



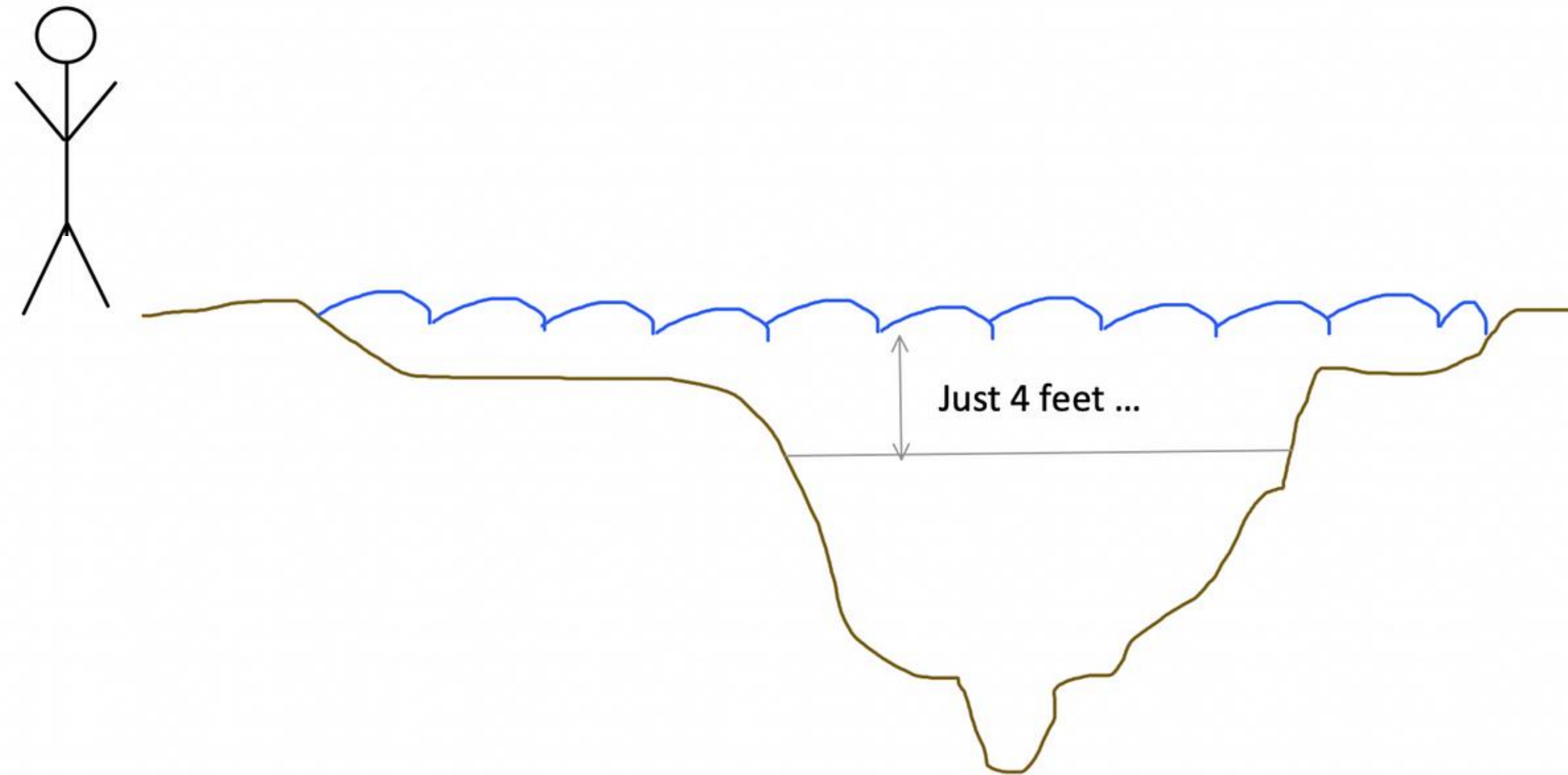
JOHNS HOPKINS
BLOOMBERG SCHOOL
of PUBLIC HEALTH

Probabilistic Risk Assessment

Alexandra Maertens, Ph.D.



Point Estimates of Complex Phenomena Are Problematic

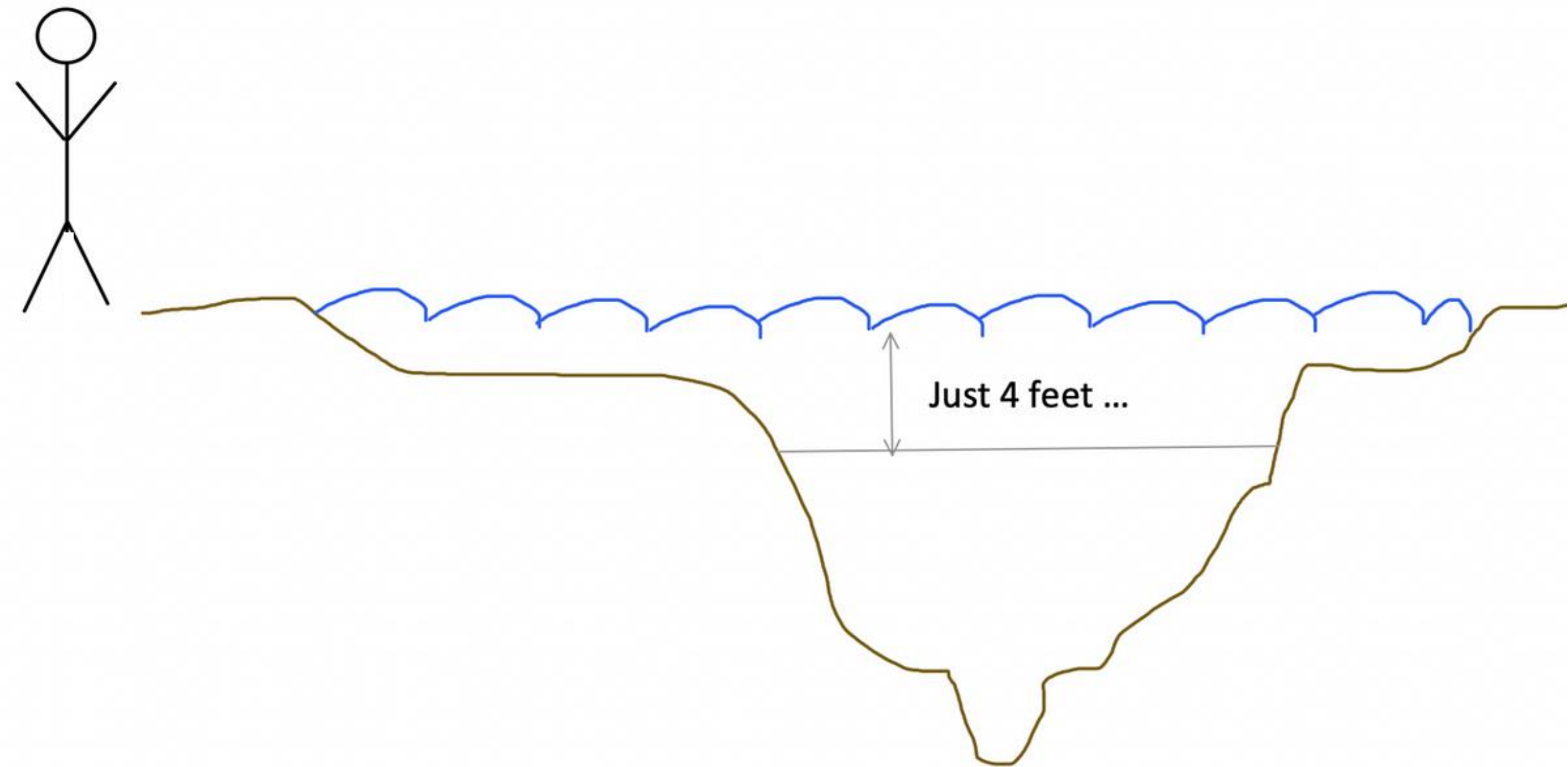


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Dealing with uncertainty like a toxicologist

Assume a depth of 4 ft, then add an uncertainty factor of 10 for geological variability and an additional 10 for database uncertainty, thus assumed an estimated depth of 400 ft - the river is uncrossable.

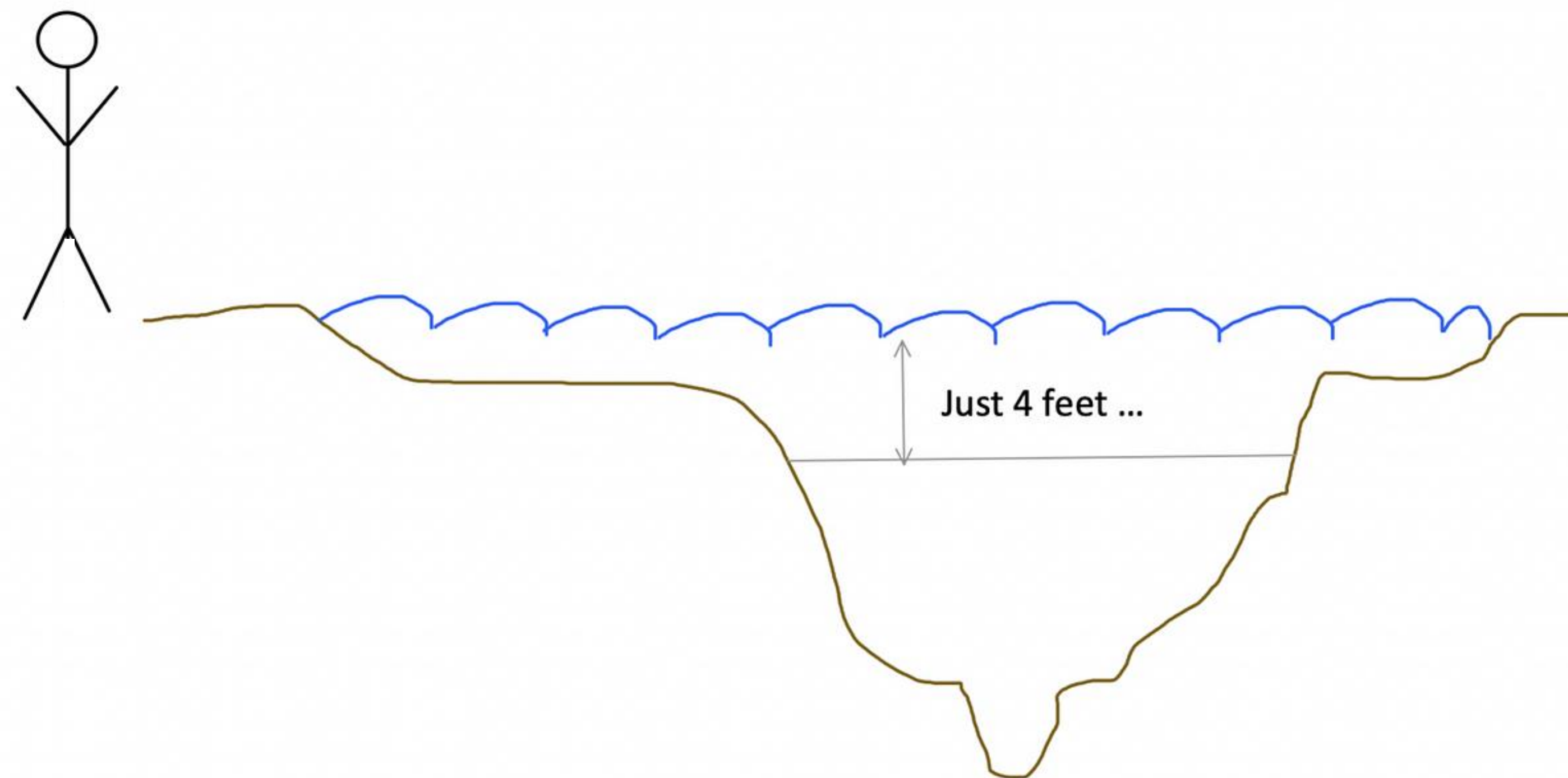


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Hazard Based Screening Assessment

Water is neither a carcinogen, mutagen nor reproductive toxicant. No sensitization potential. The river is safe!



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Probabilistic Risk Assessment

Environment

EPA Approved a Fuel Ingredient Even Though It Could Cause Cancer in Virtually Every Person Exposed Over a Lifetime

made it possible for ProPublica to calculate the lifetime cancer risk from breathing air pollution that comes from a boat engine burning the fuel. That calculation, which was confirmed by the EPA, came out to 1.3 in 1, meaning every person exposed to it over the course of a lifetime would be expected to get cancer.

 **PROPUBLICA**
Investigative Journalism in the Public Interest

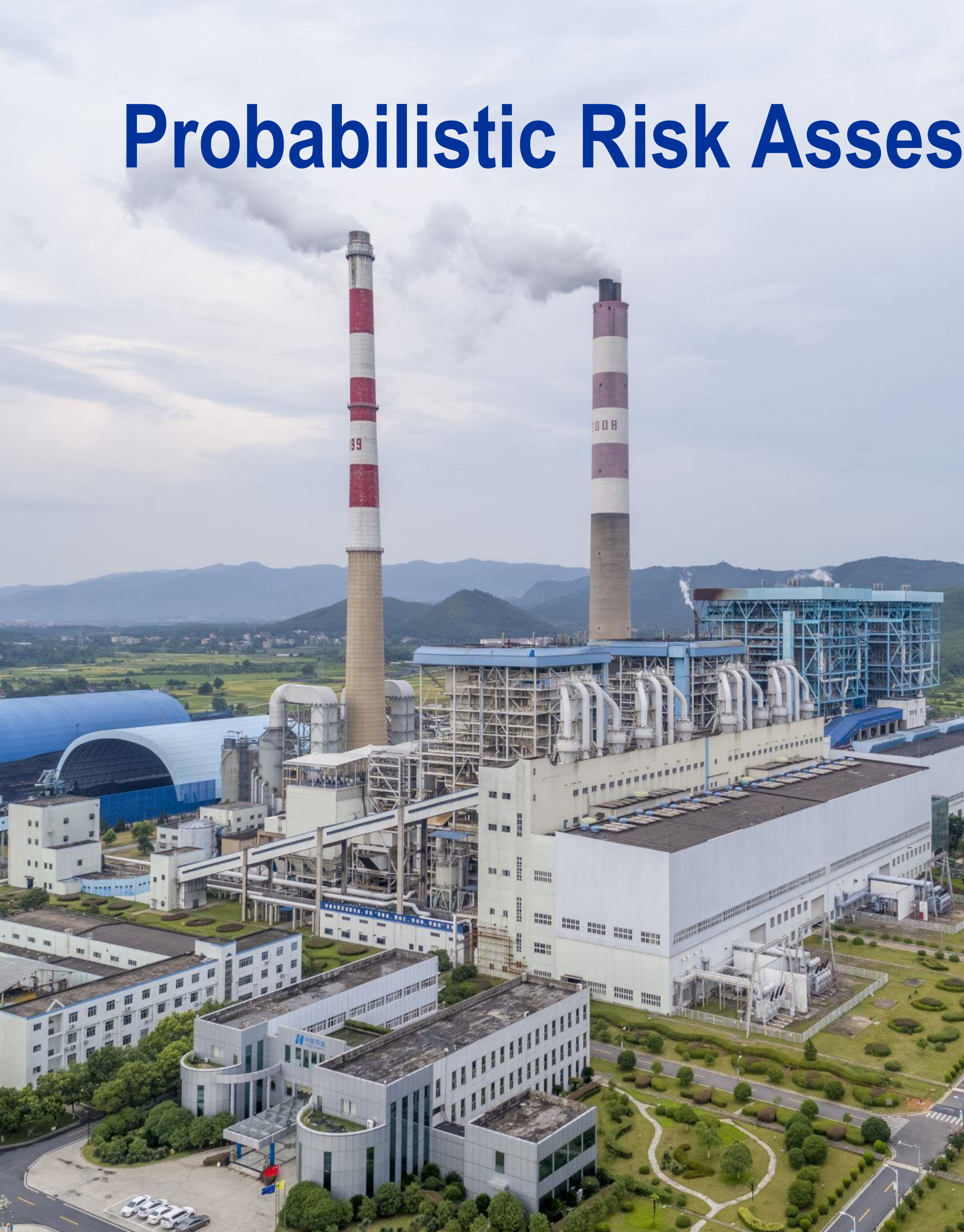
ProPublica



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Probabilistic Risk Assessment

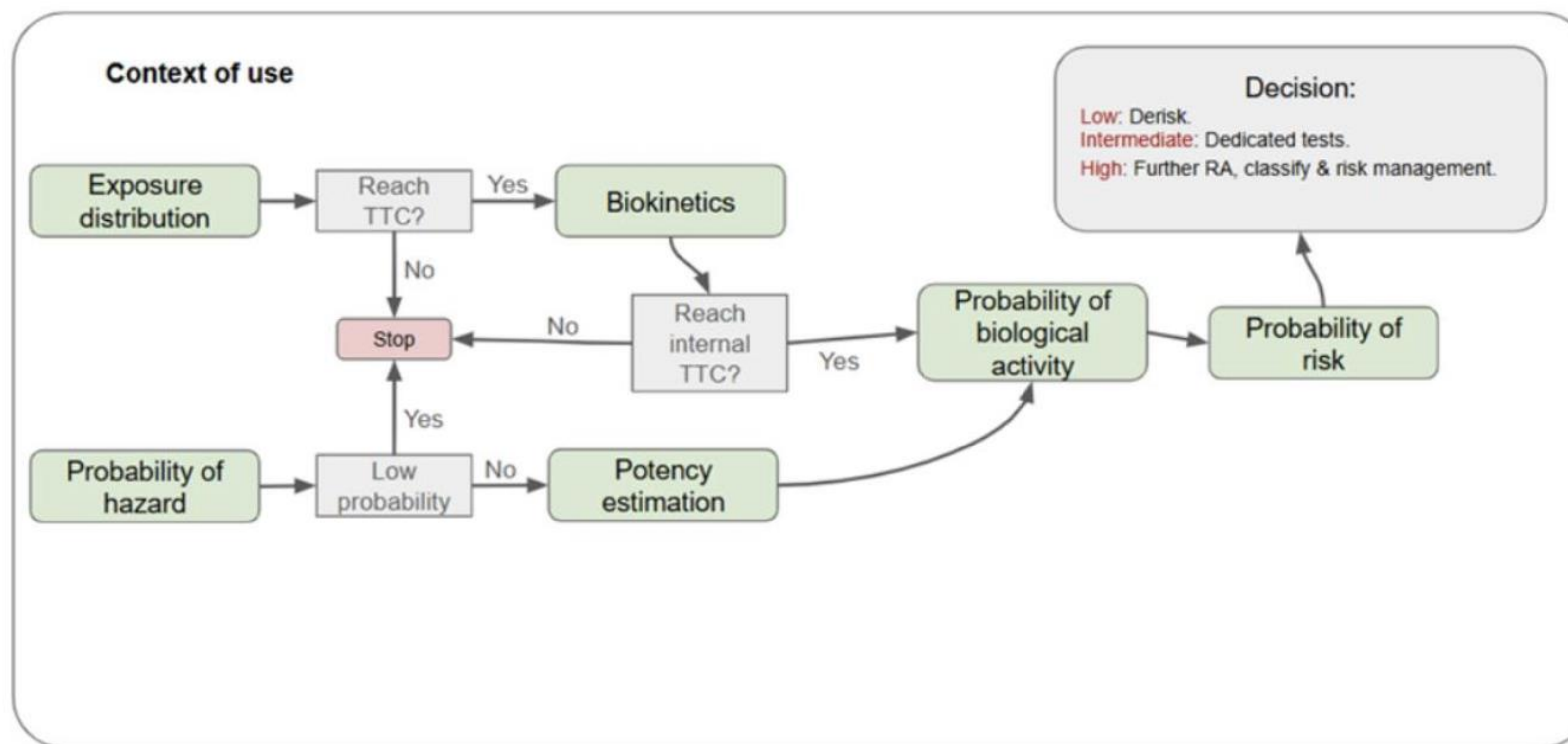
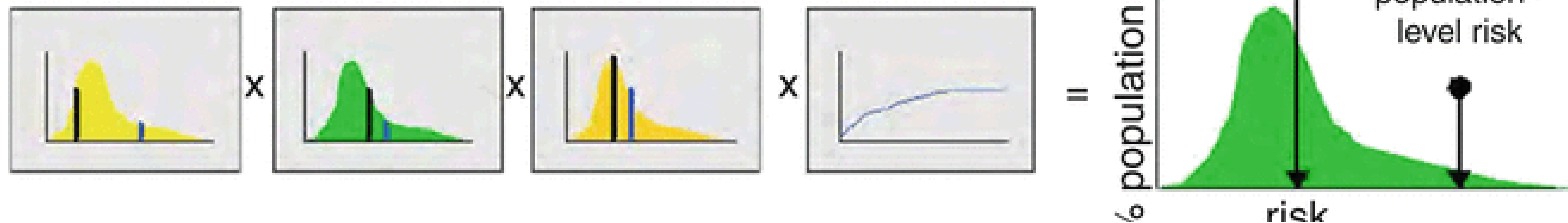


EPA said that the cancer risk estimates were “**extremely unlikely** and reported with **high uncertainty**.”

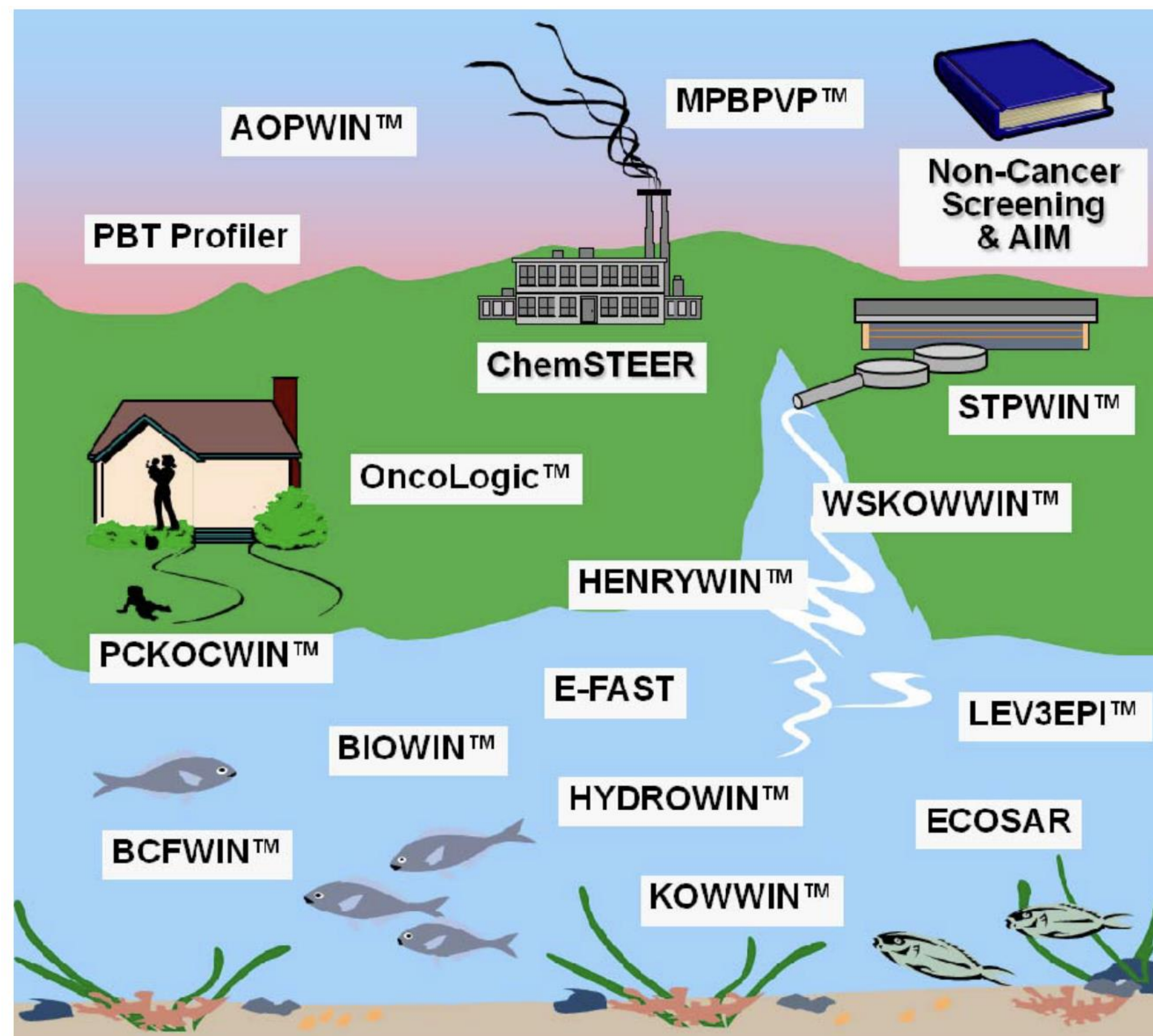
- Key endpoint is often derived from the most toxic substance in the mixture (for fuels this often benzene)
- Exposure scenarios are very conservative – for example, assumes that planes are idling through a full tank of gas per day
- Assumes residents nearby would breathe the exhaust round the clock, every day over their lifetime



$$\left[\text{Concentration in environment} \right] \times \left[\text{Exposure Duration} \right] \times \left[\text{Ingestion or Inhalation Rate} \right] \times \left[\text{Toxicity Factor} \right] = \text{RISK}$$



Environmental Fate



Physicochemical Properties

- Water solubility
- Octanol-water partition coefficient (K_{ow})
- Vapor pressure
- Henry's Law constant
- Boiling & melting points

Environmental Fate

- Biodegradation rates & pathways, including waste water treatment
- Hydrolysis products & rates
- Atmospheric oxidation
- Bioconcentration factors (BCF)
- Soil & sediment sorption (K_{oc})
- Multimedia fugacity model



EPISUITE

Application: Compares predicted values against regulatory criteria (EPA, REACH) to flag chemicals requiring detailed assessment

Persistence (P)

Biodegradation half-lives in water, soil, sediment, and air - numerical criteria

Bioaccumulation (B)

BCF estimates and potential for biomagnification - numerical criteria

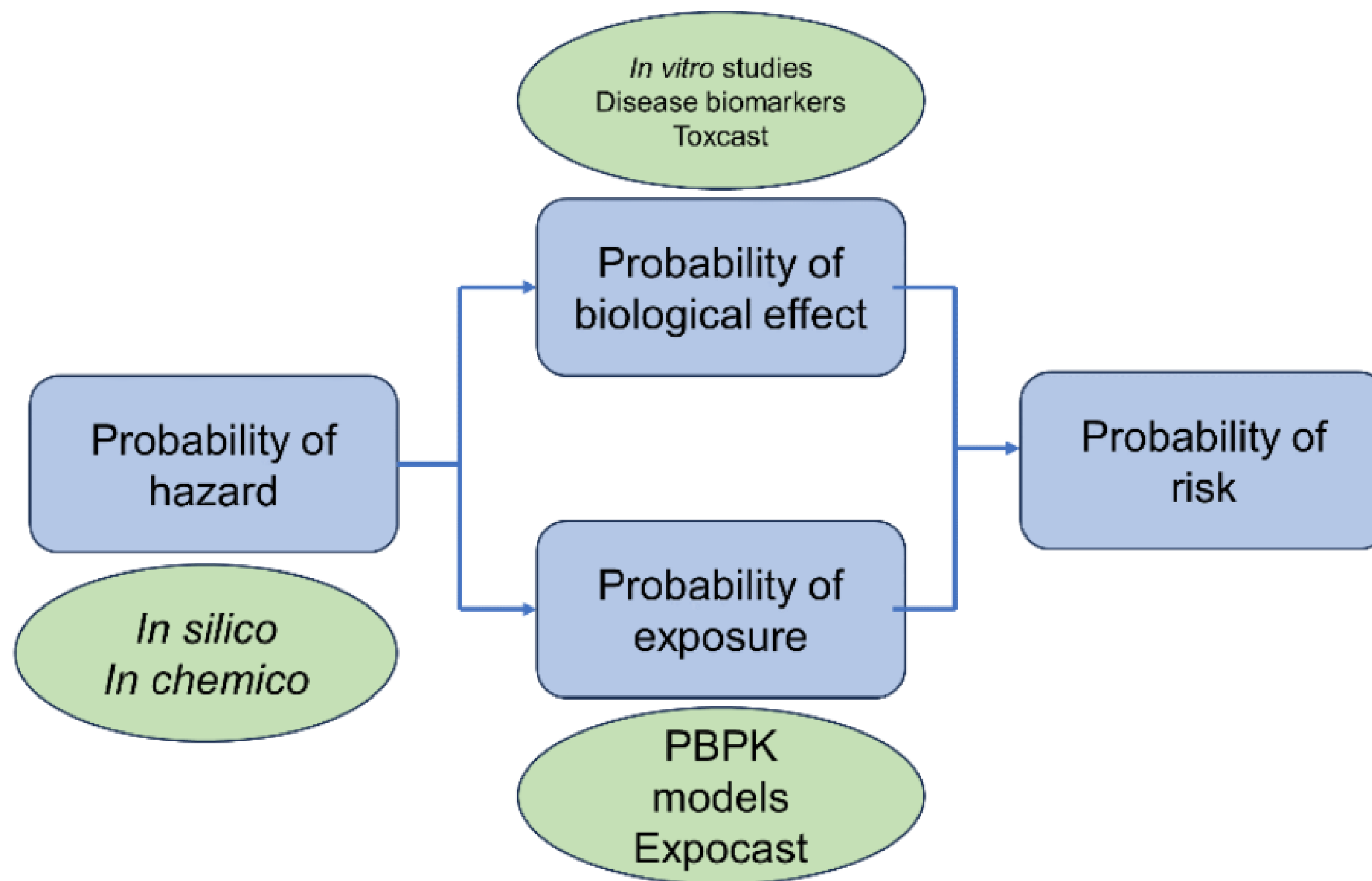
Toxicity (T)

Acute and chronic aquatic toxicity predictions ??



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Skin Sensitization

Models over all have 80-85 percent accuracy

Similar range to reproducibility of animal tests as well as the extent to which animal tests predict each other

Many of the inaccuracies are caused by weak sensitizers
– several commonly used topical skin agents have sporadic reports of sensitization and are therefore classified as skin sensitizers

Tab. 1: Classification agreement on chemicals with at least two sensitization studies in REACH dossiers from 2008-2014
Studies found by searching for all studies with *studytype* = Buehler, GPMT, Patch-Test or LLNA.

	Buehler	GPMT	Patch-test	LLNA
Buehler	95.1% (344 chem.)	91.8% (364 chem.)	87.8% (58 chem.)	76.8% (212 chem.)
GPMT		93% (624 chem.)	90.5% (107 chem.)	77.4% (403 chem.)
Patch-test			92.1% (24 chem.)	78.3% (40 chem.)
LLNA				88.5% (296 chem.)



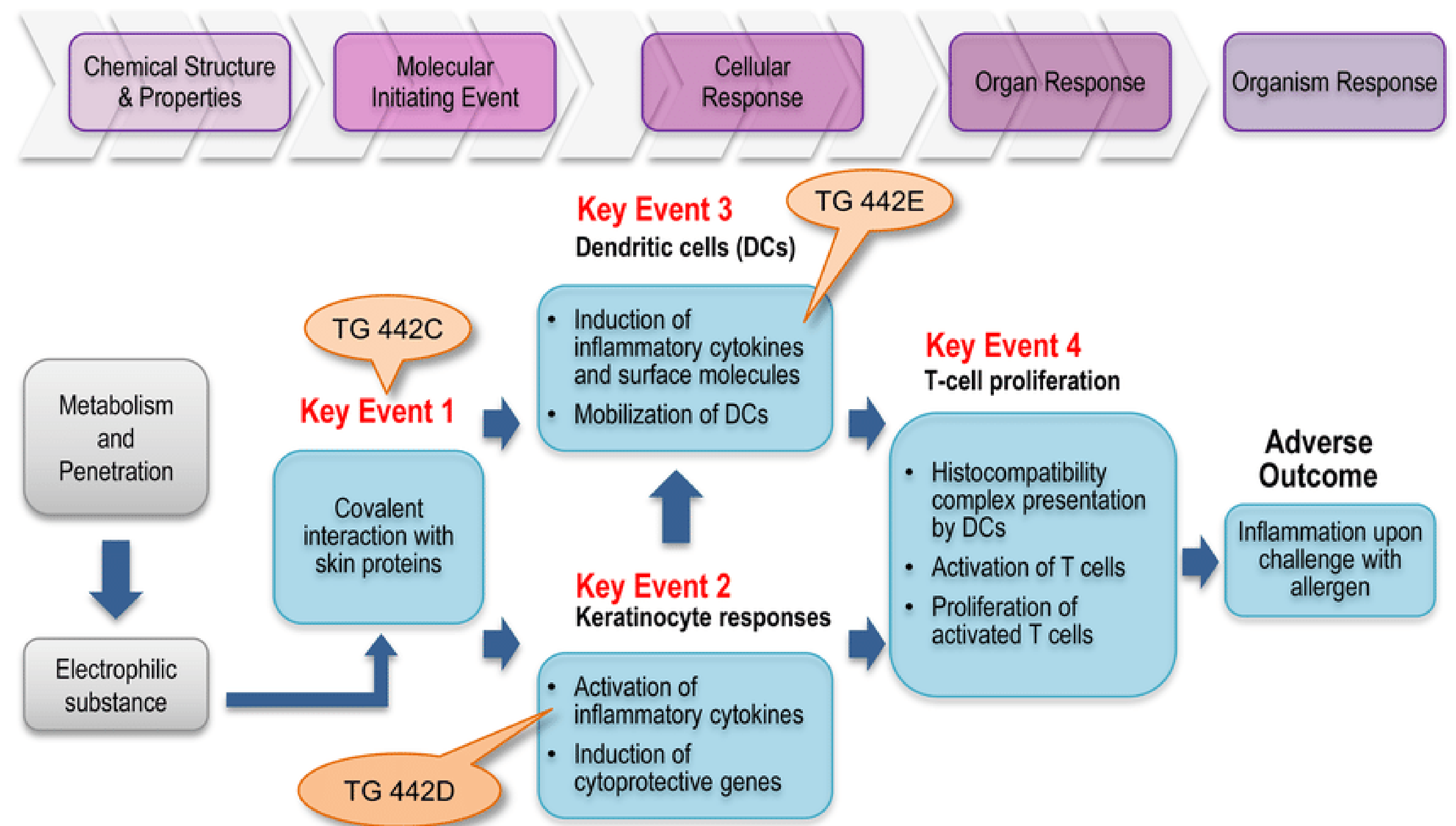
Research article

Probabilistic hazard assessment for skin sensitization potency by dose–response modeling using feature elimination instead of quantitative structure–activity relationships

Thomas Luechtefeld, Alexandra Maertens, James M. McKim, Thomas Hartung✉, Andre Kleensang, Vanessa Sá-Rocha

We think of each event in the AOP mechanistically, but in fact for each event is highly dependent on

- Potency factor of chemicals
- Exposure patterns
- Genetic background
- Stochastic element





... **structure will never be enough**



Structure provides information about molecular properties - reactivity, protein binding, mechanism.

But both potency and individual response emerge from the interaction of those properties with biological context.

QSAR (and other structure-based approaches) gives us the chemistry; **we need to add the biology**



Structure Gives Us Different Information in Different Contexts



1. Structure → Consistent Potency, Variable Response

- Cyanide, some direct-acting toxicants
- Biological variability can be understood mechanistically (e.g. organophosphates there is a 40-fold difference in efficiency of detoxifying enzymes)
- **Chemical similarity metrics that focus on RFG will predict potency**

2 Structure → Reactivity Potential, Context-Dependent Potency

Skin sensitizers, systemic toxicity

- Structure predicts electrophilicity/reactivity
- But potency depends on:
 - Penetration (skin barrier)
 - Bioactivation (local enzymes)
 - Protein binding (tissue context)
 - Immune competency (individual status)
- **QSAR can't predict potency, only reactive potential** - and this can't be fixed easily be fixed with better chemoinformatics that focuses on reactive functional groups.



Mechanism matters for variability



Direct DNA damage → Low variability (works in all cells)

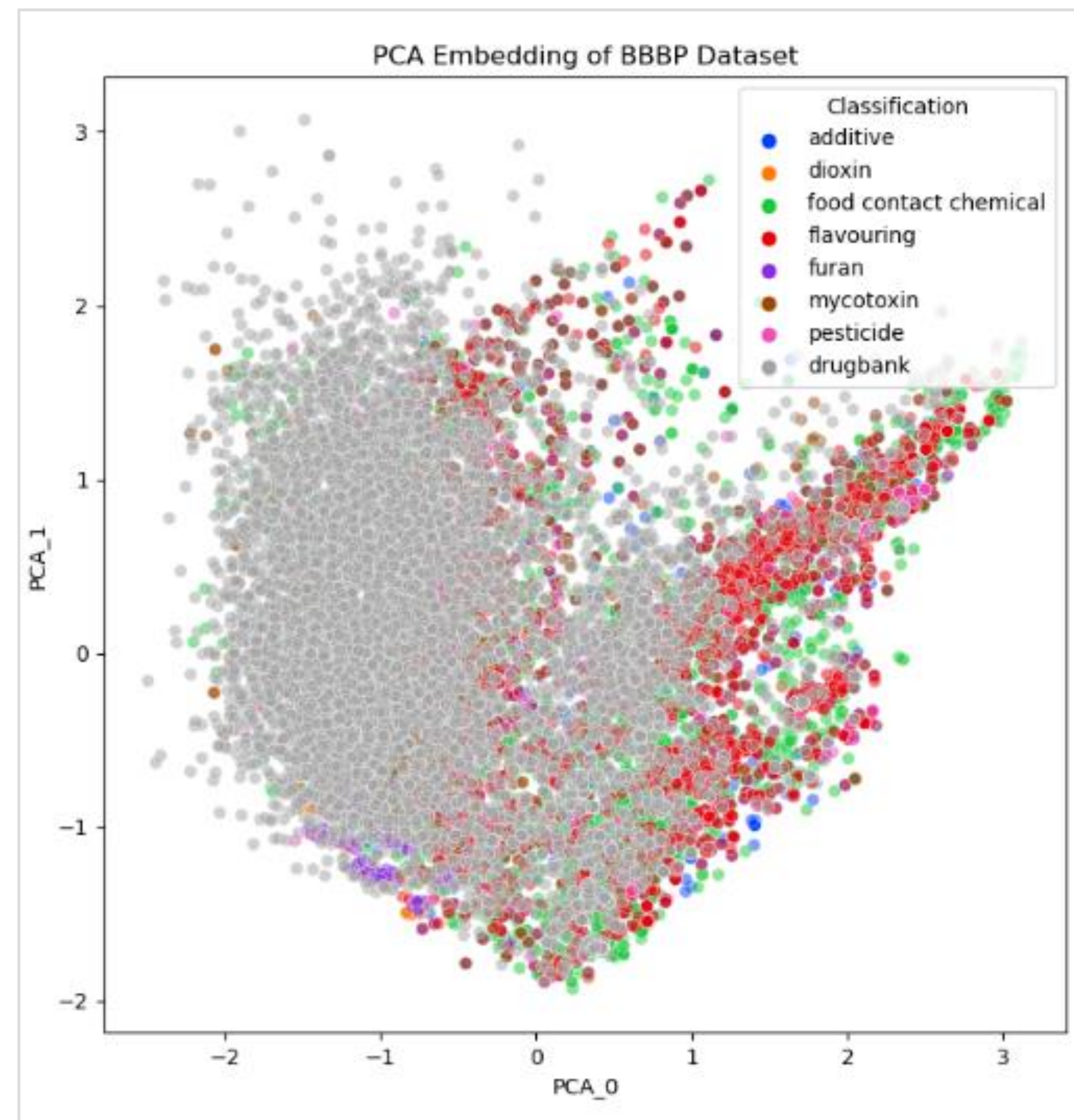
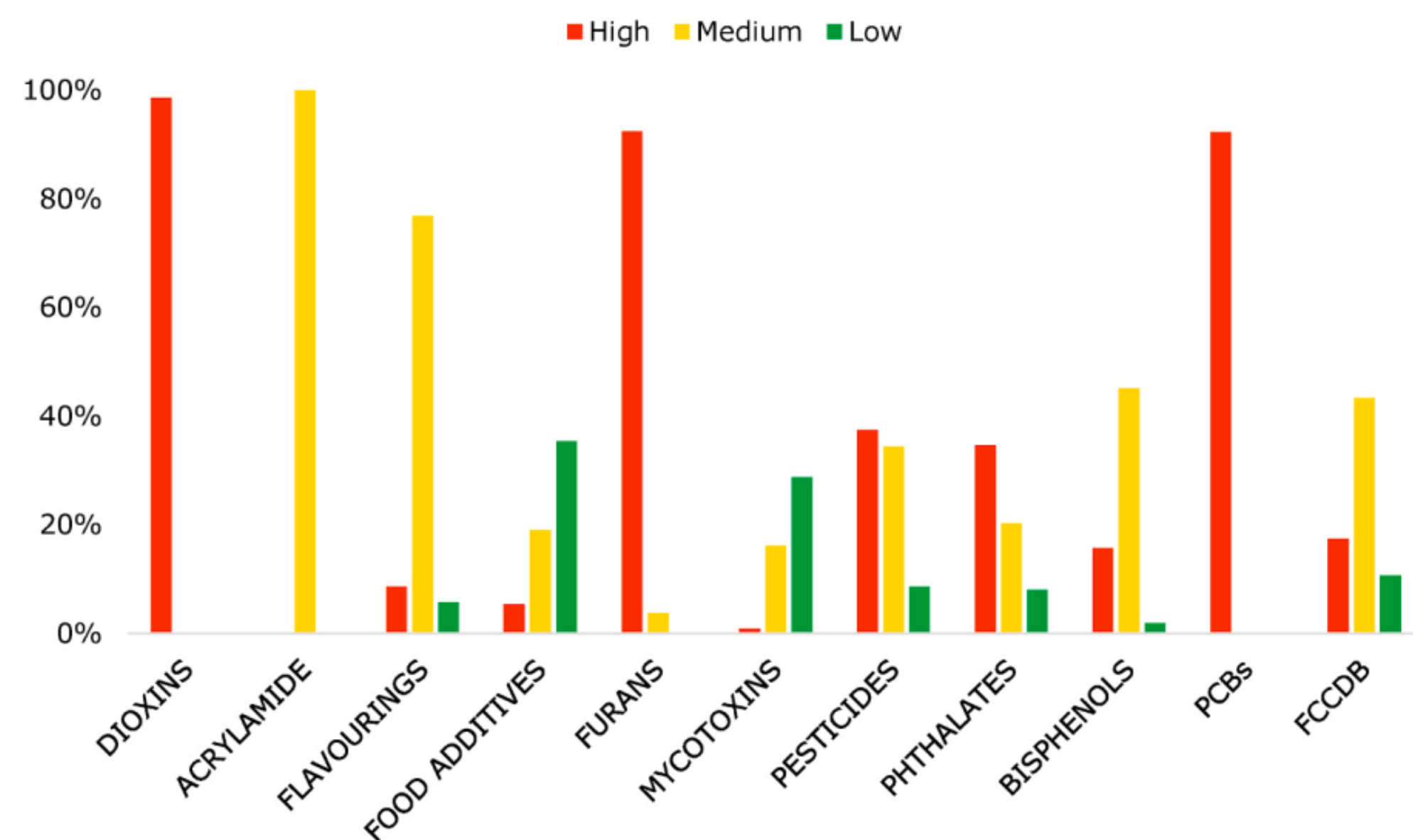
Receptor-mediated → Moderate variability (receptor polymorphisms and biological context)

Metabolic activation → Moderate variability (enzyme polymorphisms)

Immune-mediated → Very high (HLA types, immune status)

Probability of Hazard

Uncertainty surrounding molecular initiating event – many chemicals in a food contact database could bind to greater than 15 nuclear receptors



Endocrine Disruption

© 2016 DBCLS TogoTV / CC-BY-4.0

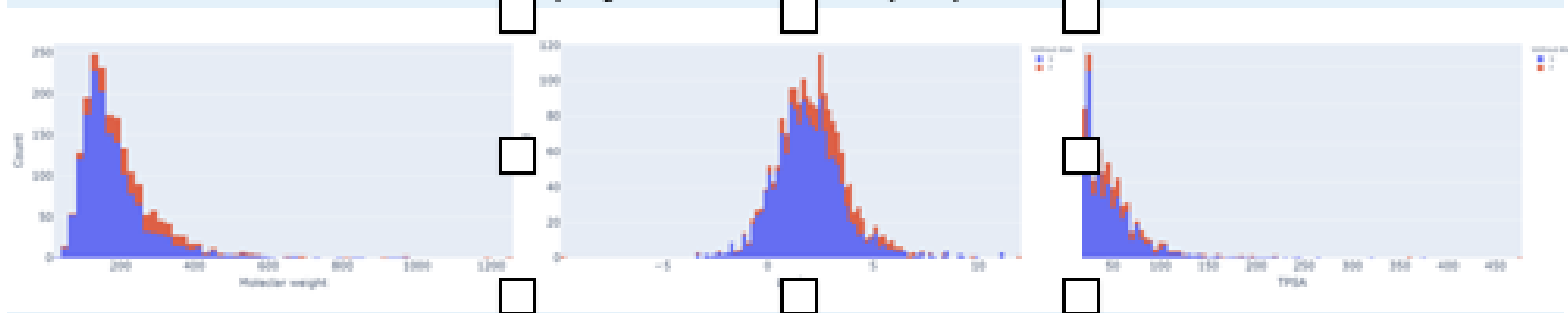
Table 1. Endpoints of each model

ER	AR	TR	Steroidogenesis
■ : Assay results not included NVS_NR_bER, NVS_NR_hER, NVS_NR_mERa, OT_ER_ERaERa_0480, OT_ER_ERaERa_1440, OT_ER_ERaERb_0480, OT_ER_ERaERb_1440, OT_ER_ERbERb_0480, OT_ER_ERbERb_1440, OT_ERa_GFPERaERE_0120, OT_ERa_GFPERaERE_0480, TOX21_ERa_BLA_Agonist_ratio, ACEA_ER_80hr, TOX21_ERa_BLA_Antagonist_ratio	NVS_NR_hAR, NVS_NR_cAR, NVS_NR_rAR, OT_AR_ARSRC1_0480, OT_AR_ARSRC1_0960, ATG_AR_TRANS, OT_AR_ARELUC_AG_1440, TOX21_AR_BLA_Agonist_ratio, TOX21_AR_LUC_MDAKB2_Agonist, TOX21_AR_BLA_Antagonist_ratio, TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881	TOX21_TR_LUC_GH3_Agonist, TOX21_TR_LUC_GH3_Antagonist, CCTE_Simmons_AUR_TPO, CCTE_Simmons_GUA_TPO, CCTE_GLTED_hDIO1, CCTE_GLTED_hDIO2, CCTE_GLTED_hDIO3, CCTE_GLTED_hIYD, CCTE_GLTED_hTPO, CCTE_GLTED_hTTR, TOX21_TSHR_HTRF_Agonist_ratio, TOX21_TSHR_HTRF_Antagonist_ratio, TOX21_TSHR_wt_Agonist_HTRF_ratio, NVS_NR_hTRa_Antagonist, <u>TOX21_TR_LUC_GH3_Antagonist_viability</u>, <u>TOX21_TRHR_HEK293_agonist</u>, CPHEA_Stoker_NIS_Inhibition_RAIU, CPHEA_Stoker_NIS_Cytotoxicity, NVS_GPCR_rTRH, CCTE_Simmons_CellTiterGLO_HEK293T, <u>CCTE_Simmons_Quantilum_inhib_2</u>	H295R cell assay Progestagens: <u>17a-hydroxypregnenolone</u>, progesterone, <u>17a-hydroxyprogesterone</u> Corticosteroids: <u>11-deoxycortisol</u>, Corticosterone, Cortisol, Deoxycorticosterone Androgens: Androstenedione, Testosterone Estrogens: Estrone, Estradiol CYP19A1 ERF_PL_ENZ_hCYP19A NVS_ADME_hCYP19A1

Endocrine Disruption

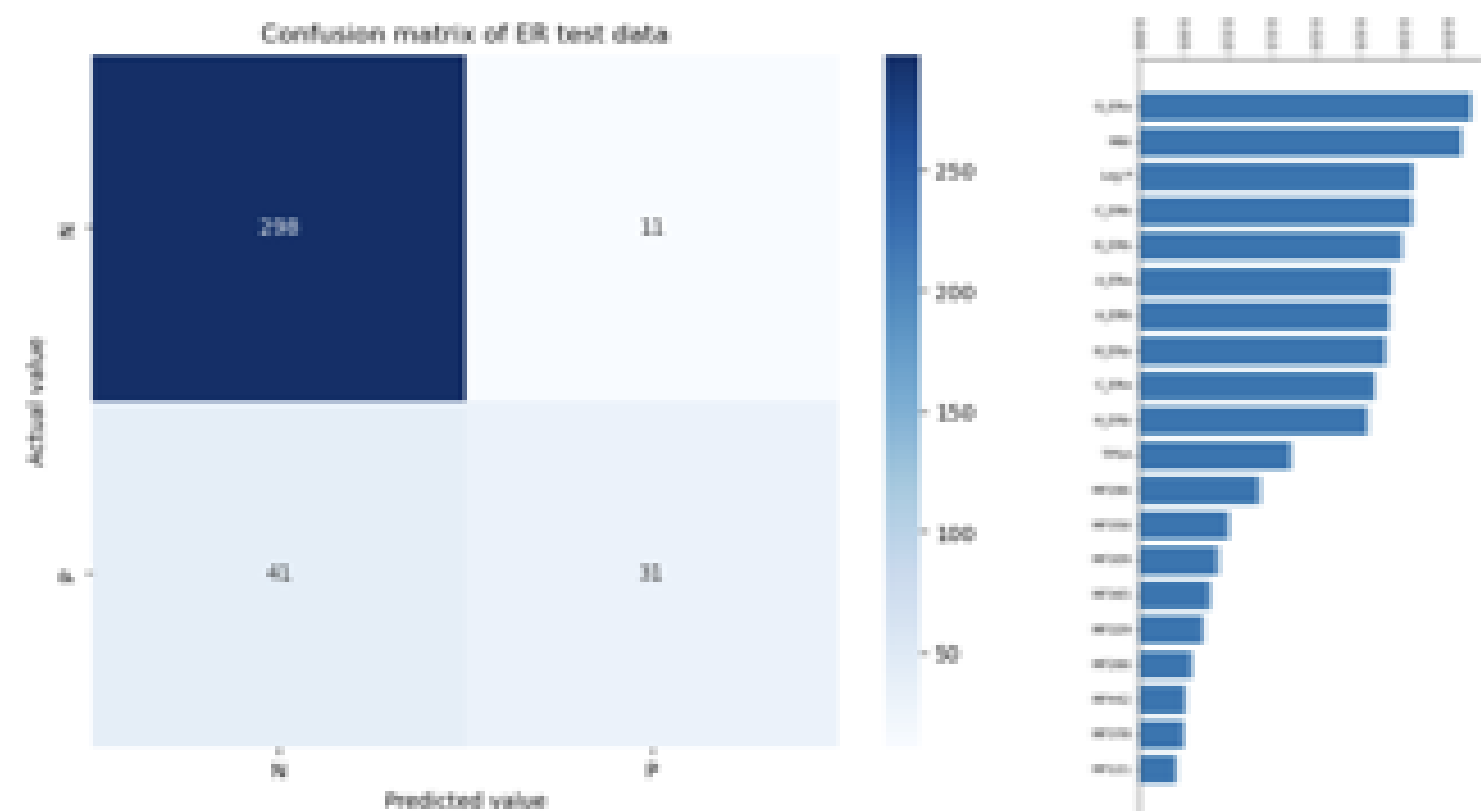
ER Model Results

Distribution of physicochemical properties in data set



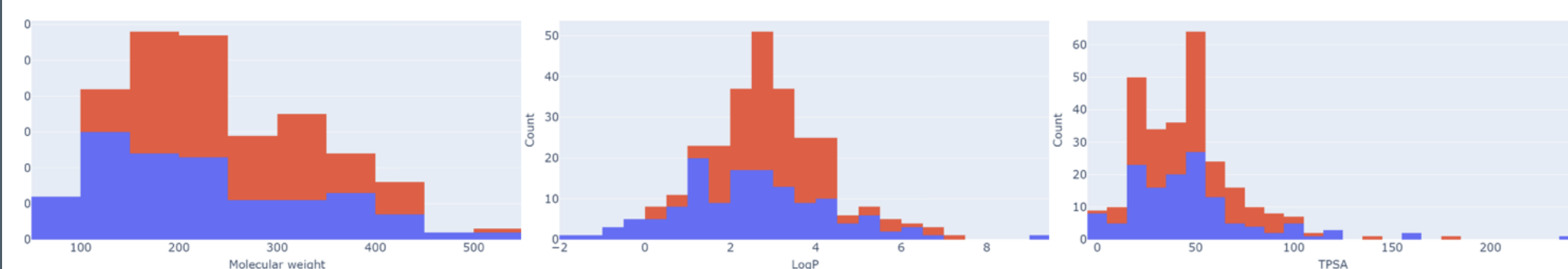
Results of model performance evaluation by test set

Item		Score
Accuracy		0.86
AUC		0.79
N	Precision	0.88
	Recall	0.96
	F-1	0.92
P	Precision	0.74
	Recall	0.43
	F-1	0.54
Balanced Accuracy		0.70



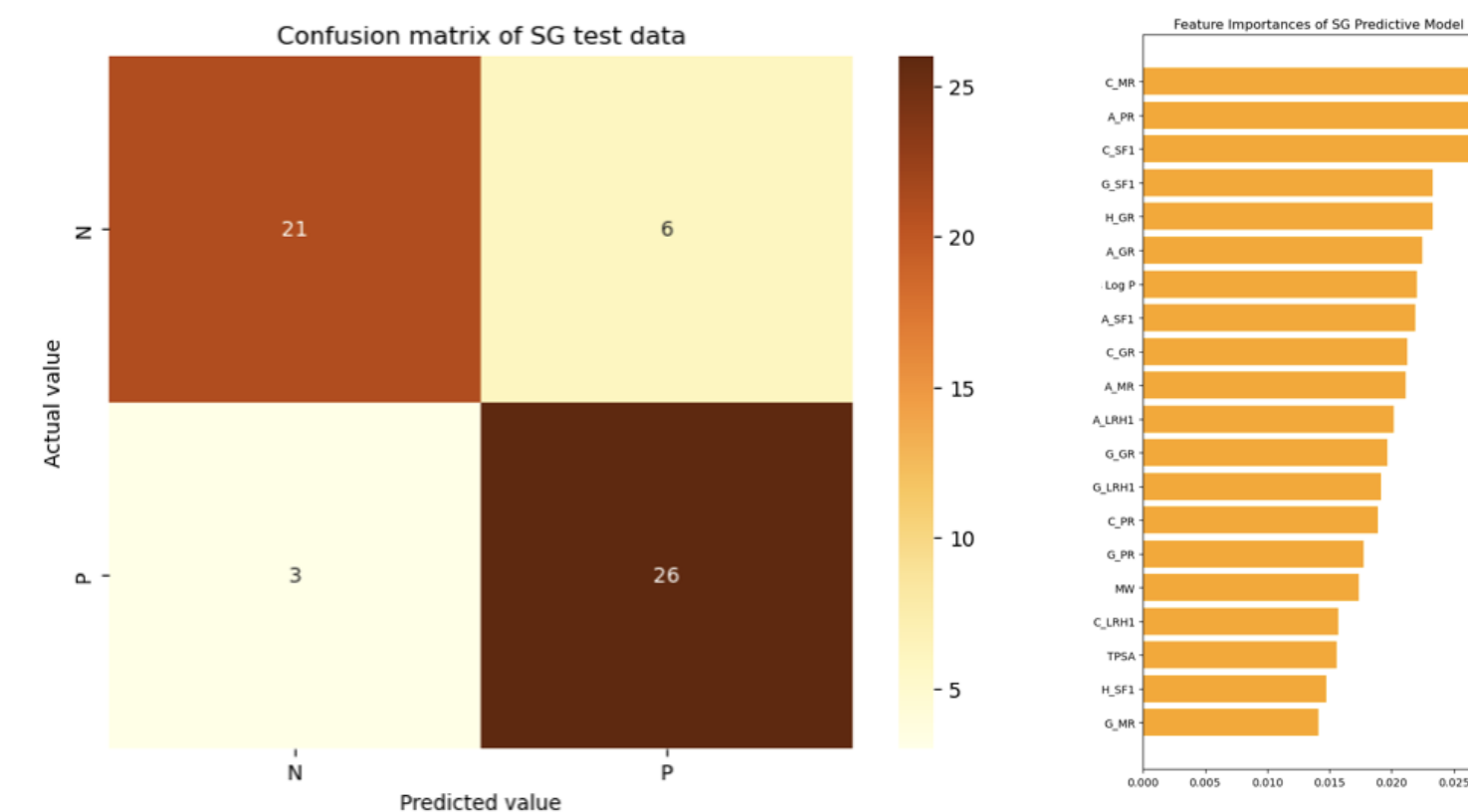
SG Model Results

Distribution of physicochemical properties in data set



Results of model performance evaluation by test set

Item		Score
Accuracy		0.84
AUC		0.90
N	Precision	0.88
	Recall	0.78
	F-1	0.82
P	Precision	0.81
	Recall	0.90
	F-1	0.85
Balanced Accuracy		0.84



Mechanism grounded models



High predictive value for simple pathways (steroidogenesis). . . but lower for more complicated pathways.

Some *in vitro* endpoints were too noisy (e.g. transcriptomics)

Binding predictions alone performed poorly, but combined with other data (and multiple predictions for the same target) **binding data consistently was the top feature**

Random Forest outperformed neural nets and deep learning based methods - **for sparse toxicology datasets, simpler models often win.**

Can Transfer Learning Make Models More Biologically Grounded?

✓ What It CAN Do

- **Better mechanistic predictions** - identify protein targets, metabolic pathways, reactive metabolites
- **Flag high-variability chemicals** - learn patterns like "metabolism-dependent = high variability"
- **Improved uncertainty quantification** - better epistemic uncertainty estimates
- **Enable read-across for variability** - group chemicals by likely variability profiles

✗ What It CANNOT Do (Yet)

- **Predict individual variability** without training data that includes response distributions
- **Create information that wasn't measured** - if variance isn't in the data, models can't learn it
- **Replace explicit biological modeling** - needs mechanistic grounding, not just patterns
- **Overcome biased data** - amplifies existing gaps in who/what we measure



Probability of biological effect: Will target tissue concentrations lead to an adverse outcome?

What We Measure In Vitro

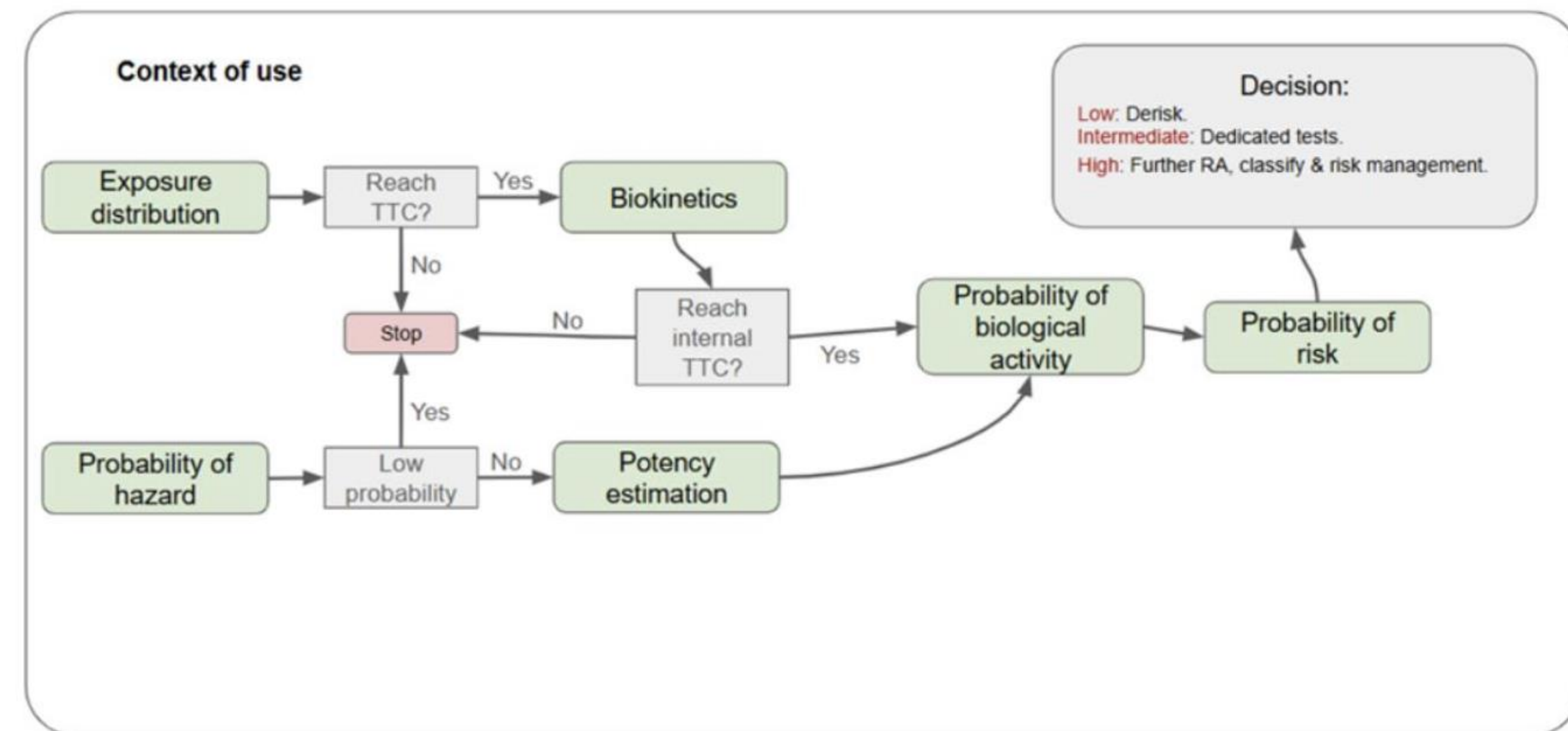
- Receptor binding
- Gene expression changes
- Pathway perturbations
- Cell viability/stress markers
- High-throughput screening hits



THE GAP

Apical Endpoints We Care About

- Systemic toxicity
- Organ damage
- Disease
- Developmental effects
- Reproductive harm



We Need: Quantitative AOPs

Why This Gap Is Hard to Bridge

- **In vitro data is noisy** - thousands of genes change, which matter? High false positive rates in HTS
- **Adaptive vs. adverse** - receptor binding $\neq$ toxicity; gene expression changes $\neq$ disease
- **No established thresholds** - what magnitude of perturbation causes harm?



> ALTEX. 2025;42(3):413-434. doi: 10.14573/altex.2501291. Epub 2025 May 26.

From cellular perturbation to probabilistic risk assessments

Alexandra Maertens¹, Breanne Kincaid¹, Eric Bridgeford², Celine Brochot³, Arthur de Carvalho E Silva⁴, Jean-Lou C M Dorne⁵, Liesbet Geris^{6,7}, Trine Husøy⁸, Nicole Kleinstreuer⁹, Luiz C M Ladeira^{10,11}, Alistair Middleton¹², Joe Reynolds¹², Blanca Rodriguez¹³, Erwin L Roggen¹⁴, Giulia Russo¹⁵, Kris Thayer¹⁶, Thomas Hartung¹

Class Based Assessments

Flame retardants exposures are ubiquitous and can be very high in children
Over 500 discrete chemicals currently identified as flame retardants

[Front Toxicol.](#) 2023; 5: 1216802.

Published online 2023 Oct 16. doi: [10.3389/ftox.2023.1216802](https://doi.org/10.3389/ftox.2023.1216802)

PMCID: PMC

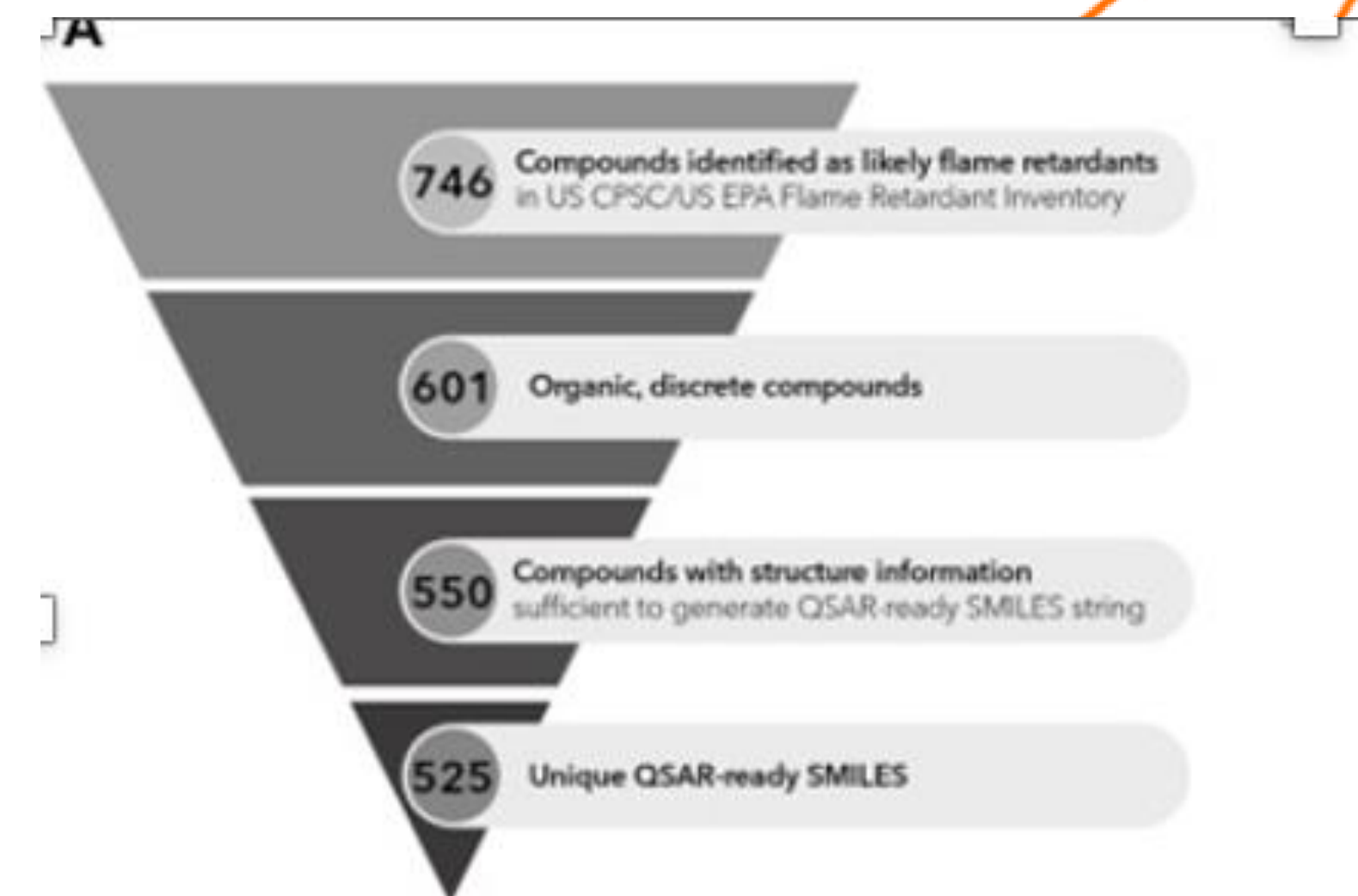
PMID:

Using *in silico* tools to predict flame retardant metabolites for more informative exposomics-based approaches

[Breanne Kincaid](#),¹ [Przemyslaw Piechota](#),¹ [Emily Golden](#),¹ [Mikhail Maertens](#),¹ [Thomas Hartung](#),^{1,2} and [Alexandra Maertens](#)^{1,*}



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C

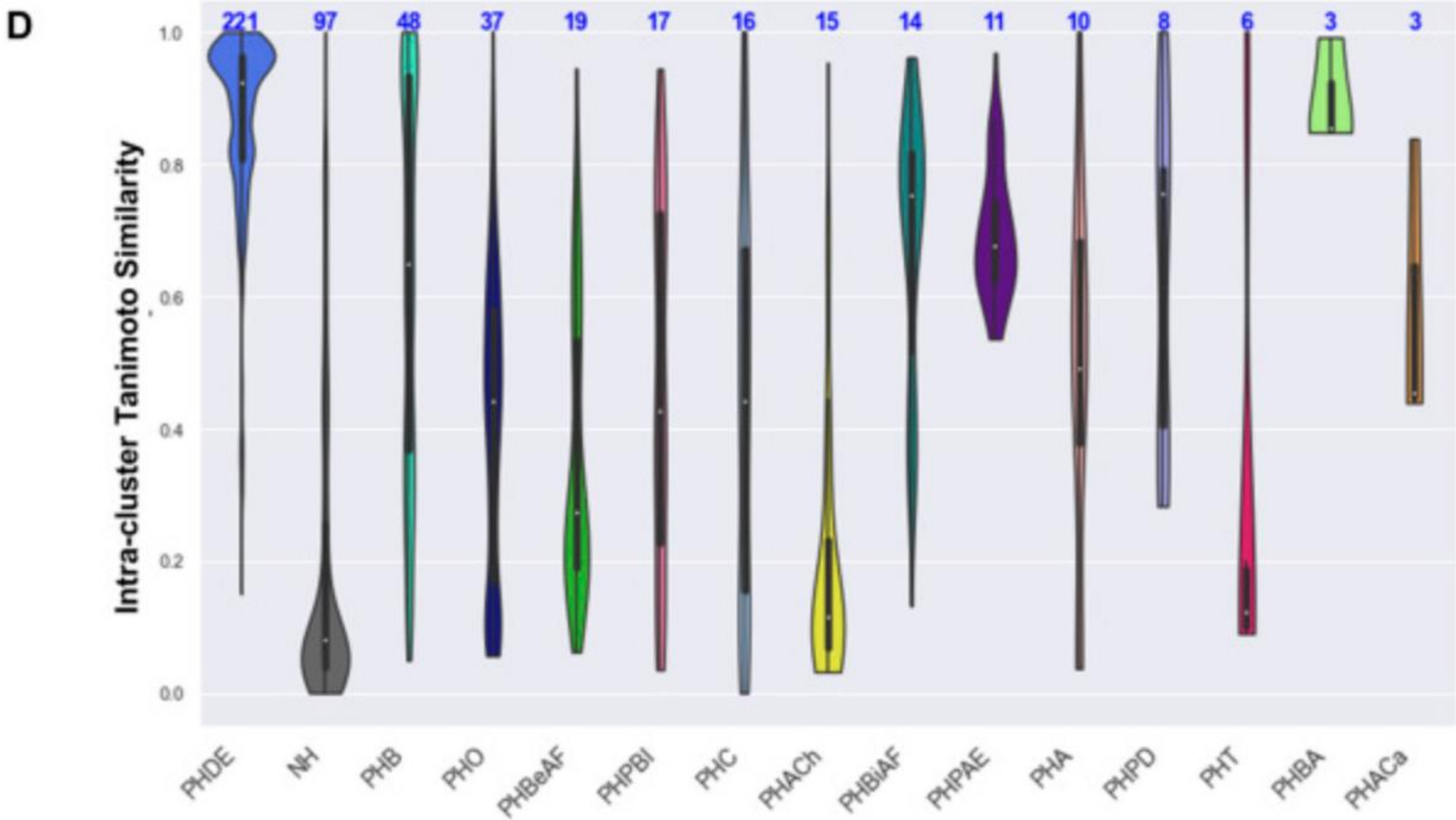
B

Flame Retardant Parent Compound Subclass	Abbreviation
Nonhalogenated	NH
polyhalogenated alicycles	PHA
polyhalogenated aliphatic carboxylate	PHACa
polyhalogenated aliphatic chains	PHACh
polyhalogenated benzene alicycles	PHBA
polyhalogenated benzene aliphatics and functionalized	PHBeAF
polyhalogenated benzenes	PHB
polyhalogenated bisphenol aliphatics and functionalized	PHBiAF
polyhalogenated carbocycles	PHC
polyhalogenated diphenyl ethers	PHDE
polyhalogenated organophosphates	PHO
polyhalogenated phenol derivatives	PHPD
polyhalogenated phenol-aliphatic ether	PHPAE
polyhalogenated phthalates/benzoates/imides	PHPBI
polyhalogenated triazines	PHT



Class based risk assessment can obscure significant differences within a class as well as similarities between classes

Exposure data is often class-based vs chemical-based



[Front Toxicol.](#) 2023; 5: 1216802. PMCID: PMC
Published online 2023 Oct 16. doi: [10.3389/ftox.2023.1216802](https://doi.org/10.3389/ftox.2023.1216802) PMID:

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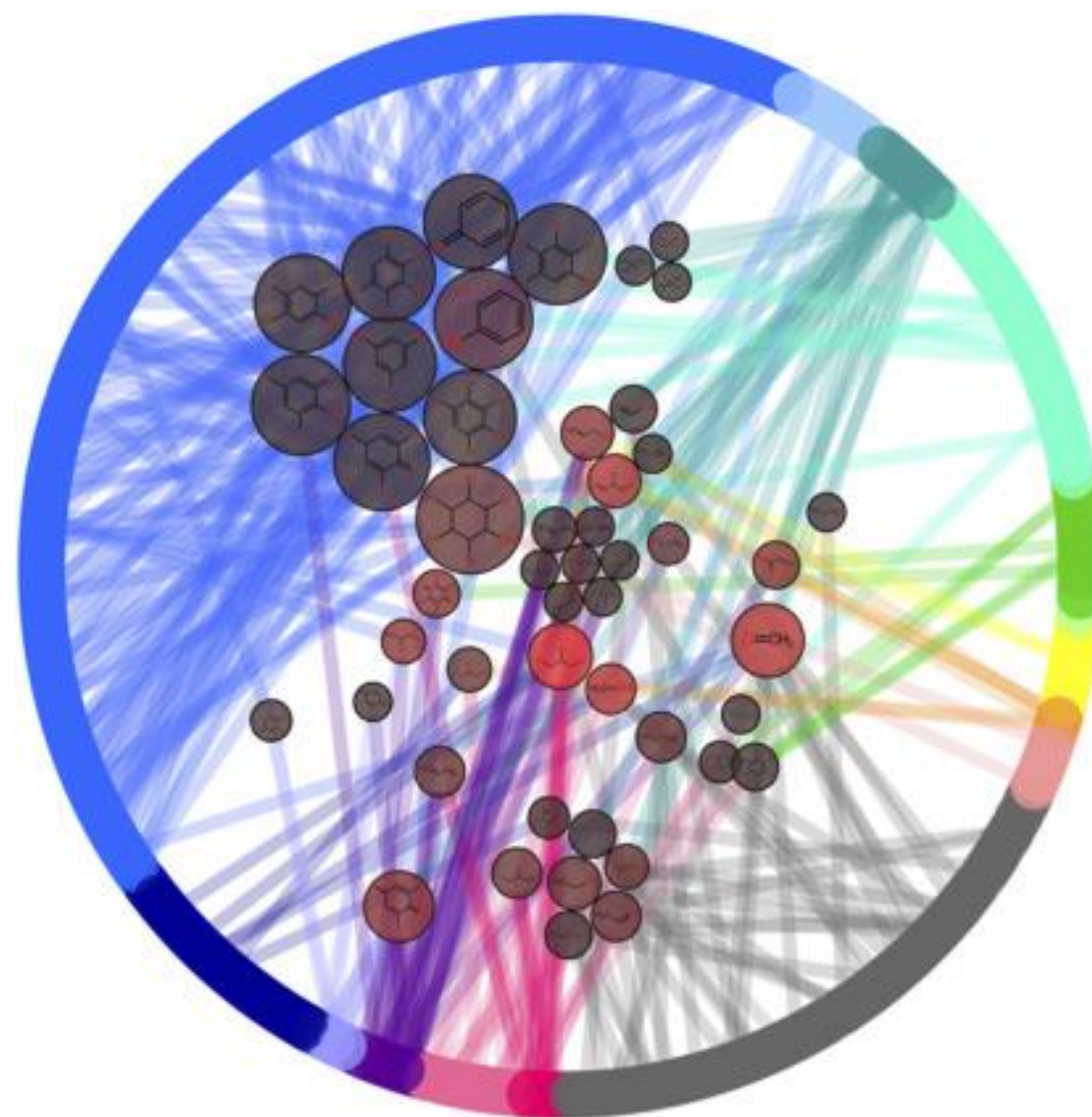
[Breanne Kincaid](#), ¹ [Przemyslaw Piechota](#), ¹ [Emily Golden](#), ¹ [Mikhail Maertens](#), ¹ [Thomas Hartung](#), ^{1,2} and [Alexandra Maertens](#) ^{1,*}

Exposomics: Metabolites

There is an extraordinary diversity of metabolites in some chemical classes – **many flame retardants are metabolized into other flame retardants**

Many of the metabolites were verified by a literature or database search – **chemoinformatics can help guide exposure science**

Phase II metabolism was more likely



Using *in silico* tools to predict flame retardant metabolites for more informative exposomics-based approaches

Breanne Kincaid¹, Przemyslaw Piechota¹, Emily Golden¹, Mikhail Maertens¹,
Thomas Hartung^{1,2}, Alexandra Maertens¹

Current Framework

Probability of Hazard

In silico / In chemico

- QSAR → Single LD50 value
- Reactivity assays → Single EC50
- Model prediction intervals only
- **Epistemic uncertainty** (how sure is the model?)

Probability of Biological Effect

In vitro / Biomarkers / ToxCast

- Cell-based dose-response curves
- Population-level associations
- Cell line variability
- **Missing individual variability**

Proposed Framework

Probability of Hazard

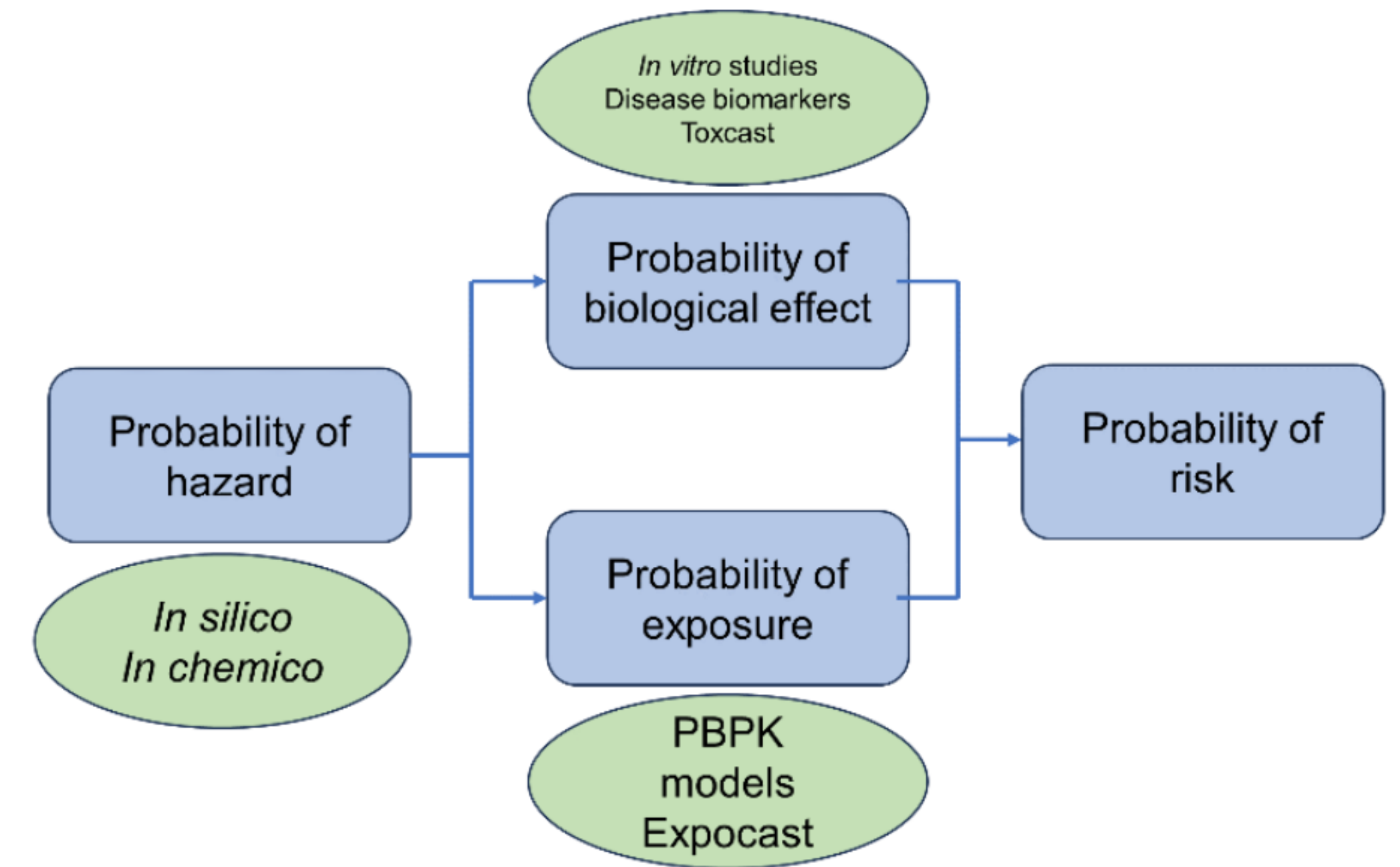
Mechanism-informed

- Structure → **Mechanism** → Potency
- Flags for high biological variability
- Separate chemical potency from population response
- **Epistemic + variability flags**

Probability of Biological Effect

Population-based

- **Population response distribution**
- Genetic polymorphisms (e.g., PON1, CYP450)
- Context-dependent factors (barrier, immune status)
- **Aleatoric uncertainty** (biological variability)





Multi-scale modeling

(Q)SAR → Molecular interactions vs complicated endpoints

PBPK → Tissue concentrations and population variance

Systems biology → Pathway perturbations considering genetic variability and biological context

Population model → Distribution of outcomes





Biased Data



Data doesn't just appear - someone has to decide **who counts**, what we **quantify**, and how **we encode that**

Because of that - **all data is biased**. We can be more thoughtful about this fact but we cannot get rid of it

AI often makes this less obvious.

For respiratory sensitization: Is asthma encoded with equal precision for men vs women? For patients of different backgrounds? Different occupations and exposure scenarios? How useful is an encoding of asthmatic/non-asthmatic?



Challenges: AI



A Can BPA be linked to respiratory sensitization?

Yes, there is scientific evidence suggesting BPA (bisphenol A) can be linked to respiratory sensitization and related allergic responses.

A Can lead be linked to respiratory sensitization?

Yes, lead exposure has been linked to respiratory sensitization and various respiratory health effects, though the mechanisms differ somewhat from other environmental toxins.

A Can mercury be linked to respiratory sensitization?

Mercury exposure has been associated with respiratory sensitization and allergic though the evidence is more complex and varied compared to some other environmental toxins.

A Can PCBs be linked to respiratory sensitization?

Yes, PCBs (polychlorinated biphenyls) have been linked to respiratory sensitization and various respiratory health effects through multiple studies.



Challenges: AI



Image:Lesswrong.com

AI: Challenges - Mining the Literature



Illuminating how chemicals affect human health.

Comparative Toxicogenomics Database

Home

Search

Analyze

Download

Commercial Users

Help

 Respiratory Hypersensitivity

Basics

Chemicals

Genes

Phenotypes

Comps

Pathways

Exposure Studies

Exposure Details

References

These chemicals are associated with *Respiratory Hypersensitivity* or its descendants. A chemical has either a curated association to the disease ( marker/mechanism and/ or tion.

Filter by

Association type

curated

Filter

1-50 of 471 results.

First

Previous

1

2

3

4

5

6

7

8

Next

Last

	Chemical	Disease	Direct Evidence	Enrichment Analysis
1.	Dust	Asthma		  
2.	Ovalbumin	Asthma		  
3.	Toluene 2,4-Diisocyanate	Asthma		  
4.	Nitrogen Dioxide	Asthma		  
5.	Antigens, Dermatophagoides	Asthma		  
6.	Carbon Monoxide	Asthma		  
...				
	Dietary Fats	Asthma		  

AI Challenges - Mining the Literature

- Inferred connections between asthma and 23,457 chemicals

These chemicals are associated with *Respiratory Hypersensitivity* or its descendants. A chemical has either a curated association to the disease (M marker/mechanism and/or T the

Filter by

Association type

inferred only

Filter

1-50 of 23,457 results.

First

Previous

1

2

3

4

5

6

7

8

Next

Last

	Chemical	Disease	Direct Evidence	Enrichment Analysis
1.	4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)imidazole	Asthma		  

- How many genes does Bisphenol A interact with? - Most of the human genome!

Comparative Toxicogenomics Database

Home

Search

Analyze

Download

Commercial Users

Help

←

Revise query:

CHEMICAL=name:bisphenol A

&

ORGANISM=TAXON:9605

Gene Query

1-50 of 18,999 results.

First

Previous

1

2

3

4

5

6

7

8

Next

Last

1.

A1BG

alpha-1-B glycoprotein

How many diseases is it implicated in? 5,830

Filter by

Disease category

ALL

Association type

ALL

Filter

1-50 of 5,830 results.

First

Previous

1

2

3

4

5

6

7

8

Next

Last

	Disease	Direct Evidence	Enrichment Analysis	Inference Network	Inference Score	References
1.	Liver Cirrhosis, Experimental	M	GO	829 genes	551.29	97
2.	Prostatic Neoplasms	M	GO	630 genes	263.80	332
3.	Autism Spectrum Disorder	M	GO	336 genes	209.65	46
4.	Weight Loss	M	GO	49 genes	203.15	73
5.	Necrosis	M	GO	60 genes	193.13	64
6.	Edema	M	GO	32 genes	185.92	35
7.	Kidney Diseases	M	GO	99 genes	138.92	125
8.	Hypotension	M	GO	54 genes	137.34	81
9.	Poisoning	M	GO	16 genes	132.46	20

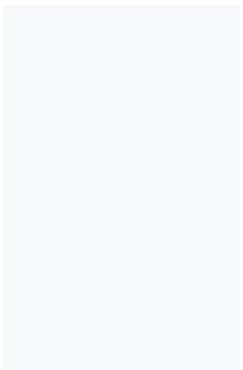
Communicating Risk in the Era of Exposomics, NGRA and Probabilistic Risk Assessment Will Be a Challenge

Pregnancy

Researchers link asthma risk to folic acid during pregnancy

Physician's Weekly
Female Sex Linked With Asthma Prevalence & Control

Jan 4, 2024



The BMJ
Paracetamol use linked to asthma

May 1, 2000



U.S. News & World Report
Night Shift Associated With Asthma Risk In Women

Jun 18



Docwire News
Below-Average Temperatures During Winter Linked to Increased Risk of Asthma

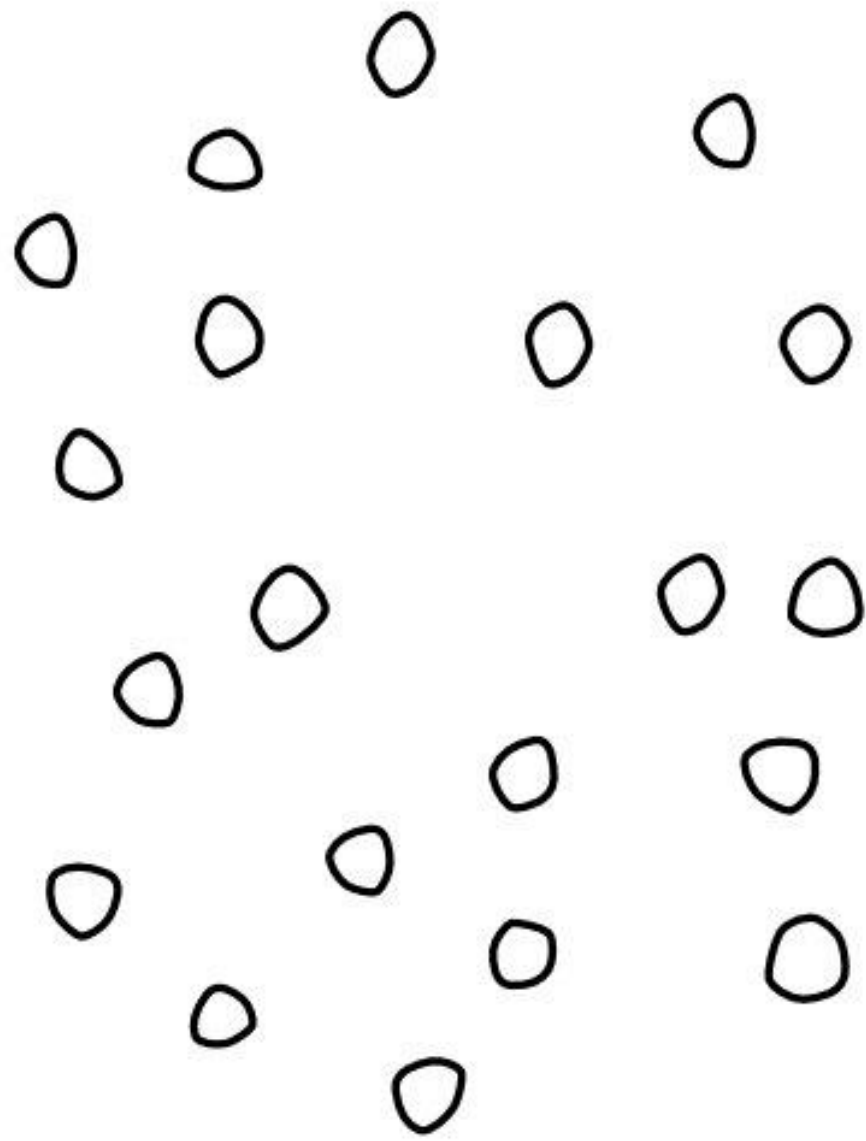


Communicating Risk in the Era of Exposomics, NGRA and Probabilistic Risk Assessment Will Be a Challenge

- As a community, we need to think more carefully about how we report our findings:
 - Emphasize the importance of **casual inference** - "linking" an exposure to an outcome disguises that correlating to variables from (often high-dimensional) data
 - Honest about **uncertainty** and explore ways to meaningfully communicate this
 - Focus on meaningful health impacts - the difference between a shift in population means vs adversity will be critical going forward

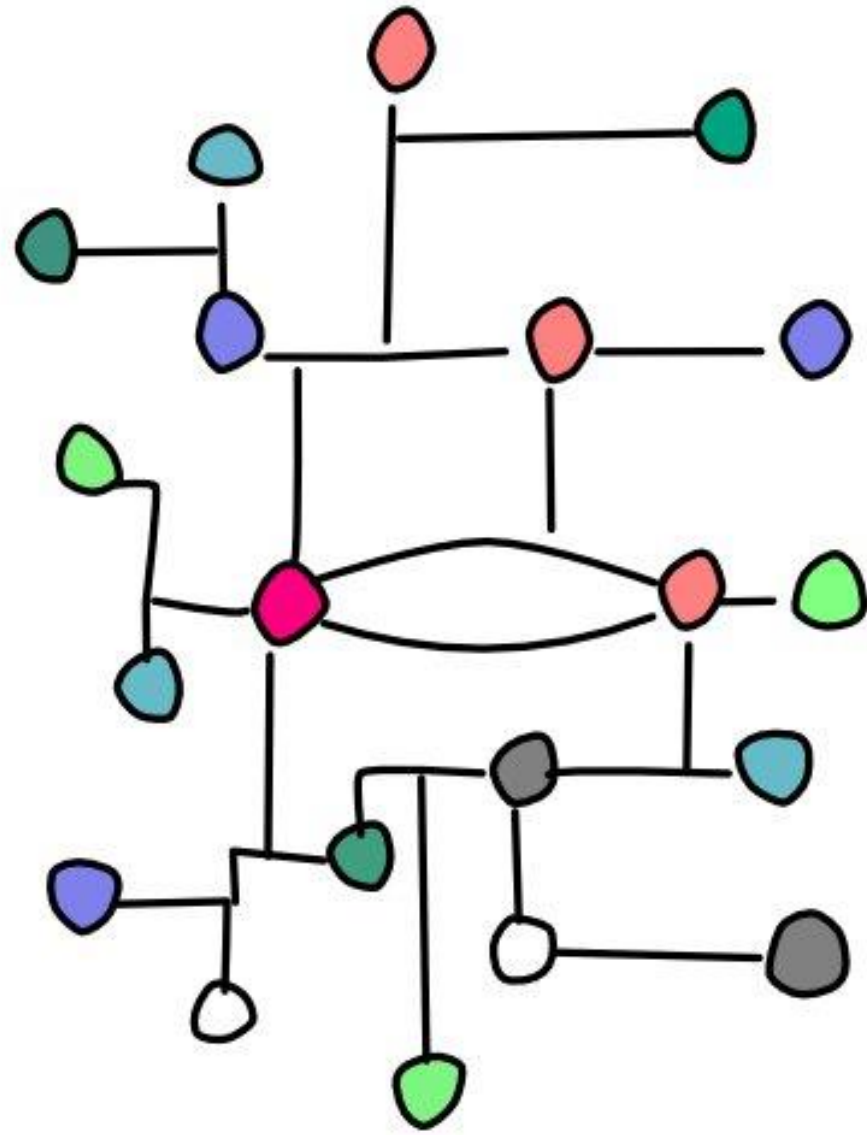
Data isn't enough...

Data



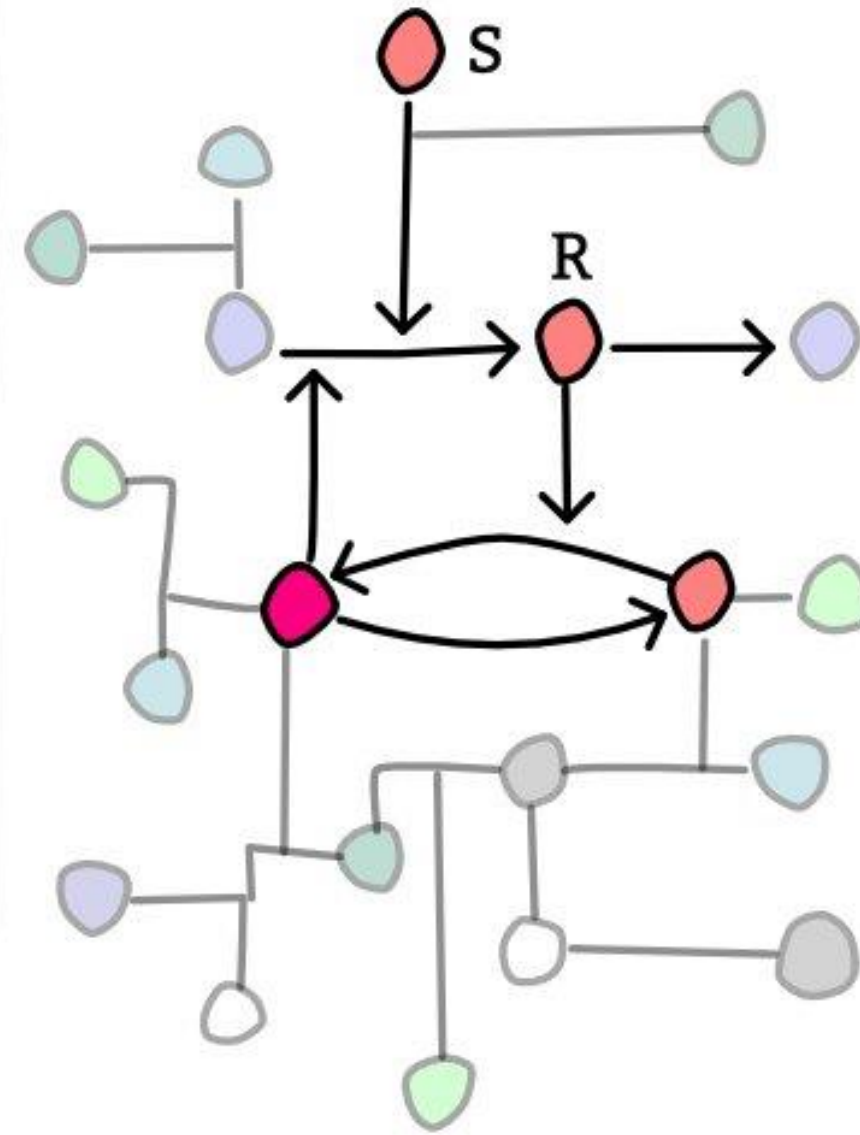
... from a molecular system

Information



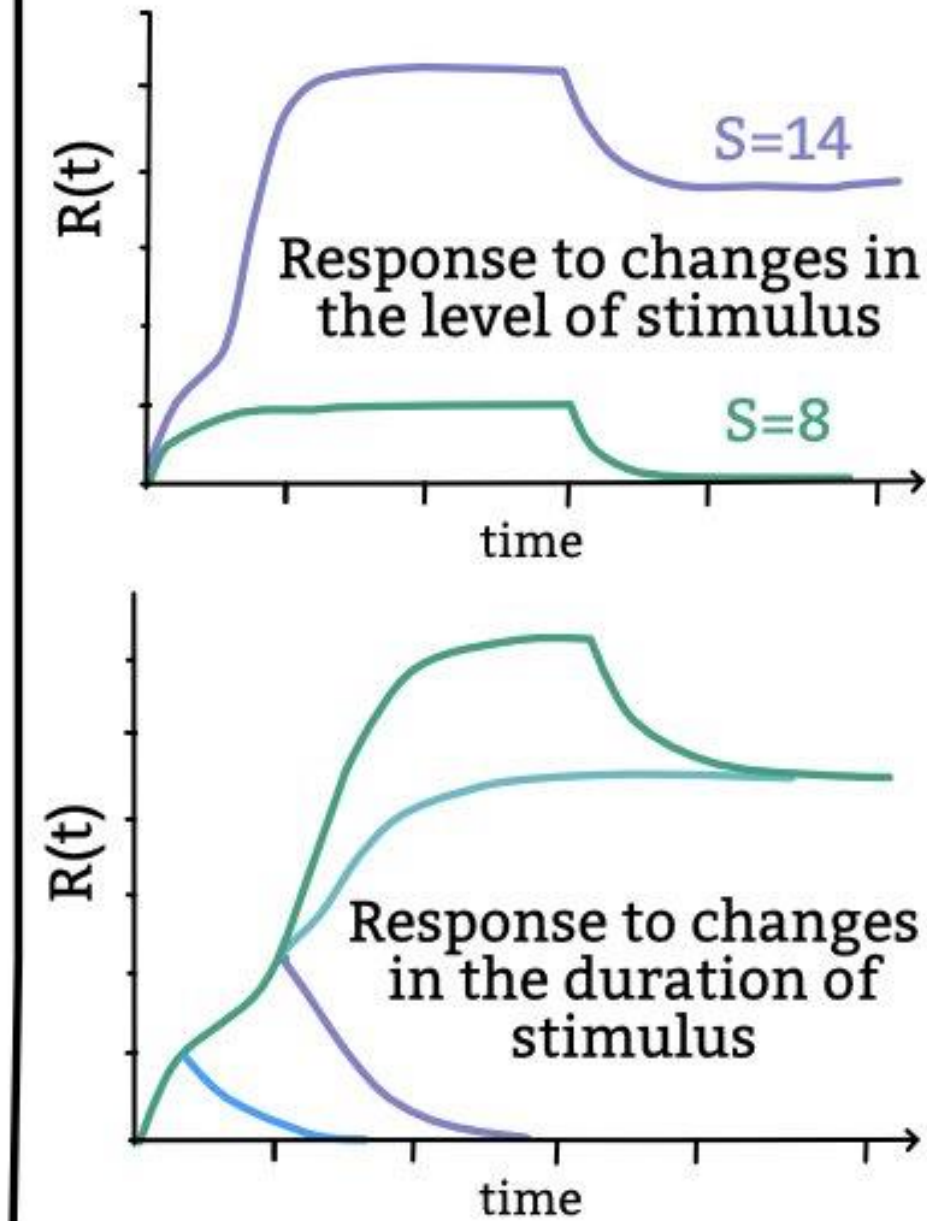
... enriched with bioinformatics

Modelling



... using a systems biology approach

Simulation

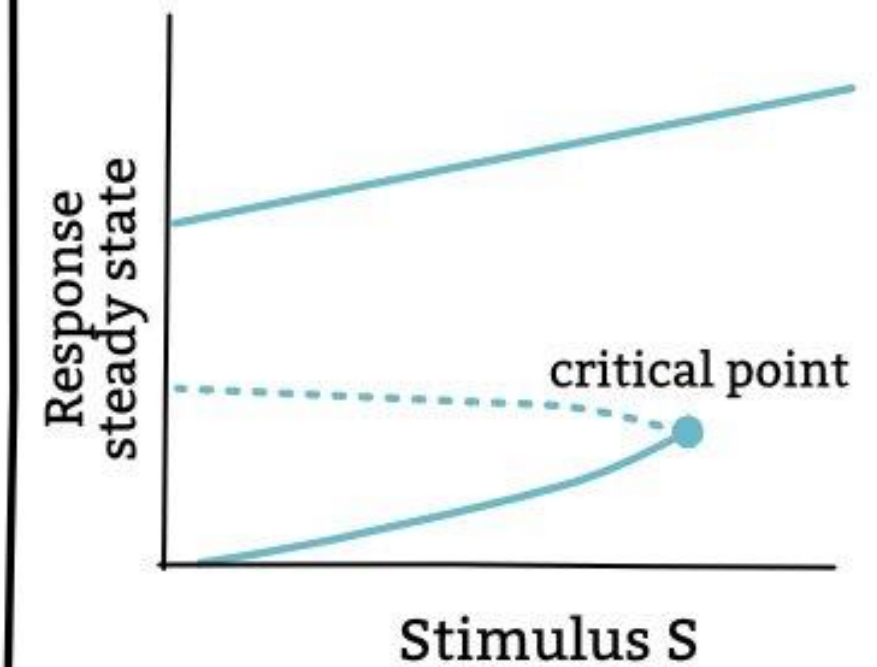


... to understand its (functioning) dynamics

Explanation

„The core regulatory system is driven by a positive *feedback mechanism*.“

„The system is *bistable*: Its behaviour depends on the level and duration of stimulus. Once pushed across a critical point, the behaviour is irreversibly changed.“



@OlafWolkenhauer