Deriving a no expected sensitization induction level for fragrance ingredients without animal testing: An integrated approach applied to specific case studies

Andreas Natsch

16.5.2018
1. Defined approach (DA) + Data Interpretation procedure (DIP)
   1. Potency based on kinetic peptide reactivity and quantitative KeratinoSens data and Regression models

2. Domain and global assessments

3. IATA: Targeted additional testing

4. Uncertainty assessment

5. Adjustment of NESIL based on uncertainty assessment

6. Types of case studies

7. Case study Citral

8. Case studies: Molecules with high quality LLNA and human data

9. Case studies new molecules
Overall approach

• Determine «**most likely LLNA EC3 value**» as **Point of departure** (PoD) with a defined approach (DA) using a data integration procedure (DIP)
  • Global model for all chemicals
  • Use a domain-model for prediction if available

• (Opt:) Refine prediction with targeted **additional testing** based on domain of molecule: Integrated approach for testing and assessment (IATA), requires some expert input

• Search for analogues in database with *in vitro* and *in vivo* data: Predict with same approach
  • Determine **uncertainty** based on prediction accuracy

• Determine an **adjustment factor** based on uncertainty analysis

• **Divide PoD by adjustment factor to arrive at a final NESIL**
**Overall approach: Schematic – details to follow…..**

**New chemical**

- Perform KeratinoSens and reactivity assays (adduct formation, kinetic depletion)

**TIMES: Structural alert for reactivity? Peptide adduct consistent with alert?**

- YES
  - Local model available on related molecules with same mechanism?
    - YES
      - Predict with local model in parallel
    - NO
      - Use global model only

- NO
  - NO
    - Use global model only
    - Predict with local model in parallel

**Defined approach based on standard information sources (KeratinoSens, peptide reactivity and TIMES)**

- Regression equations are used as *Data Integration procedures (DIP)*

**Specific additional tests for this mechanistic domain available?**

- YES
  - Perform additional mechanistic tests *
  - Refine prediction
- NO
  - IATA taking into account structural information and additional mechanistic tests

**Search for closely related molecules with *in vivo* and *in vitro* data**

- Predict potency with same scheme

**High certainty of prediction by local and/or global model**

- YES
  - Prediction fits with *in vivo* data?
    - YES
      - Predict potency with same scheme
    - NO
      - Adjust NESIL
- NO
  - Adjust NESIL
Defined approach (DA) : Potency based on kinetic peptide reactivity and quantitative KeratinoSens data

- **Standard input data for all molecules in DA:**
  - Dose response from KeratinoSens: EC1.5, EC3, IC50
  - Kinetic peptide reactivity (Rate constant for depletion)
  - Peptide adduct formation for reaction mechanism
  - TIMES for attribution to structural domains

- Data interpretation procedure (DIP): Regression equations to predict **Likely LLNA EC3** as point of departure (PoD)

**Global model:**

\[
p_{EC3} = 0.04 + 0.38 \times \log K_{norm} + 0.25 \times \log EC1.5_{norm} + 0.25 \times \log IC50_{norm} - 0.19 \times \log VP_{norm}
\]

- **Peptide reactivity**
- **KeratinoSens**
- **Volatility**


Published also as OECD case study Nr. 7 in ENV/JM/MONO(2016)29/ANN1
Domain and global assessments

- Based on TIMES SS and experimental peptide adduct data: Attribute chemicals to a domain (if applicable)
  - Global model for all chemicals
  - Use a domain-model for prediction if available

```
New chemical

TIMES: Structural alert for reactivity? Peptide adduct consistent with alert?
YES
- Local model available on related molecules with same mechanism?
  YES
  - Predict with local model in parallel
  NO
  - Use global model only

NO

Defined approach based on standard information sources (KeratinoSens, peptide reactivity and TIMES)

Regression equations are used as Data Integration procedures (DIP)
```

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IATA: Targeted additional testing

- Depending on the structure / domain, specific tests may help to refine the potency prediction.

  Specific additional tests for this mechanistic domain available? YES → Perform additional mechanistic tests *
  NO → Refine prediction

IATA taking into account structural information and additional mechanistic tests

- Examples:

  - A) **Aldehydes**: Reactivity test using butylamine to measure rate of SchiffBase formation
    ⇒ Local model combined with KS data

  - B) **Phenolic prohaphtens**: KS or peptide reactivity with activation system

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Uncertainty assessment

• Search for closely related molecules with existing in vivo data in database with similar substructure for the putative reactive part of the molecule

• Perform same assessment (DA / DIP /IATA)

• **Compare outcome to in vivo situation**

  • This helps to assess uncertainty for the very specific subdomain of chemicals
  • Based on the uncertainty assessment, NESIL may be adjusted
Adjustment of NESIL based on uncertainty assessment

- The predicted PoD (likely EC3 value) is transformed into a NESIL
- If uncertainty is low ⇒ Proposed adjustment factor = 2
  - Note: NESIL is defined as a NOEL
  - LLNA is extrapolated between NOEL and LOEL – 3-fold proliferation is already an ‘effect’
- If uncertainty is high – adjust based on uncertainty assessment
- If no uncertainty assessment possible – adjust based on precision of global model

**Diagram:**
- **Close analogues in database?**
  - **YES**
  - **NO**
  - Analogues predicted with < 2-fold error?
  - **YES**
  - **NO**
  - Prediction on conservative side?
    - **YES**
    - **NO**
    - Adjustment factor = 2
    - Adjustment factor based on error for analogues
  - Adjustment based on (global) model uncertainty (e.g. 90% percentile)

**Graphs:**
- Human NOEL, low end of dose-response
- LLNA EC3, Extrapolated from increasing dose response
Four types of case studies done:

• 15 molecules with mainly congruent LLNA and human data, with human NOEL and LOEL (No /Lowest observed effect dose) data
  • Allows direct comparison of derived NESIL with human and animal derived NESIL
• 7 molecules with partly discordant human and LLNA data / missing human LOEL values
  • Indicates how DA /IATA compares against LLNA or human data for difficult cases
• 3 new molecules – tested as case studies and later challenged by LLNA
  • Molecules tested when REACH still considered LLNA as mandatory, unique opportunity to challenge predictions by *in vivo* data
• 4 new molecules, no LLNA data available nor currently planned
  • Demonstrates approach to risk assessment in absence of animal data
Case study Citral

- One infocard covers all steps for each molecule; same info card generated for each molecule to be assessed

**Case Study on Citral**

<table>
<thead>
<tr>
<th>a) Data, assessment with DIP and additional mechanistic tests</th>
</tr>
</thead>
</table>
| **Name:** Citral | **DFRA:** Cys-depletion: 85.7 \%  
Sey-depletion: 16.9 \%  
Positive in high category |
| **Structure:** | **KeratinoSens:** EC 1.5: 23 \( \mu \)M  
IC 50: 183 \( \mu \)M  
Positive |
| **TIMES parent:** Strong sensitizer, Di-substituted \( \alpha \beta \)-unsaturated aldehydes | **Prediction global model:** EC 5.2 \% |
| **TIMES metabolite:** Weak sensitizer, hydroperoxide | **Prediction Local model:** EC 3.6 \% |
| **LC-MS:** Cortisol depletion: 27.2 \%  
Adduct: direct Michael Acceptor (MA)  
adduct 8.1 \%;  
Peptide oxidation predominant | **Additional mechanistic tests:** Reactivity with amine groups to test for Schiff Base MoA |
| **Domain attribution:** Michael acceptor | **Results mechanistic test:** Low amine reactivity, local model with RA-test indicates lower Sensitization potential (EC3 = 11.6 \%); MA MoA confers stronger sensitization potential, assess with MA model |

**b) Analysis of close analogues for uncertainty assessment**

<table>
<thead>
<tr>
<th>Close analogue:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Farnesal</td>
<td>Olfanil</td>
</tr>
</tbody>
</table>

| Rationale for selecting close analogue: | \( \beta \)-alkyl-substituted \( \alpha \beta \)-unsaturated aldehydes  
Di-substituted \( \alpha \beta \)-unsaturated aldehydes |
| Prediction close analogue global model: | EC 3.2 \%  
EC 3.1 \% |
| Prediction close analogue local model (MA): | EC 3.6 \%  
EC 3.4 \% |
| In vivo results close analogue: | EC 11.7 \%  
EC 3.5 \% |
| Prediction accuracy analogues: Local model predicts within 2-fold error; on conservative side |

**c) IATA assessment and discussion**

**Weight of evidence assessment:** Directly reactive Michael acceptor based on LC-MS, aldehyde MoA of lower potency. Take EC3 = 6.8 \% from local MA model, moderate sensitizer, PoD: 1700 \( \mu \)g/cm².

**Uncertainty assessment based on close analogues:** Predictions with local model for close analogues indicate high certainty, predictions on conservative side. Adjustment factor to derive NESIL = 2.

**In vivo results:** LLNA EC3 5.5 \% (1425 \( \mu \)g/cm²; weighted average 11 studies[10]), 9.3 \% (Median 6 studies[31]), PoD LLNA and human: 1400 \( \mu \)g/cm², LOEL human 3870 \( \mu \)g/cm²

**WoE and conclusions**

**DA and DIP results**

**IATA: additional tests and results**

**Uncertainty analysis:** Close analogues with DA / DIP results and in vivo data
Case study Citral: Prediction by DA and IATA

- Local Michael acceptor model predicts EC3 of 6.8%
- Close to global model (EC3 = 5.2%) 
- IATA: SchiffBase formation alternative MoA

  - Amine reactivity would indicate weaker activity – Michael acceptor MoA confers stronger reactivity and sensitization: **Use local MA model**

<table>
<thead>
<tr>
<th>Name:</th>
<th>Citral</th>
<th>DPRA:</th>
<th>Cys-depletion: 85.7 %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lys-depletion : 16.9 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Positive in high category</td>
<td></td>
</tr>
<tr>
<td>Structure:</td>
<td></td>
<td></td>
<td>Prediction global model:</td>
</tr>
<tr>
<td>TIMES parent:</td>
<td>Strong sensitizer, Di-substituted αβ-unsaturated aldehydes</td>
<td>Prediction global model:</td>
<td>EC3 5.2 %</td>
</tr>
<tr>
<td>TIMES metabolite:</td>
<td>Weak sensitizer, hydroperoxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC-MS:</td>
<td>Cor1C420 depletion: 27.2 %</td>
<td>Additional mechanistic tests:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adduct: direct Michael Acceptor (MA) adduct 8.1%; Peptide oxidation predominant</td>
<td></td>
<td>Reactivity with amine groups to test for Schiff Base MoA</td>
</tr>
<tr>
<td>Domain attribution:</td>
<td>Michael acceptor</td>
<td>Results mechanistic tests:</td>
<td>Low amine reactivity, local model with BA-test indicates lower Sensitization potential (EC3 = 11.6%); MA MoA confers stronger sensitization potential, assess with MA model.</td>
</tr>
</tbody>
</table>

**TIMES indicates MA acceptor, which is verified by LC-MS based protein binding test**

Low amine reactivity, local model with BA-test indicates lower Sensitization potential (EC3 = 11.6%); MA MoA confers stronger sensitization potential, assess with MA model.
Case study Citral: Uncertainty assessment

- Related $\beta$-branched, $\alpha\beta$-unsaturated aldehydes assessed
- Local MA models predicts EC3 within 2-fold error, on conservative side
- **Indicates high certainty of the prediction for Citral**

<table>
<thead>
<tr>
<th>Close analogue:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Farnesal</td>
<td>$\beta$-alkyl-substituted $\alpha\beta$-unsaturated aldehydes</td>
<td>Safranal</td>
</tr>
<tr>
<td>$\beta$-alkyl-substituted $\alpha\beta$-unsaturated aldehydes</td>
<td>Di-substituted $\alpha\beta$-unsaturated aldehydes</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rationale for selecting close analogue:</th>
<th>Prediction close analogue global model:</th>
<th>Prediction close analogue local model (MA):</th>
<th>In vivo results close analogue:</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-alkyl-substituted $\alpha\beta$-unsaturated aldehydes</td>
<td><strong>EC3 2.3%</strong></td>
<td><strong>EC3 6.9 %</strong></td>
<td><strong>EC3 11.7 %</strong></td>
</tr>
<tr>
<td>Di-substituted $\alpha\beta$-unsaturated aldehydes</td>
<td></td>
<td><strong>EC3 3.4 %</strong></td>
<td><strong>EC3 7.5 %</strong></td>
</tr>
</tbody>
</table>

**Prediction accuracy analogues:**
Local model predicts within 2-fold error; on conservative side
Case study Citral: Conclusions

• IATA assessment and discussion

Weight of evidence assessment: Directly reactive Michael acceptor based on LC-MS, aldehyde MoA of lower potency. Take EC3 = 6.8% from local MA model, moderate sensitizer, PoD: 1700 µg/cm²

Uncertainty assessment based on close analogues: Predictions with local model for close analogues indicate high certainty, predictions on conservative side. Adjustment factor to derive NESIL = 2.

In vivo results: LLNA EC3 5.7% (1425 µg/cm², weighted average 11 studies[16]), 9.3% (Median 6 studies[31]), PoD LLNA and human: 1400 µg/cm², LOEL human 3870 µg/cm²

Discussion: In vitro prediction vs. in vivo data: PoD derived from in vitro tests close to LLNA and human PoD, below human LOEL.

• Final NESIL: PoD / adjustment factor of 2: 850 µg/cm²
• NESIL human data: 1400 µg/cm²
• NESIL LLNA data: 1400 µg/cm²
Case studies: Molecules with high quality LLNA and human data

- 15 fragrance molecules with human NOEL, LOEL and LLNA EC3
- The PoD (= predicted LLNA EC3) is compared to LLNA and human data
  - Overall good correlation of IATA PoD with Human LOEL, PoD 0.29 Log units (=2-fold) below LOEL
  - Similar correlation between LLNA EC 3 and human LOEL
Case studies: Molecules with high quality LLNA and human data

- For illustration: Summary of seven case studies

Table 1. Case studies 1-7 on sensitizers with congruent human and LLNA data leading to similar NESIL<sup>1,2</sup>)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>NESIL human NOEL (µg/cm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Human LOEL (µg/cm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>NESIL/EC3 LLNA (µg/cm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>PoD IATA (µg/cm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Uncertainty assessment IATA PoD</th>
<th>Adjustment factor to derive NESIL</th>
<th>IATA derived NESIL (µg/cm&lt;sup&gt;2&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citral</td>
<td>1400</td>
<td>3876</td>
<td>1414</td>
<td>1700</td>
<td>high certainty</td>
<td>2</td>
<td>850</td>
</tr>
<tr>
<td>Phenylacetaldehyde</td>
<td>590</td>
<td>1180</td>
<td>962</td>
<td>1250</td>
<td>high certainty</td>
<td>2</td>
<td>625</td>
</tr>
<tr>
<td>Cinnamic aldehyde</td>
<td>591</td>
<td>775</td>
<td>262</td>
<td>575</td>
<td>high certainty</td>
<td>2</td>
<td>288</td>
</tr>
<tr>
<td>Cinnamic alcohol</td>
<td>3000</td>
<td>4724</td>
<td>5250</td>
<td>5425</td>
<td>high certainty, predictions of analogues on conservative side</td>
<td>2</td>
<td>2712</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>250</td>
<td>775</td>
<td>498</td>
<td>400</td>
<td>limited; analogues well predicted</td>
<td>2 if taking conservative model</td>
<td>200</td>
</tr>
<tr>
<td>2-phenylpropionaldehyde</td>
<td>388</td>
<td>1938</td>
<td>1575</td>
<td>2400</td>
<td>high certainty</td>
<td>2</td>
<td>1200</td>
</tr>
<tr>
<td>2-hexyliden cyclopentanone</td>
<td>300</td>
<td>500</td>
<td>600</td>
<td>1100</td>
<td>high certainty</td>
<td>2</td>
<td>550</td>
</tr>
</tbody>
</table>
## Case studies on new molecules: \(\alpha\)-methyldamascone

### a) Data, assessment with DIP and additional mechanistic tests

| Name: | \(\alpha\)-methyl-\(\delta\)-damascone \[(E)-2-methyl-1-((1S,2R)-2,6,6-trimethylcyclohex-3-en-1-yl)but-2-en-1-one]\ | DPRA: | Cys-depletion: 4.4 %  
Lys-depletion: 0.2 %  
Negative in minimal category, <0.1% peptide adduct |
|---|---|---|---|
| Structure: | | KeratinoSens: | EC 1.5: >1000 \(\mu\)M  
IC50: 69.6 \(\mu\)M  
Negative |
| TIMES parent: | strong sensitizer, \(\alpha,\beta\)-Carbonyl compounds with polarized double bonds | Prediction global model: | EC3 60.2% |
| TIMES metabolite: | strong sensitizer, \(\alpha,\beta\)-Carbonyl compounds with polarized double bonds | Prediction Local model: | EC3 58.1% |
| LC-MS: | Cor1C420 depletion: 6.8 %; Adduct: trace (< 0.5%) direct MA adduct | Additional mechanistic tests: | Kinetic profiling of adduct formation vs. benchmarks, see Figure 4 main document |
| Domain attribution: | Michael acceptor | Results mechanistic tests: | 4000-fold reduction in kinetic reaction rate vs. damascones |

Better characterize reactivity of close damascone analogue.
α-methyldamascone: Kinetic adduct formation

- Low reactivity cannot be accurately quantified based on depletion
- Additional test to quantify and verify low reactivity: Kinetic adduct formation

![Graph showing the formation of adducts over time with different markers for δ-damascone, γ-damascone, 2,6-dimethyl-cyclohexyl-crotonate, and α-methyl-δ-damascone. The graph indicates a 4000-fold reduced reactivity vs. benchmark.](image-url)
Case studies on new molecules: \(\alpha\)-methyldamascone

a) **Analysis of close analogues for uncertainty assessment**

<table>
<thead>
<tr>
<th>Close analogue:</th>
<th><img src="image1" alt="Methylionone" /></th>
<th><img src="image2" alt="Delta-damascone" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rationale for selecting close analogue:</td>
<td>(\alpha,\beta)-Carbonyl compounds with polarized double bonds</td>
<td>(\alpha,\beta)-Carbonyl compounds with polarized double bonds</td>
</tr>
<tr>
<td>Prediction close analogue global model:</td>
<td><em>Negative, EC3 34.6% by cytotoxicity</em></td>
<td>EC3 1%</td>
</tr>
<tr>
<td>Prediction close analogue local model (MA):</td>
<td><em>Negative, EC3 63.3% by cytotoxicity</em></td>
<td>EC3 2.7%</td>
</tr>
</tbody>
</table>
| In vivo results close analogue: | EC3 21.8\%  
HRIP 
> 70'866 \(\mu\)g/cm\(^2\) | EC3: 9.6/0.9/5.2; *Median* 5.2\%  
HRIP LOEL 500 \(\mu\)g/cm\(^2\) |
| Prediction accuracy analogues: | Good prediction with local model, esp. for human data | |
α-methyldamascone: IATA assessment and discussion

• **Weight of evidence assessment:**
  - Hazard assessment 2 out of 3: Negative (Negative KS and negative DPRA)
  - Very low residual reactivity observed by adduct formation
  - Predicted very weak sensitizer, EC3 60%; PoD 15’000 µg/cm²

• **Uncertainty assessment based on close analogues:** Prediction with local model for close analogues indicate high certainty, esp. for human data
  - Note: Methylionone has equal cytotoxicity (IC50 = 58 µM), highly similar structure
  - Methylionone is non-reactive and negative in human tests at high conc.; positive LLNA at EC3 21% could be due to irritation.

• **In vivo results:** **Negative, EC3 >25%**
  - LLNA performed after this prediction was made

• **Discussion**
  - **In vivo** data congruent with prediction and observation of very low reactivity
  - **In vitro** and **in vivo** data overrule the TIMES alert: TIMES sees 2D alerts, steric effects not taken into account!
Case studies: Two other new molecules, later challenged by LLNA

- Two molecules:
  - A) Crotonate: Predicted weak sensitizer, low direct reactivity observed
  - B) Oxime ether: Parent non sensitizer, weak sensitizer predicted due to metabolic activity

Table 3. Risk assessment for three new molecules without animal data – later challenged by LLNA

<table>
<thead>
<tr>
<th>Chemical structure</th>
<th>TIMES prediction</th>
<th>KS result</th>
<th>Peptide reactivity</th>
<th>PoD IATA (µg/cm²)</th>
<th>Uncertainty assessment IATA PoD</th>
<th>Adjustment factor to derive NESIL</th>
<th>IATA derived NESIL (µg/cm²)</th>
<th>LLNA result ¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,6-dimethylcyclohexyl-crotonate</td>
<td>weak sensitizer, α,β-Carbonyl / polarized double bonds</td>
<td>negative</td>
<td>Cor1C420: 5% direct MA adduct; DPRA low category</td>
<td>EC3 30 – 40%; 11’000 µg/cm²</td>
<td>low uncertainty</td>
<td>2</td>
<td>5500</td>
<td>Positive, EC3 21%; 5450 µg/cm²</td>
</tr>
<tr>
<td>(E)-3-ethoxy-4-hydroxybenzaldehyde O-methyl oxime</td>
<td>Parent: Non-sensitizer Metabolite: Strong sensitizer, Quinoid oxime structure</td>
<td>negative</td>
<td>Cor1C420: 5.7 % depletion; no adduct; DPRA negative</td>
<td>EC3 30 – 50 %, 7500 µg/cm².</td>
<td>High certainty for four tested analogues; Remaining uncertainty due to metabolic activation</td>
<td>2</td>
<td>3750</td>
<td>Negative, EC3 &gt;25%; &gt;6250 µg/cm²</td>
</tr>
</tbody>
</table>

¹) Determined after IATA assessment was made
Case study: Oxime ether, potential prohapten

<table>
<thead>
<tr>
<th><strong>Data, assessment with DIP and additional mechanistic tests</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name:</strong> (E)-3-ethoxy-4-hydroxybenzaldehyde O-methyl oxime</td>
</tr>
<tr>
<td><strong>DPRA:</strong> Cys-depletion: 7.3 %</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Structure:</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>KeratinoSens:</strong> EC 1.5: &gt;1000 µM</td>
</tr>
<tr>
<td>IC50: &gt;1000 µM</td>
</tr>
<tr>
<td><strong>TIMES parent:</strong> Non-sensitizer</td>
</tr>
<tr>
<td><strong>Prediction global model:</strong> Non-sensitizer; EC3 &gt;100 %</td>
</tr>
<tr>
<td><strong>TIMES metabolite:</strong> Strong sensitizer; Quinone methide(s)/imines, Quinoide oxime structure, Nitroquinone</td>
</tr>
<tr>
<td><strong>Prediction Local model:</strong></td>
</tr>
<tr>
<td><strong>LC-MS:</strong> Cor1C420 depletion: 5.7 %</td>
</tr>
<tr>
<td>Adduct: no adduct</td>
</tr>
<tr>
<td><strong>Additional mechanistic tests:</strong> Test in presence of metabolic system (LC-MS and KS)</td>
</tr>
<tr>
<td><strong>Domain attribution:</strong> Quinone methide precursor</td>
</tr>
<tr>
<td><strong>Results mechanistic tests:</strong> Small trace of peptide adduct in presence of microsomes, positive in KeratinoSens with S9</td>
</tr>
</tbody>
</table>

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### Case study: Oxime ether, potential prohapten

- **Analysis of close analogues for uncertainty assessment**

<table>
<thead>
<tr>
<th>Close analogue:</th>
<th>Isoeugenol</th>
<th>Eugenol</th>
<th>Ethylvanillin</th>
<th>Benzaldoxime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rationale for selecting close analogue:</td>
<td>Quinone methide precursor</td>
<td>Quinone methide precursor</td>
<td>Substructure of target</td>
<td>Aromatic oxime; Substructure of target</td>
</tr>
<tr>
<td>Prediction close analogue global model:</td>
<td>EC3 1.6 %</td>
<td>EC3 14.1 %</td>
<td>EC3 41 %</td>
<td>EC3 29.8 %</td>
</tr>
<tr>
<td>Prediction close analogue local model:</td>
<td>EC3 7.9 %</td>
<td>EC3 16.2 %</td>
<td>EC3 49 %; &gt;100% model with BA-test</td>
<td>No model</td>
</tr>
<tr>
<td>In vivo results close analogue:</td>
<td>EC3 1.8 %</td>
<td>EC3 12.9 %</td>
<td>&gt; 50%</td>
<td>&gt; 20%</td>
</tr>
<tr>
<td>Prediction accuracy analogues:</td>
<td>Good prediction with local and global model, better accuracy for global model in case of isoeugenol</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Case study on new material: Risk assessment without LLNA

- New molecule predicted as sensitizer by TIMES, KeratinoSens, DPRA and LC-MS assay

<table>
<thead>
<tr>
<th>a) Data, assessment with DIP and additional mechanistic tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name:</strong> ethyl (Z)-2-acetyl-4-methyltridec-2-enoate</td>
</tr>
<tr>
<td><strong>DPRA:</strong> Cys-depletion: 27.8 %</td>
</tr>
<tr>
<td>Lys-depletion: 1.3 %</td>
</tr>
<tr>
<td><strong>Positive in low category,</strong> ca. 6.6% <strong>direct adduct</strong> with Cys-peptide</td>
</tr>
<tr>
<td><strong>Structure:</strong></td>
</tr>
<tr>
<td><strong>KeratinoSens:</strong> EC 1.5: 7.95 µM</td>
</tr>
<tr>
<td>EC3 not reached due to cytotoxicity</td>
</tr>
<tr>
<td>IC50: 13.2 µM</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
</tr>
<tr>
<td><strong>TIMES parent:</strong> strong sensitizer, αβ-Carbonyl compounds with polarized double bonds</td>
</tr>
<tr>
<td><strong>Prediction global model:</strong> EC3: 5.1 %</td>
</tr>
<tr>
<td><strong>TIMES metabolite:</strong> strong sensitizer, αβ-Carbonyl compounds with polarized double bonds</td>
</tr>
<tr>
<td><strong>Prediction Local model:</strong> EC3: 14 %</td>
</tr>
<tr>
<td><strong>LC-MS:</strong> Cor1C420 depletion: 14 %</td>
</tr>
<tr>
<td><strong>direct MA adduct</strong></td>
</tr>
<tr>
<td>Peptide oxidation predominant</td>
</tr>
<tr>
<td><strong>Additional mechanistic tests:</strong> Not needed</td>
</tr>
<tr>
<td><strong>Domain attribution:</strong> Michael acceptor</td>
</tr>
<tr>
<td><strong>Results mechanistic tests:</strong> n/a</td>
</tr>
</tbody>
</table>
Case study on new material: Risk assessment without LLNA

• Uncertainty assessment:
  • Related analogues: Michael acceptors with the double bond activated by two carbonyl groups
  • Well predicted by global and local model, here global model more accurate and on conservative side
  • Use global model for conservative assessment

<table>
<thead>
<tr>
<th>Close analogue:</th>
<th><img src="image" alt="Diethylmaleate" /></th>
<th><img src="image" alt="ethyl (Z)-2-acetyldic-2-enoate" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rationale for selecting close analogue:</td>
<td>Double activated MA-ester</td>
<td>Double activated MA-ester, substructure of target</td>
</tr>
<tr>
<td>Prediction close analogue global model:</td>
<td>EC3 1.4%</td>
<td>EC3 3%</td>
</tr>
<tr>
<td>Prediction close analogue local model (MA):</td>
<td>EC3 3.8 %</td>
<td>EC3 5.6 %</td>
</tr>
<tr>
<td>In vivo results close analogue:</td>
<td>EC3 2.1 %</td>
<td>EC3 2.6 %</td>
</tr>
<tr>
<td>Prediction accuracy analogues:</td>
<td>Good prediction with local and global model, better accuracy for global model for these double activated MA-esters</td>
<td></td>
</tr>
</tbody>
</table>
ethyl (Z)-2-acetyl-4-methyltridec-2-enoate: IATA assessment and discussion

- **Weight of evidence assessment:**
  - Hazard assessment 2 out of 3: Positive (Positive KS and positive DPRA)
  - Directly reactive Michael acceptor
  - Conservative assessment takes EC3 from global model
  - EC3 = 5.1%; PoD 1250 µg/cm²

- **Uncertainty assessment based on close analogues:**
  - Prediction with global model for close analogues indicates high certainty
  - adjustment factor to derive NESIL = 2, since conservative assessment from global model taken

*In vivo results:*
- No LLNA planned, use NESIL from this assessment
- **NESIL = 625 µg/cm²**
Overall approach: Hopefully clear by now …..

**New chemical**

1. Perform KeratinoSens and reactivity assays (adduct formation, kinetic depletion)
2. TIMES: Structural alert for reactivity? Peptide adduct consistent with alert?
   - **YES**
     - Local model available on related molecules with same mechanism?
     - **YES**
       - Predict with local model in parallel
     - **NO**
       - Use global model only
   - **NO**
     - Predict with local model in parallel

**Defined approach** based on standard information sources (KeratinoSens, peptide reactivity and TIMES)

Regression equations are used as **Data Integration procedures** (DIP)

- **Specific additional tests for this mechanistic domain available?**
  - **YES**
    - Perform additional mechanistic tests *
  - **NO**
    - Refine prediction

- **Search for closely related molecules with in vivo and in vitro data**
  - **YES**
    - Predict potency with same scheme
  - **NO**
    - Adjust NESIL

**Predict with high certainty of prediction by local and/or global model**

Uncertainty assessment based on read accross
Discussion and Conclusion

- Structured approach with clearly defined data sources
- Takes chemical information into account
- Uses continuous variables from *in vitro* tests
- Read across to chemicals with known *in vivo* and *in vitro* data helps to assess uncertainty
  - Clearly possible in the data-rich domain of fragrance molecules – may be more difficult in other use sectors!
- Adjustment based on uncertainty assessment to transform PoD into NESIL for risk assessment
- Good prediction for fragrance molecules with high quality animal and human *in vivo* data
- Good prediction for three new molecules which were only later tested in LLNA
- **Approach deemed fit-for-purpose and now used on our latest four market candidates with no animal data**
Thank you