Analytical Challenges Related to the Analysis of Processing Contaminants in Foods and Impacts on Risk Assessment

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Objectives

- Highlight some key challenges in developing analytical methods for the analysis of contaminants in foods
- Discuss how these challenges can impact exposure assessments and risk characterization
- Provide specific examples of these analytical challenges by taking a look at current methods for contaminant analysis
Role of the Analytical Chemist in Risk Characterization

• Risk characterization process:

  Hazard Identification
  Chemical present in food—
  is it harmful to health?

  Exposure Assessment
  What are the exposure
  levels in the population?

  Risk Characterization
  What is the risk to health in
  an exposed population?

  Toxicity Assessment
  What are the health effects
  at different doses?

• Where does the analytical chemist come in to play in the process of risk characterization?
Challenges Related to Risk Assessment

- Toxicity assessments
  - Available data for chemical contaminant? Resources available to carry out tox assessment? Time constraints?

- What about challenges related to exposure assessments?
  - Contaminant occurrence data in foods required for exposure assessments
  - Accuracy of occurrence data vital piece of the puzzle in determining accurate exposure
  - Analytical methods for determination of contaminant levels are essential!
Analytical Challenges Related to Contaminant Analysis

Exposure Assessment Challenges (Analytical)

(1) Reliability of Methods
- Are they official? Validated?

(2) Complexity of the Analytical Method
- Availability of methods in multiple food matrices?
- Methods carried out easily/efficiently?

(3) Method LODs/LOQs
- Detection at occurrence levels?
- Chasing zero – are MDLs “too low”?
- Detection at regulatory levels?
Challenge 1: Reliability of Methods

- Official methods aren’t always necessary, but using an official method typically provides greater confidence in results.

- What if an official method is not available? Can single/multi-lab validated methods be used?

- Reliability can sometimes be called into question.
Example: Analysis of MCPDE/GE

3-MCPD (monochloropropanediol) esters

R = various fatty acids

2-MCPD esters

Glycidyl ester

Hydrolyzed upon ingestion

3-MCPD

2-MCPD

Glycidol

Group 2B carcinogen (possibly carcinogenic to humans)¹

No classification; limited tox data

Genotoxic; Group 2A carcinogen (possibly carcinogenic to humans)²

Refined oils

Primary fat source in infant formula

MCPDE/GE contaminants also present in formula

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Primary fat source in infant formula

MCPDE/GE contaminants also present in formula
Methods for MCPDE/GE in Infant Formula

- No official methods for MCPDE/GE determination in infant formula
- Some single-lab validated methods:
  - Direct detection of 3-MCPDE/GE by LC-MS/MS
  - Acidic indirect detection of free 3-MCPD/glycidol by GC-MS/MS
  - Alkaline indirect detection of free 3-MCPD/glycidol by GC-MS
Unintended Reactions During Analysis

- In some instances, methods can produce variable results:

Indirect analysis (free forms of compounds)

Some conditions can produce unwanted side reactions

Even with validated methods, confirmation of accuracy still necessary!
Reliability of Methods: When Methods Aren’t Available in Matrix of Interest

- Methods of analysis not always available for wide-ranging food matrices (particularly processed foods)

- Can determination of contaminant levels in a food ingredient accurately provide an exposure assessment for a given food?

- Analysis of ingredients/components for estimation of exposure is tricky
Analysis of Ingredients for Exposure Estimates?

- MCPDE/GE example again—no official method for the MCPDE/GE determination in infant formula—and some validated methods can produce inaccurate results.

- Can the specific oils used in the manufacture of infant formula be analyzed instead?

Refined Oils

Primary fat source in infant formula

Infant Formula

3-MCPD diester
3-MCPD monoester (sn-1)
3-MCPD monoester (sn-2)
2-MCPD diester
2-MCPD monoester
Glycidyl ester
Comparison of Results in Ingredients and Finished Product

Bound glycidol determination in each product

Several official methods of analysis that provide reliable/trusted results:
- AOCS Cd 29a-13 / ISO 18363-3
- AOCS Cd 29b-13 / ISO 18363-2
- AOCS Cd 29f-2021 / ISO 18363-4

No official methods of analysis; some methods can produce unreliable results for infant formula

Comparison of bound glycidol levels in oil blend used in infant formula and final infant formula product

Bound Glycidol (µg/g fat)

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Analysis of Ingredients for Exposure Estimates in Food Challenging

- Glycidol analysis of ingredients and final product produce different results
- Processing of infant formula results in degradation/conversion of contaminant of interest
- In this example, *analysis of ingredient(s) would not be sufficient for infant formula exposure assessment*
Challenge 2: Complexity of Methods

- Contaminant detection can involve the analysis of a large number of related compounds
- This can make the analytical method inherently more difficult to carry out
- Some methods require high levels of expertise to carry out—can be a challenge for some laboratories
Example: Analysis of MOSH/MOAH

**MOSH**

**Mineral Oil Saturated (Aliphatic or Cyclic) Hydrocarbons**

**Examples:**
- octane
- 2-methyl-heptane
- iso-octane
- naphthenes

**Toxicity:**
- Can bio-accumulate in the body
- Possible microgranuloma formation

**MOAH**

**Mineral Oil Aromatic Hydrocarbons**

**Examples:**
- aromatics

**Toxicity:**
- Some compounds suspected carcinogenic and/or genotoxic
- Specifically, 3-7 ring PACs
Potential sources of contamination:

- Migration from food contact materials (particularly recycled materials)
- Lubricating oils from processing machinery
- Certain food additives (such as separating agents, coating agents, etc.)

Analysis of potentially 100s of compounds!
Number of compounds makes analysis inherently difficult:

- LC-GC-FID for routine analysis
- Integration “hump” gives total MOSH/MOAH levels
- Interpretation of chromatograms requires high level of expertise

Further Challenges: Separation of Other Compounds from Analytes of Interest

Further complexities:

• Biogenic substances difficult to separate from MOAH

• Important consideration for regulatory limits—e.g., draft 2 ppm EU limit for MOAH

• More sophisticated methods of analysis necessary—e.g., GCxGC-TOF/MS

Complexity of analyses makes obtaining accurate results challenging!

https://info.leco.com/blog/using-gcxgc-to-see-mosh-and-moah
Challenge 3: Appropriate Method
LODs/LOQs

- Limit of detection (LOD)/limit of quantitation (LOQ)
- LODs/LOQs important—appropriate for measuring relevant contaminant levels
- Reliable methods may be available, but are the LODs/LOQs low enough to detect a contaminant?
- Detection of contaminants at levels of toxicological importance? Meet regulatory limits?
Challenge of MCPDE/GE Detection at Relevant Contaminant Levels

- Some method LOQs insufficient for determination of contaminant occurrence:

*Updated occurrence of 3-monochloropropene-1,2-diol esters (3-MCPD) and glycidyl esters in infant formulas purchased in the United States between 2017 and 2019*

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>n</th>
<th>Bound 3-MCPD (µg g⁻¹ powder)</th>
<th>Bound Glycidol (µg g⁻¹ powder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>45</td>
<td>0.12, 0.050–0.67</td>
<td>0.019, &lt;LOQ-0.089</td>
</tr>
<tr>
<td>B</td>
<td>125</td>
<td>0.070, 0.013–0.95</td>
<td>0.033, &lt;LOQ-0.20</td>
</tr>
<tr>
<td>C</td>
<td>27</td>
<td>0.035, 0.018–0.12</td>
<td>0.019, &lt;LOQ-0.057</td>
</tr>
<tr>
<td>D</td>
<td>25</td>
<td>0.63, 0.36–0.81</td>
<td>0.22, 0.10–0.37</td>
</tr>
</tbody>
</table>

- Bound MCPD LOQ: ~1.5 µg g⁻¹
- Bound Glycidol LOQ: ~10 µg g⁻¹
Challenges of Contaminant Detection at Regulatory Levels

Example: MOSH/MOAH

EU Reference Method for MOSH/MOAH Analysis

**BS EN 16995:2017**

Foodstuffs. Vegetable oils and foodstuff on basis of vegetable oils. Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis

According to the results of the interlaboratory studies, the method has been proven suitable for MOSH- and MOAH mass concentrations each above 10 mg/kg. In case of suspected interferences from natural sources, the mineral origin of the MOSH and MOAH fraction can be verified by examination of the pattern by GC-MS.

However

Draft EU levels for max. sum concentrations of MOAH:
- 0.5 mg/kg for dry foods with a low fat/oil content (≤ 4% fat/oil)
- 1 mg/kg for foods with a higher fat/oil content (> 4% fat/oil)
- 2 mg/kg for fats/oils

Reference method suitability above 10 ppm

Draft regulatory limit lower than method LOQ!

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Considerations for Setting Limits

- Toxicity Assessment
- Regulatory Limits
- Capability of Methods (LODs/LOQs)
A Final Note—“Chasing Zero”

• At times, there is a push to continually decrease MDLs—even when these levels are no longer of toxicological relevance to human health

• Not only complicated from an analytical standpoint, but can have negative impacts on how the public perceives the presence of a contaminant (even if there is no risk)
Summary

- Risk characterization a complicated process
- Challenges related to toxicity assessment not the only considerations
- Analytical methods must be available, reliable, and applicable in order to produce accurate occurrence data—which are essential for accurate exposure assessments
- Important to consider the analytical methodology for accurate risk characterization!
References


Questions

US FDA, Center for Food Safety and Applied Nutrition (CFSAN), College Park, MD