Revision of the Cramer Decision Tree

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The Cramer/Ford/Hall Decision Tree

Published in 1978

Food Cosmet. Toxicol. (1978) **16**, 255-276

Screening for toxicity testing

Classified substances :

- ➢ 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)

The Original Cramer/Ford/Hall Decision Tree
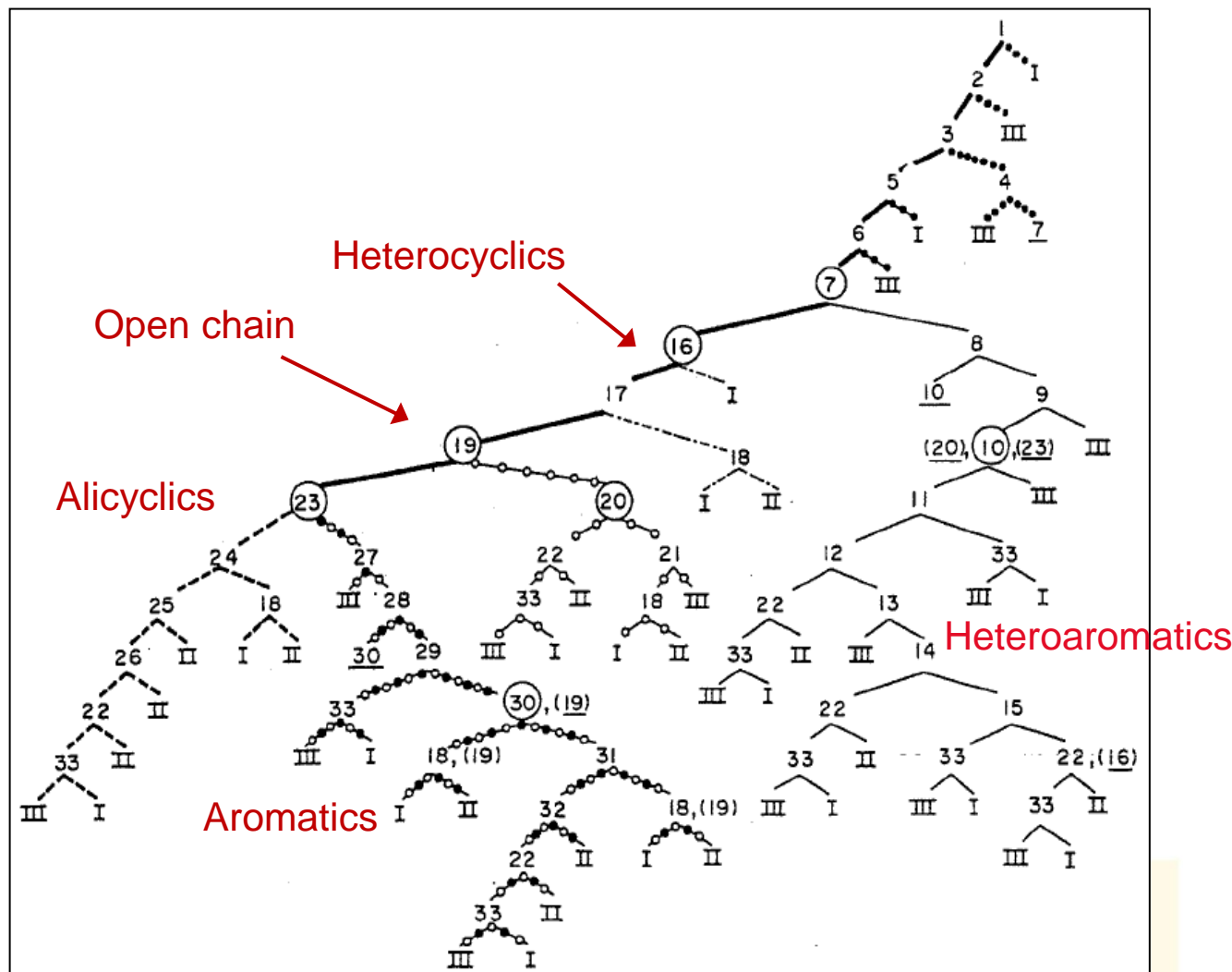
Class Structure

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 presumptions of safety, or may suggest significant toxicity. These substances deserve the highest priority for investigation

**International Organization
of the Flavor Industry**

Relation of DT Class to Threshold of Toxicological Concern (TTC) (Munro, 1996)

| Structural Class (examples)	TTC (µg/person/day)
I (ethyl butyrate, cinnamaldehyde)	1800
II (3,6-dimethylpyrazine, pulegone)	544
III (estragole, anethole)	90

5

Role of the Decision Tree within Flavoring Safety Assessments

- Close to 3000 flavoring substances have been evaluated in procedures that rely on the Cramer/Ford/Hall DT as 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
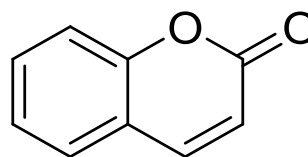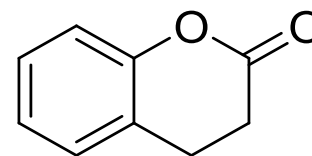
DT: Reconciling New Knowledge
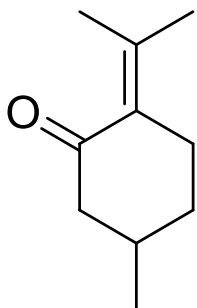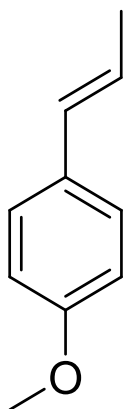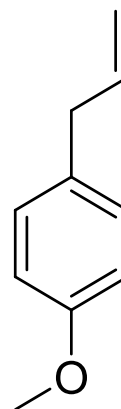
Pulegone
DT Class II
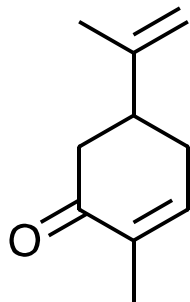
Carvone
DT Class II

Coumarin
DT Class III

Dihydrocoumarin
DT Class III

Anethole
DT Class III

Estragole
DT Class III

Flavor Industry Work on Revising Cramer/Ford/Hall DT

- In a co-funded project, IOFI and FEMA have developed suggestions for strengthening the Cramer/Ford/Hall DT "Package"

- Some proposed reworking of the DT questions/routing

- Updating of the database that supports the application of the TTC concept to the DT
  - Expanded Munro database
  - Opportunity to further strengthen and even expand current TTC classes

Proposed Revisions

- Revise "trunk" of the tree steps
- Steps lacking biochemical basis eliminated
    - Biological normality Step 1
    - Common component of food Step 22
        - Remove need for 'lookup' lists
- Increase elements
    - Other than C, H, O, N, divalent S, to include higher oxidation state S, Cl, F, and P in a biologically stable oxidation state

**Factors taken into consideration during revision**

- Skeletal structure

- Functional group

- Presence or absence of other functional groups

- Extent of conjugation

- Impact of electron donating groups

- Positional & geometric isomers

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

myrcene
(FEMA 2762)   CYP450 →   oxidation/hydroxylation
or
epoxidation/hydrolysis
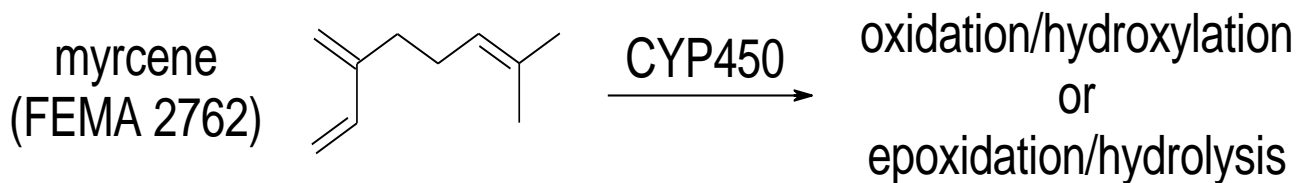
- High Concern Substances: weak substrates for low capacity detoxication enzyme pathways leading to stable, excretable metabolites or metabolites of unknown stability
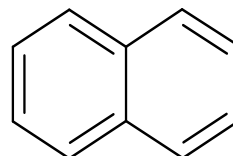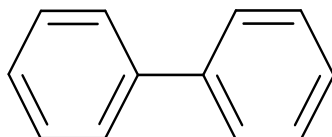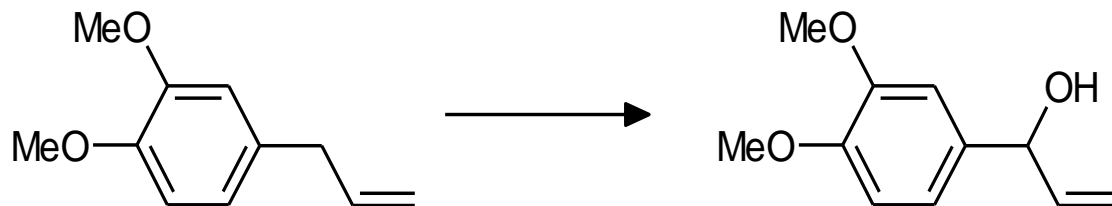
Even Higher Biochemical Concern
Could Argue for Additional Structural Classes

- "weak" substrates for low capacity intoxication pathways leading to reactive metabolites
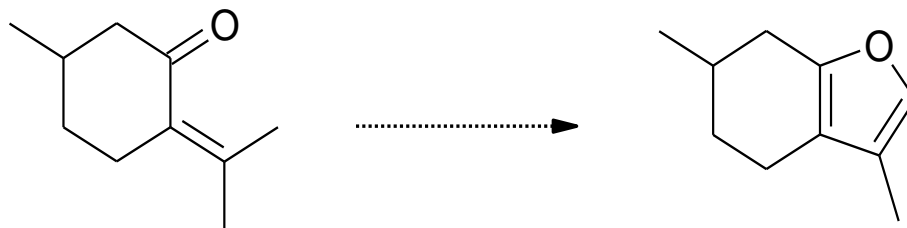
- "excellent" substrates for low capacity intoxication pathways leading to reactive metabolites
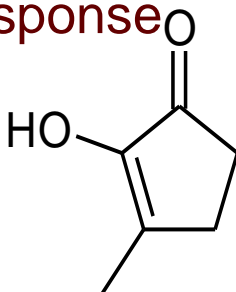
Consideration of Chemistry

- ## Low Concern Substances/metabolites w/functional moieties

  – Substance/metabolites unlikely to react with biomolecules *in vivo* leading to adverse response
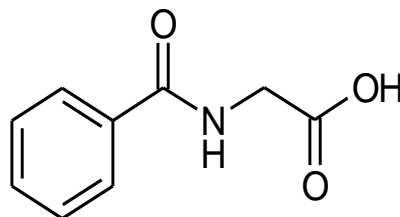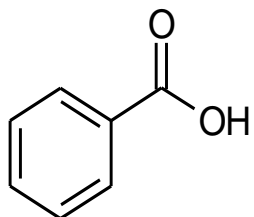
- ## Medium Concern/metabolites

  – 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
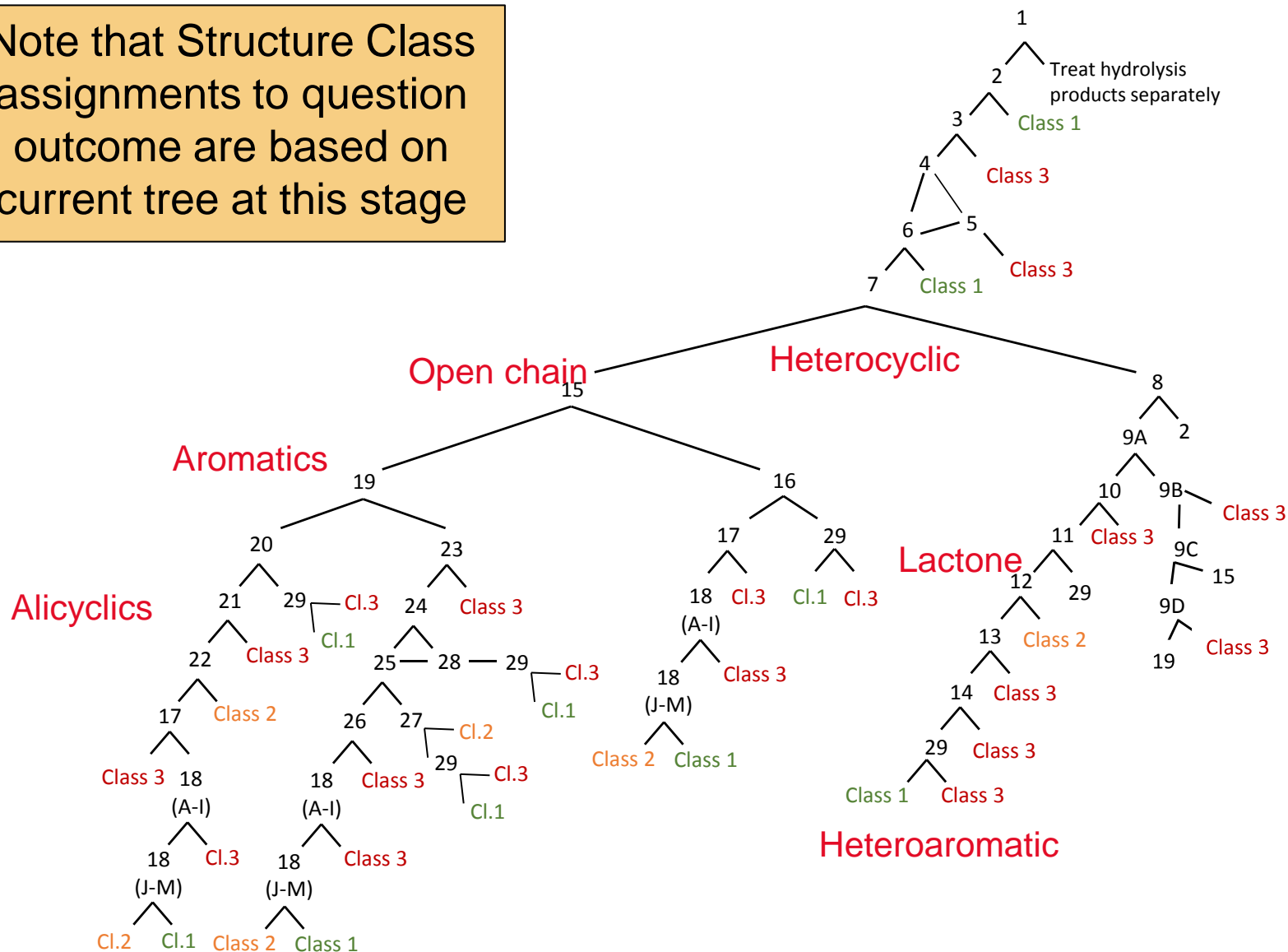
Proposed Revisions: Work Continues!

- Relation of new question outcomes to Structure Class Assignments?

Improving and Refining the Munro 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/NOAEL 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 oral studies added

(Some studies suggest the validity of using a conversion factor to equate NOAEL values between studies of differing lengths.)

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