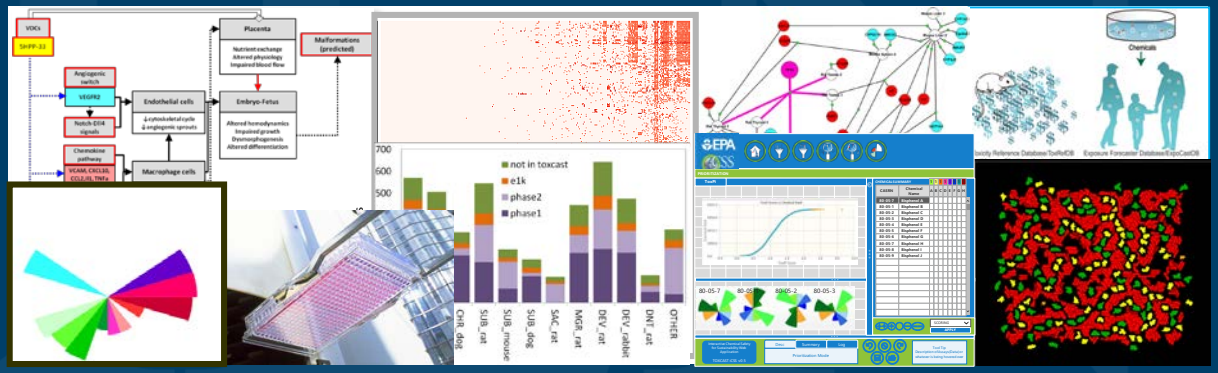
EPA United States Environmental Protection Agency

Moving Towards an Integrated Alternative Testing Paradigm to Identify Carcinogenic Hazards



ToxForum Winter Meeting
January 29, 2018

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Director
National Center for Computational Toxicology

Predicting Chemical Carcinogenesis is a Tall Order

Relevance: High
Amt of Data: Low

Relevance: Low
Amt of Data: High

Limited Predictivity Across Species

TABLE 2. Predictive Value of Carcinogenicity Study Outcomes between Species

N = 259 compounds tested in both sexes: all tumor outcomes[a]

		Carcinogenicity in rat (test)					
		Positive	Negative	% Positive Prevalence	39	% Negative Prevalence	61
Carcinogenicity in mouse (outcome)	Positive	53	47	% Specificity	68	% Sensitivity	53
	Negative	51	108	% Positive predictivity	51	% Negative predictivity	70
				% Added to positive prevalence	12	% Added to negative prevalence	9
						% Exclusion	60
		Carcinogenicity in mouse (test)					
		Positive	Negative	% Positive Prevalence	40	% Negative Prevalence	60
Carcinogenicity in rat (outcome)	Positive	53	51	% Specificity	70	% Sensitivity	51
	Negative	47	108	% Positive predictivity	53	% Negative predictivity	68
				% Added to positive prevalence	13	% Added to negative prevalence	8
						% Exclusion	61

N = 259 compounds tested in both sexes: only malignant tumor outcomes[a]

		Carcinogenicity in rat (test)					
		Positive	Negative	% Positive Prevalence	21	% Negative Prevalence	79
Carcinogenicity in mouse (outcome)	Positive	10	44	% Specificity	87	% Sensitivity	19
	Negative	26	179	% Positive predictivity	28	% Negative predictivity	80
				% Added to positive prevalence	7	% Added to negative prevalence	1
						% Exclusion	86
		Carcinogenicity in mouse (test)					
		Positive	Negative	% Positive Prevalence	14	% Negative Prevalence	86
Carcinogenicity in rat (outcome)	Positive	10	26	% Specificity	80	% Sensitivity	28
	Negative	44	179	% Positive predictivity	19	% Negative predictivity	87
				% Added to positive prevalence	5	% Added to negative prevalence	1
						% Exclusion	79

Carcinogenicity study data were extracted from the U.S. EPA Toxicity Reference database. Tumor outcomes were identified from the Data Evaluation Records for each chemical prior to expert committee review by the U.S. EPA Office of Pesticide Programs.

[a]There were 259 total chemicals in ToxRefDB with carcincogenicity study data in both species and sexes.

Hill *et al.*, *Tox Sci* 2017

Limited Predictivity Across Time

TABLE 3. Predictive Value of Subchronic Effects for Carcinogenicity Study Outcomes in the Rat and Mouse

N = 169 compounds tested in rat subchronic and rat carcinogenicity studies[a]

		Subchronic effect in rat (test)					
		Positive	Negative	% Positive Prevalence	43	% Negative Prevalence	57
Carcinogenicity in rat (outcome)	Positive	43	29	% Specificity	53	% Sensitivity	60
	Negative	46	51	% Positive predictivity	48	% Negative predictivity	64
				% Added to positive prevalence	6	% Added to negative prevalence	6
						% Exclusion	47

N = 175 compounds tested in rat subchronic and mouse carcinogenicity studies[b]

		Subchronic effect in rat (test)					
		Positive	Negative	% Positive Prevalence	31	% Negative Prevalence	69
Carcinogenicity in mouse (outcome)	Positive	40	15	% Specificity	42	% Sensitivity	73
	Negative	70	50	% Positive predictivity	36	% Negative predictivity	77
				% Added to positive prevalence	5	% Added to negative prevalence	8
						% Exclusion	37

N = 147 compounds tested in rat subchronic and both rat and mouse carcinogenicity studies[c]

		Subchronic effect in rat (test)					
		Positive	Negative	% Positive Prevalence	54	% Negative Prevalence	46
Carcinogenicity in rat or mouse (outcome)	Positive	52	27	% Specificity	50	% Sensitivity	66
	Negative	34	34	% Positive predictivity	60	% Negative predictivity	56
				% Added to positive prevalence	7	% Added to negative prevalence	9
						% Exclusion	44

Subchronic and carcinogenicity study data were extracted from the U.S. EPA ToxRef database. A positive subchronic signal was based on histopathologic risk factors or evidence of hormonal perturbation, as defined in methods. The number of chemicals has been filtered by dose exclusion criteria to avoid subchronic and carcinogenicity studies with large differences in dose ranges.

[a]There were 215 chemicals with rat subchronic and carcinogenicity study data in ToxRefDB. Dose exclusion criteria eliminated 46 chemicals, leaving 169 chemicals for analysis.

[b]There were 186 chemicals with rat subchronic and mouse carcinogenicity study data in ToxRefDB. Dose exclusion criteria eliminated 11 chemicals, leaving 175 chemicals for analysis.

[c]There were 184 chemicals with rat subchronic and both rat and mouse carcinogenicity study data in ToxRefDB. Dose exclusion criteria eliminated 37 chemicals, leaving 147 chemicals for analysis.

Hill *et al., Tox Sci* 2017

Limited Reproducibility Between Studies

Table 4. A comparison of the classification in the NCI/NTP and literature parts of the CPDB.

	Carcinogen[a]	Noncarcinogen[b]	Inadequate[c]	Literature
Carcinogen[a]	39	13	1	53
Noncarcinogen[b]	20	30	0	50
Inadequate[c]	10	8	0	18
NCI/NTP	69	51	1	121

Concordant classification: 69 compounds (57%); discordant classification: 52 compounds (43%).
[a]At least one experiment is evaluated as positive. [b]At least one experiment is evaluated as negative and no experiment is evaluated as positive. [c]Experiments are evaluated neither positive nor negative.

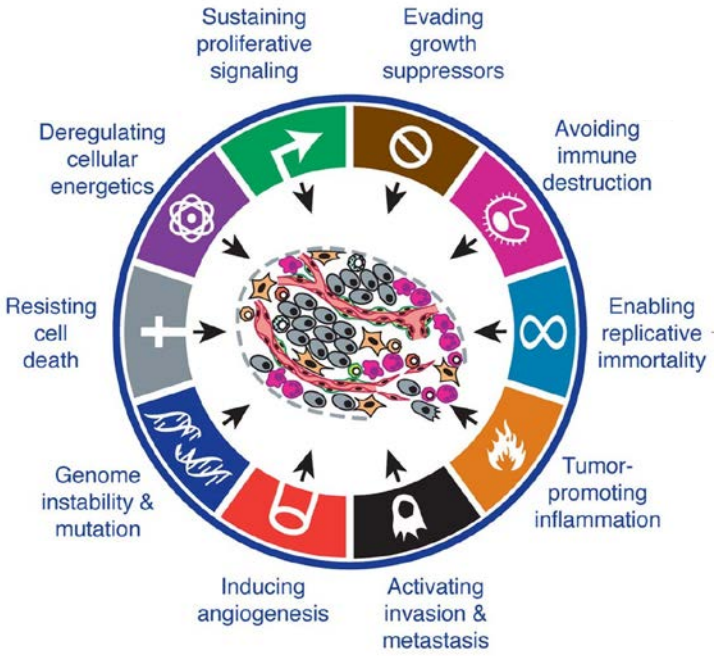
Gottmann *et al., Env Hlth Perspect* 2001

In ToxRefDB, 16 chemicals were run in repeat carcinogenicity studies for rat and mouse. Concordance for any tumor outcome was 69% (11/16 chemicals) for rat and 63% (10/16 chemicals) for mouse repeat studies.

Hill *et al., Tox Sci* 2017

So Now What…

Are Mechanistic and *In Vitro* Approaches the Answer?



Hanahan and Weinberg, *Cell* 2011

Table 1. Key characteristics of carcinogens.

Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

Smith *et al.*, *Env Hlth Perspect* 2016

Machine Learning and Simple Hit Counts of *In Vitro* Assays Do Not Seem Promising

Regulatory Toxicology and Pharmacology 90 (2017) 185–196

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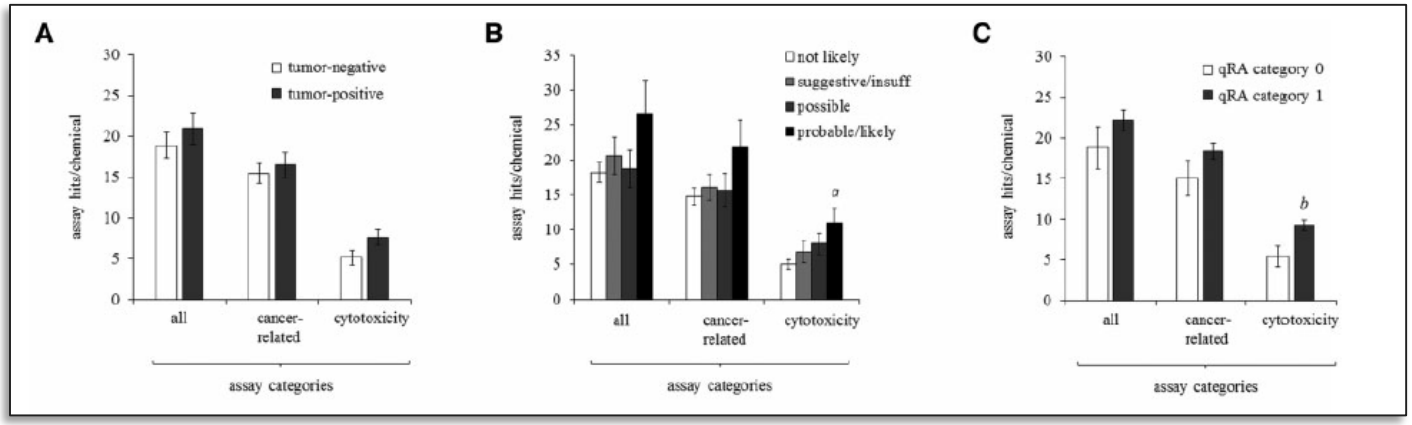
Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

How well can carcinogenicity be predicted by high throughput "characteristics of carcinogens" mechanistic data?

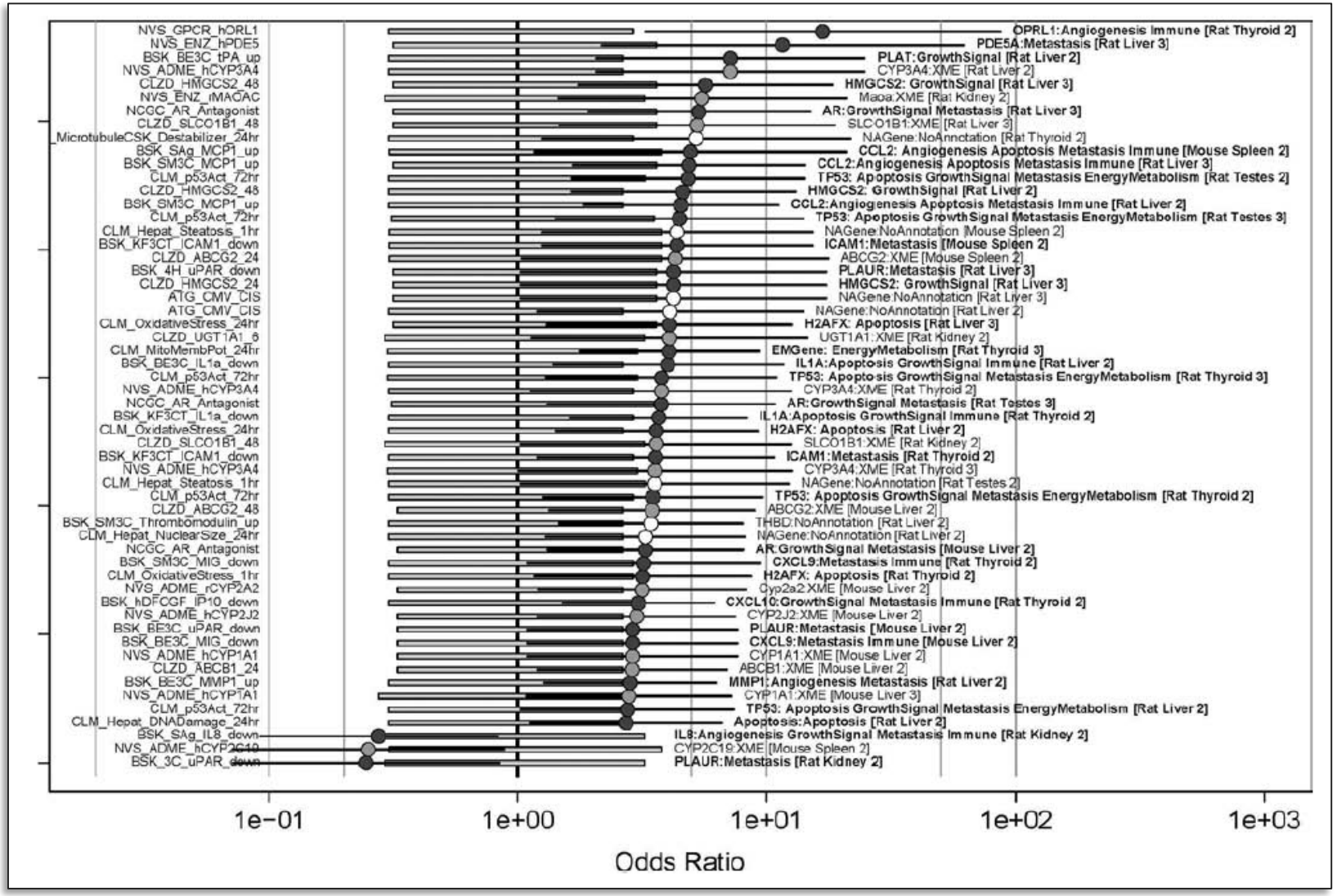
Richard A. Becker [a, *], David A. Dreier [b], Mary K. Manibusan [c], Louis A. (Tony) Cox [d], Ted W. Simon [e], James S. Bus [f]

"Using the same assignments as IARC of ToxCast/Tox21 assays to the seven key characteristics of carcinogens, the ability to predict cancer hazard for each key characteristic, alone or in combination, was found to be no better than chance."



Hill *et al., Tox Sci* 2017

Analyzing Individual Assays in Terms of Relative Risk Provides More Nuance



Kleinstruer *et al.*, *Tox Sci* 2013

But, a Knowledge Based Approach May Ultimately Prove More Useful

Aop: 220

AOP Title

Chronic Cyp2E1 Activation Leading to Liver Cancer

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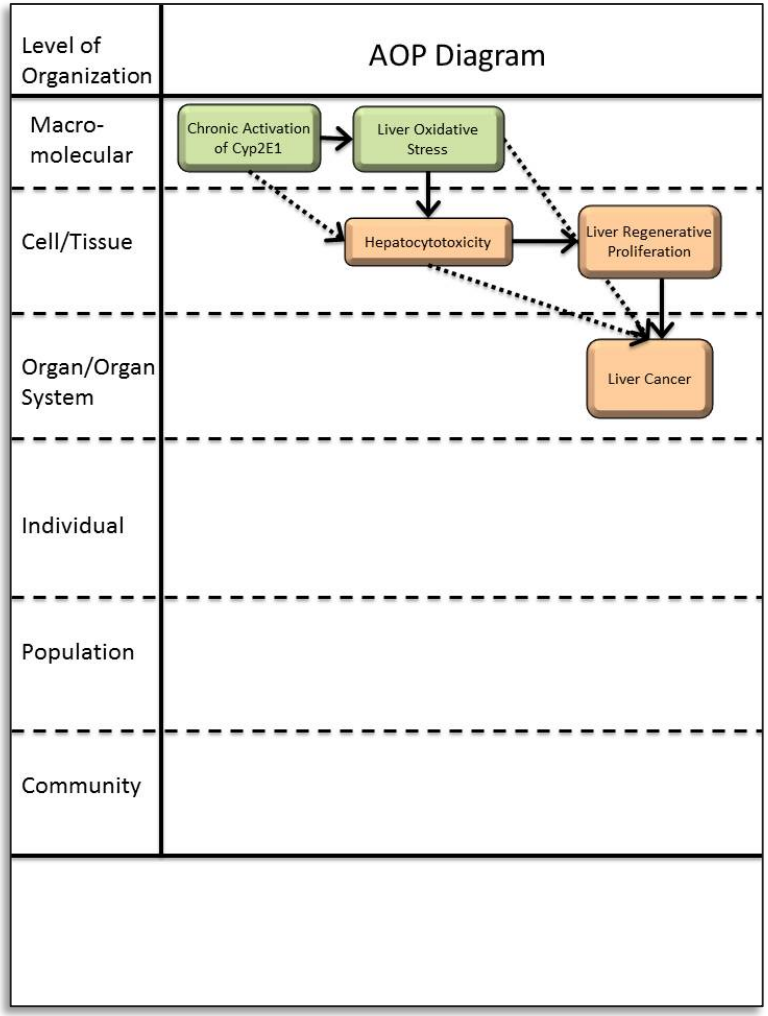
Contributors

Carole Yauk

Status

Author status	OECD status	OECD project	SAAOP status
Open for citation & comment	EAGMST Under Review	1.24	Included in OECD Work Plan

https://aopwiki.org/aops/220



Current *In Vitro* Assays Also Have Limitations Impacting Cancer Hazard Predictivity

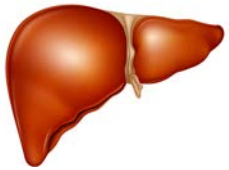
- Limited or lack of relevant metabolism
- Incomplete coverage of important pathways (i.e., biological space)
- Limited higher order biological interactions (i.e., cell-cell, tissue, and organ-level)
- Limited chemical domain of applicability (e.g., volatiles, high logP)

(not a complete list)

Current Strategy to Address Metabolic Competence

"Extracellular" Approach

Chemicals metabolism in the media or buffer of cell-based and cell-free assays



More closely models effects of hepatic metabolism and generation of circulating metabolites

"Intracellular" Approach

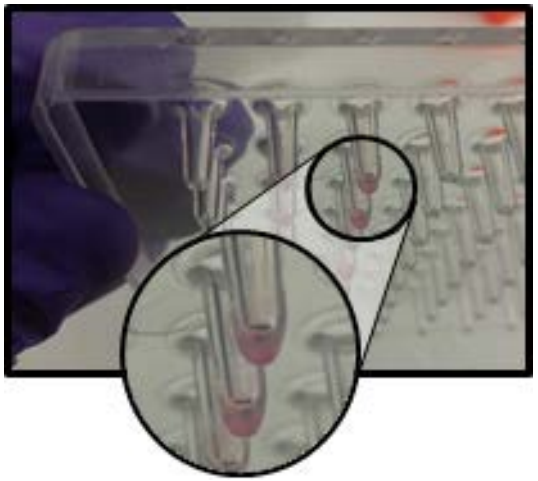
Capable of metabolizing chemicals inside the cell in cell-based assays

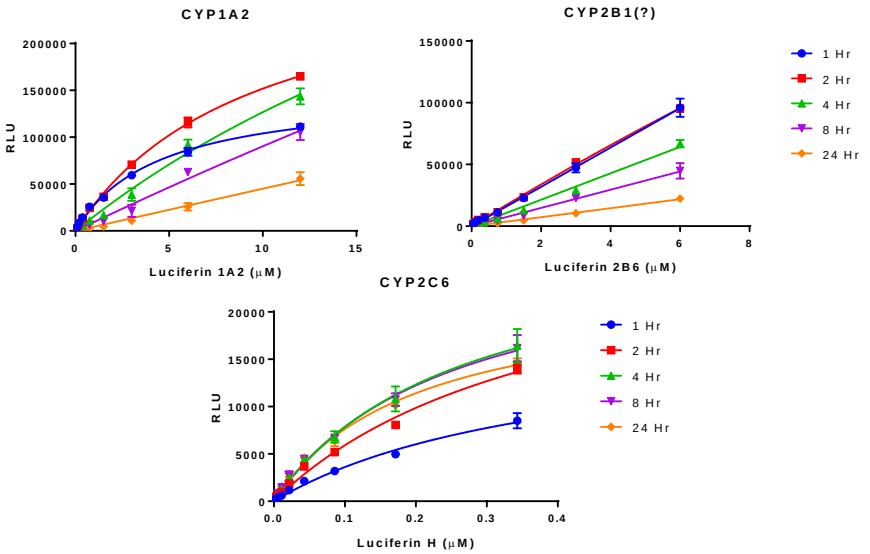
More closely models effects of target tissue metabolism

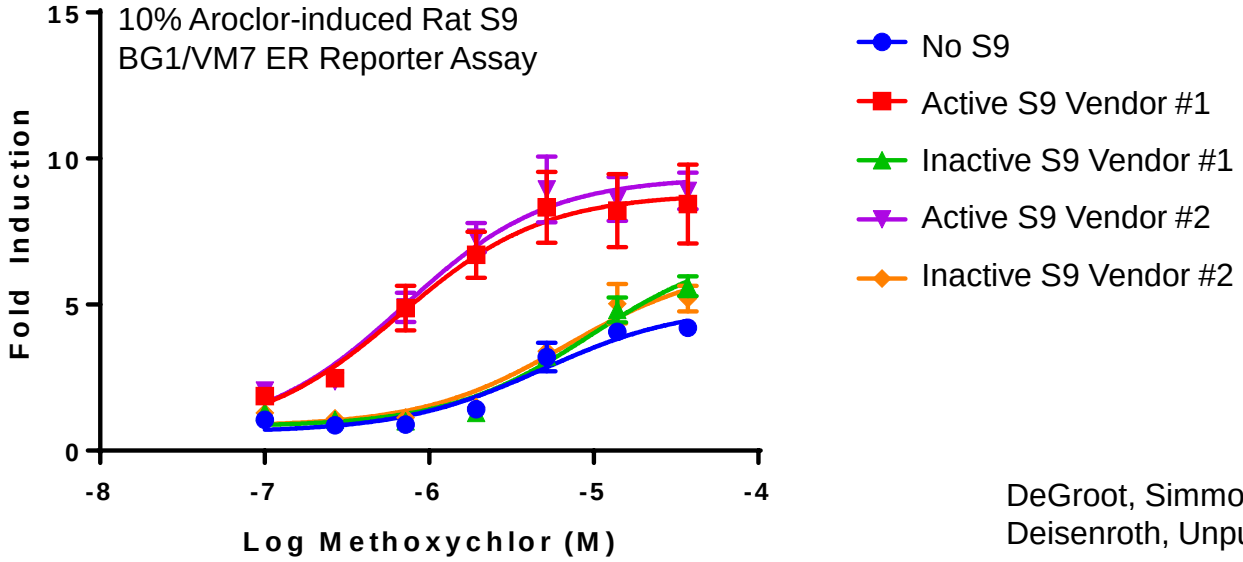
Integrated approach to model *in vivo* metabolic bioactivation and detoxification

Collaboration with Unilever

Extracellular Approach Shows Bioactivation In ER Assay

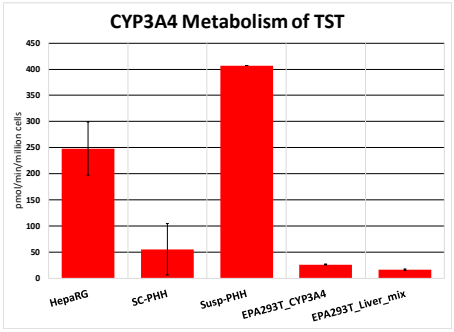


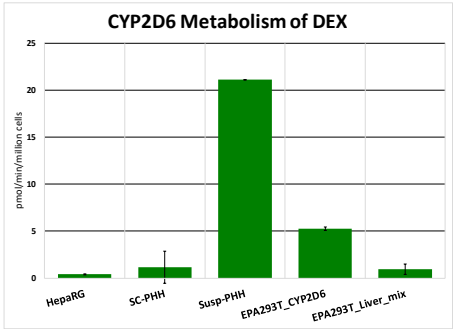


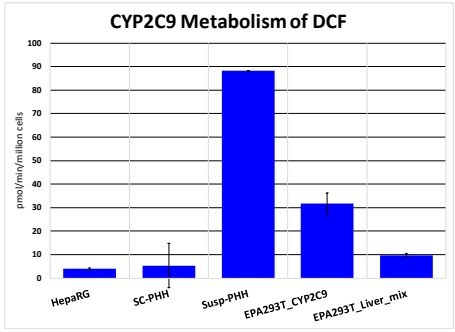


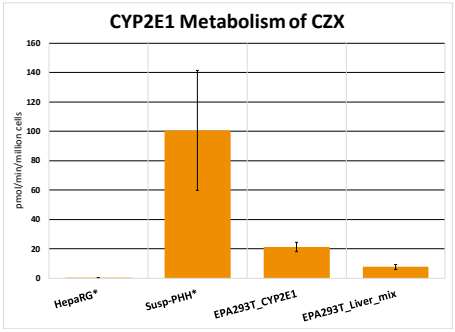
DeGroot, Simmons, and Deisenroth, Unpublished

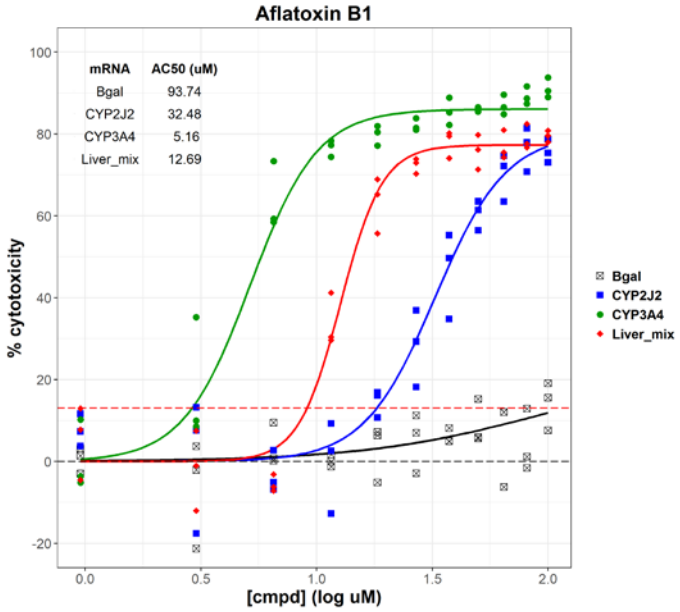
Intracellular Approach Shows Bioactivation in ER and Cytotox Assays

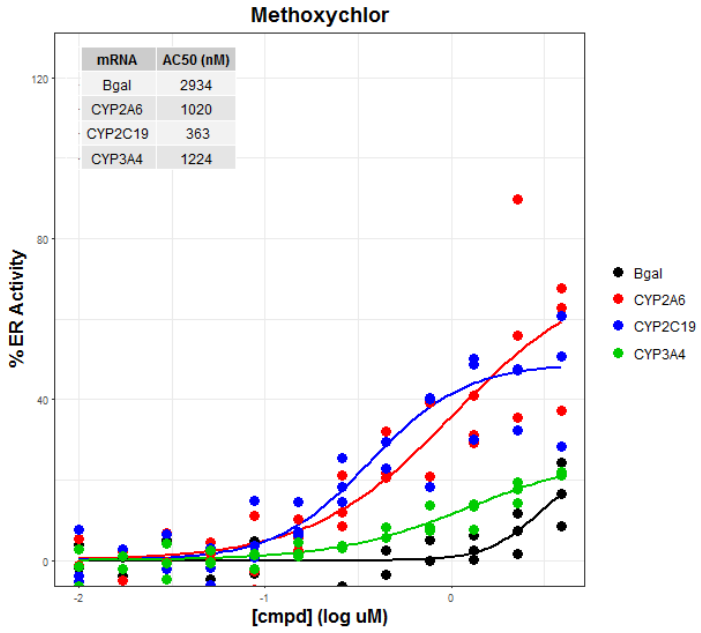






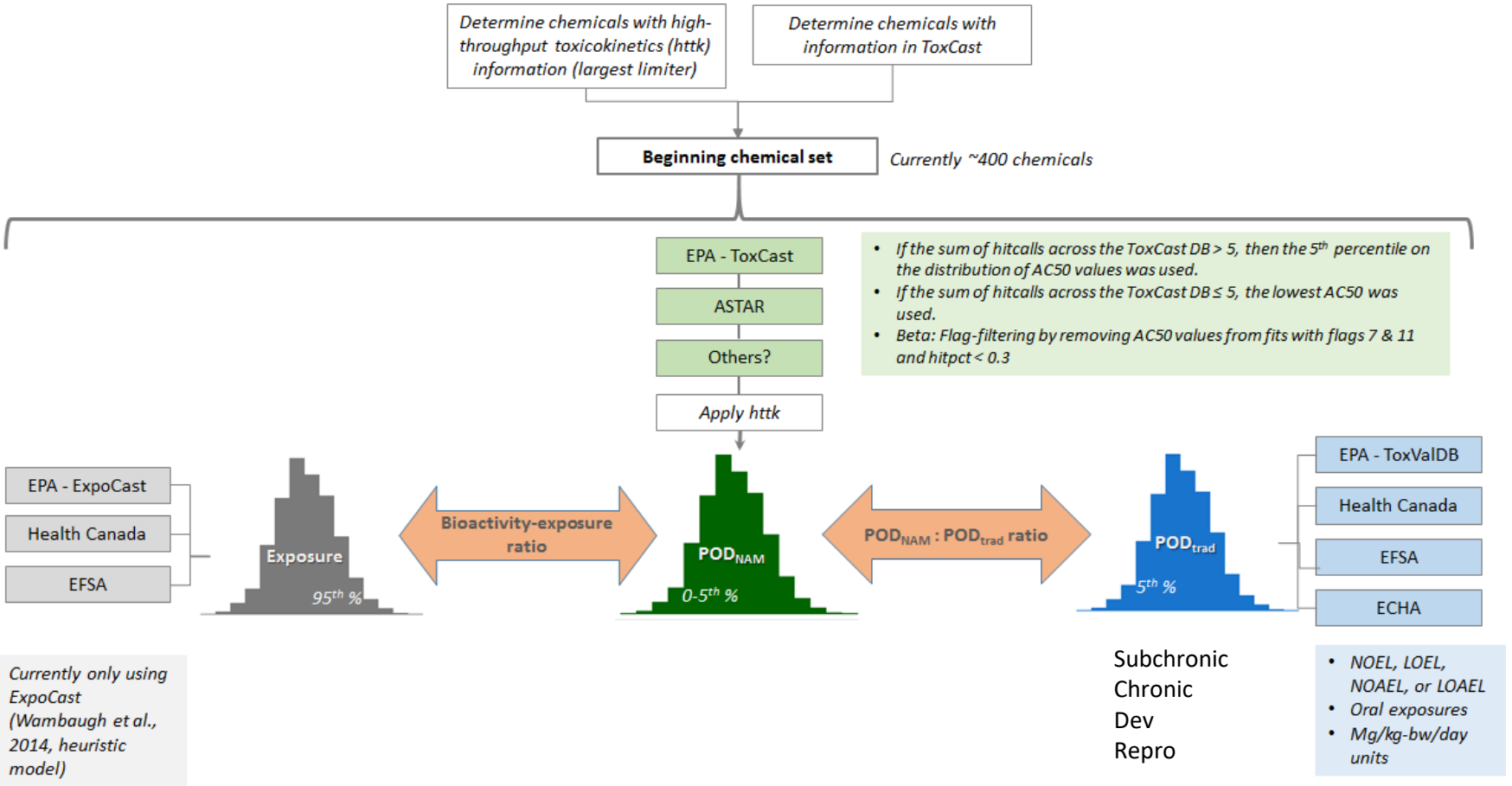






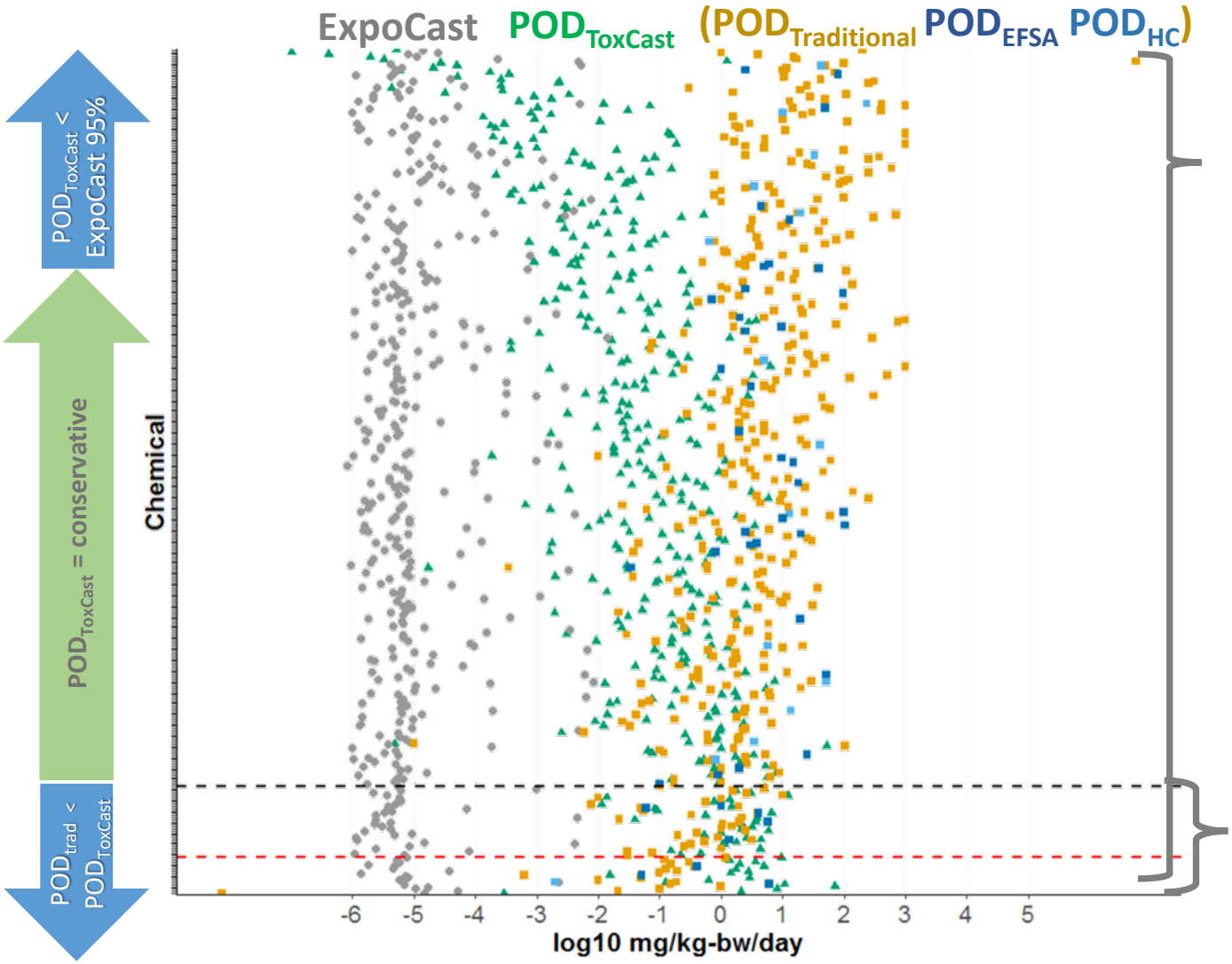
Simmons et al., Unpublished

What About Aiming for Protection Instead of Prediction?



Paul-Friedman, Unpublished
Collaboration with ECHA, EFSA, Health Canada, A*STAR, JRC, EPA

Bioactivity Provides a Conservative Estimate of a NOAEL/LOAEL



Total =

380 chemicals

httk, ToxCast data, and POD value(s) currently available

For ~91.3% of the chemicals, $POD_{ToxCast}$ was conservative.

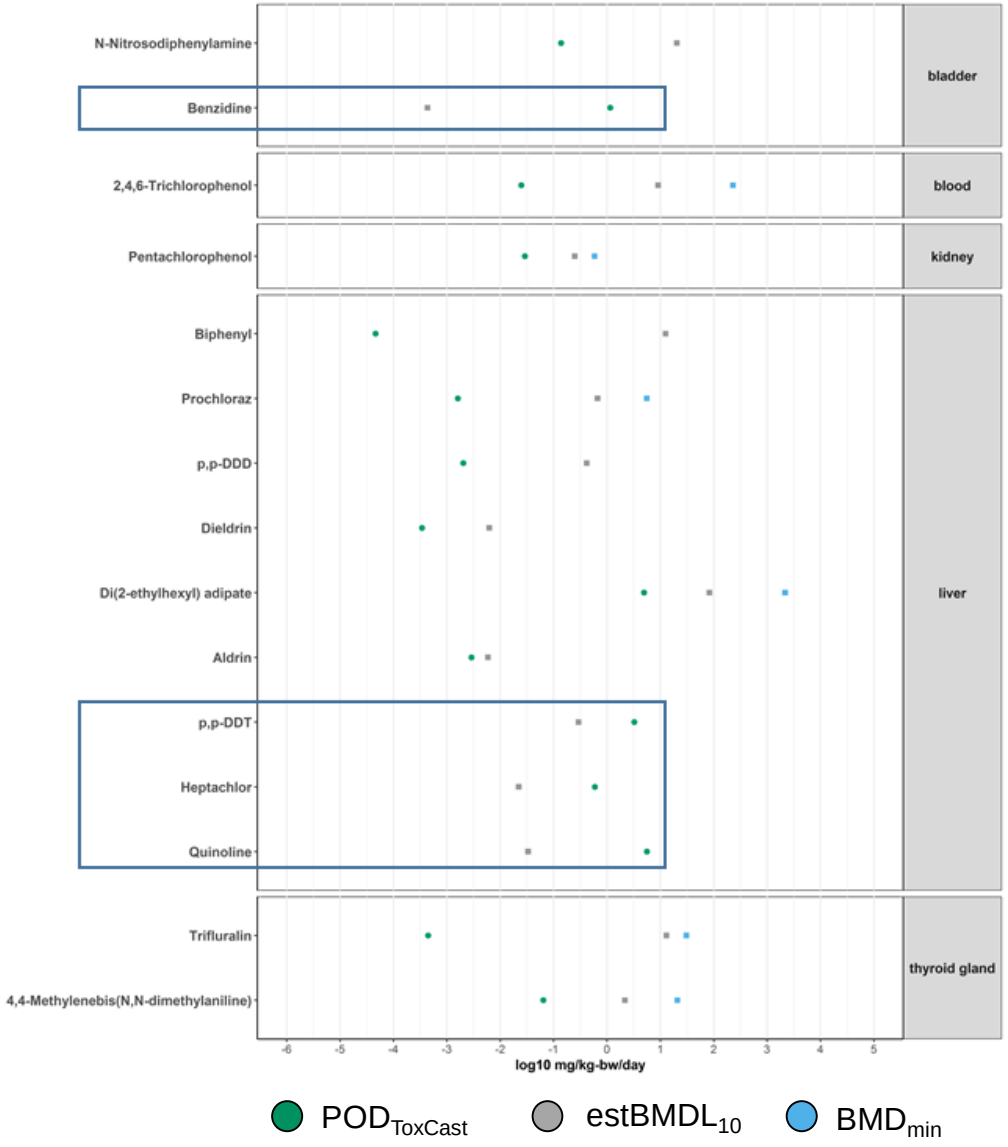
(~130-fold with human HTTK

~40-fold with rat HTTK)

Missing an important component of biology?

Is Bioactivity a Conservative Estimate for Cancer Responses?

- Chemicals with cancer slope factor from the IRIS program, and have httk and ToxCast data (15 chemicals)
- estBMDL10 = 0.1/cancer slope factor in mg/kg-day^{-1}
- minBMD = for a subset of the chemicals where available, the min(BMD) value from any effect data in ToxRefDB that was BMDS-amenable (6/15)
- $POD_{ToxCast}$ < estBMDL10 (11/15 chems)



Take Home Messages…

- The prediction of cancer hazard is confounded by limited data in relevant species
- Using rodent cancer bioassay data as a benchmark is confounded by significant cross-species differences, temporal dependence, and limited reproducibility
- Mechanistic approaches that use machine learning and simple assay count methods have shown little promise to predict cancer hazard
- Limitations in current *in vitro* assays related to cancer mechanisms are being addressed (e.g., metabolic competence)
- Estimating protective doses based on relevant biological activity may provide a feasible path forward for application to cancer risk assessment

Acknowledgements and Questions

Tox21 Colleagues:
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- Health Canada

EPA's National Center for Computational Toxicology