Welcome to the American College of Toxicology's Webinar Series

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British Toxicology Society

SOT | Biological Modeling Specialty Section

Society of Toxicologic Pathology

Teratology Society

Computational Toxicology: A useful tool for hazard identification and risk assessment?

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Presenter

Russell Naven, PhD

- Senior Principal Scientist, Molecular Informatics Worldwide Medicinal Chemistry, Pfizer Inc., US
  - Identify safety risks early in the drug discovery process
  - Development of predictive *in silico* and *in vitro* models of *in vivo* toxicity
- Principal Scientist, Lhasa Limited, UK
  - Developed structure-activity relationships for various toxicological endpoints for inclusion in Derek for Windows (Derek Nexus)
- Senior Synthetic Chemist, AstraZeneca, UK
  - Design and synthesis in oncology and inflammation research

Presentation

- What is computational toxicology?
- Traditional applications e.g. prediction of mutagenicity
  - Challenges in modelling *in vitro* data
  - Assessing predictive performance
- The prediction of complex toxicological endpoints
- Development of robust safety screening paradigms
- Summary

What is Computational Toxicology?

- Understanding the relationship between the properties of a compound and its toxicological activity

Structural
- Molecular weight
- Number of atoms
- Reactive chemical groups (nitro, aromatic amines)

Features
- Lipophilicity
- Solubility
- ADME properties (permeability, metabolism)
- Results from *in vitro* assays
  - Cytotoxicity
  - Mitochondrial dysfunction

- Build predictive models for toxicity prediction

What is Computational Toxicology?

- Computational Toxicology is essential to improving the risk assessment process and identifying safety hazards across many industries

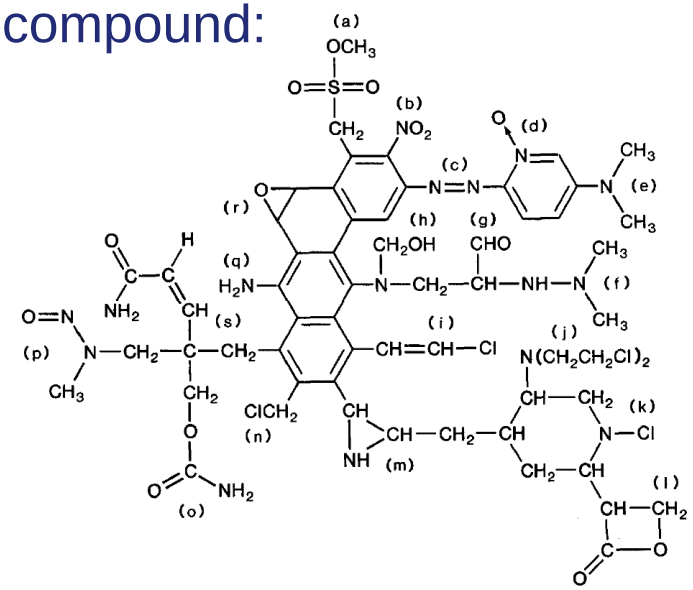
in silico | **in chemico** | **in vitro** | **ex-vivo/in vivo**

Structural Alerts | Physicochemical Properties | Reactivity | Stability | Biochemical | ADME | Cellular | Organ | Zebrafish

in silico

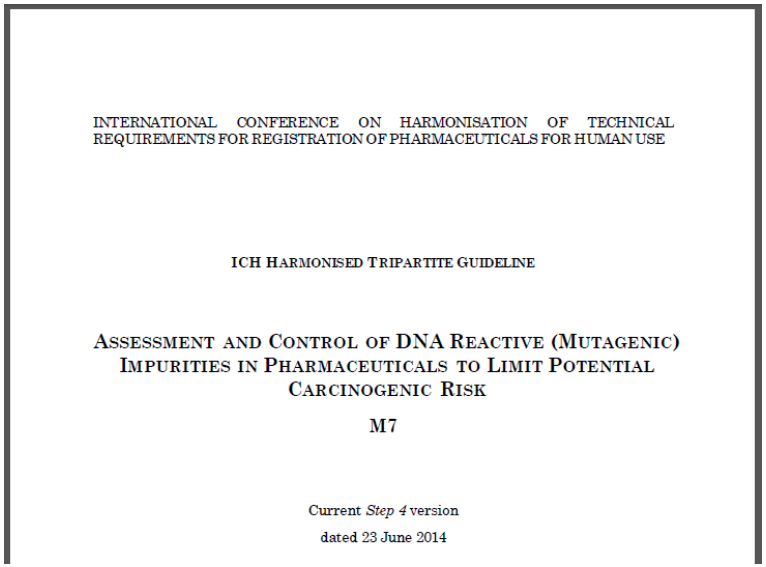
Modelling Mutagenic Activity

- Mutagenicity is hereditary DNA damage and can be observed in the Ames test
- In 1991 – Published a structural alert based electrophilic model compound:



Tennant and Ashby, Mut. Res. 1991, 257, 209-227

- 2014 – ICH M7 guidelines for use of *in silico* models
- Predictions may be accepted *in lieu* of experimental data



INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

ASSESSMENT AND CONTROL OF DNA REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO LIMIT POTENTIAL CARCINOGENIC RISK

M7

Current *Step 4* version

dated 23 June 2014

Modelling Mutagenic Activity

- Relatively simple mechanisms of activity enable the development of robust models

1. Guanine is an electron-rich base that can react with many electron-poor compounds

2. Bases may oxidize in the presence of reactive oxygen species

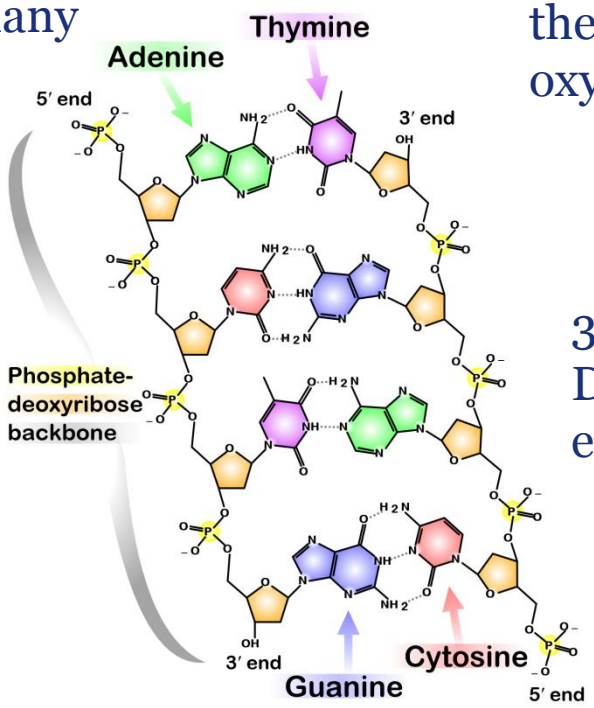
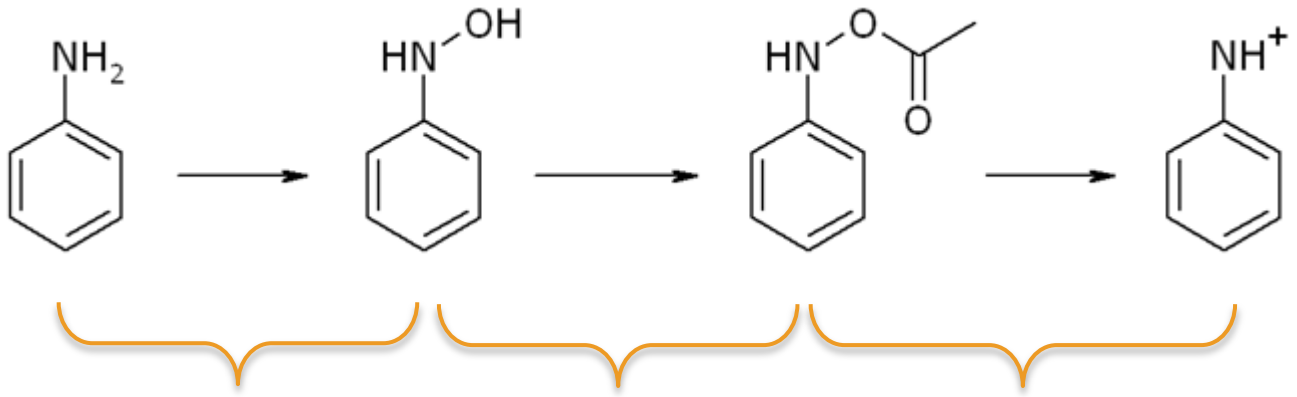


Fig: Madprime (wiki)

3. Intercalation can disrupt DNA synthesis, repair and enhance mechanisms 1&2

Aromatic Amine Mutagenicity

- General mechanism involves formation of reactive intermediates:



Phase I Metabolism

oxidation

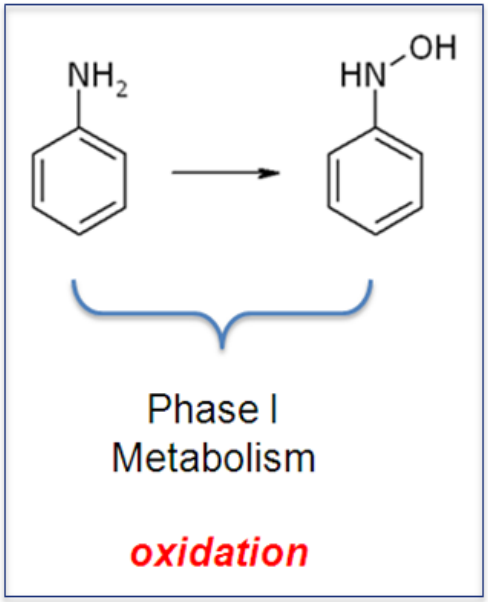
Phase II Metabolism

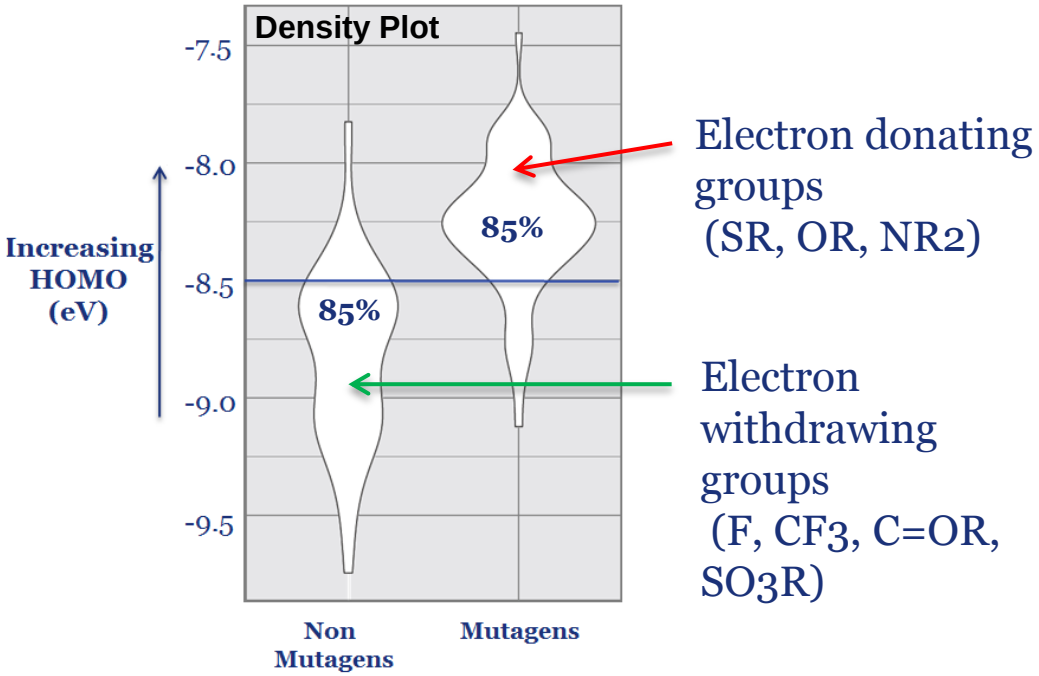
sulphonation
acetylation

Nitrenium ion formation

What Features Drive Aromatic Amine Mutagenicity

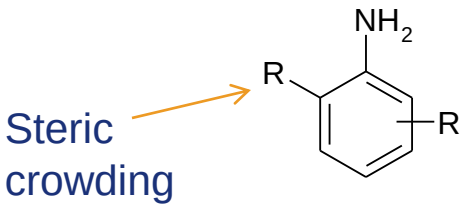
- Not all aromatic amines are mutagenic
- Mutagenicity correlates strongly with Highest Occupied Molecular Orbital (HOMO)
- HOMO reflects ability of amine to be oxidized by Cytochrome P450s



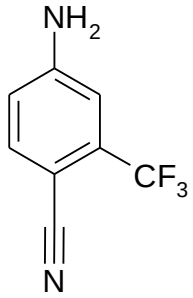


Mispredicted Compounds

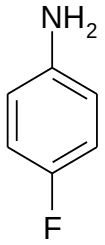
- Despite >85% predictive performance, HOMO energy does not describe mutagenic activity of all chemical space



Non-Mutagenic



Mutagenic[1]
HOMO = -9.27 eV



Mutagenic[2]
HOMO = -8.55 eV

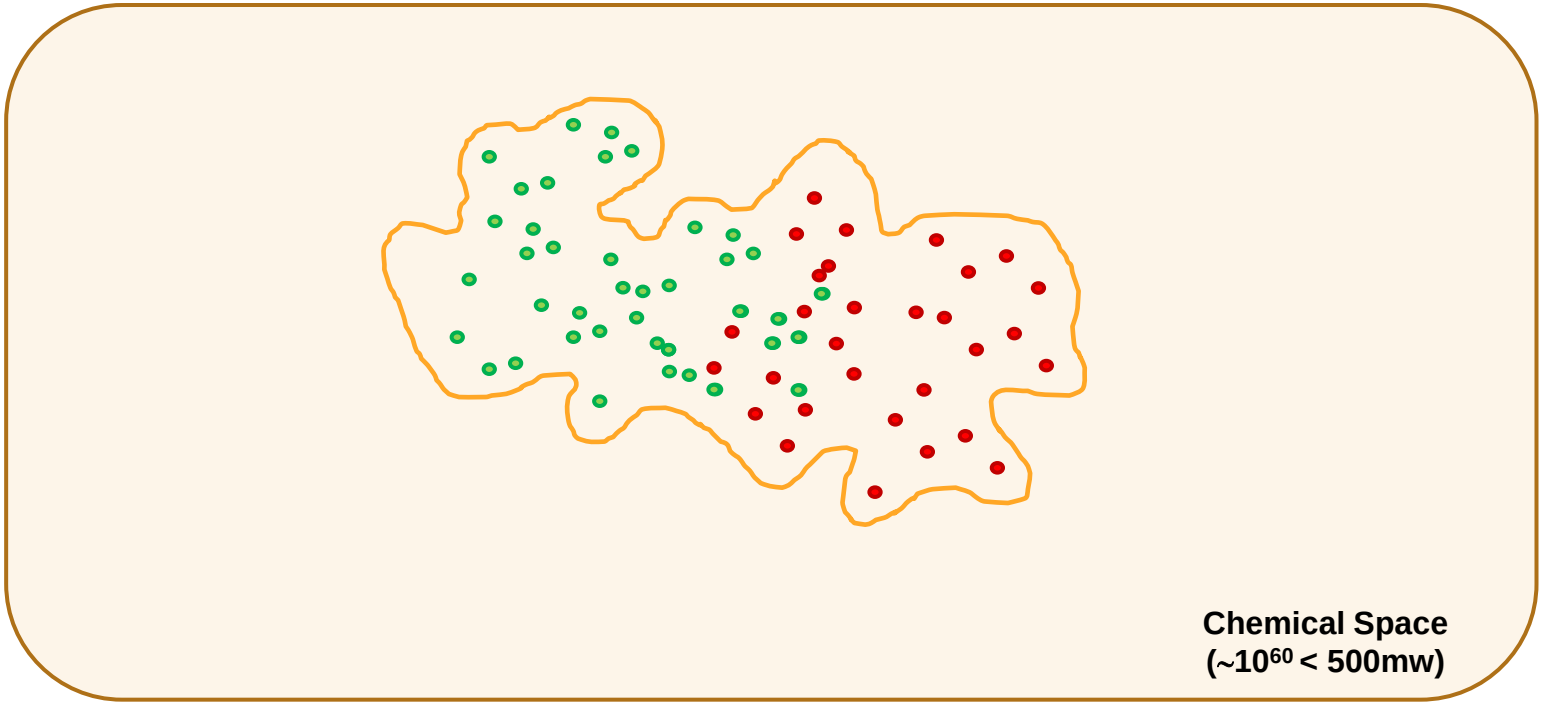
- These compounds require additional features within the model to describe their activity
- Should be investigated if we are to fully understand the predictive performance of the model

[1]Sutter *et al.* Reg Toxicol Pharm, 2013, 67, 39-52

[2]Bentzien *et al.* J Chem Inf & Mod, 2010, 50, 274-297

The Applicability Domain of a Model

- Describes the chemical space upon which the model has been created



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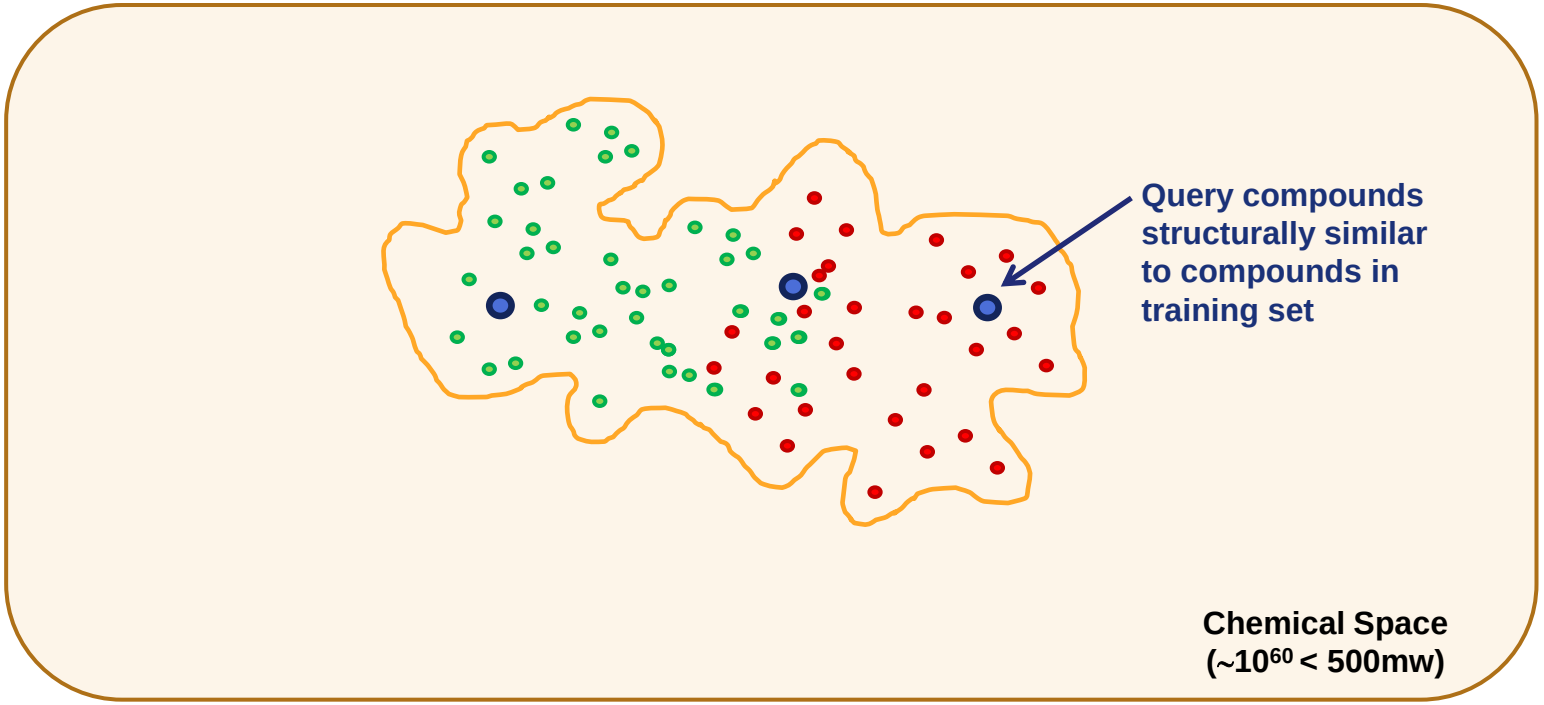

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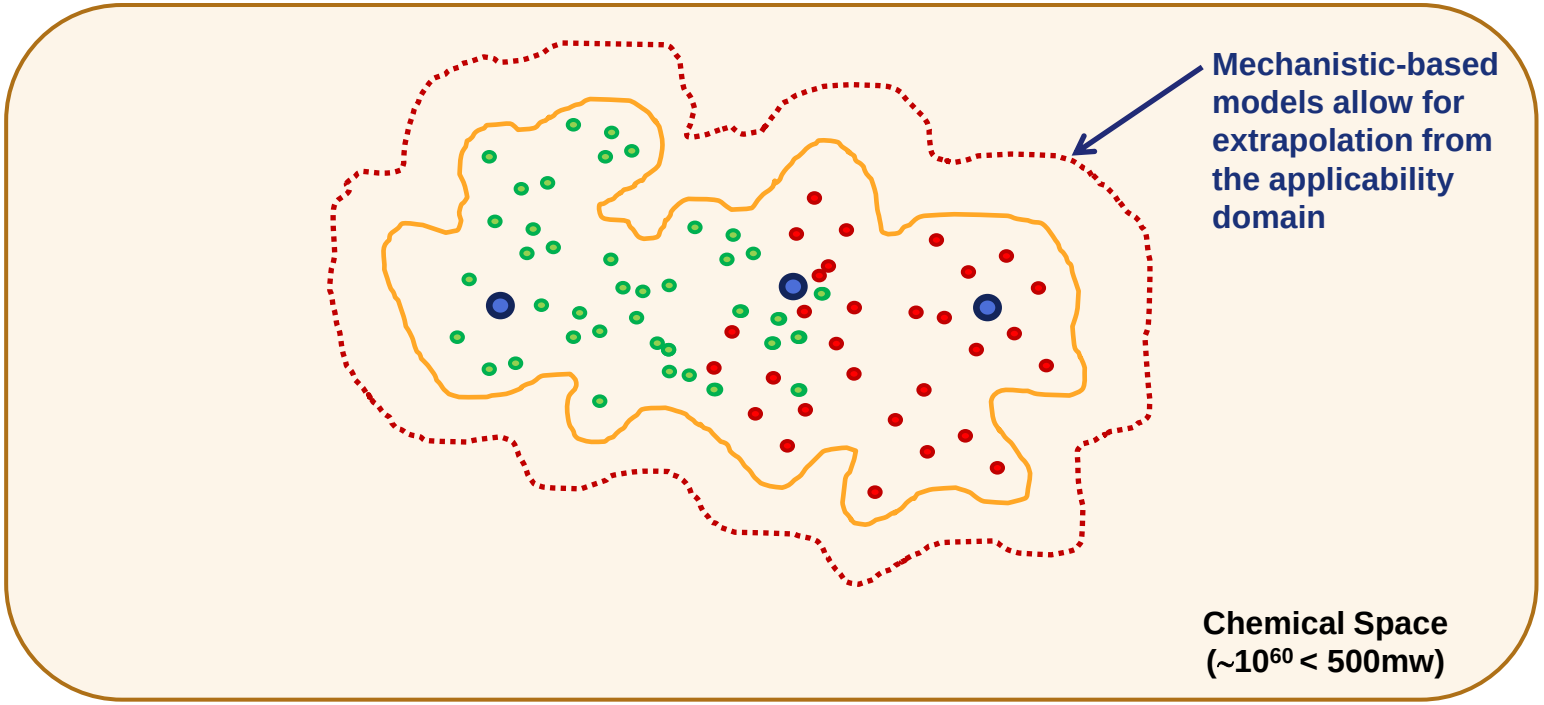

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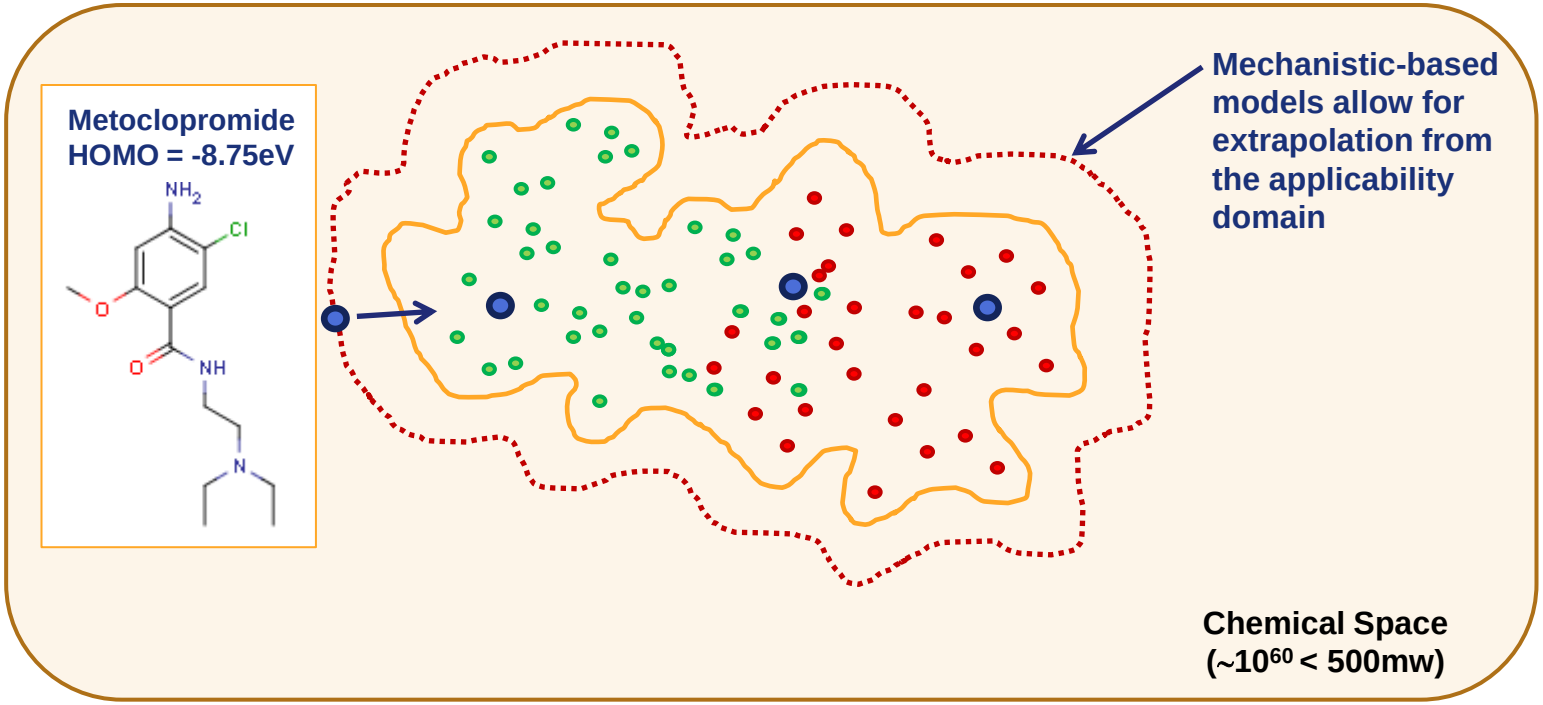
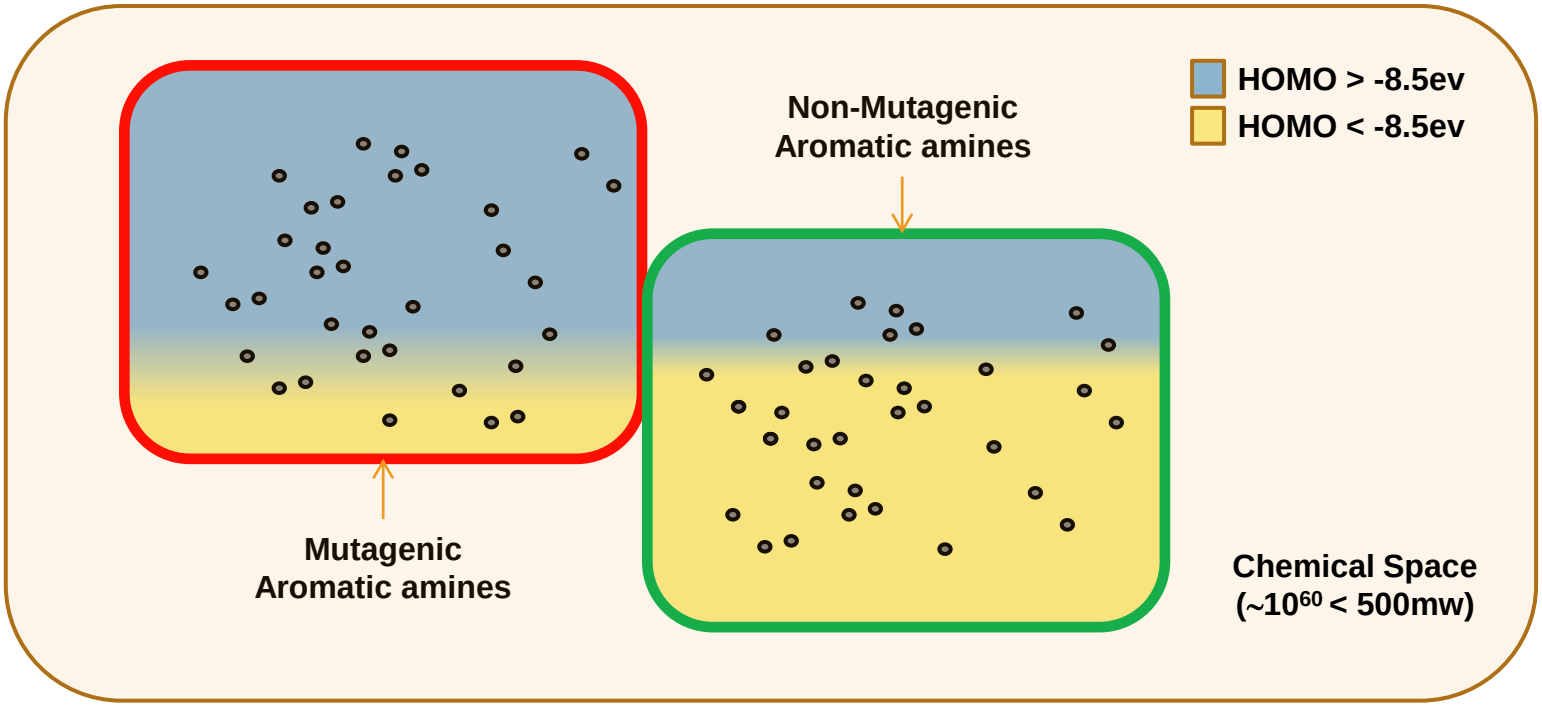


Model Validation

- Hypothetical pictorial view of aromatic amine dataset:



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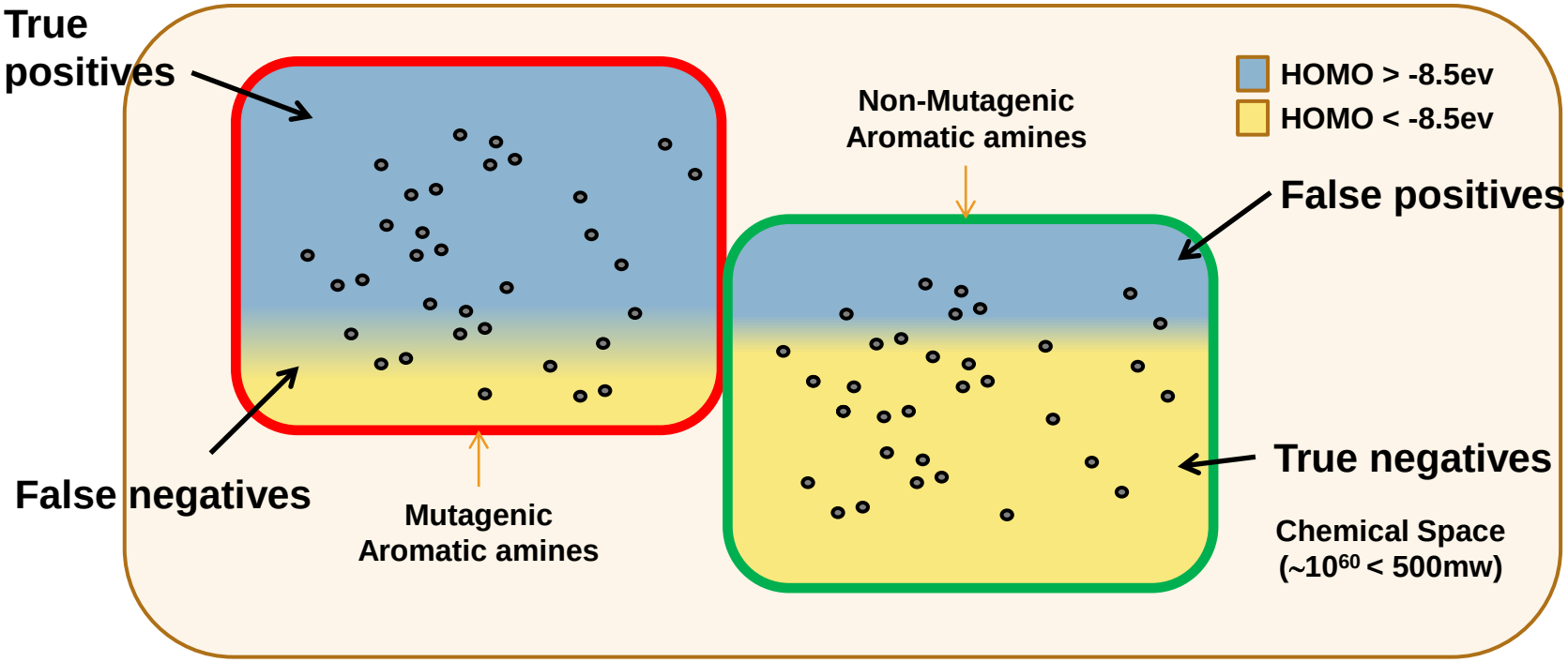


Truth Tables

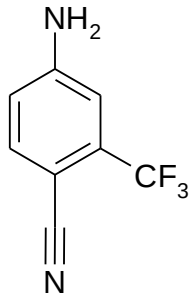
	Ames POS	Ames NEG
Model POS	True POS	False POS
Model NEG	False NEG	True NEG

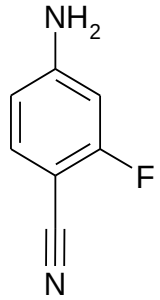
- Statistics help in assessing how good the model is at predicting the training set (applicability domain)
- Real value is highlighting mispredicted compounds!

Understanding Mispredicted Compounds

- Outliers, false positives and false-negatives highlight *potential* knowledge gaps within models

Mutagenic
HOMO = -9.27 eV





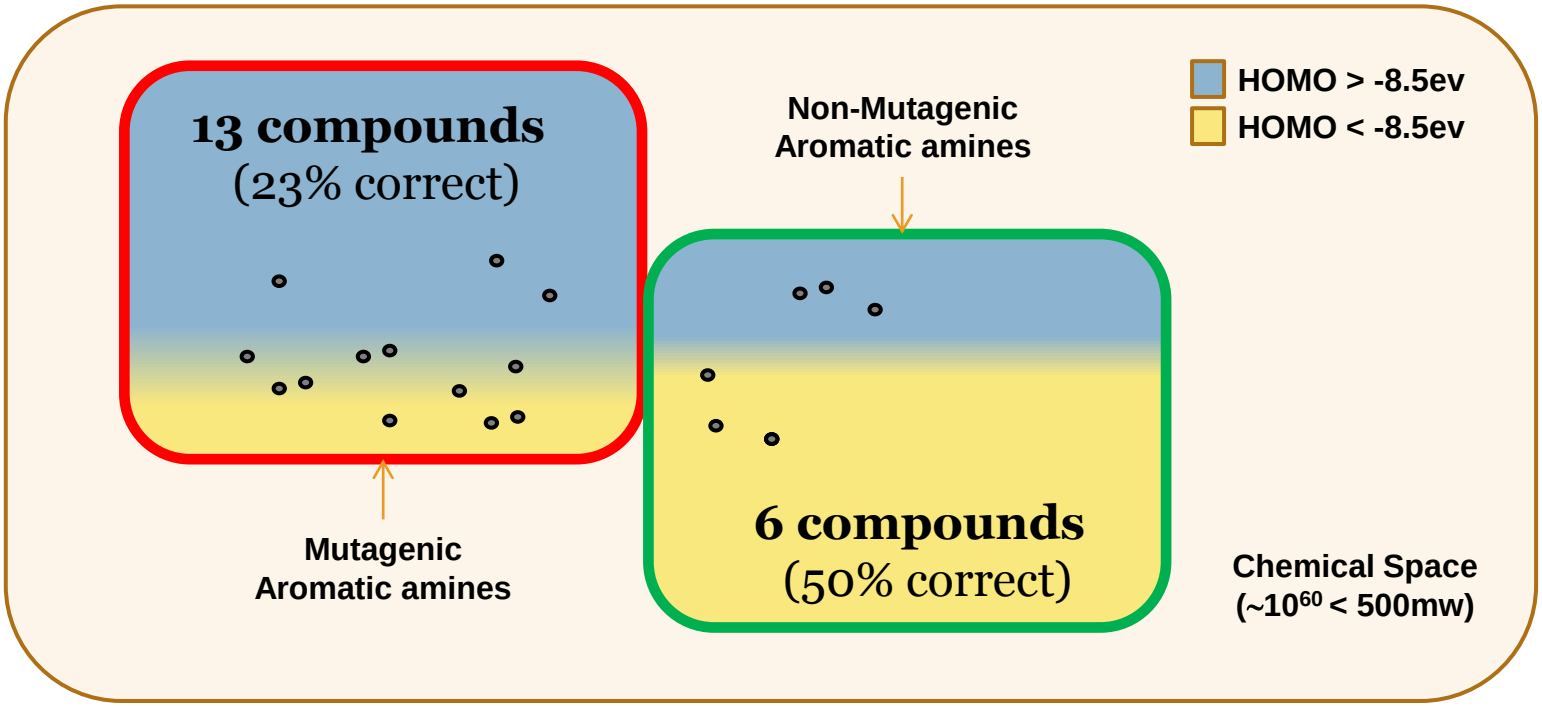
?

HOMO = -9.06 eV

- Focused testing in the future must prioritize these areas
- Understanding the applicability of a model is essential to understanding relevance of external validation statistics

Model Validation

- Performance is dependent upon chemical space of test set
- e.g. test set of 19 compounds



The Real Value of Truth Tables

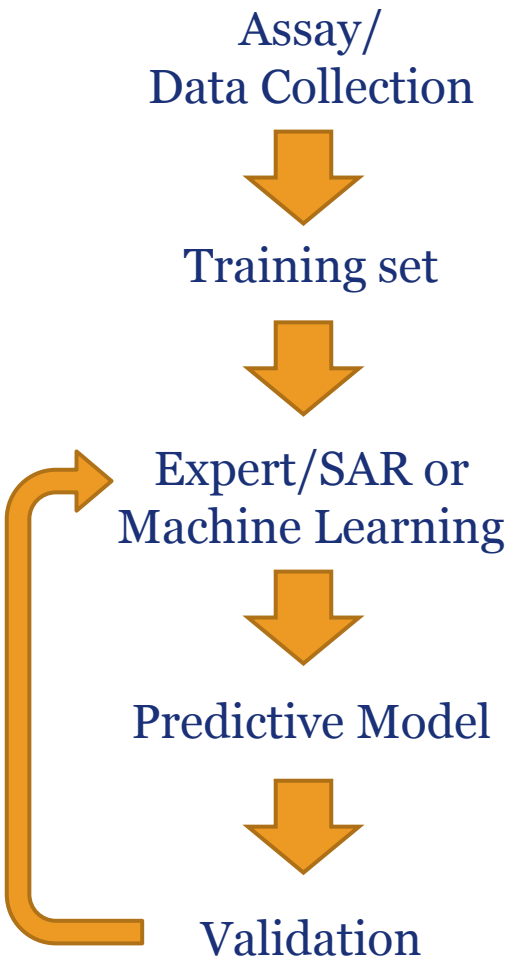
	Ames POS	Ames NEG
HOMO POS	Correlative	1) Steric crowding 2) Cyp-deactivation 3) Diverted metabolism
HOMO NEG	1) Another mechanism 2) Impurity?	useful in SAR determination

How Useful is My *in silico* Model?

- Performance is in the eye of the test set holder
  - Performance depends upon chemical space of the test set
- Transparency is key
  - How was your prediction derived?
  - Do you have access to the training set?
  - Is the model based on a mechanism that enables confident extrapolation outside of the applicability domain?
  - Aware of the limitations of the model?

in silico Summary

- This process largely works for toxicological endpoints based on structure-related mechanisms of action, e.g.
  - Mutagenicity
  - Skin sensitization
  - Genotoxic carcinogenicity
- Improvements can be made through investigating toxicological knowledge gaps within our datasets

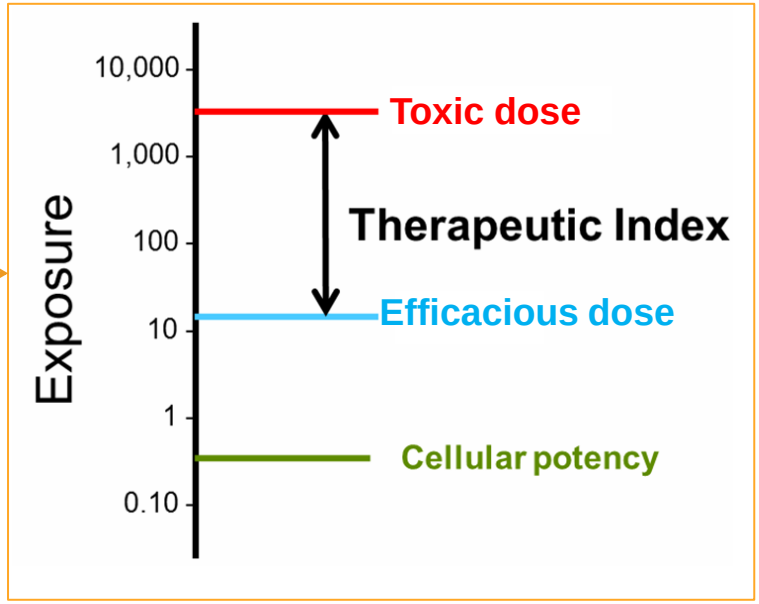


Can we apply computational toxicology to predict *in vivo* toxicity?

Can We Predict *in vivo* Toxicity?

- Yes - all compounds are toxic
- Calculated human LD50 values:[1]
  - Water – 6 liters
  - Caffeine – 118 coffees
  - Alcohol – 13 shots

- Focus on toxicity observed at therapeutically relevant levels

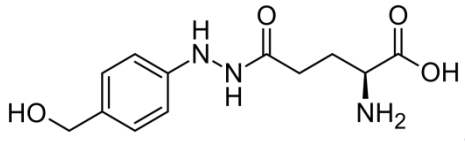


[1]www.compoundchem.com/2014/07/27/lethaldoses

What Drives *in vivo* Toxicity?

- Exposure

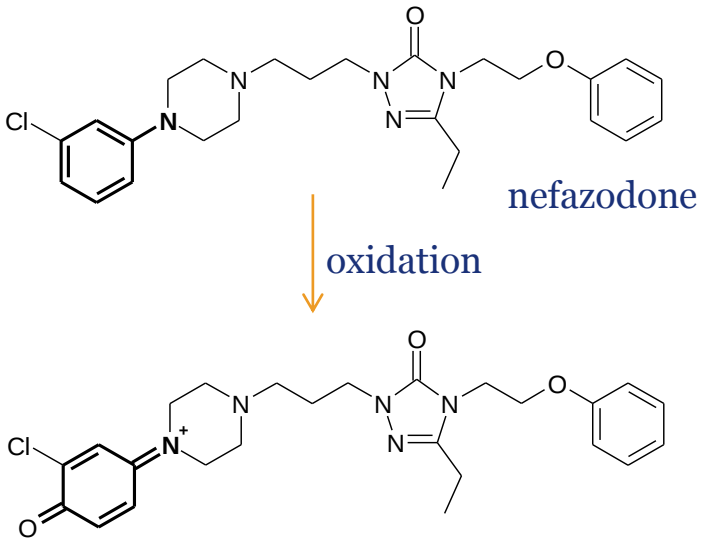
- Toxic Potential



agaritine

Example - Nefazodone

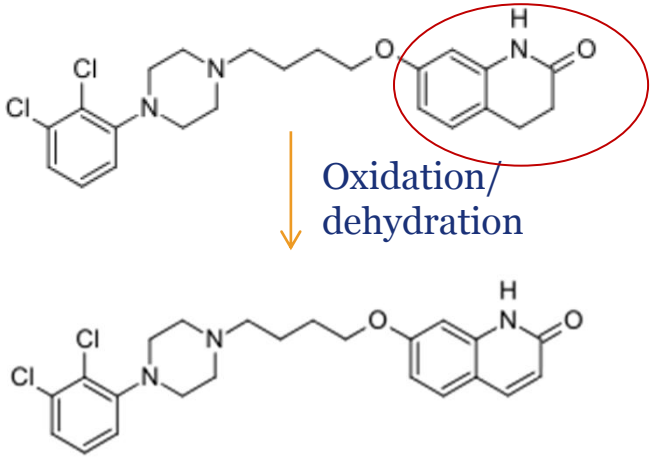
- Potent 5-HT_{2A} receptor antagonist and antidepressant
- Withdrawn in 2003 owing to very rare, but severe, liver toxicity
- Has multiple safety liabilities
  - Contains structural alert (aniline)[1]
  - Metabolic liabilities[2]
  - Inhibitor bile-salt export pump[3]
  - Cytotoxic[4]
  - Mitochondrial dysfunction[4]
  - High dose: >200mg/day



Refs
1. Stepan *et al*., Chem. Res. Toxic., 2011, 24, 1345-1410.
2. Kalgutkar *et al.*, Drug Metab. Disp., 2005, 33, 243-253
3. Kostrubsky *et al*, Toxicol. Sci., 2006, 90, 451-459
4. Dykens *et al*., Toxicol. Sci., 2008, 103, 335-345.

What About Aripiprazole?

- Structurally similar, yet successfully marketed drug
  - ***No*** reports of acute hepatotoxicity
- Has multiple liabilities
  - Contains structural alert (aniline) [1]
  - Metabolic liabilities[2]
  - Cytotoxic and lysosomotropic[3]
  - *Low dose: 10-20 mg/day*

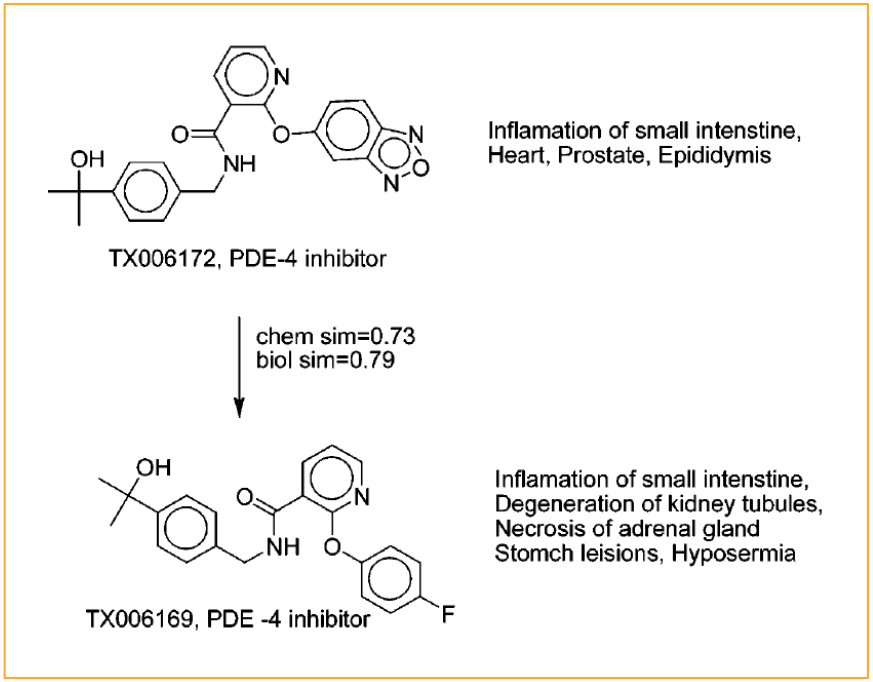


- Why is aripiprazole not hepatotoxic?
  - Related to different pharmacological profile?
  - Different metabolic profile?
  - Low dose?

Refs
1. Stepan *et al.*, Chem. Res. Toxic., 2011, 24, 1345-1410.
2. Bauman *et al.*, Drug Metab. Disp., 2008, 36, 1016-1029.
3. Nadanaciva *et al.*, Toxicol. in Vitro, 2011, 25, 715-723.

Do Similar Compounds Have Similar Toxicological Profiles?

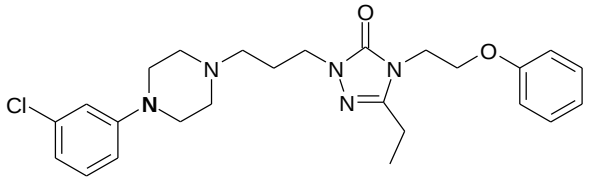
- Similar compounds with a similar *in vitro* toxicity profile may not express similar *in vivo* findings…



Shah and Greene, Chem Res &Tox, 2014, 27, 86-98

Challenges of Modelling *in vivo* Toxicology Data

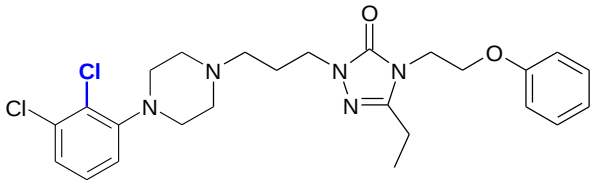
- Nefazodone



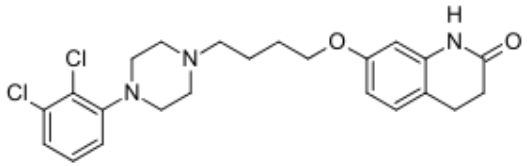
‘**hepatotoxic**’

Dose = 200-400 mg/day

- Hepatotoxic at 20mg/day?
- Can we predict:



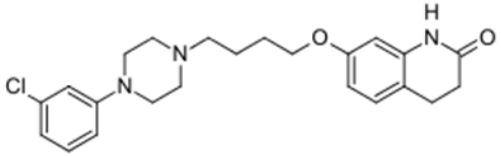
- Aripiprazole



‘**non-hepatotoxic**’

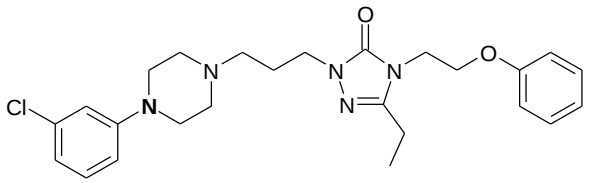
Dose = 10-30 mg/day

- Hepatotoxic at 200 mg/day?
- Can we predict:



Challenges of Validating *in silico/in vitro* Models

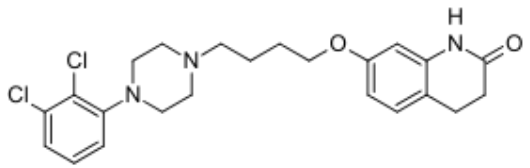
- Nefazodone



‘**hepatotoxic**’

- Aniline structural alert for hepatotoxicity?
  - True positive ✓
- Cytotoxicity assay
  - True positive ✓

- Aripiprazole



‘**non-hepatotoxic**’

- Aniline structural alert for hepatotoxicity?
  - False positive ✘
- Cytotoxicity assay
  - False positive ✘

Validating Models of *in vivo* Data

- Performance is in the eye of the test set holder
- For *in vivo* endpoints, performance is dependent upon:
  - Appropriate annotation of toxicological data
  - Understanding of exposure and pharmacokinetic profile

	in vivo toxic	*in vivo* clean
Model/assay positive	**True POS**	**False POS**
Model/assay negative	**False NEG**	**True NEG**

Validating Models of *in vivo* Data

- Performance is in the eye of the test set holder
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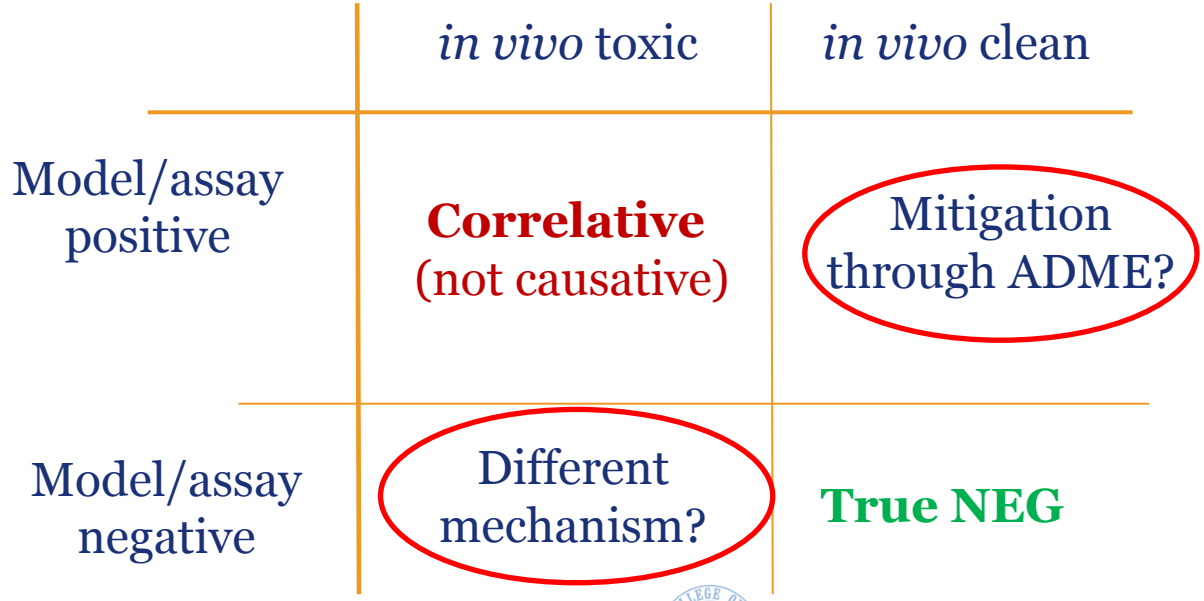
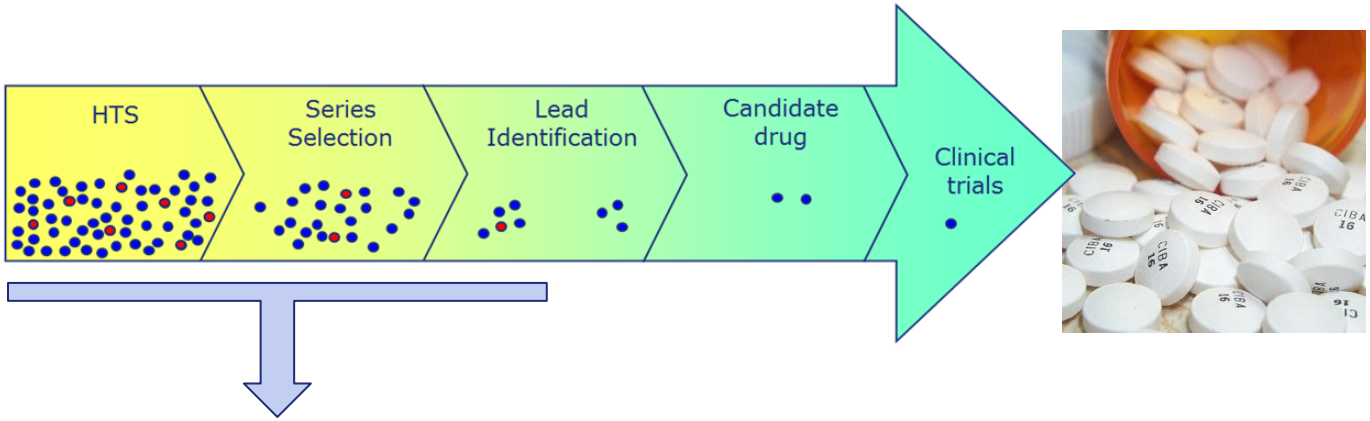


The Role of Early Screening Paradigms

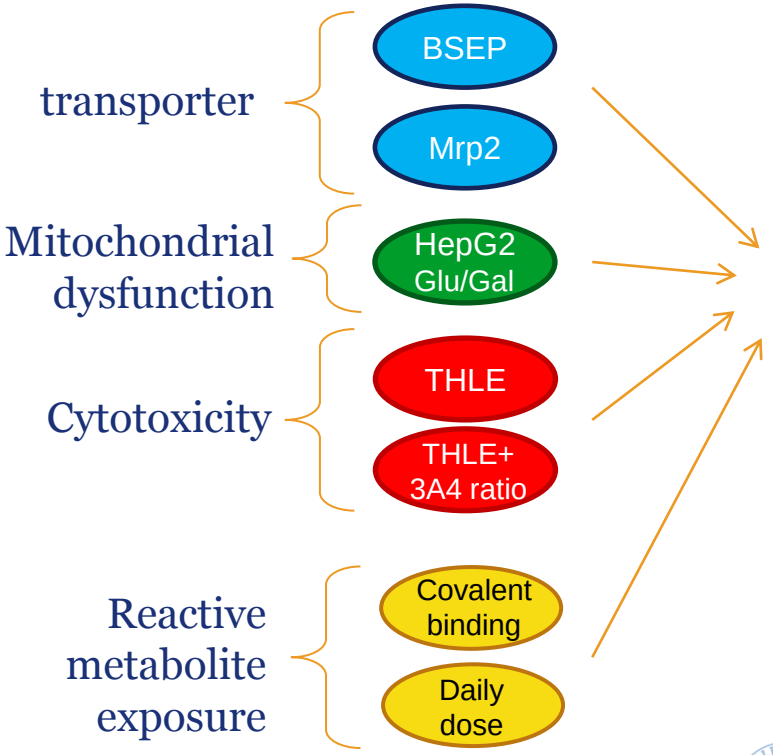
- *in vivo* Toxicology is complex to predict



- Identify potential risk early using *in silico* and *in vitro* models
- Recognize that *in vitro-in vivo* translation may not be possible without in-depth, costly, exposure-related studies

Early Screening Cascades: Example

- Platform of assays developed to identify risk of idiosyncratic drug reactions for 36 compounds with liver toxicity profiles



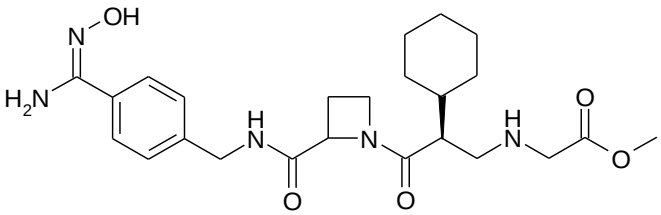
Integrated risk score:

- Able to differentiate 27 idiosyncratic liver toxicants from 7 of the 9 clean compounds

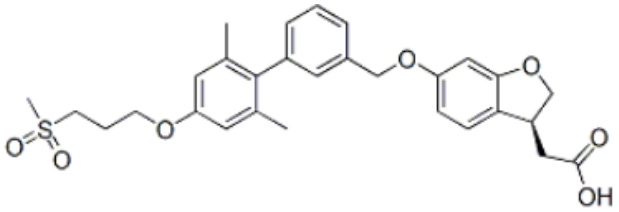
Thompson et al; Chem. Res. Toxicol., 2012, 25 (8), pp 1616–1632

Recently Discontinued Drugs

- Withdrawn owing to liver safety signals in Phase III



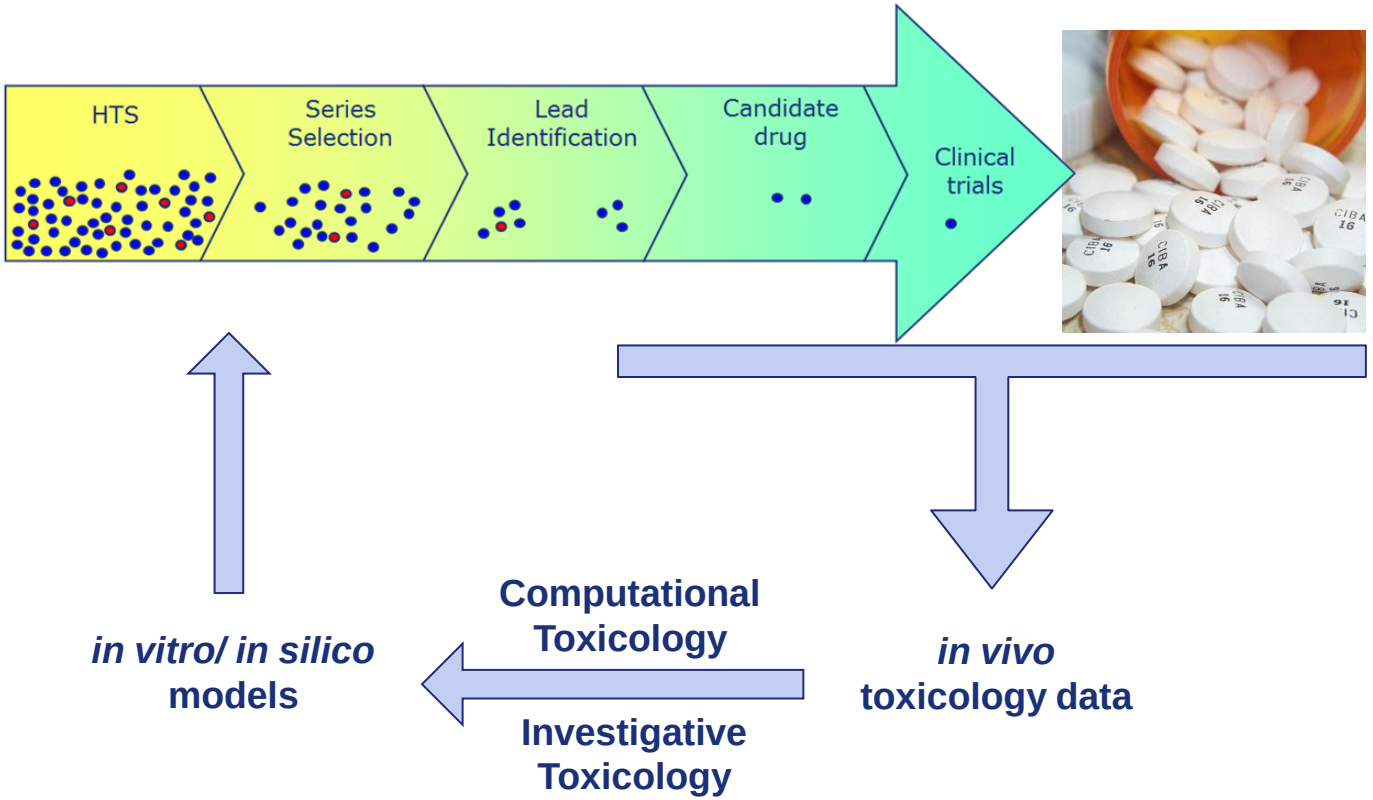
Ximelagatran
- Thrombin Inhibitor
- Withdrawn 2006



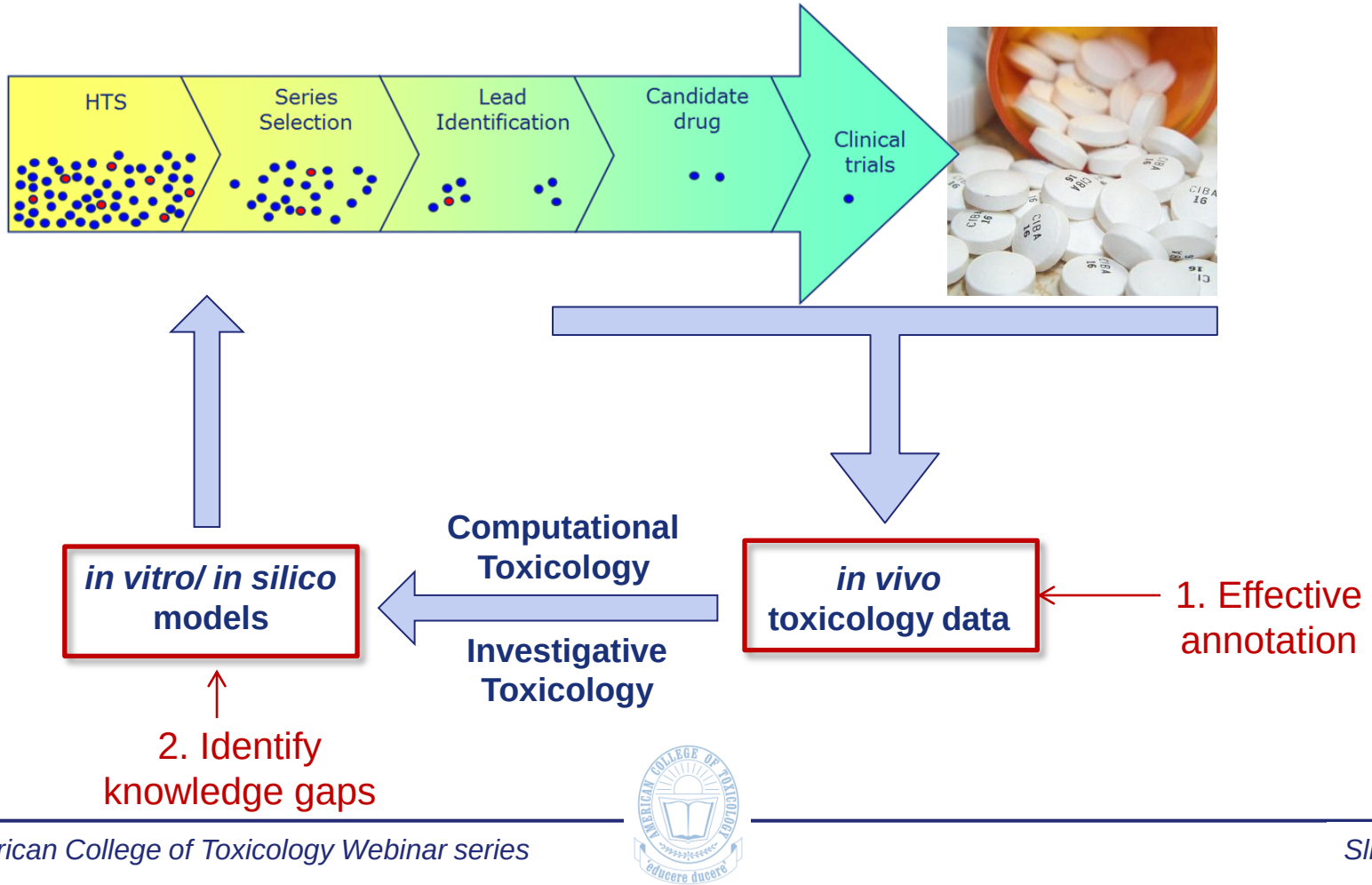
Fasiglifam
- GPR40 agonist
- Withdrawn 2013

- Why were these safety liabilities not caught early?

Improving *in vivo* Toxicity Prediction



Improving *in vivo* Toxicity Prediction



Features That Are Predictive of *in vivo* Toxicity

- Study 1: 207 preclinical candidates investigated
  - Compounds were annotated against the observation of any *in vivo* toxicity findings at 10µM (total plasma exposure)
  - Odds of toxicity established for various physicochemical properties

Tox@10µM	TPSA>75	TPSA<75
ClogP<3	0.39 (57)	1.08 (27)
ClogP>3	0.41 (38)	2.4 (85)

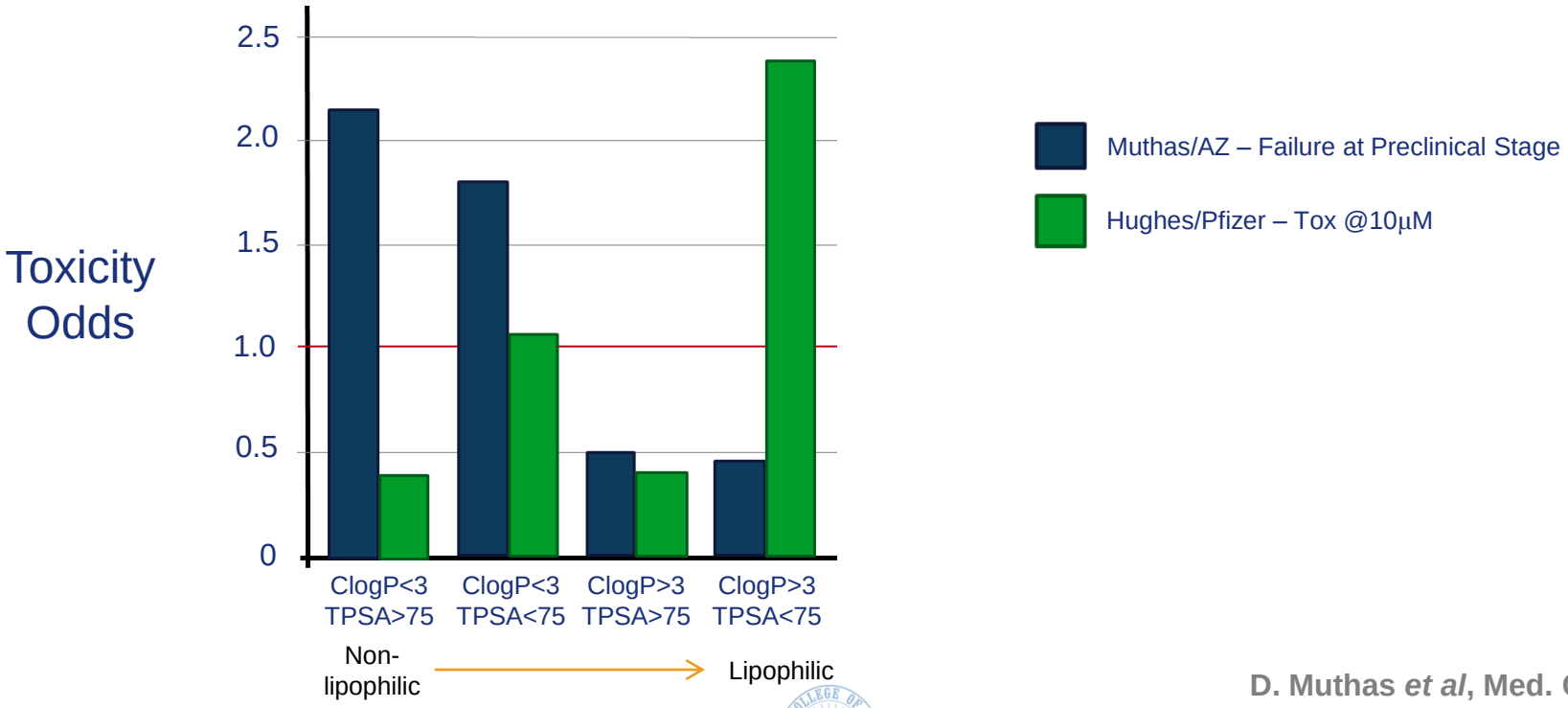
TPSA and ClogP are calculated measures of lipophilicity

⇨ Study conclusions: likelihood of toxicity increases with lipophilicity

Hughes *et al*, Bioorg&Med Chem Lett, 2008,18, 4872–4875

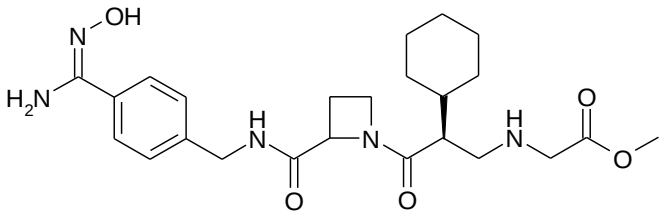
Features That Are Predictive of *in vivo* Toxicity

- Study 2: Odds of failure in preclinical/Phase 1 studies
  - Authors found that compounds were more likely to fail due to toxicity in non-lipophilic space:



D. Muthas *et al*, Med. Chem. Commun., 2013, 4, 1058

Which Thresholds Can You Believe In?



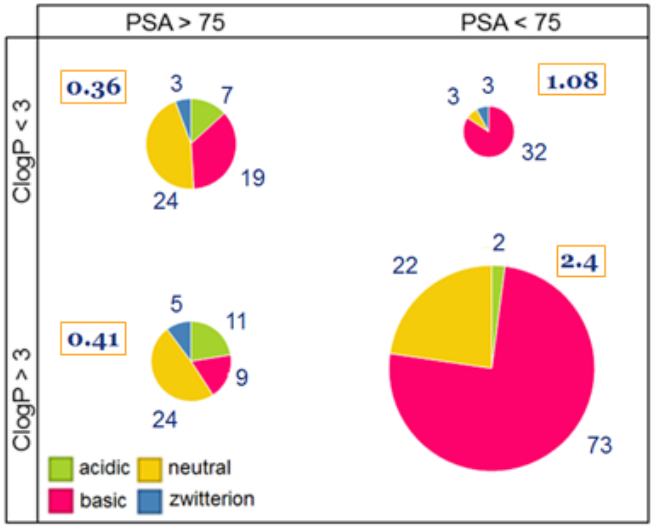
Ximelagatran

- ClogP = **1.8** ✓ - Hughes
- PSA = **144** ✘ - Muthas

- All models are useful… but *only* for a portion compounds in the training set
- It is essential to understand in which chemical space the model works and where it doesn't

Understanding The Applicability Domain

- Hughes training set is dominated by lipophilic basic drugs

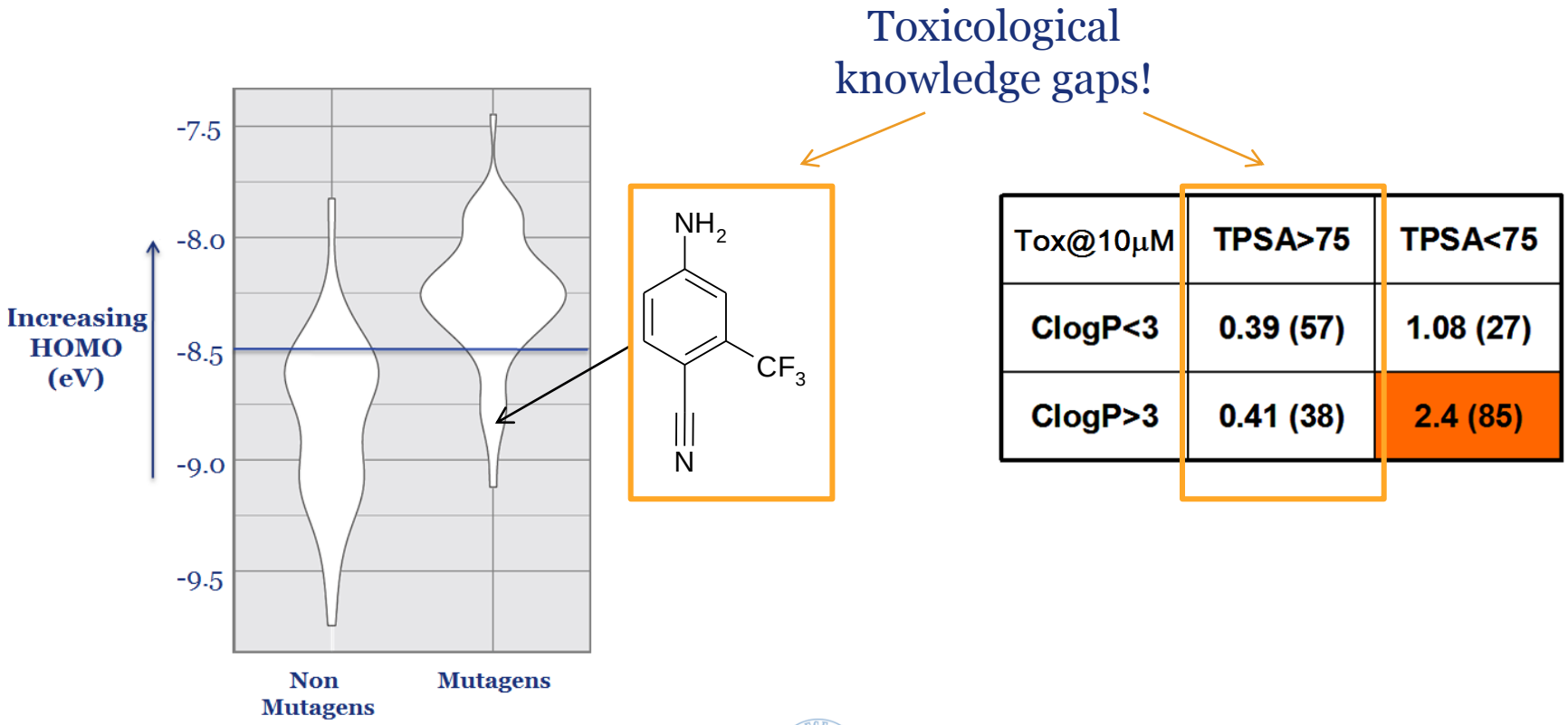


- Lipophilic basic drugs cause general toxicity, e.g. through lysosomal dysfunction and disruption of ion channels
- What factors drive toxicity of neutral +acidic compounds?

Hughes *et al*, Bioorg&Med Chem Lett, 2008,18, 4872–4875

Identifying Toxicological Knowledge Gaps

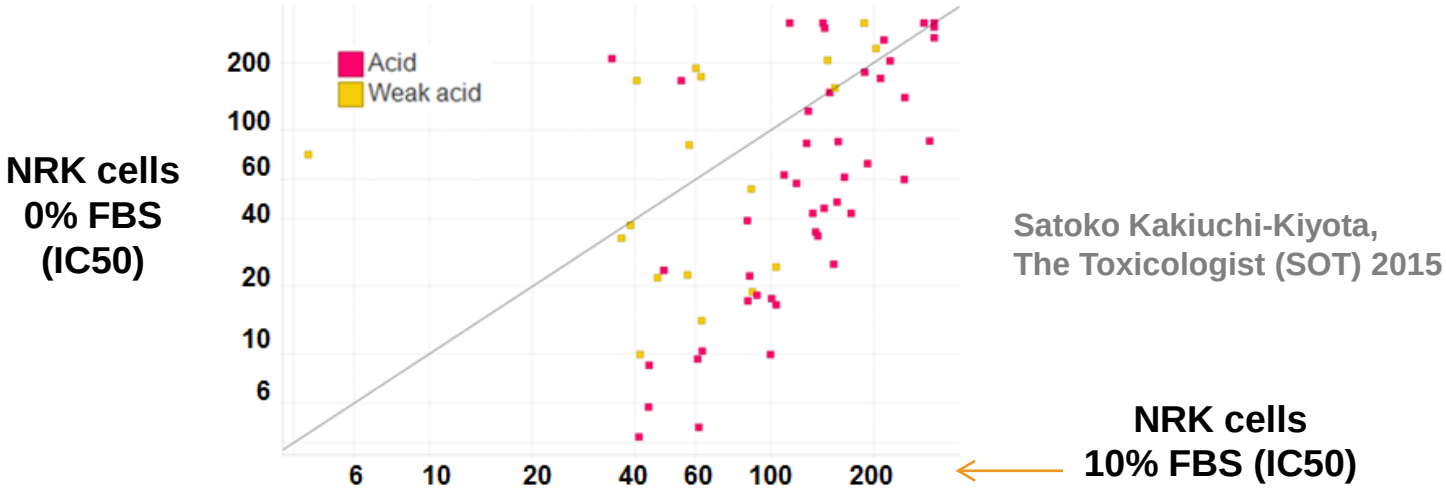
- Applicability enables the identification of knowledge gaps in your assay or model



Tox@10μM	TPSA>75	TPSA<75
ClogP<3	0.39 (57)	1.08 (27)
ClogP>3	0.41 (38)	2.4 (85)

Toxicity and Acidic Compounds

- Acidic compounds tend to have low cytotoxicity in cytotoxicity assays
- Acids tend to be highly protein bound
- Is toxicity mitigated by high protein binding to assay serum?

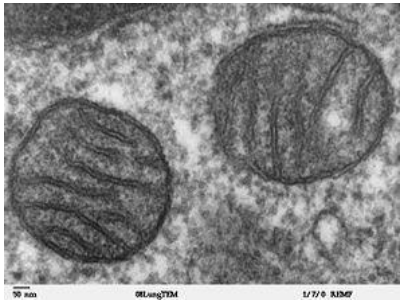


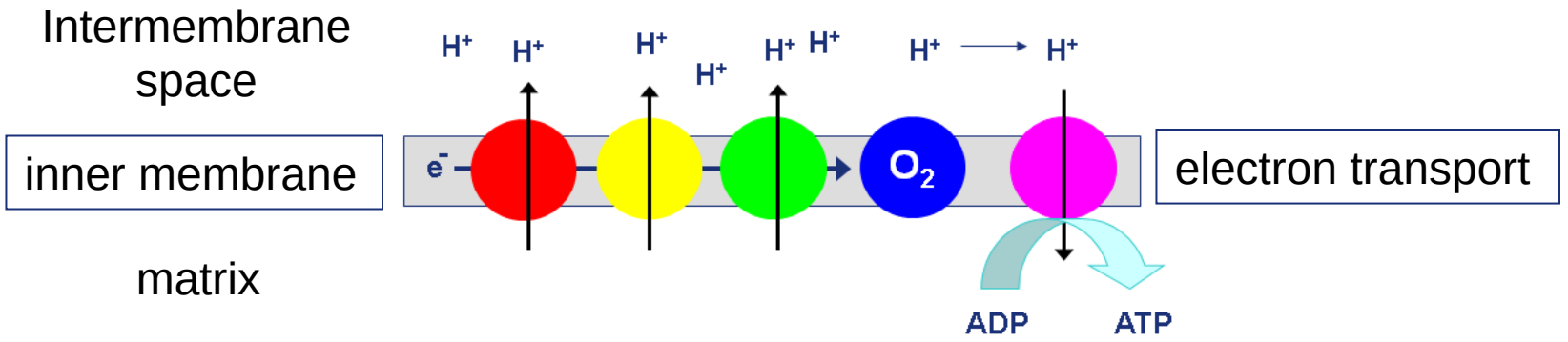
Satoko Kakiuchi-Kiyota,
The Toxicologist (SOT) 2015

- The impact of this result is not clear without an assessment of the toxicological and ADME profile of compounds in the dataset

Disruption of Oxidative Phosphorylation

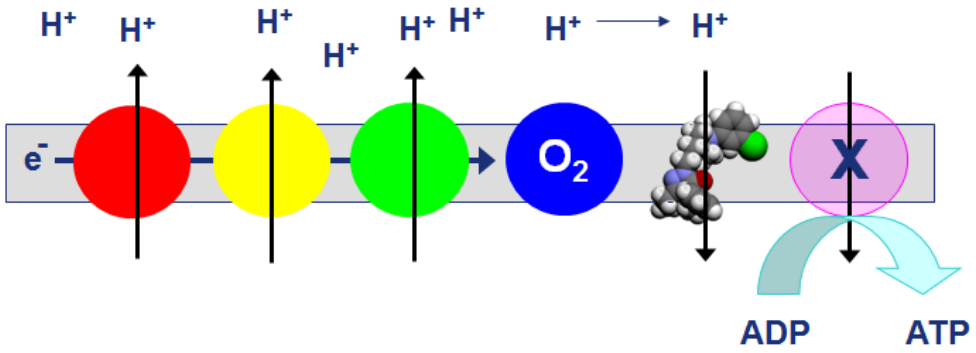
- Oxidative Phosphorylation occurs in mitochondria
  - Provides cellular energy (ATP)
  - Disruption linked to idiosyncratic organ toxicity



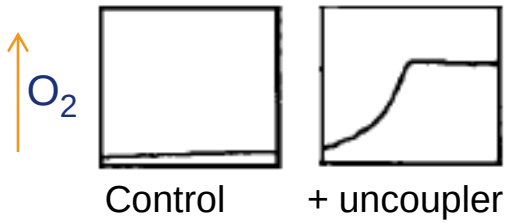


Mechanism of Uncoupling

- Modelling suggests lipophilicity and acidity is essential for protonophoric uncoupling
  - The uncoupler needs to reside in the membrane (be lipophilic)
  - Be able to shuttle protons across the membrane (have an acidic group)



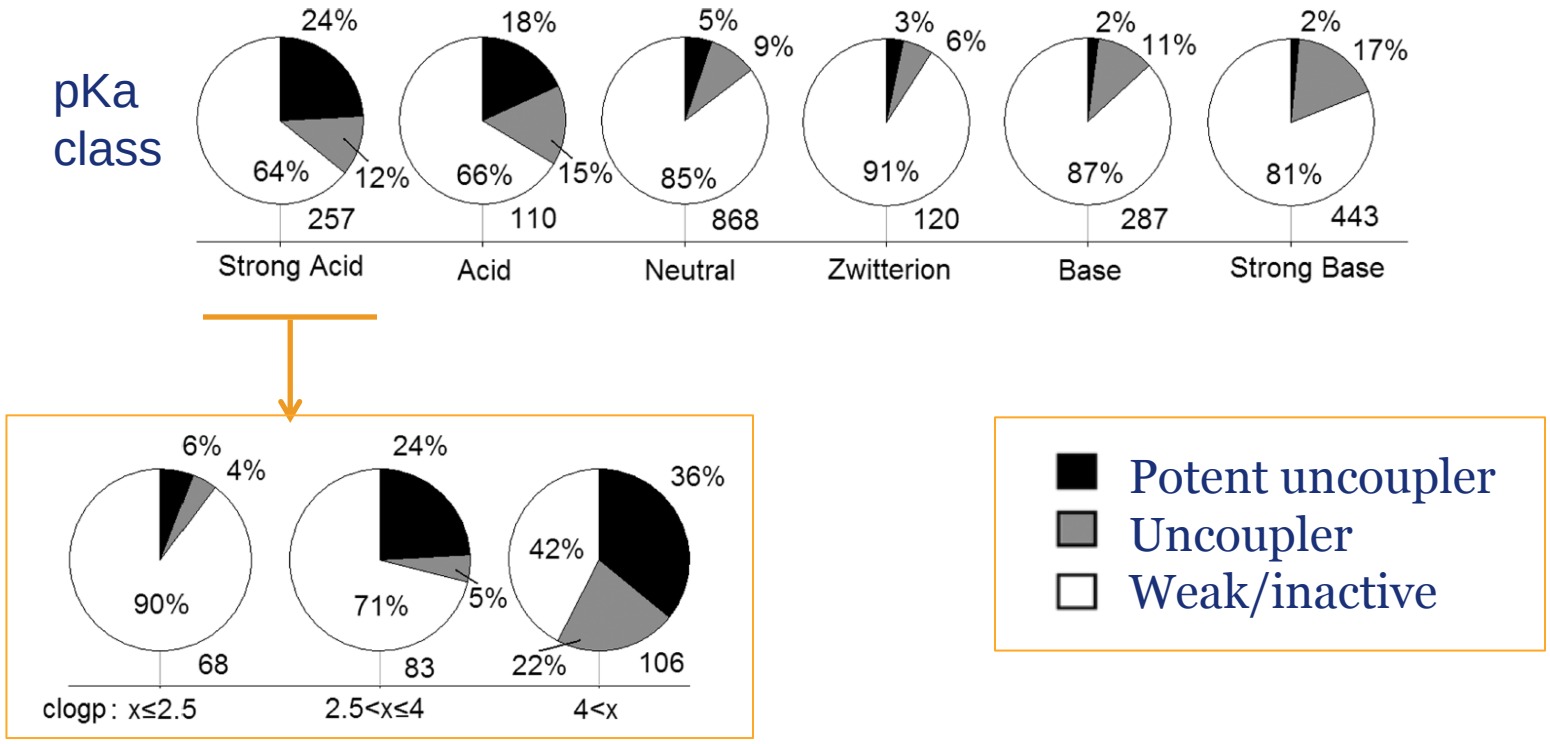
Detect uncoupling by measuring oxygen consumption



Hynes *et al*, Toxicol. Sci., 2006,92, 186-200

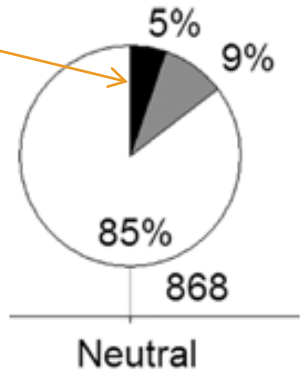
SAR Studies on 2000+ Compounds

- Uncoupling is highly dependent upon lipophilicity and acidity

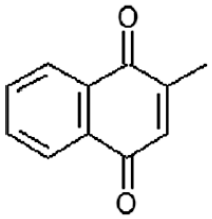


Outliers and Falsely-Predicted Compounds

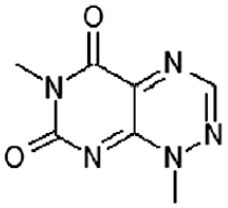
- "False Negatives"



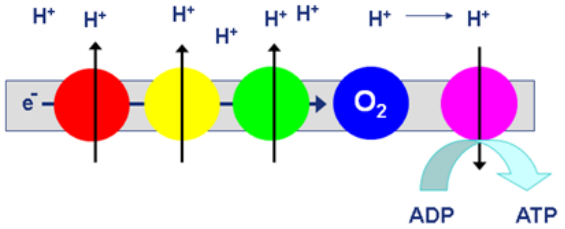
- Redox Cyclers
  - Neutral, non-lipophilic compounds



Menadione

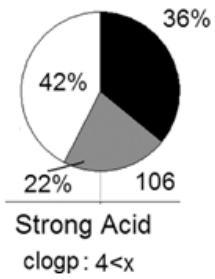


Toxoflavin

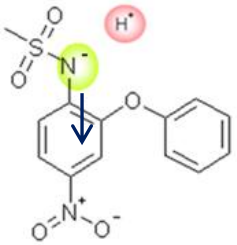


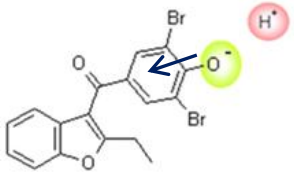
Outliers and Falsely-Predicted Compounds

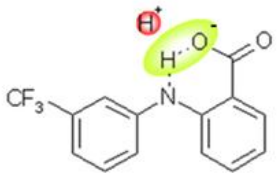
- 42% of lipophilic, acidic compounds were not uncouplers

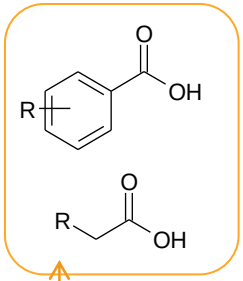


- Potent uncoupling requires stabilization of the negative charge:


Nimesulide


Benzbromarone


Flufenamic acid

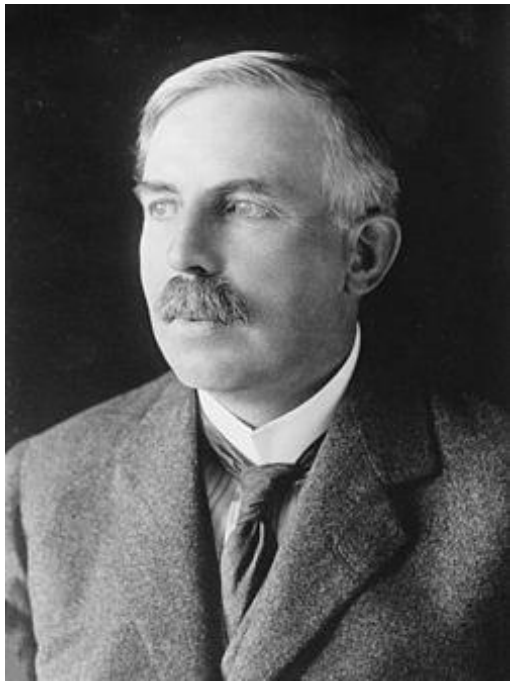


Unlikely to be uncouplers

Key Points Summary

- Computational Toxicology is essential to developing effective risk assessment strategies and models
- *in vivo* Toxicology is complex and we need to understand what our assays and models are telling us
- Recognize the utility and limitations of current predictive tools
  - *in silico*
  - *in vitro*

  } Identify and address toxicological knowledge gaps
- Move beyond broad annotations of *in vivo* toxicology data and include exposure assessment, if possible



Ernest Rutherford

- If your experiment needs statistics, you ought to have done a better experiment

Acknowledgements

- Satoko Kakiuchi-Kiyota, Pfizer, Groton, USA
  - Serum Free Assay
- Yvonne Will and Rachel Swiss, Pfizer
  - Uncoupling assay
- Compound Safety Prediction Group, Pfizer
  - Nigel Greene

Thank you for your participation in the American College of Toxicology Webinar!

We hope to see you at the 36th Annual Meeting of the American College of Toxicology
Red Rock Resort, Summerlin, Nevada,
November 8–11, 2015