Comparison of Cramer Classification between Toxtree, OECD QSAR Toolbox and Expert Judgement

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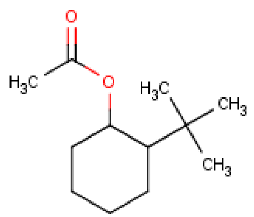
Background

- The Concept of TTC is useful to evaluate materials for their potential toxicity
- The Cramer decision tree is used for categorizing chemicals in order to determine their TTC thresholds based on different Cramer classes
- Therefore, assigning the appropriate Cramer class to a chemical of interest that lacks toxicity data is a crucial step to its risk assessment

Background (Cont.)

- *In silico* tools such as, Toxtree (TT) and OECD QSAR Toolbox (TB), may be used to readily get the Cramer class in an automated way
  - However, discrepancies between these tools were observed
- At RIFM, we manually assigned Cramer classes for >1000 fragrance ingredients by experienced experts and compared the results with *in silico* programs
  - Discrepancies were observed
- Further analysis were conducted and our suggestions were proposed

Cramer Classification using *in silico* Tools



Menthyl acetate (CAS # 89-48-5)
Cramer Class I, based on Chemists

TT	TB
Q1. Normal constituent of the body? No, → Q2 Q2. Any of these functional groups: aliphatic sec-amine, cyano, N-nitroso, diazo, triazeno, or quaternary N? No, →Q3 Q3. Any elements other than C, H, O, N, divalent S? No, →Q5 Q5. Is it a simply branched acyclic aliphatic hydrocarbon or a common carbohydrate? No, → Q6 Q6. Is it a benzene derivative…? No, Q7 Q7. Is it heterocyclic? No, → Q16 Q16. Is it a common terpene? No, → Q17 Q17. Is it readily hydrolysed to a common terpene, -alcohol, -aldehyde or -carboxylic acid? Yes, giving acetic acid (→ Q19) and menthol (→ Q 18) Residue 1: menthol Q18.One of the list (see explanation) **Yes** Class Intermediate (Class II) Residue 2: acetic acid Q19.Open chain **Yes** Q20.Aliphatic with some functional groups (see explanation) **Yes** Q21. 3 or more different functional groups **No** Q18.One of the list (see explanation) **No** Class Low (Class I) O=C(OC(C(C(C)(C)C)CCC1)C1)C 'Residue 1'	Q1. Normal constituent of the body? **No,** → Q2 Q2. Any of these functional groups: aliphatic sec-amine, cyano, N-nitroso, diazo, triazeno, or quaternary N? **No**, → Q3 Q3. Any elements other than C, H, O, N, divalent S? **No**, → Q5 Q5. Is it a simply branched acyclic aliphatic hydrocarbon or a common carbohydrate? **No,** → Q6 Q6. Is it a benzene derivative…? **No**, Q7 Q7. Is it heterocyclic? **No**, → Q16 Q16. Is it a common terpene? **No,** → Q17 Q17. Is it readily hydrolysed to a common terpene, -alcohol, -aldehyde or -carboxylic acid? **No** Q19. Open chain → **No** Q23. Aromatic → **No** Q24. Monocarbocyclic with simple substituents → **Yes** → Class I

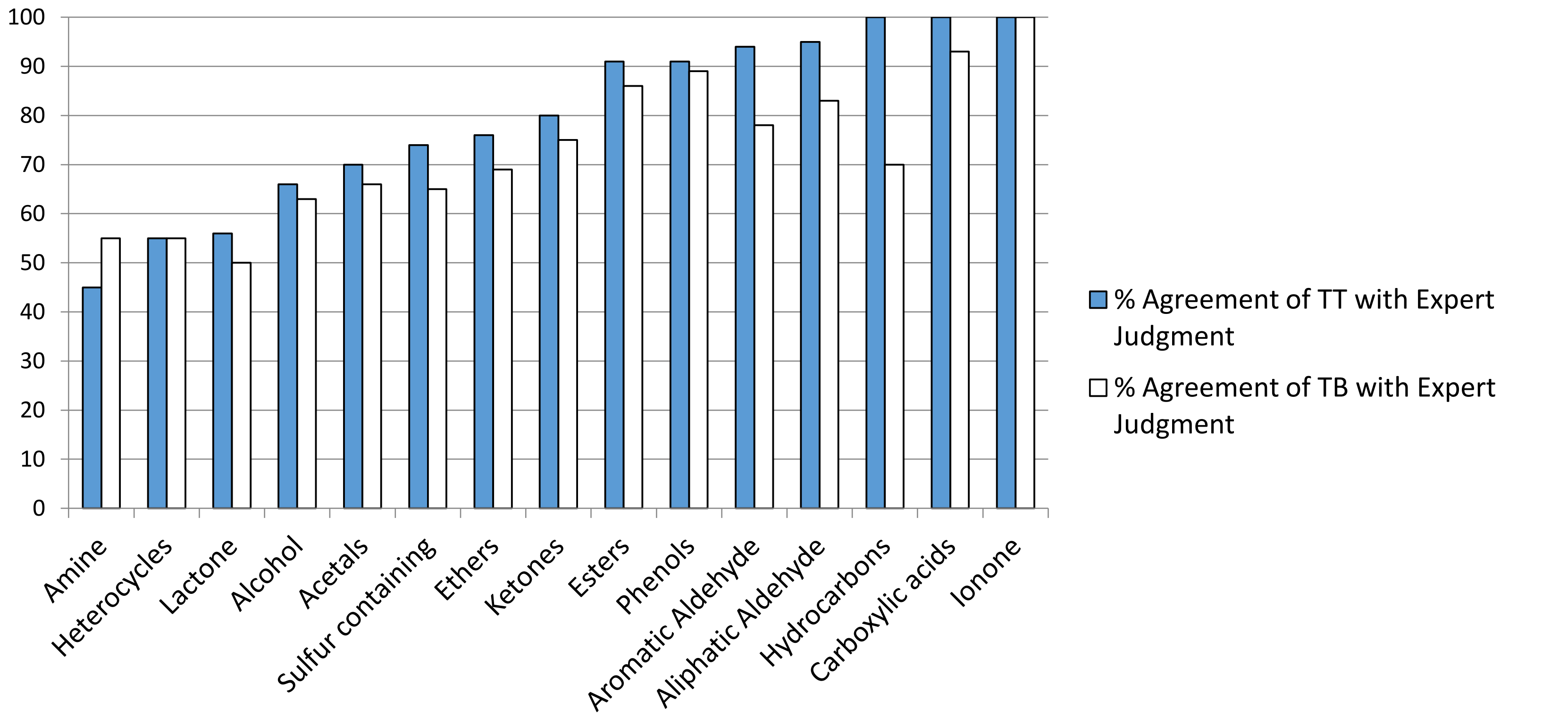
Disconcordance between TT and TB

16% of testing material (1016) differed between TT and TB

		TT Version 2.6		
		Class I	Class II	Class III
TB Version 3.1	Class I	602	45	7
	Class II	28	94	36
	Class III	36	13	155

Discrepancies of TT and TB, compared with Experts Judgement

- 17% of fragrance ingredients were misclassified by TT
- 22% of fragrance ingredients were misclassified by TB



Examples of Misclassified Materials

CAS No.	Material Name	Generic Class	Expert judgment	TT (v 2.6)	TB (v 3.1)	Misinterpreted rule in TT	Misinterpreted rule in TB
65505-24-0	Isobutyl N-methyl anthranilate	Amines	III	III	II		Q32
611-13-2	Methyl 2-furoate	Heterocycles	II	III	III	Q22	Q11
6066-49-5	3-n-Butyl phthalide	Lactone	III	III	I		Q9
25524-95-2	5-Hydroxy-7-decenoic acid δ-lactone	Lactone	I	II	I	Q18	
639-99-6	Elemol	Alcohol	I	III	II	Q24	Q18
100-86-7	α,α-Dimethylphenethyl alcohol	Alcohol	I	III	II	Q30	Q18
13254-34-7	2,6-Dimethyl-2-heptanol	Alcohol	I	III	II	Q20	Q18
1113-21-9	Geranyl linalool	Alcohol	I	III	II	Q20	Q18
619-01-2	Dihydrocarveol (isomer unspecified)	Alcohol	I	I	II		Q18
107-74-4	Hydroxycitronellol	Alcohol	I	III	II	Q20	Q18
88-15-3	2-Acetyl thiophene	Sulfur-containing	II	III	III	Q22	Q22
141-97-9	Ethyl acetoacetate	Ketones	I	I	III		Q21
4695-62-9	d-Fenchone	Ketones	II	III	III	Q26	Q26
4674-50-4	Nootkatone	Ketones	II	III	III	Q26	Q26
4728-82-9	Allyl cyclohexaneacetate	Esters	II	II	III		Q24
105-95-3	Ethylene brassylate	Esters	I	I	III		Q8
21834-92-4	5-Methyl-2-phenyl-2-hexenal	Aromatic Aldehyde	II	II	I		Q30
107-75-5	Hydroxy citronellal	Aliphatic Aldehyde	I	III	II	Q20	Q18
116-26-7	2,6,6-Trimethyl cyclohexa-1,3-dienyl methanal	Aliphatic Aldehyde	I	I	II		Q24
122-59-8	Phenoxy acetic acid	Carboxylic acids	III	III	I	Q30	Q25

Taking Home Messages

- Assigning Cramer Class using *in silico* tools could bring discrepancies
- Using different tools can help identify misclassified materials, but not all
    - ➢More than 50% of misclassified materials have the same assignment from TT and TB
- Some of the Cramer Rules are difficult to be interpreted correctly by computers
    - ➢Look-up list rules(e.g. Q1, Q22)
    - ➢Structure-based rules (e.g. Q18, Q20)
    - ➢Other rules (e.g. Q16, Q17)
- Using expert judgement when the Cramer classification is important to you
- **When validating TTC thresholds, be cautious if *in silico* tools are used to derive the Cramer class**

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Thank you!

Questions or Comments?