Towards an MIE Atlas

The roads not taken: avoiding adverse outcome pathways

Compound → Molecular Initiating Event → Adverse Outcome Pathway

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Modern humans are exposed to hundreds of chemicals in their everyday lives.

To ensure the safety of these chemicals, toxicity testing must be carried out.

In the past 50 years, the in vivo approach using laboratory animals has changed little – particularly in the pharmaceutical industry.

These tests are expensive, time consuming, ethically unsound and animal models do not effectively reflect human responses.
In 2007 the NRC published a report highlighting these drawbacks and setting in motion the TT21C drive.

Advances in *in silico* and *in vitro* methods pave the way for greater understanding of the mechanisms behind toxicological effects, moving toxicology away from a predominantly observational craft towards a science based on understanding.
One such approach is the Adverse Outcome Pathway (AOP) framework for risk assessment.

The aim is to build understanding of a compounds effects across all levels of biological organisation.

The **Molecular Initiating Event (MIE)** can be thought of as the gateway to the AOP – the initial chemical interaction.

**Chemistry is key to understanding the MIE** – What is it about these molecules that allow them to do this?

Using knowledge of the chemical characteristics that govern these interactions, a **greater understanding of why chemicals cause toxic effects can be gained.**

The Adverse Outcome Pathway

The beginning of the project focused on searching for existing information on MIEs to see what could be pieced together.

Searching toxicological databases provided little information as these were frequently poorly populated, and did not contain information relevant to our study.

As such a literature search was performed for well understood molecules to gain a detailed picture of their toxicity.

Several structurally and toxicologically diverse molecules were chosen for this search.
Acetaminophen

NAPQI

- Binds to Cellular Proteins/Lipids/Nucleic acids
- Inhibition of Ca/Mg ATPase
- Increased Concentration of Calcium Ions
- Release of Cytokines
- Plasma Membrane Degradation
- Depletes Glutathione
- Oxidative Stress
- Binds to Glutathione
- ROS/RNS
- DNA Damage
- Cell Necrosis
- Cell Death
- Liver Failure

Acetaminophen
- Glucuronate Metabolite
- Sulfate Metabolite
- Inhibition of COX

Inhibition of Complex I
Inhibition of Complex II
Inhibition of Complex III
ATP Reduction

JNK Activation
Cytochrome C
SMAC
Endo G
AIF
DNA Fragmentation

Acetaminophen

Acetaminophen $\xrightarrow{\text{P450 Oxidation}}$ NAPQI $\xrightarrow{\text{Binding to Glutathione}}$ MIE

Acetaminophen SAR

Acetaminophen

Para-aminophenol fragment
Acetaminophen SAR

Acetaminophen → Para-aminophenol fragment

Phenacetin

Amiodiaquine
Acetaminophen SAR

Acetaminophen

Para-aminophenol fragment

Phenacetin

Amiodiaquine

Antiarhythmia SAR

Antiarhythmia SAR

![Chemical structures of Amiodarone and Chlorpromazine]

- **Amiodarone**
- **Chlorpromazine**

**Compound** → **Molecular Initiating Event** → **Adverse Outcome Pathway**

**hERG Channel Inhibition** → **Antiarhythmia**

Antiarhythmia SAR

Amiodarone

Chlorpromazine

Amiodarone → hERG Channel Inhibition → Antiarrhythmia

Chlorpromazine → hERG Channel Inhibition → Antiarrhythmia
Antiarrhythmia SAR

Amiodarone

Chlorpromazine

MIE

hERG Channel Inhibition

Antiarrhythmia

Compound → Molecular Initiating Event → Adverse Outcome Pathway
Antiarhythmia SAR

Aromatic Amine Fragments

\[
\begin{align*}
\text{Compound} & \rightarrow \text{Molecular Initiating Event} & \rightarrow \text{Adverse Outcome Pathway}
\end{align*}
\]
Antiarhythmia SAR

Aromatic Amine Fragments

- Imipramine
- Citalopram
- Chloroquine
Antiarhythmia SAR

Aromatic Amine Fragments

- Imipramine
- Citalopram
- Chloroquine

A single definition of the MIE has yet to reach acceptance. Different defections stem from different fields that have focused on a specific type of interaction.

With experience from the atlas of many interactions we were able to combine the best features of existing definitions to form a unified definition.

The MIE is the initial interaction between a molecule and a biomolecule or biosystem that can be causally linked to an outcome via a pathway.

This definition focuses on the initial interaction, relates the interaction to a measurable outcome, includes a multitude of different interactions, and does not focus the term entirely in toxicological research.
To prove the concept of predictive toxicology using the chemistry of molecules, models needed to be built and tested using knowledge we had gained so far.

As such the principles of the MIE Atlas were used to design a model approach.

**Characteristics of molecules that are associated with a certain MIE** will be used to build the models. While many characteristics can be evaluated, black box models with little mechanistic or theoretical background lead to confusion and fail to provide new understanding.

*As such we aim to build clear models using fragment structural alerts in 2D, and a range of chemically sound molecular descriptors.*

This will allow additional understanding to be gained about the MIE itself and help our models at a regulatory stage.
The NIH Tox Data Challenge 2014 was initiated by the US NIH over the summer to “crowdsource” data analysis and evaluate computational models for toxicity prediction.

Data on several receptors and *in vitro* tests were provided as training sets and test sets will be evaluated by the NIH to determine a score for submitted models.

The **Nuclear Receptor binding data provided a good dataset for MIE based models** to be developed and tested.

Receptors included are the Androgen Receptor (AR), the Estrogen Receptor (ER), the Aryl hydrocarbon Receptor (AhR), Aromatase, and PPAR-gamma. There are also ligand binding domains for the Androgen and Estrogen receptors (AR-LBD and ER-LBD).
AR-LBD Results

Positive

CID 371180

Hg

Mercury

Trimethyl Tin

Steroidal Core

Negative

Anisole

Benzaldehyde

Dimethylethylamine
AR-LBD Results

Positive

CID 371180
Mercury
Trimethyl Tin

Set A

Set B

Steroidal Core

Negative

Anisole
Benzaldehyde
Dimethylethylamine
AR-LBD Results

**Positive**

<table>
<thead>
<tr>
<th>Compound</th>
<th>SE</th>
<th>SP</th>
<th>Q</th>
<th>MCC</th>
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<tbody>
<tr>
<td>Mercury</td>
<td>62.0</td>
<td>99.1</td>
<td>97.8</td>
<td>0.657</td>
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<tr>
<td>Trimethyl Tin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Set A**

CID 371180

**Set B**

Steroidal Core

**Negative**

<table>
<thead>
<tr>
<th>Compound</th>
<th>SE</th>
<th>SP</th>
<th>Q</th>
<th>MCC</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benзaldehyde</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dimethylethylamine</td>
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**Train** (SET A) Or (NO NEG + SET B)

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<tr>
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<th>FN</th>
<th>TN</th>
<th>FP</th>
<th>SE</th>
<th>SP</th>
<th>Q</th>
<th>MCC</th>
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<td>115</td>
<td>8223</td>
<td>73</td>
<td>62.0</td>
<td>99.1</td>
<td>97.8</td>
<td>0.657</td>
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**Test (int)** (SET A) Or (NO NEG + SET B)

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<th>TN</th>
<th>FP</th>
<th>SE</th>
<th>SP</th>
<th>Q</th>
<th>MCC</th>
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<tbody>
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<td>7</td>
<td>816</td>
<td>8</td>
<td>63.2</td>
<td>99.0</td>
<td>98.2</td>
<td>0.606</td>
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**AR-LBD Results**

**Positive**

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<th>Model</th>
<th>TP</th>
<th>FN</th>
<th>TN</th>
<th>FP</th>
<th>SE</th>
<th>SP</th>
<th>Q</th>
<th>MCC</th>
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<td>3</td>
<td>247</td>
<td>1</td>
<td>25.0</td>
<td>99.6</td>
<td>98.4</td>
<td>0.346</td>
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</table>

**Negative**

- Anisole
- Benzaldehyde
- Dimethylethylamine

**Set A**

- CID 371180
- Mercury
- Trimethyl Tin

**Set B**

- Steroidal Core

**Balanced Accuracy:** 62.3

**Best Score:** 65.0

**2nd Best in Top 10**
In order to apply our approach to a large dataset, a number of appropriate receptors were chosen, based on our previous research and a paper on pharmacological anti-targets (Bowes 2012).

This set includes a wide variety of targets, including Enzymes, GPCRs, Ion Channels, Nuclear Receptors, and Transporters.

Receptor binding data has been obtained for analysis from the open source database ChEMBL.

This data is being used to elucidate 2D fragments associated with toxicity, to be built into MIE reports.
Histamine Receptors

Histamine H1 Receptor Fragments

HH1R Frag 1 12%

HH1R Frag 2 13%

1-Methylpiperidine 37%

Piperidine 38%

3-Phenoxy-1-propanamine 20%

4-Phenoxy piperidine 13%

Monochlorobenzene 30%

Diphenylmethane 16%

CID 587118 9%
Histamine Receptors

Histamine H2 Receptor Fragments

HH2R Frag 1 20%

1,3-Dimethyl-indole 22%

1-Ethylpiperidine 28%

Indole 27%

CID 158749 19%

CID 25719674 13%

2-Butylguanidine 31%

1-Methylguanidine 38%
Histamine Receptors

The Endogenous Ligand – Histamine

\[
\text{Histamine}
\]

Greater Structural Similarity to H2 Fragments than H1.

CID 158749

\[
\text{Indole}
\]

1-Methylguanidine
Histamine Receptors

Histamine H2 Receptor Specific Antagonists

- **Ranitidine**
- **Cimetidine**
- **Famotidine**
- **Nizatidinie**

Bradshaw, J., et al. (1979) *P. BPS.*; 464P.
Histamine Receptors

Histamine H2 Receptor: Histamine-like MIE

- Imidazole
- Guanidine
- Diamino ethylene
- Indole
Histamine Receptors

Histamine H2 Receptor: Histamine-like MIE

- Imidazole
- Guanidine
- Diamino ethylene
- Indole

48/141  54/141  1/141  38/141
Histamine Receptors

Histamine H2 Receptor: Histamine-like MIE

- Imidazole: 48/141
- Guanidine: 54/141
- Diamino ethylene: 1/141
- Indole: 38/141
Histamine Receptors

Histamine H2 Receptor: Histamine-like MIE

- Imidazole: 48/141
- Guanidine: 54/141
- Diamino ethylene: 1/141
- Indole: 38/141
Histamine Receptors

Histamine H2 Receptor: Histamine-like MIE

- Imidazole: 48/141
- Guanidine: 54/141
- Diamino ethylene: 1/141
- Indole: 38/141

36/141

5/141
# Histamine Receptors

## Histamine H2 Receptor: Histamine-like MIE

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>3DSA</th>
<th>3DVol</th>
<th>ALogP</th>
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<tbody>
<tr>
<td>Imidazole</td>
<td>299</td>
<td>504</td>
<td>489</td>
<td>1.36</td>
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<tr>
<td>Guanidine</td>
<td>390</td>
<td>637</td>
<td>618</td>
<td>1.80</td>
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<tr>
<td>Indole</td>
<td>422</td>
<td>628</td>
<td>612</td>
<td>2.19</td>
</tr>
</tbody>
</table>
Histamine Receptors

Histamine H1 Receptor Specific Antagonists

- Diphenhydramine
- Loratadine
- Clemastine
- Fexofenadine
- Cetirizine

Histamine Receptors

Histamine H1 Receptor Crystal Structure

A flexibly attached basic nitrogen is protonated to form a charge-charge interaction.

A large hydrophobic pocket is occupied by the tricyclic system.

Doxepin

Histamine Receptors

Histamine H1 Receptor: Doxepin-like MIE

Doxepin

Diphenylmethane
Tertiary Amine
Histamine Receptors

Histamine H1 Receptor: Doxepin-like MIE

Doxepin

Diphenylmethane

Tertiary Amine
Histamine Receptors

Histamine H1 Receptor: Doxepin-like MIE

Doxepin

Diphenylmethane

Tertiary Amine

63/499
Histamine Receptors

Histamine H1 Receptor: 4-Phenoxy piperidine-like MIE

HH1R Frag 1

HH1R Frag 2

4-Phenoxy piperidine
Histamine Receptors

Histamine H1 Receptor: 4-Phenoxy Piperidine-like MIE

HH1R Frag 1

HH1R Frag 2

4-Phenoxy Piperidine

67/436
Histamine Receptors

Histamine H1 Receptor: 4-Phenoxy piperidine-like MIE

HH1R Frag 1

HH1R Frag 2

4-Phenoxy piperidine

No Overlap with Doxepin MIE

67/436
Thymidylate Synthase

Thymidylate Synthase Fragments (Train 178, Test 61)

- N-Benzoylglutamic acid 22%
- N-Acetyl-DLglutamic acid 25%
- CID 8858953 11%

- Benzamide 28%
- Thiophenol 49%
- Thioanisole 39%
- Benzylaniline 17%
- N,N'-Dimethylimidodifomamide 38%
Thymidylate Synthase

Endogenous Reaction

\[ \text{dUMP} \rightarrow \text{dTMP} \]

5,10-Methylenetetrahydrofolate

Dihydrofolate
Thymidylate Synthase

Known Binders

- Raltitrexed
- Fluorouracil
- Nolatrexed

Thymidylate Synthase

Two Analogue Compound Classes

Folate Analogues

Fluoropyrimidines

Folate Pharmacophore

Uracil

Thymidylate Synthase

Two Analogue Compound Classes

Folate Analogues

Fluoropyrimidines

8 Matches

45 Matches

Both: 4 Matches

2 Matches
Thymidylate Synthase

Two Analogue Compound Classes

Folate Analogues

- MIE 1
  - Folate Pharmacophore
  - 8 Matches

Fluoropyrimidines

- MIE 2
  - Uracil
  - 2 Matches
  - Both: 4 Matches

45 Matches
8 Matches
2 Matches
Towards an MIE Atlas

These reports are the initial finding that will lead towards a large number of well characterised MIEs, across a diverse set of important pharmacological receptors, for publication and use in developing SAR tools.

- Endogenous Ligand
- Receptor Type
- Typical Binders
- Associated Tox.
- MIE Classification
“Here’s a new molecule – is it safe?”
“Here’s a new molecule – is it safe?”

Exposure Data
The Big Question

“Here’s a new molecule – is it safe?”

Exposure Data

Pharmacokinetic Modelling
“Here’s a new molecule – is it safe?”

- Exposure Data
- Pharmacokinetic Modelling
- MIE Tool
“Here’s a new molecule – is it safe?”

Exposure Data

Pharmacokinetic Modelling

MIE Tool

Biosystem Disturbance
The Big Question

“Here’s a new molecule – is it safe?”

- Exposure Data
- Pharmacokinetic Modelling
- MIE Tool
- Biosystem Disturbance
- Understanding of Downstream Effects
The Big Question

“Here’s a new molecule – is it safe?”

Exposure Data
Pharmacokinetic Modelling
MIE Tool
Biosystem Disturbance
Understanding of Downstream Effects

MIE-AOP Research
“Here’s a new molecule – is it safe?”

“Here’s a new molecule – what MIEs can it activate?”
“Here’s a new molecule – is it safe?”

“Here’s a new molecule – what MIEs can it activate?

2D Fragments

Hydroxyamphetamine
48%
The Big Question

“Here’s a new molecule – is it safe?”

“Here’s a new molecule – what MIEs can it activate?”

2D Fragments

Literature Knowledge

Hydroxyamphetamine 48%

Morphine

H-bond donor

H-bond acceptor

Hydrophobic Region

Basic Nitrogen

Covalent bond Forming Alcohol
The Big Question

“Here’s a new molecule – is it safe?”

“Here’s a new molecule – what MIEs can it activate?”
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