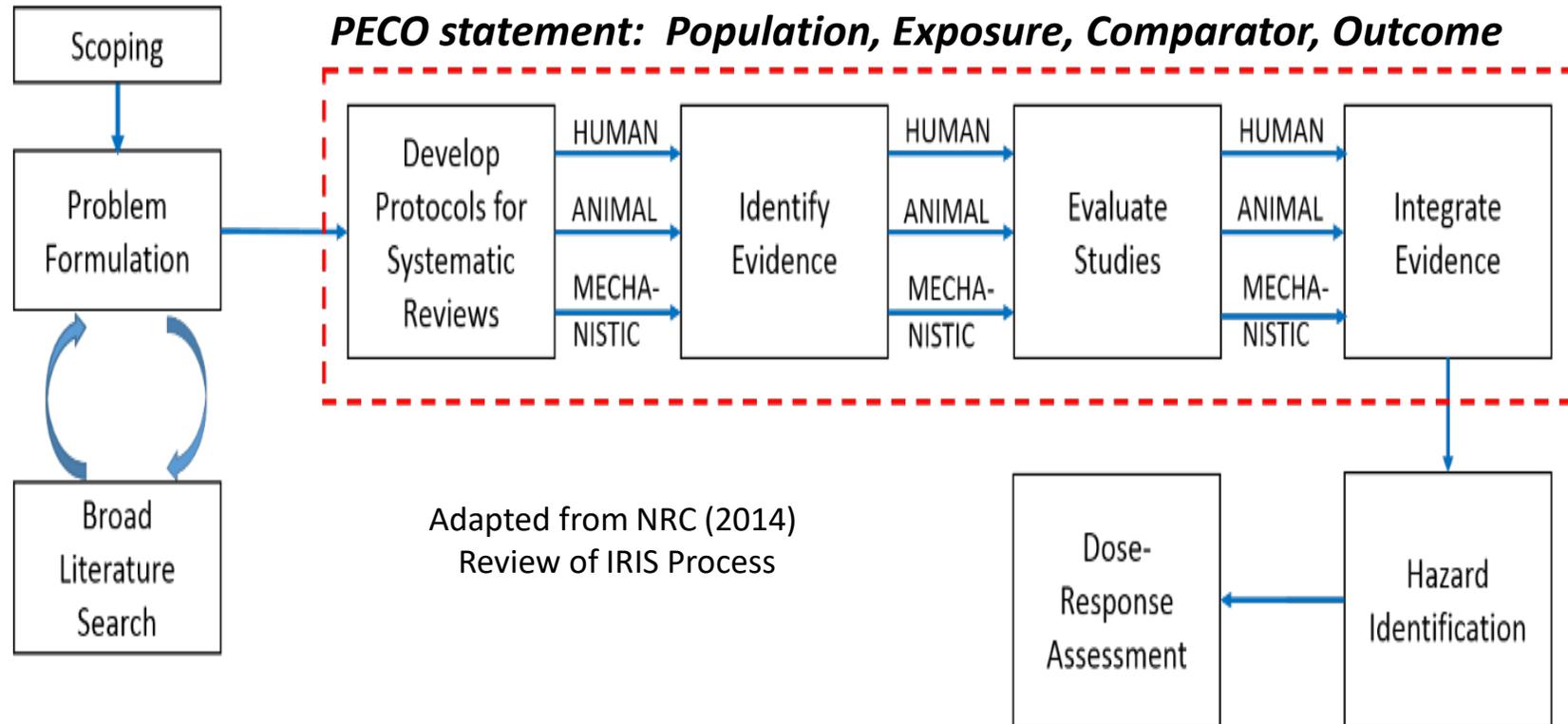
*Disclaimer: These views are those of the author and do not represent US EPA policy.*

# Risk Assessment Landscape



- Problem formulation: Fit for purpose
- Different data sources and strategies across landscape
- ***Mechanistic approach can create coherent context***

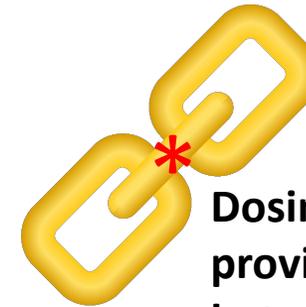
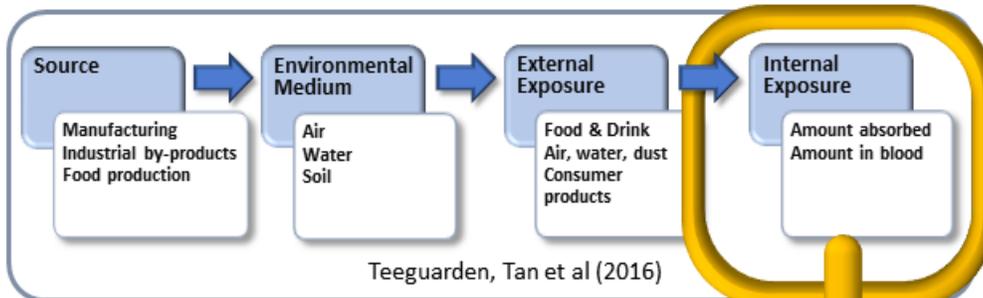
# Challenge: Evidence Integration



- Diverse exposure systems
- Dose at different levels of biological organization
- Various types of outcomes and modeling approaches
- Mechanistic data not considered in an integrated structure

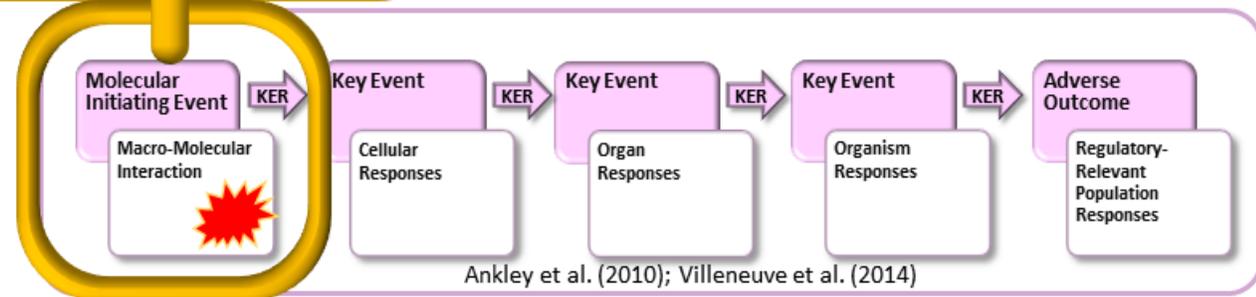
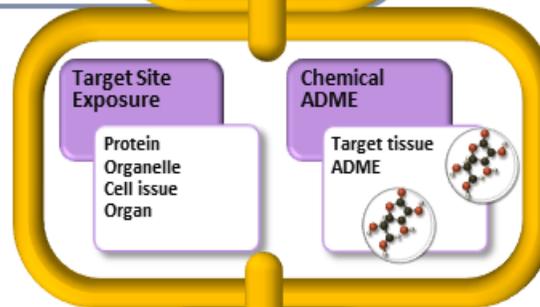
# Transitions: Comprehensive Characterization

## Aggregate Exposure Pathway (AEP)



**Dosimetry modeling provides critical link between exposure and key events of response**

**TSE = Target Site Exposure**



## Adverse Outcome Pathway (AOP)

# Transitions: Novel Approach Methods (NAMs)

- EPA Strategic Plan published June 22, 2018  
(<https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/strategic-plan-reduce-use-vertebrate-animals-chemical>)
- EPA views the term New Approach Methodologies (NAMs) as equivalent to alternative test methods and strategies (the language in the statute)

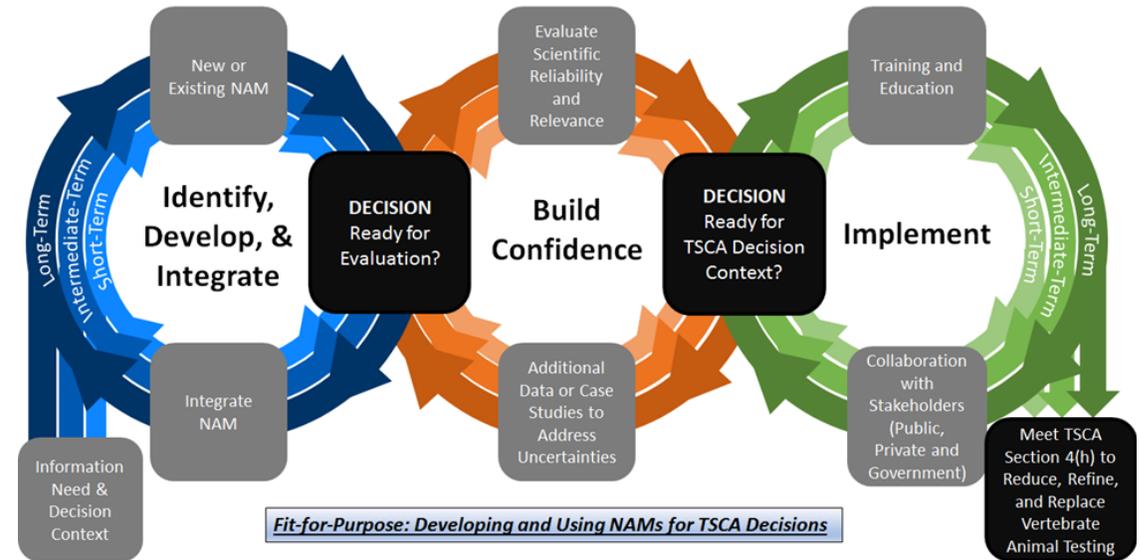


- EPA Work Plan for Reducing Use of Animals in Chemical Testing published June 2021  
(<https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing>)

# NAMs: Strategy for Success

- **Strategic plan components**
  - ID, Develop, Integrate
  - Build confidence
  - Implement
- Demonstrated approach for skin sensitization adapted to inhalation
- **Create context** to advance understanding
  - **Target *in vitro* assays to evaluate key events** in various AOP
  - **Bridge acute to chronic pathogenesis**

Fig. 1 Core Components of EPA Strategic Plan to Develop and Implement New Approach Methodologies (NAMs) in TSCA



Toxicology in Vitro 52 (2018) 131–145



Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: [www.elsevier.com/locate/toxinvit](http://www.elsevier.com/locate/toxinvit)



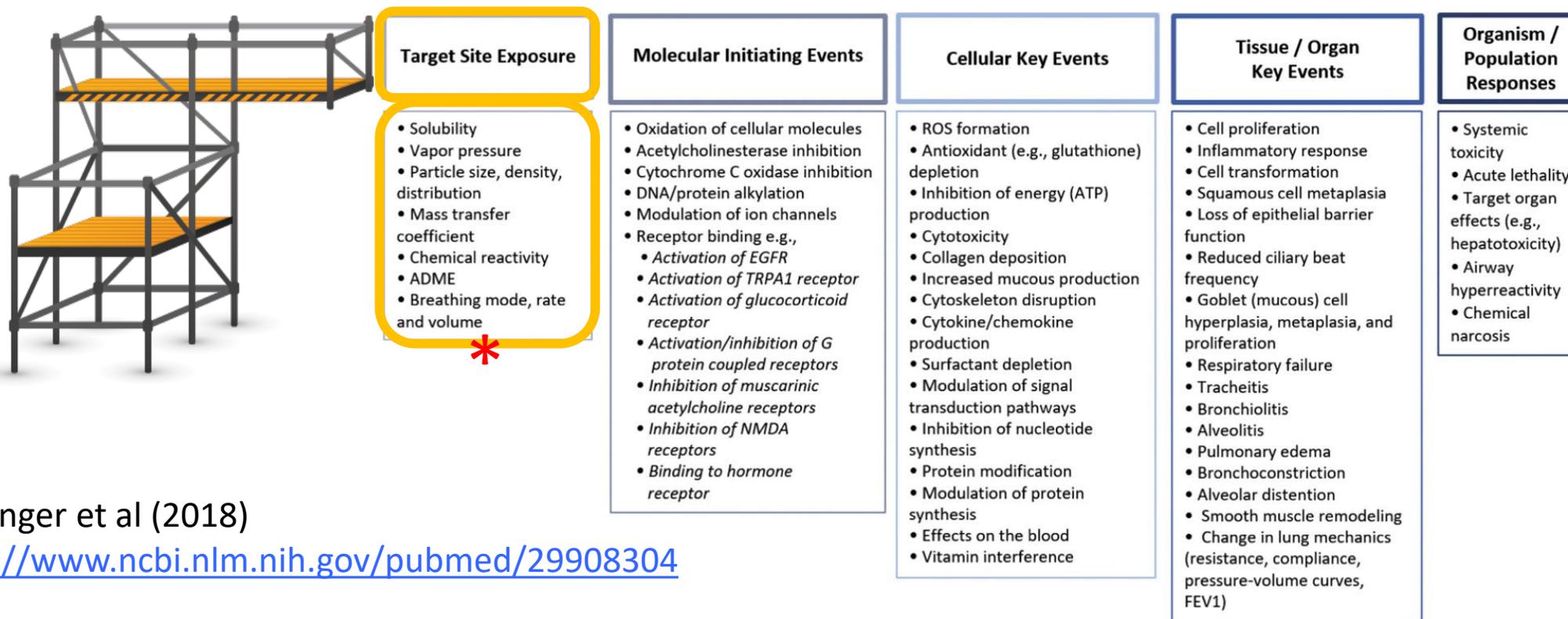
Review

Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity



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# Translation: AOP as Mechanistic Scaffold

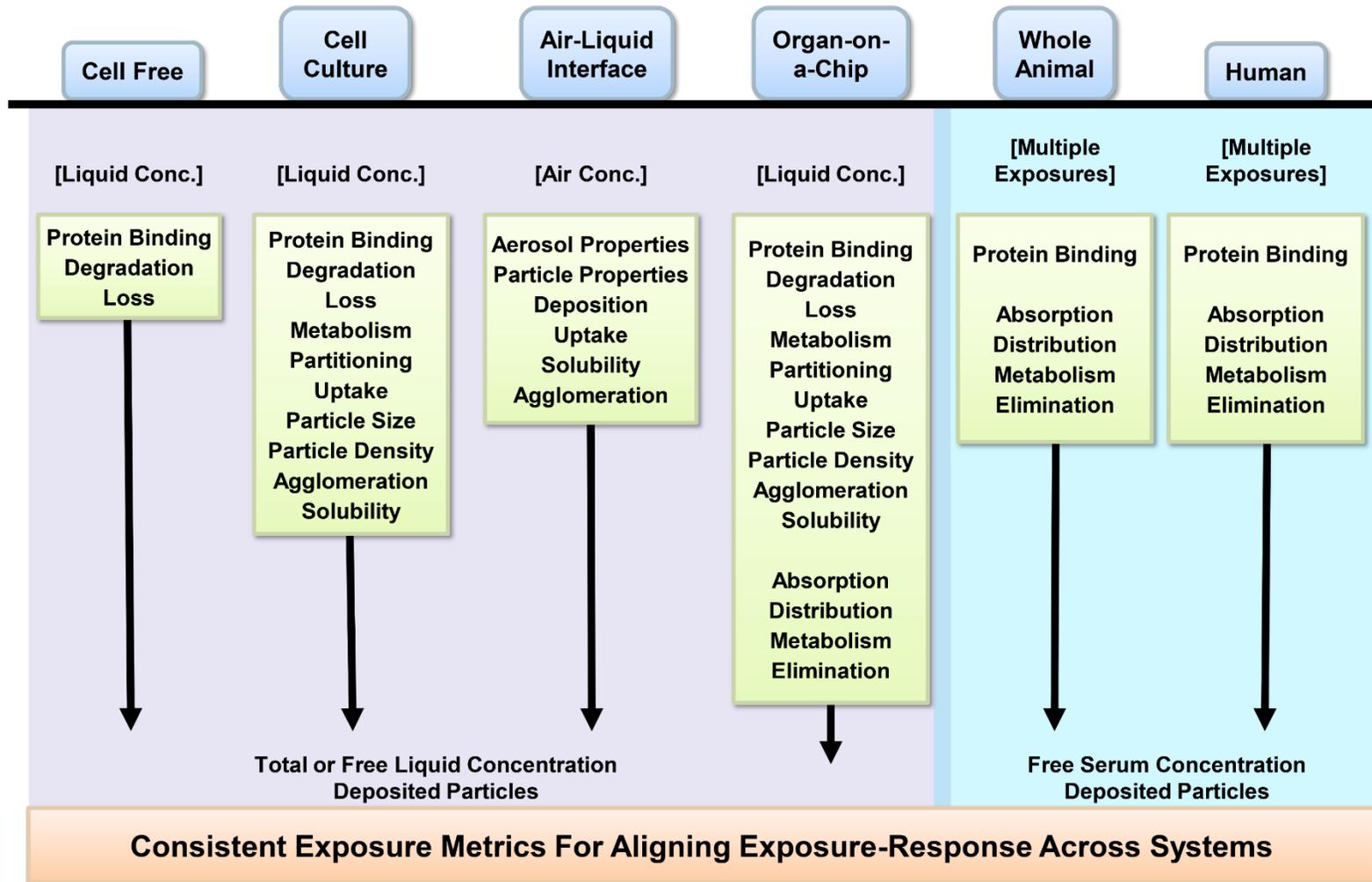


Clippinger et al (2018)

<https://www.ncbi.nlm.nih.gov/pubmed/29908304>

- **Mechanistic data to describe dose characterize key events (KE)**
- **Transition assays** from prioritization / hazard ID to **quantitative AOP (qAOP)** for *in vitro* to *in vivo* extrapolation (**IVIVE**)

# Translation: Exposure Alignment



NAS (2017). Using 21st Century Science to Improve Risk-Related Evaluations

<http://www.nap.edu/24635>

# Dosimetry Models in Risk Assessment

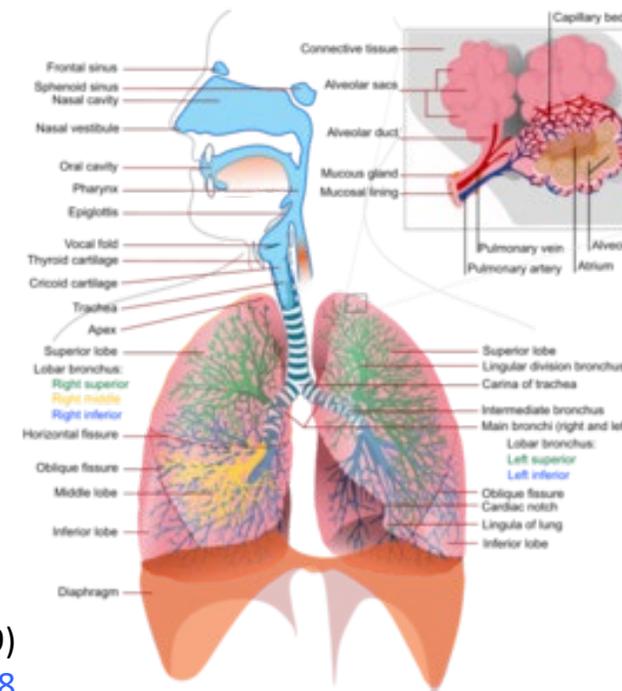
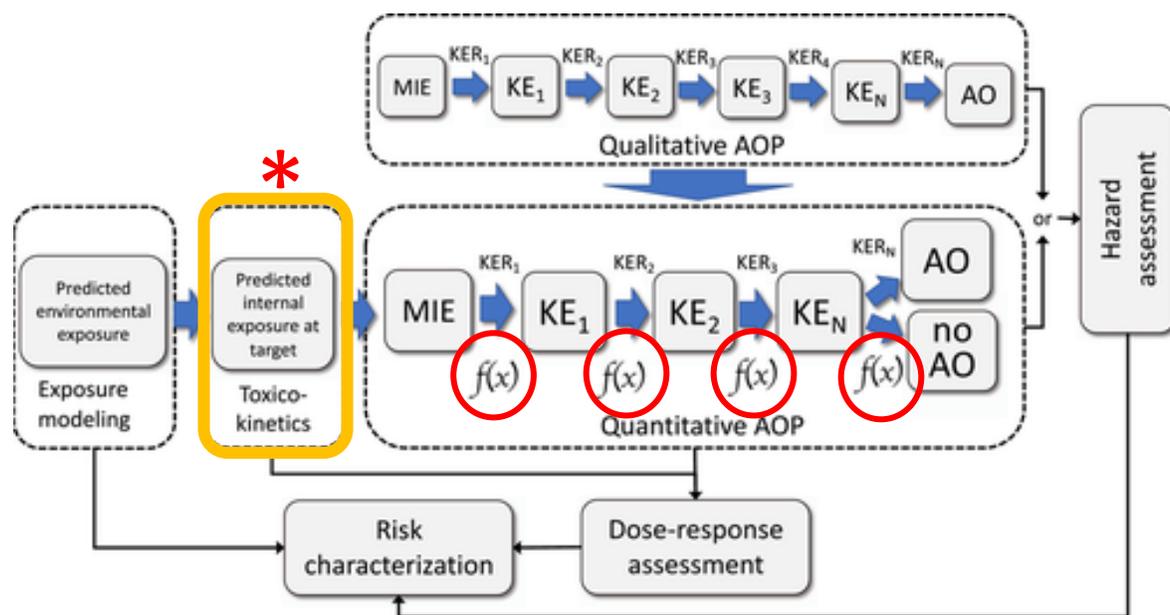
- **“Dose”**
  - Exposure versus internal amount at target site of exposure (e.g., deposited or retained; tissue / cell / molecular)
  - *Defined best as causal or at least a metric best associated (correlated) with toxicity or key event / endpoint used to evaluate “dose-response” relationship*
- **“Metric”**
  - Measurement: mass, surface area (SA), number (#); peak concentration, AUC
  - Scale of metric should be same as observation or the key event used as response endpoint (e.g., lung region versus local, specific cell type)
  - Motivate based on understanding of mode of action
- **“Model”**
  - Conceptual or quantitative description of important processes
  - Simulate different exposure scenarios and experimental designs

# Translation: Mechanistic Modeling

- **Evolves empirical modeling** (observations of **WHAT**) → to **HOW** and **WHY** they occur
  - **Qualitative agreement** with current **biological understanding** of ADME and pathogenesis processes
  - **Quantitative agreement** with **test measures of key events**
- Incorporates important **physicochemical properties**
- **Translates** dose across various **experimental designs** to improve **data integration**
- **Addresses differences** between test systems, species and humans to **refine inferences**
- **Quantifies** and explores properties **systematically and consistently**

# Translation: TSE Alignment and Quantitative AOP

- Account for **key characteristics of exposure**
- Address **physicochemical properties** as determinants of internal dose
- **Characterize** anatomical or physiological parameters and processes determining **dosimetry / ADME**
- Describe ***quantitative*** relationships among key events (KE) in an AOP

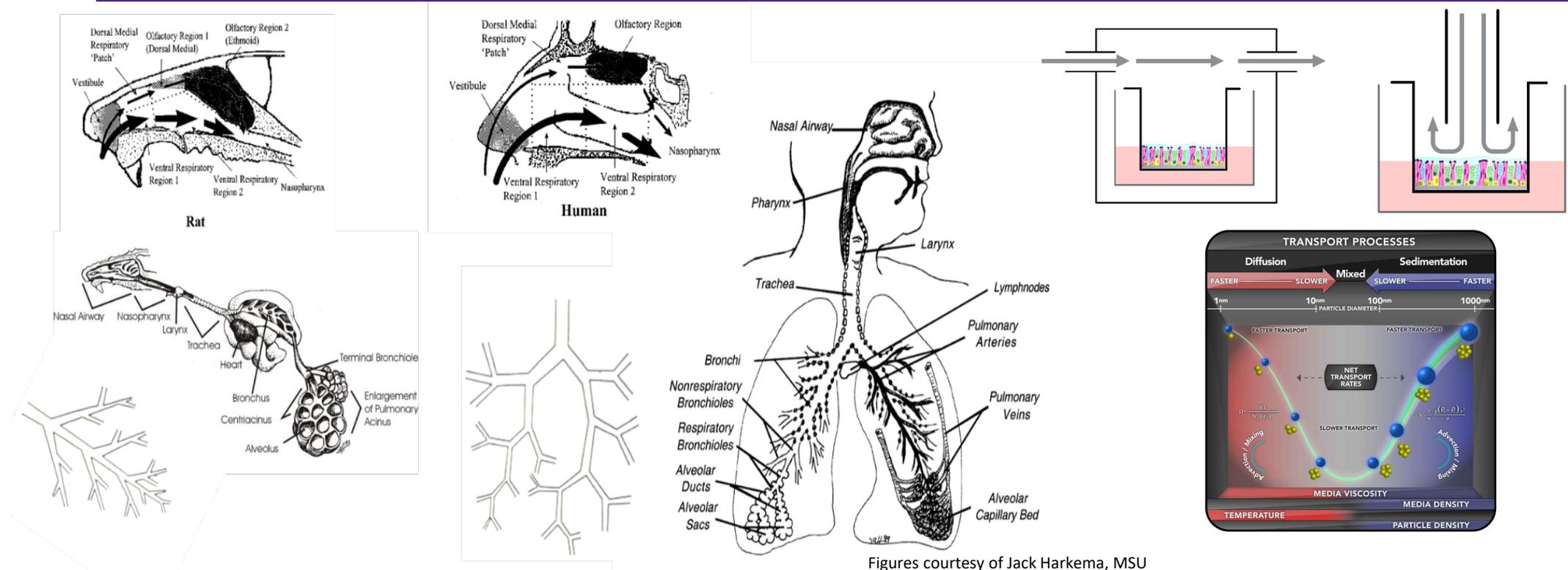


Perkins et al (2019)

<https://www.ncbi.nlm.nih.gov/pubmed/31127958>

# Conceptual Basis of Extrapolation

Not to scale



Figures courtesy of Jack Harkema, MSU

- To **integrate** human / laboratory animal and *in vitro* data need to **systematically** account for differences in
  - **Exposure systems and regimen** (e.g., occupational vs laboratory vs *in vitro*)
  - **Anatomy** (e.g., species and age-specific architecture)
  - **Physiology** (e.g., breathing mode and ventilation activity pattern)

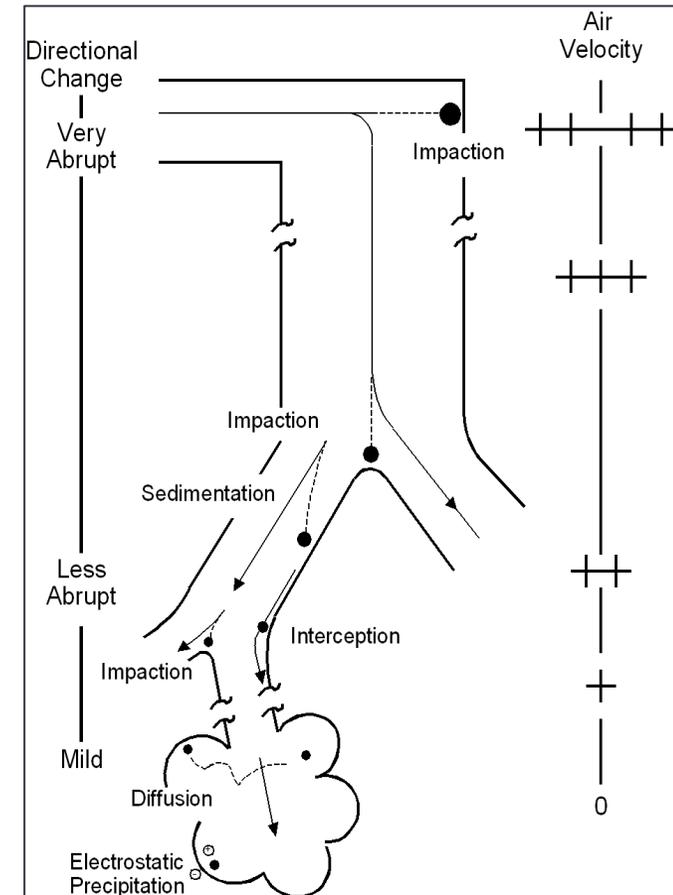
# Physicochemical Properties

## Particle / Fibers / Manufactured Nanomaterials

- Density / Dimensions and Distribution
- Hygroscopicity
- Shape and surface area
- Agglomeration state
- Solubility and dissolution rate
- Crystal structure
- Chemical composition (spatially averaged (bulk) and heterogenous)
  - Physiosorption or chemisorption of biomolecules (e.g., proteins)
  - Biochemically-induced changes in surface chemistry
- Surface chemistry
- Surface charge (Zeta potential)
- Porosity

*Determine aerodynamics and deposition*

**Exposure ≠ internal dose**



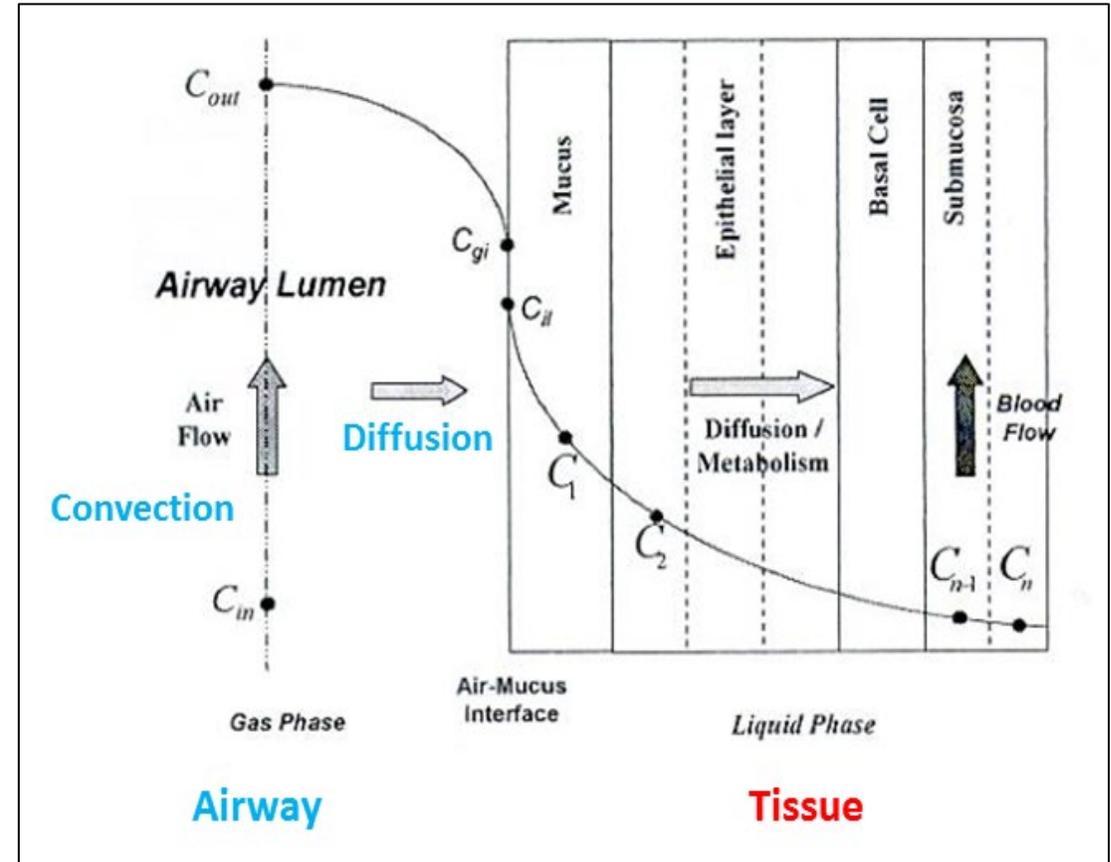
**Retained burden = (Inhalability + Deposition) - Clearance**

**Note: Relative contribution of each mechanism is different in each region of respiratory tract**

# Physicochemical Properties

## Gases

- Molecular diffusivity
- Reactivity
  - Hydrolysis
  - Protein binding
  - Metabolism / tissue reactions
- Solubility
  - Blood:air and blood:tissue partition coefficients



Bogdanffy and Jarabek (1995). *Toxicol Lett* 82-83:919-32.

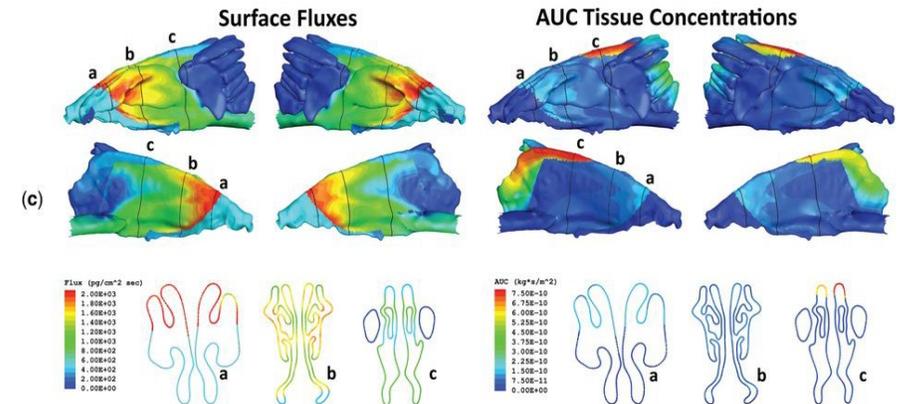
<https://www.ncbi.nlm.nih.gov/pubmed/8597163>

Bogdanffy et al. (1999). *Toxicol Sci* Sep;51(1):19-35.

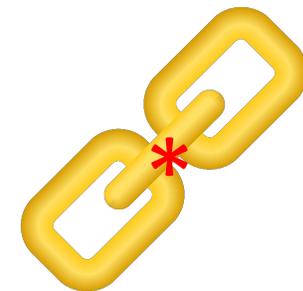
<https://www.ncbi.nlm.nih.gov/pubmed/10496674>

# Dosimetry Deployed to Compute the TSE

- **Range** from default to sophisticated forms
- Differ by **physicochemical property**
  - Particle: MPPD and CFD
  - Gas: CFD, PBPK, hybrid PBPK-CFD
- Account for **key characteristics of exposure**:
  - Concentration, duration, and frequency
  - Regimen: Acute, episodic, ambient (constant), workplace
- Characterize **anatomical and physiological determinants** of ADME
  - Breathing rate, mode (oral, nasal), ADME and metric
- **Determine dose** in exposure test system
  - Submerged vs. air-liquid interface
  - Choice of cell type



Corley et al. *Toxicol. Sci.* 2015;146:65-88



# Translate TSE to Human Equivalent Concentration (HEC)

- **Account** for PC and ADME **determinants in test system**
  - Mass per volume of cell media and surface area differs across transwell sizes
  - $[\text{Toxicant}]_{\text{reported}} \neq [\text{Toxicant}]_{\text{applied}} \neq [\text{Toxicant}]_{\text{aqueous}}$  due to analytical issues and losses to media, plate, etc.
- **Adjust** relative to human target and conditions: **Ratio to appropriately normalize**
- Illustrated for **regional deposited dose (RDD)** of particles in animals (A) **or in vitro (\*)** and humans (H) but can be calculated for any other **particle dose metric** (SA, #) or **normalizing factor** (# epithelial cells, # alveolar macrophages)
- **Minute volume** can be age-specific and incorporate a ventilatory activity pattern reflecting breathing mode (nasal, mouth, oronasal)

$$(\text{RDD})_r = \frac{(\text{RDD})_{A^*}}{(\text{RDD})_H} = \frac{(C_1)_{A^*}}{(C_1)_H} / \frac{(\text{Normalizing Factor})_{A^*}}{(\text{Normalizing Factor})_{\ddagger H}} \times \frac{(\dot{V}E)_{A^*}}{(\dot{V}E)_H} \times \frac{(F_r)_{A^*}}{(F_r)_H}$$

$(\dot{V}E)$  = Minute volume (ventilation rate)

$F_r$  = fraction of mass deposited in region predicted with model

$r$  = Region of observed toxicity for extrapolation

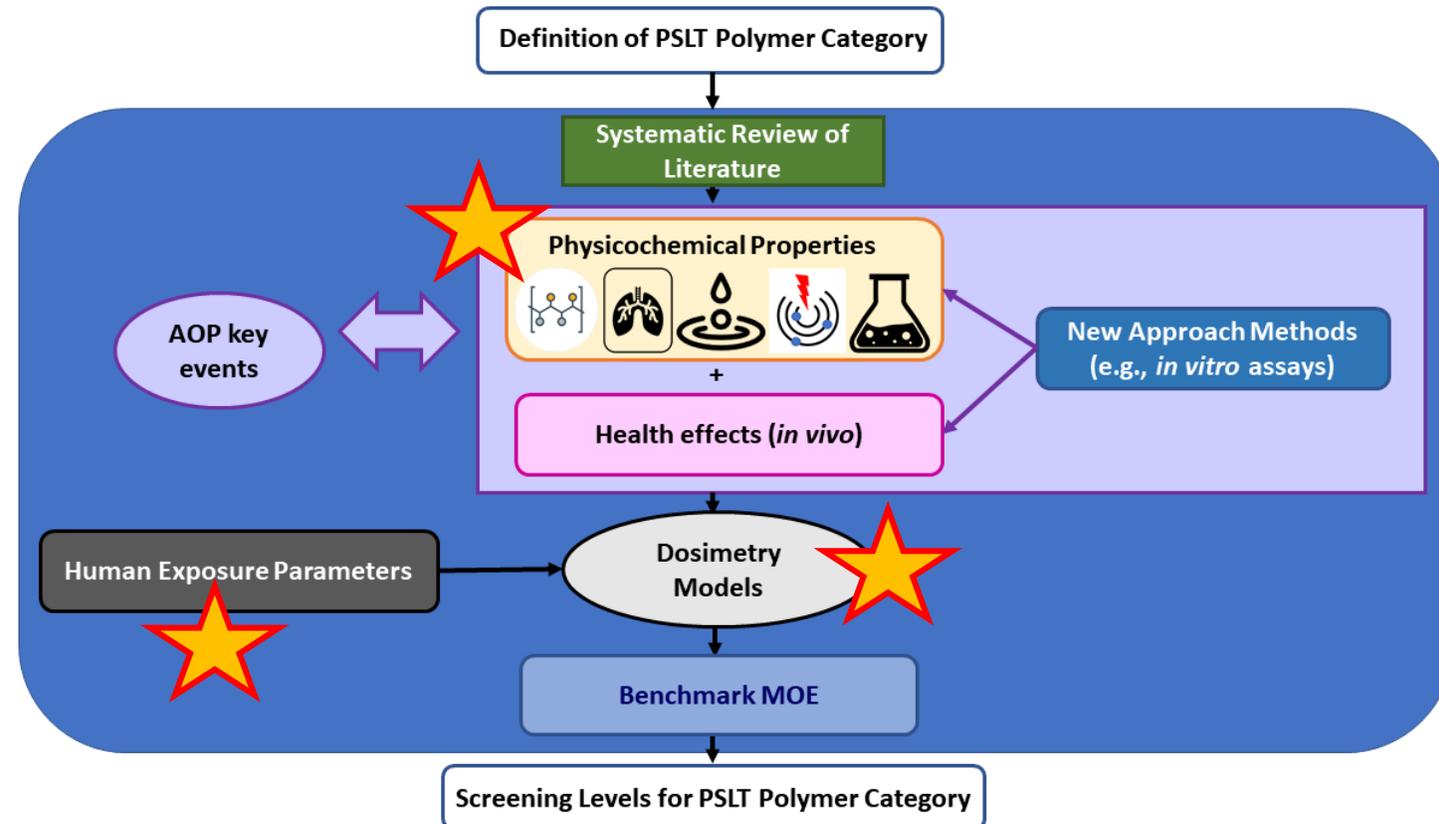
$\ddagger$  = Surface area (SA) for respiratory effects and body weight (BW) for remote effects

# Case Study: New Chemical Substances (NCS) under TSCA

- Section 5 of TSCA does not require upfront testing for NCS; only extant data need be submitted
- Various methods used to assess risks with limited data
  - Chemical categories based on comparator chemicals
  - “Read across” approaches using analogues
- Newly proposed integrated approach to testing and assessment (IATA) based on dosimetry modeling and AOP-inspired NAMs (SOT 2021)
  - General surfactants (Henry et al.; SOT Poster #2583)
  - Poorly soluble low toxicity (PSLT) polymers (Jarabek Stedeford et al.; SOT Poster #2593)
- Manuscripts undergoing re-submission to Chemical Research Toxicol

# Integrated Approach to Testing and Assessment (IATA)

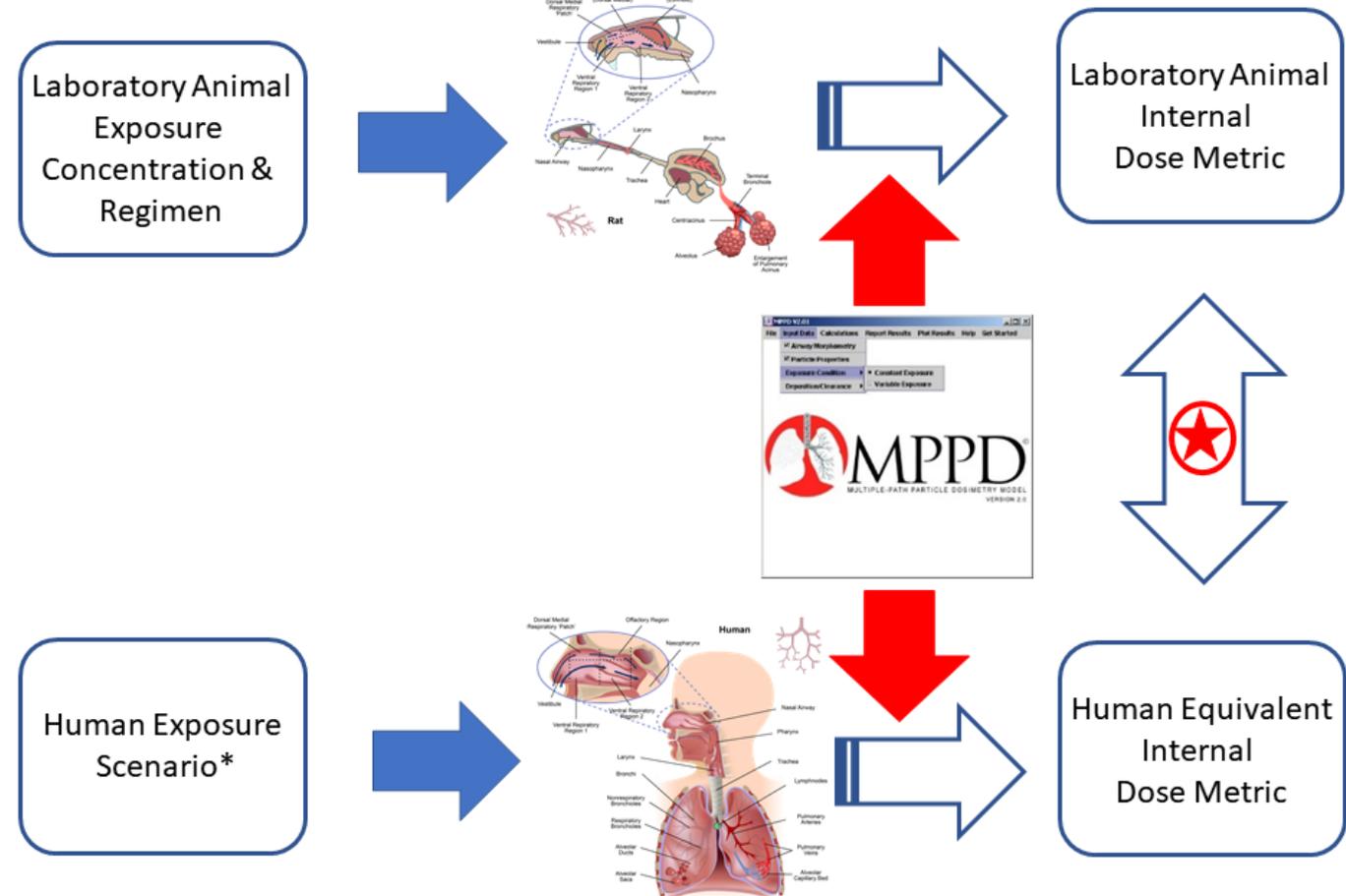
- Dosimetry plays critical role in strategy for evidence integration and evaluation to aid assessments
  - Inclusion criterion based on physicochemical (PC) properties
  - Translation of dose across experimental platforms
  - Target specific exposures
- NAMs can provide data to
  - Inform both PC properties and health effects based on AOP
  - Refine model parameters (e.g., solubility rates)



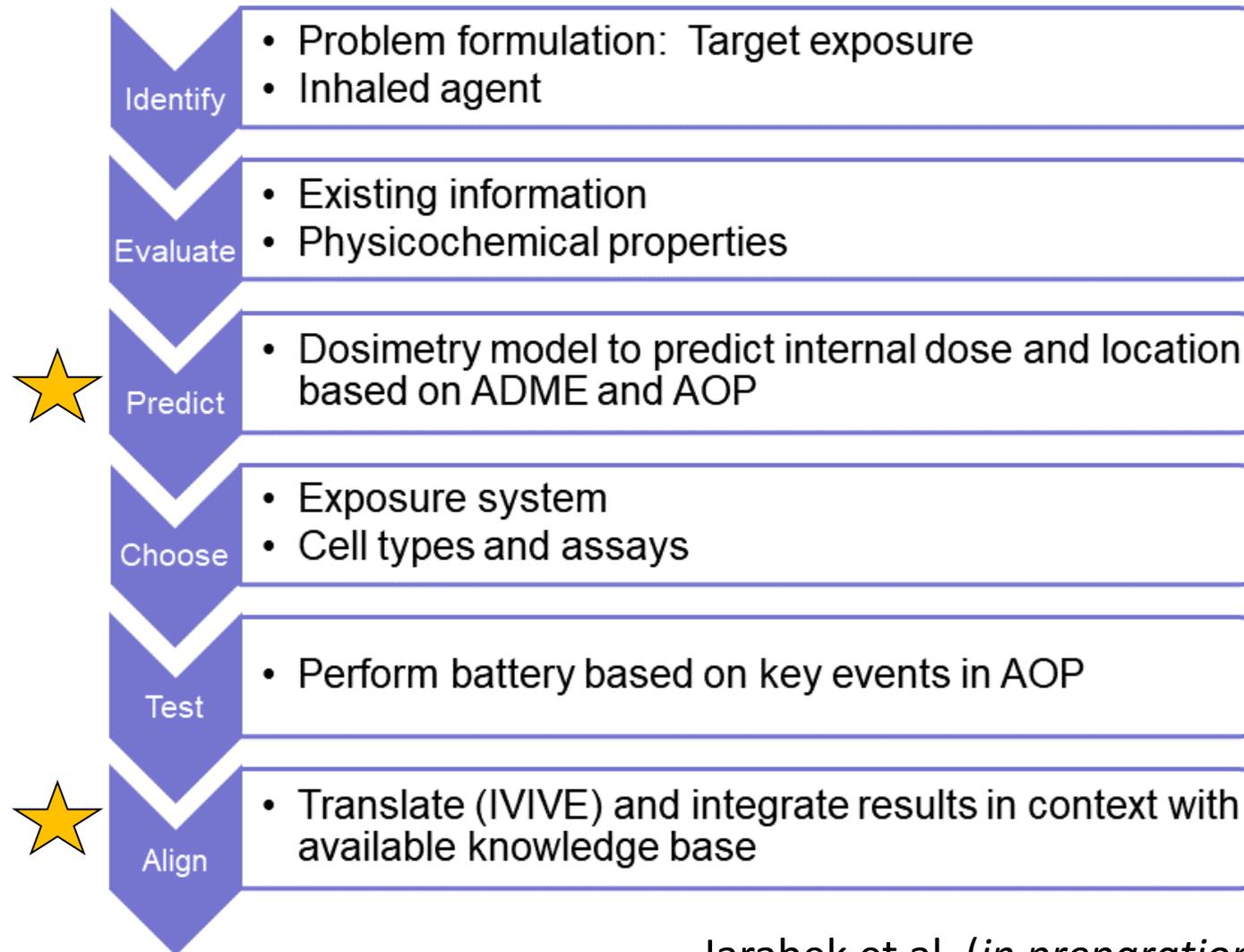
Jarabek Stedeford et al. (accepted)

# MPPD Model to Calculate HEC: PSLT Polymers

- **Human equivalent concentration (HEC)** based on **extrapolation** of laboratory animal data
- **Multiple-path particle dosimetry (MPPD)** model deployed to **simulate** both the laboratory animal **exposure regimen** (e.g., 6 hr/day and 5 days/week for 28 days) and the human exposure scenario (e.g., occupational 8 hr/day and 5 days/week for 40 years)
  - Different particle distribution
  - Various ventilation parameters
- Human exposure scenario can be **default or targeted (\*)** with specific data



# AOP-*Inspired* Integrated Approach to Testing and Assessment



Jarabek et al. (*in preparation*)

- **Data sharing: Standards**

- MIAME: Minimum Information About a Microarray Experiment
- SEND: Standard for Exchange of Non-clinical Data

- **FAIR Principles: Findable / Accessible / Interoperable / Reusable**

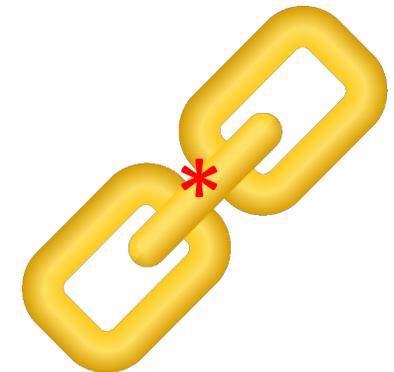
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4792175/pdf/sdata201618.pdf>

- **Translate TSE across exposure systems to aid evidence integration**

- Exposure system operating parameters and conditions
- Rationale for choice of cells and assays
- Modular, multi-scale dosimetry to support interoperability

- **Data pipelines and analytical work flows: Meta data**

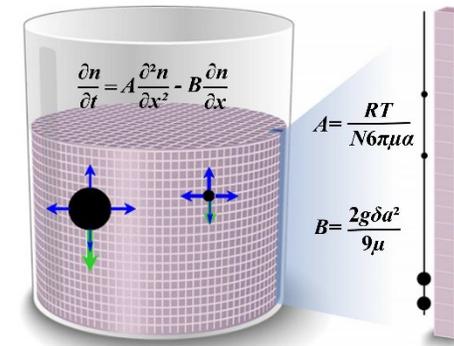
- Experimental annotation: **WHAT / HOW / WHY**
- Curation and consistency: Domain expertise and detail
- Interdisciplinary dialogue
- **Repurposing: Applicability**



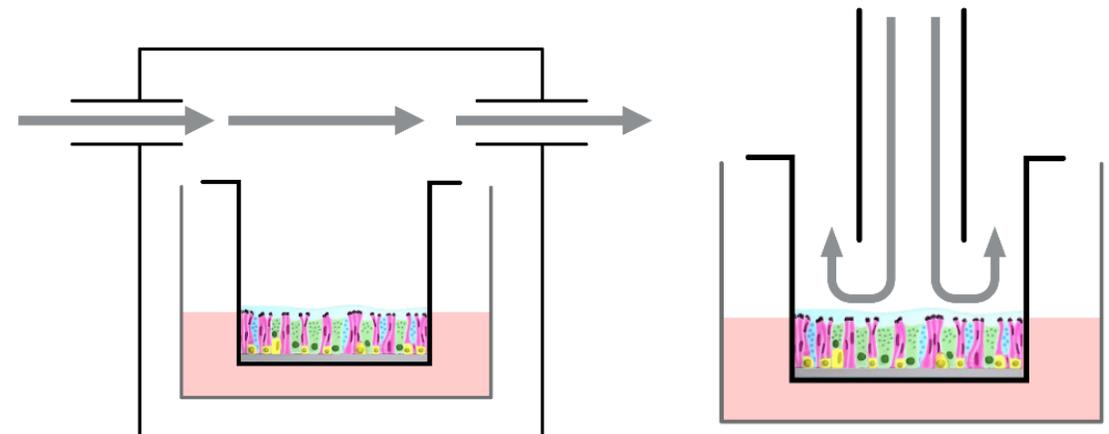
# Reporting Standards: Exposure Systems

- **Generation system and specifications**
  - Dimensions and volume
  - Air flow rate
  - Delivery mechanism(s)
  - Plate size and number, inserts
- **Concentration** (delivered relative to nominal should be consistent)
- **Analytical methods**
- **Temperature**
- **Humidity**
- **Relevance to target scenario**
  - Regimen and duration
  - Physicochemical characteristics
    - Gas: Mass transfer determinants
    - Particle: Deposition mechanisms

Jarabek et al. (*in preparation*)



Hinderliter et al. 2010. *Part Fibre Toxicol.* 7(1) 36  
<https://nanodose.pnnl.gov/default.aspx?topic=ISDD>



# Reporting Standards: Cell Systems

- **Culture system**
  - Demonstrated reliability
- **Cell type(s)**
  - Source(s)
  - Metabolic competency
  - Rationale for choice (e.g., relevance to target scenario)
- **Media**
  - Type (components / lot #)
  - Location (epithelial or endothelial)
  - Volume
- **Viability**
  - Evaluation
  - Duration

- **Assays**

Jarabek et al. (*in preparation*)

- Relevance to key events and respiratory tract
- Established performance and variability
- Response levels and rationale

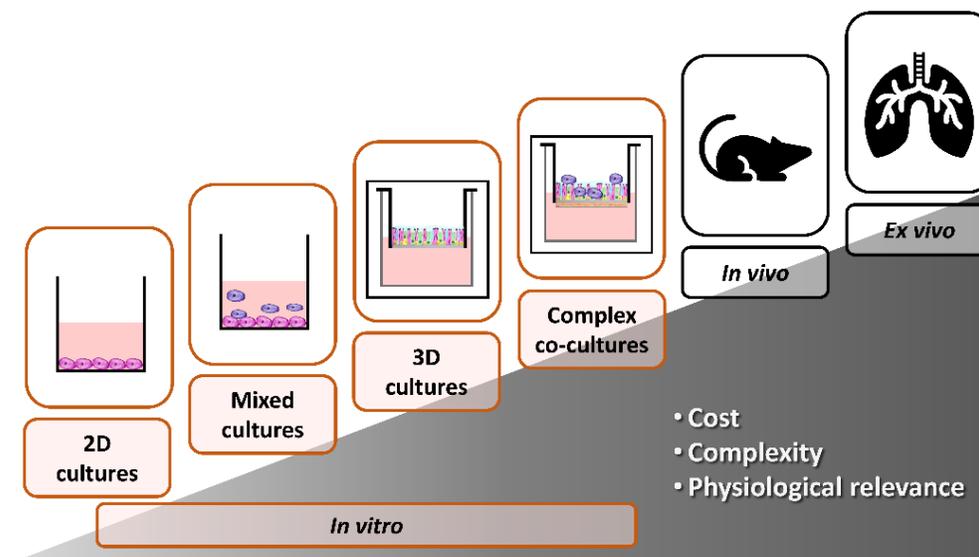


Figure adapted from Lacroix et al. (2018). *Appl in vitro Tox*, 4(2), 91 – 106.

<https://www.liebertpub.com/doi/full/10.1089/aivt.2017.0034>

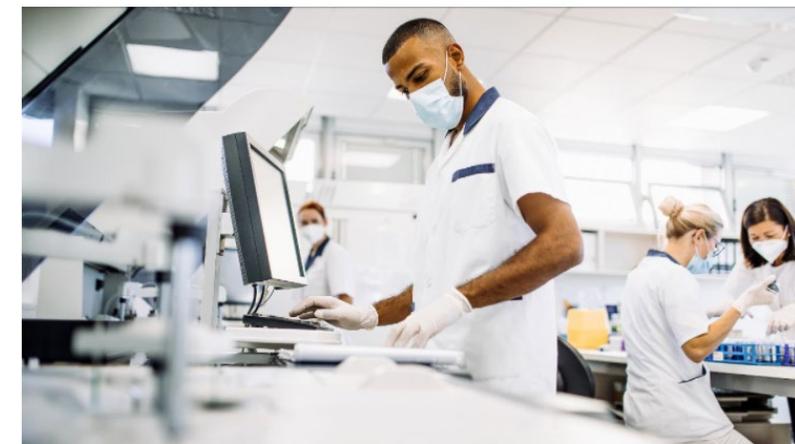
# Characterization: Translation Factors

- **Traditional factors of uncertainty and variability**
  - **Intrahuman:** Variability within the human population, including susceptible subpopulations, due to differences in life stage, disease states, and other determinants of TK or TD
  - **Interspecies (across experimental systems):** Differences in TK and TD
  - **Duration:** Use of acute data to predict episodic or chronic exposure outcomes
  - **Severity:** Nature of effect and prognostic value
  - **Database:** Coverage to comprehensively address potential effects
- **Novel translations:** Cell system as target tissue / system surrogate
  - Target tissue specificity and viability
  - Spatial representation and variability of sample
  - Metabolic competency and variability



*New Study Committee Announcement:*  
**Variability and Relevance of Current Laboratory Mammalian Toxicity Tests and Expectations for New Approach Methods (NAMs) for use in Human Health Risk Assessment**

DEADLINE: Sunday, August 29, 2021

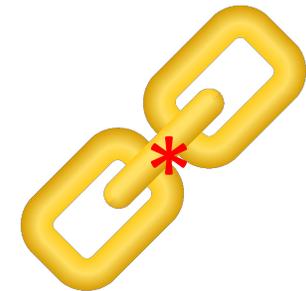


# Impacts: Inferences and Integration

- Clarify terminology
  - “Model”
  - Effects, relationships and outcomes
- Evaluate new data resources
- Incorporate computational outputs
- Rectify units
- Elucidate study quality and utility
- Inform “causality” considerations
- Illuminate assumptions
- Support reusability and interoperability
- ***TRANSFORM translation and improve evidence integration***

# Summary: Advancing NAMs

- **Evolve empirical modeling** (observations of **WHAT**) → to **MECHANISTIC MULTISCALE MODELS** (*HOW* and *WHY*)
- Bridge to systems biology with **Integrated Approaches to Testing and Assessment (IATA)**: **key events** of pathogenesis and *quantitative* AOP (qAOP)
  - **Characterize** dose and effects at different **levels of observation**
  - **Understand** various **dimensions of disease** and relationships (e.g., early or late)
- **Translate target site exposure (TSE) across exposure systems** to aid and transform **evidence integration: develop ANALYTIC WORKFLOWS**
  - **Align** human and animal **exposures**
  - **Refine** in vitro to in vivo extrapolation (**IVIVE**)
- Facilitate **interdisciplinary dialogue**
  - **Transparency** re: assumptions and foundational data
  - Appreciate **assumptions and impacts**
  - Support **modularity for interoperability** with other models



# Thanks and Contact Information

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919-637-6016

**SOT Poster #2583 | Surfactants Category: An Integrated Approach to Testing and Assessment (IATA) Including New Approach Methods (NAMs) for Assessing Inhalation Risks under the Toxic Substances Control Act (TSCA)**

*T.R. Henry<sup>1</sup>, K.D. Salazar<sup>1</sup>, M.P. Hayes<sup>2</sup>, W. Kennedy<sup>3</sup>, A.M. Keene<sup>3</sup>, A.M. Jarabek<sup>4</sup>, O.T. Price<sup>5</sup>, S. Moors<sup>6</sup>, L. Jovanovich<sup>7</sup>, J.L. Rose<sup>8</sup>, A. Tveit<sup>9</sup>, R.T. Tremblay<sup>10</sup>, R.A. Becker<sup>11</sup>, S. Osman-Sypher<sup>11</sup>, P.D. McMullen<sup>12</sup>, S.D. Slattery<sup>12</sup>, W. Irwin<sup>1</sup>, M. Odin<sup>13</sup>, J. Melia<sup>13</sup>, M. Sharma<sup>14</sup>, A.O. Stucki<sup>14</sup>, A.J. Clippinger<sup>14</sup>, and T. Stedeford<sup>1</sup>.* <sup>1</sup>US EPA, Washington, DC; <sup>2</sup>Procter & Gamble, St. Bernard, OH; <sup>3</sup>Afton Chemical Corporation, Richmond, VA; <sup>4</sup>US EPA, Research Triangle Park, NC; <sup>5</sup>Applied Research Associates, Inc., Arlington, VA; <sup>6</sup>BASF Corporation, Duesseldorf, Germany; <sup>7</sup>Stepan Company, Northfield, IL; <sup>8</sup>Procter & Gamble, Mason, OH; <sup>9</sup>BASF Corporation, Florham Park, NJ; <sup>10</sup>Procter & Gamble, Strombeek-Beaver, Belgium; <sup>11</sup>American Chemistry Council, Washington, DC; <sup>12</sup>ScitoVation, Durham, NC; <sup>13</sup>SRC Inc., North Syracuse, NY; and <sup>14</sup>PETA Science Consortium International e.V., Stuttgart, Germany.

**SOT Poster #2593 | Poorly Soluble, Low Toxicity (PSLT) Polymer Category: An Integrated Approach to Testing and Assessment (IATA) Including New Approach Methods (NAMs) under the Toxic Substances Control Act (TSCA)**

*A.M. Jarabek<sup>1</sup>, T. Stedeford<sup>2</sup>, G.S. Ladics<sup>3</sup>, O.T. Price<sup>4</sup>, A. Tveit<sup>5</sup>, M.P. Hayes<sup>6</sup>, R.T. Tremblay<sup>7</sup>, S.A. Snyder<sup>8</sup>, K.D. Salazar<sup>2</sup>, S. Osman-Sypher<sup>9</sup>, W. Irwin<sup>2</sup>, M. Odin<sup>10</sup>, J. Melia<sup>10</sup>, H. Carlson-Lynch<sup>10</sup>, M. Sharma<sup>11</sup>, A.O. Stucki<sup>11</sup>, A.J. Clippinger<sup>11</sup>, S. Anderson<sup>3</sup>, and T.R. Henry<sup>2</sup>.* <sup>1</sup>US EPA, Research Triangle Park, NC; <sup>2</sup>US EPA, Washington, DC; <sup>3</sup>IFF, Wilmington, DE; <sup>4</sup>Applied Research Associates Inc., Arlington, VA; <sup>5</sup>BASF Corporation, Florham Park, NJ; <sup>6</sup>Procter & Gamble, Mason, OH; <sup>7</sup>Procter & Gamble, Strombeek-Beaver, Belgium; <sup>8</sup>Covestro LLC, Pittsburgh, PA; <sup>9</sup>American Chemistry Council, Washington, DC; <sup>10</sup>SRC Inc., North Syracuse, NY; and <sup>11</sup>PETA Science Consortium International e.V., Stuttgart, Germany.