# Cramer classes in the TTC — fit for purpose ?

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# APPLICATION OF TTC APPROACH WHAT INFORMATION DO WE NEED?

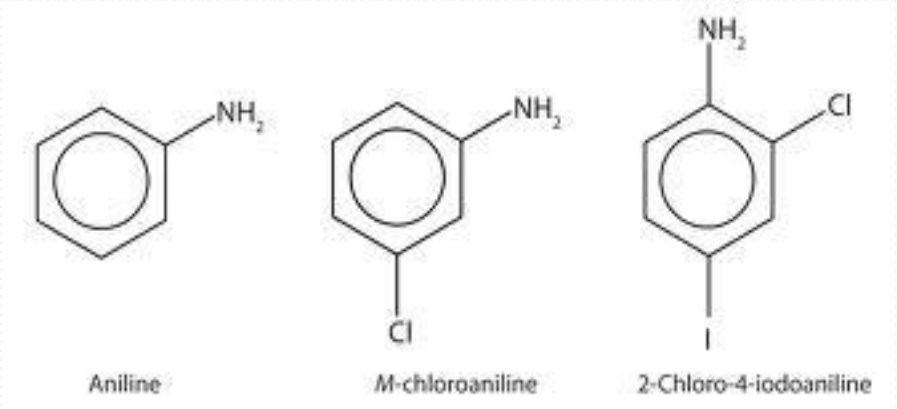
- Chemical structure

- 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

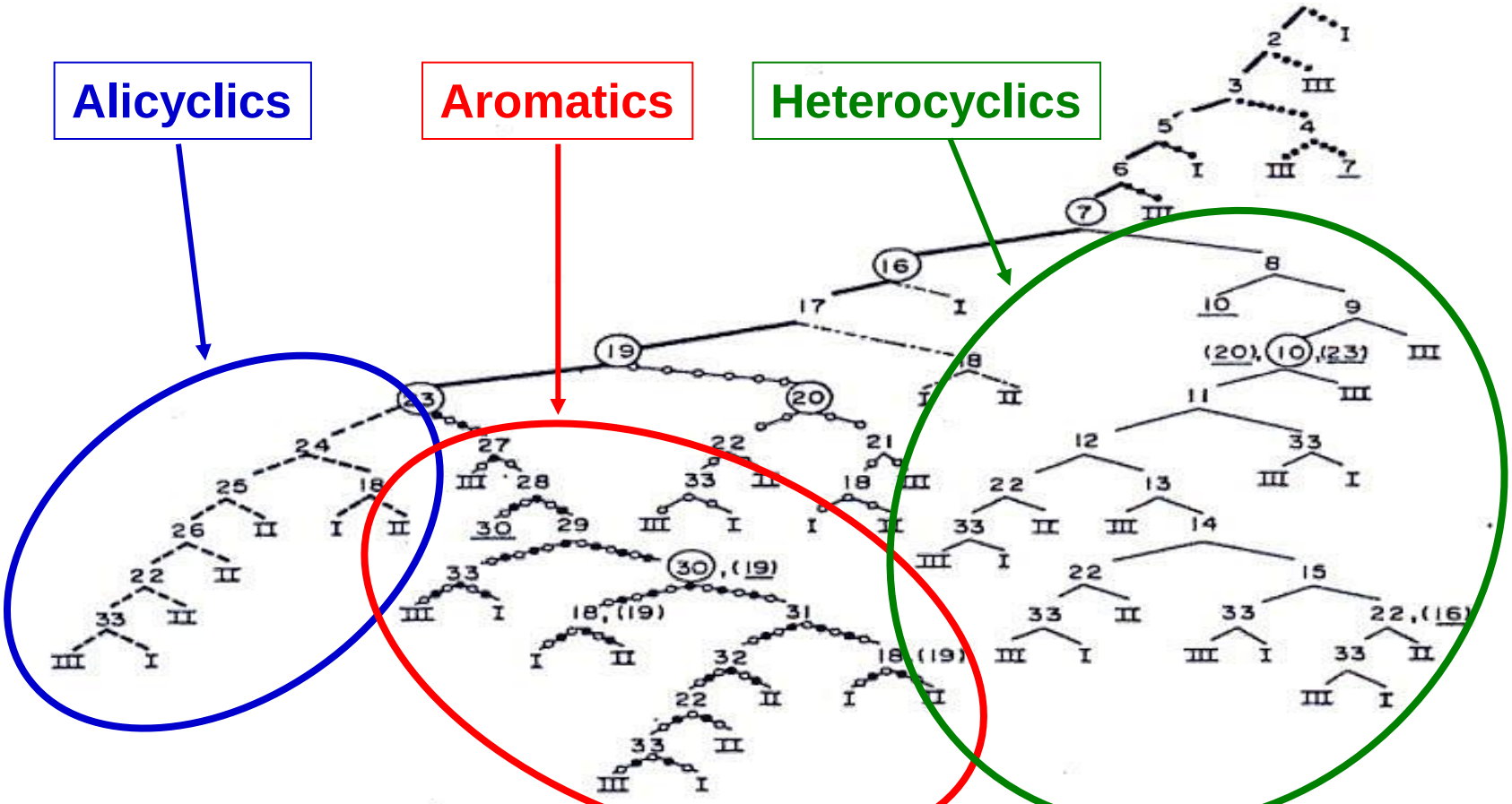
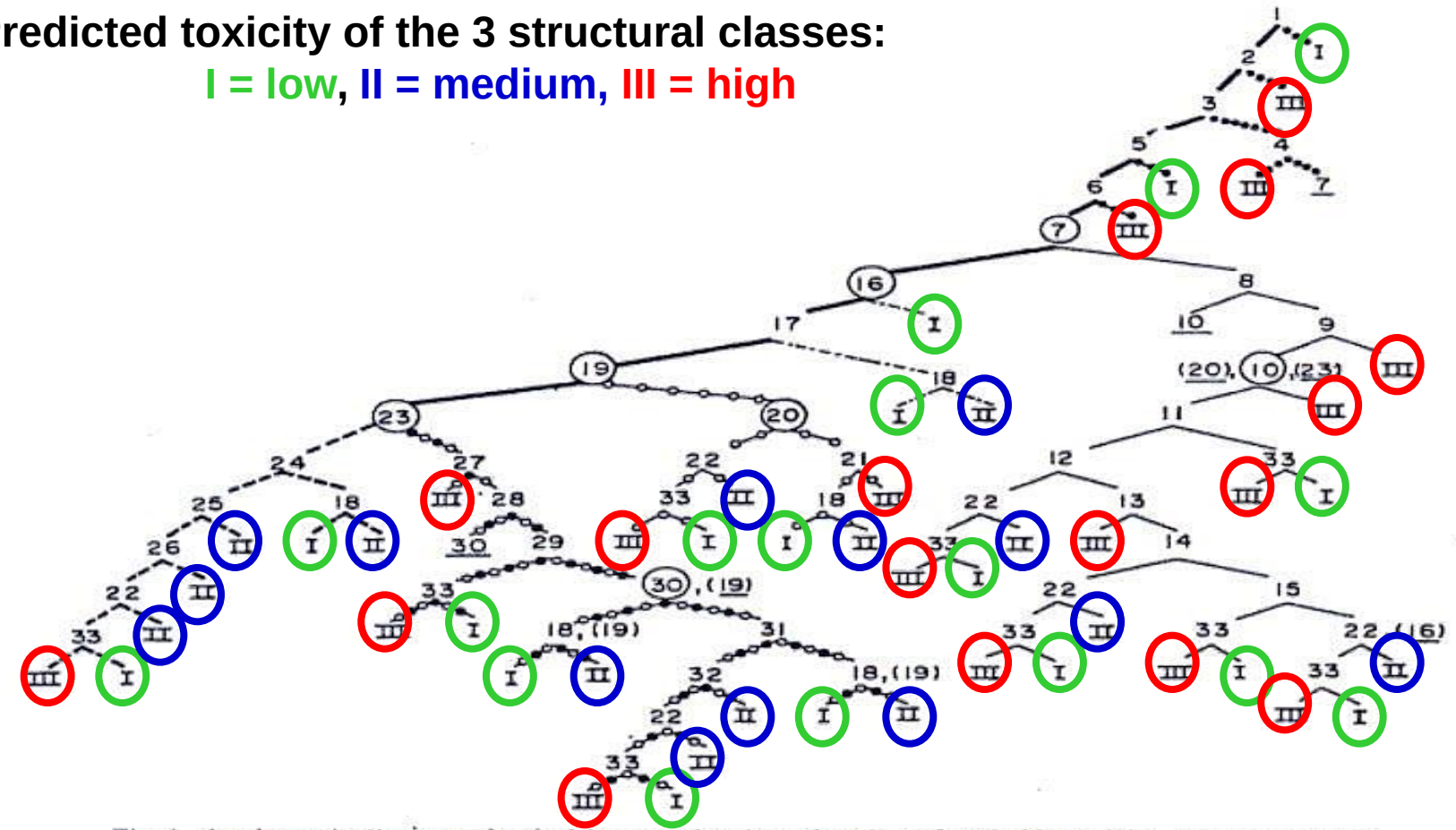



Fig. 1. A schematic diagram of a decision tree for the estimation of probable toxicity. Assessors should (a) start with question 1, (b) proceed by 'no' or 'yes', (c) move from any underscored number encountered to same circled number and (d) proceed to final classes I, II or III. Working downwards through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

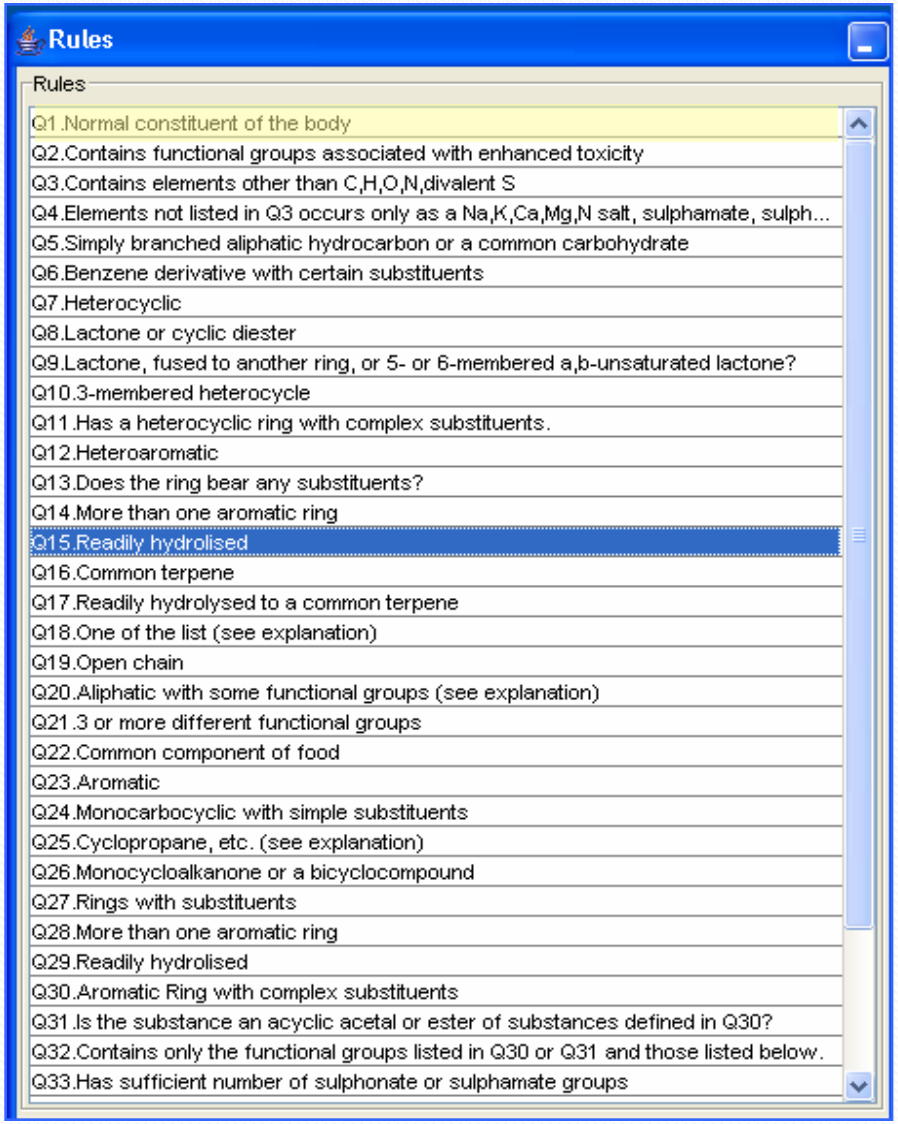
- No – there is **Toxtree** software to help:
  - freely-available, downloadable, user-friendly
  - runs on Microsoft and other platforms
  - can be edited or modified to suit
- Software developed by Idea Consult under contract to the EC Joint Research Centre (JRC)
- Toxtree first version 2005, now running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

**http://sourceforge.net/projects/toxtree/**

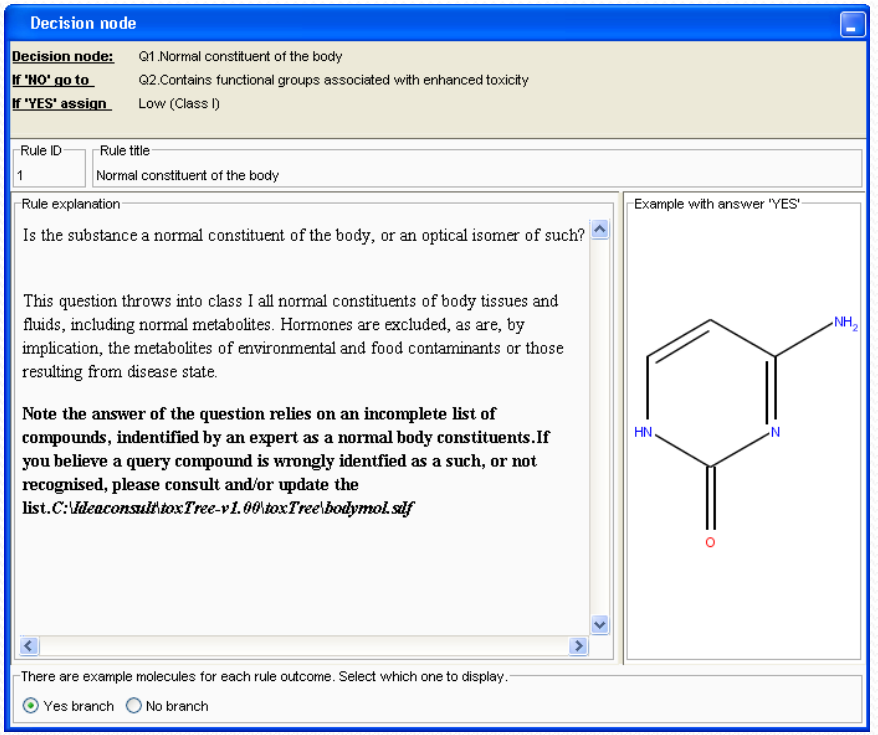
# IS THE CRAMER DECISION TREE DIFFICULT TO USE ?

- 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




## 1. Normal constituent of the body?

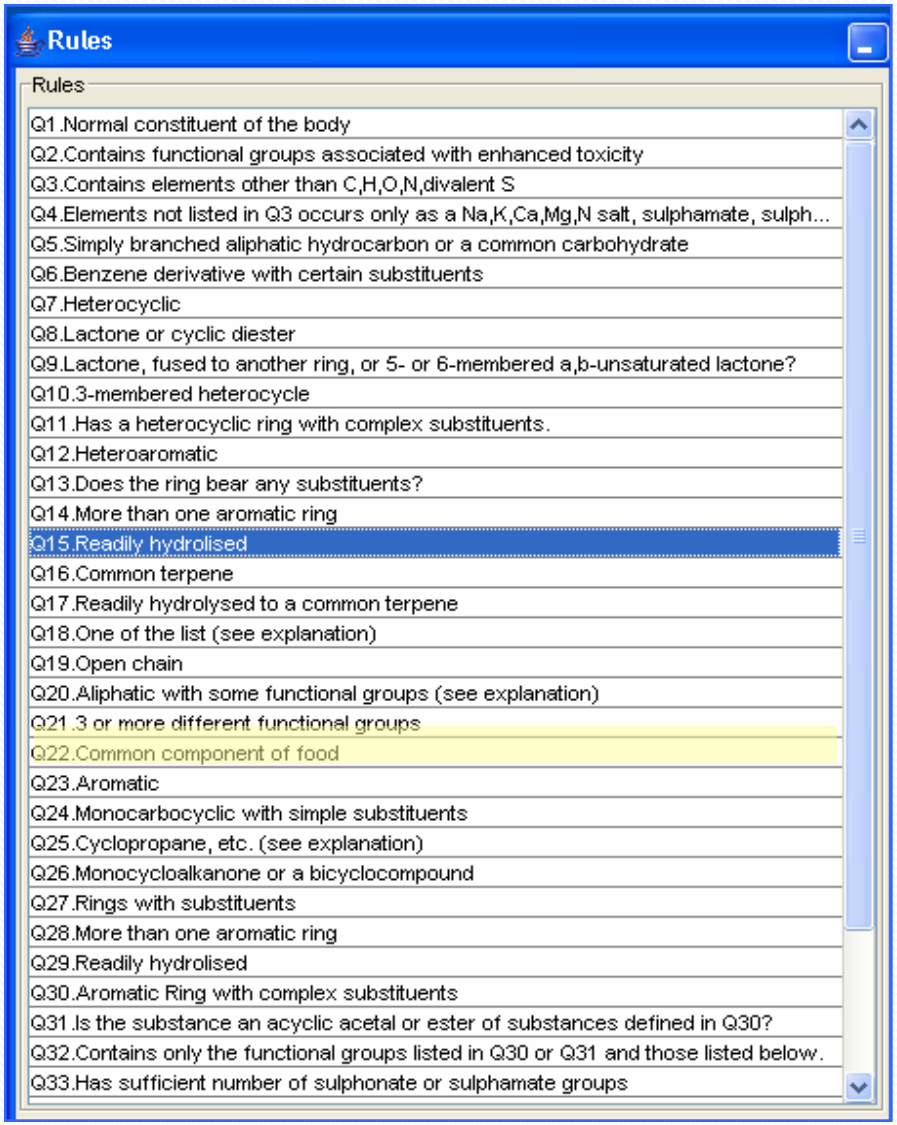


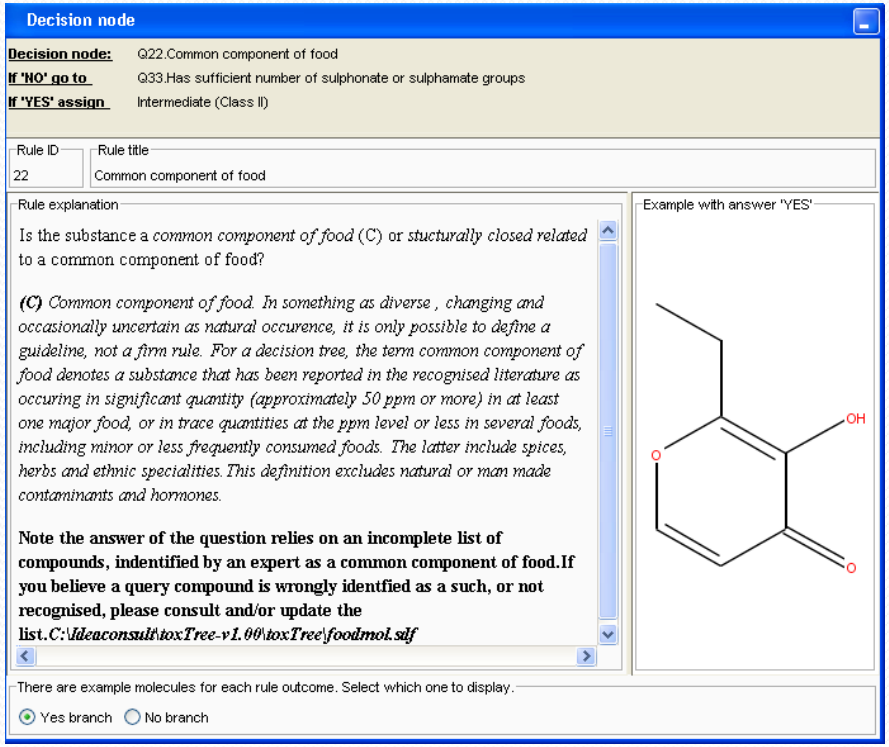

***Yes* - cytosine: *Class I (low concern)***

***No proceed down the tree (Q2)***

Slide from Andrew Worth JRC

# APPLYING QUESTIONS TO A QUERY SUBSTANCE




## 22. Common component of food?

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

***No proceed down the tree***

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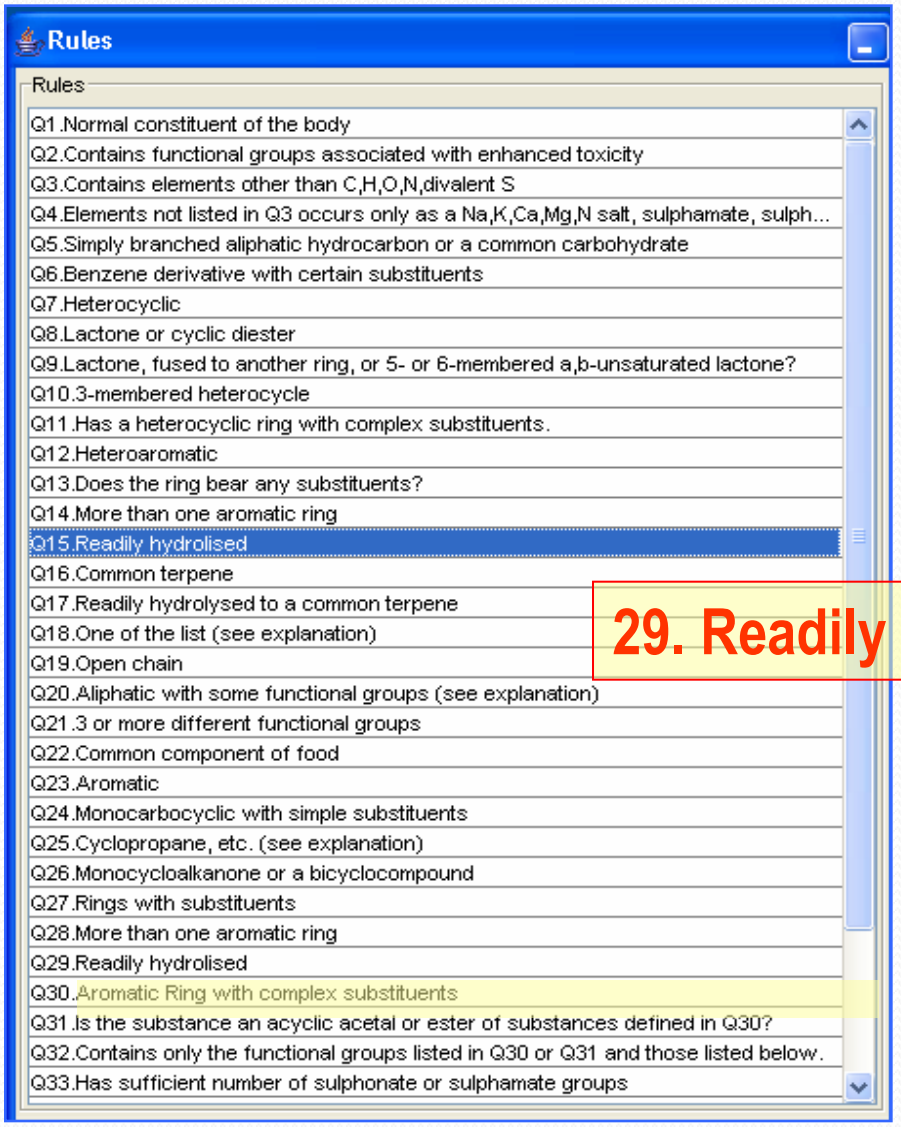
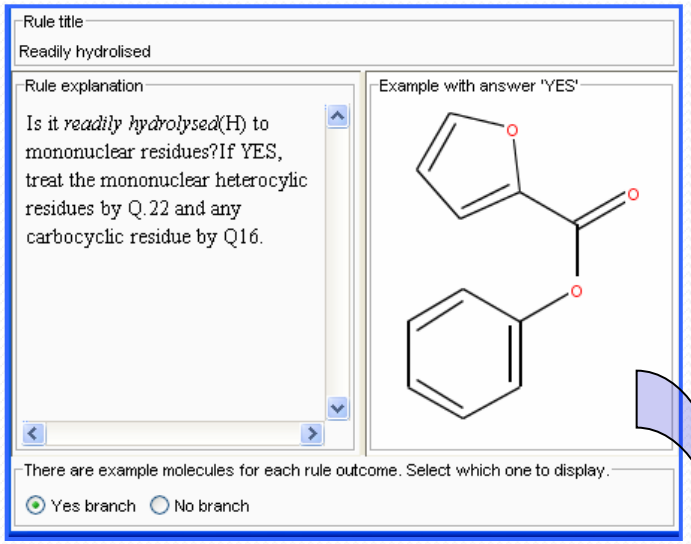
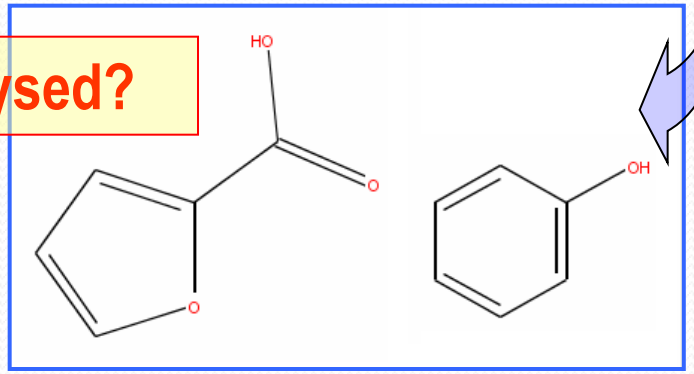
# APPLYING QUESTIONS TO A QUERY SUBSTANCE




**29. Readily hydrolysed?**

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

***No Proceed down the tree***

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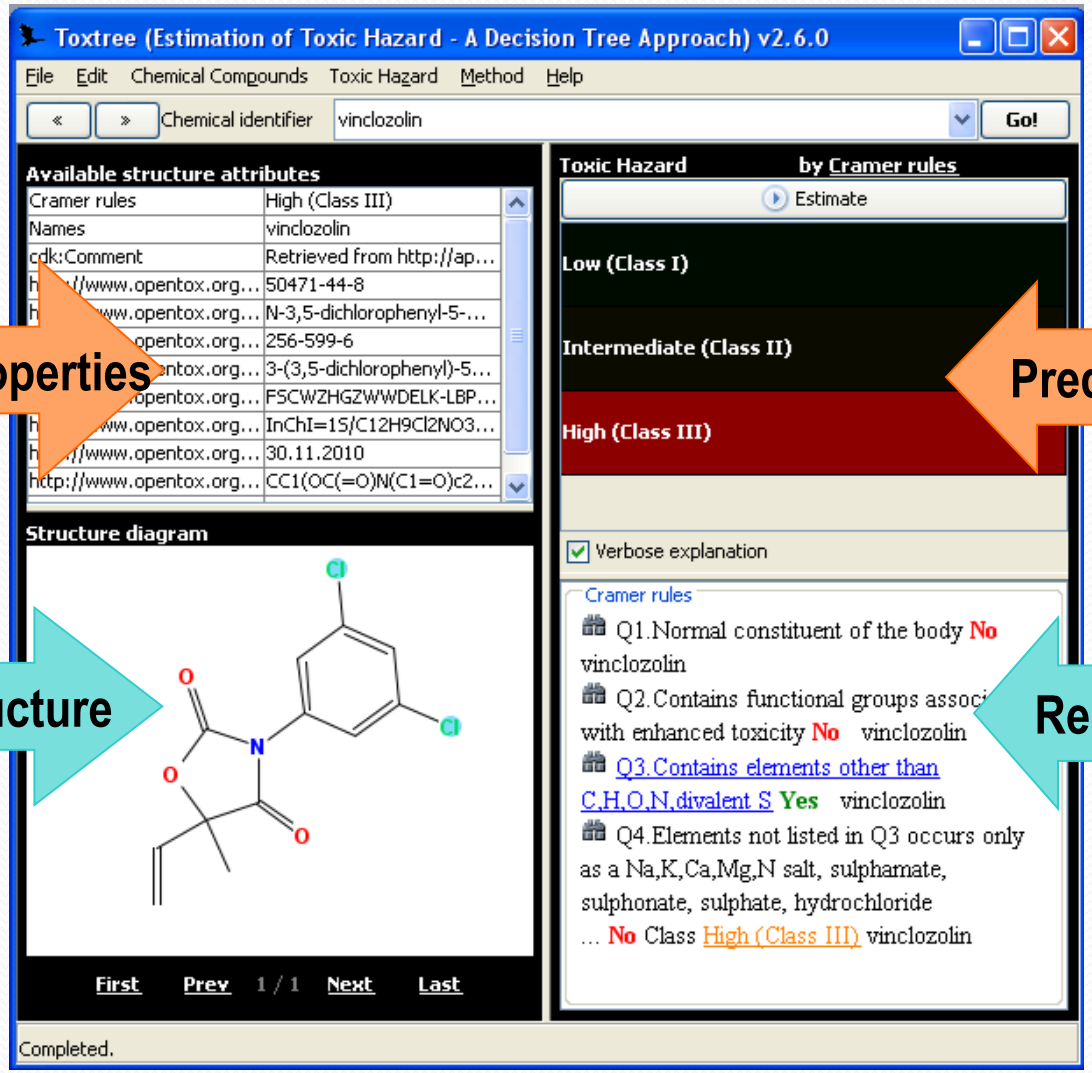
# TOXTREE MAIN SCREEN: EXAMPLE VINCLOZOLIN




**Compound properties**

**Compound structure**

**Prediction**

**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 ANLYSIS 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 Log(1/NOEL) values

| Hazard level | Log(1/NOEL) (mol/kg/day) | Experimental hazard class | # structures |
|---|---|---|---|
| Low hazard | Log(1/NOEL) < 0.2 | 1 | 168 |
| Medium hazard | 0.2 ≤ Log(1/NOEL) < 1.5 | 2 | 227 |
| High hazard | Log(1/NOEL) ≥ 1.5 | 3 | 192 |

**Slide from *S-IN***

# *S-IN* CRAMER SCHEME EVALUATION

**Munro dataset**

| Experimental Hazard classes | Cramer hazard classes | | | |
|---|---|---|---|---|
| | Class I (low hazard) | Class II (medium hazard) | Class III (high hazard) | Total |
| 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

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# *S-IN* CRAMER SCHEME EVALUATION

## CPDB dataset experimental classification

Categorisation of the Log(1/TD50) values combined with Salmonella test results

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

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# *S-IN* CRAMER SCHEME EVALUATION

**CPDB dataset**

| Experimental Hazard classes | Cramer hazard classes | | | |
|---|---|---|---|---|
| | Class I (low hazard) | Class II (medium hazard) | Class III (high hazard) | Total |
| 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

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# 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**

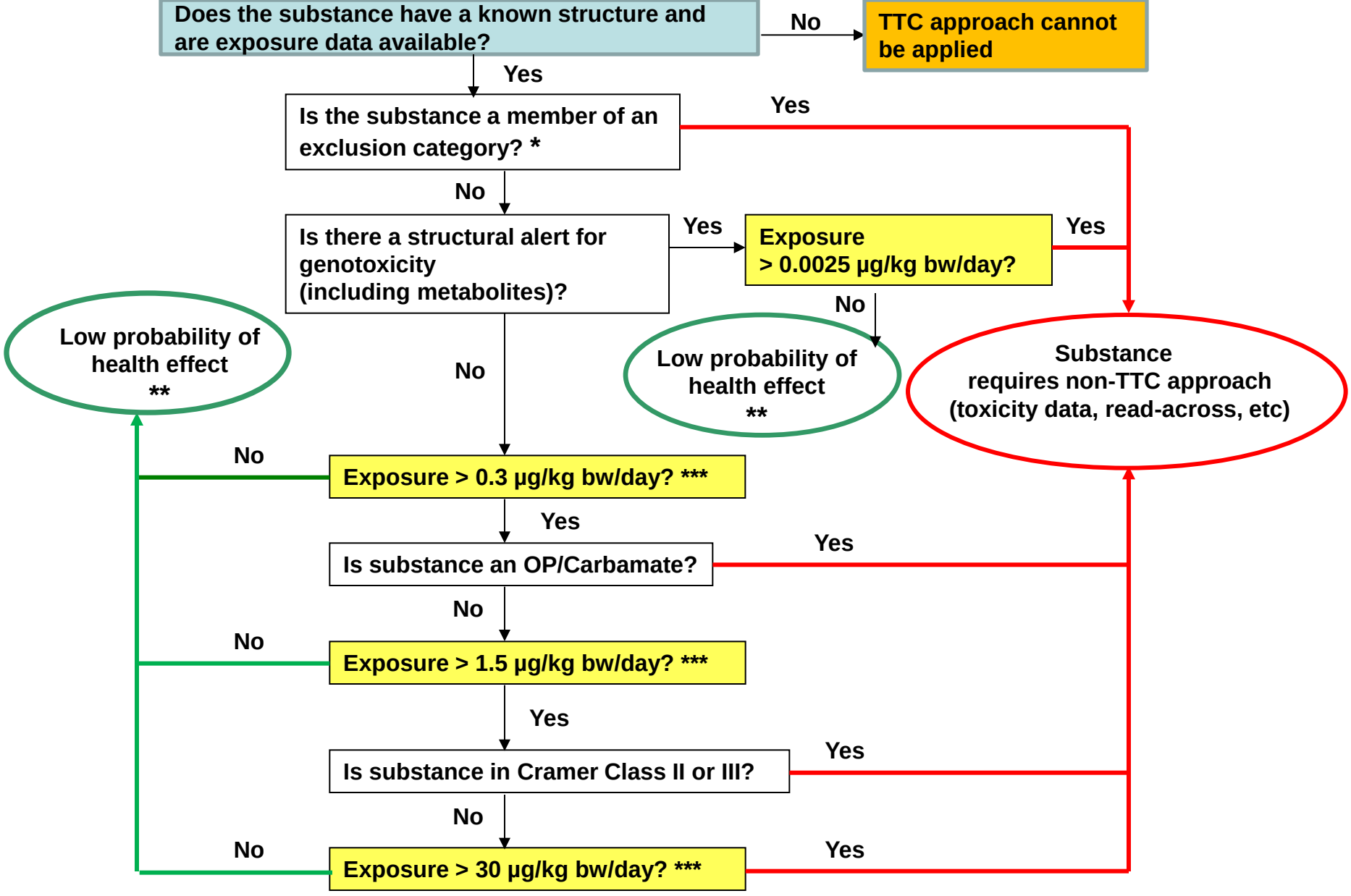
# EFSA 2012 RECOMMENDATIONS ON CRAMER CLASSIFICATION SCHEME

- 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**




*** 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