Green Chemistry Education Webinar Series

Integrating Toxicity Information into Chemical Design

March 18, 2014

GREEN CHEMISTRY & COMMERCE COUNCIL

Business Mainstreaming Green Chemistry

Today's Speakers

Martin J. Mulvihill

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Executive Director

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Sustainability A to Z

Chief Scientific Officer

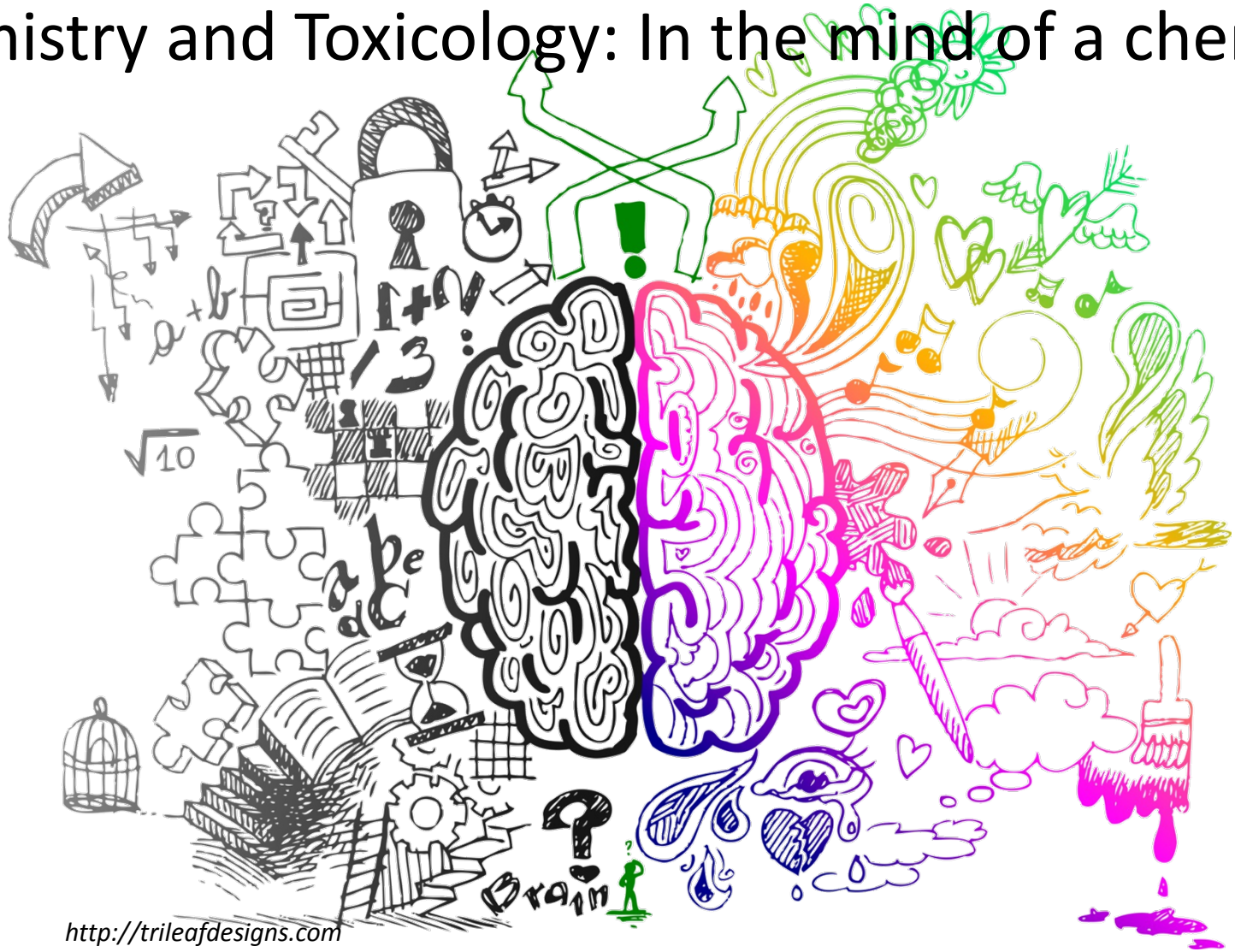
Nigel Greene

Pfizer

Associate Research Fellow

Compound Safety Prediction Group

Chemistry and Toxicology: In the mind of a chemist



http://trileafdesigns.com

Marty Mulvihill, UC Berkeley Center for Green Chemistry

How do we train the next generation of chemists to consider hazard during the design of new chemicals and materials?

How do we promote the adoption and commercial success of safer chemicals and products?

What you will find when you "Ask an Expert"

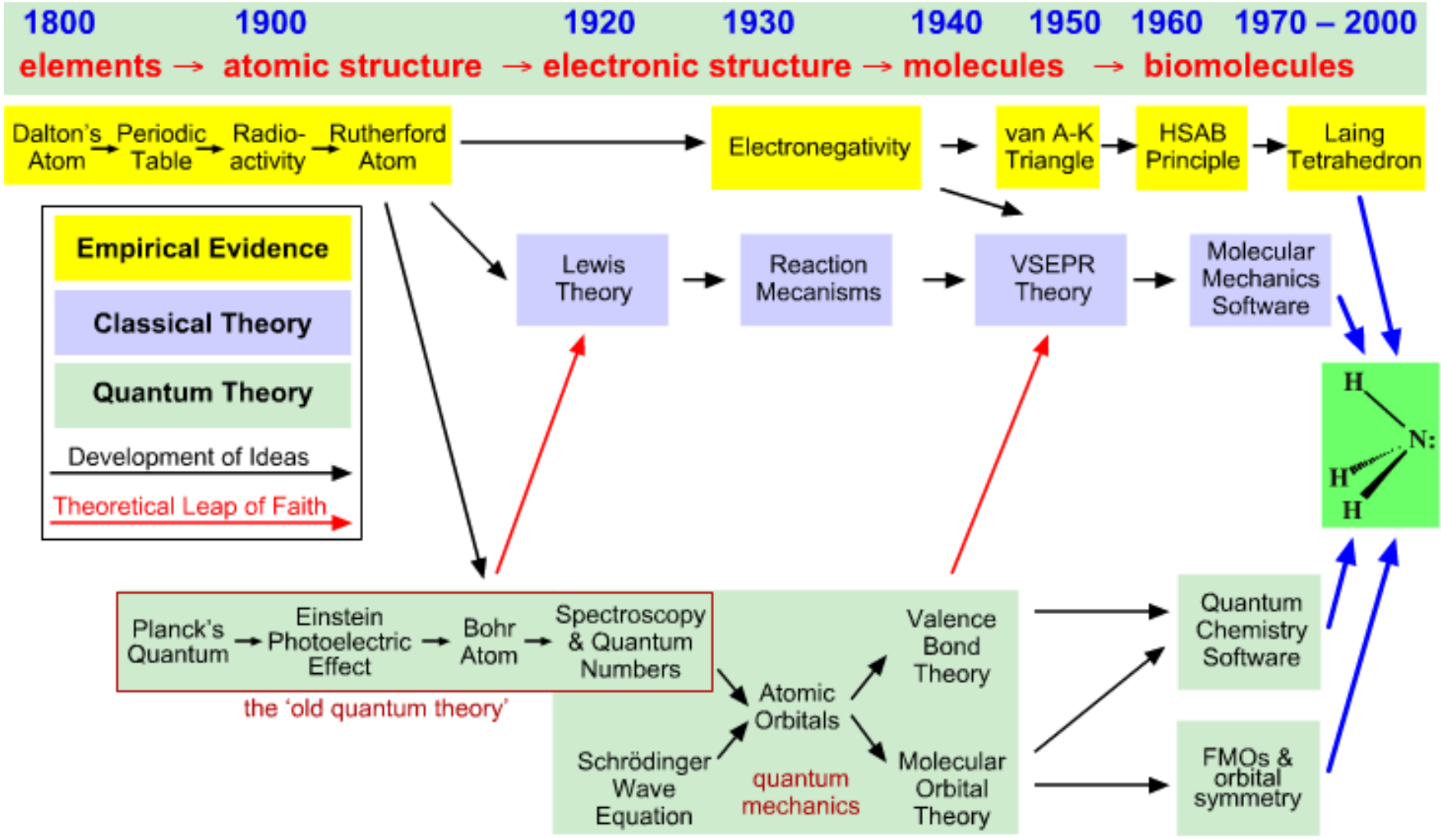
The following is based on a real exchange on the DOE "Ask an scientist" webpage:

A North Carolina teacher asks for a substitute for toluene for a high school chemistry lab on polarity.

Answers:

1. Are you substituting because you can'find any? Go to a hardware store and get paint thinner.
2. Xylene
3. MTBE
4. The closest substitute solvents for toluene (solubility index of 2.4) are 1. xylene (SI = 2.5); 2. Methyl-t-butyl ether (MTBE; SI = 2.5) and 3. diisopropylether (SI = 2.2). Numbers 1 (0.02%) and 3 (0%) have the closest water miscibility as toluene (0.05%) and MTBE has a much higher miscibility with water (4.8%)

History of Modern Chemistry: Understanding Matter



www.meta-synthesis.com

History of Modern Chemistry: Turning Waste into Gold

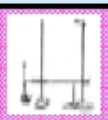
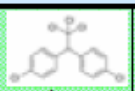
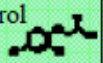
BASF 1880's Coal Tar to dyes.

Dow 1900's Salt water to Bleach (Chloroalkali process)

1800's DuPont, Black Powder

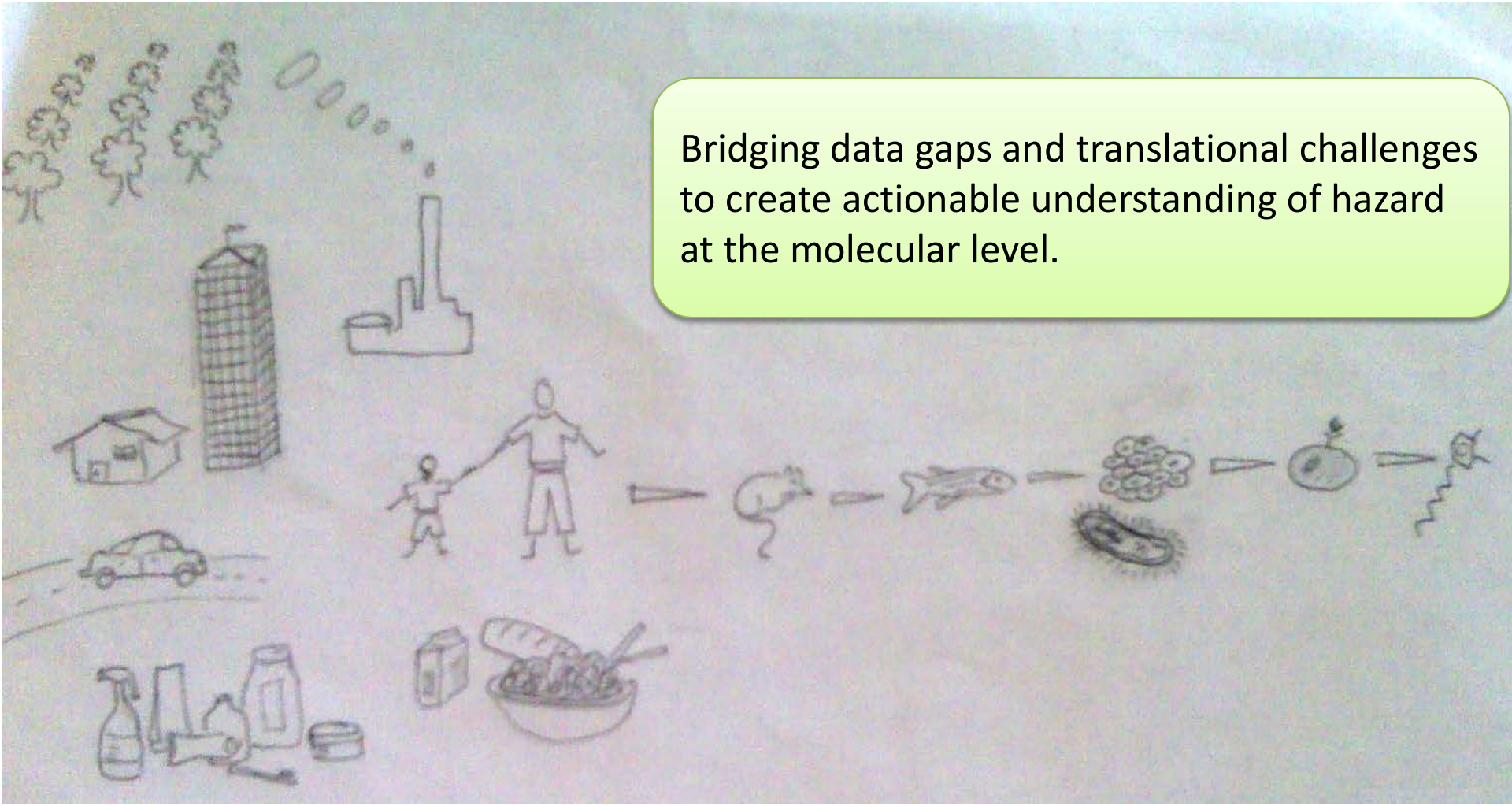
Chemistry education today still focuses on developing the knowledge and skills needed to help transform relatively simple feedstocks into well-defined and well-controlled high value products with desired properties.

History of Modern Toxicology: Characterizing Poisons

1800s	**Thomas de Quincey (1785-1859)**  English writer became addicted to opium in early 1800's and published *Confessions of an Opium Eater* in 1821.	**James Marsh (1794-1846)**  Chemist developed and perfected the Marsh test for arsenic. The improved Marsh test was used forensically for the first time in 1840 during the trial of Marie Lafarge.	**Robert Christison (1797-1882)**  Toxicologist at University of Edinburgh wrote *Treatise on Poisons* in 1829; invented poison harpoon for whaling that contained prussic acid.	**Claude Bernard (1813-1878)**  French physiologist studied the effects of carbon monoxide and curare. Influenced by Francoise Magendie.
1900-1930s	**Upton Sinclair (1878-1968)**   Published *The Jungle* in 1905. Chronicled the unsanitary conditions in meat packing industry in Chicago.	**Pure Food and Drugs Act - 1906**  Harvey Washington Wiley, M.D. (1844-1930). Law prevents production or trafficking of mislabeled, adulterated or poisonous foods, drugs, medicines, and liquors.	**Chemical Warfare A Reality 1915**  German chemist Fritz Haber (1868-1934) developed blistering agents used in WWI; chlorine and cyanide gases.	**U.S. Prohibition 1919-1933**  Law that made the production and sale of alcoholic beverages illegal but very profitable.
1940-1960s	**DDT – 1939**  Recognized as insecticide by the Swiss scientist Paul Hermann Müller, who was awarded the 1948 Nobel Prize in Physiology and Medicine. Banned in 1972. 	**2,4-D – 1946**  Developed during WW II at British Rothamsted Experimental Station, by J.H. Quastela and sold commercially in 1946. Used to control broadleaf plants. 	**Minimata Japan (1950's)**  Minimata Bay contaminated with mercury by chemical industry. Thousands adults and children were poisoned from eating fish contaminated with methyl mercury.	**Poison Control Centers 1953**  First, Chicago 1953, second at Duke University, NC in 1954, and third opened in Boston 1955.
1970-2006	**Mr. Yuk 1971**  Symbol adopted by the Pittsburgh Poison Center at The Children's Hospital in 1971. Used to educate children and parents about poisons and to prevent accidental poisonings.	**Iraq – Mercury 1971**  Pink-colored seed grain coated with a mercury fungicide was tragically consumed by Iraqis tragically affecting over 40,000 people.	**Bangladeshi 1970s Arsenic poisoning**  Tubewells, drilled to provide clean drinking water, are contaminated by arsenic resulting in millions of people harmed.	**First Modern Toxicology Textbook 1975**  Louis J. Casarett & John Doull edited, *Toxicology: The Basic Science of Poisons*, in 1975.

http://www.toxipedia.org/display/toxipedia/History+of+Toxicology

One approach to bridging chemistry and toxicology focuses on translating information from the macroscopic health effects to molecular design.



Bridging data gaps and translational challenges to create actionable understanding of hazard at the molecular level.

Meg Schwarzman

Strategies for Improved Molecular Design

Reduce Persistence

- Design for greater biodegrability;

Reduce Bioaccumulation

- Understand the role of K_{ow} and biodegradation;

Reduce Toxicity

- Design molecules to have low bioavailability;
- Avoid structural features known to bestow toxicity;
- Infer structural modifications expected to reduce toxicity;
  - from mechanism of toxicity information;
  - from structure-activity (toxicity) information.
- Isosteric substitution of molecular substituents responsible for observed toxicity.

Design for Degradation

Help Degradation:

- Esters
- Oxygen (except ethers)
- Unsubstituted Linear alkyl chains

"All rules of thumb are half-truths some are useful."

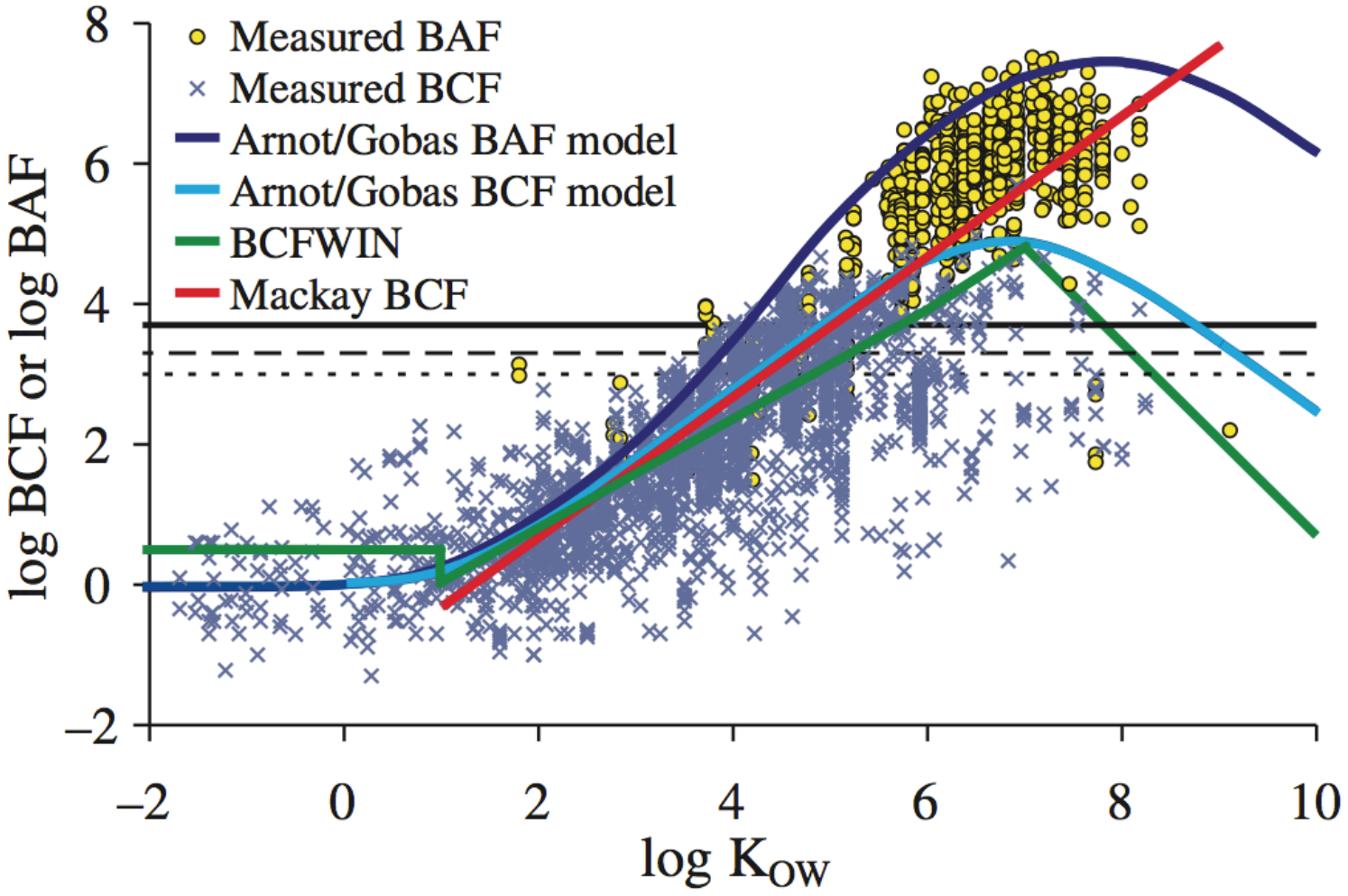
Boethling, et al. Chem. Rev. **2007**, 2207.

Hinder Degradation:

- **halogens**, especially chlorine and fluorine and especially if there are more than three in a small molecule (iodine and (probably) bromine contribute to a lesser extent);
- **chain branching** if extensive (quaternary C is especially problematic);
- **Nitrogen**: tertiary amine, nitro, nitroso, azo, and arylamino groups;
- **polycyclic residues** (such as in polycyclic aromatic hydrocarbons), especially with more than three fused rings;
- **heterocyclic residues**, for example, imidazole;
- **aliphatic ether bonds** (except in ethoxylates)

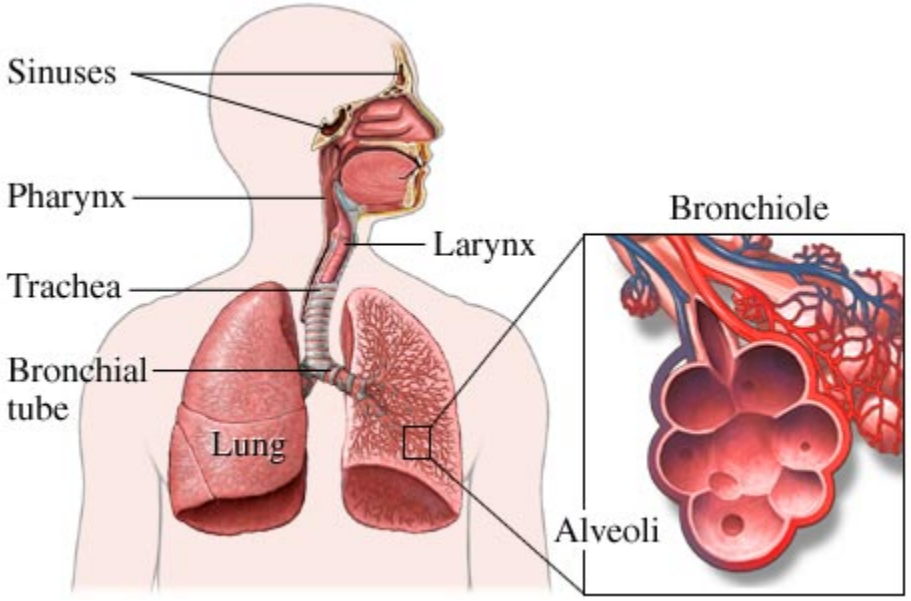
B

Fish BCF and BAF are correlated with K_{ow}



Arnot and Gobas, "A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments of organic chemicals in aquatic organisms," *Env. Rev.* **2006**, *14*, 257-297.

Absorption in Respiratory Tract



Parameters to Consider

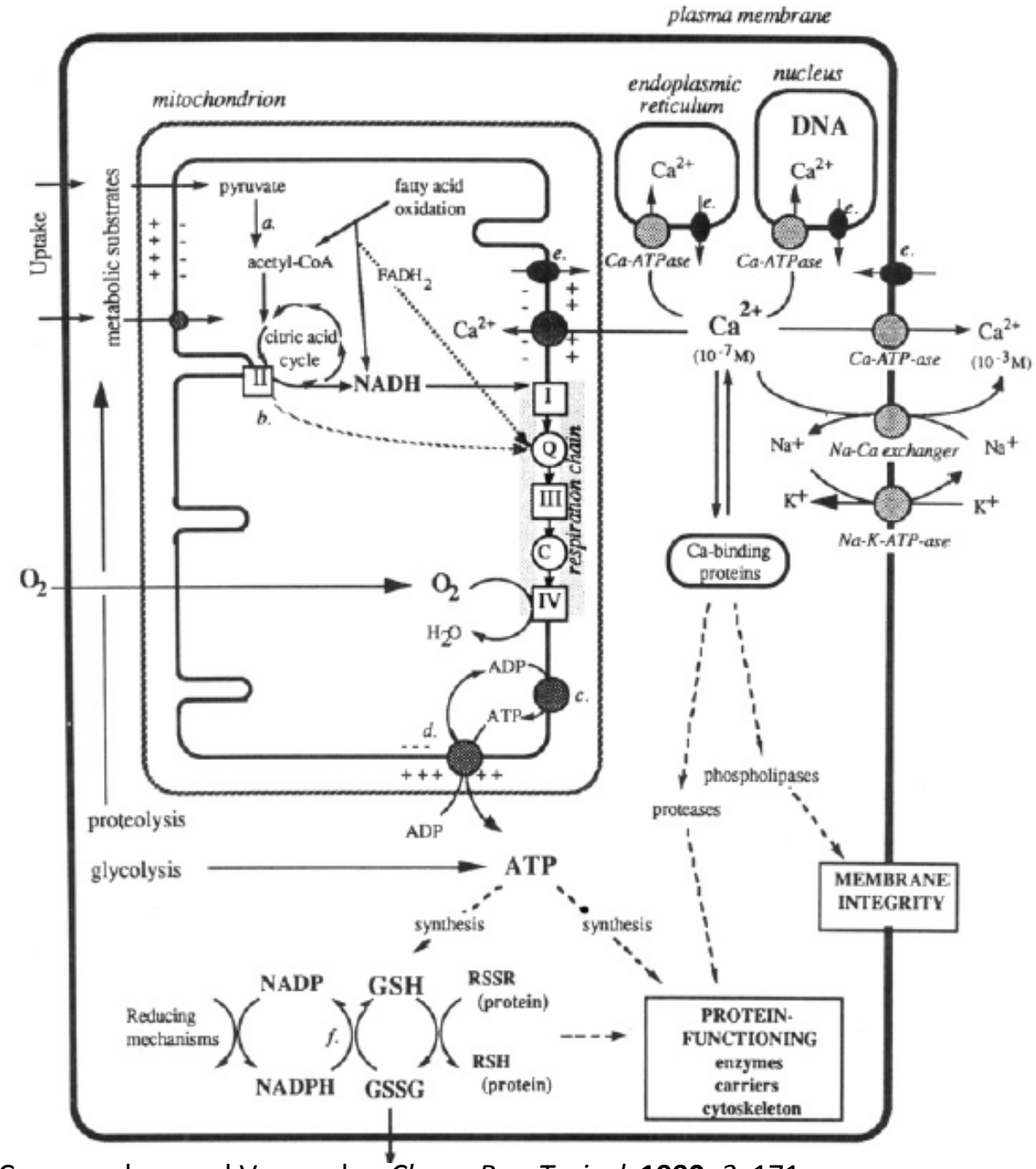
Particles: > 5 um mass median aerodynamic diameter.

Blood to Gas Partitioning P_{BG}*:* < 1

Molecular Weight: > 400 Da (more importantly is a low Vapor pressure!)

Vapor Pressure: < 0.001 mmHg

Chem. Rev. **2010**, 110, 5845.



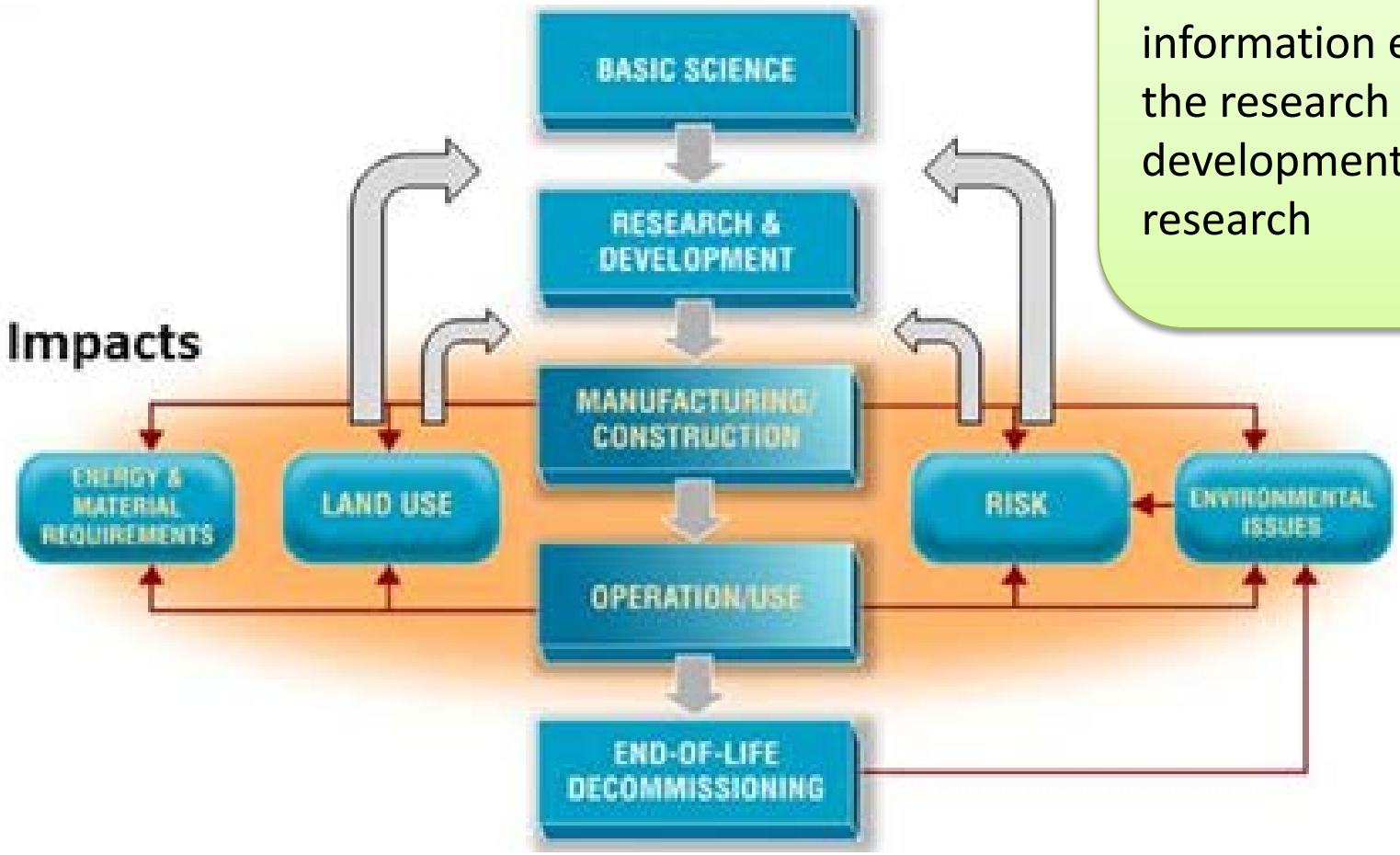
Where and how do chemicals act in a cell?

1. Electrophiles
2. Radicals
3. Reactive Oxygen Species
   - O_2^{*-}
   - H_2O_2
   - OH^*
4. Heavy Metals
5. Organic cations
6. Chelators/Ligands

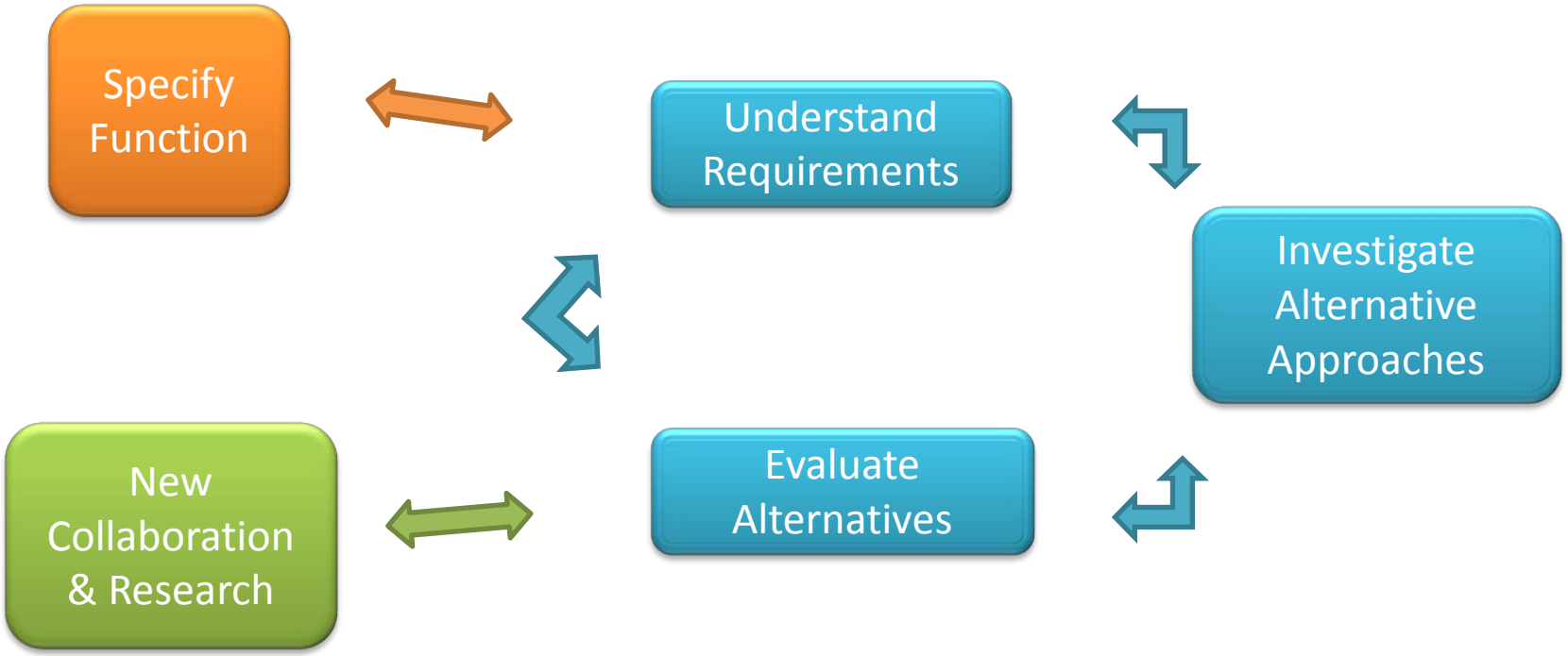
Commandeur and Vermeulen *Chem. Res. Toxicol.* **1990**, *3*, 171.

Another approach to safer design focuses on considering chemical hazards early in the product design process.

Work to introduce hazard and other impact information earlier into the research and development stages of research



Our approach focuses on iterative design and evaluation



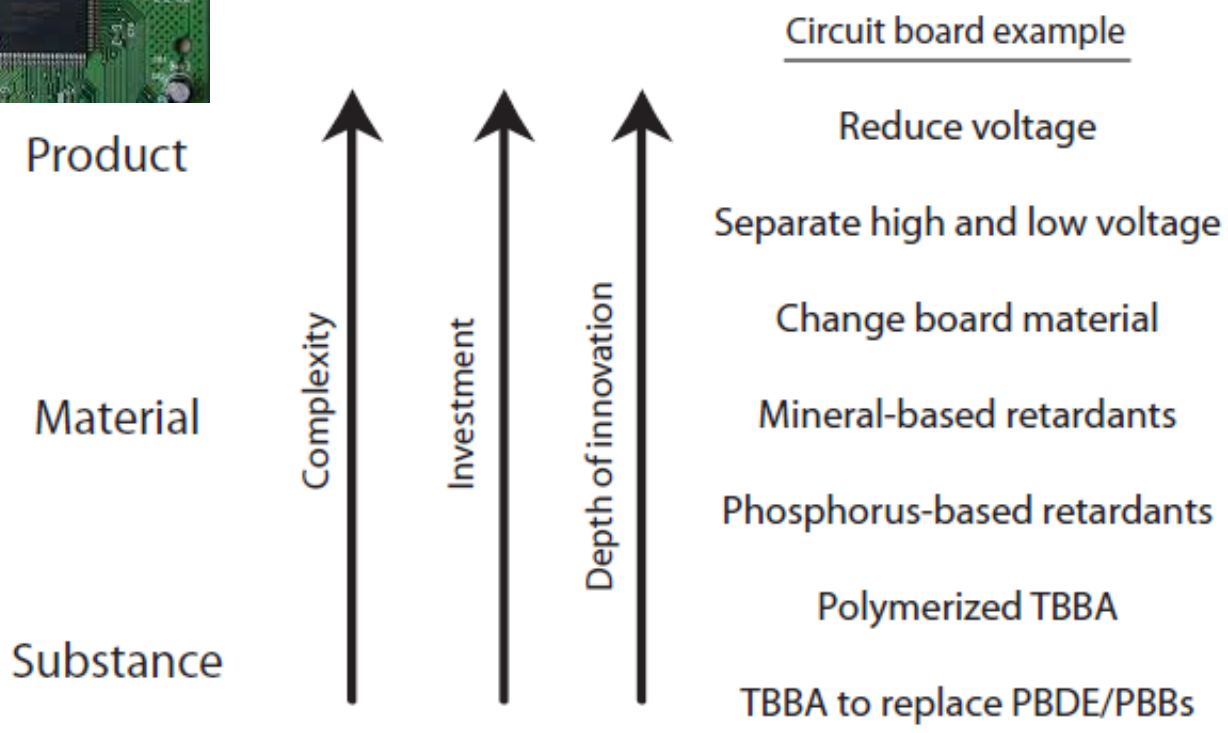
Consider the broadest range of opportunities for innovation

<u>Incremental</u>
Minimal Investment
Quick Adoption

<u>Disruptive</u>
Significant Investment
New Markets are needed
Long term shift in company structure

Disruptive vs. Incremental Change

Challenge: Remove Polybrominated diphenyl ethers (PBDEs) flame retardants from circuit boards.



Move beyond drop-in substitution: Invest in product redesign and basic research.

4 step process for identifying hazard data

1. Identify compounds of interest
2. List screening: Search for hazard information based on 'authoritative' lists
   - Obtain detailed info from the source lists
3. Literature review: Search for information on chemicals not listed by authoritative bodies
   - Go to the primary literature
4. Fill gaps: For chemicals with little or no hazard data, consider functional group analysis, chemical class information, and analogies to similar chemicals/materials

Step 1: For each potential solution consider the types of chemical or material are you would use

Plastic	Mineral/Metal	Chemical/Molecule
Factors influencing overall hazard of a material		
Feedstock Monomers Additives Breakdown Products	Size Oxidation State Compound Form	Structural Features Partitioning Related Compounds
Notes about available information		
Additives and Monomers are small molecules if you can find the information. (Often only general information is available) Search Literature	Consider health and environmental endpoints. Must use situation specific information to assess relevance of toxicity literature.	Can use models when information is unavailable. These are more reliable for persistence and bioaccumulation.

Step 2: Search authoritative sources

Chemicals that are recognized as hazardous by authoritative bodies

- governmental, regulatory or international consensus groups

Ready source of information on well-studied chemicals *not necessarily* indication of highest hazard

- Variety of endpoints
- A wide range of methods, cutoffs, priorities
- Looking for keys by the lamppost

Information just needs to be retrieved

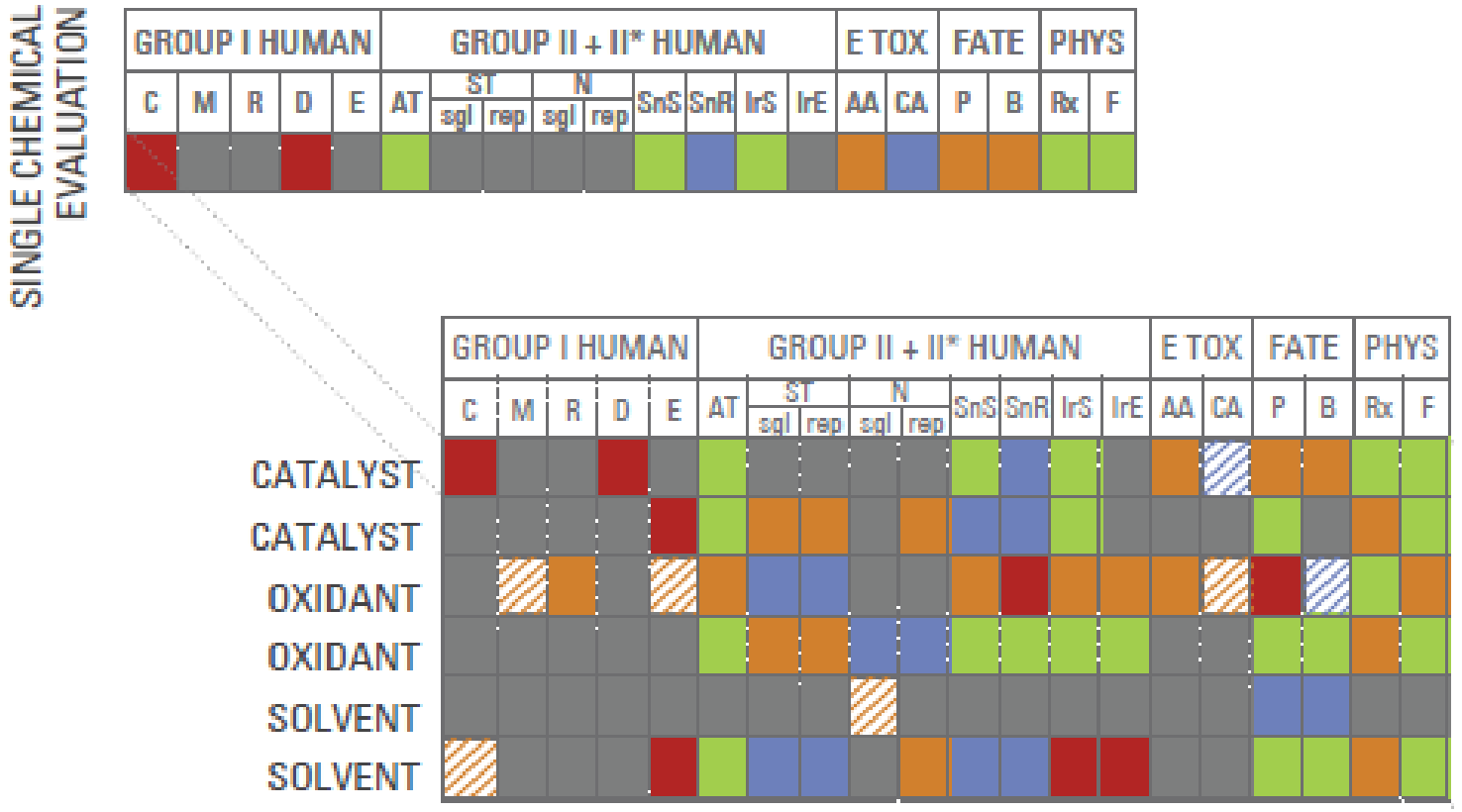
- Search www.pharosproject.net to find authoritative evaluations
- From pharos, go to source listing (IARC, NIOSH, NTP, etc) for more details on associated endpoint

Step 3: Search literature for information on unlisted chemicals

If substance is not listed on an 'authoritative source', search the literature

- Wikipedia, etc. for general information
- PubMed (or Web of Science)
  - Search for review papers
  - Intimidated? Read several abstracts to get an impression
- HSDB (via toxnet http://toxnet.nlm.nih.gov/index.html) Use with caution!
  - Avoid "toxicity summaries" (computer generated)
  - Beware outdated information
- Others (e.g., CTD)

Move beyond Red-lists to Heath Performance Characteristics



Understand that hazard is relative, and comparisons should be made within functional use space.

Two ways to think about designing and improving the safety of chemicals and materials:

1) Molecular design- building chemical intuition

2) Incorporating hazard analysis into design

Don't assume chemists or manufactures are thinking about hazard.

Be explicit, and help translate the current understanding of hazard and toxicity.

Empower people with options and a path toward continuous improvement.

Computational Approaches to Designing Safer Chemicals

Jakub Kostal, PhD
Sustainability A to Z
jakub@sustainabilityatoz.com

Green Chemistry Principle #4

Chemical products should be designed to preserve efficacy of function while reducing toxicity and other environmental hazards.

Anastas, P.; Warner, J. *Green Chemistry: Theory and Practice*, Oxford Press 1998

Identification of Toxic Chemicals vs. Design for Minimal Toxicity

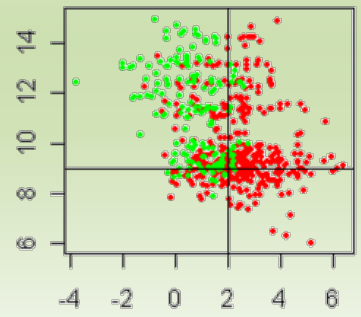
Value of Reactive Approach

- Identify hazardous chemicals from those already in existence
- Evaluate chemical alternatives

Value of Proactive Approach

- Redesign an existing chemical to minimize biological activity
- Design a new chemical that has a superior safety profile to chemicals in the market

Physico-chemical properties and molecular attributes



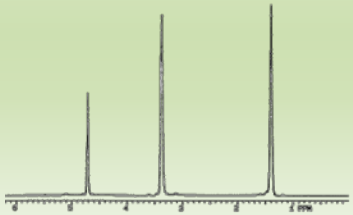
1

In chimico or *in vitro* assays of chemical reactivity

3

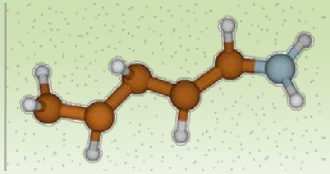
Molecular Design Guidelines

Experimental spectroscopic data



2

In silico modeling of chemical reactivity



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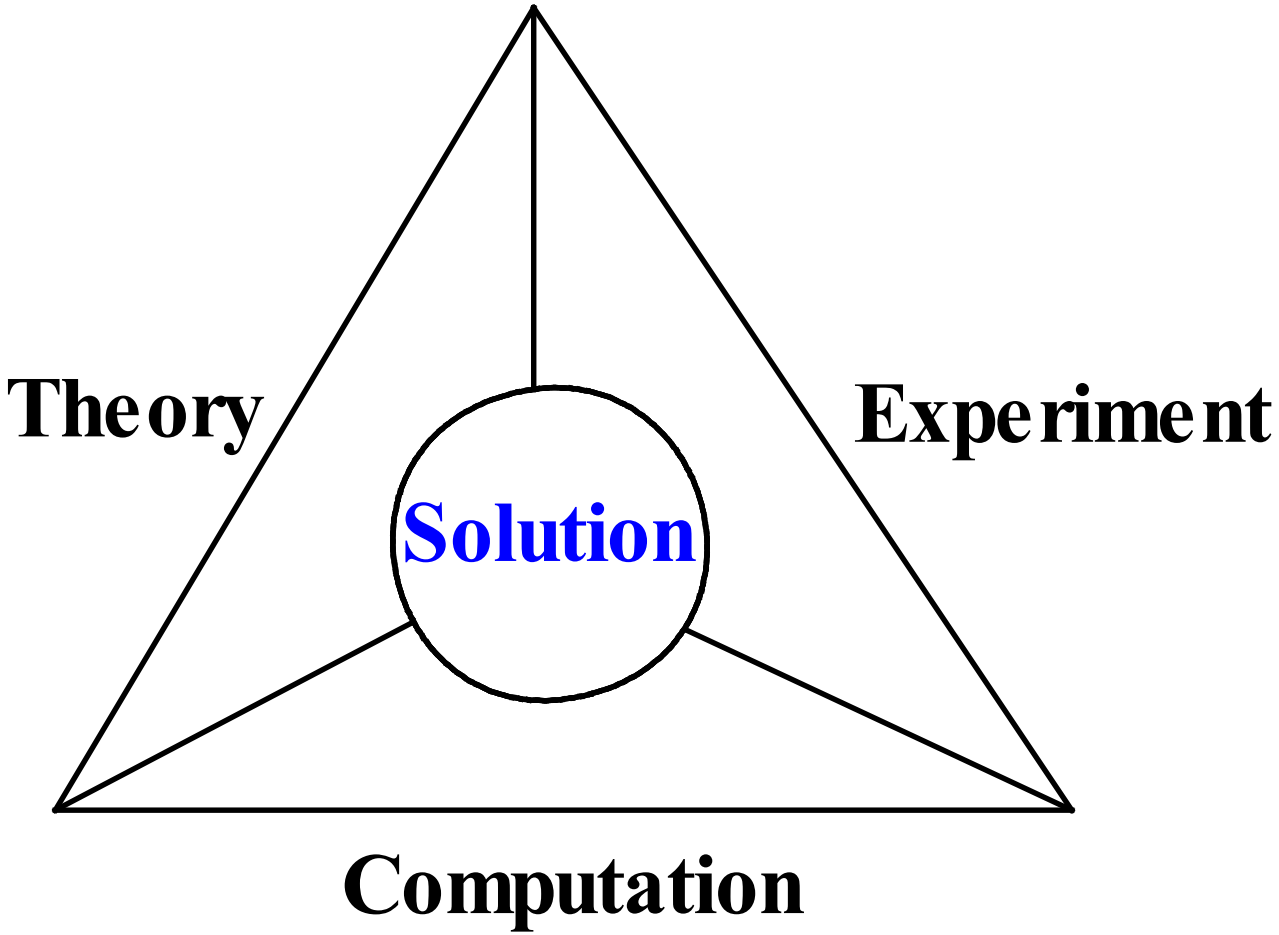
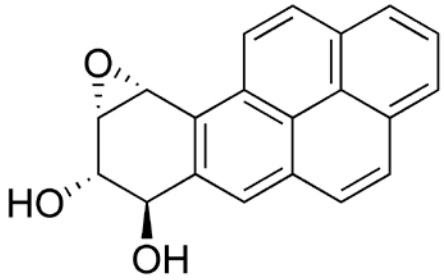

Theory
Experiment
Solution
Computation

Central Dogma of Computational Chemistry

benzo[a]pyrene diol epoxide



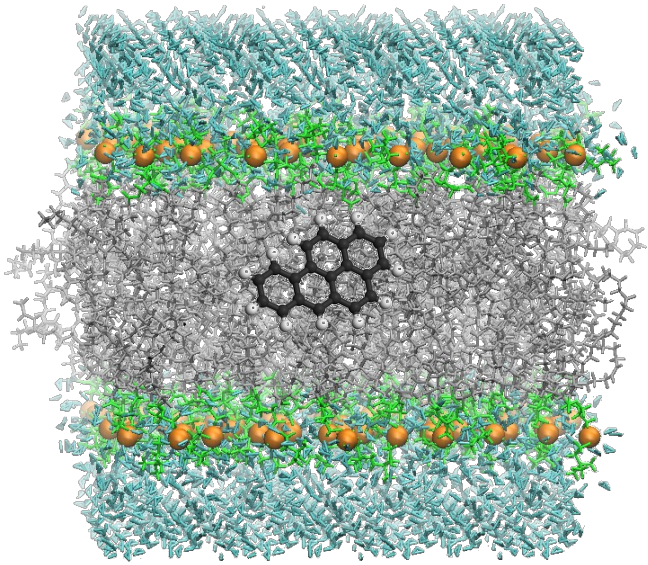
STRUCTURE

↓

DYNAMICS

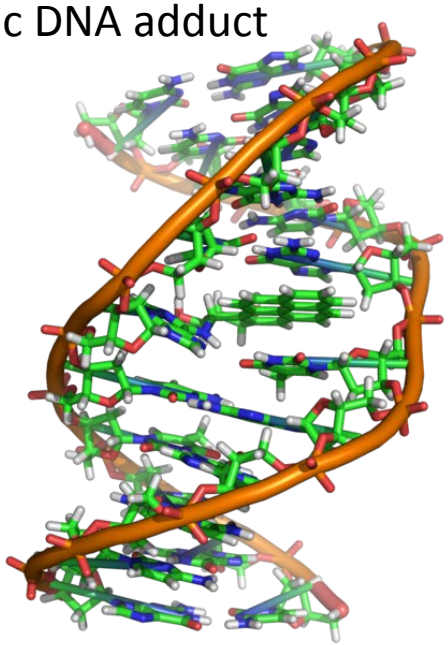
↓

REACTIVITY

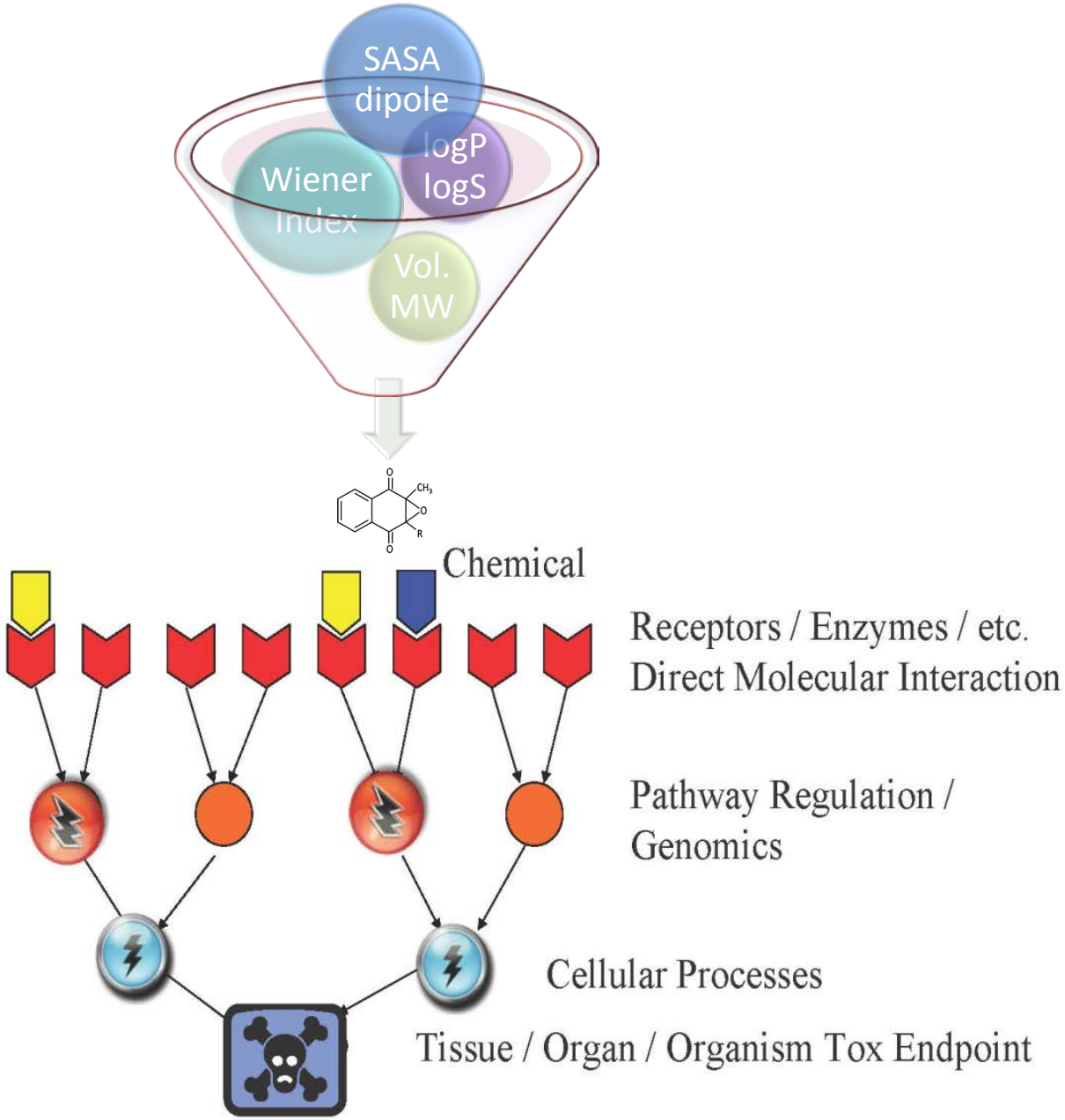


Phospholipid bilayer

Mutagenic DNA adduct



Approach of property-based filters



Design Guidelines for Reduced Aquatic Toxicity: Identifying key properties

logP/D$_{(o/w)}$

$$\log\ P_{\mathrm{oct/wat}} = \log\left(\frac{[\mathrm{solute}]_{\mathrm{octanol}}}{[\mathrm{solute}]^{\mathrm{un-ionized}}_{\mathrm{water}}}\right)$$

$$\log\ D_{\mathrm{oct/wat}} =$$

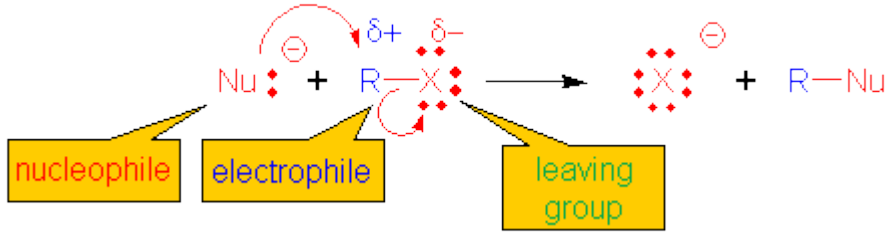
$$\log\left(\frac{[\mathrm{solute}]_{\mathrm{octanol}}}{[\mathrm{solute}]^{\mathrm{ionized}}_{\mathrm{water}} + [\mathrm{solute}]^{\mathrm{neutral}}_{\mathrm{water}}}\right)$$

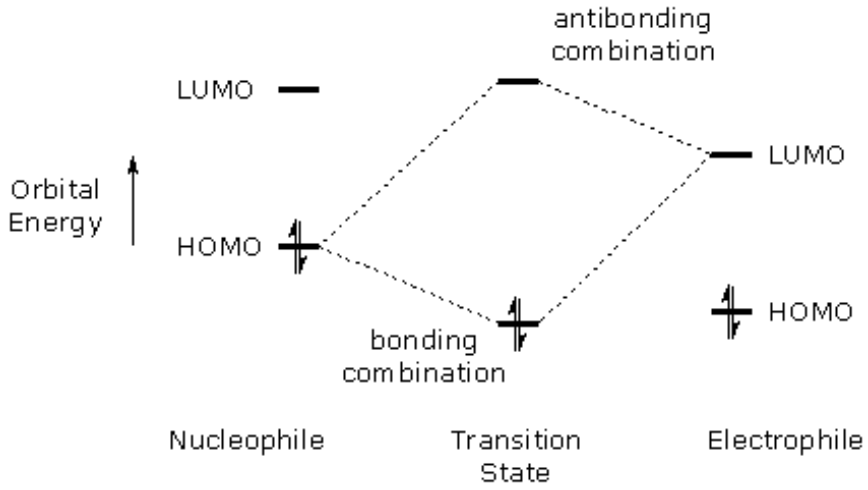
Ionizability of organic chemicals strongly affects bioavailability

- 10 +10

hydrophilic lipophilic

Frontier orbitals





Low LUMO energies and **high** HOMO energies promote chemical reactivity

Aquatic Toxicity Model Systems

Fathead minnow
LC_{50}, 96-h assay

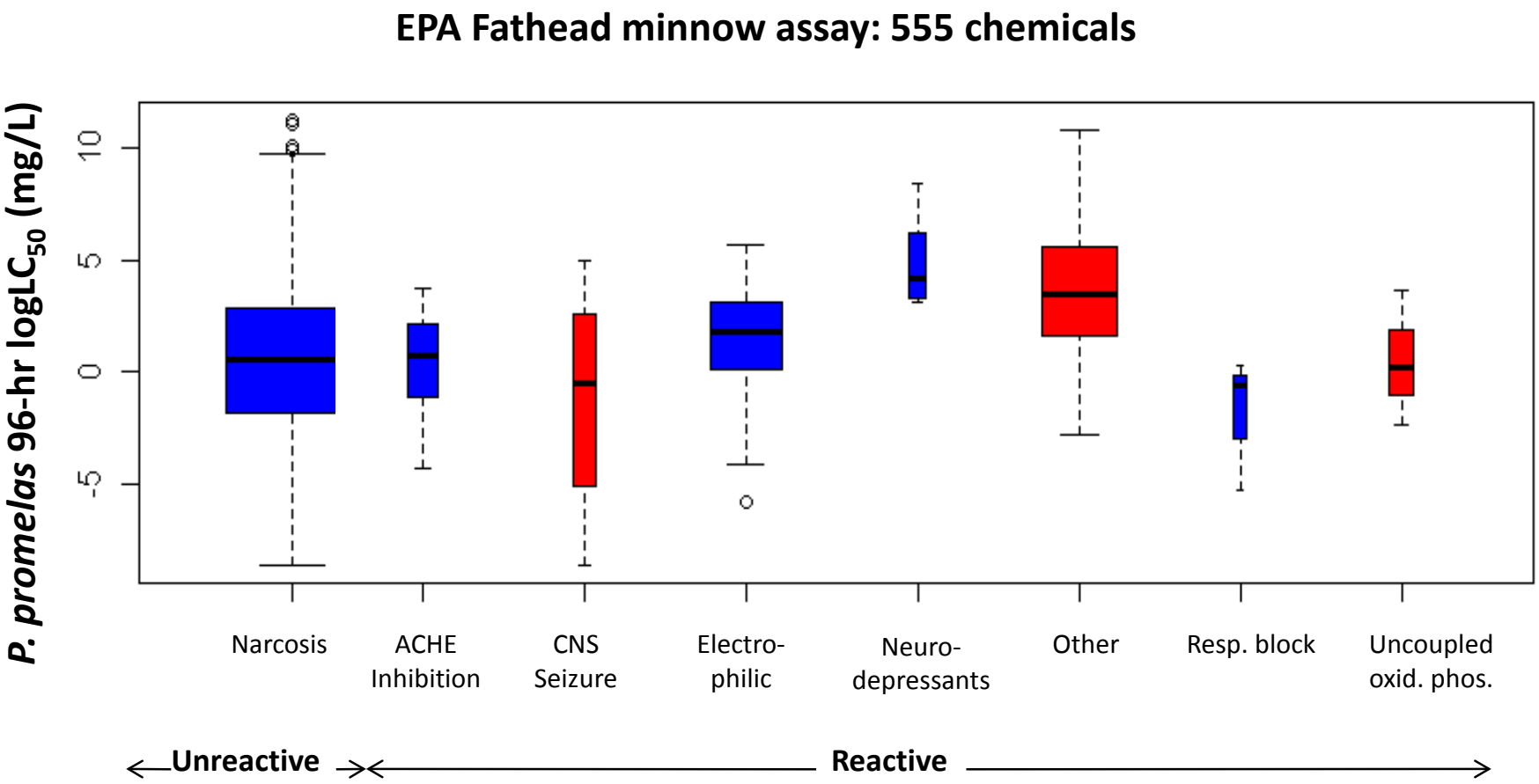
U.S. E.P.A.

555 chemicals

4 categories guided by EPA thresholds of concern for acute aquatic toxicity (LC_{50}/EC_{50}:)

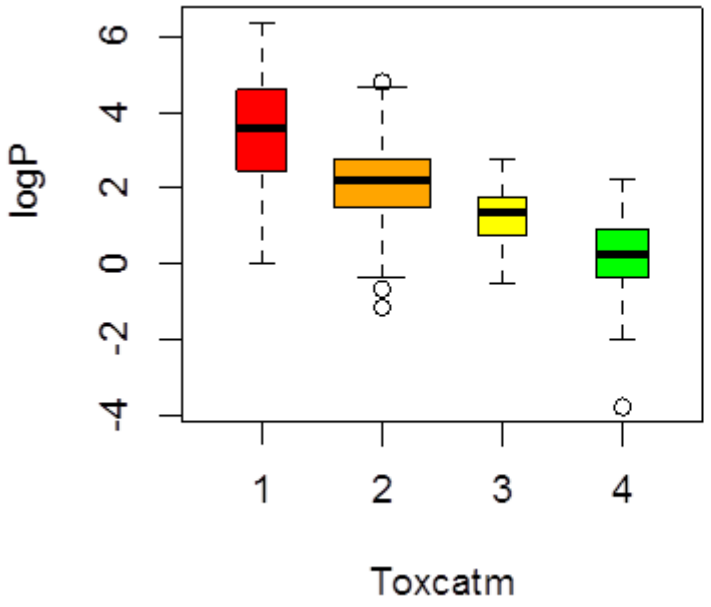
<1 mg/L < 0.0067 mmol/L	1–100 mg/L 0.0067 - 1.49 mmol/L	100–500 mg/L 1.49-3.32 mmol/L	> 500 mg/L >3.32 mmol/L

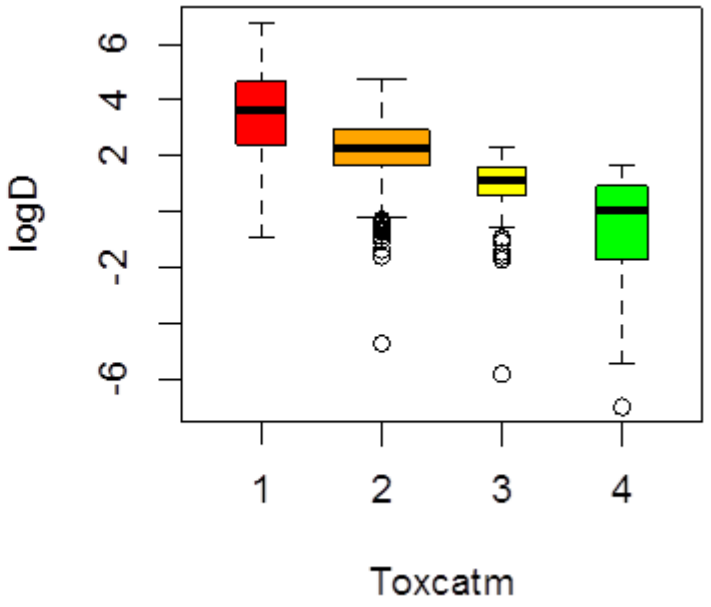
Acute Aquatic Ecotoxicity by MOA



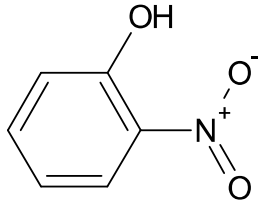
log P and log D

- log D/log P is not sufficient as sole descriptor of aquatic toxicity:

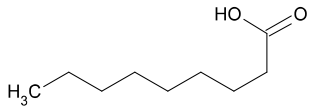




15% of the compounds are ionized at pH 7.4

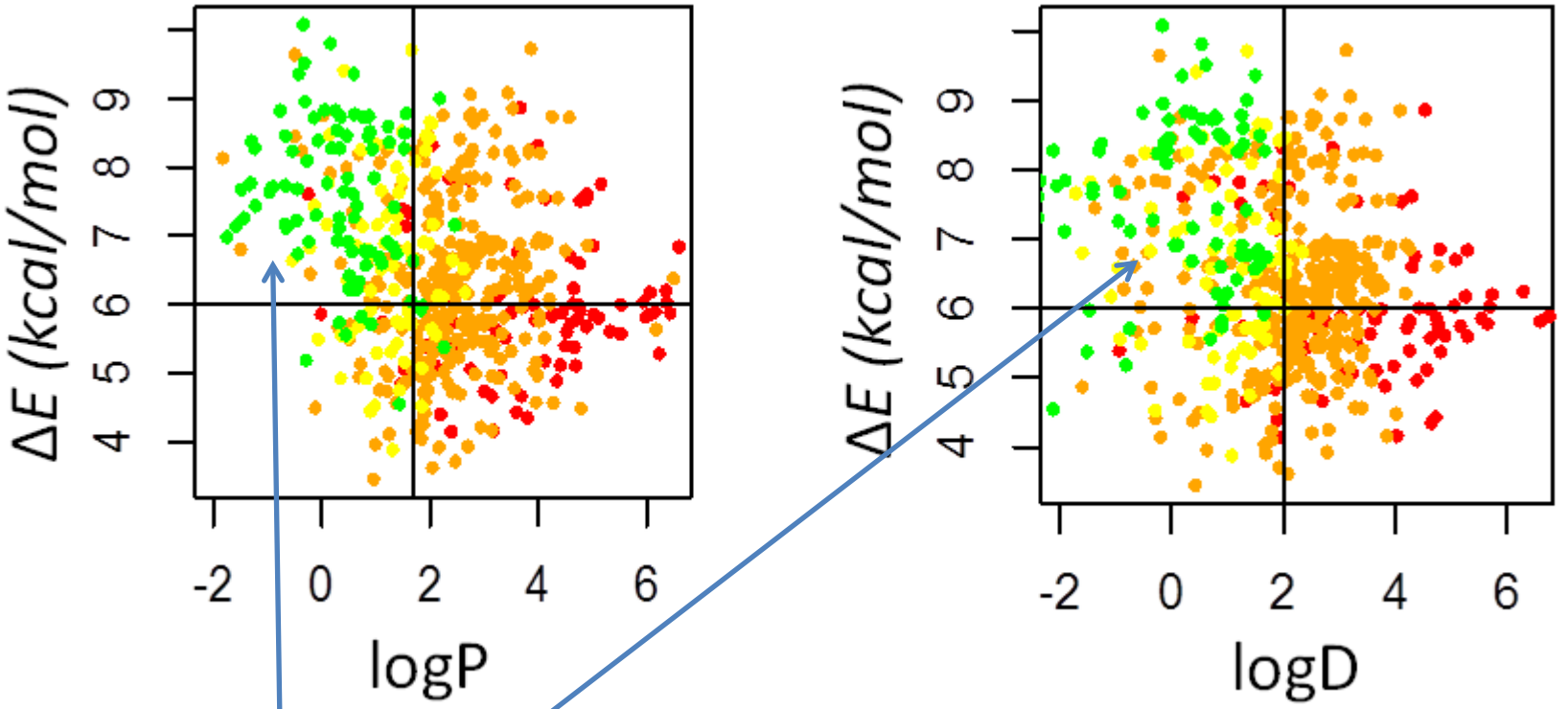


2-Nitrophenol



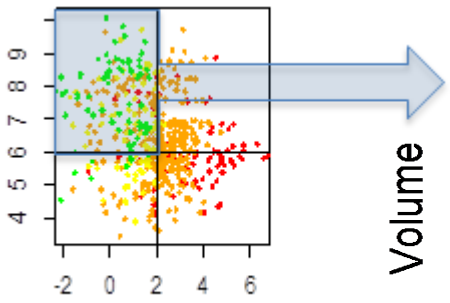
Nonanoic acid

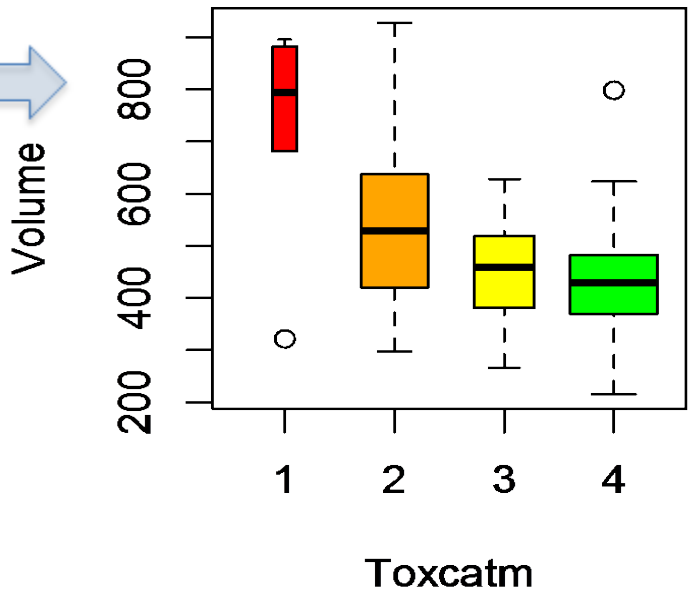
“Safer space” definition based on logD/logP and HOMO-LUMO gap (Δ*E*)

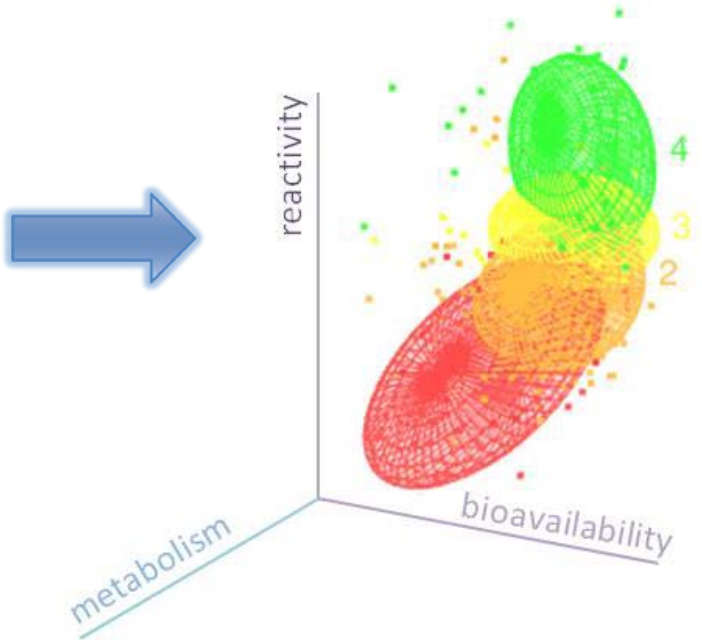


“safer chemical space”: log$D_{o/w}$ <1.7, Δ*E*>6 eV

	logP	Δ*E* (eV)
BPA	1.10	3.48
phthalate	-4.47	4.45
atrazine	1.18	5.62
PBDE	6.33	4.36







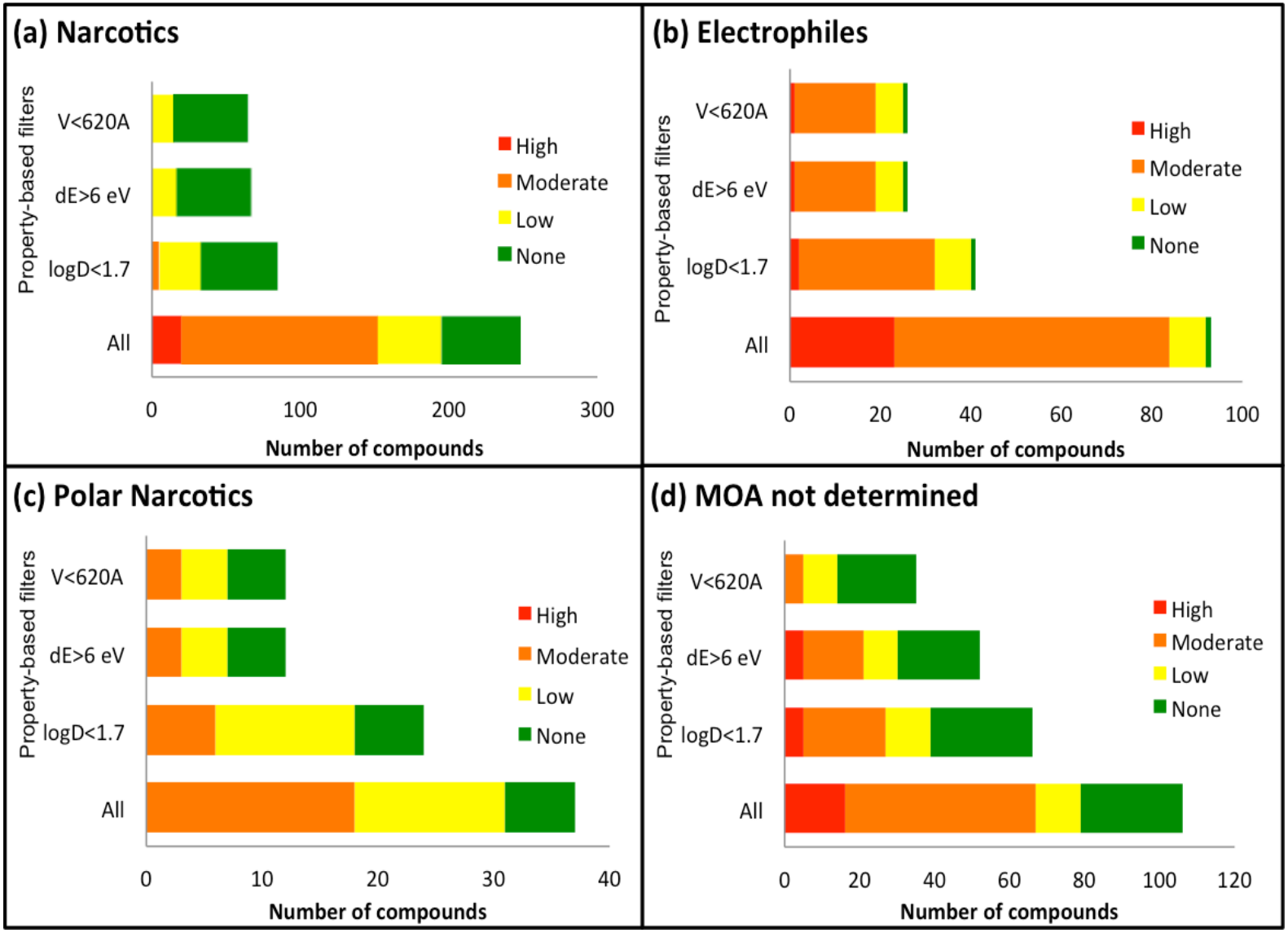
	Acute Aquatic Toxicity Concern Category	High	Moderate	Low	None	Mean LC_{50} of compounds in safer chemical space (mg/L)	Mean LC_{50} of compounds in safer chemical space (mmol/L)
Property-based filter	none	15%	55%	15%	15%	999	0.155
	log$D_{o/w}$<1.7	12 %	27%	80%	100%	2247	1.29
	log$D_{o/w}$<1.7; ΔE>6 eV	7 %	15 %	48%	89%	3006	2.71
	log$D_{o/w}$<1.7; ΔE>6 eV; V<620 Å^3	**1 %**	**11%**	**45%**	**88%**	**3405**	**3.65**

How good are these design guidelines?

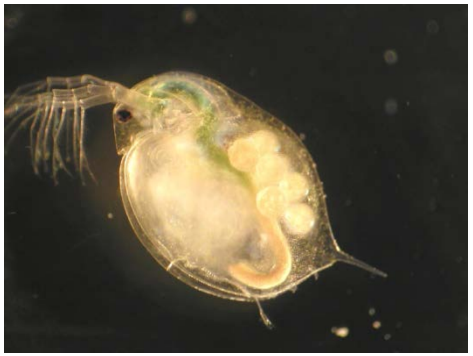
- Compounds that meet the property-based criteria are **10 times more likely to have no or low acute aquatic toxicity** compared to compounds that do not meet these criteria. These results are mechanistically rationalized.
- **Less than 1% chance** that chemicals belonging to high concern category for aquatic toxicity are included in the "safer" chemical space

Kostal, J.; Voutchkova, A. et al. *Proc. Natl. Acad. Sci.* ***2014***, In Press.

Design guidelines by MOA



Validation of the "Rule of Three"



Daphnia magna
EC_{50}, 48-h assay
Japan Ministry of Environment
363 chemicals

Acute Aquatic Toxicity Concern Category	High	Moderate	Low	None
$\log D_{o/w}$<1.7; ΔE>6 eV; V<620 Å^3	1 %	11%	45%	88%
$\log D_{o/w}$<1.7; ΔE>6 eV; V<620 Å^3	5 %	14%	55%	67%

In conclusion…

- We can build “simple” guidelines for reduced toxicity that can be applied to the design of new chemicals
- We do not need a multitude of descriptors, as commonly seen in many QSAR models, to obtain valuable probabilistic information regarding chemical’s toxicity
- The simplicity of these guidelines provides additional benefit to designing around toxicity while retaining functionality

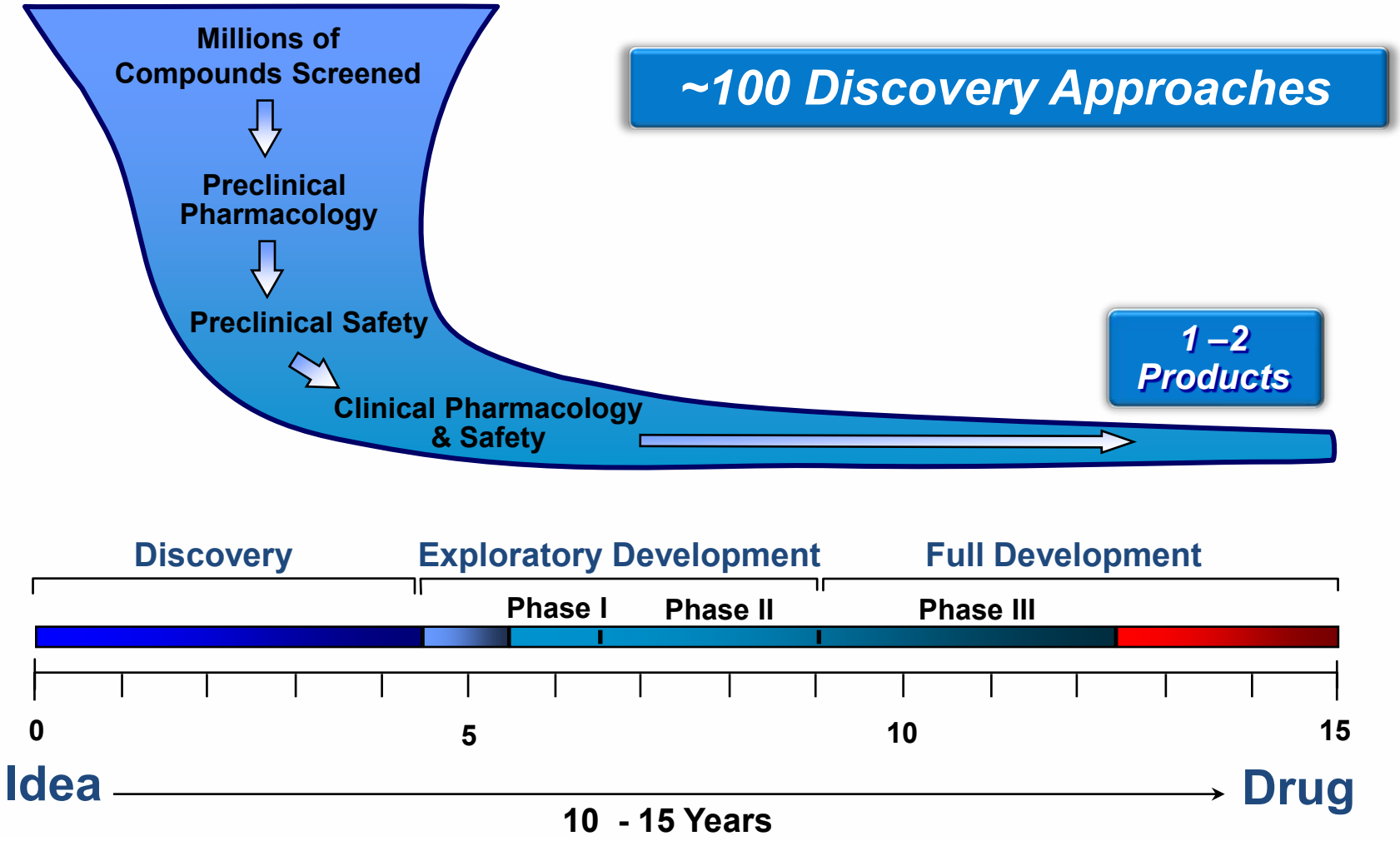
Incorporating Safety into Early Drug Design

Nigel Greene

GC3 Green Chemistry Education Webinar Series
March 18th 2014

Pfizer
WORLDWIDE RESEARCH & DEVELOPMENT
Medicinal Chemistry

Attrition is High in the R&D Process

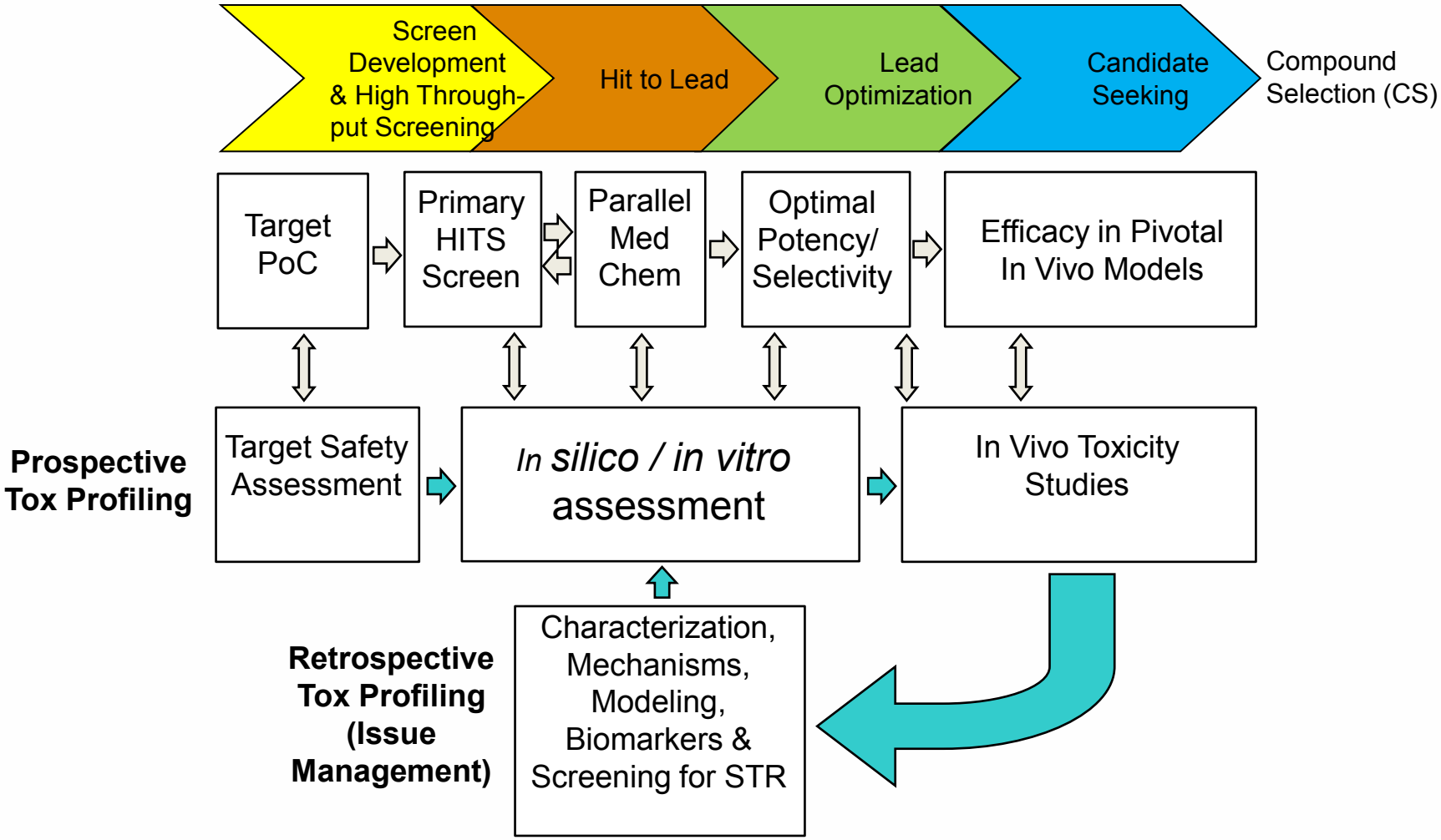


*Source: DiMasi & Grabowski, Managerial Decision Econ, 2007;28:469-479

Drugs Discovery is Time Consuming, Risky and Expensive

- Average Cost of Developing a New Medicine = $1.3B
- Average Time from Discovery to Patient = 10-15 Years
- 1 in 5,000-10,000 Compounds Approved by FDA

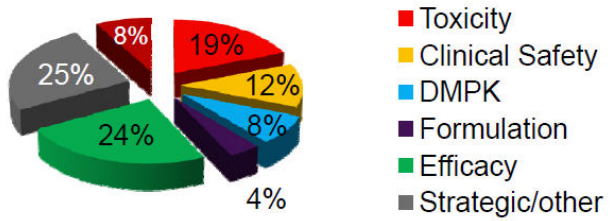
Toxicity Profiling in Drug Discovery



Attrition Causes

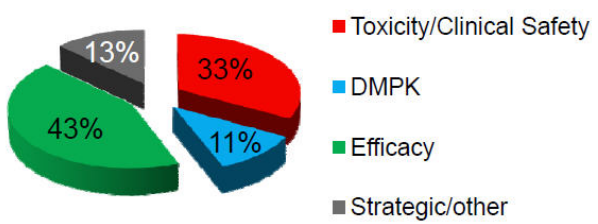
Causes for Drug Attrition: Changing?

Kola & Landis, *Nature Reviews Drug Discovery*, **2004**, *3*, 711

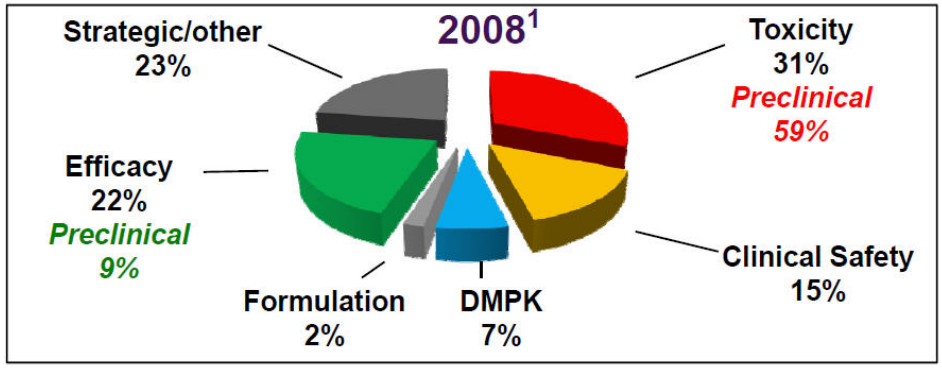


2000

Schuster et al, *Curr. Pharm. Des.*, **2005**, *11*, 3545



1992-2002



Presented at 2010 ACS (Boston) by J Empfield, AZ

"Approximately 10% of new chemical entities (NCEs) show serious adverse drug reactions (ADRs) after market launch."[2]

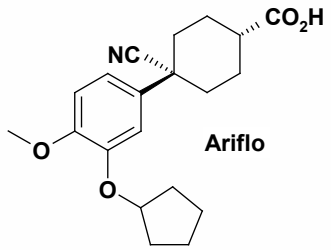
1. Pharmaceutical Benchmarking Forum Study 2008
2. Schuster, D., Laggner, C., and Langer, T. In *Antitargets*, Vaz, R. J., Klabunde, T. Ed.; Methods and Principles in Medicinal Chemistry; Wiley-VCH; **2008**, Ch.1, p3.

The Basic Question

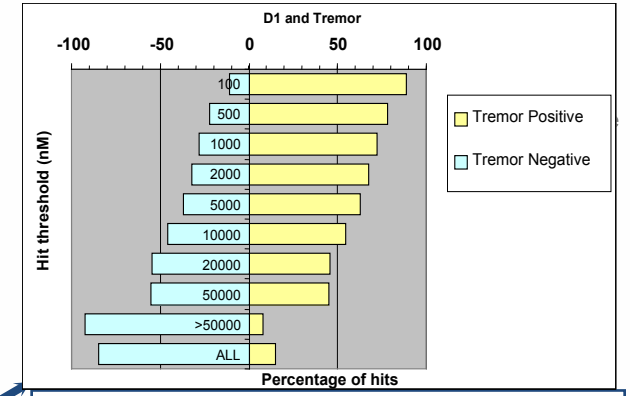
What design features signpost risk?

Factors that Influence Safety Profiles

PDE-4 inhibitors are linked to emesis and vasculitis



D1 activity is linked to tremor



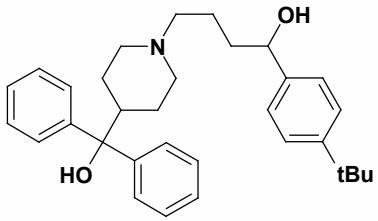
Primary pharmacology

Secondary pharmacology

Origins of adverse safety profile

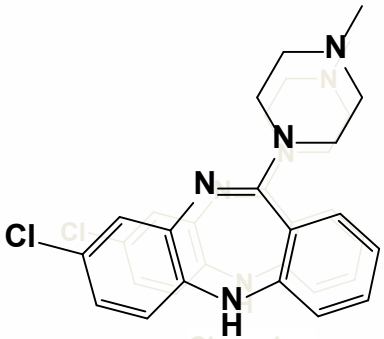
Physicochemical properties

Chemical structure



Terfenadine

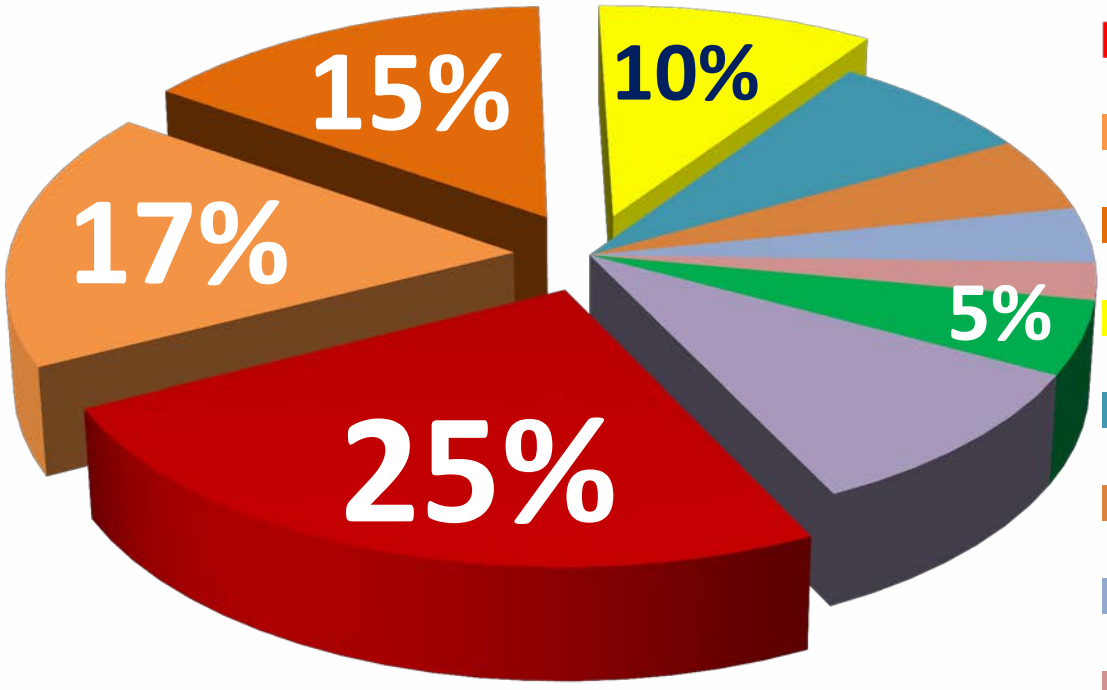
Lipophilic basic compounds at risk of:
Phospholipidosis
QT interval prolongation



Clozapine

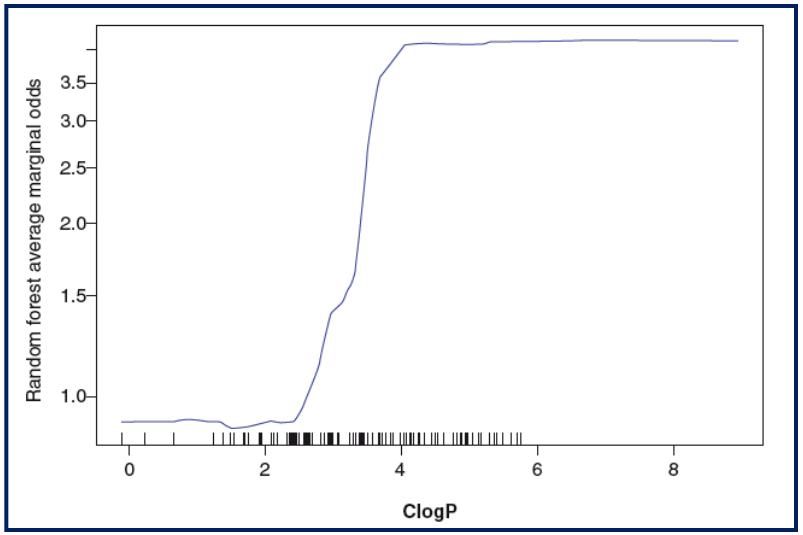
Clozapine causes agranulocytosis and forms reactive metabolites

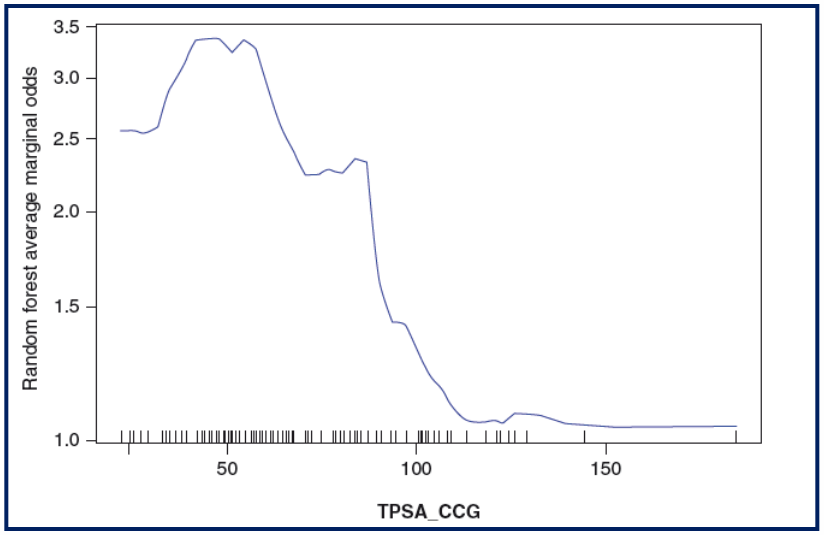
Structural Alerts: 81 drugs withdrawn for idiosyncratic toxicity reasons



- **aniline group**
- **quinone group** **67%**
- **e rich Ar group**
- **acyl glucuronide group**
- quinolone
- hydrazide/hydrazine
- Michael
- benzodioxolane
- ***none group***
- other STAs (singletons)

The role of physiochemical properties



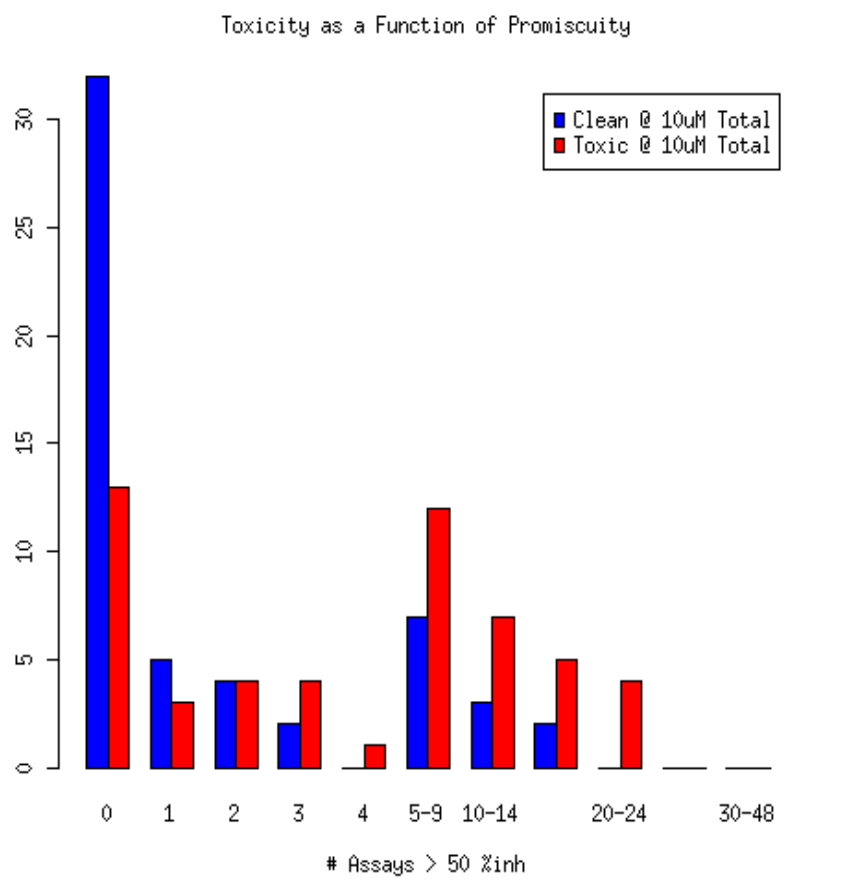


Total Drug	TPSA < 75	TPSA>75
ClogP > 3	2.4 (85)	0.41 (38)
ClogP < 3	1.08 (27)	0.39 (57)

A compound that flags both properties is **~six times** more likely to cause findings in a IVT study at Cmax<10μM than a compound that does not flag in either of these properties.

Expert Opin. Drug Metab. Toxicol. (2009) **5**(8)

Off Target promiscuity



Ratio of promiscuous to non-promiscuous compounds

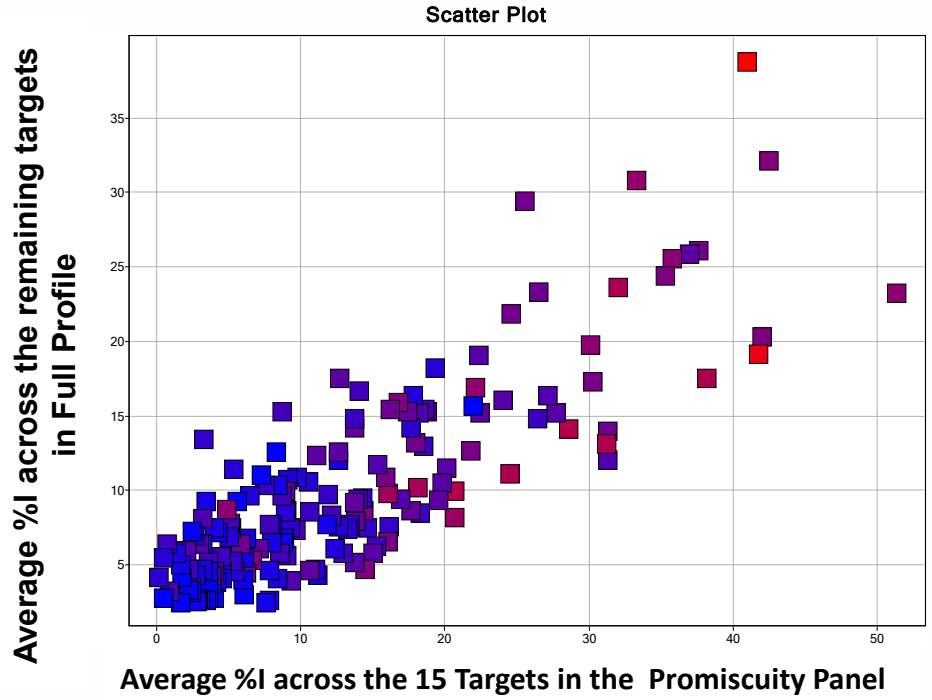
Cerep	TPSA < 75	TPSA>75
ClogP > 3	6.25 (29)	0.44 (13)
ClogP < 3	0.80 (18)	0.25 (25)

Odd Ratio = 25 X

- promiscuity defined as >50% activity in >2 Bioprint assay out of a set of 48 (selected for data coverage only)

Efficiently Characterizing Promiscuity

- Selected subset of 15 targets – The Promiscuity Panel
- Covers GPCRs, Ion channels, PDEs, transporters

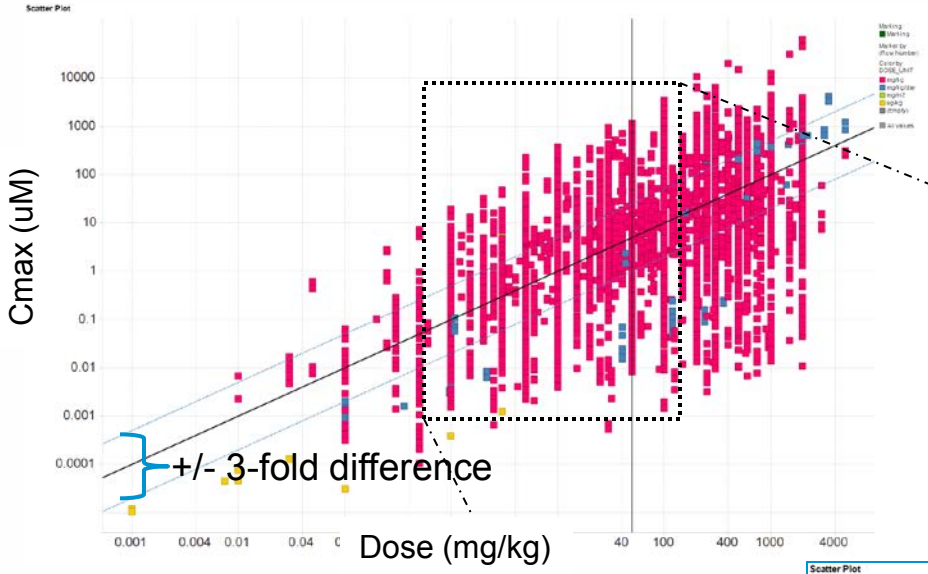


Modified Gini-Coefficient:
Comparing Measures of Promiscuity and Exploring Their Relationship to Toxicity

Xiangyun Wang and Nigel Greene
Molecular Informatics , in Press

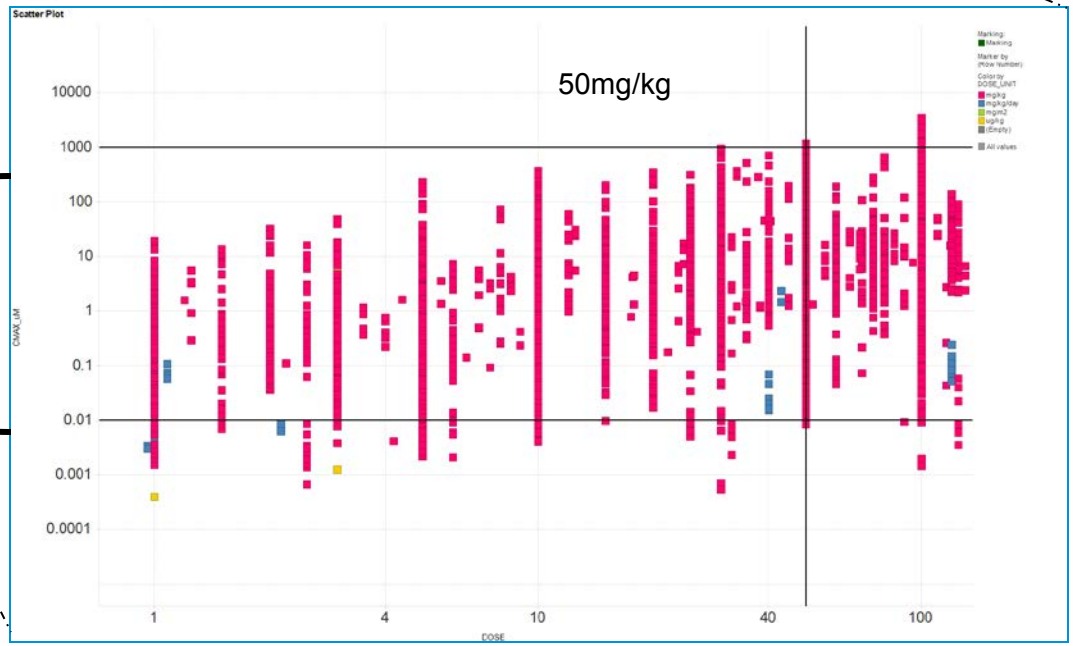
Average inhibition of the 15 targets generally correlates well with overall promiscuity

Dose vs. Exposure



Looking at ~850 compounds across 1600 rat, oral studies

A 50mg/kg dose can give C_{max} concentrations that can span ***5 orders of magnitude*** depending on the compound! (AUC gives a similar picture)

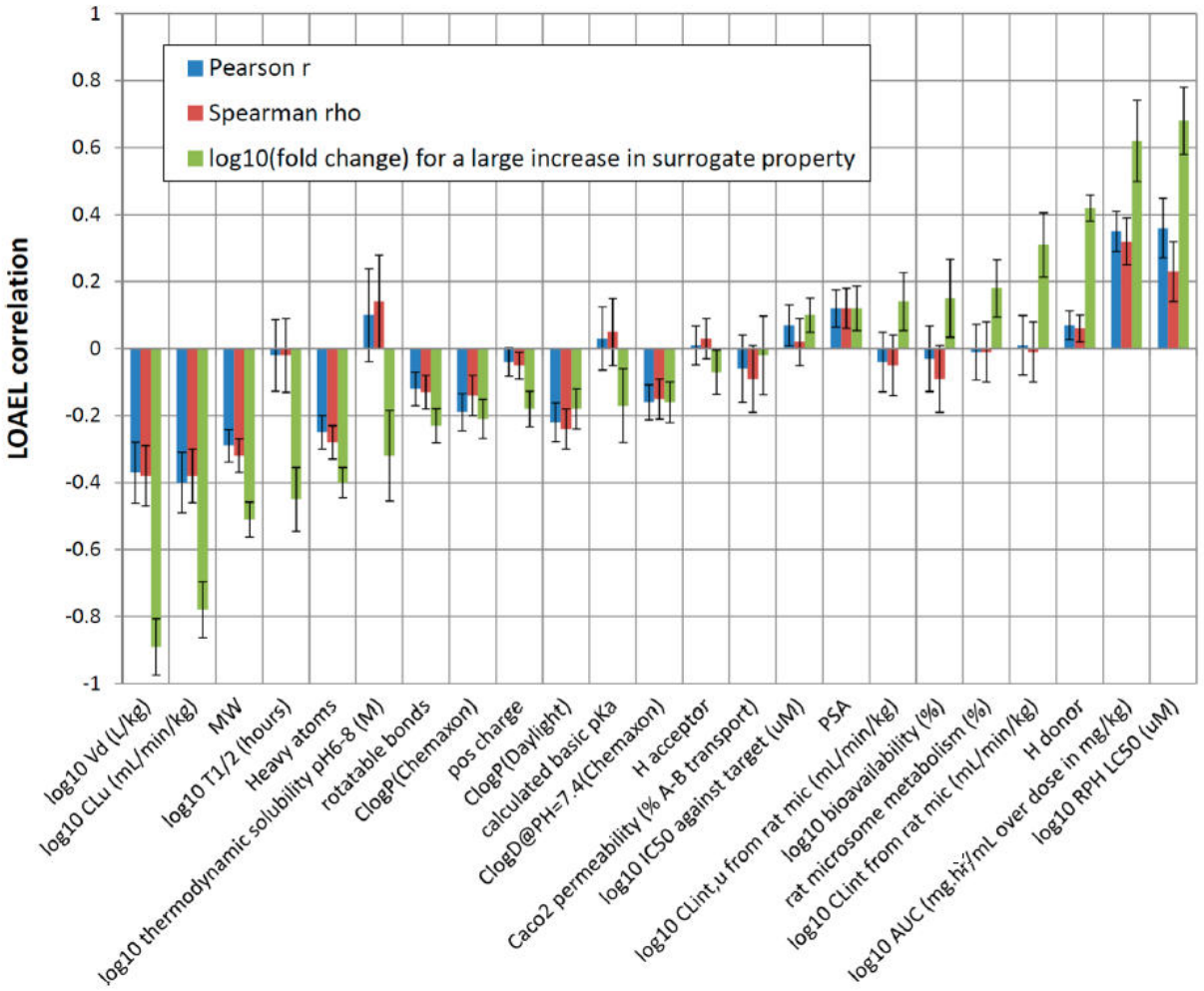


Properties related to LOAEL

Sutherland, J.J., *et al.*, J Med Chem, 2012. **55**(14): p. 6455-66.

LOAEL = Lowest Observable Adverse Effect Level

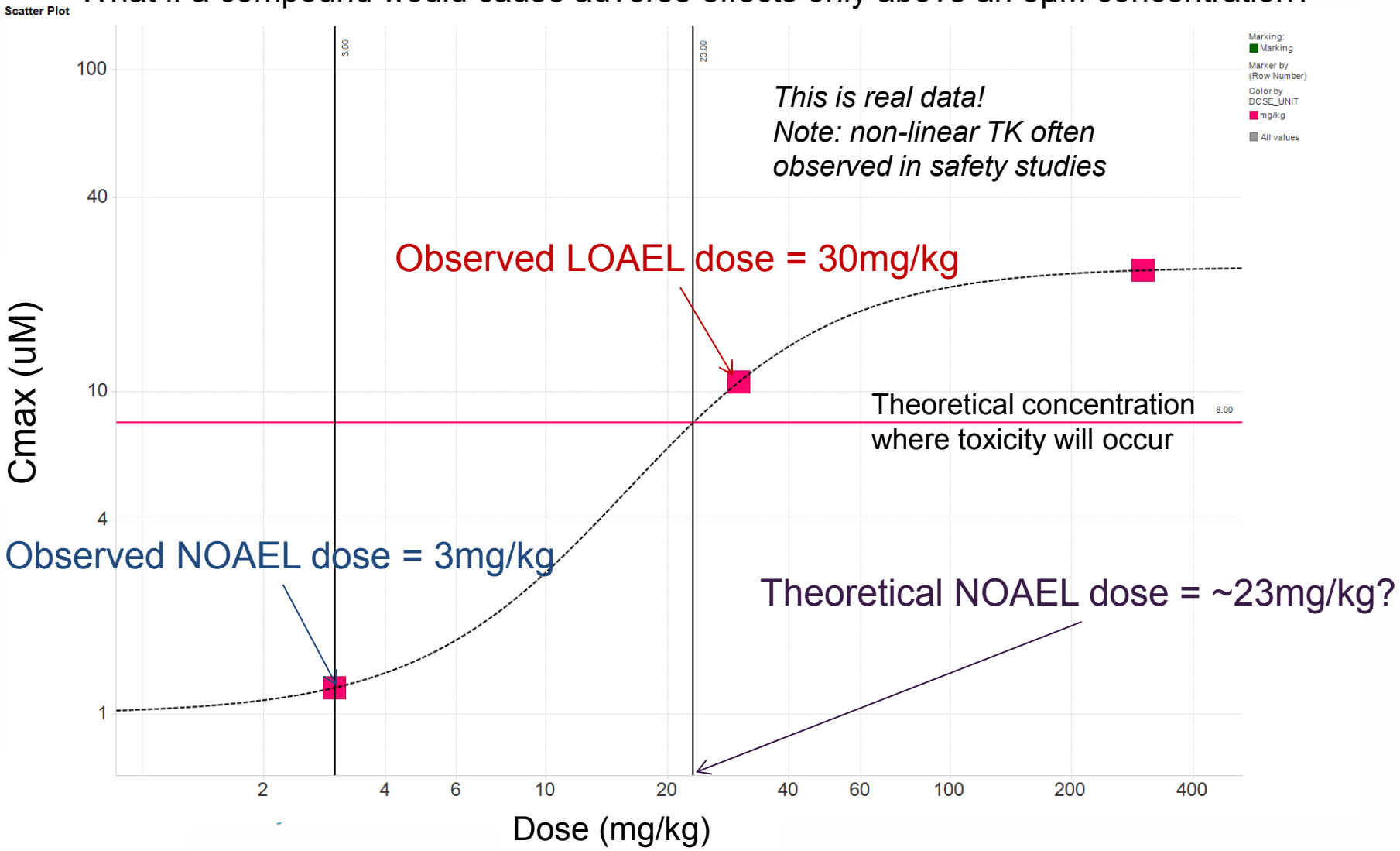
- Volume of distribution and cytotoxicity had largest impact on LOAEL in a rodent study.
  - Increase in Vd → Decrease in LOAEL
  - Increase in LC50 → Increase in LOAEL



The Problem with LOAELs

The observed NOAEL and LOAEL are heavily reliant on where doses are set in a study.

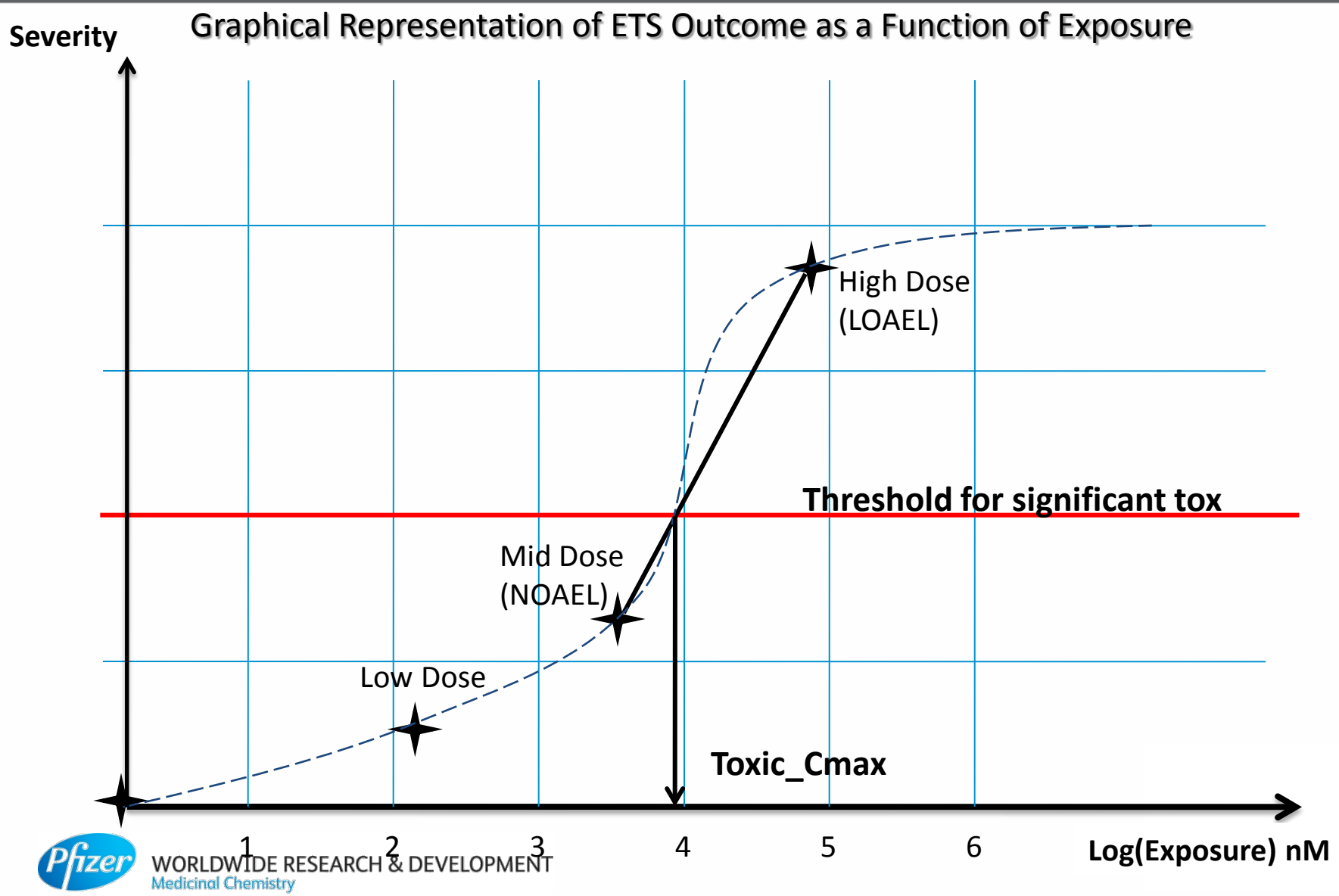
What if a compound would cause adverse effects only above an 8µM concentration?



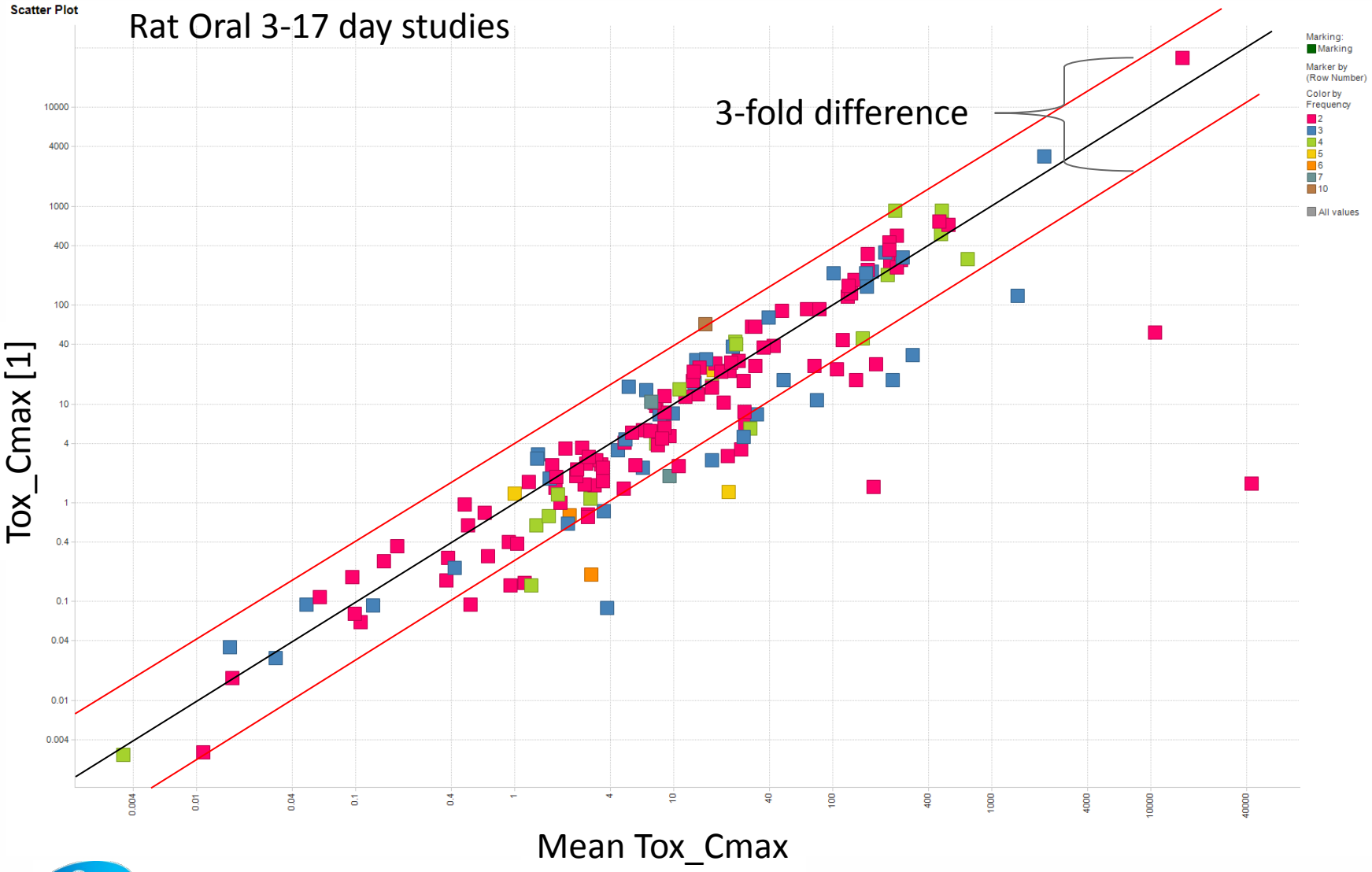
A New Classification System

- Uses a scoring system to grade the severity of toxicity seen at each dose in the study
    - Arbitrary scale based on impact of each finding
    - Redness (1); inflammation (10); degeneration (100); death (1000)
    - A cumulative score of ≥ 100 considered to be “significant” level of toxicity
- Using a threshold of 100, estimate what Cmax would give rise to significant toxicity for each compound
- Use this Toxic_Cmax to rank order compounds
    - Now a continuous scale rather than two-bucket system
- No extrapolation for studies where significant toxicity not observed

Toxic Cmax Approach

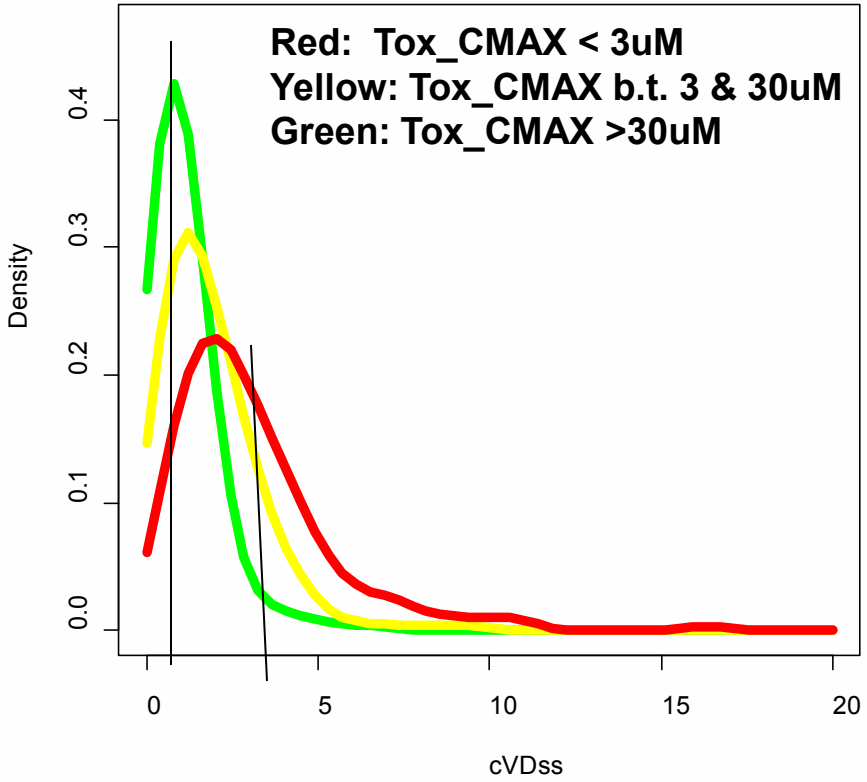


Variability in Toxic Cmax

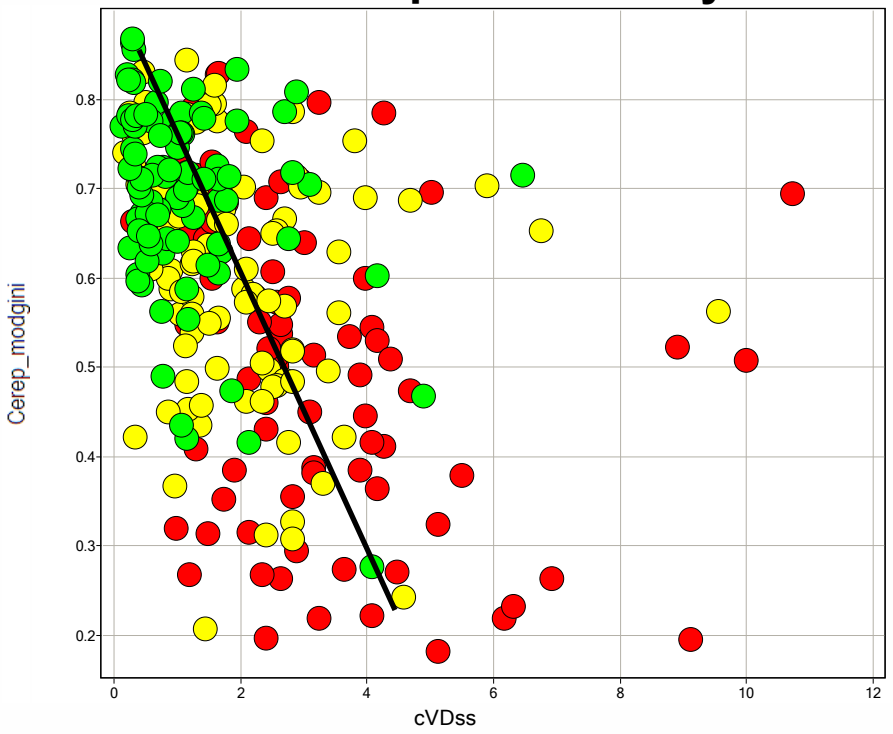


Correlations to ToxicCmax

Density Distribution Plot of cVDSS



cVDSS ~ Cerep Promiscuity

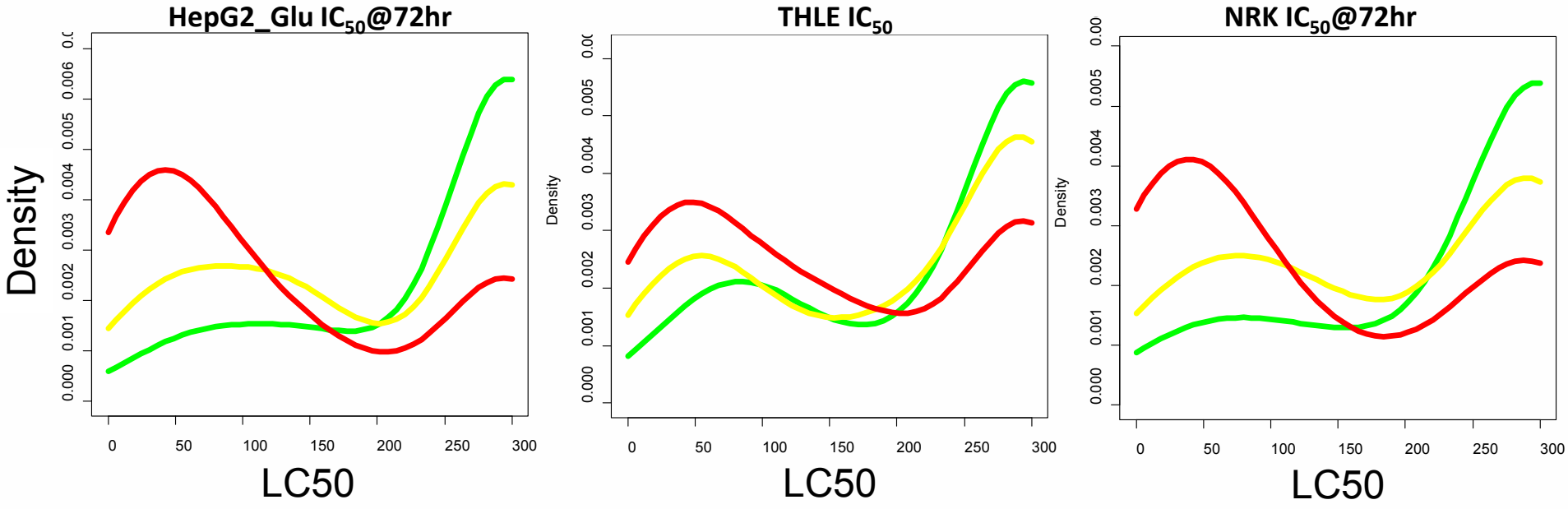


c.f. Relating Molecular Properties and in Vitro Assay Results to in Vivo Drug Disposition and Toxicity Outcomes
J. Med. Chem., 2012, *55* (14), pp 6455–6466

Comparing Assays to Toxic Cmax

Red line: Compounds where ToxicCmax < 3uM
Yellow line: Compounds where ToxicCmax between 3 & 30uM
Green line: Compounds where ToxicCmax >30uM

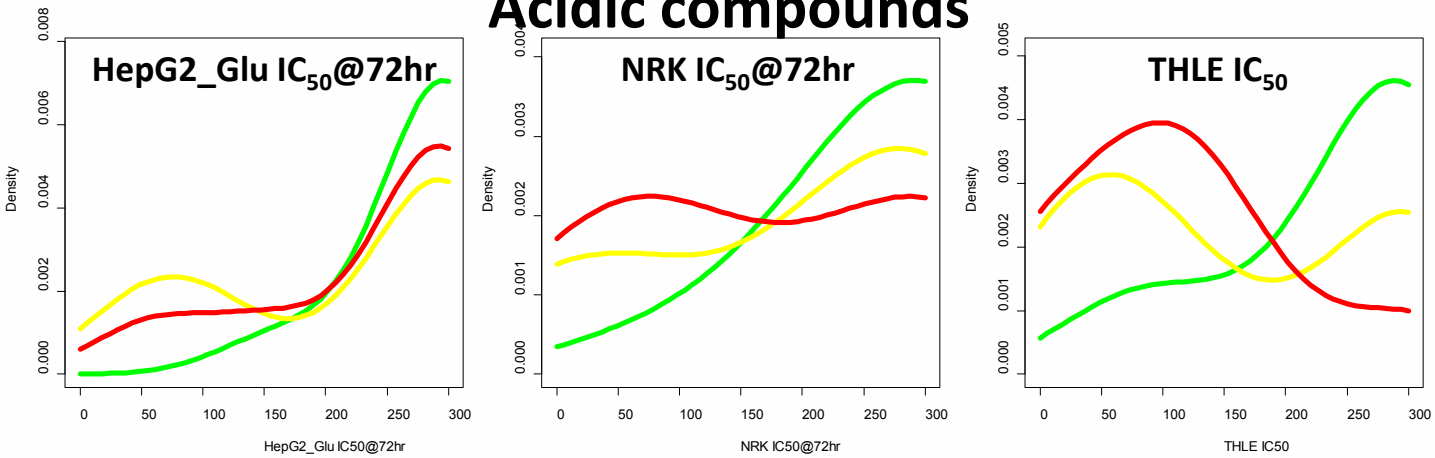
Cell line:



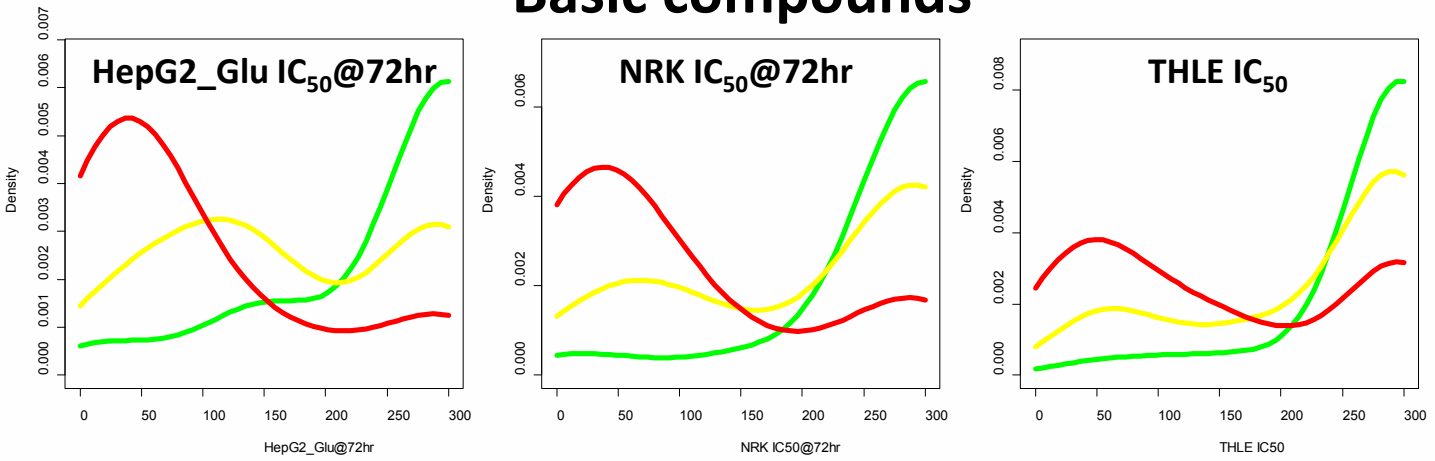
- "Diverse" dataset combining of basic, netrual and acidic compounds

The Importance of Ionization State

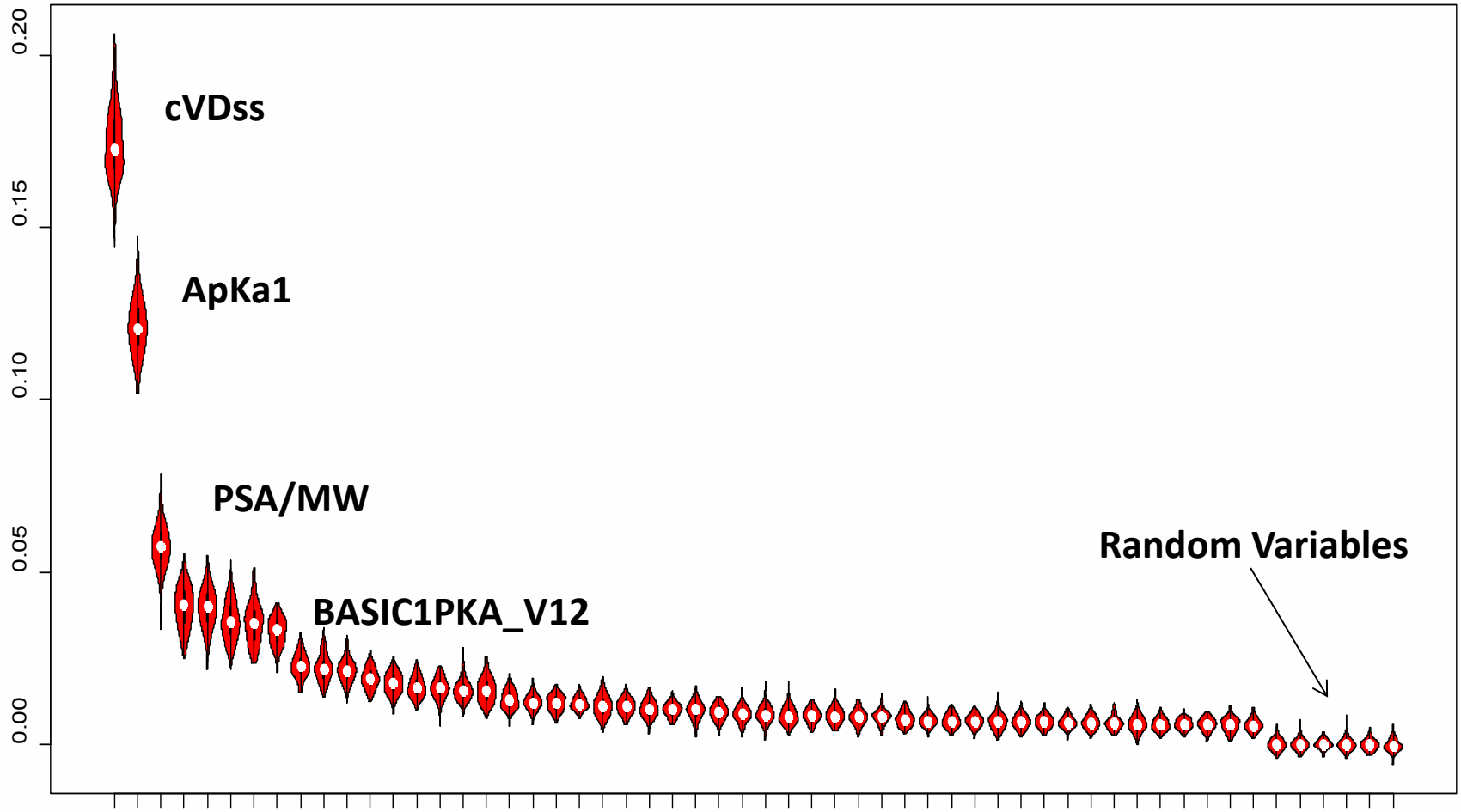
Acidic compounds



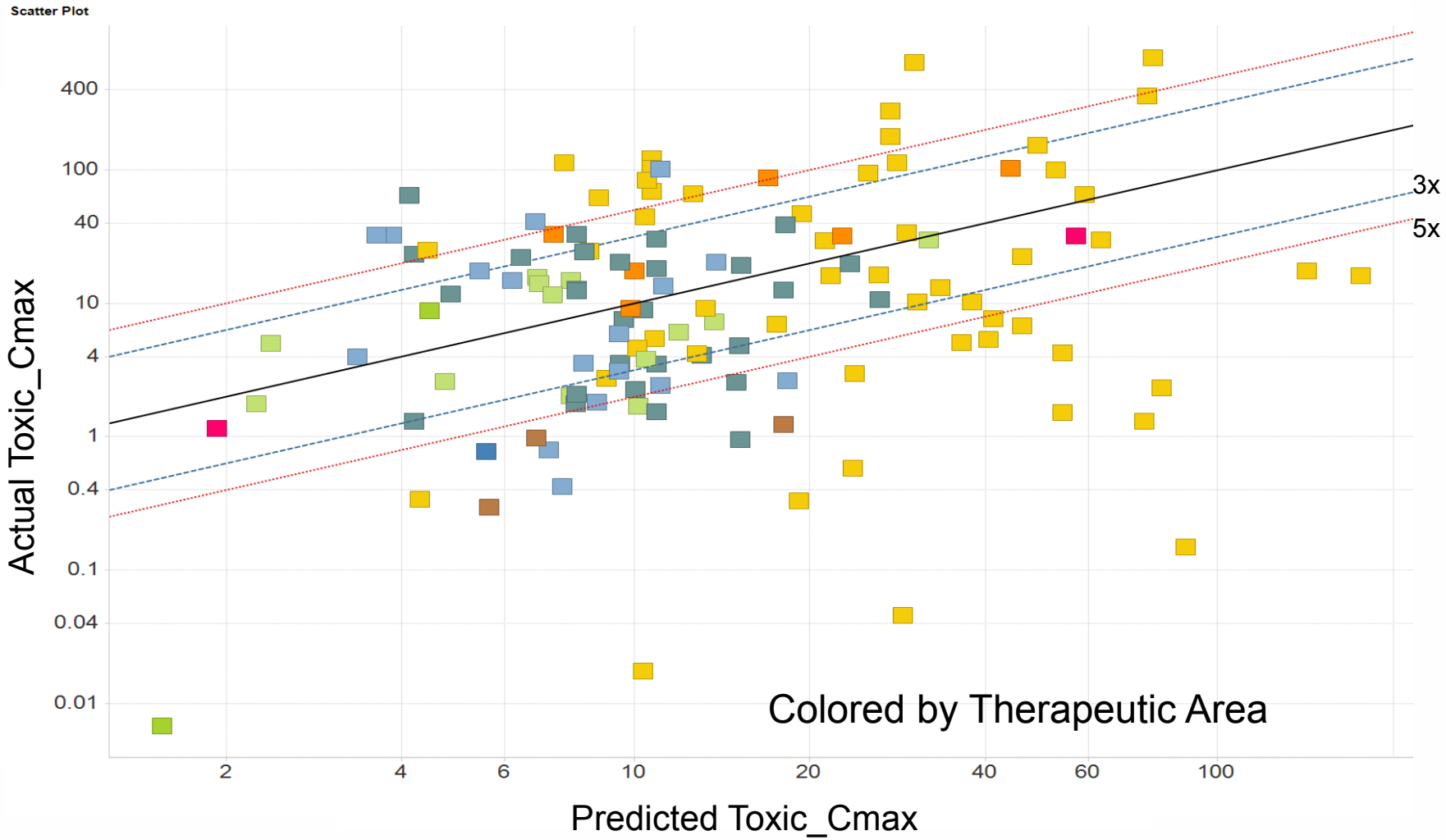
Basic compounds



Variable Importance from modeling Toxic_Cmax



Predicted vs. Actual Toxic_Cmax

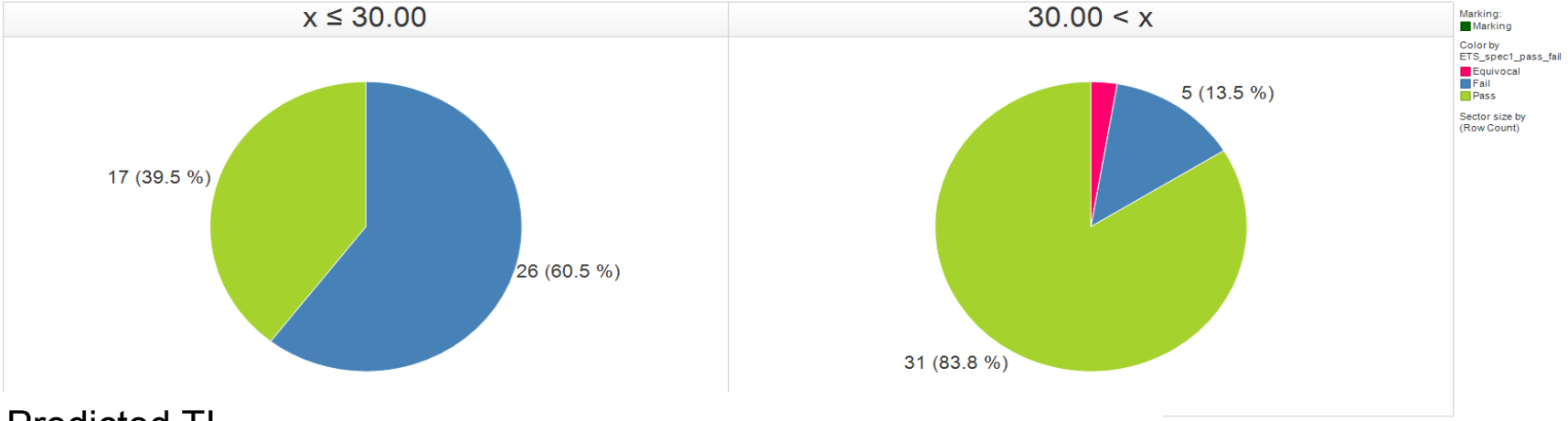


Therapeutic Index

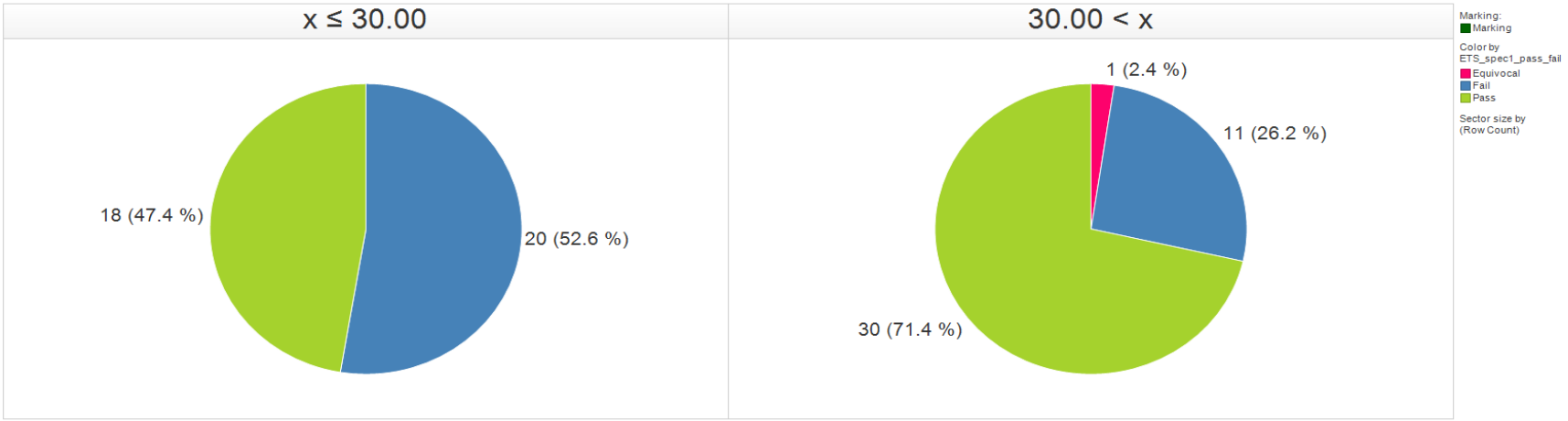
- Most decisions in drug development are based on a therapeutic index (TI)
  - The difference between the efficacious concentration and the toxic concentration
- An adequate TI determines if compound progresses in development (pass) or is stopped (fail)
- Acceptable levels for TI are often situational depending on many factors
  - Indication
  - Duration of treatment
  - Patient population, etc

Figure 6b: Distribution of compounds by pass or fail call that have a TI <30 or TI>30

Actual TI

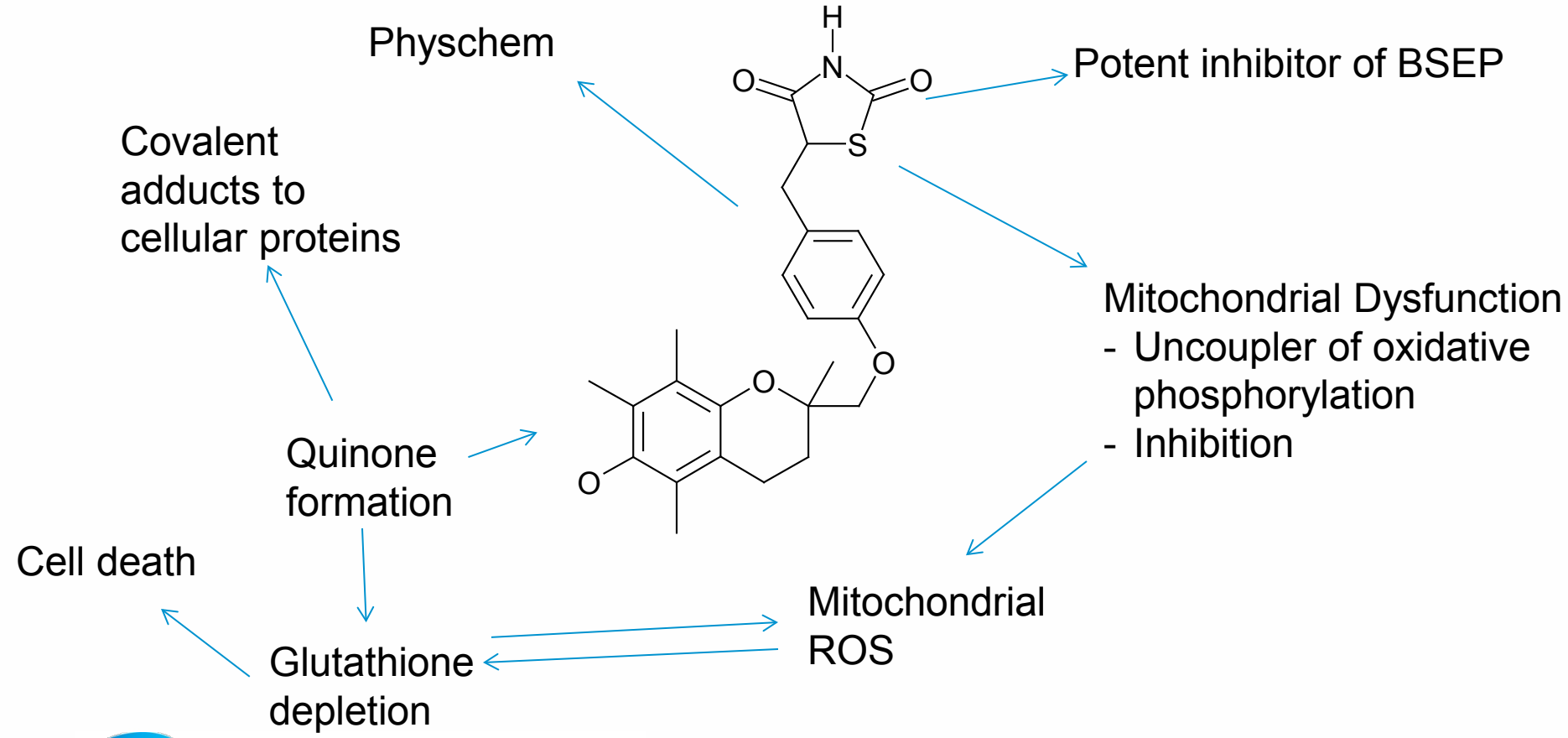


Predicted TI



In Vivo Toxicity is (mostly) Multifactorial

- Troglitazone – withdrawn for liver failure



Summary

- In our small molecule discovery programs we employ a predictive platform which detects around **60% of the compounds which cause low dose toxicity** in preclinical species (with a <10% false positive rate).
- In 2013 Pfizer utilized this approach to help guide the early chemistry efforts on >70 discovery projects. This approach **initiates safety considerations early** in projects, and is a framework for evaluating the predictivity of new assays.
- Building such a tool **relies heavily on well characterized training compound sets** and excellent engagement across biologists, chemists and computational scientists.
- Our current focus for this approach is to address the impact of dose projection, and to model severity of toxicity.
- Value is in **steering away from no hope chemistry**, better survival and resource utilization

Thanks for joining us!

For more educational webinars
or to learn about the GC3:

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