

Cramer classes in the TTC — fit for purpose ?

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Food Packaging Forum Workshop

Zurich 17 October 2013



APPLICATION OF TTC APPROACH

WHAT INFORMATION DO WE NEED?

- Chemical structure
- Estimate of human exposure that is not an underestimate



CRAMER STRUCTURAL CLASSES FOR CHEMICALS

THE THINKING BEHIND CRAMER CHEMICAL CLASSES

Based on similarities in toxicity of structurally-related chemicals
the toxicity of untested members of a closely-related group
can be predicted



Aniline and its many of its derivatives cause methaemoglobinaemia and haemolysis due to common hydroxylamine metabolites

THE THINKING BEHIND CRAMER CHEMICAL CLASSES



Can this approach be extended to the world of chemicals to predict likely toxic potency without animal testing?

THE THINKING BEHIND CRAMER CHEMICAL CLASSES

For chemicals sharing broadly similar functional groups

- the nature of their toxicity cannot be predicted
- but can they be separated into groups of low, medium and high concern?

ASSIGNING CHEMICALS TO STRUCTURAL CLASSES: CRAMER DECISION TREE

The Cramer Decision Tree allows chemicals to be classified into three structural classes, based on:

- Toxicity conferred by certain structural groups
- Whether the substance occurs naturally in food
- Whether it is naturally present in the body
- What is known about its metabolism

*Cramer, Ford and Hall (1978) Food Cosmet. Toxicol. **16**, 255-276*

CRAMER DECISION TREE: STRUCTURAL CLASSES

Class I

Substances with simple structure with efficient metabolism
suggesting a low order of toxicity

Class III

Substances with structures that permit no strong initial
presumption of safety or which suggest significant toxicity

Class II

Anything that cannot be put into Class I or Class III

CRAMER DECISION TREE

- The decision tree is a series of 33 questions that are applied in sequence
- Logic of the questions based on the then-available knowledge on chemicals and toxicity and how substances are metabolised in the body
- The questions relate to chemical features known to be associated with toxicity but it is not an expert system or a (Q)SAR system designed to predict the nature of the toxicity, only the likelihood of toxicity

Cramer decision tree separates chemicals into 3 structural classes via a series of questions

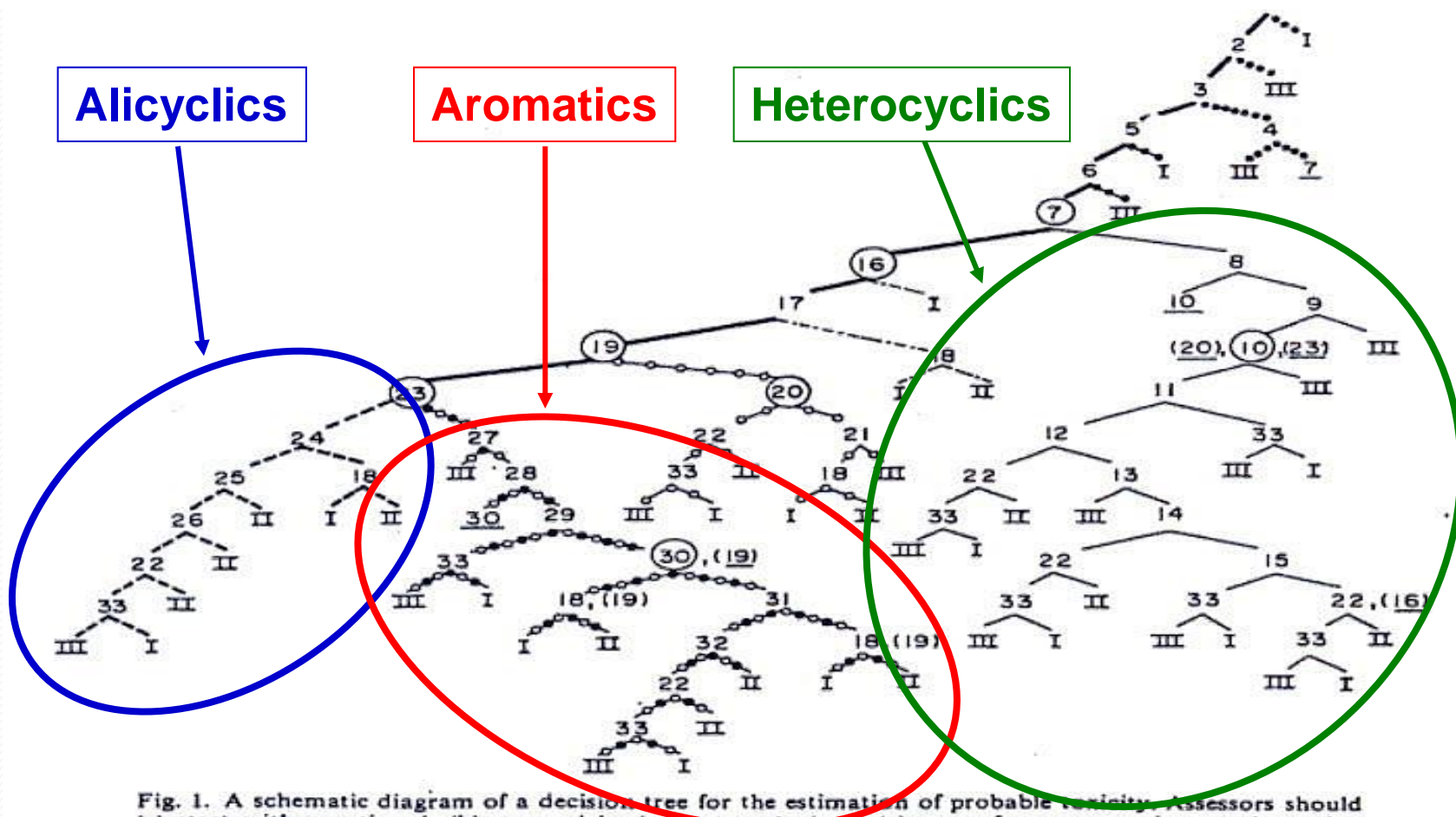


Fig. 1. A schematic diagram of a decision tree for the estimation of probable toxicity. Assessors should (a) start with question 1, (b) proceed by 'no' or 'yes', (c) move from any underscored number encountered to same circled number and (d) proceed to final classes I, II or III. Working downwards through the tree, the symbols designate the following groupings: biological normality (●●●), high and low toxicity (●—●); heterocyclics (—); terpenoids (---); aliphatics (—○—○—○); alicyclics (---).

Predicted toxicity of the 3 structural classes:

I = low, II = medium, III = high

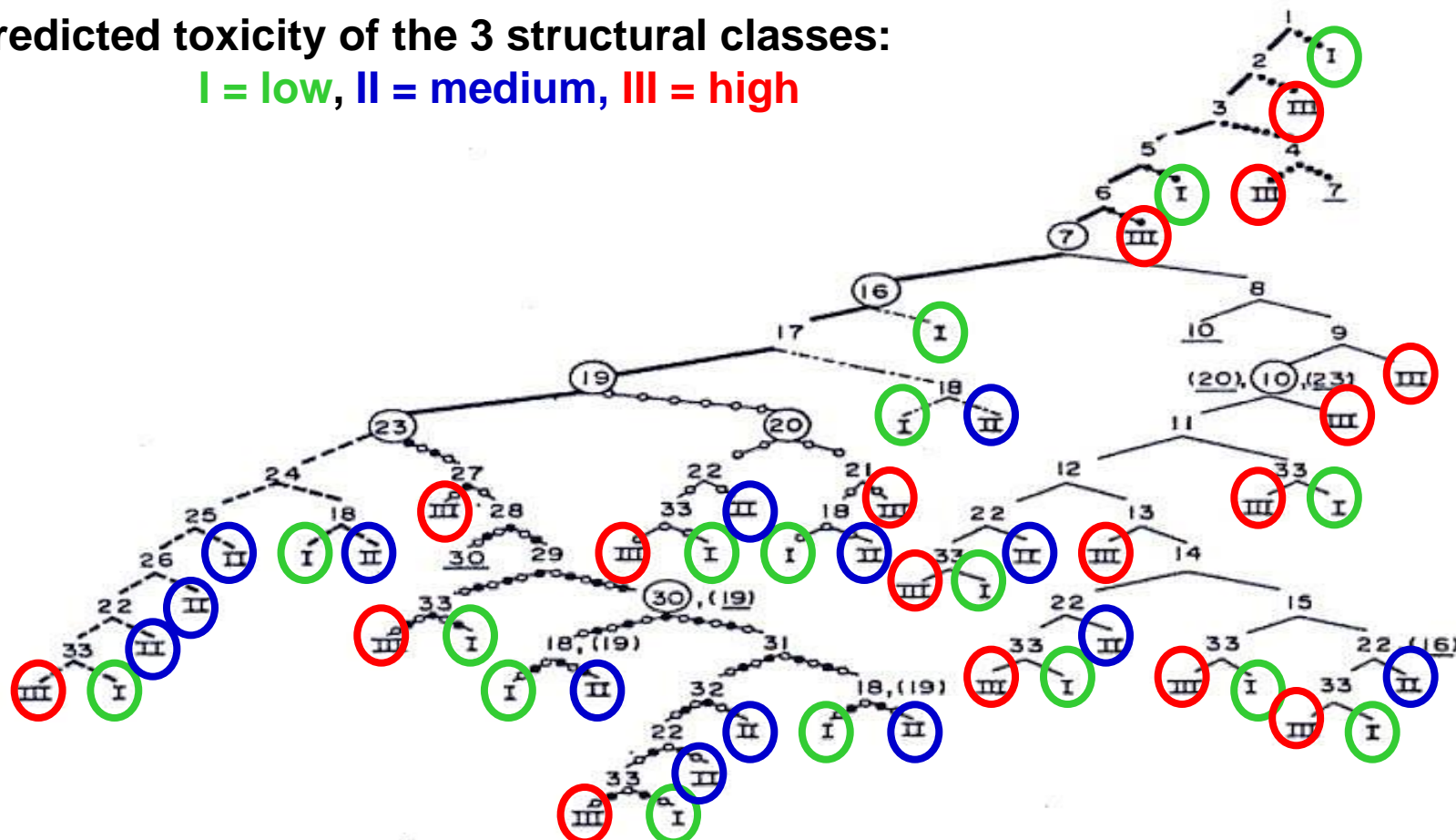


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EXAMPLES OF HOW THE DECISION TREE CLASSIFIES SUBSTANCES

Cramer Class I

- Normal constituents of the body, excluding hormones
- Simply-branched, acyclic aliphatic hydrocarbons
- Common carbohydrates
- Common terpenes
- Substances that are sulphonate or sulphamate salts, without any free primary amines

Any substance containing something other than C, H, O, N, divalent S, is excluded from Class I

EXAMPLES OF HOW THE DECISION TREE CLASSIFIES SUBSTANCES

Cramer Class II

- Common components of food
- Substances containing no functional groups other than alcohol, aldehyde, side-chain ketone, acid, ester, or sodium, potassium or calcium sulphonate or sulphamate, or acyclic acetal or ketal and it is either a monocycloalkanone or a bicyclic compound with or without a ring ketone

EXAMPLES OF HOW THE DECISION TREE CLASSIFIES SUBSTANCES

Cramer Class III

- Structures that contain elements other than carbon, hydrogen, oxygen, nitrogen or divalent sulphur
- Certain benzene derivatives
- Certain heterocyclic substances
- Aliphatic substances containing more than three types of functional groups.

IS THE CRAMER DECISION TREE DIFFICULT TO USE ?

- No – there is **Toxtree** software to help:
 - freely-available, downloadable, user-friendly
 - runs on Microsoft and other platforms
 - can be edited or modified to suit
- Software developed by Idea Consult under contract to the EC Joint Research Centre (JRC)
- Toxtree first version 2005, now running as version 2.6.0 (July 2013)

<http://sourceforge.net/projects/toxtree/>

IS THE CRAMER DECISION TREE DIFFICULT TO USE ?

- It allows a drawn chemical structure to be imported, or can use chemical name, CAS No or SMILES code
- It takes the structure sequentially through the questions until it gives an answer that allows the structure to be classified in either Cramer Class I, Class II or Class III

APPLYING QUESTIONS TO A QUERY SUBSTANCE

Rules

Rules

- Q1.Normal constituent of the body
- Q2.Contains functional groups associated with enhanced toxicity
- Q3.Contains elements other than C,H,O,N,divalent S
- Q4.Elements not listed in Q3 occurs only as a Na,K,Ca,Mg,N salt, sulphamate, sulph...
- Q5.Simply branched aliphatic hydrocarbon or a common carbohydrate
- Q6.Benzene derivative with certain substituents
- Q7.Heterocyclic
- Q8.Lactone or cyclic diester
- Q9.Lactone, fused to another ring, or 5- or 6-membered a,b-unsaturated lactone?
- Q10.3-membered heterocycle
- Q11.Has a heterocyclic ring with complex substituents.
- Q12.Heteroaromatic
- Q13.Does the ring bear any substituents?
- Q14.More than one aromatic ring
- Q15.Readily hydrolysed
- Q16.Common terpene
- Q17.Readily hydrolysed to a common terpene
- Q18.One of the list (see explanation)
- Q19.Open chain
- Q20.Aliphatic with some functional groups (see explanation)
- Q21.3 or more different functional groups
- Q22.Common component of food
- Q23.Aromatic
- Q24.Monocarbocyclic with simple substituents
- Q25.Cyclopropane, etc. (see explanation)
- Q26.Monocycloalkanone or a bicyclic compound
- Q27.Rings with substituents
- Q28.More than one aromatic ring
- Q29.Readily hydrolysed
- Q30.Aromatic Ring with complex substituents
- Q31.Is the substance an acyclic acetal or ester of substances defined in Q30?
- Q32.Contains only the functional groups listed in Q30 or Q31 and those listed below.
- Q33.Has sufficient number of sulphonate or sulphamate groups

1. Normal constituent of the body?

Decision node

Decision node: Q1.Normal constituent of the body

If 'NO' go to: Q2.Contains functional groups associated with enhanced toxicity

If 'YES' assign: Low (Class I)

Rule ID: 1 Rule title: Normal constituent of the body

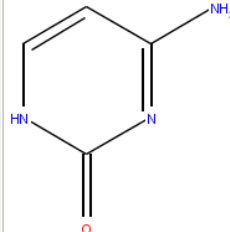
Rule explanation:

Is the substance a normal constituent of the body, or an optical isomer of such?

This question throws into class I all normal constituents of body tissues and fluids, including normal metabolites. Hormones are excluded, as are, by implication, the metabolites of environmental and food contaminants or those resulting from disease state.

Note the answer of the question relies on an incomplete list of compounds, identified by an expert as a normal body constituents. If you believe a query compound is wrongly identified as a such, or not recognised, please consult and/or update the list. C:\Ideas\consult\toxTree-v1.00\toxTree\bodymol.slf

Example with answer 'YES'



There are example molecules for each rule outcome. Select which one to display.

☒ Yes branch ☐ No branch

Yes - cytosine: Class I (low concern)
No proceed down the tree (Q2)

Slide from Andrew Worth JRC

APPLYING QUESTIONS TO A QUERY SUBSTANCE

Rules

Rules

- Q1.Normal constituent of the body
- Q2.Contains functional groups associated with enhanced toxicity
- Q3.Contains elements other than C,H,O,N,divalent S
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- Q9.Lactone, fused to another ring, or 5- or 6-membered α,β -unsaturated lactone?
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- Q17.Readily hydrolysed to a common terpene
- Q18.One of the list (see explanation)
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- Q20.Aliphatic with some functional groups (see explanation)
- Q21.3 or more different functional groups
- Q22.Common component of food**
- Q23.Aromatic
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- Q26.Monocycloalkanone or a bicyclic compound
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- Q32.Contains only the functional groups listed in Q30 or Q31 and those listed below.
- Q33.Has sufficient number of sulphonate or sulphamate groups

Decision node

Decision node: Q22.Common component of food

If 'NO' go to: Q33.Has sufficient number of sulphonate or sulphamate groups

If 'YES' assign: Intermediate (Class II)

Rule ID: 22 Rule title: Common component of food

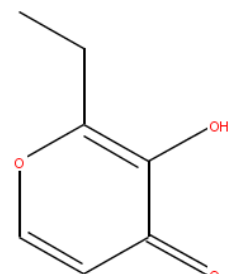
Rule explanation:

Is the substance a *common component of food* (C) or *structurally closed related* to a common component of food?

(C) Common component of food. In something as diverse, changing and occasionally uncertain as natural occurrence, it is only possible to define a guideline, not a firm rule. For a decision tree, the term common component of food denotes a substance that has been reported in the recognised literature as occurring in significant quantity (approximately 50 ppm or more) in at least one major food, or in trace quantities at the ppm level or less in several foods, including minor or less frequently consumed foods. The latter include spices, herbs and ethnic specialities. This definition excludes natural or man made contaminants and hormones.

Note the answer of the question relies on an incomplete list of compounds, identified by an expert as a common component of food. If you believe a query compound is wrongly identified as a such, or not recognised, please consult and/or update the list. *C:\ideaconsult\toxTree-v1.0\toxTree\foodmol.sdf*

Example with answer 'YES'



There are example molecules for each rule outcome. Select which one to display.

☒ Yes branch ☐ No branch

22. Common component of food?

Yes - ethyl maltol (flavour): Class II (intermediate class)

No proceed down the tree

APPLYING QUESTIONS TO A QUERY SUBSTANCE

Rules

Rules

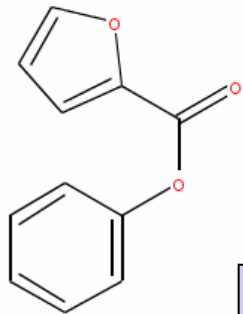
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29. Readily hydrolysed?

Rule title
Readily hydrolysed

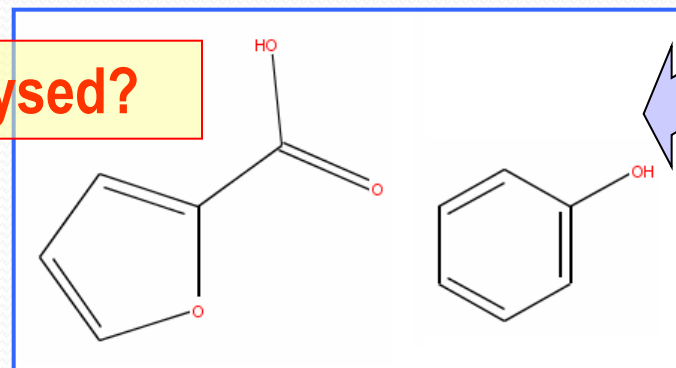
Rule explanation
Is it *readily hydrolysed*(H) to mononuclear residues? If YES, treat the mononuclear heterocyclic residues by Q.22 and any carbocyclic residue by Q16.

Example with answer 'YES'



There are example molecules for each rule outcome. Select which one to display.

☒ Yes branch ☐ No branch



Yes Treat the individual aromatic residues by Q30, and any other residues by Q19

No Proceed down the tree

TOXTREE MAIN SCREEN: EXAMPLE VINCLOZOLIN

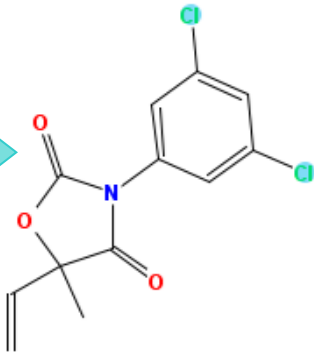
Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) v2.6.0

File Edit Chemical Compounds Toxic Hazard Method Help

Chemical identifier: vinclozolin **Go!**

Available structure attributes	
Cramer rules	High (Class III)
Names	vinclozolin
cdk:Comment	Retrieved from http://ap...
http://www.opentox.org...	50471-44-8
http://www.opentox.org...	N-3,5-dichlorophenyl-5-...
http://www.opentox.org...	256-599-6
http://www.opentox.org...	3-(3,5-dichlorophenyl)-5-...
http://www.opentox.org...	F5CWZHGZWDELK-LBP...
http://www.opentox.org...	InChI=1S/C12H9Cl2NO3...
http://www.opentox.org...	30.11.2010
http://www.opentox.org...	CC1(OC(=O)N(C1=O)c2...

Structure diagram



Toxic Hazard by Cramer rules

Estimate

Low (Class I)

Intermediate (Class II)

High (Class III)

☒ Verbose explanation

Cramer rules

- Q1. Normal constituent of the body **No** vinclozolin
- Q2. Contains functional groups associated with enhanced toxicity **No** vinclozolin
- Q3. Contains elements other than C,H,O,N,divalent S **Yes** vinclozolin
- Q4. Elements not listed in Q3 occurs only as a Na,K,Ca,Mg,N salt, sulphamate, sulphonate, sulphate, hydrochloride ... **No** Class **High (Class III)** vinclozolin

First Prev 1 / 1 Next Last

Completed.

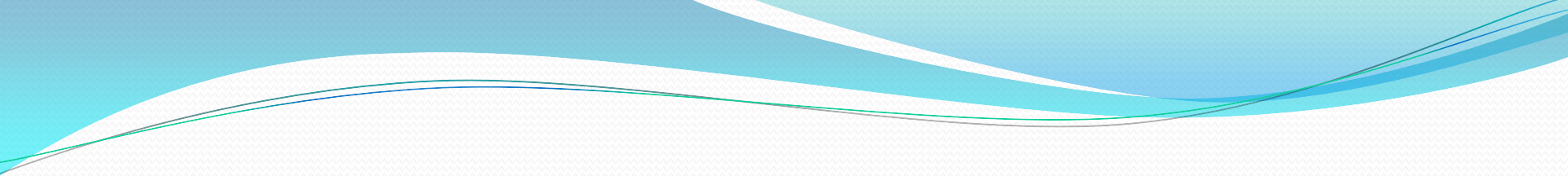
Compound properties

Prediction

Compound structure

Reasoning

Slide from
Andrew Worth
JRC



VALIDATIONS OF THE CRAMER DECISION TREE

VALIDATION BY CRAMER ET AL

- In 1978 Cramer, Ford & Hall validated their decision tree against NOELs of 81 substances with data on toxicological properties (pesticides, drugs, food additives, industrial chemicals)
- The NOEL distributions of the three classes were reasonably well separated, with some overlap
- They acknowledged the questions were a compromise between simple discrimination and complexity and that the decision tree could be further refined

JRC EVALUATION OF TOXTREE-CRAMER

Survey of Toxtree users (Lapenna & Worth, 2011, JRC report EUR 24898 EN)

- Many original Cramer rules are written in a confusing and inter-dependent way, which leads to difficulties in rationalising the predictions they make
- Two rules are not based on chemical features, but simply make reference to look-up lists of chemicals (Q1, normal body constituents; Q22, common food components)
- Some rules make ambiguous references to chemical features (e.g. steric hindrance) which need to be clarified and possibly revised/deleted
- Several studies have identified outliers (e.g. Class I compounds that have low NOELs). A revised/alternative classification scheme should be more discriminating in terms of NOEL values

→ need to update Cramer classification scheme

JRC MODIFICATIONS TO CRAMER DECISION TREE

Introduced an extended rulebase as an option in Toxtree because the original Cramer rulebase misclassifies some substances in Class I or II despite low NOELs (high toxicity) and vice versa

Extended Cramer rule base

- Recognises more substances as natural constituents of the body (67→400)
- Allows harmless phosphates to be identified (no longer automatically assigned to Class III)
- Classifies more benzene-like substances as Class III (i.e. any benzene ring with 0 – 6 single atom substituents)
- Recognises potential toxicity of non-natural divalent sulfur-containing compounds by assigning to Class III
- Classifies α,β unsaturated compounds as Class III instead of Class I or II

***S-IN* SOLUZIONE INFORMATICHE ANALYSIS OF CRAMER DECISION TREE**

- Used experimental data on chronic toxicity of chemicals in the Munro et al. database and the Carcinogenic Potency Database
- An experimental classification was obtained by categorising chronic toxicity NOEL values (Munro) or TD50 values (CPDB) according to arbitrary defined thresholds, designed so that classes were roughly homogeneously populated

S-IN CRAMER SCHEME EVALUATION

Munro dataset experimental classification

Categorisation of the $\text{Log}(1/\text{NOEL})$ values

Hazard level	$\text{Log}(1/\text{NOEL})$ (mol/kg/day)	Experimental hazard class	# structures
Low hazard	$\text{Log}(1/\text{NOEL}) < 0.2$	1	168
Medium hazard	$0.2 \leq \text{Log}(1/\text{NOEL}) < 1.5$	2	227
High hazard	$\text{Log}(1/\text{NOEL}) \geq 1.5$	3	192

Slide from *S-IN*

S-IN CRAMER SCHEME EVALUATION

Munro dataset

Experimental Hazard classes	Cramer hazard classes			Total
	Class I (low hazard)	Class II (medium hazard)	Class III (high hazard)	
Class 1 (low hazard)	80	11	77	168
Class 2 (medium hazard)	37	16	177	227
Class 3 (high hazard)	10	3	179	192
Total	127	27	433	587

- **74%** (433/587) classified in Class III (High hazard)
- **Less than 5%** (10/192) of the experimentally high hazard structures are classified as low hazard

S-IN CRAMER SCHEME EVALUATION


CPDB dataset experimental classification

Categorisation of the $\text{Log}(1/\text{TD50})$ values combined with Salmonella test results

Experimental class	Experimental hazard class code	Salmonella and $\text{Log}(1/\text{TD50})$ values	# structures
Non mutagen in Ames test and low potency carcinogen	1	Negative Ames test AND $\text{Log}(1/\text{TD50}) < 0$	65
Non mutagen in Ames test but high potency carcinogen	2	Negative Ames test AND $\text{Log}(1/\text{TD50}) > 0$	117
Mutagen in Ames test	3	Positive Ames test	279
Total			461

S-IN CRAMER SCHEME EVALUATION

CPDB dataset

Experimental Hazard classes	Cramer hazard classes			Total
	Class I (low hazard)	Class II (medium hazard)	Class III (high hazard)	
Class 1 (non mutagen in Ames; low carcinogen)	25	2	38	65
Class 2 (non mutagen in Ames; high carcinogen)	 10	2	105	117
Class 3 (mutagen in Ames)	12	1	266	279
Total	47	5	409	461

- **89%** (409/461) classified in Class III (High hazard)
- **8.5%** (10/117) of the experimentally carcinogenic structures are classified as low hazard

CONCLUSIONS OF S-IN CRAMER SCHEME EVALUATION

- The Cramer scheme is highly conservative
- It performs better in identifying high hazard compounds than low hazard ones
- Misclassification is possible but Cramer scheme minimises number of experimentally high hazard structures classified as low hazard (less than 5% in both datasets)
- Use of structural subclasses within Cramer I and III, or use of a ranking classification model were not significantly better than Cramer scheme

CRAMER CLASSIFICATION SCHEME COMPARED WITH GHS

Kalkhof et al. Arch. Toxicol. 86, 17-25, 2012

- For over 800 substances tested according to standard OECD 28-day and 90-day toxicity tests, they compared Cramer classification with UN Globally Harmonised System of classification and labelling based on NOAELs /LOAELs
- 90% were classified in Cramer Class III
- Only 22% were classified by GHS in highest toxicity category

Cramer over-predicts toxicity, illustrating it is conservative

EFSA 2012 RECOMMENDATIONS ON CRAMER CLASSIFICATION SCHEME

- Cramer classification scheme should be revised and refined in light of knowledge since 1978
- Cramer Class II substances should be treated as Class III because Class II TTC value based on very few substances
- OPs and carbamates (Class III) should be identified and a lower TTC value for that class applied
- Nevertheless, application of the existing Cramer classification scheme is conservative and therefore protective of human health



EFSA GENERIC SCHEME FOR TTC

Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC)

EFSA Journal 2012;10(7):2750

Does the substance have a known structure and are exposure data available?

No

TTC approach cannot be applied

Yes

Is the substance a member of an exclusion category? *

Yes

No

Is there a structural alert for genotoxicity (including metabolites)?

Yes

Exposure > 0.0025 µg/kg bw/day?

Yes

No

Low probability of health effect **

Substance requires non-TTC approach (toxicity data, read-across, etc)

Low probability of health effect **

No

No

Exposure > 0.3 µg/kg bw/day? ***

Yes

Is substance an OP/Carbamate?

Yes

No

No

Exposure > 1.5 µg/kg bw/day? ***

Yes

Is substance in Cramer Class II or III?

Yes

No

No

Exposure > 30 µg/kg bw/day? ***

Yes

*** Exclusion categories**

High potency carcinogens; Inorganic substances; Metals and organometallics; Proteins; Steroids; Substances known/predicted to bioaccumulate; Nanomaterials; Radioactive substances; Mixtures.

**** If exposure of infants < 6 months is in range of TTC
→ consider if TTC is applicable**

***** If exposure only short duration
→ consider margin between human exposure & TTC value**

SUMMARY & CONCLUSIONS

- Using the Cramer decision tree, the chances of misclassifying a high hazard substance as low hazard range from zero to 5%
- The decision tree could undoubtedly be improved by some further revisions and refinements
- Refinement by subdivision of the structural classes into many other classes, each with their own TTC value, would become read-across rather than a general tool
- Cramer decision tree is sufficiently conservative that it can be used in its original form (or with Toxtree extended rulebase) for the TTC approach