Decision Tree (DT) Approach & Threshold of Toxicological Concern (TTC)

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The author declares no conflicts of interest.
Range of Safe Levels

Lethal by oral, dermal, and inhalation routes
NOEL: $\sim 1 \mu g/p/d \approx 1$ quadrillion molecules

Water hemlock

Safe level: 4 Liters
$\approx 100$ billion quadrillion molecules

Water

NOEL: No Observed Effect Level
Table of Contents

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- Application of DT and TTC
- Current and Past Issues
- Proposal: “Expanded” DT (EDT) Database
  - Criteria – Data Collection
    - ADME
    - Toxicity
- Development of EDT sequence
  - Broader scope of structures - Chemical grouping
  - Common “mode of action”
  - Species and sex- differences
Birth of the Decision Tree

- **1960-1972** US FDA performs toxicity studies on ~50 of 1100 flavor ingredients
- **1974** US FDA awards contract to Flavor & Extract Manufacturers Assoc. (FEMA) to collect safety data on flavor ingredients in use
- **1974-1979** FEMA publishes data on ~1100 flavoring ingredients organized into 65 scientific literature reviews (SLRs)
  - SLR based on structural features, data on metabolic fate and toxicity
- **1978** FEMA designs sequence of structure-based questions based on data in SLRs and other sources. DT is born.
Distribution of NOELs vs. DT Class

Class I: 5th % 3 mkd = TTC 1800 µg/d
Class II: 5th % 0.91 mkd = TTC 544 µg/d
Class III: 5th % 0.15 mkd = TTC 90 µg/d

Fig. 1. Empirical cumulative distributions of NOELS of compounds in the reference database and log-normally fitted cumulative distributions (solid lines). Compounds have been grouped into the structural classes I, II and III of Cramer et al. (1978).

Munro et al., 1996
Comparison of intake with thresholds of toxicological concern

- The Threshold of Toxicological Concern (TTC) concept - widely applied and used in JECFA safety evaluation
- TTC's derived for each of the three Cramer Decision Tree Classes (Munro, et al., 1996)
Other Low Exposure Applications of DT and TTC

- Flavoring Agents (JECFA 1996)
- Food Additives (Kroes et al., 2004)
- Chemical Mixtures (Smith et al., 2005)
- Cosmetics and Fragrances (Kroes et al., 2007)
- Industrial Chemicals (inhalation) (Escher et al., 2008)
- Industrial Chemicals (Kalkhof et al., 2011)
- Flavoring Agents (EFSA, 2002-2015)
Critical Analysis of Cramer et al. DT & TTC

- Phillips et al., 1987
- Lapenna and Worth, 2011
- Tluczkiewicz et al., 2011
- Roberts et al., 2015
- WHO/EFSA, 2016
  - Eliminate non-structure-based questions
  - Specify metabolism & toxicity underpinning DT questions
  - Update questions to reflect current “state of scientific knowledge”
  - Document with “mode of action”/species difference
Expanded Decision Tree (EDT)

Principle Objectives:

- Scientific update of the Cramer et al., DT
- Increase chemical space for EDT development
  - Food contact, pesticide residues, contaminants, monomers
- EDT DB – Integrate structure, ADME & toxicity data
- Lowest NOAEL (or NOEL) and LOAEL data for the most sensitive, “relevant” species of longest duration
  - Alpha-2-µ-globulin, peroxisome proliferation
  - Relevance of forestomach effect in rodents
Criteria for EDT NOELs

- **Duration factor**: 4 for studies of 28-90 days; 3 for studies of 91-98 days; 1 for studies of >98 days; if maternal or paternal NOEL in repro/teratology study, use duration factors.
- **Relevant sex/species** based on recognized mode of action.
- **Single dose NOEL** if structurally related substances exhibit similar NOAEL range.
- **Replace** original NOEL in Munro *et al.*, 1996, if study provides lower NOEL.
- **Expand chemical space** by increasing the number of NOELs (to ≈2000) Structural variation (key factor), not no. of NOELs.
- **Validate against NOEL data** reported by authoritative bodies (e.g., EPA/EFSA/ECHA/FDA/IARC/JECFA/NTP/OECD).
- **Use the unit** of mmole/kg body weight (bw)/day.
“Mode of action” Criteria for EDT DB

- Biochemical map w/ reactive intermediate, perturbation of cellular homeostasis (pathway to toxicity), etc.
- Sort out functional groups or moiety based on ADME and toxicity

<table>
<thead>
<tr>
<th>Substance</th>
<th>Structure</th>
<th>Key metabolite</th>
<th>Target Organ</th>
<th>NOEL mmoles/kg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furan</td>
<td>1-4-but-2-endial (reacts w/protein)</td>
<td>Liver</td>
<td>&lt;0.013</td>
<td></td>
</tr>
<tr>
<td>Furfuryl alcohol</td>
<td>Furoic acid glycine conj. (excreted in urine)</td>
<td>Liver</td>
<td>&gt;1.5</td>
<td></td>
</tr>
<tr>
<td>Furfuryl mercaptan</td>
<td>Furfuryl mercaptan</td>
<td>Blood (hemolysis)</td>
<td>0.026</td>
<td></td>
</tr>
</tbody>
</table>
# Cramer et al., DT vs EDT

<table>
<thead>
<tr>
<th></th>
<th>Cramer DT</th>
<th>Munro TTC</th>
<th>EDT</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Questions</td>
<td>33</td>
<td></td>
<td>47</td>
</tr>
<tr>
<td># of Structure-based decisions</td>
<td>≈ 50</td>
<td></td>
<td>≈ 120</td>
</tr>
<tr>
<td># of Chemicals in DB</td>
<td>247</td>
<td>613</td>
<td>1,800</td>
</tr>
<tr>
<td># of Classes</td>
<td>3</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td># of TTCs</td>
<td></td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>
Cramer et al. DT Schema

Cramer et al. DT:
- 33 questions
- 50 structural classifications
EDT Schema

Expanded DT:
- 47 Questions
- 120 structural classifications
- ~1800 substances in DB
- 6 structural classes

- N & S FGs
- Simple salts
- Simple aliphatic HCs
- 10-22. Heterocycles
- 23-27. Acyclics
- 29-32. Alicyclics
- 33-46. Aromatics ring
- X≠C,H,O,N,S

- Alkenylbenzenes
- Phenols
- Hydroquinones
- Aromatic diamines
- Aromatic monoamines

- Food nutrients
- Organophosphorous
- 3. & 4. Halogenated compounds
- Natural toxins
- Simple salts
- N & S FGs
- Simple aliphatic HCs
- 10-22. Heterocycles
- 23-27. Acyclics
- 29-32. Alicyclics
- 33-46. Aromatics ring

- 20 reactive moieties

SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety
Six TTC Classes:

- **Class I**: non-toxic, participate in pathways used by food nutrients (e.g., ethyl laurate) form CO$_2$, H$_2$O,…….
  - animals $\approx$ humans

- **Class II**: low toxicity, participate in Phase I and II pathways of detoxication (e.g., menthol, cinnamaldehyde)
  - animals $\neq$ humans but only at $\uparrow\uparrow$ exposure

- **Class III**: intermediate toxicity, some show sex/species differences (e.g., polysulfides, furfural)
  - animal $\neq$ humans
Class IV: structural features of parent and/or metabolites suggest toxicity-(e.g., pyridine, acrolein)
- animals≈ humans?

Class V: toxicity at very low levels (10^{-3} to 10^{-5} mmole/kg) (e.g., bidrin or fumonisin B1)
- animals≈humans

Class VI: toxicity at very low levels (<10^{-5} mmole/kg) over short duration (select organophosphates, Na & K channel blockers, natural toxicants)
- animals≈ humans
Preliminary TTC Comparisons

![Graph showing Preliminary TTC Comparisons]

- **EDT**
  - Class I
  - Class II
  - Class III
  - Class IV
  - Class V
  - Class VI

- **Cramer**
  - Class I
  - Class I
  - Class II
  - Class III
  - Class III
  - Class III

Legend:
- New TTC
- JECFA
EDT: Question Development

- Review range of structural variations for chemical group (phenols, phosphites, aromatic primary amines)
  - Grouping relates to metabolic options, reactive moiety and common toxic endpoint
- Identify/predict dose-dependent metabolic pathways active in animal models over dose range: compare to humans?
- Effect of additional functional groups and structural skeletal changes
- Develop structure-based questions that account for metabolic options and toxicity NOEL changes.
  - “Relevance to humans”
Metabolism of Substituted Phenols

- **Phase I Metabolism**
  - p-hydroxylation to hydroquinone (HQ), followed by oxidation to p-quinone *(Intox)*

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**Diagram:**

- **Phenol** → **Hydroquinone** *(detox)* → **Phase II Conjugation** → **Quinone** *(detox)*
Metabolism of Substituted Phenols

- **Phase II Metabolism**
  - sulfate or glucuronide conjugation *(Detox)*
    - No ortho substitution
    - Small o-alkyl substituent (e.g., methyl)
    - p-alkyl substituent

<table>
<thead>
<tr>
<th>Phenol</th>
<th>NOEL: 5.31 mmol/kg bw/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Methylphenol</td>
<td>NOEL: 1.56 mmol/kg bw/day</td>
</tr>
<tr>
<td>4-tert-Butylphenol</td>
<td>NOEL: 1.33 mmol/kg bw/day</td>
</tr>
</tbody>
</table>
Substituted Phenols and NOELs

2,6-di-tert-Butylphenol
NOEL: 0.0182 mmol/kg/d

2-Hexadecan-2-yl-4,6-Methylphenol
NOEL: 0.0096 mmol/kg/d

2-tert-Butyl-4-[1-(tert-butyl-4-hydroxy-2-methylphenyl)butyl]-5-methylphenol
NOEL: 0.0044 mmol/kg/d
### Species Differences for 2,6-di-t-Butylphenol

<table>
<thead>
<tr>
<th>Metabolic Route</th>
<th>Rat</th>
<th>Dog</th>
<th>Rabbit</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugation</td>
<td>0</td>
<td>2%</td>
<td>12%</td>
<td>40%</td>
</tr>
<tr>
<td>p-Hydroxylation/conj.</td>
<td>55%</td>
<td>49%</td>
<td>39%</td>
<td>27%</td>
</tr>
</tbody>
</table>

2,6-di-t-tert-Butylphenol
# Substituted Phenols

<table>
<thead>
<tr>
<th>Structure</th>
<th>Phenol</th>
<th>2-Methylphenol</th>
<th>4-tert-Butylphenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOAEL (mmol/kg/day)</td>
<td>5.31</td>
<td>1.56</td>
<td>1.33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Structure</th>
<th>2,6-di-tert-Butylphenol</th>
<th>2-Hexadecan-2-yl-4,6-methylphenol</th>
<th>2-tert-Butyl-4-[1-(tert-butyl-4-hydroxy-2-methylphenyl)butyl]-5methylphenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOAEL (mmol/kg/day)</td>
<td>0.018</td>
<td>0.0096</td>
<td>0.0044</td>
</tr>
</tbody>
</table>
## Sorting of Phenols: Cramer et al. vs EDT

<table>
<thead>
<tr>
<th>Substance</th>
<th>CDT Class</th>
<th>EDT Class</th>
<th>NOEL mmole/kg/d</th>
<th>Species-Adjusted NOEL mmol/kg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-tert-Butyl-4-[1-(-tert-butyl-4-hydroxy-2-methylphenyl)butyl]-5-methylphenol</td>
<td>III</td>
<td>III</td>
<td>0.0044</td>
<td>0.044</td>
</tr>
<tr>
<td>2-Hexadecan-2-yI-6-methylphenol</td>
<td>II</td>
<td>III</td>
<td>0.0096</td>
<td>0.096</td>
</tr>
<tr>
<td>2,6-di-tert-Butylphenol</td>
<td>I</td>
<td>III</td>
<td>0.018</td>
<td>0.18</td>
</tr>
<tr>
<td>4-tert-Butylphenol</td>
<td>I</td>
<td>II</td>
<td>1.33</td>
<td>13.3</td>
</tr>
<tr>
<td>2-Methylphenol</td>
<td>I</td>
<td>II</td>
<td>1.56</td>
<td>15.6</td>
</tr>
<tr>
<td>Phenol</td>
<td>I</td>
<td>II</td>
<td>5.31</td>
<td>53.1</td>
</tr>
</tbody>
</table>
Conclusions

- “New Science” allows for greater specificity in questions relating structure to toxicity
- ADME data underpinned EDT questions that link structure to endpoint toxicity
- Inclusion of more elements and moieties increases applicability of EDT to greater scope of chemicals present in food
- EDT better delineates TTC classes compared to Munro classes and provides better understanding of structure activity relationships
References


References


14) European Food Safety Authority (EFSA)

15) The Joint FAO/WHO Expert Committee on Food Additives (JECFA)
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Questions