Estimation of Toxic Hazard- A Revised Cramer-Ford-Hall Decision Tree

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Outline

- The Cramer-Ford-Hall Decision Tree
  - Description
  - Class structure
  - Relationship to TTC Concept
- Considerations for revising the Decision Tree
- Key features of updated Decision Tree
- Supporting database
- Proposed Steps Forward
- Significance of work
Publication in 1978

Screening for toxicity testing

Classification of substances using the following data:

- Structure (key)
- Metabolism
- Toxicology
- Natural occurrence (in body and/or food)

Validated against chemicals with data on biological and toxicological properties (pesticides, drugs, food additives, industrial chemicals, flavorings, fragrances)

Cramer/Ford/Hall Decision Tree

Decision Tree Scheme

- Heterocyclics
- Open chain
- Alicyclics
- Aromatics
- Heteroaromatics
Class structure:

Class I
Structures and related data suggest a low order of oral toxicity. If combined with low human exposure, require a low priority for investigation.

Class II
Less clearly innocuous than Class I, but no firm indication of toxicity or the lack thereof.

Class III
Structure and related data permit no initial presumption of safety, or may suggest significant toxicity. These substances deserve the highest priority for investigation.
The Threshold for Toxicologic Concern (TTC) concept utilizes the Cramer/Ford/Hall DT to classify substances.

Munro Study – 611 chemical studies with oral repeated-dose studies
- Plotted DT Class versus NOELs
- Identified 5\textsuperscript{th}% NOEL for each Class
- Assumed 100-fold safety factor
- Defined Human Exposure Threshold (HET):
  \[
  \text{HET(\mu g/p/d)} = 5\% \text{NOEL(\mu g/kg/d)} \times 100/60\text{kg/d}
  \]

<table>
<thead>
<tr>
<th>Structural Class (examples)</th>
<th>TTC (\mu g/p/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (ethyl butyrate, cinnamaldehyde)</td>
<td>1800</td>
</tr>
<tr>
<td>II (3,6-dimethylpyrazine, pulegone)</td>
<td>544</td>
</tr>
<tr>
<td>III (estragole, anethole)</td>
<td>90</td>
</tr>
</tbody>
</table>

Munro, I.C. \textit{et al.} (1996) “Correlation of Structural Class with No-Observed-Effect Levels: A Proposal for Establishing a Threshold of Concern”, \textit{Food and Chemical Toxicology.}, 34, 829 - 867.
Close to 3000 flavoring substances have been evaluated in procedures that rely on the Cramer/Ford/Hall DT as a critical component to guide the safety assessment

- JECFA
- EFSA

US-based Flavor and Extract Manufacturers Association (FEMA) Expert Panel has utilized the Cramer/Ford/Hall DT as critical part of safety assessment process for the constituent - based assessment of natural complex substances used as flavoring ingredients since publication of a paradigm in 2005.

This has given us the opportunity to better understand the strength and also some of the weaknesses of the Cramer decision tree.
**Project Goal:** Incorporate new data on metabolism, toxicity and biochemistry

Factors for consideration:

- Functional group
- Skeletal structure
- Functional moiety
- Presence or absence of other functional groups
- Extent of conjugation
- Impact of electron donating groups
- Positional & geometric isomers

IOFI and FEMA co-funded project
Reconciling New Knowledge

- **Pulegone**
  - DT Class II
  - NOAEL = 0.44 mg/kg bw per day

- **Carvone**
  - DT Class II
  - NOAEL = 125 mg/kg bw per day

- **Coumarin**
  - DT Class III
  - NOAEL = 10 mg/kg

- **Dihydrocoumarin**
  - DT Class III
  - NOAEL = 400 mg/kg

- **Anethole**
  - DT Class III
  - NOAEL 240-300 mg/kg bw per day

- **Estragole**
  - DT Class III
  - NOAEL 37.5 mg/kg bw per day
Consideration of Biochemistry

Low Concern Substances: predominantly undergo detoxication or complete metabolism via high capacity enzyme-catalyzed pathways oxidation in the TCA cycle, fatty acid pathway, cytosolic carbonyl reduction, etc.

Medium Concern Substances: excellent substrates for low capacity detoxication pathways leading to stable, excretable metabolites

High Concern Substances: weak substrates for low capacity detoxication enzyme pathways leading to stable, excretable metabolites or metabolites of unknown stability

Myrcene (FEMA 2762)

\[
\text{Myrcene} \quad \xrightarrow{\text{CYP450}} \quad \text{oxidation/hydroxylation or epoxidation/hydrolysis}
\]

Revised Decision Tree (RDT)
Consideration of Chemistry

- Low Concern Substances/metabolites *w/functional moieties*
  - Substance/metabolites unlikely to react with biomolecules *in vivo* leading to adverse response

- Medium Concern/metabolites
  - Interact with biomolecules → products unlikely to result in adverse biological response
Proposed Revisions would lead to Revised Structure

Note that Structure Class assignments to question outcome are based on current tree at this stage.
Revised Decision Tree – Overall Goals

Consistent with WHO/EFSA Report – “no scientifically-based justification for major restructuring of the decision tree”

“The Expert group recommended minor changes to modify the Cramer decision tree to remove ambiguity, improve clarity and to harmonize with the electronic tool Toxtree”.

<table>
<thead>
<tr>
<th>Improvement of the Decision Tree - Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Questions</td>
</tr>
<tr>
<td>Unchanged</td>
</tr>
<tr>
<td>Minor changes</td>
</tr>
<tr>
<td>Major change/expansion of question</td>
</tr>
<tr>
<td>Eliminated</td>
</tr>
<tr>
<td>Added</td>
</tr>
</tbody>
</table>
Improving and Refining the Decision Tree

Summary of Updates:

New Q1: Predicts hydrolysis/reduction of substances in gut/intesting. Metabolites processed down the DT.

Expanded Chemical Space: Several questions appended/changed to include more functionalities, including phosphate esters, halogenated compounds and salts.

Incorporate New Information: New questions concerning lactones and heterocyclic compounds.

Questions Updated: Current toxicological and metabolic data used to update questions in the DT.

Remove and compensate for questions reliant on reference lists: Compounds classified based on being common terpenes, endogenous to the human body or commonly occurring in food in the original DT will be classified based on structure in the revised DT.
<table>
<thead>
<tr>
<th>CDT Question</th>
<th>WHO/EFSA Comment</th>
<th>Incorporation into RDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q3</td>
<td>Include language specific for simple ionic phosphate esters (free acid and Na, K, Ca, Mg or NH₄ salts) of the form R-O-PO₃²⁻.</td>
<td>Q4 adds qualification for “pentavalent P bonded only to O with at least one –P-O-M⁺ or P-OH moiety”</td>
</tr>
<tr>
<td>Q11 and Q18</td>
<td>Include thioesters in the list of constituent groups under consideration.</td>
<td>Thioesters and the hydrolysis of thioesters are incorporated.</td>
</tr>
<tr>
<td>Q20</td>
<td>Account for the reduction of disulfide groups.</td>
<td>The reduction of di- and poly-sulfides are incorporated.</td>
</tr>
<tr>
<td>Q22</td>
<td>Add an additional condition that for flavoring agents and other chemicals added to food, the ratio between natural occurrence and the amounts should be &gt;10.</td>
<td>Q22 has been eliminated to avoid the need for reference lists.</td>
</tr>
<tr>
<td>Q29</td>
<td>For a chemical with greater than 2 aromatic rings, can it be hydrolyzed or reduced to mononuclear residues</td>
<td>This is now considered in Q1.</td>
</tr>
<tr>
<td>Q31</td>
<td>Modify to allow for the reduction of alkylaryl disulfides</td>
<td>This is now considered in Q1.</td>
</tr>
<tr>
<td>Cramer Extention</td>
<td>Comment</td>
<td>Incorporation into RDT</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Toxtree rule ID 4 and Rule ID 40</td>
<td>Adds phosphate to list of elements that do not automatically go to Class 3.</td>
<td>Q4 adds qualification for “pentavalent P bonded only to O with at least one –P-O-M+ or P-OH moiety”</td>
</tr>
<tr>
<td>Toxtree Rule ID 42</td>
<td>Assigns to Class 3 possibly harmful analogs of benzene that “consist of one aromatic ring with at most one heavy atom connected to each aromatic atom”.</td>
<td>Both substituted and unsubstituted aromatic compounds are considered</td>
</tr>
<tr>
<td>Toxtree rule ID 43</td>
<td>assigns “possibly harmful divalent sulfur” to Class 3</td>
<td>WHO/EFSA found this comment unclear and does not recommend implementation</td>
</tr>
<tr>
<td>Toxtree Rule ID 44</td>
<td>assigned any free alpha-beta-unsaturated heteroatom (including ketones and alcohols) to Class 3.</td>
<td>Q18 – new section: alpha-beta-unsaturated aldehyde or ketone with no or one beta carbon substituent is assigned to Class 2 (if not parsed into Class 3 earlier in the tree.)</td>
</tr>
</tbody>
</table>
Improving and Refining the Database

**Chemical Space:**

Foundation: Munro Database (~ 600 chemically defined compounds)  
Additions (~1400 chemically defined compounds):  
1. Flavoring Substances  
2. Compounds with published studies  
   - National Toxicology Program (NTP) Studies (USA)  
   - Environmental Protection Agency (EPA) Studies (USA)  
   - Scientific Literature

NOEL/NOEAL Values determined for each study are extracted.

Munro, I.C. *et al.* (1996) “Correlation of Structural Class with No-Observed-Effect Levels: A Proposal for Establishing a Threshold of Concern”, *Food and Chemical Toxicology.*, 34, 829 - 867.
Improving and Refining the Database

**Studies:**

*Foundation*: Munro Database
- Exclusively oral (dietary, gavage) studies
- Mainly chronic studies (short term and acute studies not included)

*Additions*:
- Chronic and short term studies oral added
- (Some studies suggest the validity of using a conversion factor to equate NOAEL values between studies of differing lengths.)

Munro, I.C. *et al.* (1996) “Correlation of Structural Class with No-Observed-Effect Levels: A Proposal for Establishing a Threshold of Concern”, *Food and Chemical Toxicology.*, 34, 829 - 867.
• IOFI/FEMA has contracted the development of a new Toxtree module encoding the RDT.

• RDT-Toxtree: Appearance and function similar to current Toxtree

• Input in SMILES format for beta-testing
• Displays structure and summarizes steps
• Open Source
• The development of RDT along with a new Toxtree module will aid in the harmonization.
Revised Toxtree Module (v 3.0.0.1777)
Analysis of Menthol
Significance of Toxtree Project

Open Source – Updates can be made by scientific community

Standardizes Application of the DT Tool:

Currently, results can differ between analyses derived from:

a. Expert judgement using 1978 publication
b. Toxtree module
c. QSAR toolbox

The revised DT Toxtree module being developed with the goal that results will be the same as expert judgement.
Conclusions

• Overall, we trust that both the review of the Cramer Decision Tree and strengthening the Munro Database will increase the robustness of the TTC concept and we are working/planning towards sharing our data with the scientific community including the WHO/EFSA review project on TTC