Exploring potential refinements to existing Threshold of Toxicological Concern values for environmentally-relevant chemicals

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Background and Objectives

- The Threshold of Toxicological Concern (TTC) is an exposure threshold below which there is expected to be no appreciable risk to human health
- Munro et al (1996) developed TTCs based upon non-cancer effects
- To achieve this chemicals were grouped using the Cramer decision tree, a distribution was fitted to associated No Observable (Adverse) Effect Level (NO(A)EL) data from repeat dose toxicity studies, finally 5th percentile values were calculated and adjusted using a default safety factor of 100
- TTC was originally developed to facilitate assessments of food additives, flavourings, and contact materials
- Recently, Patlewicz et al (2018) utilised TTC, in conjunction with high-throughput exposure estimates, to prioritise large numbers of chemicals based upon their concern level
- In this study, we wanted to address several questions regarding whether the previously developed TTC values were relevant for the types of chemicals of interest to EPA
- To do this we extracted data from US EPA's Toxicity Values (ToxVal) database, which aggregates *in vivo* testing data from over 40 sources including US federal and state agencies, as well as international agencies such as the European Chemicals Agency and the World Health Organisation (Williams et al, 2017)
- ToxVal is available via the US EPA's CompTox Chemicals Dashboard (comptox.epa.gov/dashboard)

- Using these data our objectives were:
  - Reproduce the TTC values developed by Munro et al (1996)
  - Follow the Kroes et al (2004) workflow to assign substances present in ToxVal to their respective Cramer classes and use the associated repeat dose toxicity data to derive new TTC values
  - Evaluate whether the TTC values from ToxVal and Munro are statistically equivalent
  - Derive confidence intervals for the new TTC values
  - Compare and contrast the chemistry of the two data sets to rationalise any (dis)similarities in TTC values

Methods and Analysis

TTC Class	Nº. chemicals
Cramer Class I	1,476
Cramer Class II	162
Cramer Class III	1,673
Alert for genotoxicity	1,025
OPs and carbamates	102
Not Applicable	114

Table 1. Number of chemicals from ToxVal with QSAR ready SMILES that were profiled into the different TTC classes. For the remainder of the study we only focus on those chemicals profiled into one of the three Cramer classes

Datasets

1. US EPAs ToxVal
2. Munro et al (1996)

Study Inclusion Criteria

- Study duration:
  - (Sub)-chronic,
  - Reproductive,
  - Developmental, or
  - Multigenerational
- Route of Exposure:
  - Oral
- Species:
  - Rodents
- Units:
  - mg/kg-day

Chemical collection and profiling (ToxVal)

- 4,554 chemicals with QSAR ready SMILES were extracted from ToxVal
- These chemicals were profiled in each of five modules using Toxtree(v3.1.0):
  - Cramer (original)
  - Kroes
  - Carbamates
  - Organophosphates (OPs)
  - Steroids
- The last three modules were developed *ad hoc* for Patlewicz et al (2018)

Data extraction and removal of outliers

- Chemicals assigned to Cramer Class I, II, or III were separated and data were extracted from ToxVal that met study criteria from Munro et al (1996)
- Sub-chronic data were divided by a factor of 3 per Munro et al (1996)
- Extreme outliers were removed (Figure 1)
- Minimum NO(A)EL taken for each chemical

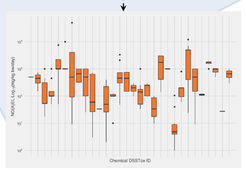

Figure 1. Distribution of NO(A)EL values from ToxVal for chemicals in Cramer Class II. Points were removed as lying outside of Tukey fence (1.5x IQR)

Estimation of 5th percentile NO(A)EL

- Cumulative distribution plotted for each Cramer class and fitted with lognormal distribution (Figure 2)
- Kolmogorov-Smirnov (K-S) test used to identify if distributions differed significantly between Cramer classes from ToxVal data
- Identified 5th percentile NO(A)EL for each Cramer class from ToxVal (Table 2)
- The associated TTC values can be calculated by dividing the 5th percentile values by 100

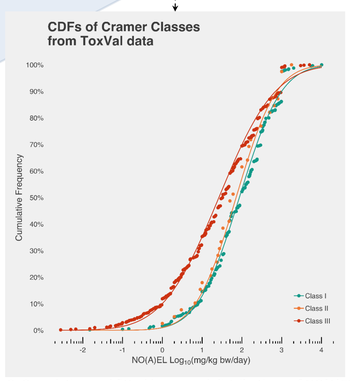

Figure 2. Cumulative and fitted lognormal distributions of NO(A)EL values from ToxVal for chemicals in Cramer Classes I, II, and III. Only the distributions for Cramer classes I and III differ significantly ($p < 0.05$).

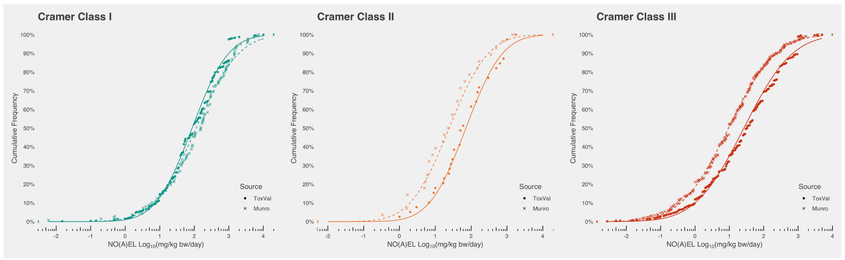

Figure 3. Comparison of cumulative and fitted lognormal distributions for ToxVal and Munro NO(A)EL data for each Cramer class. Only the distributions for Cramer class III were seen to be significantly different between the two data sets ($p < 0.05$)

Cramer Class	Nº. chemicals (ToxVal)	ToxVal 5th %ile (mg/kg-day)	No. chemicals (Munro)	Munro 5th %ile (mg/kg-day)
Class I	565	3.73 (2.97-4.79)	137	3.0 (1.71-5.31)
Class II	39	3.46 (1.5-8.63)	28	0.91 (0.32-3.02)
Class III	700	0.39 (0.3-0.53)	448	0.15 (0.11-0.22)

Table 2. Comparison of 5th percentile values for each Cramer class for ToxVal and Munro data sets (with 95% confidence intervals in parentheses).

Comparison between ToxVal and Munro

- K-S test used to identify if distributions for each Cramer class between the ToxVal and Munro data sets differed significantly (Figure 3)
- Used R (v3.5.1) to compare 5th percentile values for each Cramer class between ToxVal and Munro
- Performed bootstrap sampling to calculate confidence intervals around the 5th percentile values for each data set and Cramer class (Figure 4)

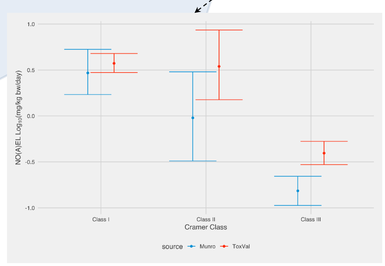

Figure 4. Fifth percentile values identified for each Cramer class from ToxVal and Munro, including confidence intervals calculated using 5000 bootstrap samples. Cramer class III 5th percentile values differ significantly between the two data sets ($p < 0.05$)

Method used for separation	Number of chemicals retained	Re-derived 5th percentile (mg/kg-day)	Number of chemicals removed	Removed chemical 5th percentile (mg/kg-day)	Statistically different from ToxValDB class III 5th percentile
Chemotype enrichment	306	0.22	142	0.075	No
Original SMARTS	386	0.2	62	0.056	No
Updated SMARTS	397	0.23	51	0.037	No

Table 3. Comparison of the 5th percentile values for the Munro Cramer class III chemicals that were retained and removed after utilising different methods.

Investigation of Cramer class III 5th percentile discrepancies between datasets

- Used three methods:
  - Chemotype enrichment
  - OP/carbamate SMARTS from Patlewicz et al (2018)
  - Generation of more specific OP/carbamates SMARTS using OPs/carbamates manually identified by Leeman et al (2014)
- Investigated Cumulative Distribution Functions (CDFs) of retained and removed chemicals to determine whether there was an overlap in distribution (Figure 5)
- Calculated 5th percentile of retained and removed chemicals (Table 3)
- Used bootstrapping to compare these values to those from ToxVal Cramer class III
- Presence of OP/carbamates in Munro class III largely explained the difference in 5th percentiles between data sets
- Refined SMARTS were better able to identify OP/carbamate insecticides that act via AChE inhibition than the original SMARTS

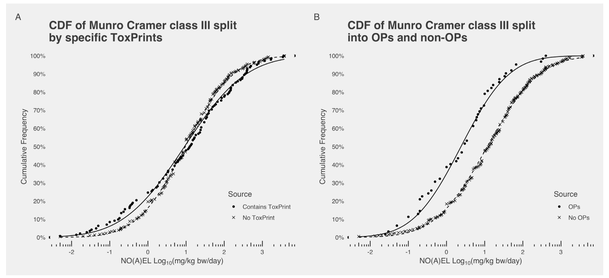

Figure 5. Cumulative distribution function and fitted lognormal distribution for the Munro Cramer class III chemicals after being split using A) ToxPrints identified using chemotype enrichment (not significantly different) and B) the OP/carbamates modules developed by Patlewicz et al (2018) (significantly different).

Conclusions and Future Directions

- The original Munro et al TTC values remains consistently lower than the thresholds derived from the 5th percentile NO(A)EL values identified in this study
- Bootstrap sampling enabled us to calculate the confidence interval surrounding the 5th percentile values, allowing for observation of the uncertainty around these values for both ToxVal and Munro data sets
- The presence of OP/carbamates in the Munro Cramer class III set largely explained the difference in 5th percentile values
- Refinements were made to the SMARTS in Toxtree that were originally used to identify OPs and carbamates
- Refined SMARTS were used to profile a large dataset of 45,000 chemicals and assign their Cramer class
- Utilising other data present in ToxVal we plan to extend this work to other routes and/or durations of exposure to calculate different TTC values

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