Refinement of TTC values - Identification of outliers in Cramer class I - III

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Introduction

- The TTC approach is envisaged to be used for exposure based waiving to reduce animal testing e.g. under REACH
- **TTC** = **T**hreshold of **T**oxicological **C**oncern
  - Threshold values for compounds with **known chemical structure** but **unknown toxicity** ➡ maximum human intake below which no risk for human health is assumed (ILSI Europe 2005)
- Aim of the current project: Analysis of outliers to reduce the overlap of Cramer class I (non toxic) to Cramer class III (toxic) (Cramer et al. 1978)

Method

- Combination of databases RepDose and Munro; focus on repeated dose studies with rats/mice; exclusion of genotoxic compounds using the software ToxTree
- Assessment factors for LOEL-NOEL, species and study duration extrapolation were applied to standardize L(N)OEL values and to reduce data variability
- Outliers are identified by applying cut-off values to exclude high toxic chemicals from Cramer class I and low toxic chemicals from Cramer class III
  - Class I: N = 122; cut-off value: NOELs ≤ 0.1 mmol/kg bw/d (~ 12 mg/kg bw/d); $N_{outlier}$ = 57
  - Class III: N = 266; cut-off value: NOELs ≥ 0.01 mmol/kg bw/d (~ 2 mg/kg bw/d); $N_{outlier}$ = 124

Results I

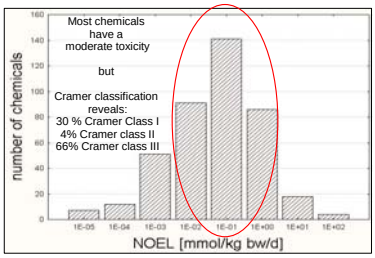


Fig.1 Distribution of toxicity for the oral pathway

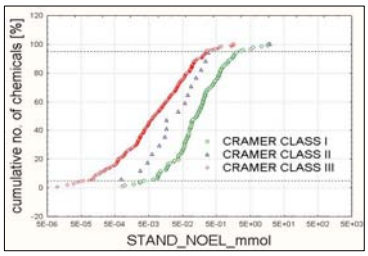


Fig.2 Cumulative distribution of oral NOELs within the three Cramer classes

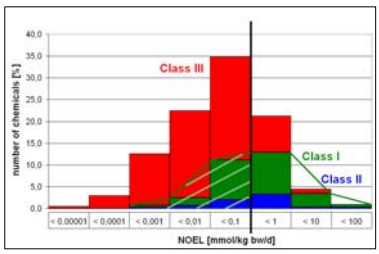


Fig.3 Histogram of oral NOELs within the three Cramer Classes and outlier of class I

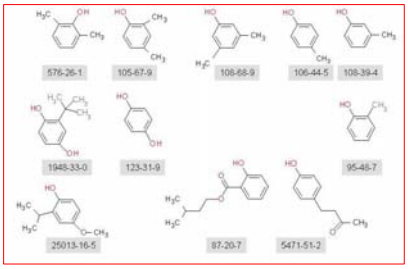
- Analysis of the combined databases RepDose and Munro reveals for oral exposure:
  - Most chemicals have a moderate toxicity, but only 4% of the analysed substances belong to Cramer class II (Fig. 1)
  - The three Cramer classes of low, intermediate and high toxicity are not clearly distinguished
    - ➡ this is due to chemicals with very high toxicity in Cramer class I and chemicals with low toxicity in Cramer class III
- If cumulative distribution of data are shown (Fig.2), Cramer classes appear to be quite separated, but Fig. 3 shows a great overlap of Cramer class I, II and III

Analysis of outliers in Cramer class I

- Cramer class I outliers were visualized and analyzed with the OECD (Q)SAR Application Toolbox
- 11 **phenol derivatives** were identified as one group of outliers
- "Similarity search" within Cramer class I identified 10 **"other" phenols**
- These two groups have different structural characteristics (as follows):

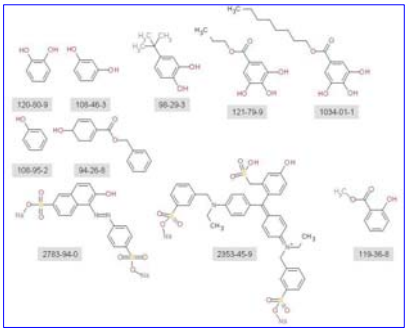
- Phenol derivatives:
  - Alkylated phenols in meta, ortho or para position
  - ➡ steric hindrance
  - Phenols with two hydroxy groups in para position
  - ➡Toxicity can be explained by oxidative stress

N = 11

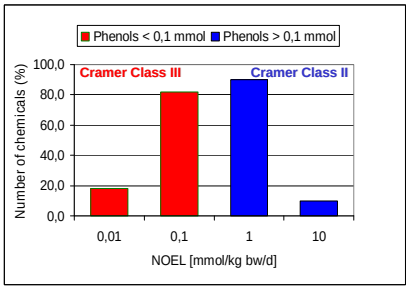


- "Other" phenols:
  - Non alkylated phenol
  - ➡ First pass effect
  - Two or more hydroxy groups in meta position (oxidative stress not possible – less toxic than para position)
  - Two sulphonated substances, that are readily excreted by the organism

N = 10



- Toxicity-analysis:
  - Shows 2 groups of toxicity
  - The outlier phenols have NOELs between 0.01 and 0.1 mmol/kg bw/d and can be allocated to Cramer class 3
  - The "other" phenols have NOELs between 0.1 and 10 mmol/kg bw/d and can be allocated to Cramer class 2



Results II - outlier analysis

- Analysis of "outliers" using structural and toxicity data is a successful strategy to refine the Cramer classes
- Revised data show shifted graphs for all classes (Fig.4)
- Due to the "new classified" phenolic compounds class II and III comprise more data (higher graph) and class I less

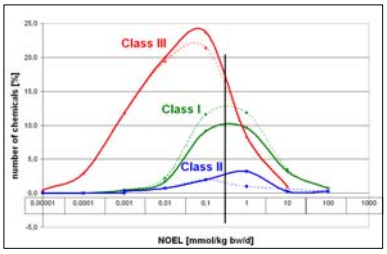


Fig.4 Histogram of the new classified data (old data showed with dashed lines)

Results III - OECD guideline specific thresholds

Tab.1: 5th percentiles of standardized data for NOEL values and related calculated thresholds

Study duration	OECD Guideline	N_{NOEL}	5th NOEL [mmol]	Threshold NOEL [µmol/person*d]	General threshold [µg/person/day]	Lowest threshold [µg/person/day]
Oral NOELs						
Subacute	407	152	$1.3*10^{-4}$	0.31	54.4	37.6
Subchronic	408	376	$2*10^{-4}$	0.48	83.6	
Chronic	451/452/453	311	$9*10^{-5}$	0.22	37.6	
Inhalation NOELs						
Subacute	412	77	$2.1*10^{-4}$	0.5	87.6	12.4
Subchronic	413	125	$3*10^{-5}$	0.07	12.4	
Chronic	451/452/453	72	$4*10^{-5}$	0.1	16.8	

- Question: Are OECD related TTC values useful for e.g. exposure based waiving?
- Lowest threshold for all endpoints is ~ 13 mg/person/d (3fold lower than oral TTC)
- Analysis revealed no major differences in thresholds for different study durations
- Influence of the chemical on the TTC is higher than the influence of study durations

Perspectives

- Exclude more groups of outliers with moderate toxicity from Cramer class III (toxic)
- "Fill up" the underrepresented Cramer class II (moderate toxic)
  - ➡ to display the distribution of toxicity within the databases more realistic
- The "grouped" outliers can then be used to refine critical classifications of the Cramer decision tree

Acknowledgement

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