



# **New computational profiling approaches to identify inhaled sensory irritants**

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**New computational  
profiling approaches to  
identify inhaled sensory  
irritants**

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Dan Wilson (retired)***



**Dr. Jon Hotchkiss  
1954 - 2022**

# Agenda

- Sensory irritation
  - TRP channels, RD50
- Computational profiling
  - TRPV1, TRPA1
- Application of the models
  - RD50 database
- Future work

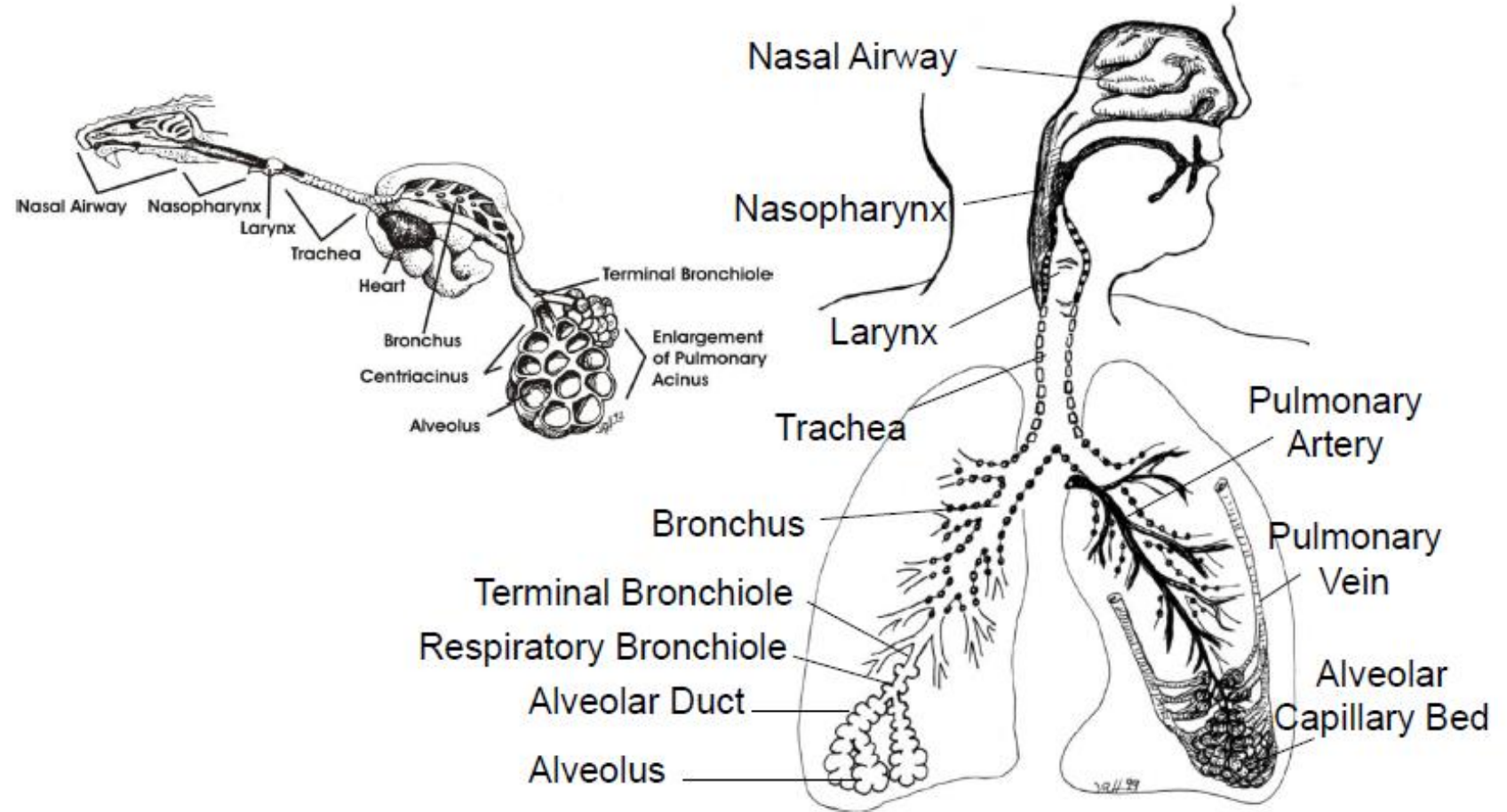
# Inhaled toxicants

- Inhalation is a major route of human exposure
- Unique interface between environment and systemic circulation
  - Upper (URT) and lower (LRT) respiratory tract important
- Exposure-response data for hazard identification
  - Integration of material properties, deposition, absorption, transport, metabolism and elimination
- Identify critical responses to inhaled materials
  - Portal of entry effects
    - Cells and tissues of URT and LRT
  - Systemic effects
    - Internal organs and tissues



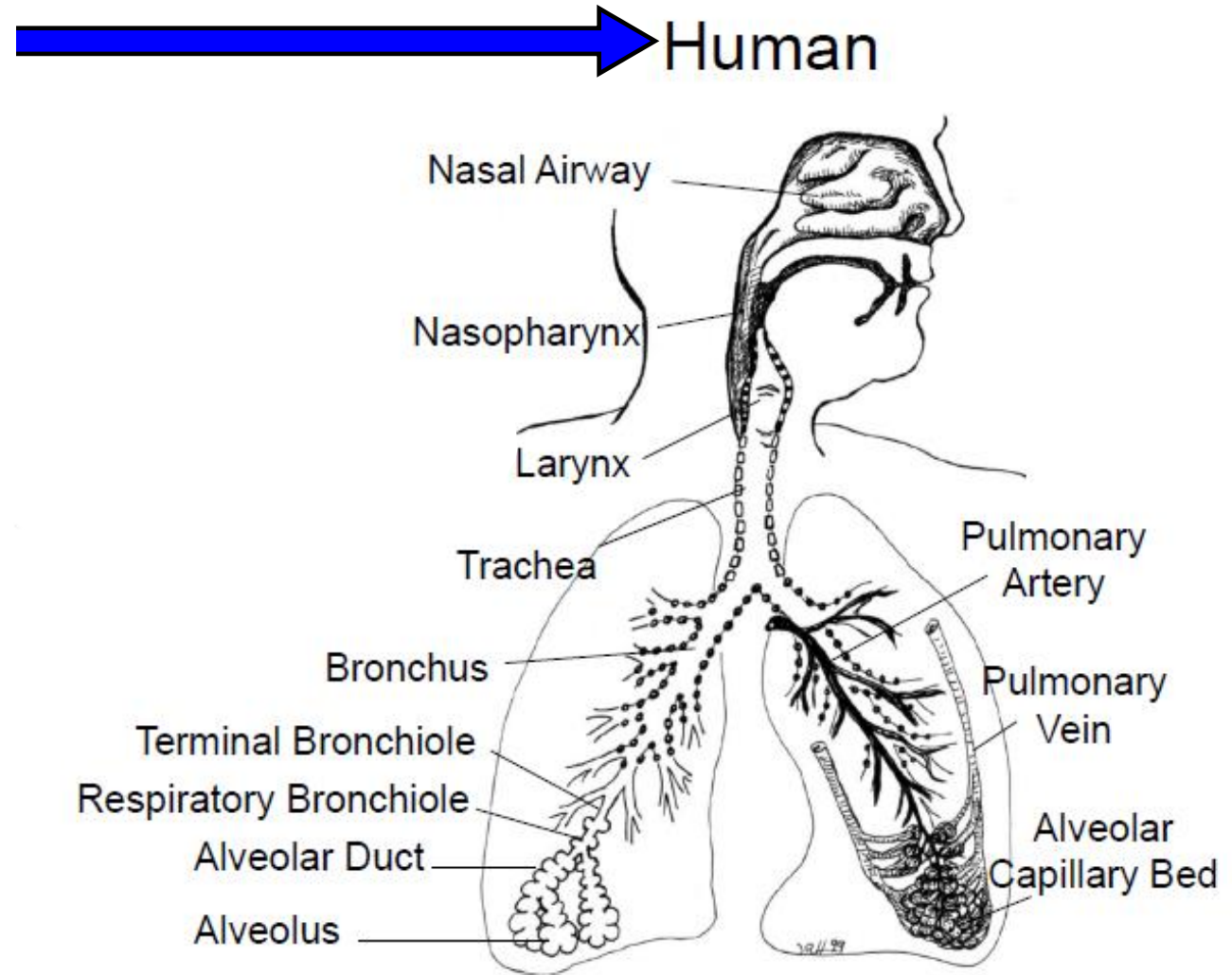
# What is the goal?

*In Vivo*  Human



# What is the goal?

*In Silico*  
*In Vitro*



# Sensory irritation

- Perceived irritation in the nose, throat and eyes
- Common manifestation of exposure to irritants
- Primarily from stimulation of receptors on sensory nerves
  - Transient Receptor Potential (TRP) channels
- Frequently used as a critical effect in setting airborne occupational exposure limits (OELs)

# Nociception

- Occupational exposure limits (OELs) & threshold limit values (TLVs) often set based on sensory irritation of nose, throat and eyes. This is due to nociception...
- Latin nocere 'to harm or hurt' - nervous system's process of encoding noxious stimuli
- Potentially damaging mechanical (e.g., cutting, crushing), thermal (heat and cold), and chemical (e.g., capsaicin or other irritants), stimuli detected by specialized nociceptors in sensory nerves
- Triggers variety of physiological and behavioral responses - perception of pain or irritation
- Evolutionarily protective physiological response, including protection against irritants



# Transient Receptor Potential (TRP) Channels

- 28 types of  $\text{Ca}^{2+}$  channels – similar structure & function, not same mechanism of activation
- Ubiquitously expressed in many cells and tissues on plasma membrane, including sensory nerves
- At least 2 types important for sensory irritation
  - *Transient receptor potential vanilloid type 1 protein (TRPV1; capsaicin receptor)*
  - *Transient receptor potential ankyrin subfamily type 1 protein (TRPA1; allyl isothiocyanate receptor)*
- Warn against irritants - cause coughing, sneezing, watery eyes, etc.

# Biological Relevance of Sensory Irritation/ TRP Channels

- Impairment of ability to escape
  - Frequently used as a critical effect in setting airborne OELs
- Rodent-specific nociceptive adaptations: decreased respiration, thermoregulation (GD 39)
  - Rodent toxicity studies → sensory irritation → potential for downstream effects that can be misconstrued as systemic chemical toxicity/adverse effects
  - Respiratory depression (RD) data may be used to estimate test chemical concentrations that may be irritating to rodents and humans
- Targets (esp TRPV1) for next-gen pesticides and pharmaceuticals

# Current Cheminformatics Models/Profilers

## NON-SPECIFIC

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Facile chemical reactivity \*

Chelation

Small alcohols

Surfactants

Hydrocarbons

## DOSIMETRY PREDICTION

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Acute oral toxicity (\*)

## RECEPTOR/TARGET-SPECIFIC

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Acetylcholinesterase inhibitors \*

Muscarinic cholinergic receptor \*

Nicotinic cholinergic receptor \*

Serotonin Cys loop & GPCRs

GABA Cys loop and GPCRs \*

Glycine Cys loop receptor

Pyrethroids

TRPV1 & TRPA1 receptors \*

Mitochondria inhibitors \*

Anticoagulants

Ah receptor agonists

Estrogen receptor (\*)

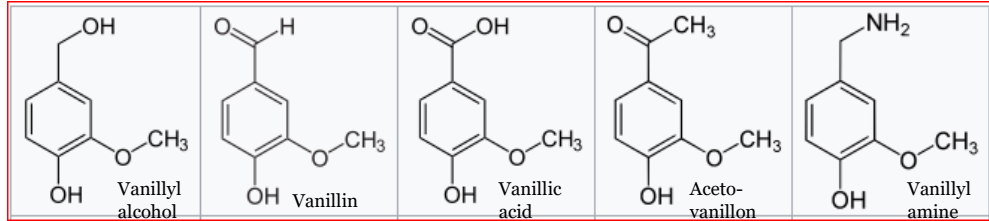
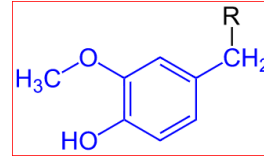
Aromatase

# Why build a computational model for sensory irritants?

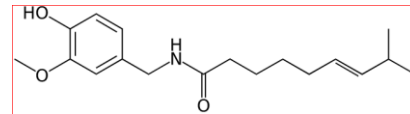
- OELs & TLVs often set based on sensory irritation of nose, throat and eyes
  - Activation of specific receptors located in trigeminal sensory nerve endings
  - Tearing, sneezing, coughing, pain, mucus secretion
  - Inflammation of the airways (trachea, bronchitis, and bronchiolitis)
- Currently no *in silico* models for sensory irritants exist
- Preclude or decrease the need for conduct of *in vivo* sensory irritation and perhaps other rodent inhalation studies
- Inform appropriate model for subsequent *in vitro* assessment
- TRPV1 and TRPA1 two important biological targets for sensory irritation and potential commercial targets (pharma, crop protection)

# Transient Receptor Potential Vanilloid-1 (TRPV1)

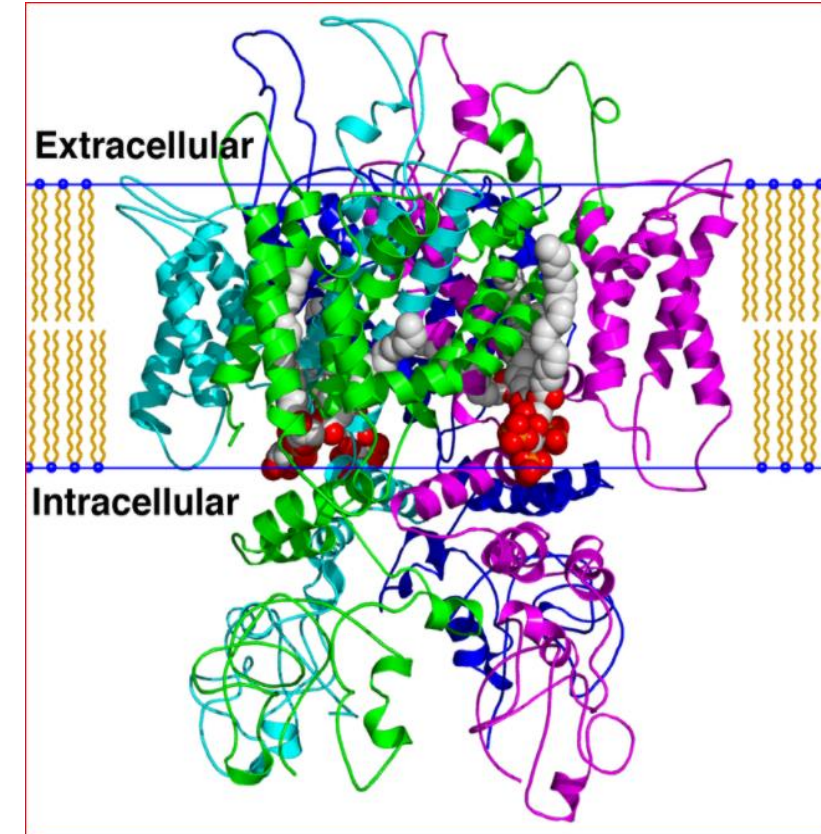
- Known as capsaicin receptor
- Capsaicin is a member of the vanilloid family



- Binds to a vanilloid receptor subtype 1 (TRPV1)
- Capsaicin
  - Capsaicinoids produced by chili peppers
  - a.i. in personal defense pepper spray agents
  - causes burning sensation when contacts mucous membranes
- Binding to TRPV1 in sensory neurons allows  $\text{Ca}^{2+}$  ions to pass through neuron cell membrane resulting depolarization and afferent stimulation
- Sequelae from activation of sensory C-fibers includes inflammatory bronchoconstriction and pulmonary edema



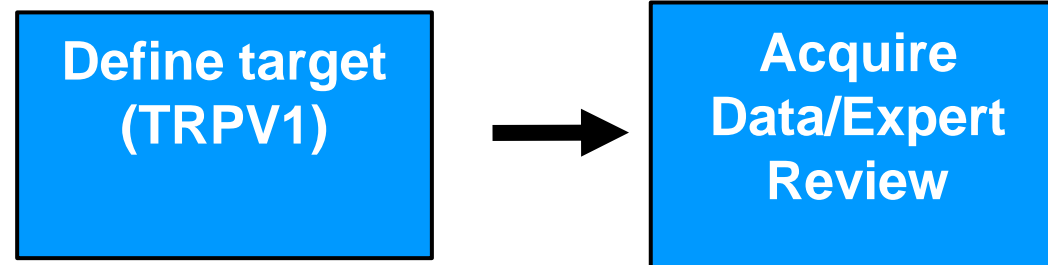
TRPV1 consists of tetramers arranged around an ion conduction pore selective for  $\text{Ca}^{++}$



<https://en.wikipedia.org/wiki/TRPV#:~:text=TRPV%20is%20a%20family%20of,type%2C%20and%20in%20some%20fungi>



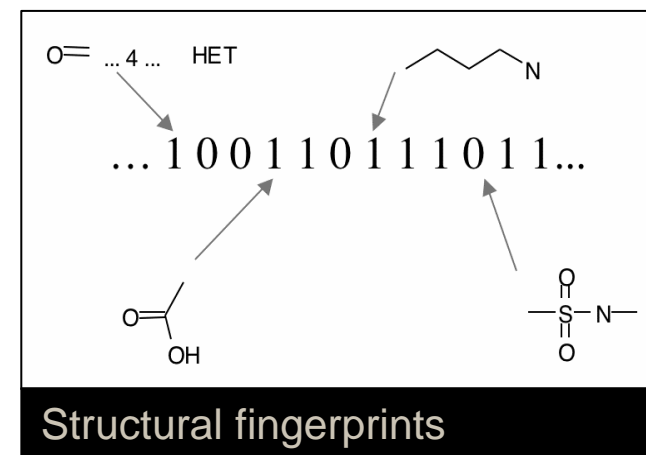
# TRPV1 DB curation



- TRPV1 not included in ToxCast or Tox21 HTS screening programs
- Curated 3,934 actives and 9493 controls from public sources
  - PubChem, ExCAPE-DB, ChEMBL, BindingDB, ZINC15

# TRPV<sub>1</sub> Model

- Fingerprints look for predefined structural features
  - Each feature queried in each Pos & Neg compound
  - Each query = a bit
    - Present (1)
    - Absent (0)
- Used MACCS public fingerprint model
- Query Pos and Neg training sets, use that information to predict whether an unknown will be Pos or Neg

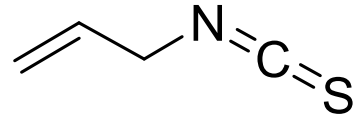


# TRPV<sub>1</sub> Model

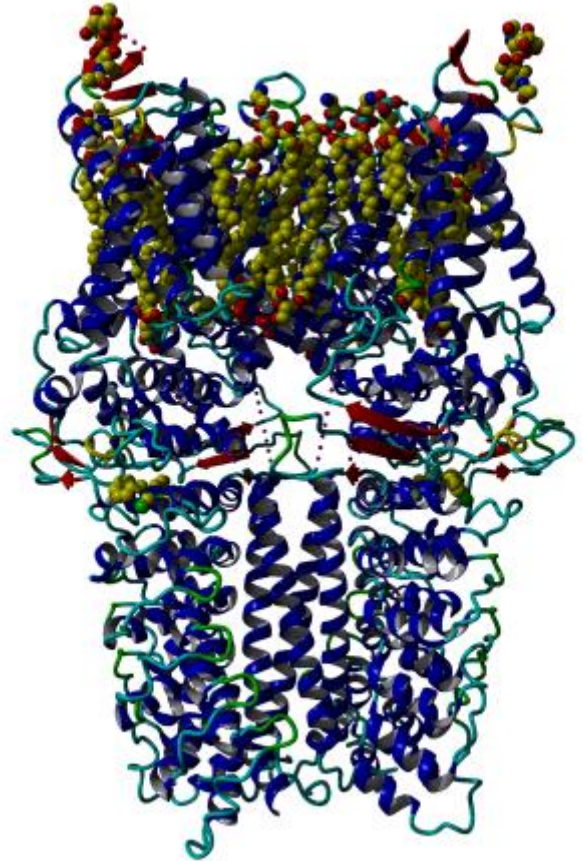
- Developed a machine-learning prediction model for TRPV<sub>1</sub> using default random-forest capabilities in KNIME
- Developed model statistics by training model with 90% and challenging it with remaining 10%
  - Data highly unbalanced (far more controls)
  - Best model statistics derived by training model separately 5x with equal random number of controls each time
- **Machine learning model has high sensitivity (96.9%) and balanced accuracy (97.9%), with a very high specificity (98.9%)**

# Transient Receptor Potential Ankyrin-1 (TRPA1)

- Known as Wasabi receptor
  - Allyl isothiocyanate (AITC)
- Functions in the detection, integration and initiation of pain signals in the PNS
- Ligand binding site contains a Cys moiety
- Considered a more likely target for xenobiotic electrophiles than TRPV1

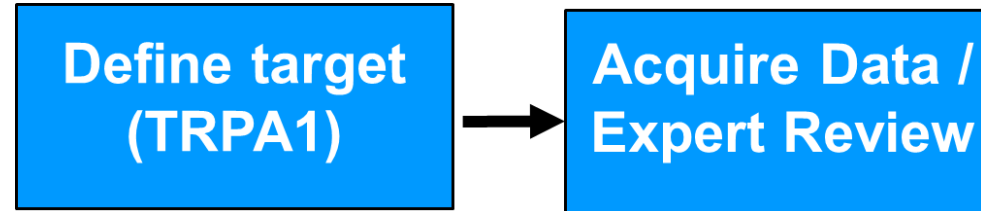


TRPA1 consists of tetramers arranged around an ion conduction pore selective for  $\text{Ca}^{++}$



PDB ID: 6PQP

# TRPA1 DB curation



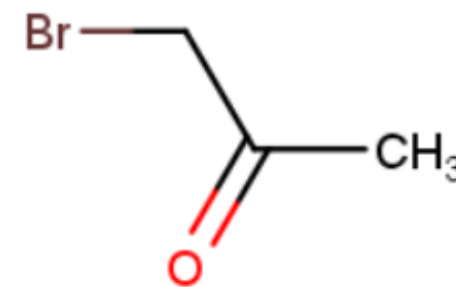
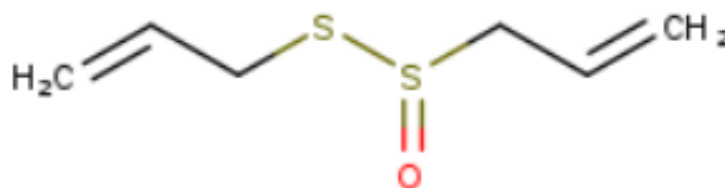
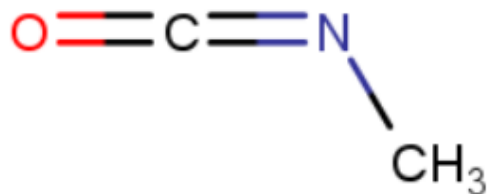
- TRPA1 not included in ToxCast or Tox21 HTS screening programs
- Curated 1,796 actives from public sources
  - PubChem, ExCAPE-DB, ChEMBL, BindingDB, ZINC15
- Mechanistic profiler based on conserved structural motifs (scaffolds)
  - Compounds that bind TRPA1 with high affinity
    - half-maximal inhibition/binding constants < 50  $\mu\text{M}$



# TRPA1 profiler



- 1,796 actives clustered and scaffolds determined
- Maximal common substructures (“scaffolds”) calculated for each cluster
- Yielded 120 unique scaffolds covered all molecules in DB



# Limitations of the models

## 1) Binary (YES/NO) without potency

- TRPA1 profiler: should not be used to assign lack of binding due to the minimal number of non-interacting compounds available for this target ( $n \sim 52$ )

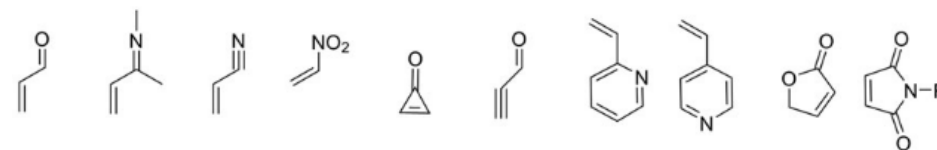
## 2) Applicability domain limitations

- Pharma-like DB
- MW limitations

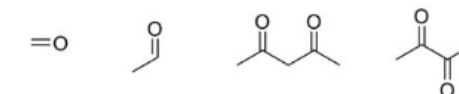
# Facile Reactivity Profiler

- Identifies electrophiles that can covalently adduct to biological nucleophiles (thiols and amines in amino acids [e.g., Cys and/or Lys] and/or DNA) under normal physiological conditions without need for enzymatic catalysis

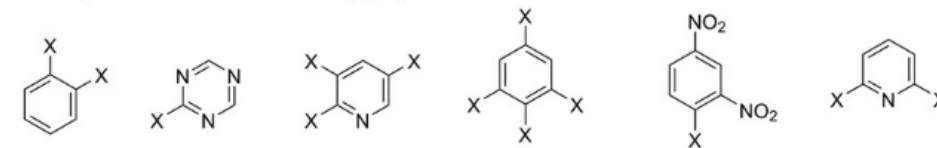
## Michael Acceptors



## Schiff Base Formers

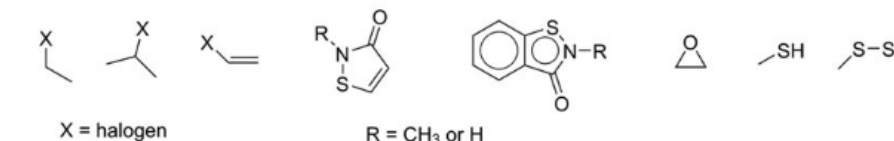


## Nucleophilic aromatic substitution ( $S_NAr$ )



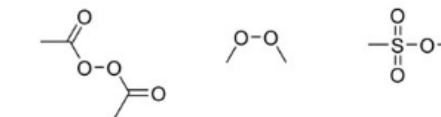
X = halogen or other EWG

## $S_N2$

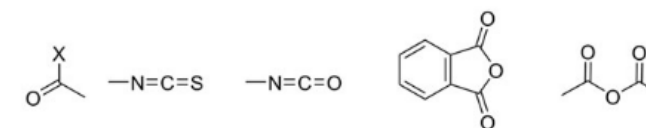


X = halogen

R = CH<sub>3</sub> or H



## Acyating agents



X = halogen or other EWG

# Application of the Models



Designation: E 981 – 04

## Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals<sup>1</sup>

This standard is issued under the fixed designation E 981; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

### 1. Scope

1.1 This laboratory test method provides a rapid means of determining sensory irritant potential of airborne chemicals or mixtures. It may also be used to estimate threshold limit values (TLV) for man. However, it cannot be used to evaluate the relative obnoxiousness of odors.

3.1.1 It provides positive recognition of sensory irritants of widely varying potencies.

3.1.2 It is sufficiently simple to permit the testing of large numbers of materials.

3.1.3 This test method is capable of generating concentration-response curves for purposes of compound com-

- Use of the mechanistic model for the TRPV1 receptor and TRPA1 and facile reactivity profilers to assess a rodent RD<sub>50</sub> database
- RD<sub>50</sub> – concentration causing a 50 percent reduction in respiration rate, gold standard *in vivo* rodent assay
- Historically, many human threshold limit values (TLVs) have been set to fall within 0.01 - 0.1 of the RD<sub>50</sub> in rodents
- Initial DB courtesy of Alarie and Schaper

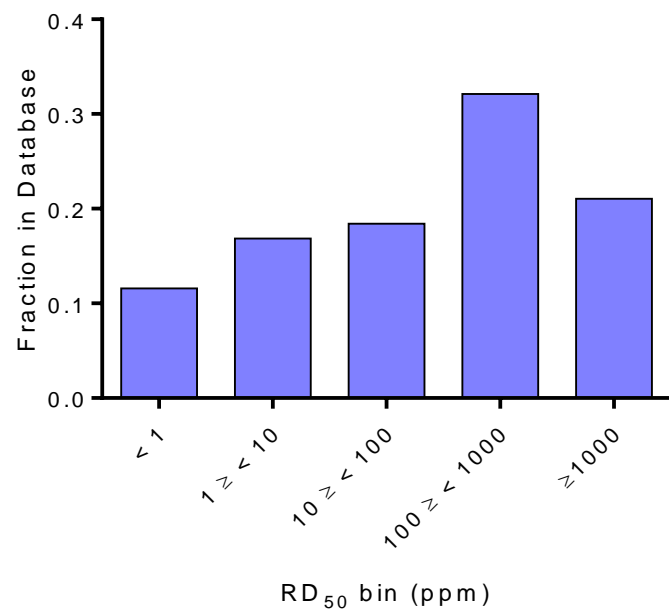
# RD50 Database

- Compiled DB of 600 compounds with rodent inhalation RD50 values from public sources
- Compounds without defined RD50 values removed
- Salts and metals removed
- Where multiple RD50 values were available for a given compound, the lowest (most potent) value was retained
- **Final database of 190 compounds with a unique RD50 values**

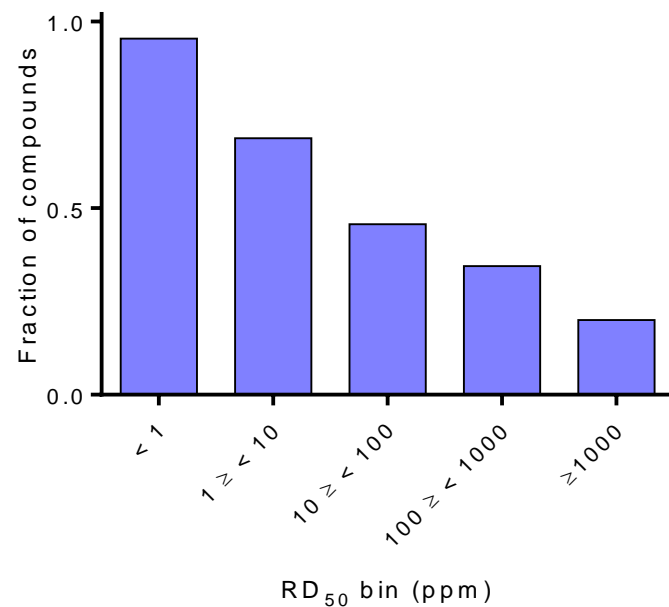


# Profiling results

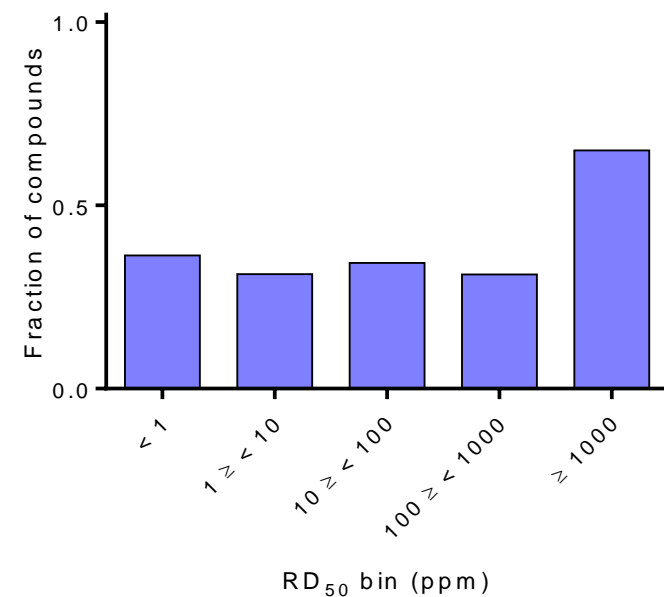
**A** Distribution of compounds in RD50 DB



**B** Fraction profiled for TRPA1 vs. RD50 bin

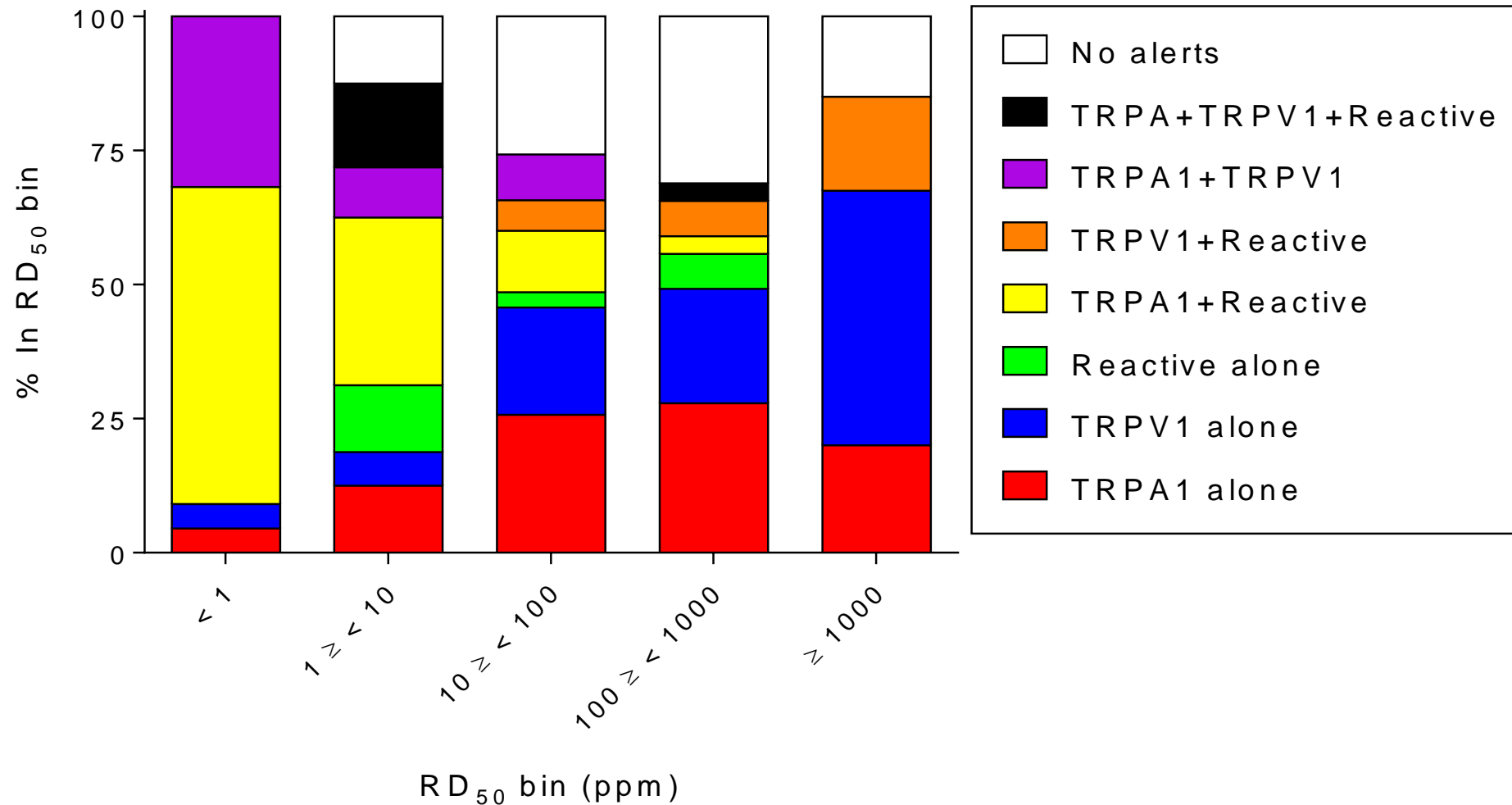


**C** Fraction profiled for TRPV1 vs. RD50 bin



# Profiling Results

Distribution of alerts for TRPA1, TRPV1 and/or Reactive



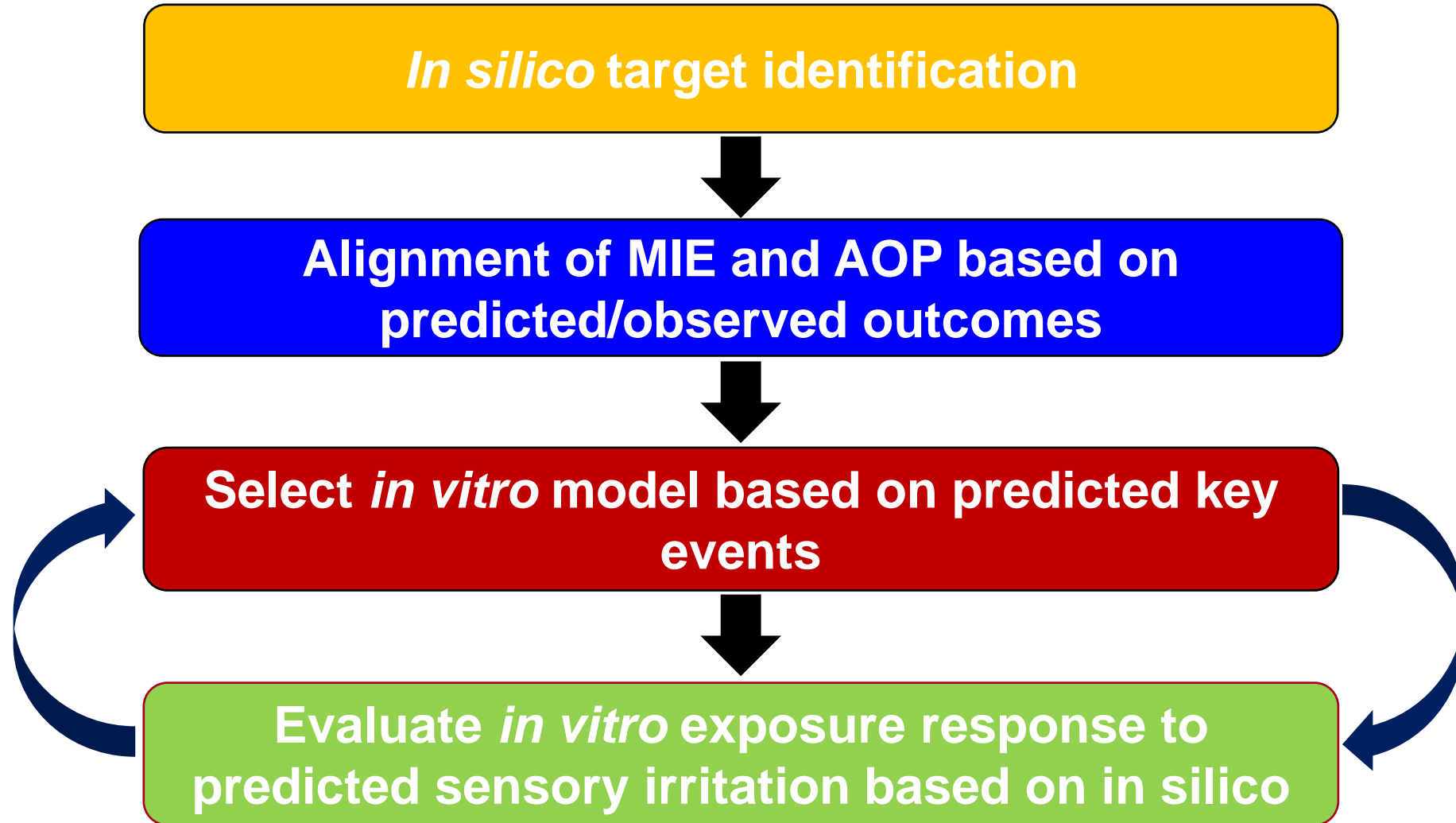
# Summary and Next Steps

- We have built the only available computational models for TRPV1 and TRPA1
- Profiling of a database of sensory irritants with potency values (RD50) was conducted to assess the potential relationship between mechanistic target and RD50 potency

## Next steps:

- Assess use of computational models (TRPV1, TRPA1, facile chemical reactivity) in informing OELs and emergency response values
- Advocate for establishment of in vitro assays, HTS data
- Collaboratively explore use of these approaches in sensory irritation IATA

# Sensory Irritation IATA





Thank you