



A Defined Approach to Skin Sensitisation: Integrating Derek Nexus with *In Chemico/In Vitro* Assays Based on Exclusion Criteria

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Agenda

- Skin sensitisation
- Legislation
- Non-animal approaches
- Lhasa's defined approach
- Conclusions.



What is skin sensitisation?

- Common occupational disease
- Estimated to cost the EU €600 million and 3 million lost working days per year
- Not life-threatening but is life-long
- Traditionally assessed using *in vivo* assays (GPMT¹ & LLNA²).



1. OECD 1992, Test No. 406: Skin Sensitisation

2. OECD 2010, Test No. 429: Skin Sensitisation: Local Lymph Node Assay

Legislation



EU REACH
Cosmetics Regulation



Korea REACH



Turkey KKKDIK



China REACH

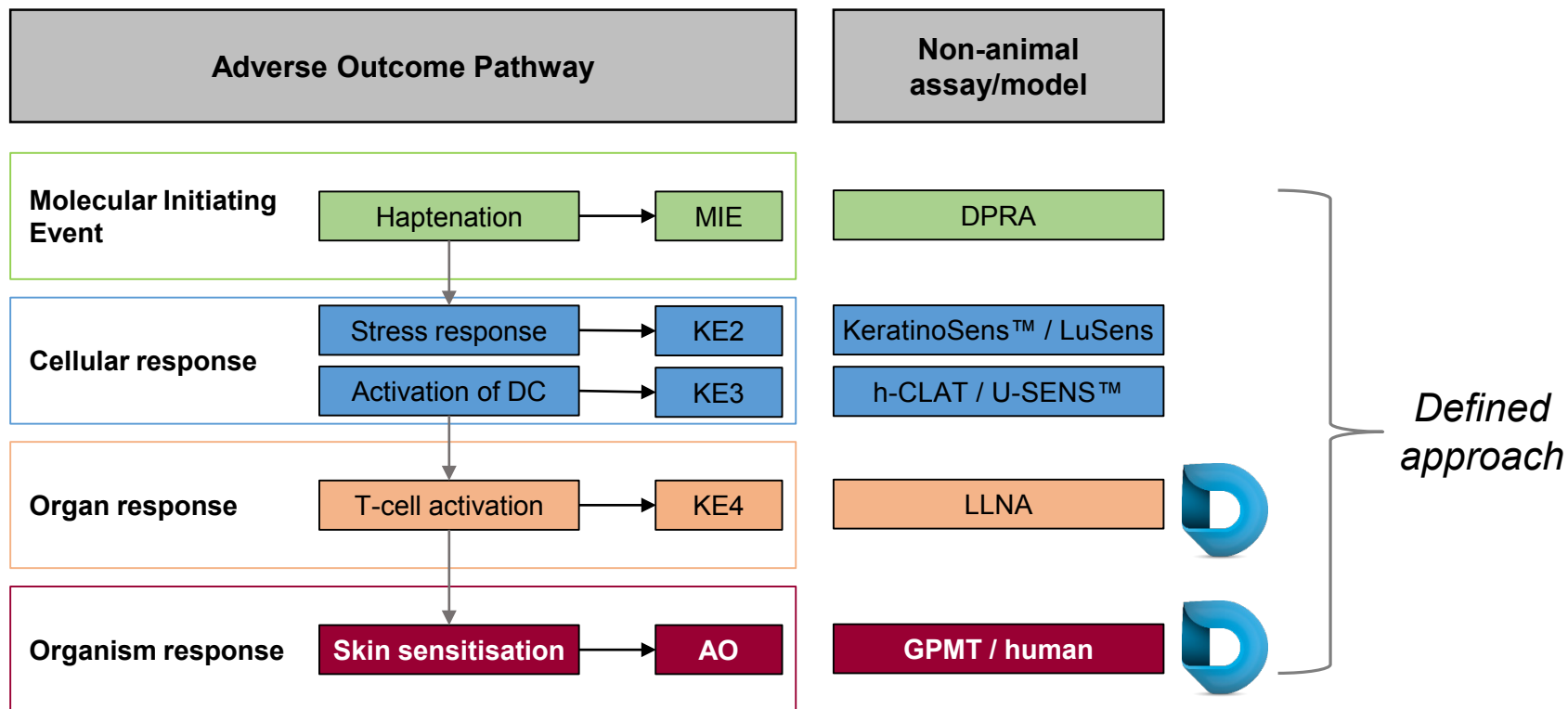


Brazil cosmetics ban



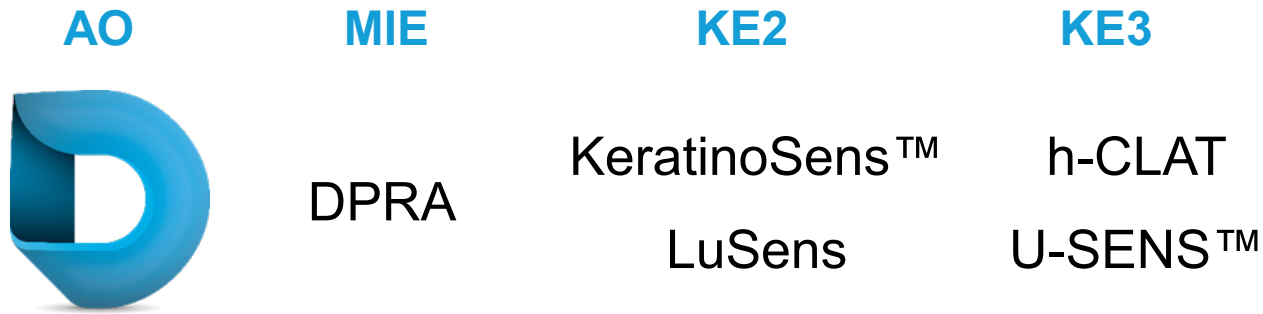
USA – Amended TSCA

AOP and non-animal approaches



- *In chemico/in vitro* assays can't be used in isolation
- *In silico* predictions may provide valuable information.

Lhasa's defined approach



- Our hypothesis:
 - Use Derek information alongside assay data (grouped into key events in the AOP)
 - Apply **exclusion criteria** to take into account assay limitations and confidence in Derek predictions
 - Ensure the most relevant information source(s) are used for specific chemicals.

Summary of exclusion criteria

| Exclusion criteria | | Derek | MIE | KE2 | KE3 | Comment |
|---------------------|------------------------|-------|-----|-----|-----|---|
| Metabolism | Prohaptens | ✓ | ✗ | ✓ | ✓ | Assays lacking metabolic competency are deprioritised as they are less likely to predict prohaptens well |
| logP | > 3.5 | ✓ | ✓ | ✓ | ✗ | Cell-based assays are deprioritised for chemicals with a logP > 3.5 (KE3) and logP > 5 (KE2) as more lipophilic chemicals may lack high solubility in these cell-based assays |
| | > 5 | ✓ | ✓ | ✗ | ✗ | |
| Lysine reactive | Exclusive | ✓ | ✓ | ✗ | ✓ | The Nrf2-ARE pathway is associated with cysteine binding - lysine-reactive chemicals may not be reliably predicted |
| Likelihood | Equivocal | ✗ | N/A | | | Alerts with a likelihood of equivocal have less evidence of skin sensitisation potential than other likelihoods (e.g. certain) and are thus deprioritised |
| Negative prediction | Misclassified features | ✗ | N/A | | | Negative predictions with 'misclassified features' or 'unclassified features' are deprioritised as these are associated with higher uncertainty. |
| | Unclassified features | ✗ | N/A | | | |

Making use of Derek

- Metabolism
- Lipophilicity
- Lysine reactivity
- Likelihood level
- Negative predictions

▼ Comments
Skin sensitisation: murine local lymph node assay (LLNA), guinea pig Freund's complete adjuvant test
Potential mechanism: Prohaptens producing electrophilic Michael acceptor [Aptula and Roberts]

| Fact/Property | Value |
|------------------------|--------|
| LogP | 2.8 |
| LogKp | -1.74 |
| Average Molecular Mass | 165.23 |

▼ Comments
Skin sensitisation: guinea pig Buehler test, guinea pig maximisation test (GPMT)
Potential mechanism: Haptens acting as an electrophilic thiocarbamylating agent [Payne and Walsh]

▼ Skin sensitisation
▼ mamma - EQUIVOCAL
▼ Alert - 712: Terpenoid
EC3 LLNA EC3: 44% (weak sensitiser) - [Derek EC3 Model - 1.2.0]

▼ Skin sensitisation
▼ mammal - NON-SENSITISER
Contains misclassified feature(s)

Hazard prediction

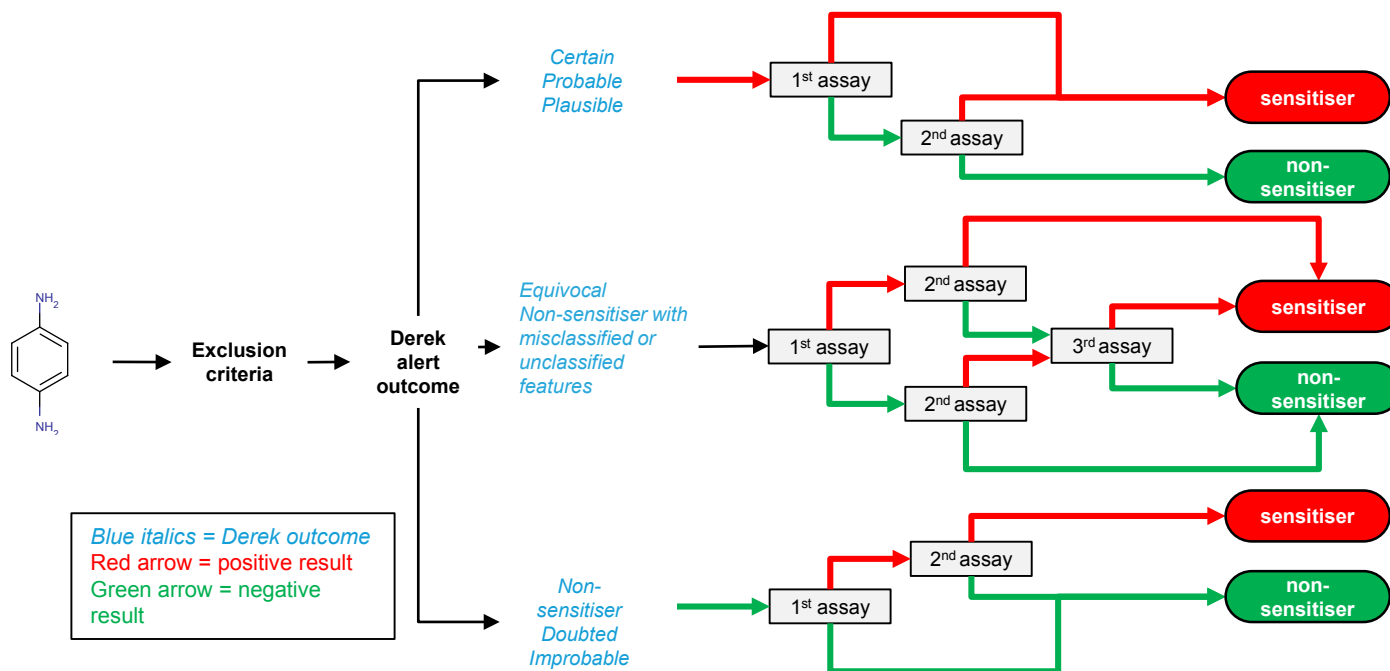
Chemical of interest

Prioritise *in chemico/in vitro* assays using exclusion criteria

Use Derek outcome to determine decision tree branch

Run *in chemico/in vitro* assays in order of AOP (MIE → KE2 → KE3) unless de-prioritised by exclusion criteria

Hazard prediction using '2 out of 3' approach



Potency prediction

Chemical of interest

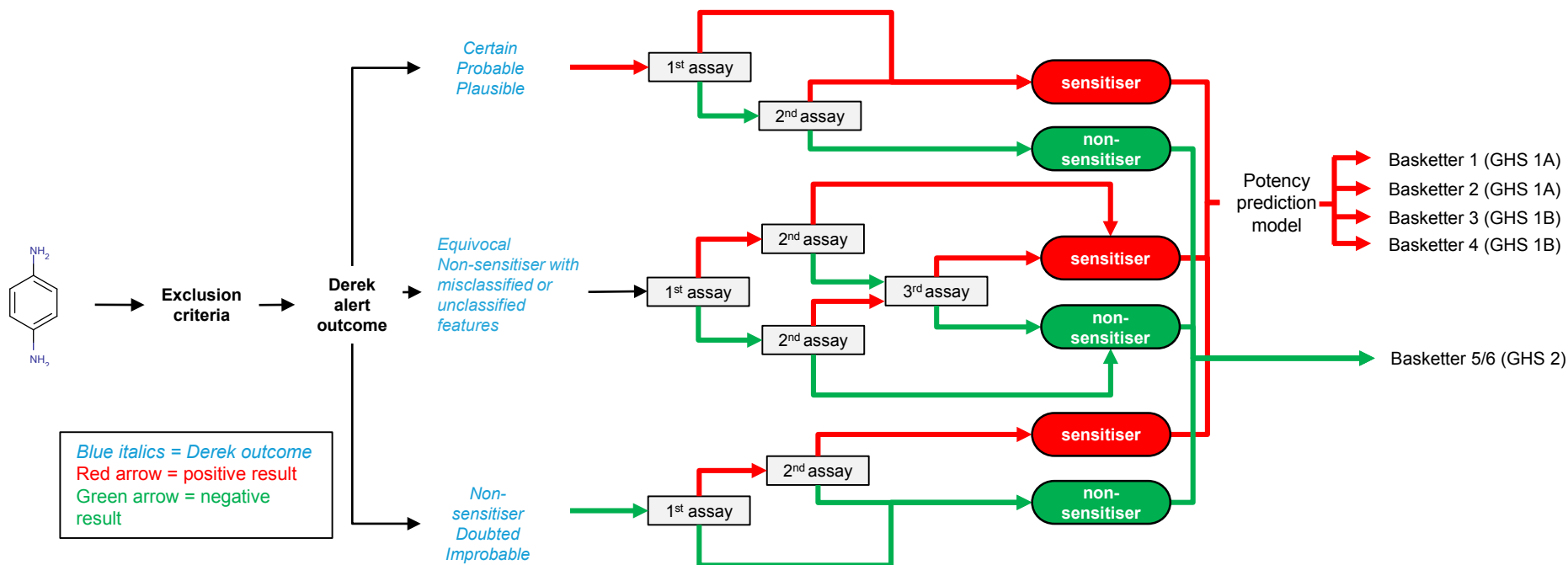
Prioritise *in chemico/in vitro* assays using exclusion criteria

Use Derek outcome to determine decision tree branch

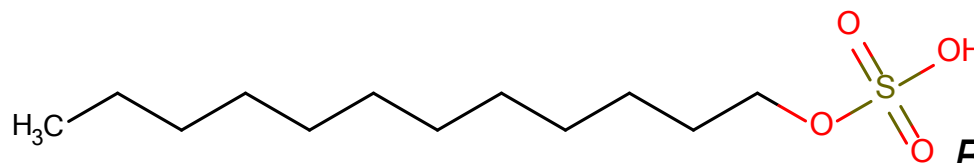
Run *in chemico/in vitro* assays in order of AOP (MIE → KE2 → KE3) unless de-prioritised by exclusion criteria

Hazard prediction using '2 out of 3' approach

Potency prediction using k-nearest neighbours model



Example



in vivo
Non-sensitiser
Basketter category 5/6
GHS 2

| Fact/Property | Value |
|---------------|-------|
| LogP | 4.99 |

- ↘ Skin sensitisation
 - ↘ mammal - **NON-SENSITISER**
 - No misclassified or unclassified features

| Exclusion criteria | Chemical property | Assay(s) excluded |
|---------------------------|--|-------------------|
| Metabolism | n/a | - |
| Lipophilicity | 4.99 | KE3 |
| Lysine reactivity | n/a | - |
| Derek likelihood | n/a | - |
| Derek negative prediction | No misclassified or unclassified features | - |

Example

Chemical of interest

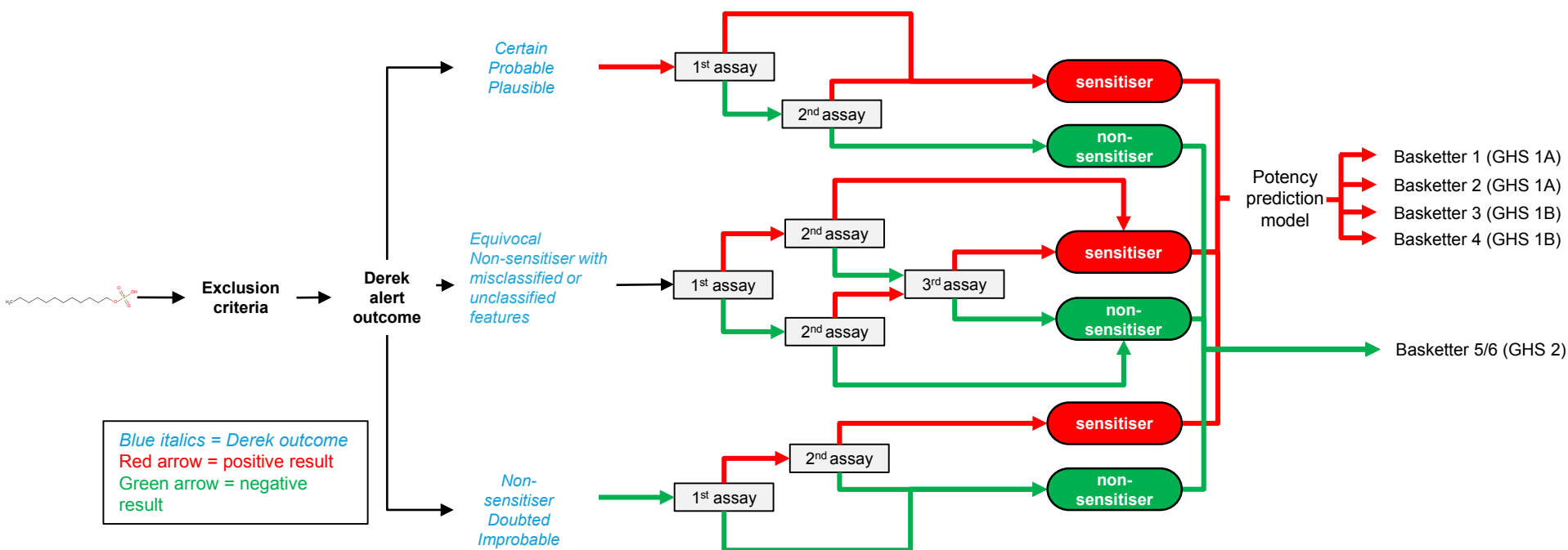
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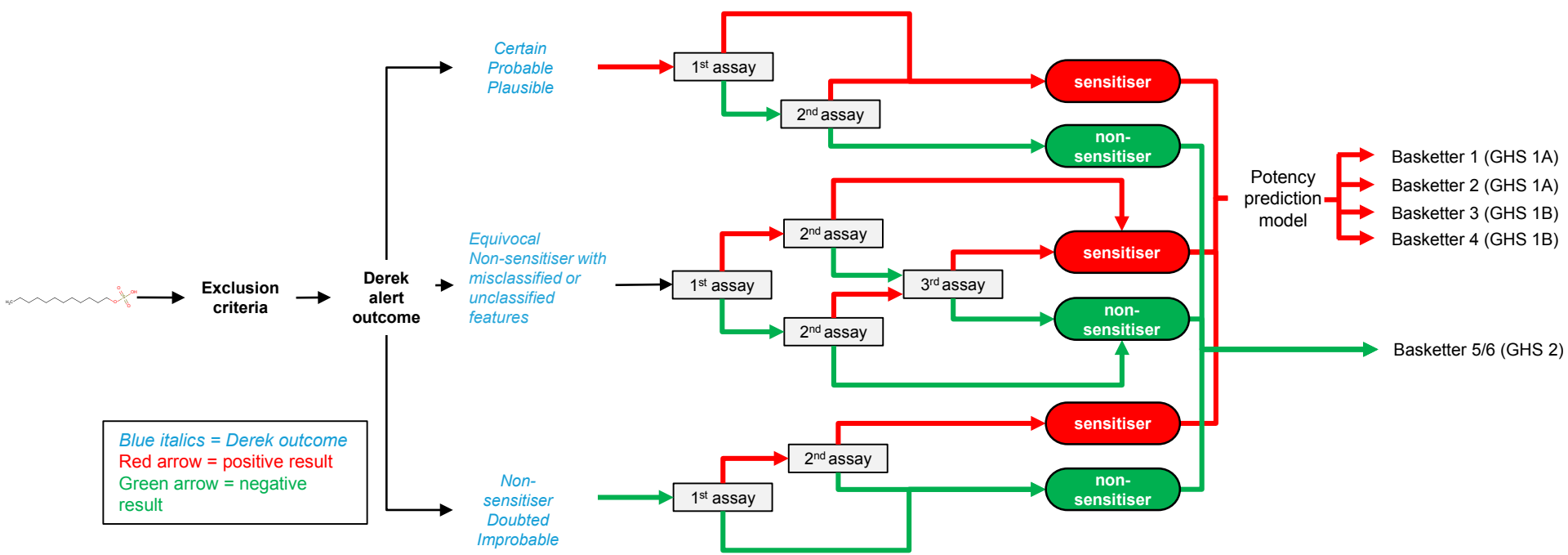
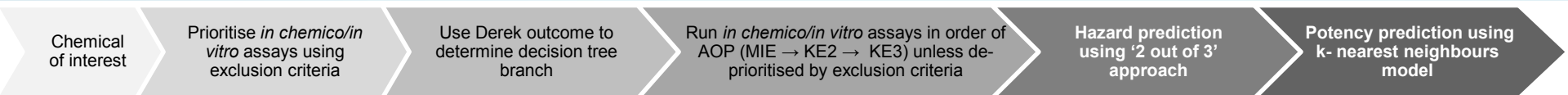
Hazard prediction using '2 out of 3' approach

Potency prediction using k-nearest neighbours model



| AOP event | Assay / model | Outcome |
|-----------|------------------------|-----------------------|
| AO | DX | Non-sensitizer |
| KE1 | DPRA | positive |
| KE2 | KeratinoSens™ / LuSens | negative |
| KE3 | deprioritised | |

Example

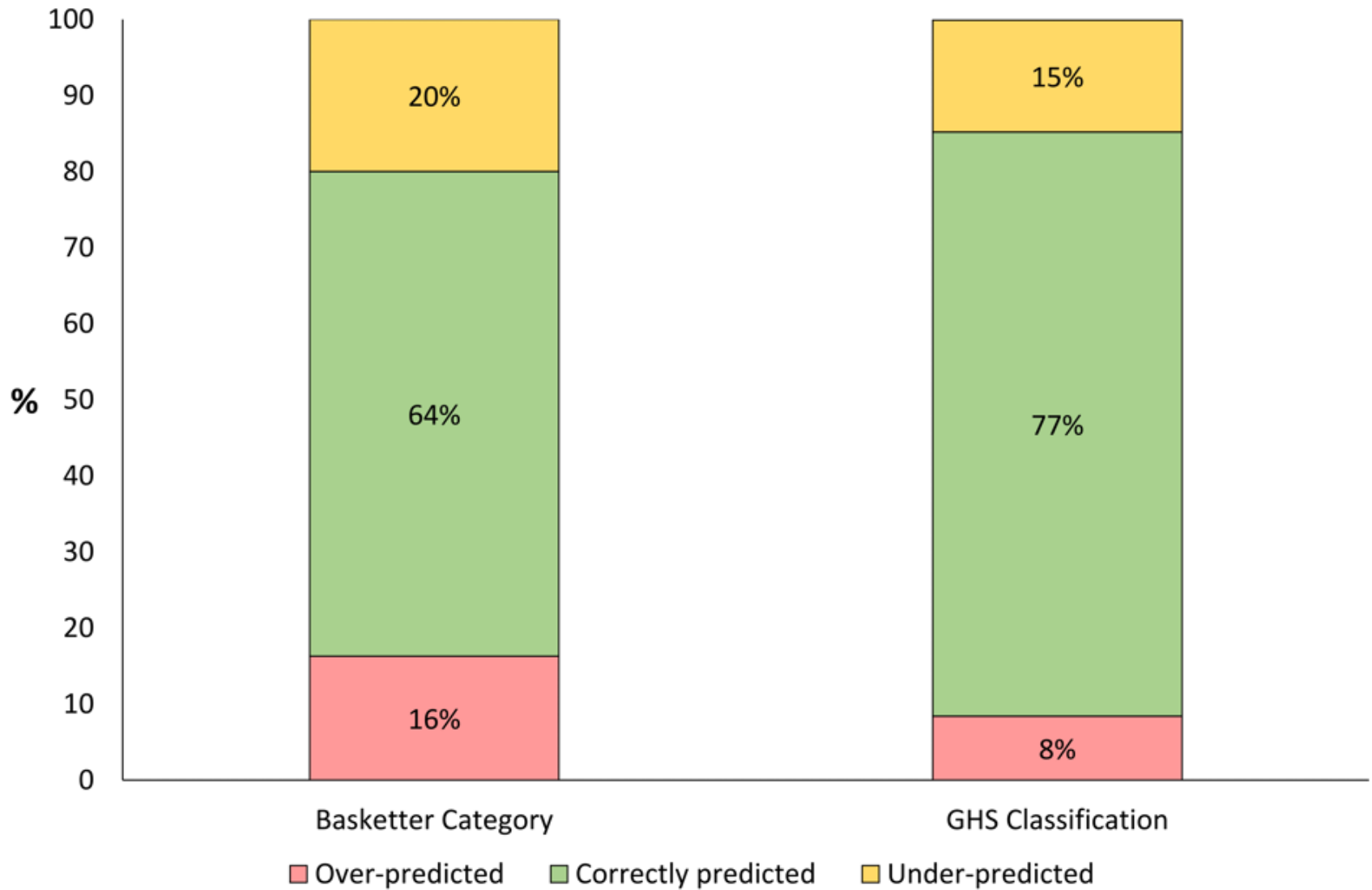


in vivo
Non-sensitiser
 Basketter category 5/6
 GHS 2

Defined approach
Non-sensitiser
 Basketter category 5/6
 GHS 2



Results



Conclusions

- A decision tree defined approach has been designed using exclusion criteria based on known limitations of *in chemico/in vitro* assays and Derek Nexus
- When assessed against a dataset of 210 chemicals, the defined approach predicts both the Basketter category and GHS classification well
- We are currently seeking collaborations in order to validate the approach using new *in chemico/in vitro* data.