Establishing the level of safety concern for chemicals in food without the need for toxicity testing

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Food is chemicals

- proteinase inhibitor
- methylbutanal
- asparagine
- iron
- chaconine
- phenylalanine
- amylase
- oxalic acid
- ascorbic acid
- DDT
- lead
- propham
- potassium
- dimethylpyrazine
- styrene
- globulin
- phytate
- lectin
- glycophosphate
- 4-nonylphenol
- cadmium
- acrylamide
- furan
- lead
- isopropylthioxantone
- albumin
- fufural
- dimethylpyrazine
- methylbutanal
- 4-nonylphenol
- hydoxymethyhfurfural
- etc...
200 tropane alkaloids

350 pyrrolizidine alkaloids

6000 substances potentially used in inks

HEATOX: 800 process contaminants
Food is chemicals: Current understanding

- 5,000,000 man made chemicals known
- 80,000 chemicals in commercial use today
- 100,000 naturally occurring substances

Establish level of safety concern:
- Incident/crisis management
- Priority setting (for further testing or management)
- Optimize the use of animals
- Optimize R&D project management
Alternative solution: *In silico/*structure-activity relationship

Structure determines toxicological properties:

- Direct use of data from well characterized substances
- Predictive modelling

- Comparison
- Statistical analysis
The application of *in silico* methods are increasingly recognized.

### Use of computational tools in the field of food safety

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  - Structure Activity Relationship (SAR)

**Abstract**

In this article we give an overview of how computational methods are currently used in the field of food safety by national regulatory bodies, international advisory organisations and the food industry. Our results show that currently the majority of stakeholders in the field of food safety do not apply computational methods on a routine basis, mainly because of a lack of in-house expertise. Some organisations, however, are very experienced in their use and have developed specialised in-house approaches. Despite this variable situation, computational tools are widely perceived to be a useful tool to support regulatory assessments and decision-making in the field of food safety. Recognised, however, is a widespread need to develop guidance documents and software tools that will promote and harmonise the use of computational methods together with appropriate training.
Predicting toxicity: The food context

✓ “Global”:
  - Broad chemical diversity

✓ Reliable/performant:
  - Protective,
  - Not overtly conservative

✓ Relevant:
  - Risk assessment

✓ Quantitative
  - NOAEL, LOAEL, TD50
  - Not only qualitative (yes-no answer)

Establish a margin of exposure (MoE)
Margin of exposure (MoE)

Risk characterization: **standard.**

\[
ADI = \frac{\text{NOAEL}_{\text{pivotal}}}{\text{UFs}}
\]

- Uncertainty factors (UFs):
  - Inter-species differences
  - Inter-individual differences
  - Limitations of the database

**Margin of Safety (MoS) = ADI/Exposure**

Risk characterization: **alternative.**

\[
\text{MoE} = \frac{\text{Tox.value}}{\text{Exposure}}
\]

- Uncertainties
  - Inter-species differences
  - Inter-individual differences
  - Extrapolation LOAEL/NOAEL
  - Exposure duration
  - ....

**MoE = Margin of exposure**
• Find a relationship (model) between the chemical structures of compounds and a given property

<table>
<thead>
<tr>
<th>comp</th>
<th>LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>comp 1</td>
<td>80</td>
</tr>
<tr>
<td>comp 2</td>
<td>20</td>
</tr>
<tr>
<td>comp 3</td>
<td>77</td>
</tr>
<tr>
<td>comp 4</td>
<td>30</td>
</tr>
<tr>
<td>comp 5</td>
<td>200</td>
</tr>
<tr>
<td>comp 6</td>
<td>5000</td>
</tr>
<tr>
<td>comp 7</td>
<td>3000</td>
</tr>
<tr>
<td>comp 8</td>
<td>20</td>
</tr>
<tr>
<td>comp 9</td>
<td>300</td>
</tr>
<tr>
<td>comp 10</td>
<td>4000</td>
</tr>
<tr>
<td>....</td>
<td>.....</td>
</tr>
<tr>
<td>comp n</td>
<td>30</td>
</tr>
</tbody>
</table>
Predicting rat chronic toxicity LOAEL-rat model

- 567 Lowest Observed Adverse Effect Levels, chronic, rat, 445 substances
- “Leave-one-out” cross-validation

<table>
<thead>
<tr>
<th>Prediction ...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. error</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>64%</td>
</tr>
<tr>
<td>85%</td>
</tr>
<tr>
<td>99%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>average experimental variability</th>
<th>average model error</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4</td>
<td>5.4</td>
</tr>
</tbody>
</table>

In silico-QSAR models:
Other chronic toxicity endpoints

• *MRTD (Human maximum recommended therapeutic dose):

<table>
<thead>
<tr>
<th></th>
<th>within applicability domain</th>
<th>all predictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean error</td>
<td>0.47 (log)</td>
<td>0.59</td>
</tr>
<tr>
<td>Predictions within 1 log unit</td>
<td>89%</td>
<td>82%</td>
</tr>
</tbody>
</table>


• TD_{50} (carcinogenic potency)

<table>
<thead>
<tr>
<th>Author (Method)</th>
<th>Rat</th>
<th>≤1-fold (%)</th>
<th>≤2-fold (%)</th>
<th>≤5-fold (%)</th>
<th>≤10-fold (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bercu et al. (VISDOM)</td>
<td>RAT</td>
<td>59</td>
<td>64</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>MOUSE</td>
<td>66</td>
<td>81</td>
<td>88</td>
<td>97</td>
</tr>
<tr>
<td>Contrera (SciQSAR)</td>
<td>RAT</td>
<td>48</td>
<td>65</td>
<td>78</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>MOUSE</td>
<td>56</td>
<td>75</td>
<td>78</td>
<td>97</td>
</tr>
<tr>
<td>(Lazar**)</td>
<td>RAT</td>
<td>43</td>
<td>57</td>
<td>71</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>MOUSE</td>
<td>48</td>
<td>76</td>
<td>86</td>
<td>93</td>
</tr>
</tbody>
</table>

Example: Isopropylthioxanthone (ITX)

- Photoinitiator, UV-cured inks
- Detected in milk bricks: 70-600 ppb
- Calculated exposures: 3 et 50 μg/kg/d
- Non genotoxic

<table>
<thead>
<tr>
<th></th>
<th>TOPKAT</th>
<th>Rat LOAEL</th>
<th>Human LOAEL</th>
<th>*Experimental (LOAEL 28 d rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITX (tox pred.)</td>
<td>32 (mg/kg)</td>
<td>15 (mg/kg)</td>
<td>0.59 mg/kg</td>
<td>50 mg/kg</td>
</tr>
<tr>
<td>MoE*</td>
<td>700-10000</td>
<td>325-5000</td>
<td>12-200</td>
<td>1000-17000</td>
</tr>
</tbody>
</table>

*MoE (margin of exposure) = ratio tox value/human exposure

MoE interpretation:

- Rat LOAEL/TOPKAT:
  - Conversion LOAEL-NOAEL (3-10)
  - Interspecies differences (10)
  - Interindividual differences (10)

- Human LOAEL:
  - Conversion LOAEL-NOAEL (10)
  - Interindividual differences (2-10)

MoE: >6000 (10x10x10x6)

MoE: >100

• Unknown activity for a compound can be extrapolated from the activities of similar compounds.

• Establishing similarity is complex:
  – Chemical structure
  – Physico chemical properties
  – Metabolism
  – Mechanism of action
  – Availability of reliable data on analogues

  ➢ Based on expert judgments/choices
Docking: predicting ligand-receptor interactions

Collaboration with University of Parma (publication in preparation)


Docking to serve read-across

**Table 1. In vitro results**

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC_{50} Redistribition Assay</th>
<th>EC_{50} CALUX Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>0.14 nM</td>
<td>4.6 pM</td>
</tr>
<tr>
<td>hypothemycin</td>
<td>n. b.</td>
<td>n. b.</td>
</tr>
<tr>
<td>α ZOL</td>
<td>0.22 nM</td>
<td>9.6 pM</td>
</tr>
<tr>
<td>α ZAL</td>
<td>0.20 nM</td>
<td>19 pM</td>
</tr>
<tr>
<td>β ZAL</td>
<td>0.79 nM</td>
<td>280 pM</td>
</tr>
<tr>
<td>ZEN</td>
<td>4.27 nM</td>
<td>490 pM</td>
</tr>
<tr>
<td>β ZOL</td>
<td>48.69 nM</td>
<td>2500 pM</td>
</tr>
</tbody>
</table>

**Table 2. In silico results of ZEN, reduced metabolites and positive (estradiol) and negative (hypothemycin) control**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experimental rank</th>
<th>HINT score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>1</td>
<td>1380.22</td>
</tr>
<tr>
<td>hypothemycin</td>
<td>/</td>
<td>-1314.35</td>
</tr>
<tr>
<td>α ZOL</td>
<td>2</td>
<td>648.39</td>
</tr>
<tr>
<td>α ZAL</td>
<td>3</td>
<td>632.53</td>
</tr>
<tr>
<td>β ZAL</td>
<td>4</td>
<td>505.31</td>
</tr>
<tr>
<td>ZEN</td>
<td>5</td>
<td>499.77</td>
</tr>
<tr>
<td>β ZOL</td>
<td>6</td>
<td>469.55</td>
</tr>
</tbody>
</table>

**Table 3. In silico results of oxidized metabolites**

<table>
<thead>
<tr>
<th>Compound</th>
<th>HINT score</th>
<th>Predicted activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-OH-ZEN</td>
<td>-47.27</td>
<td>Negative</td>
</tr>
<tr>
<td>15-OH-ZEN</td>
<td>545.20</td>
<td>Positive</td>
</tr>
<tr>
<td>8aOH-ZEN</td>
<td>-37.74</td>
<td>Negative</td>
</tr>
<tr>
<td>6aOH-ZEN</td>
<td>197.87</td>
<td>Positive</td>
</tr>
<tr>
<td>8bOH-ZEN</td>
<td>212.76</td>
<td>Positive</td>
</tr>
<tr>
<td>6bOH-ZEN</td>
<td>280.99</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Collaboration with University of Parma *(publication in preparation)*
In silico tools are valuable to establish level of concern

- Require adequate information on structure.
- Many different tools available:
  - Cover various modes/mechanisms of action and effects:
    - Yes/No
    - Ranking/potency
  - Provide quantitative information (for MoE)
- Use models validated according to international standards

➤ Integration of different tools
A decision tree was developed to aid integrating exposure and predicted toxicological values (ILSI-Europe).

Predictions:
- Read across
- QSAR
- Docking

Weight of Evidence:
- Quality
- Relevance
- Reliability
- Consistency
- ....

Level of confidence

"Establishing the level of safety concern for chemicals in food without the need for toxicity testing",
Application of the DT
Uncertainties

- Application of DT brings uncertainties similar to those associated with classical RA:
  - Exposure assessment
  - Hazard assessment (relevance for human, MOA)

- Relying on predicted tox values introduce additional uncertainties:
  - QSAR (performance of the models, domain of applicability)
  - Read-across (suitability of analogues, quality of tox data, extrapolation from analogues)

- Unlikely to significantly impact the “degree of conservatism” of the assessment:
  - Errors of the models not systematic (under- or over- estimate tox)
  - Different models based on independent training datasets
  - Different approaches involved (read-across, QSAR)
  - Introduction of mechanistic considerations
Safety assessment of packaging: A role for *in silico* toxicology?

Migration study:
- Food simulant(s)

Raw materials
- Existing and new packaging
- EU/Nestlé compliance:
  - IAS and known NIAS (target analysis)
  - unknown NIAS (non-target analysis)

Suppliers

Compliance

Hazard identification/Characteristics NIAS

Risk assessment

Risk management

Supplier Compliance

Chemicals to be assessed
- Exposure rough estimates
- Existing exp. tox data, SML
- TTC, *CompTox*

Level of concern
- low
- high

Management decisions:
- Generate exposure data
- Generate exp. tox data
- Mitigation

Specifications
- Suitable

QA

Reject
• Hazard characterization can be conducted based on predictive models/approaches:
  – Several independent models should be applied in an integrated way
  – Models should be properly validated

• If properly applied, the ILSI-DT is likely to bring a reasonable degree of conservatism.

• The application of the ILSI-DT is likely to be a valuable tool allowing decisions while ensuring health protection.

• It can play a significant role in the safety assessment of packaging materials.

• Should be used to address the concern of large number of non-tested substances.
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