Biomarkers in risk assessment:
Application for chemical contaminants

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Head of Contaminants Unit
Overview

- Introduction
- Risk assessment of contaminants and challenges
- Biomarkers using cadmium as example
- Outlook biomarkers in risk assessment of contaminants
EFSA's mission

• EFSA is the European Union's scientific risk assessment body for food and feed safety, providing the scientific basis for risk management decisions in this area (Regulation (EC) 178/2002).

• Provide scientific and technical advice on all matters within these fields.

• Communicate all scientific outputs publicly (communication task is shared with EC/MS).

EFSA: http://www.efsa.europa.eu
Introduction

How does EFSA work?

Receipt of the request

European Commission

European Parliament

Member States

EFSA ("self mandate")

Question?

Risk Assessment
Risk Communication

10 Scientific Panels
1 Scientific Committee
17 Scientific Units

Examination
Acceptance
Allocation to CONTAM Panel
Register of questions
Introduction

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)

Since 2003 >2000 scientific outputs
Mandate of the CONTAM Panel

Deliver scientific opinions on contaminants in food and feed, associated areas and undesirable substances such as natural toxicants, mycotoxins and residues of non authorised substances not covered by another Panel.
Risk assessment - contaminants

HAZARD IDENTIFICATION

HAZARD CHARACTERISATION

Toxicokinetic (ADME), acute/sub/chronic toxicity, human data, genotoxicity, mode/mechanism of action, dose-response for critical effect, derivation of a health based guidance value (ARfD, TDI)

EXPOSURE ASSESSMENT

Occurrence in food × Food consumption

dietary EXPOSURE in EU

Relevant food groups, adults and specific groups of the population,

RISK CHARACTERIZATION

Relating exposure to health-based guidance value or Margin of Exposure

Vulnerable groups (children – high level consumers)
Risk assessment - contaminants: challenges

• The CONTAM Panel deals with general risk assessments and relies on scientific information which is in the public domain or being provided to the CONTAM Panel.

• The CONTAM Panel has to overcome challenges in the risk assessment such as incomplete toxicological databases and time-consuming data collection and data analysis.
EFSA calls for occurrence data

Occurrence data to be submitted to EFSA in standardised format:
• electronic format (XML)
• Excel reporting format

EFSA Comprehensive European Food Consumption Database:

- Data for adults from 22 EU Member States
- Data for children from 13 EU Member States (EXPOCHI article 36 project)
- The most recent data within the country
- The most complete and detailed data currently available in EU

Pan-European data “EU Menu”:

- Pilot project started in 2011 first data due by Dec. 2011
Human exposure assessment

Chemical Occurrence ➔ Exposure Assessment ➔ Food consumption

1. Hazard Identification
2. Hazard Characterisation
3. Exposure Assessment
4. Risk Characterisation
Exposure assessment for different population groups

Mean consumers
Vegetarians, diabetics, ...

High consumers

Small children

Pregnant women

Infants

Elderly
Three classes of biomarkers are identified (WHO 1993):

- "biomarker of exposure": an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism;

- "biomarker of effect": a measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease;

- "biomarker of susceptibility" - an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance".
CONTAM Panel: 87 scientific outputs since 2003

<table>
<thead>
<tr>
<th>FOOD</th>
<th>44</th>
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<tbody>
<tr>
<td>Metals</td>
<td>6</td>
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<tr>
<td>Mycotoxins</td>
<td>3</td>
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<tr>
<td>Persistent organic pollutants</td>
<td>5</td>
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<td>Marine biotoxins</td>
<td>13</td>
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<tr>
<td>Food processing</td>
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<td>Other</td>
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<th>FEED</th>
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<tr>
<td>Metals</td>
<td>4</td>
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<tr>
<td>Mycotoxins</td>
<td>5</td>
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<td>Persistent organic pollutants</td>
<td>10</td>
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<td>Plant toxicants</td>
<td>9</td>
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<td>Coccidiostats</td>
<td>11</td>
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<tr>
<td>Others</td>
<td>4</td>
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</table>
## Use of biomarkers in risk assessments by the CONTAM Panel

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Biomarker type</th>
<th>Marker</th>
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<tbody>
<tr>
<td>Lead (EFSA, 2010)</td>
<td>Exposure</td>
<td>Lead in blood</td>
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<tr>
<td>Cadmium (EFSA, 2009)</td>
<td>Exposure</td>
<td>Cadmium in urine</td>
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<td>- “ - ”</td>
<td>Effect</td>
<td>Beta-2-microglobulin</td>
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<td>Arsenic (EFSA, 2009)</td>
<td>Exposure</td>
<td>Inorganic arsenic in urine/hair/nail and total arsenic in blood</td>
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<tr>
<td>Aflatoxin (EFSA, 2007)</td>
<td>Exposure</td>
<td>Aflatoxin (and metabolites) in urine</td>
</tr>
<tr>
<td>Ochratoxin A (EFSA, 2006)</td>
<td>Exposure</td>
<td>Ochratoxin A in serum/plasma/urine</td>
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<tr>
<td>Acrylamide (EFSA, 2005)</td>
<td>Exposure</td>
<td>Adducts of acrylamide and glycidamide in urine</td>
</tr>
<tr>
<td>Methylmercury (EFSA, 2004)</td>
<td>Exposure</td>
<td>Mercury in hair and blood (ratio)</td>
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</tbody>
</table>
Cadmium occurs naturally in the environment as a result of volcanic emissions and weathering of rocks.

Cadmium is released into the environment via anthropogenic activities e.g. metal production, waste incineration, burning of fossil fuels, and use of phosphate and sewage sludge fertilisers.

Cadmium is toxic to the kidney, particularly to the proximal tubular cells, and can cause renal dysfunction.

Food is the major source of exposure to cadmium for the non-smoking general population.
• Assess whether PTWI of 7 µg/kg b.w. is still appropriate

• Updated exposure assessment on cadmium
  – Exposure from food including drinking water
  – Importance of other non-dietary sources (e.g. air, cigarette smoke etc.)
  – Exposure in specific group of population (e.g. infants and children, people following specific diets) and indication of age group most exposed to cadmium

• Take into account biomonitoring data
• To confirm whether the tolerable weekly intake (TWI) for cadmium of 2.5 µg/kg body weight (b.w.) established by the CONTAM Panel in 2009 is still considered appropriate or whether any modifications are needed.
Dataset used in the cadmium assessment

- A large number of studies on health effects of cadmium exposure in humans in different countries investigated the relationship between urinary cadmium levels and tubular effects.

- Tubular damage is the earliest effect of cadmium exposure, thus the CONTAM Panel based its risk assessment on this effect.
The earliest signs of tubular toxicity are decreased tubular reabsorption of low molecular weight proteins (LMWP) and increased excretion of markers of cell shedding.
Biomarkers of effects

Tubular markers
- Urinary excretion of LMWP and some enzymes have been used to assess tubular dysfunction
- Examples for these markers: beta-2-microglobulin (B2M), retinol-binding protein, alpha-1-microglobulin, N-acetyl-beta-glucosaminidase (NAG).

Glomerular markers
- Urinary excretion of high molecular weight proteins (HMWP) and glomerular filtration rate (GFR) have been used to assess the integrity of glomerular sieve.
- Examples for these markers: Albumin, GFR.

The CONTAM Panel selected B2M in relation to tubular effects as the most useful biomarker.
The CONTAM Panel carried out a meta-analysis to evaluate the dose-response-relationship between the urinary cadmium (marker of exposure) and B2M (marker for effects).

A meta-analysis refers to the analysis of analyses, this means to the statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings.

No individual data were available, but a total of 165 matched pairs of summary data were available from 35 studies.

Number of subjects varied between 3 and 1,300 per group.
Two components used by the CONTAM Panel

- Concentration-effect modelling of epidemiological studies assessing the relationship between urinary cadmium concentration and B2M levels, a protein biomarker for renal tubular effects.

- Toxicokinetic modelling relating urinary cadmium concentrations to dietary cadmium intake.
• The CONTAM Panel used a hybrid benchmark dose approach in which the Hill model was fitted through the data to estimate an extra risk of 5% of producing a specified change in the urinary B2M level.

• The CONTAM Panel chose two cut-off points:
  - a biological cut-off of 300 µg B2M/g creatinine
  - a statistical cut-off (211 or 374 µg B2M/g creatinine)

• B2M values over 300 µg B2M/g creatinine have been associated with an age-progressive loss of renal function.
BMD and BMDL (in mg Cd/g creatinine) estimates for various cut-offs leading to extra risks of 5% in the total population, and non-occupationally exposed subjects > 50 years adjusted for Caucasian ethnicity.

<table>
<thead>
<tr>
<th>Statistical cut-off(^a) for U-beta-2-microglobulin (µg/g creatinine)</th>
<th>U-beta-2-microglobulin &gt;300 µg/g creatinine</th>
<th>U-beta-2-microglobulin &gt;1000 µg/g creatinine</th>
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<tbody>
<tr>
<td>BMD5</td>
<td>BMDL5</td>
<td>BMD5</td>
</tr>
<tr>
<td>U-Cd (µg/g creatinine) from the whole population</td>
<td>3.98</td>
<td>3.62</td>
</tr>
<tr>
<td>U-Cd (µg/g creatinine) from non-occupationally exposed subjects over 50 years</td>
<td>5.28</td>
<td>4.89</td>
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</table>

\(^a\) 211 and 374 for whole and subjects over 50 years, respectively.

In 2009 the CONTAM Panel identified a group-based BMDL\(_5\) of 4 µg cadmium / g creatinine as reference point.

In 2011 the CONTAM Panel confirmed this value.
CONTAM Panel recognized the problem of having a large within group variability in absence of individual data.

Scatter plot of data from all studies linking urinary cadmium to B2M using a different colour for each study and illustrating within group variability. Each study population is represented by an ellipse on the log scale, with log(GSD) (Figure 13 of EFSA 2009)
The BMD approach applied by the CONTAM Panel to the matched pairs of summary data accounted for most sources of variability, but not for variability in urinary cadmium concentrations within each dose group, due to the fact that group means were used.

The CONTAM Panel noted that some of the inter-individual variability may have been already accounted for in the BMD analysis, but it is not possible to determine to what extent (EFSA, 2011).

Based on the results of a simulation exercise by AMU, the CONTAM Panel concluded that the BMDLs estimated on group means were likely to be higher then if calculated with individual data ((EFSA, 2011).
Variability and uncertainty in the concentration-effect model

- Therefore an adjustment factor was calculated based on the estimated coefficient of variation of inter-individual variability of urinary cadmium concentrations within the sub-groups.

- According to WHO-IPCS (2005) this factor can be defined as
  \[ AF = \frac{\text{95th Percentile (BMD)}}{\text{Median (BMD)}} \]

- Since concentrations have been assumed to be lognormal
  \[ AF = \exp(1.64 \ln(1+CV^2)^{1/2}) \]

- For a CV of 100 %, an adjustment factor of **3.9** can be calculated to cover 95 % of the population.
In 2011 the CONTAM Panel reiterated its previous view that it was necessary to apply the adjustment factor of 3.9 to the reference point (BMDL$_5$) of 4 µg cadmium/g creatinine.

Therefore the CONTAM Panel re-confirmed the value of 1 µg cadmium/g creatinine in urine as modified reference point to be used for the derivation of the health based guidance value for cadmium.
EFSA’s CONTAM Panel used a one compartment model in which urinary cadmium concentration is proportional to dietary cadmium intake.

This model was fitted to the data from the population-based Swedish Mammography Cohort study comprising 680 never smoking Swedish women, 56-70 years age, in order to estimate dietary cadmium exposure corresponding to different urinary cadmium levels for different proportions of the population.
In order to keep 95% of the population by age 50 below a urinary concentration of 1 µg cadmium/g creatinine, the average daily dietary cadmium intake should not exceed 0.36 µg/kg b.w.

This daily intake leads to a TWI of 2.5 µg/kg b.w. for cadmium.

<table>
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<tr>
<th>Proportion of the population below 1 µg urinary cadmium/g creatinine</th>
<th>Dietary cadmium exposure</th>
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<tbody>
<tr>
<td></td>
<td>µg/kg b.w. per day</td>
</tr>
<tr>
<td>50 %</td>
<td>0.78</td>
</tr>
<tr>
<td>90 %</td>
<td>0.42</td>
</tr>
<tr>
<td>95 %</td>
<td>0.36</td>
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General

• Chemical contaminants comprise environmental contaminants (POPs, metals) but also compounds formed naturally in food and during thermal food processing.

• Biomarkers will not replace traditional approaches used in risk assessment, but are an additional tool.

• Validated biomarkers are useful in reducing uncertainty in risk assessments.

• Validated biomarkers require both laboratory and epidemiological studies and bioinformatics and advanced statistical methods.
Exposure

• Information on dietary intake (FFQ and 24 hr recall) and biomarker of exposure from the same subject is needed.

Effects

• Biomarkers are often non-specific for a certain exposure and may not necessarily be adverse themselves, but can indicate potential health impairment.
Thank you very much for your attention!