Safety assessment of food contact materials

Use of the Threshold of Toxicological Concern principle

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Outline

- Difficulties in safety assessment of food packaging materials
- Threshold of Toxicological Concern (TTC) principle
- Innovative approach based on the TTC principle
- Future challenges in the development of this approach
What do we know of chemical food safety?

- Our food is estimated to contain at least hundreds of thousands of different substances, that are either natural, chemical, or present due to processing, contamination or migration from packaging.

  ![Typical chromatogram for foods visualising the substances present in a specific food product](image)

- The large majority of components present in a food matrix is unidentified and little or nothing is known about their toxicological properties.
What do we know of chemical food safety?

- Adverse effects of chemicals in food often become manifest only after many years.

- Non FCM Example: acrylamide
Safety assessment is a bottleneck for innovation

- To guarantee safe food/food packaging for future generations there is a need for more rapid development and introduction of innovations in food production
  
  e.g. new sources, use of by-products, new processing techniques

- The safety assessment of such novel products is expensive and time- and animal-consuming as each individual substance should be assessed based on toxicological information

- Legislation on new products is strict (e.g. EU regulation for Novel Foods and Food contact materials)

Exposure-based approaches like the Threshold of Toxicological Concern principle are helpful for a more efficient safety assessment
Threshold of Toxicological Concern (1)

- The Threshold of Toxicological Concern (TTC) is a pragmatic risk assessment tool that is based on the principle of establishing an exposure threshold value for all substances below which there is a very low probability of an appreciable risk for humans (Kroes, R. et al., 2004)

- TTC has been developed to assess safety of substances for which structural information is available, but toxicological information is lacking
Threshold of Toxicological Concern (2)

- Based on a large database containing chronic toxicity and carcinogenicity data of about 600 chemicals

- Three structural classes of chemicals (Cramer et al. 1978)
  - CLASS I = simple structures efficiently metabolized to innocuous products; anticipated low order of oral toxicity
  - CLASS II = intermediate structures (less innocuous than substances in Class I, but no positive indication of toxic potential)
  - CLASS III = complex structures; metabolism to reactive products suggestive of potential toxicity

- Threshold based on 5th percentile of No Observed Effect Levels (NOELs) per class
TTC – decision tree

- Excluded substances
  - Aflatoxin-, azoxy- and nitroso-like substances
  - Proteins
  - Non-essential metals
  - Dioxin-like substances

- Structural alerts for genotoxicity => 0.15 µg/person/day

- Organophosphate or carbamate => 18 µg/person/day

- Cramer class III (most substances) => 90 µg/person/day

- Cramer class II => 540 µg/person/day

  EFSA: Cramer class III threshold is applicable

- Cramer class I => 1800 µg/person/day
TTC is step forward, but

What to do with the assessment of substances which cannot be identified in a (complex) food matrix, like Non-Intentionally Added Substances (NIAS) in FCM?

Is there a more pragmatic approach possible to assess safety of a complex food matrix containing many substances?

TNO Complex Matrix Safety Assessment Strategy
Identify & quantify all components
Hazard & safety assessment for each individual component
Unidentified substances cannot be assessed

Focus on full identification

- Identify & quantify all components
- Hazard & safety assessment for each individual component
- Unidentified substances cannot be assessed

Focus on toxicological relevance

- Targeted analysis for certain groups of (highly) potent components
- Exclude genotoxicity
- Identification and safety assessment only for substances above exposure threshold
CoMSAS

- Exposure driven safety assessment
- Step-wise strategy combining analytical techniques with the TTC concept
- Exposure threshold and strategy is based on the TTC decision tree (Kroes et al 2004) updated according to latest insights (e.g. Munro et al, 2008 and EFSA, 2012)
STEP 1
Translate response into intake and identify peaks corresponding with intakes of more than exposure threshold

Rennen et al. 2011

STEP 2, exclude:
- proteins (or assess safety)
- non-essential/heavy metals
- metal containing compounds
- dioxin-like chemicals
- high potent genotoxic compounds
- Organophosphates/carbamates

STEP 3, exclude:
(structural alerts for) genotoxicity

STEP 4
Identify and assess compounds with intakes > exposure threshold and non-excluded compounds

STEP 5, assess allergenicity

1 based on Cramer class III
Step 1: General analytical screening

<table>
<thead>
<tr>
<th>Combination of techniques covering broad spectrum of substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Volatile substances                                      Headspace/SPME GC-MS</td>
</tr>
<tr>
<td>• Semi-volatile subst.                                      GC-FID/MS</td>
</tr>
<tr>
<td>Medium polar/apolar subst.</td>
</tr>
<tr>
<td>• Non/semi-volatile subst.                                  Derivatisation* GC-FID/MS</td>
</tr>
<tr>
<td>Small polar/medium polar subst.</td>
</tr>
<tr>
<td>• Non volatile subst.                                       LC-UV/light scattering/MS</td>
</tr>
<tr>
<td>Polar – apolar subst.</td>
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</tbody>
</table>

*silylation makes non-volatile substances more volatile
Step 1: Conversion to estimated intake/ feasibility CoMSAS

- Estimated intake per ‘peak’: using estimated concentration of detected substances and food consumption data of the total food product

- Based on the ratio of peaks above and below the exposure threshold of 90 µg/day decide whether CoMSAS is an efficient approach for this case

  - **Note**: majority of substances exceeding intake of 90 µg/day are the constituents of the food matrix which are known/ intended to be present (like sugars, nutrients, water etc)
Step 2: exclude known high toxic compounds and other TTC excluded classes

<table>
<thead>
<tr>
<th>Class of substances</th>
<th>Analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>LC-MS methods</td>
</tr>
<tr>
<td>Azoxy substances</td>
<td>Targeted analysis</td>
</tr>
<tr>
<td>N-nitroso substances</td>
<td>LC, GC, Thermal Energy Analyser</td>
</tr>
</tbody>
</table>

Exclusion based on available information, expert judgment and/or targeted analysis

| Non-essential metals         | Inductively coupled plasma-mass spectrometry (IPC-MS)  |
| High MW substances           | Size exclusion chromatography and LC-MS               |
| Proteins                     | LC-MS/ ELISA                                          |
| Organophosphates/ carbamates | Nitrogen/phosphorous detector (NPD) for GC and LC with orbitrap or FT-MS |
Step 3: Exclude (structural alerts for) genotoxicity

Chemical analysis
Excluding genotoxicity by chemical analysis very difficult (~28 structural alerts).

Bioassays
Conventional assays
AMES, MLA, CA not developed for complex matrices (higher assay sensitivity required)

New developments; e.g. Bluescreen
• Luminescent assay (sensitive)
• Sensitive for gen mutations, clastogenicity and aneugenicity
• High throughput! (96 well-format)
• Assay validated for pharmaceutical formulations
• Test protocol developed for complex matrices (e.g. using extraction and fractionation techniques)
Step 4: Safety assessment of substances excluded from CoMSAS

- Concerns substances
  - Exceeding intake of 90 µg/day
  - Detected in step 2 or 3

- Determine substance specific threshold
  - Based on substance-specific toxicological data
  - TTC threshold for specific substance (if Cramer class I)
  - Legal limit values (e.g. in case of heavy metals, aflatoxins etc)
  - Toxicological data from comparable substances (in structure and mode of action)
Step 5: Assess allergenicity

- Proteins might give allergic responses in sensitive people and should therefore be evaluated
- If considered relevant screening for known allergens
- Safety assessment for the probability of an allergic response of a sensitive individual
- Eventually labelling of the food product
From theory to practice…

- CoMSAS demonstrated to be an efficient method for safety assessment of food contact materials (e.g. Non Intentionally Added Substances (NIAS)), natural food supplements and processing of herbs
- Publication for use CoMSAS in safety assessment carton food contact material in preparation (Koster et al)
- ILSI guidance on NIAS in preparation

- Currently, in collaboration with partners working on other CoMSAS democases in food
Challenges for applying TTC approach to unknowns?

Combination toxicity

- Synergistic effects only when 2 or more compounds are above effect level (not likely at low TTC exposure)
- Dose addition at low concentrations cannot be excluded
- But…
- Cumulative effect is depending on potency
- TNO has assessed the relative potency for acute and chronic effects for certain classes of substances (e.g. organophosphates, triazoles)
- Conclusion: Health relevance of possible cumulative effects at 90 µg/day is considered to be low, need for correction factor very low to absent

Leeman et al. 2013
Challenges for applying TTC approach to unknowns?

**Bio-accumulating substances**

- Log Po/w as ‘marker’ for accumulation

Three studies where no relation was found between log Po/w and NOAEL:

- Ravenzwaay (2011): 111 NOAELs from developmental rat studies (Log Po/w: -4.3 to 15; median 2.12)
- Ravenzwaay (2012): 104 NOAELs from developmental rabbit studies (Log Po/w: -13 to 15)
- Kalkhof (2012): 824 NOAELs from (28/90 day) repeated dose studies (Log Po/w: -2.76 to 7.1 [5th/95th Percentile]; median 2.36)
  - Health relevance of accumulation at low exposure???
    (polyhalogenated and metals already excluded)
Challenges for applying TTC approach to unknowns?

*Exposure threshold*
- Exposure threshold CoMSAS = Cramer class III (90 µg/day)

- TNO has assessed chronic toxicity dataset underlying Cramer class III (and II) substances to assess whether on a scientifically valid bases other thresholds can be derived for (sub)classes of Cramer class III substances

- Publication in preparation
CoMSAS

- Makes optimal use of existing toxicological information, by applying the Threshold of Toxicological Concern (TTC) concept (Kroes et al. 2004; Munro et al. 2008)

- Enables quick safety screening, e.g. for selection of raw materials, determine show stoppers during innovation, measure effect of changes in processing, assess product deviations

- Conclusions on feasibility can be drawn early in assessment process; no full analysis required

- Safety assessment possible with a running time of 5-10 days
Thank you for your attention!

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