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

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Conflict of Interest Statement

The author declares no conflicts of interest.

Range of Safe Levels

Water hemlock

Lethal by oral, dermal, and inhalation routes

NOEL: ~1µg/p/d ≈ 1 quadrillion molecules

Water

Safe level: 4 Liters

≈100 billion quadrillion molecules

NOEL: No Observed Effect Level

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- Proposal: “Expanded” DT (EDT) Database
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    - ✓ 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 flavour 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

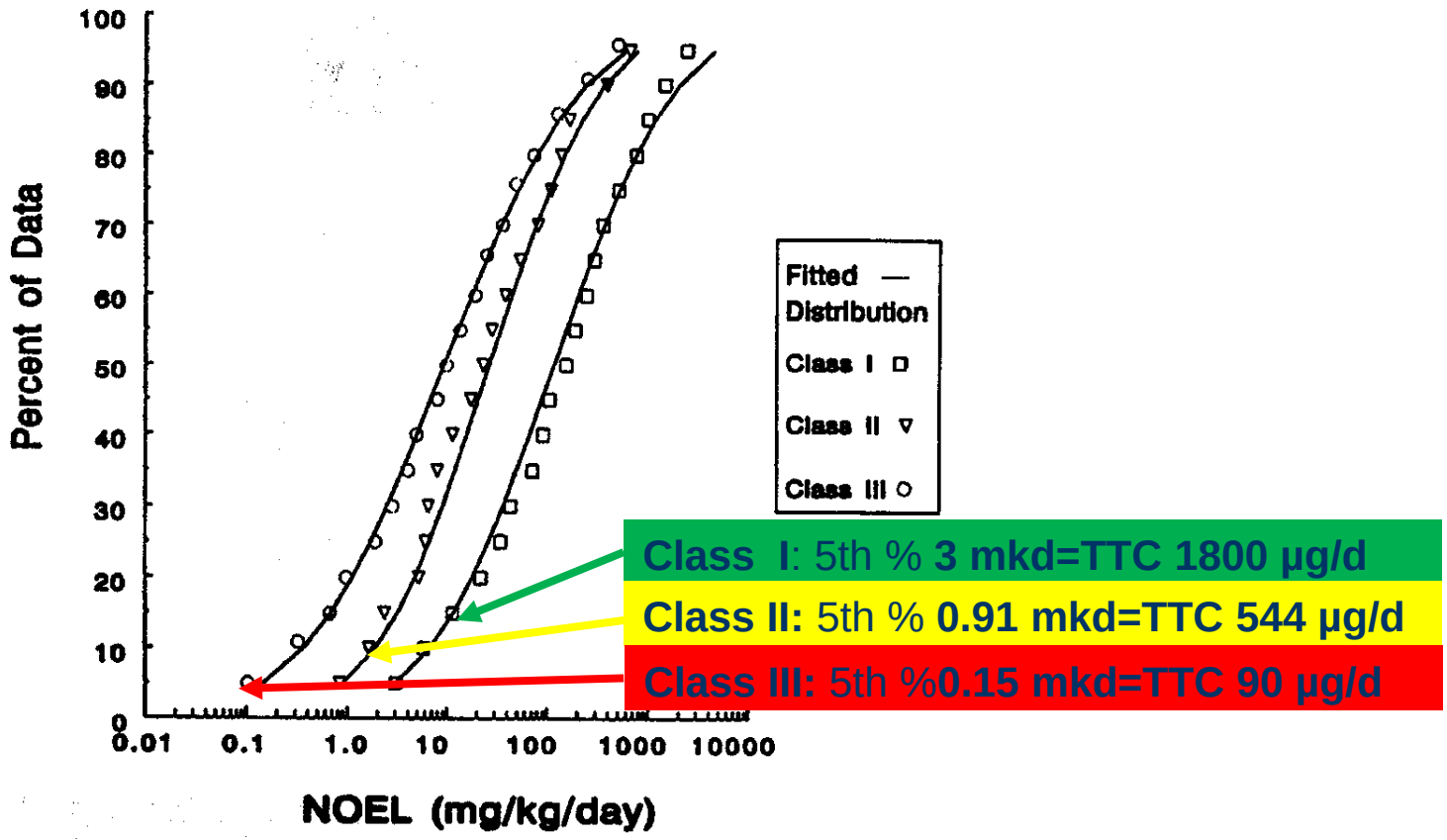


Fig. 1. Empirical cumulative distributions of NOELS of compounds in the reference database and log-normally fitted cumulative distributions (solid lines). Compounds have been grouped into the structural classes I, II and III of Cramer *et al.* (1978).

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

Substance	Structure	Key metabolite	Target Organ	NOEL mmoles/kg/d
Furan		1-4-but-2-endial (reacts w/protein)	Liver	<0.013
Furfuryl alcohol		Furoic acid glycine conj. (excreted in urine)	Liver	>1.5
Furfuryl mercaptan		Furfuryl mercaptan	Blood (hemolysis)	0.026

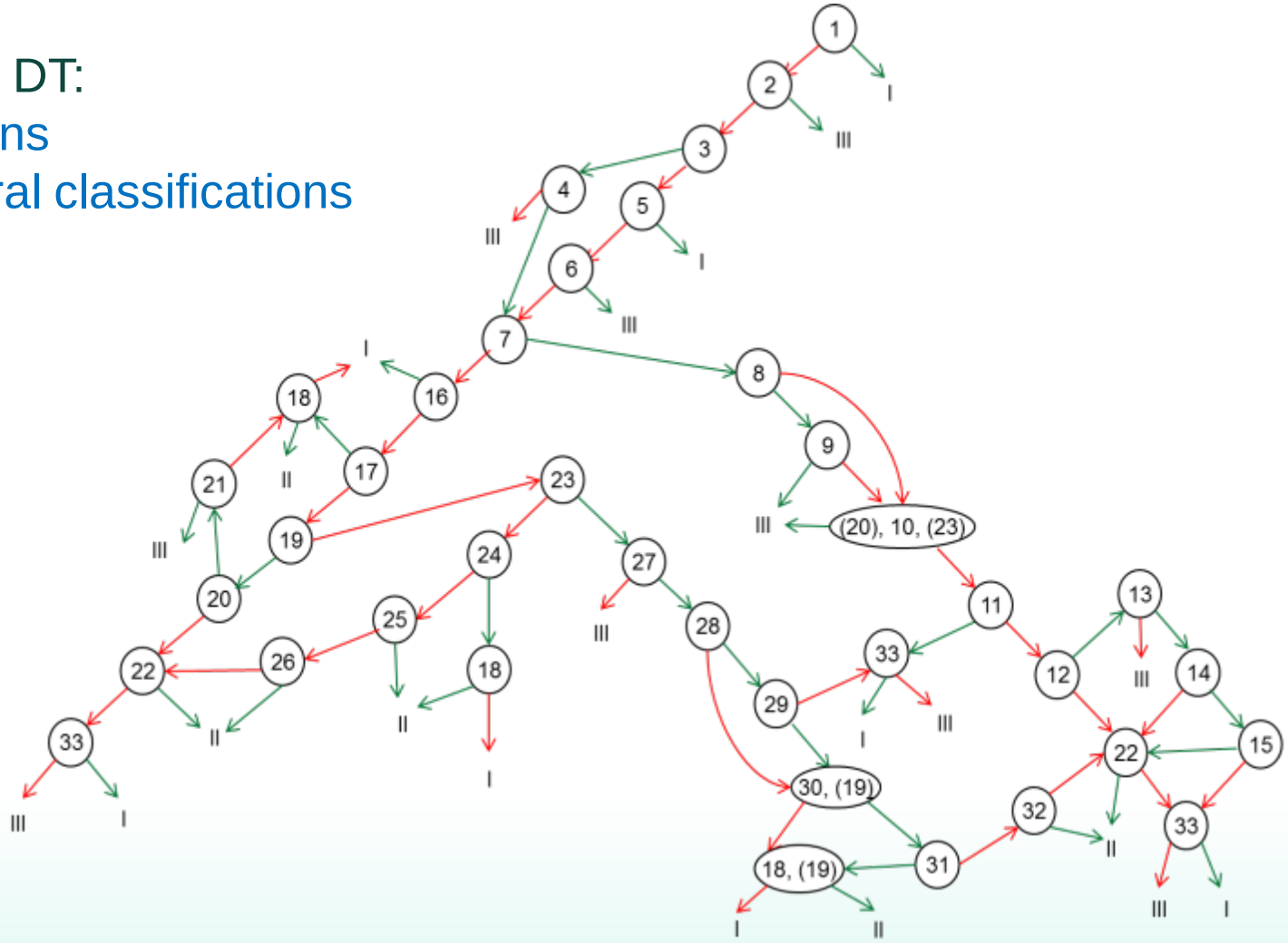
Cramer *et al.*, DT vs EDT

	Cramer DT	Munro TTC	EDT
# of Questions	33		47
# of Structure-based decisions	≈ 50		≈ 120
# of Chemicals in DB	247	613	1,800
# of Classes	3		6
# of TTCs		3	6

Cramer *et al.* DT Schema

Cramer et al. DT:

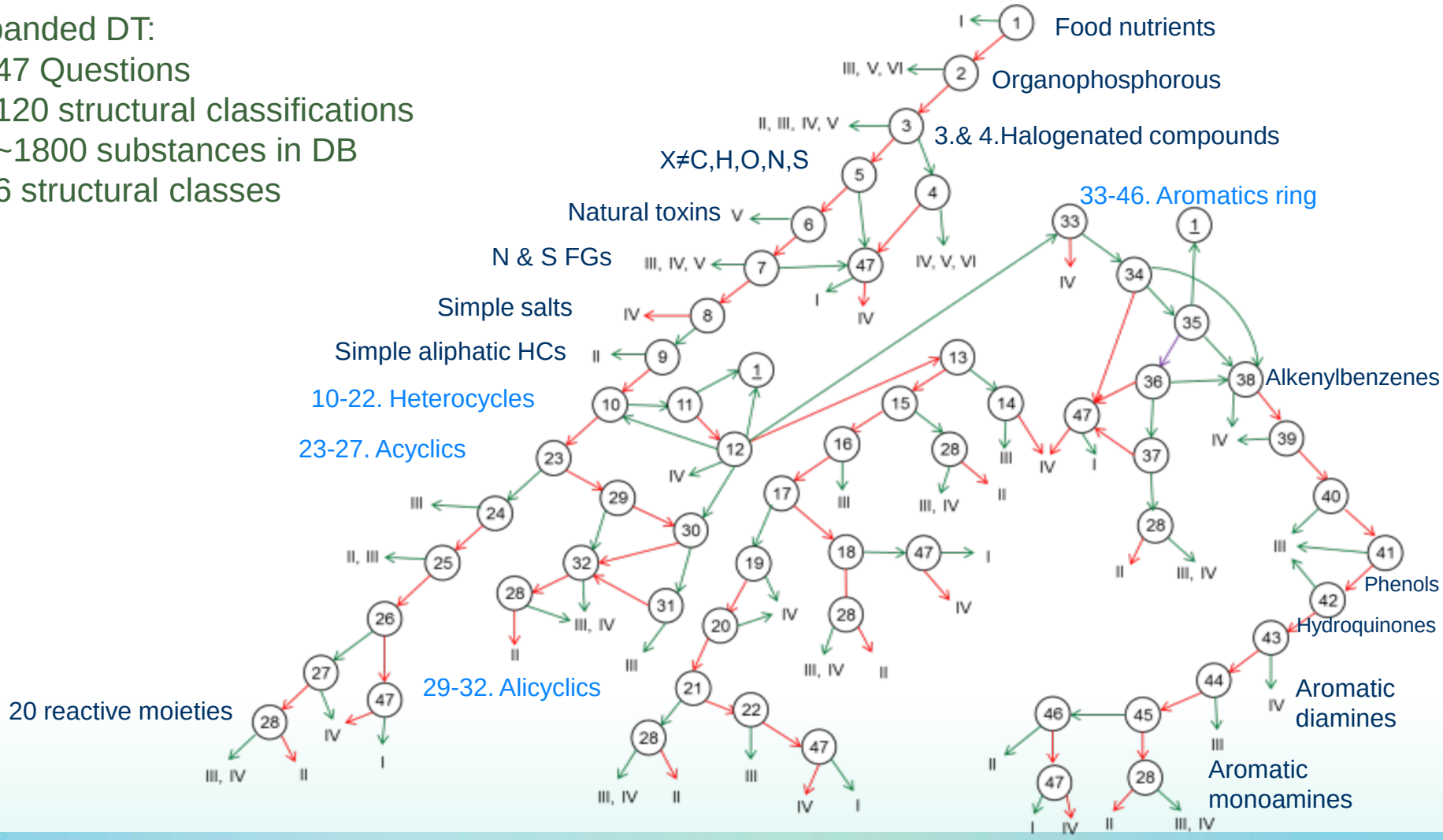
- 33 questions
- 50 structural classifications



EDT Schema

Expanded DT:

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



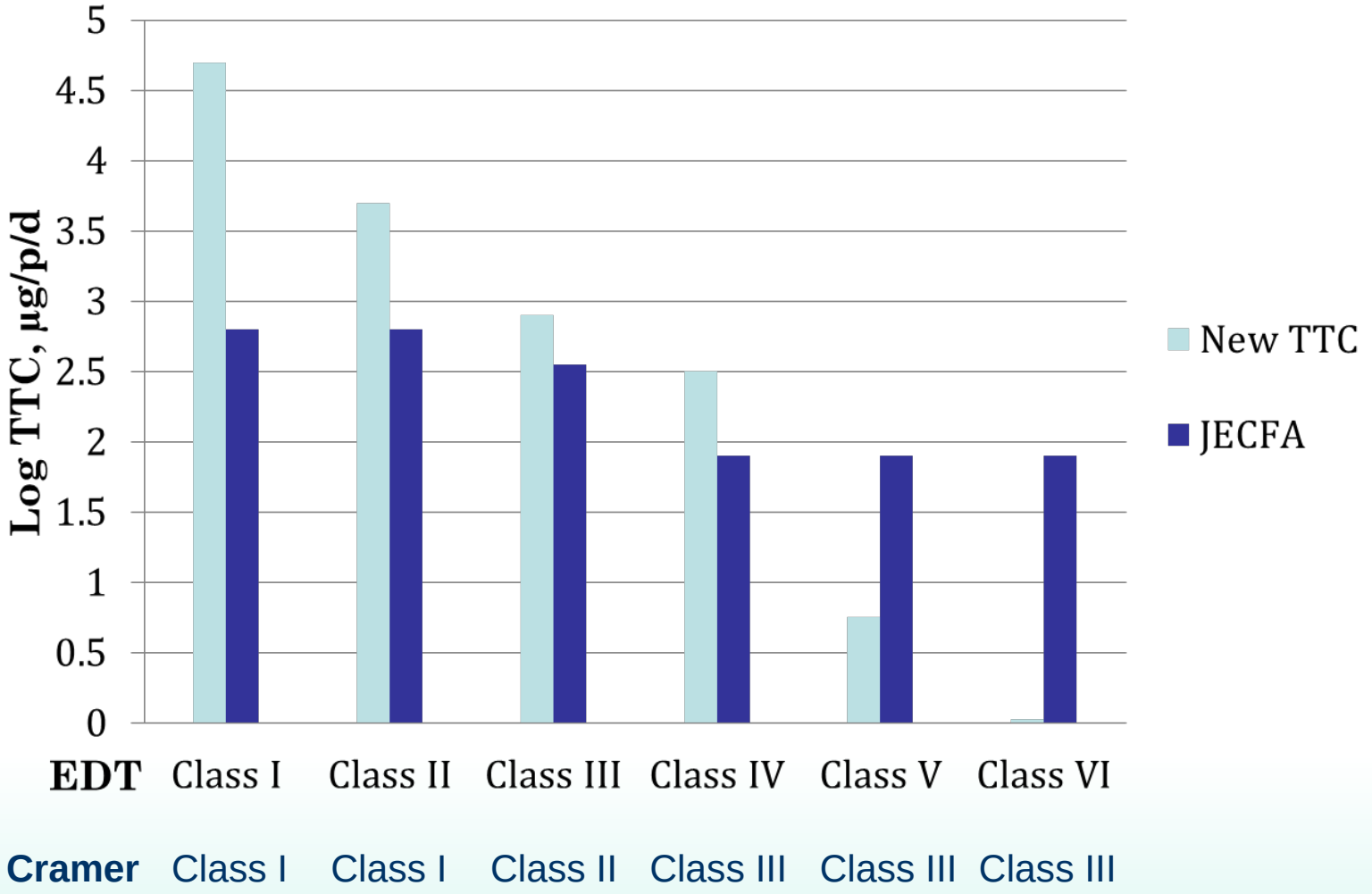
Cramer *et al*., DT vs EDT: Preliminary Results for EDT

- Six TTC Classes:
  - **Class I**: non-toxic, participate in pathways used by food nutrients (e.g., ethyl laurate) form CO_2, H_2O,…….
    - animals≈humans
  - **Class II**: low toxicity, participate in Phase I and II pathways of detoxication (e.g., menthol, cinnamaldehyde)
    - animals≠humans but only at ↑ ↑ exposure
  - **Class III**: intermediate toxicity, some show sex/species differences (e.g., polysulfides, furfural)
    - animal≠humans

Cramer *et al*., DT vs EDT: Preliminary Results for EDT

- **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

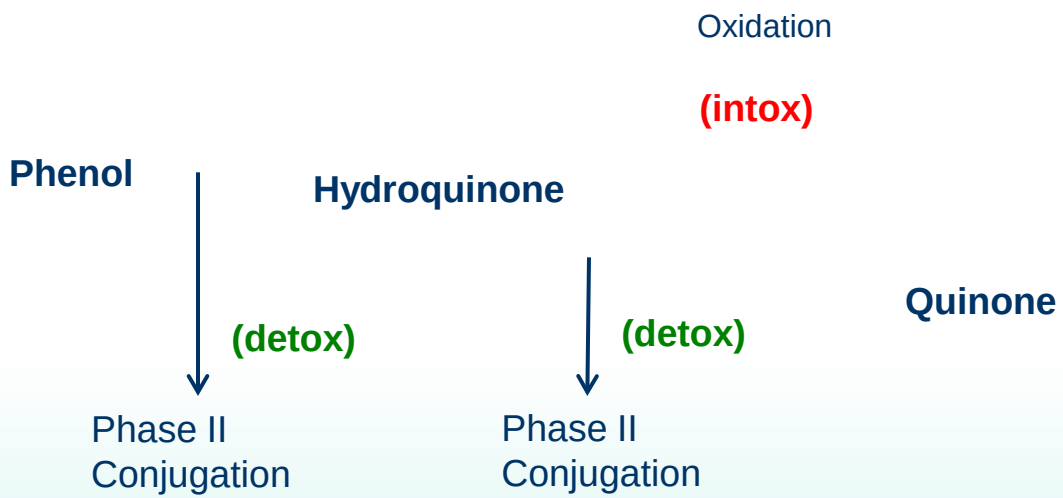


EDT: Question Development

- 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)**



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

Phenol
NOEL: 5.31
mmol/kg bw/day

2-Methylphenol
NOEL: 1.56
mmol/kg bw/day

4-tert-Butylphenol
NOEL: 1.33
mmol/kg bw/day

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

Metabolic Route	Rat	Dog	Rabbit	Human
Conjugation	0	2%	12%	40%
p-Hydroxylation/conj.	55%	49%	39%	27%

2,6-di-tert-Butylphenol

Substituted Phenols

Structure			
Name	**Phenol**	**2-Methylphenol**	**4-tert-Butylphenol**
NOAEL (mmol/kg/day)	5.31	1.56	1.33
Structure			
Name	**2,6-di-tert-Butylphenol**	**2-Hexadecan-2-yl-4,6-methylphenol**	**2-tert-Butyl-4-[1-(-tert-butyl-4-hydroxy-2-methylphenyl)butyl] -5methylphenol**
NOAEL (mmol/kg/day)	0.018	0.0096	0.0044

Sorting of Phenols: Cramer *et al.* vs EDT

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

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

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Questions