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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

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)

Datasets

- US EPA's ToxVal
- Munro et al (1996)

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

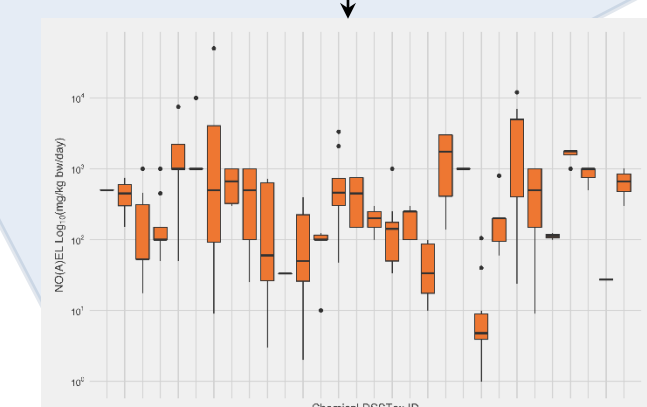


Figure 1. Distribution of NO(A)EL values from ToxVal for chemicals in Cramer Class II. Points were removed as lying outside of Tukey fence (1.5x IQR)

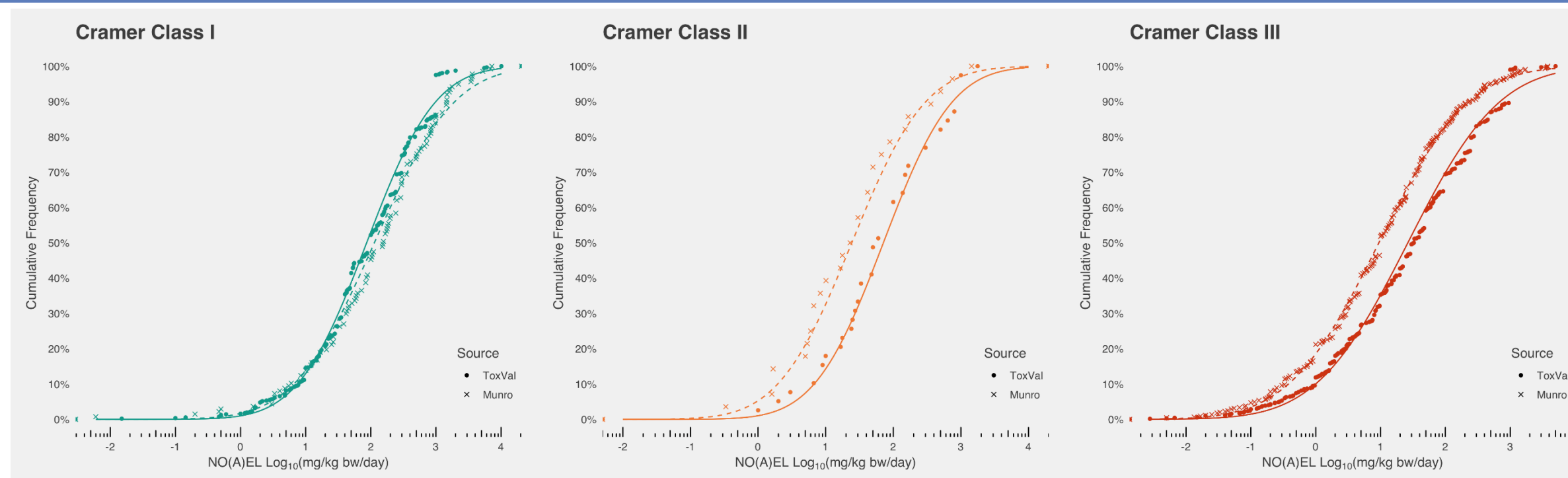


Figure 3. Comparison of cumulative and fitted lognormal distributions for ToxVal and Munro NO(A)EL data for each Cramer class. Cramer class I and II distributions between ToxVal and Munro were not significantly different ($p > 0.05$). Meanwhile, the distributions for Cramer class III were seen to be significantly different between the two data sets ($p < 0.05$)

Cramer Class	Nº. chemicals (ToxVal)	ToxVal 5 th %ile (mg/kg-day)	No. chemicals (Munro)	Munro 5 th %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. Values in parentheses are the calculated 95% confidence intervals surrounding the 5th percentile values. The large confidence intervals for the class II compounds is likely due to how few chemicals were assigned to this class.

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

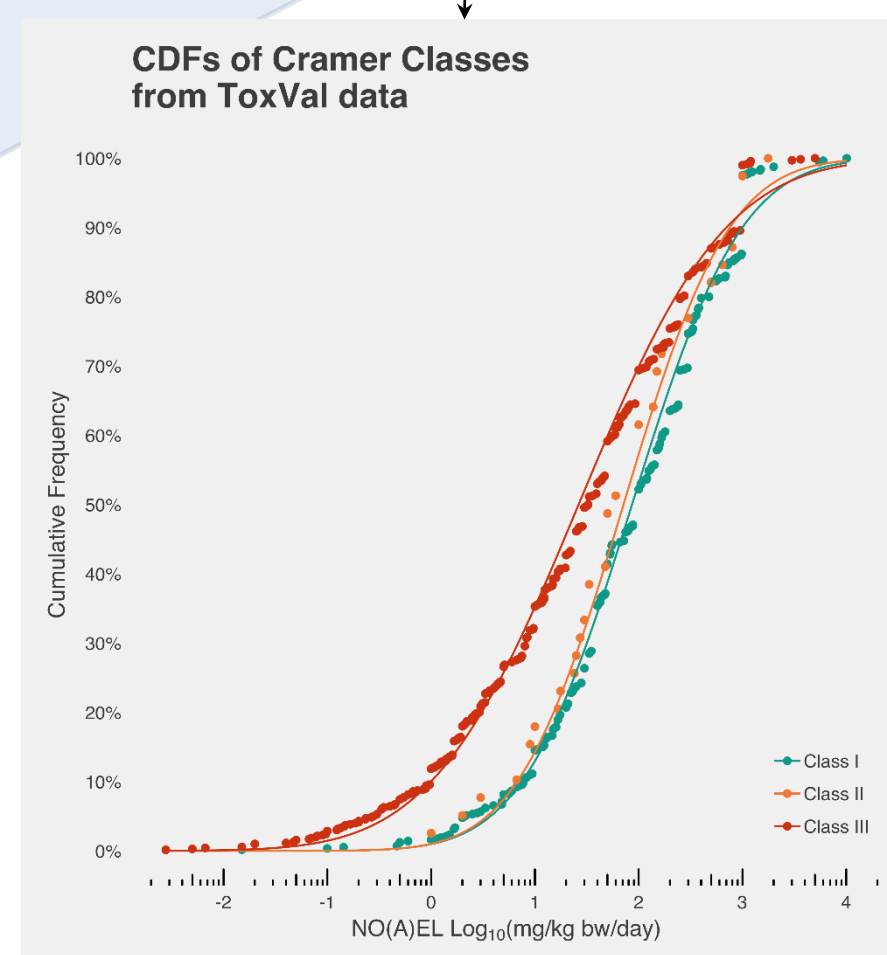


Figure 2. Cumulative and fitted lognormal distributions of NO(A)EL values from ToxVal for chemicals in Cramer Classes I, II, and III. The distributions for Cramer classes I and III differ significantly ($p < 0.05$). Whilst, Class I and Class II, and Class II and Class III not found to differ significantly ($p > 0.05$).

Comparison of ToxPrints between ToxVal and Munro

- We calculated the frequency of ToxPrints for both ToxVal and Munro Cramer class III compounds to investigate the chemical landscape and its potential impacts on the discrepancy in 5th percentile values (Figure 5)

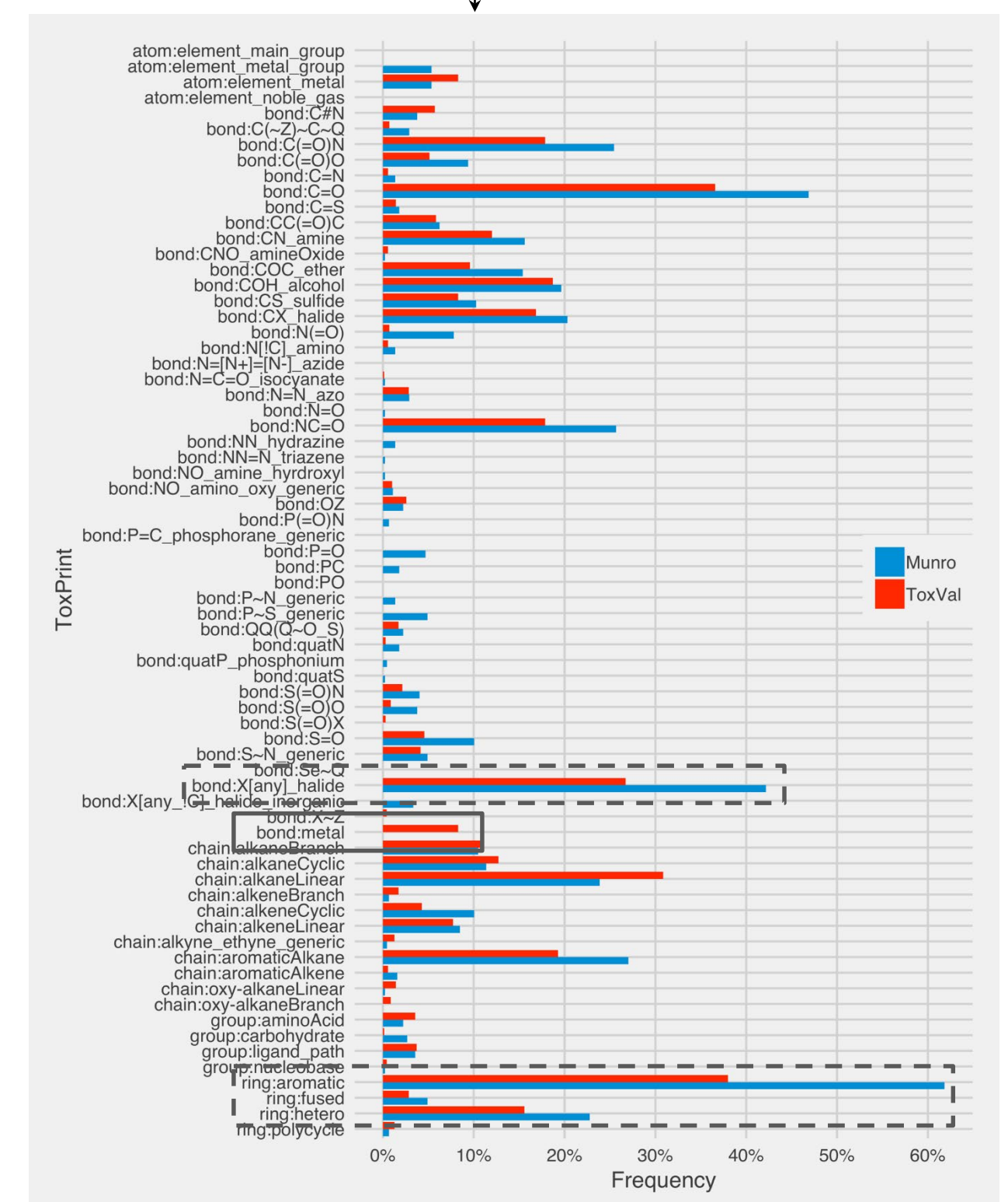


Figure 5. Comparison of frequency of ToxPrints between ToxVal and Munro Cramer class III compounds. The Munro data set contains a higher frequency of many ToxPrints, including aromatic and heterocyclic rings, and halide atoms (boxes with dashed lines). ToxVal has a higher frequency of Si and B containing compounds (box with solid lines). The majority of the amide (C(=O)N) containing compounds in ToxVal contain a carboxamide group; although there are seven carbamate containing compounds.

Conclusions and Future Directions

- Bootstrap sampling enabled us to calculate the confidence interval surrounding the fifth percentile values, allowing for observation of the uncertainty around these values for both ToxVal and Munro data sets
- 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
- Further investigation is ongoing to identify whether differences in chemical features is the reason behind the difference in class III 5th percentile values
- Utilising other data present in ToxVal we can extend this work to other routes and/or durations of exposure to calculate different TTC values for a variety of different situations

This project was supported in part by an appointment to the Internship/Research Participation Program at the Office of Research and Development, U.S. Environmental Protection Agency, administered by the Oak Ridge Institute for Science and Education through and interagency agreement between the U.S. Department of Energy and EPA. **Disclaimer:** The views expressed in this poster are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.