Read-across and QSARs in the risk assessment of food and feed

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Introducing EFSA

• Set up in 2002
• Moved to Parma 2005
• Now around 450 staff
• 1500 external experts
Who can task EFSA?

- European Commission
- EU Member States
- European Parliament
- Self-tasking
### Scientific Committee and Panels

#### Mainly opinions on applications
- Food additives and nutrient sources (ANS)
- Food contact materials, enzymes, flavourings (CEF)
- Feed additives (FEEDAP)
- Genetically modified organisms (GMO)
- Nutrition (NDA)

#### Mainly generic opinions
- Animal health and welfare (AHAW)
- Biological hazards (BIOHAZ)
- Contaminants (CONTAM)
- Plant health (PLH)
- Plant protection products (PPR)
- Scientific Committee (SC)

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Integrated approach

The Commission’s White Paper on Food Safety (2000): “… food safety policy must be based on a comprehensive, integrated approach…” meaning:

- Throughout the food chain ('farm to table')
- Across all food sectors
- Between the Member States
- At the EU external frontier and within the EU
- In international and EU decision-making
- At all stages of the policy-making cycle
How can we achieve this?

- To achieve integration in food-environment risk assessments, we must consider a number of aspects or dimensions
  - Risk assessment workflow
  - Protection goals and stressors
  - Distribution of risk (spatial, temporal, social)
  - Distribution of knowledge/expertise

- Conditions that will facilitate integration
  - Harmonised approaches and methods (incl. terminology)
  - Efficient channels for communication
  - A framework for collaboration
“The Scientific Committee shall be responsible for .... harmonisation of working methods.”
General Food Law, regulation 178/2002

- Scientific Opinion of the Scientific Committee on Existing approaches incorporating replacement, reduction and refinement of animal testing: applicability in food and feed risk assessment (2009)
- A Working Group of the Scientific Committee exploring options for providing preliminary advice about possible human health risks based on the concept of Thresholds of Toxicological Concern (also with support from PPR and CEF units)
- The PPR Panel has outsourced exploratory activities and is evaluating the toxicological relevance of metabolites and degradates of pesticide active substances in dietary risk assessment
Potential for use of non-test methods

- Legal framework (varies from area to area in EFSA’s assessment work)
- Integrated testing and risk assessment strategies may be applied
  - Exposure-driven, for substances with very low exposure
  - Chemical structure-driven, e.g. enzymes in animal feed, nutritional substances, and certain additives
- Tiered approach, *in silico* → *in vitro* → *in vivo*, e.g. food contact materials
- Prediction of ADME properties, e.g. flavourings
Current use of QSARs and read-across

Read-across
- Food contact materials
- Flavourings
- Feed additives

QSARs
- Plant protection products
  - Metabolites
  - Impurities
- Food contact materials
- Flavourings

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Some general limitations to use of QSARs

- **Required accuracy, use of model predictions**

- **Type of endpoint(s)**
  - Single mode of action (e.g., mutagenicity and baseline toxicity)

  or

  - Complex endpoints (e.g., repeated-dose, reproductive and developmental toxicity)

- **Availability of models (software) and expertise**
Example 1 – read-across

4-Methylbenzophenone

- Found in breakfast cereals
  - Germany and Belgium, Feb. 2009
  - Migration from printing inks
- Commission asked EFSA if the substance would be covered by the TDI for
  - Benzophenone
  - Hydroxybenzophenone
- EFSA statement 4 March 2009
  - EFSA Journal 243, 1-19
- CEF Panel opinion 14 May 2009
  - EFSA Journal 1104, 1-30

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Example 1 – read-across

4-Methylbenzophenone

- Little information available in the literature
- Lack of structural alerts for genetic toxicity
- Toxicological studies on a “similar compound”, benzophenone used instead
- Further justification
  - Assumed similar metabolism to benzophenone,
  - with additional oxidation of the methyl group to the carboxylic acid
Example 1 – read-across

4-Methylbenzophenone

- Inclusion into the group-TDI is not justified
- Margin of Exposure (MoE) approach is proposed
  - $BMDL_{10} \colon 3.1 \text{ mg/kg/day for benzophenone}$
- Uncertainty factors
  - Inter- and intraspecies differences 100, derived TDI $0.03 \text{ mg/kg/day}$
  - Read-across, an additional factor 2
- A highly conservative exposure scenario
  - Intake for children $15.2 \mu \text{g/kg/day}$
  - MoE $= 3.1 \times 1000 / 15.2 = 204 (>200)$
Example 2 – QSAR

- Genotoxicity of flavourings
- Flavouring Group Evaluation 210, subgroup 2.4*
  - 12 alpha,beta-unsaturated alicyclic ketones
  - 1 precursor for such ketones
  - Structural alert for genotoxicity
- QSAR predictions of mutagenicity with five models
  - ISS local model
  - MultiCASE x 4

* Many compounds have been analysed in a similar way.
## TABLE 3: (Q)SAR PREDICTIONS ON MUTAGENICITY IN FIVE MODELS FOR TWELVE KETONES FROM SUBGROUP 2.4

<table>
<thead>
<tr>
<th>Substance</th>
<th>Structural formula</th>
<th>FTMA no CoE no CAS no</th>
<th>ISS Local Model Ames Test TA100</th>
<th>MultiCASE Ames test</th>
<th>MultiCASE Mouse lymphoma test</th>
<th>MultiCASE Chromosomal aberration test in CHO</th>
<th>MultiCASE Chromosomal aberration test in CHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha-Iohone</td>
<td><img src="image" alt="alpha-Iohone" /></td>
<td>2504 141 127-41-3</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>EQU</td>
</tr>
<tr>
<td>Methyl-alpha-Iohone</td>
<td><img src="image" alt="Methyl-alpha-Iohone" /></td>
<td>2711 144 7779-30-8</td>
<td>NEG</td>
<td>NEG</td>
<td>OD</td>
<td>NEG</td>
<td>EQU</td>
</tr>
<tr>
<td>4-(2,5,6-Triazinyl-2-cyclohexenyl)-3-buten-2-one</td>
<td><img src="image" alt="4-(2,5,6-Triazinyl-2-cyclohexenyl)-3-buten-2-one" /></td>
<td>2297 145 76-60-6</td>
<td>NEG</td>
<td>NEG</td>
<td>OD</td>
<td>NEG</td>
<td>EQU</td>
</tr>
<tr>
<td>alpha-Iohenyl-isoureas</td>
<td><img src="image" alt="alpha-Iohenyl-isoureas" /></td>
<td>2714 160 127-51-5</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>EQU</td>
</tr>
<tr>
<td>Isopropyl alpha-Iohone</td>
<td><img src="image" alt="Isopropyl alpha-Iohone" /></td>
<td>2033 2040 79-78-7</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>EQU</td>
</tr>
<tr>
<td>Methyl-delta-Iohone</td>
<td><img src="image" alt="Methyl-delta-Iohone" /></td>
<td>2713 11852 7784-93-7</td>
<td>NEG</td>
<td>NEG</td>
<td>OD</td>
<td>OD</td>
<td>EQU</td>
</tr>
<tr>
<td>gamma-Iohone</td>
<td><img src="image" alt="gamma-Iohone" /></td>
<td>3175 1 79-76-5</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>EQU</td>
</tr>
</tbody>
</table>
### Example 2 – QSAR

#### Table: QSAR Data for Different Substances

<table>
<thead>
<tr>
<th>EU-ES JECFA no</th>
<th>Sub-group</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no CoE no CAS no</th>
<th>I55 Local Model Ames Test TA100</th>
<th>MultiCASE Ames test</th>
<th>MultiCASE Mouse lymphoma test</th>
<th>MultiCASE Chromosomal aberration test in CHO</th>
<th>MultiCASE Chromosomal aberration test in CHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>07.120 366</td>
<td>2.4</td>
<td>delta-Damascone</td>
<td><img src="image" alt="Structure" /></td>
<td>3622</td>
<td>IDT</td>
<td>NEQ</td>
<td>NEQ</td>
<td>NEQ</td>
<td>NEQ</td>
</tr>
<tr>
<td>07.124 365</td>
<td>2.4</td>
<td>alpha-Damascone</td>
<td><img src="image" alt="Structure" /></td>
<td>3619 31653 41652-87-5</td>
<td>NGT</td>
<td>NEQ</td>
<td>OD</td>
<td>NGT</td>
<td>OD</td>
</tr>
<tr>
<td>07.291</td>
<td>2.4</td>
<td>alpha-Damascone</td>
<td><img src="image" alt="Structure" /></td>
<td>55644-61-4</td>
<td>NEQ</td>
<td>NEQ</td>
<td>OD</td>
<td>NEQ</td>
<td>OD</td>
</tr>
<tr>
<td>07.170</td>
<td>2.4</td>
<td>beta-Icosene epoxide</td>
<td><img src="image" alt="Structure" /></td>
<td>11202 12167-87-4</td>
<td>NYA</td>
<td>NEQ</td>
<td>OD</td>
<td>OD</td>
<td>OD</td>
</tr>
<tr>
<td>07.228</td>
<td>2.4</td>
<td>1-(3-4,6-Dimethyl-2-cyclohexan-1-yl)tetra-2-en-1-ene</td>
<td><img src="image" alt="Structure" /></td>
<td>24720-09-0</td>
<td>NYA</td>
<td>NEQ</td>
<td>NEQ</td>
<td>NEQ</td>
<td>OD</td>
</tr>
</tbody>
</table>

**Columns:***
- **2.4:** alpha, beta-unsaturated aliphatic ketones.
- **55 Local Model on aldehydes and ketones, Ames TA100 (NEG: Negative; POS: Positive; OD: Out of domain; N/A: not yet assessed).**
- **MultiCASE Ames test (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).**
- **MultiCASE mouse lymphoma test (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).**
- **MultiCASE Chromosomal aberration test in CHO (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).**
- **MultiCASE Chromosomal aberration test in CHL (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).**
- **OD: out of applicability domain. Not matching the range of conditions where a reliable prediction can be obtained in this model. These conditions may be physicochemical, structural, biological, etc.
Example 2 – QSAR

• **QSAR predictions**
  – Negative results for gene mutations, but half of substance predictions out of domain for Mouse lymphoma assay
  – Diverging for chromosomal aberrations, negative for one cell type and equivocal for another

• **Genotoxicity studies**
  – Very limited *in vitro*, data do not indicate concern
  – One *in vivo* test (methyl-alpha-ionone), with limited validity produced a negative result

• **Conclusion**
  – The genotoxic potential can not be ruled out based on the data available
  – Additional data on substances representative for this subgroup should be provided
The TTC approach

- Structure-based thresholds for low-exposure chemicals
- Used for flavouring substances
- Possible to apply in other areas of risk assessment of food and feed?
  - Explored by SC Working Group on TTC
- Evaluation of relevance and reliability
  - Discriminative power of available databases
  - Range and number of chemicals
  - Routes of exposure and range of reported effects
  - Possibility to assess human exposure levels
- Recent Art. 36 report (to PPR Panel)
  - Applicability in the dietary risk assessment of metabolites, degradation and reaction products of pesticides
The TTC pesticide project

- Carried out by the Chemicals Regulation Directorate, UK (report 2009)
- Selection of scheme of Kroes et al. (2004)
- Validated against the ADI of 100 active substances, only slight changes
- Case study for 15 pesticides, 79 metabolites
  - 63 below their respective TTC
  - 16 require further consideration
- Some issues
  - QSAR predictions (genotoxicity) did not correlate well for active substances
  - Exposure prediction uncertainties
  - How to deal with acute exposure
### The TTC selection scheme

<table>
<thead>
<tr>
<th>Classification</th>
<th>TTC threshold (µg/person/d)</th>
<th>TTC threshold (µg/kg bw/d)</th>
<th>Structural features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cramer class I</td>
<td>1800</td>
<td>30</td>
<td>Simple structure + Metabolism</td>
</tr>
<tr>
<td>Cramer class II</td>
<td>540</td>
<td>9</td>
<td>Less innocuous than class I</td>
</tr>
<tr>
<td>Cramer class III</td>
<td>90</td>
<td>1.5</td>
<td>Suggestive of significant toxicity – Functional groups</td>
</tr>
<tr>
<td>Cramer class III + Neurotoxicity</td>
<td>18</td>
<td>0.3</td>
<td>Parent compound neurotoxic – Identification with QSAR</td>
</tr>
<tr>
<td>Cramer class III + Genotoxicity</td>
<td>0.15</td>
<td>0.0025</td>
<td>Parent compound genotoxic - Identification with QSAR</td>
</tr>
</tbody>
</table>
The TTC applicability project

- Carried out by the S-IN Soluzioni Informatiche, Vicenza, Italy (report expected in Jan/Feb 2011)
- Recovery of the most widely used TTC datasets
  - Carcinogen Potency Database
  - Munro dataset (non-cancer endpoints)
- Study how the TTC scheme can be improved by incorporating physical-chemical data
  - Characterization of chemical space by structural molecular descriptors and physical-chemical properties
  - Statistical analysis (e.g., PCA, clustering, multivariate calibration)
- Database development
QSARs - current state*

- **QSAR use by regulatory authorities and advisory organisations**
  - Majority does not use routinely, lack in-house expertise
  - Support wider use and request guidance

- **QSARs of potential use**
  - Genotoxicity and carcinogenicity (few organ toxicity)
  - Good identifiers for mutagens (evaluated with 700 chemicals), pairwise sensitivity ~90% (10% false neg.)

- **Some identified needs**
  - Policy decisions on requirements, applicability of software
  - Criteria, guidance and training

*Applicability of QSAR analysis to the evaluation of the toxicological relevance of metabolites and degradates of pesticide active substances for dietary risk assessment. JRC scientific report submitted to EFSA in May 2010 (question no EFSA-Q-2009-01076).
The future for *in silico* approaches

- **EFSA is committed to a proactive animal welfare approach**
  - This implies not only to minimize the use of experimental animals and any suffering, but also to work towards their replacement
- **QSARs and read-across is used regularly by EFSA, but still in a limited set of applications**
  - Several development projects have recently been completed or is near completion
  - Tiered risk assessment approaches are discussed, e.g., in the area of endocrine active substances
- **What will be the future for *in silico* methods?**
  - Depends on how the identified needs are addressed
Uncertainty – the challenge

• What validation will be required for acceptance?
• How do we assess and express the uncertainty?
  – Qualitatively
  – Quantitatively
• How will we use the QSAR predictions?
  – Weight-of-evidence
  – Replacement of testing
To achieve method integration

• **Terminology**
• **Data requirements**
  – Testing strategies
  – Review procedures
  – Default assumptions
• **Data analysis**
  – Statistics
  – Computational approaches
  – Characterisation of uncertainty
• **Work process**
  – Transparency
  – Tiered approach
  – Fit for the purpose