Extractable & Leachable Study with Integrity Testing & Toxicological Assessment

Dr. Heike Schmidt-Eisenlohr
Agenda

Project Planning
- Collection of Supplier Information
- Risk Assessment

Experimental Phase I
- Integrity Testing
- Extractable Study

Evaluation & Tox Assessment
- Data evaluation & Search for available toxicity data
- Classification in appropriate Cramer Class

Experimental Phase II
- Method development and Matrix specific validation
- Leachable Study
General Introduction

Extractables

Volatile

Leachables
Is the material uncritical for the intended use?

It depends!

Risk based approach

- Mode of application: ophtalmic, parenteral, inhalor or oral
- Daily dose: one or several, time of application
- Material: glass or plastic, labels & printing ink
- Shelf life: time of possible interaction
- Patient: adults or infants
- Country: high or low regulated
**Project Preparation**

- **Supplier Info**
  - Material
  - Additives
  - Ranking

- **Risk Assessment**
  - Evaluation of the process
  - Risk (ICH Q9)
  - Select material
  - Define AET

**Experimental Phase I**

- **Extractable Study**
  - Execute protocol
  - Identify extractables
  - Evaluate results
  - Deduce range for possible leaching

**Evaluation**

- **Tox Assessment**
  - CAS identifier
  - Search for available toxicity data
  - Classification in appropriate Cramer Class
  - Define specifications for leachable study if needed

**Experimental Phase II (if needed)**

- **Leachable Study**
  - Develop methods
  - Validate the developed methods
  - Perform a leachable study
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### Supplier Information – Declaration of Conformance

<table>
<thead>
<tr>
<th>Ref. No.</th>
<th>CAS-No.</th>
<th>Name</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>46640</td>
<td></td>
<td>BHT</td>
<td>SML = 3mg/kg</td>
</tr>
<tr>
<td>19243/21640</td>
<td></td>
<td>2-Methyl-1,3-butadien</td>
<td>SML ND (1 mg/kg in final product)</td>
</tr>
<tr>
<td>Dual Use Additive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95859</td>
<td>E905</td>
<td>Wachs, high MW</td>
<td>-</td>
</tr>
<tr>
<td>46640</td>
<td>E320</td>
<td>BHT</td>
<td>-</td>
</tr>
</tbody>
</table>

**Substances with specific migration limit should be addressed.**
Risk Assessment – Product Contact?

Which plastic parts need to be extracted?
- Reduction of workload
- Reduction of costs
- Avoid generation of data which are not required
Risk-based approach: AET definition – TTC concept

For each extractable from a device component, an Analytical Evaluation Threshold (AET) which is determined by consideration of the Safety Concern Threshold (SCT) and the specific drug product configuration needs to be determined as follows, e.g.

\[
AET = \frac{SCT \times D_t}{D_d \times m} = \frac{0.15 \frac{\mu g}{day} \times 8 \frac{doses}{component}}{4 \frac{doses}{day} \times \frac{mass}{component}} = 0.3 \text{ ppm}
\]

\(D_t\) = total labelled doses (8 per container)

\(D_d\) = doses per day (4)

\(m\) = mass of component in g

SCT = Safety Concern Threshold may be replaced by a TTC of 1.5

TTC = use of a numerical cancer risk value (1 in 100,000) and its translation into risk-based doses (TTC) according to ICH M7(R1) as a hypothetical concept which provides an estimate of safe long-term exposures for any mutagenic compound.
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## Extractable study - Integrity Testing

<table>
<thead>
<tr>
<th>Time point</th>
<th>Differences in visible and tactile examination against a fresh sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 Days</td>
<td>slightly softer</td>
</tr>
<tr>
<td>14 Days</td>
<td>slightly softer and more flexible</td>
</tr>
<tr>
<td>30 Days</td>
<td>slightly softer and more flexible</td>
</tr>
<tr>
<td>60 Days</td>
<td>softer and more flexible; yellow coloring comes off when drying the tube with a paper towel</td>
</tr>
<tr>
<td>90 Days</td>
<td>softer and more flexible; yellow coloring comes off when drying the tube with a paper towel; pink color is paler</td>
</tr>
</tbody>
</table>

Material not compatible with the product
⇒ Extractable study not required,
⇒ Choice of other material!

Minor changes of the material may be acceptable
Extractable study - Extraction of the plastic parts

Harsh Solvents, e.g.
- Toluene
- N-Hexane
- Isopropanol

Evaporation & concentration

Soft Solvents, e.g. according USP <1664>
- Water at neutral pH
- Water at high pH (e.g. 9.5)
- Water at low pH (e.g. 2.5)
- Mixture of buffer and organic modifier
- Placebo
- Different durations
- Different temperatures

Extraction & concentration
Extractable study - pictures
Extractable study – Special Case

Application of a polymer in the gastrointestinal tract

Do toxicologically relevant substances leach from this polymer into the gastrointestinal tract?
Can elemental impurities of class 1+2A (As, Cd, Pb, Hg, Co, V, Ni) relevant for oral application be detected by ICP-MS?

Extraction in media simulating human colonic fluid e.g.
• fluid simulated small intestine, fasted, pH 6.5
• fluid simulated small intestine, fed, pH 5.0
• fluid simulated stomach, fasted, pH 1.6
Extractable study – Special Case

FDA Guideline "Container Closure Systems for Packaging-Human Drugs and Biologics"

- e.g. for terminal sterilized vials with PTFE coated stoppers

  autoclaving for 121°C for 1 h

  Worst-case scenario which may be relevant to address intactness of PTFE coating after terminal sterilization
Extractable study - Analysis of the extracts

**Analysis of non-volatile Extractables (NVOCs)**

- UPLC-DAD
- HPLC-MS (single quad)
- Q Orbitrap
  I. (Semi)-quantitative Analysis
  II. Screen (Fingerprint)
  III. Structure elucidation (E+L database)

**Analysis of volatile Extractables (VOCs)**

- GC-FID
- GC-MS
- HS-GC-FID
- HS-GC-MS
  I. Fingerprint
  II. Screen for peaks above AET
  III. Structure elucidation (NIST database)
Targeted analysis
Targeted analysis of volatiles via HS-GC-FID

Reference 1: Isobutene

Reference 2: 2-Methyl-1,3-Butadiene
Screen
Extractable study - LC-MS Screen – APCI (pos. and neg.)
Orbitrap; APCI + full scan; zoomed isotopes
Structure Proposal: Tri-N-octyl trimellitate
Extractable study FID – Fingerprint
Extractable study GC-MS Screen – Chromatogram
<table>
<thead>
<tr>
<th>RT 16.34 min</th>
<th>Hexanediolic acid, bis(2-Ethylhexyl) ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT 17.72 min</td>
<td>1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)</td>
</tr>
<tr>
<td>RT 20.34 min</td>
<td>Tri-N-octyl trimellitate</td>
</tr>
</tbody>
</table>
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|                 |                   |
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| Experimental Phase II  | • Method development and Matrix specific validation  
|                     | • Leachable Study |
# Data Evaluation – Overall Analysis

Data evaluation of extractables > 5-fold AET

- Large intersecting set between NVOCs and VOCS
- Response factor for GC-FID more reliable than for LC-MS
- 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) approx. 1000-fold above AET
- Tri-N-octyl trimellitate approx. 10000-fold above AET
- Toxicological assessment required!

<table>
<thead>
<tr>
<th>Compound</th>
<th>Detection Method</th>
<th>n-fold AET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated A-4</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Chlorinated degradable</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Poly-THF (m7)</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Dibutyl sebacate</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Poly THF (m8)</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Dibutyl phthalate</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl) adipate</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Isophex 1010</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Diocyl sebacate</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Tri-N-ethyl trimellitate</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Riceoilic Acid</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>LC-MS</td>
<td>n.a.</td>
</tr>
<tr>
<td>Butylated Hydroxytoluene (Pharmaceutical Additive 01 or equivalent of Plastic Additive 09 or 11)</td>
<td>GC</td>
<td>5.0</td>
</tr>
<tr>
<td>Dodecanic acid, 1-methyl ester</td>
<td>GC</td>
<td>64.7</td>
</tr>
<tr>
<td>Benzene, 1,1-di-methylethyl-3-butynyl</td>
<td>GC</td>
<td>73.0</td>
</tr>
<tr>
<td>8-Methoxycinnamic acid</td>
<td>GC</td>
<td>7.2</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>GC</td>
<td>9.6</td>
</tr>
<tr>
<td>Hexadecylidrinic acid, bis(2-ethylhexyl) ester</td>
<td>GC</td>
<td>35.1</td>
</tr>
<tr>
<td>Pentadecene</td>
<td>GC</td>
<td>24.7</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)phthalate (Pharmaceutical Additive 01)</td>
<td>GC</td>
<td>14.2</td>
</tr>
<tr>
<td>Hexamadecane, 1-hexadecene nonanal</td>
<td>GC</td>
<td>17.1</td>
</tr>
<tr>
<td>Neocisplatinum</td>
<td>GC</td>
<td>32.1</td>
</tr>
<tr>
<td>Prickly acid, methyl neopenyl ester</td>
<td>GC</td>
<td>3.5</td>
</tr>
<tr>
<td>Trimethylpentane, 1,5-dimethyl</td>
<td>GC</td>
<td>5.0</td>
</tr>
<tr>
<td>1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (Pharmaceutical Additive 01)</td>
<td>GC</td>
<td>1120.5</td>
</tr>
<tr>
<td>Occadacene</td>
<td>GC</td>
<td>50.1</td>
</tr>
<tr>
<td>2-Ethylhexyl methyl arachidate</td>
<td>GC</td>
<td>9.0</td>
</tr>
<tr>
<td>Oleic acid, 8:1 ether, dipropyl ester</td>
<td>GC</td>
<td>5.1</td>
</tr>
<tr>
<td>Nonadecane</td>
<td>GC</td>
<td>29.0</td>
</tr>
<tr>
<td>Nonylacetone</td>
<td>GC</td>
<td>21.8</td>
</tr>
<tr>
<td>Oxime, 1,2-5(1,4-di-isocyanato)bis(isocyanato)bis-</td>
<td>GC</td>
<td>56.4</td>
</tr>
<tr>
<td>Tetradecanesulfonate</td>
<td>GC</td>
<td>11.1</td>
</tr>
<tr>
<td>Tri(2-ethylhexyl) trimellitate</td>
<td>GC</td>
<td>950.0</td>
</tr>
</tbody>
</table>
Data Evaluation

- The multiplier (e.g. 5-fold AET) does not indicate a daily exposure: when multiplied with the AET units found it corresponds to the **total extractable amount of a given constituent in the total plastic part**.

- The quantity in the total plastic part finally needs to be **converted into daily exposures** – assuming that during the use of the equipment the **totality of extractables is in fact extracted (worst-case assumption)**.

- Search for **CAS identifiers** of the assessed chemicals as well as **information on toxicity in chemical databases** such as PubChem, Chemspider and Sigma-Aldrich and REACH-database.
## Definition of Cramer Classes

<table>
<thead>
<tr>
<th>Class</th>
<th>Oral TTC [µg/day*person]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance with structural alerts (suspected to be genotoxic), SCT</td>
<td>0.15</td>
</tr>
<tr>
<td>Substances without structural alerts (“non-cancer substances”), TTC or ToR</td>
<td>1.5</td>
</tr>
<tr>
<td>Organophosphates (neurotoxins), carbamates</td>
<td>18</td>
</tr>
<tr>
<td>Cramer Class III: Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups.</td>
<td>90</td>
</tr>
<tr>
<td>Cramer Class II: Substances which possess structures which are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III.</td>
<td>540</td>
</tr>
<tr>
<td>Cramer Class I: Substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity.</td>
<td>1800</td>
</tr>
</tbody>
</table>
Flow chart for a Toxicological Assessment  

Ministry of the Environment (Elsa Nielsen and John Christian Larsen: The Threshold of Toxicological Concern (TTC) concept)

1. Is the substance a non-essential metal or metal containing compound, or is it a polyhalogenated-dibenzodioxin, -dibenzo furan, or -biphenyl?  
   - NO  
   - YES

2. Are there structural alerts that raise concern for potential genotoxicity?  
   - NO
   - YES

3. Is the chemical an aflatoxin-like-, azoxy-, or N-nitroso-compound?  
   - YES
   - NO

4. Does estimated intake exceed TTC of 0.15μg/day?  
   - YES
   - NO

5. Does estimated intake exceed TTC of 1.5μg/day?  
   - YES
   - NO

6. Is the compound an organophosphate?  
   - NO
   - YES

7. Does estimated intake exceed TTC of 16μg/day?  
   - YES
   - NO

8. Is the compound in Cramer structural class III?  
   - NO
   - YES

9. Does estimated intake exceed 90μg/day?  
   - YES
   - NO

10. Is the compound in Cramer structural class II?  
    - NO
    - YES

11. Does estimated intake exceed 540μg/day?  
    - YES
    - NO

12. Does estimated intake exceed 1800μg/day?  
    - YES
    - NO

Risk assessment requires compound-specific toxicity data

Substance would not be expected to be a safety concern

Negligible risk (low probability of a lifetime cancer risk greater than 1 in 10^6 – see text)
Hexanedioic acid-bis(-2-ethylhexyl ester)

Hexanedioic acid-bis(-2-ethylhexyl ester)
CAS: 103-23-1
Mr 370,6
Syn.
Di(2-ethylhexyl) adipate
Adipic acid bis(2-ethylhexyl) ester

- Toxicity data is available through ECHA
- The compound is non-toxic under relevant conditions. It breaks down metabolically to adipid acid (non-toxic) and a short chain alcohol, where relevant toxicity is not to be expected. The substance can be classified at worst in Cramer Class I.
- Calculated exposure of 97.95 μg/day versus a Cramer I-class threshold of 1800 μg/day => flow chart => assessment: “not expected to be a safety concern”.
Tox Assessment – Tri-(2-Ethylhexyl)-trimellitate

Tri-(2-Ethylhexyl)-trimellitate
CAS: 3319-31-1 and 82643-26-3
Mr 546.8
Syn.: Tris(2-ethylhexyl) benzene-1,2,4-tricarboxylate

- Toxicity data is available through ECHA and HSDB
- ECHA defines a DNEL value of 1.13 mg/kg/day for oral long term exposure and of 225 mg/kg/day for oral short term exposure
- With the definition of a DNEL the Cramer classification does not apply.
- Exposure calculation => Differentiation by material
  a. Gastrostomy Feeding Tubes: exposure does not exceed either DNEL; maximum of 0.313 mg/day found and 1.13 mg/kg allowed for long term use=> no safety concern
  b. Nasogastric Tubes: exposure reaches 10.12 mg/day => beyond the longterm DNEL of 1.13 mg/kg for the application in children weighing less than 10 kg. In newborns the exposure may be exceeded with respect to the long-term DNEL
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Leachable Study – Method Development and Validation

Pilot Study & Method Development
→ Validation Protocol
→ Execution in the Lab
→ Evaluation of the Data (Statistical Analysis)
→ Validation Report

Needs to be done for each putative Leachable and Matrix!
Leachable Study – inverted 30 °C / 65% RH; 15 months

Minimal differences in the GC-FID fingerprint => GC-MS to look into the peaks
Leachable Study – inverted 30 °C / 65% RH; 15 months

The only difference in the GC-MS peak pattern is an API related ester which is generated by the procedure. Not present in the blank, because the blank is mere placebo in the primary packaging.
Overview Extractable / Leachable Assessment

- Extract packaging material
- Identify extractants & determine toxicity
- Develop & validate method for leachable testing
- Put product on stability
- Establish a qualitative & quantitative correlation between profiles
- Assay for presence of leachables
- FINISHED