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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

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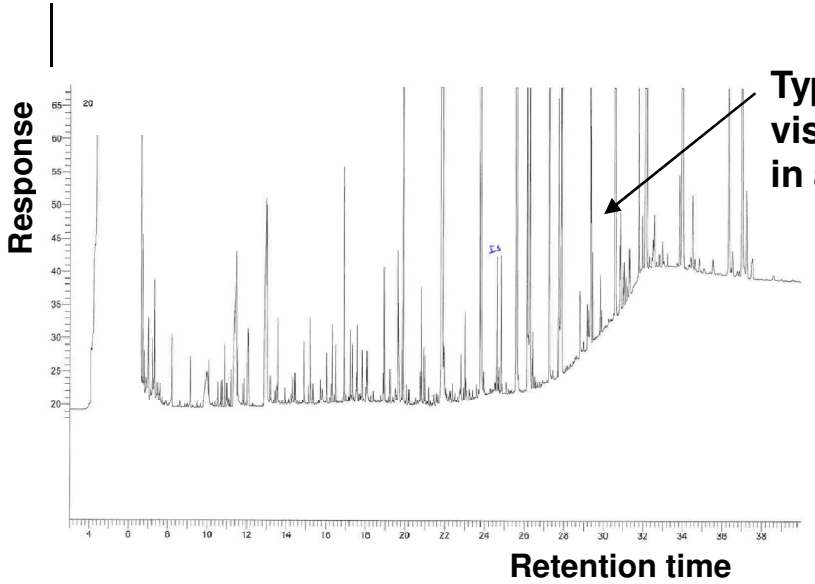
What do we know of chemical food safety?

- 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.

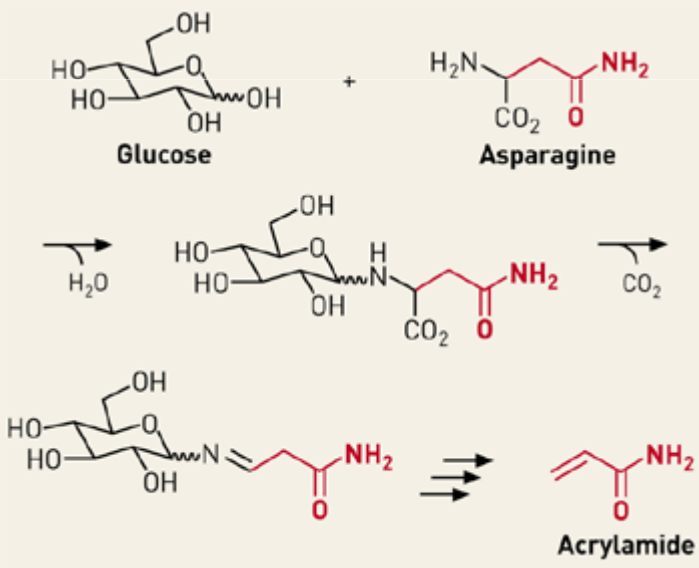


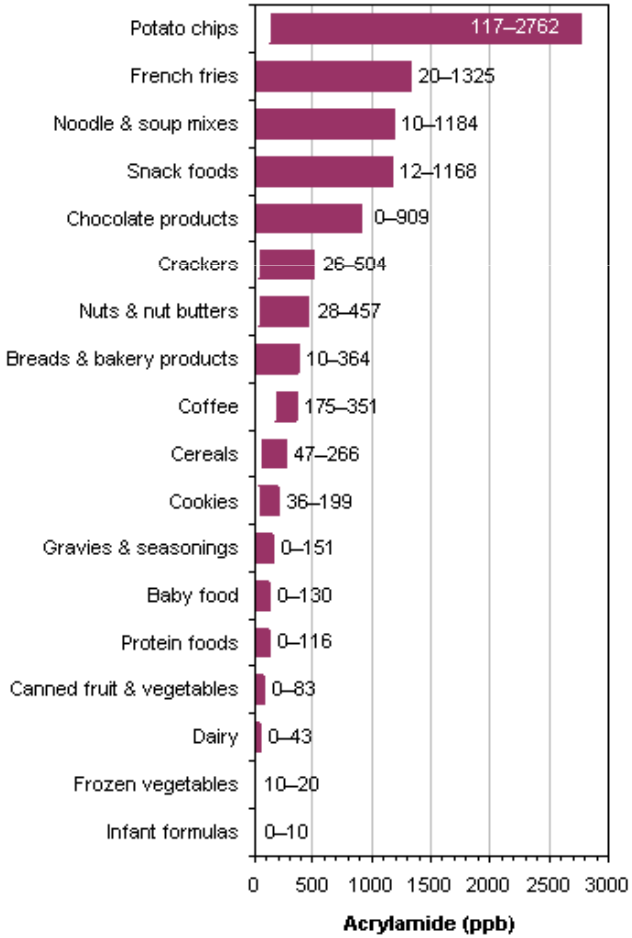
Typical chromatogram for foods visualising the substances present in a specific food product

- 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



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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


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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

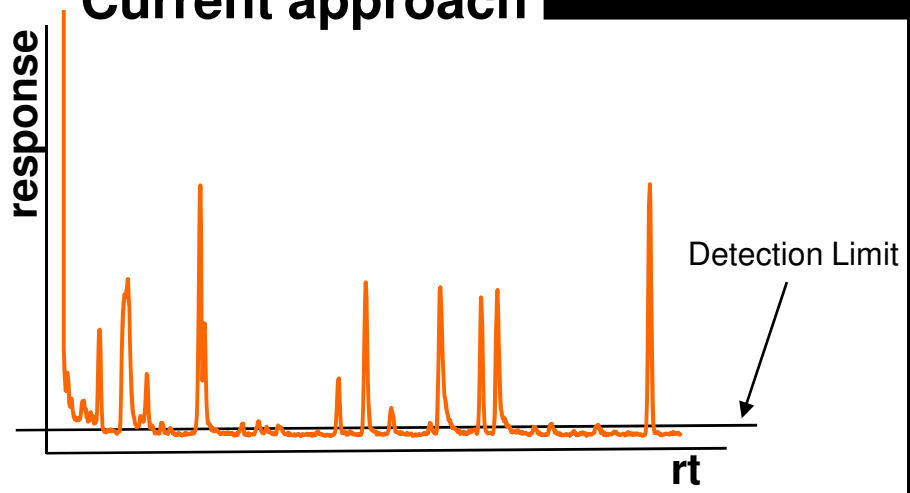

TTC is step forward, but

- 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

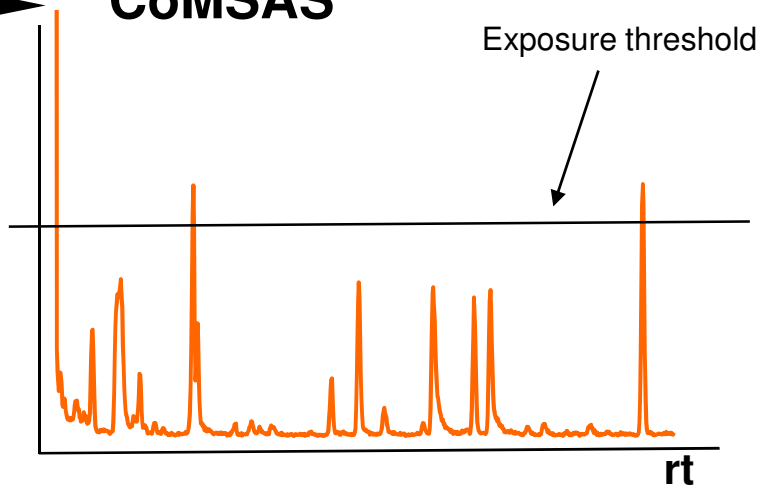
Current approach



Focus on full identification

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

CoMSAS



Focus on toxicological relevance

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

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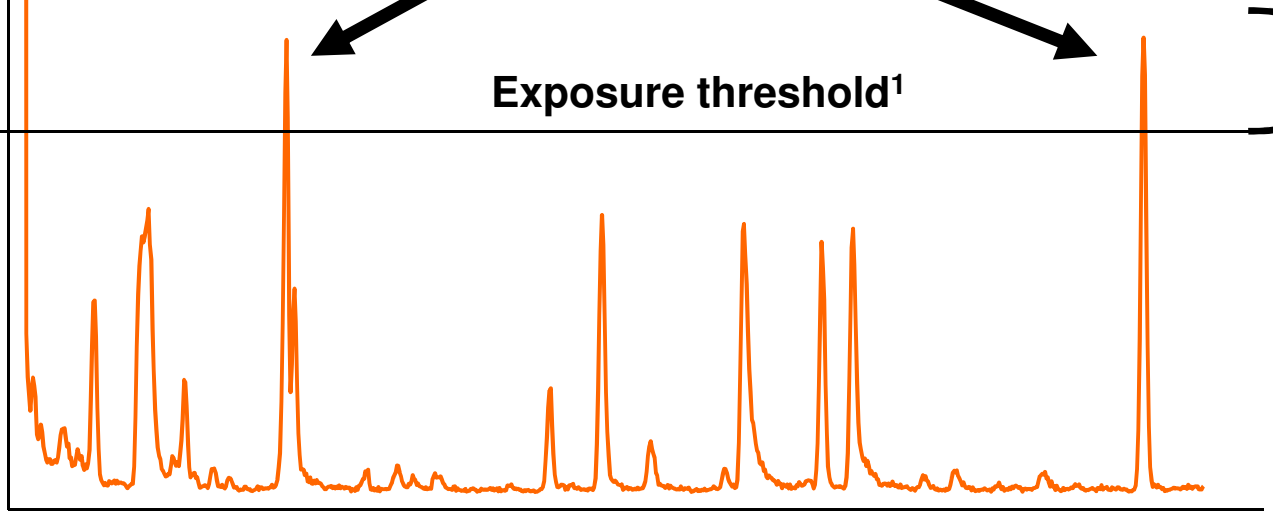
Response

Intake

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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Step 1: General analytical screening

Combination of techniques covering broad spectrum of substances

• Volatile substances	Headspace/SPME GC-MS
• Semi-volatile subst. Medium polar/apolar subst.	GC-FID/MS
• Non/semi-volatile subst. Small polar/medium polar subst.	Derivatisation* GC-FID/MS
• Non volatile subst. Polar – apolar subst.	LC-UV/light scattering/MS

*silylation makes non-volatile substances more volatile


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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

Class of substances	Analytical method
Aflatoxins	LC-MS methods
Azoxy substances	Targeted analysis
N-nitroso substances	LC, GC, Thermal Energy Analyser
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/phosporous 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)


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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)


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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


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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 [5^{th}/95^{th} 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


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