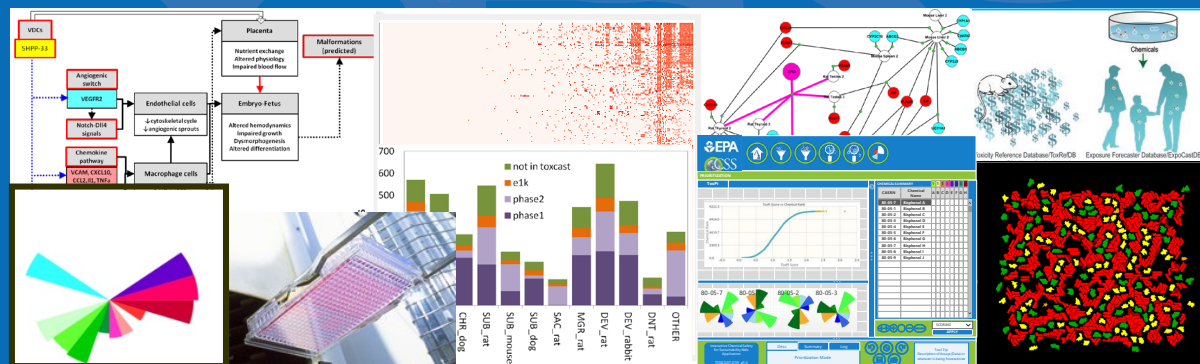# Derivation of new Threshold of Toxicological Concern (TTC) for exposure via inhalation for environmentally relevant chemicals

Grace Patlewicz and Mark Nelms
Center for Computational Toxicology and Exposure (CCTE), US EPA

Cosmetics Europe Inhalation TTC WS November 3rd-4th 2020

Outline

- **Threshold for Toxicological Concern (TTC)**
  - **Background to TTC**
 - **Proposed TTCs for inhalation**
  - **Next steps**

TTC – Threshold of Toxicological Concern

- TTC is a principle that refers to the establishment of a human exposure threshold value for (groups of) chemicals below which there would be no appreciable risk to human health

- Relies on past accumulated knowledge regarding the distribution of potencies of relevant classes of chemicals for which good toxicity data do exist

- Useful substitute for substance-specific hazard information in situations where there is exposure information which indicates that human exposure is very low and there is limited or no information on the toxicity of the chemical

TTC – Threshold of Toxicological Concern

- **Two types of TTCs:**

- **'General' TTC is based on a predicted tumour risk of 1 in a million, derived through an analysis of cancer data**

- **Structural based TTCs are based on frequency distributions (5th percentile) of NO(A)ELs of non-cancer endpoints**
  - Structural based TTCs proposed by Munro et al (1996) by assigning substances into one of 3 Cramer structural classes
  - Underlying dataset comprised 2941 NOELs for 613 substances

Cumulative Distributions of Structural Class NOELs

Percent vs NOEL (mg/kg/day)

Legend:
- Fitted Distribution —
- Class I □
- Class II ▽
- Class III ○
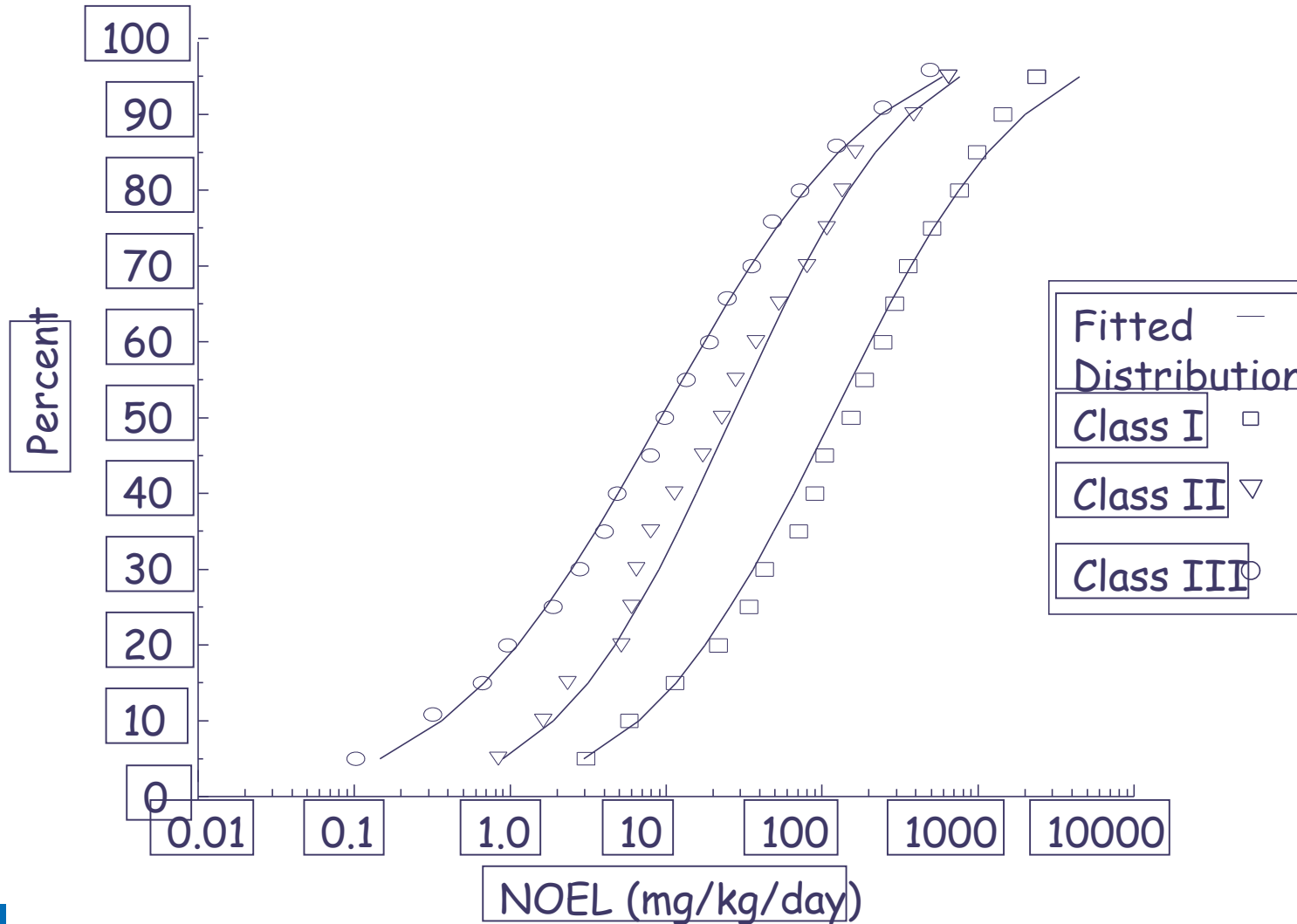
- Took min NOEL
- Fitted Log normal distribution
- Calculated 5th percentile of the fitted CDF
- Converted to TTC by dividing by a SF of 100

Kroes et al (2004) workflow

- Applying the TTC in practice
- Relies on more than the Cramer classes used by Munro et al (1996)
- Case by case basis – one chemical at a time

1. Is the substance a non-essential metal or metal containing compound, or is it a polyhalogenated-dibenzodioxin, -dibenzofuran, or -biphenyl?

NO / YES

YES → Risk assessment requires compound-specific toxicity data

2. Are there structural alerts that raise concern for potential genotoxicity?

NO / YES

5. Does estimated intake exceed TTC of 1.5µg/day?

YES / NO

3. Is the chemical an aflatoxin-like-, azoxy-, or N-nitroso- compound? YES

NO

4. Does estimated intake exceed TTC of 0.15µg/day? YES

NO

Substance would not be expected to be a safety concern

Negligible risk (low probability of a life-time cancer risk greater than 1 in 10^6 – see text)

6. Is the compound an organophosphate?

NO / YES

8. Is the compound in Cramer structural class III?

NO / YES

7. Does estimated intake exceed TTC of 18µg/day? YES

NO

9. Does estimated intake exceed 90µg/day?

YES / NO

Risk assessment requires compound-specific toxicity data

Substance would not be expected to be a safety concern

10. Is the compound in Cramer structural class II?

NO / YES

12. Does estimated intake exceed 1800µg/day?

YES / NO

11. Does estimated intake exceed 540µg/day?

NO / YES

Risk assessment requires compound-specific toxicity data

Substance would not be expected to be a safety concern

Oral TTC values

| Type of substance	µg/person/day (µg/kg-day for 60 kg adult)
Alerts for potential genotoxic carcinogenicity	Kroes: 0.15 (0.0025 µg/kg-day) ICH: 1.5 (0.025 µg/kg-day)
Acetylcholinesterase inhibitors (AChEI) Organophosphate/carbamate	18 (0.3 µg/kg-day)
Cramer Class III	90 (1.5 µg/kg-day)
Cramer Class II	540 (9.0 µg/kg-day)
Cramer Class I	1800 (30 µg/kg-day)

TTC assumes a lifetime exposure (every day for ~70 years)
TTC values that are established are for the ORAL route of entry

Investigate relevance of existing oral TTC values for substances of interest to EPA

Motivated to explore the utility of TTC for risk based prioritisation of large numbers of chemicals but were the TTC values relevant for substances of interest to EPA

Use 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

CDFs of Cramer Classes from ToxVal data

Cramer Class I

Cramer Class II
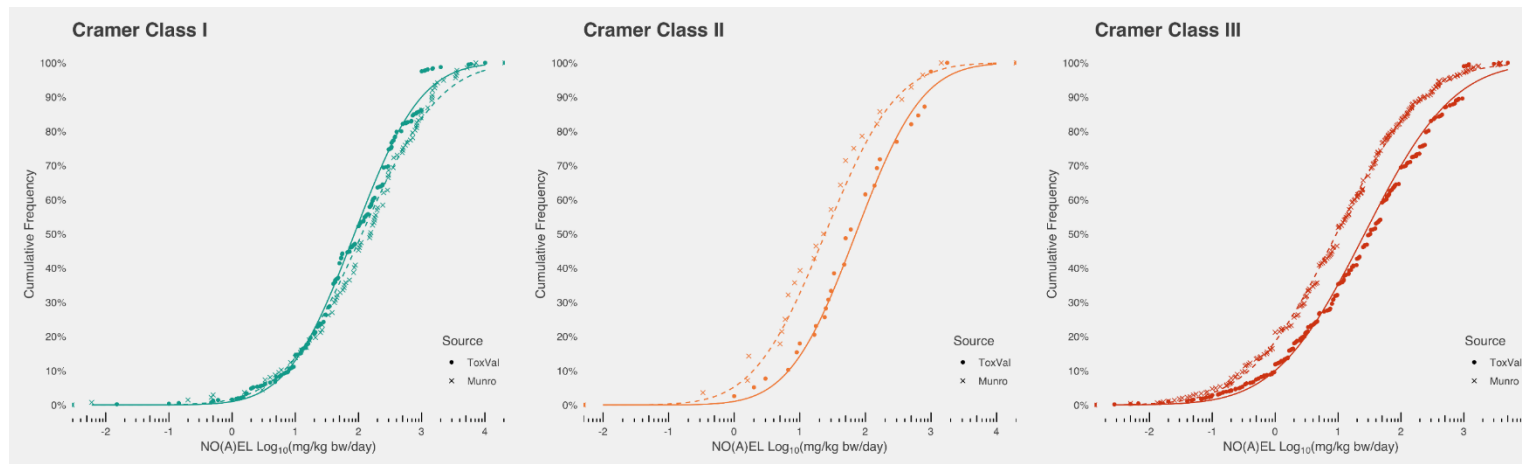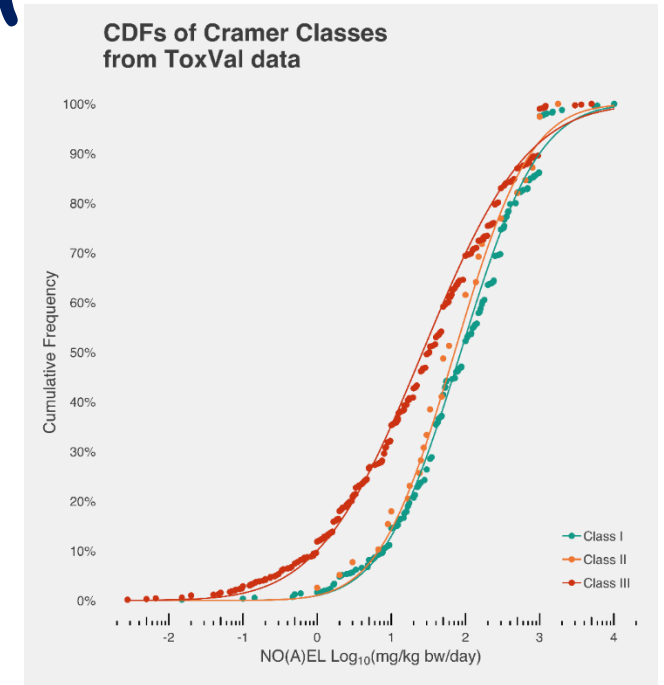
Cramer Class III

- **Bootstrap sampling used to quantify the uncertainty around the 5th percentiles values for both ToxVal and Munro data sets**
- **Differences were observed for substances assigned as Cramer Class III**
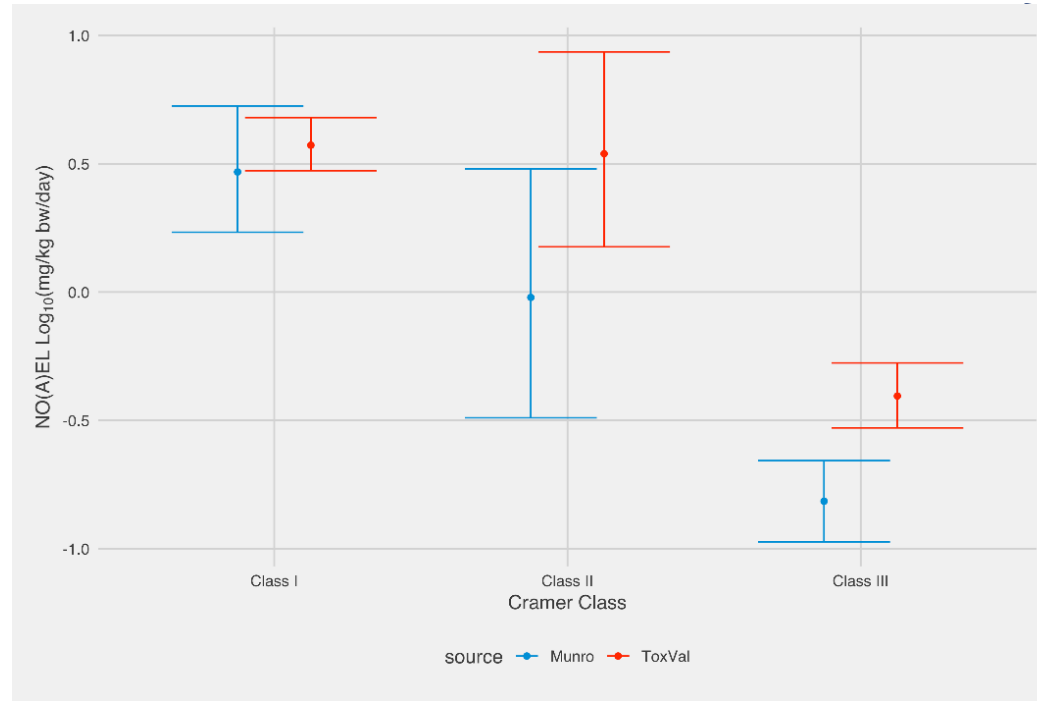
- **Presence of OP/carbamates in the Munro Cramer class III largely explained the difference in 5th percentile values**
- **Derived new modules for OPs**

Risk-Based prioritisation: inhalation route of entry

- **Whilst TTC values for oral route of exposure are well established and in routine use, the same is not true for TTC values for inhalation**

- **To that end, we investigated the feasibility of deriving new TTC values using the ToxValDB to facilitate risk-based prioritisation of large numbers of chemicals where inhalation was the relevant route of exposure**

Approach taken to derive TTCs for inhalation

- 1) Gather the chemicals and data from ToxValDB

- 2) Identify the chemical structures for all the chemicals in ToxValDB

- 3) Process the chemicals through the Kroes et al (2004) workflow but using the adhoc profilers from Patlewicz et al (2018) and Nelms et al (2019)

- 4) For substances that were assigned as belonging to the 3 Cramer classes, filter ToxValDB to identify relevant studies that met the same criteria as used by Munro et al (1996) but where the route of exposure was inhalation

- 5) Remove statistical outliers and taking the minimum NOAEL/NOAEC for each chemical as the representative value (in either mg/m3 or ppm units), and deriving the 5th percentile values

- 6) Compare the 5th percentile derived from the experimental data and their associated TTC values to those published by Carthew et al (2009) and Escher et al (2010).

- 7) Explore other means if appropriate to categorise the substances beyond Cramer designations and propose new TTC values.

Step 1: Gather relevant data from ToxValDB

- Identified study records that were tagged as subacute, subchronic, chronic, reproductive, developmental, or multigenerational study type.

- Created a study length field to help designate chronic, subchronic and reproductive studies on the basis of reported study duration and study type information.

- A short-term/repeat dose study was considered to as chronic if the "study_type" column stated it was a chronic study or if the study duration was over 100 days (or week/month equivalent).

- Similarly, a study was considered to be subchronic if the "study_type" column stated it was a subchronic study or if the study duration was >=35 days and < 100 days (or week/month equivalent). On the otherhand, a short-term/repeat dose study was only considered to be a reproductive study if the "study_type" column stated as such.

- As the dose measurements for each study was provided in either ppm- or mg/m3-related units, toxicity values was converted into common units.

Steps 2-3: Profile ToxValDB chemicals to assign TTC category

- **The 4,703 chemicals within ToxValDB, for which QSAR-ready SMILES were available, were profiled through Toxtree (v3.1.0) (IdeaConsult, Ltd) in order to assign them into the appropriate TTC category.**

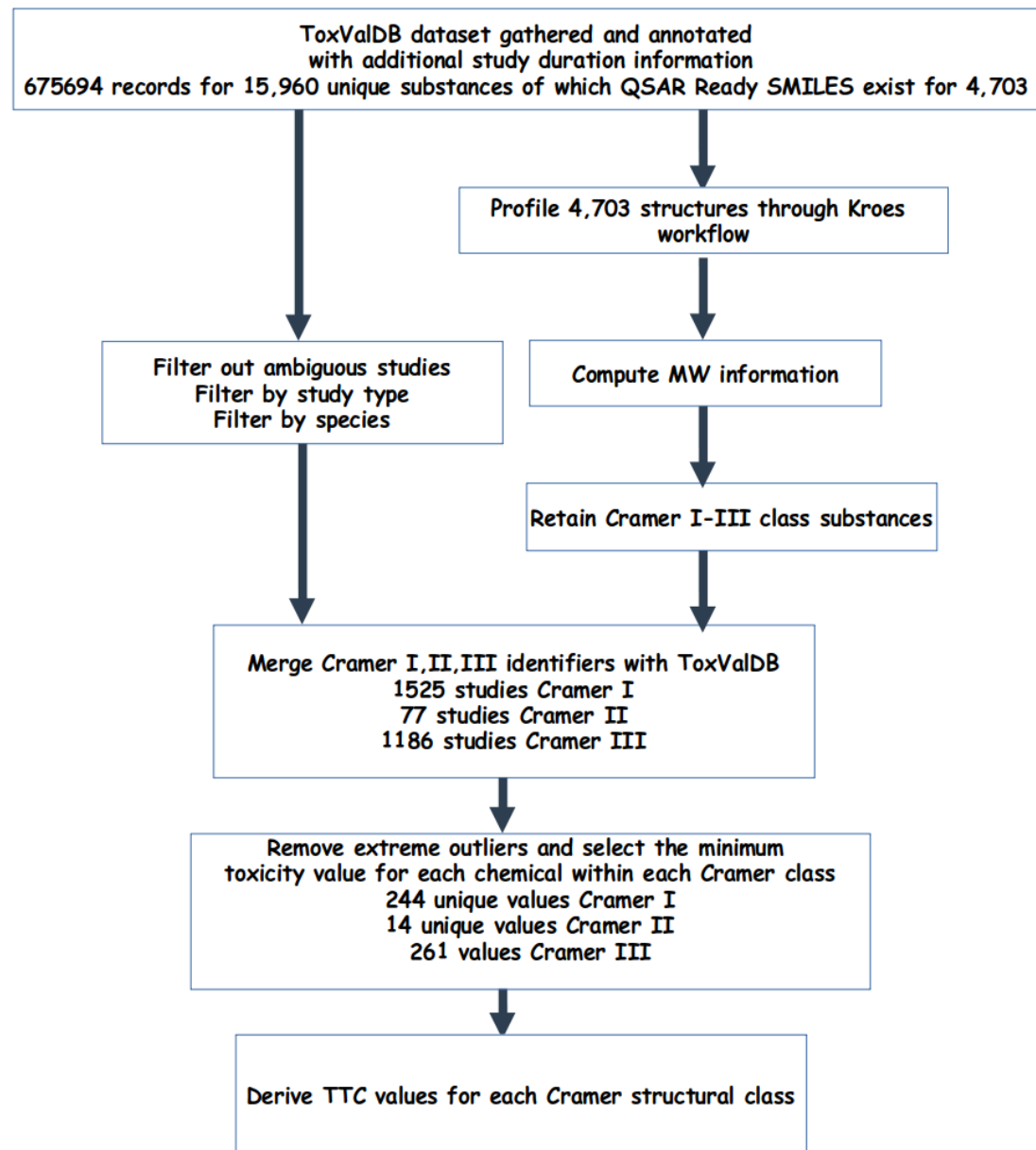| TTC Category	Number of chemicals
Not processed	2
High Potency carcinogens	18
Organophosphates	70
Carbamates	17
Steroids	0
Substances presenting a genetox structural alerts	1077
Substances not appropriate for TTC	130
Cramer Structural I	1498
Cramer Structural II	165
Cramer Structural III	1726

Step 4: Filtering ToxVal for relevant studies

- The dataset was filtered to select

- a) study length as subacute, subchronic, repeat dose, chronic, reproductive, developmental and multigenerational;

- b) exposure route as inhalation,

- c) toxval type as NO(A)EL or NO(A)EC point of departure; and

- d) species as rats, mice and rabbits

- Finally, the dataset was filtered based on the DTXSID identifier designating presence in one of the 3 Cramer structural classes.
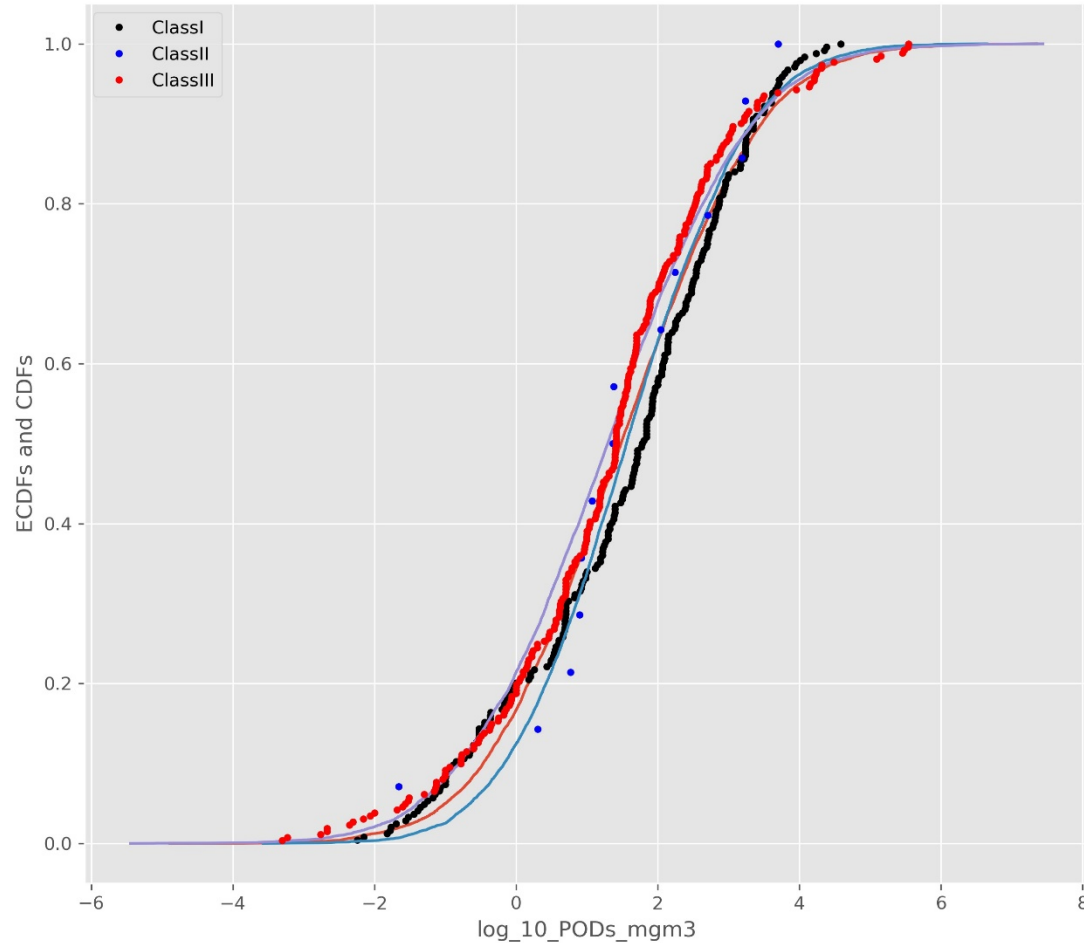
Step 5: Deriving representative values

- ToxValDB data for the 3 Cramer structural classes were processed as follows:
- 1) for substances with only 1 study, this was retained; 2) for substances with more than 1 study, extreme outliers, i.e. statistical outliers that exceeded the interquartile range were removed and the minimum value was returned.
- This was carried out for both units, mg/m3 and ppm.

Workflow summarising creation of datasets

ToxValDB dataset gathered and annotated
with additional study duration information
675694 records for 15,960 unique substances of which QSAR Ready SMILES exist for 4,703

Profile 4,703 structures through Kroes workflow

Filter out ambiguous studies
Filter by study type
Filter by species

Compute MW information

Retain Cramer I-III class substances

Merge Cramer I,II,III identifiers with ToxValDB
1525 studies Cramer I
77 studies Cramer II
1186 studies Cramer III

Remove extreme outliers and select the minimum
toxicity value for each chemical within each Cramer class
244 unique values Cramer I
14 unique values Cramer II
261 values Cramer III

Derive TTC values for each Cramer structural class

Step 5: cont..

- NOAEL/NOAEC converted to their Log10 equivalents
- Plotted ECDFs
- Not normally distributed (unlike oral NOAELs) (Based on visual inspection of ECDFs and CDFs and by using Shapiro-Wilks test)
- No separation between the Cramer I and III classes
- Insufficient chemicals in Cramer II

# Step 6: Deriving TTC values & comparing them to published values

Source	Number of chemicals	Source	TTC mg/m3	TTC ug/person/day
ToxVal	244	Cramer I	4.14E-03	8.27
	14	Cramer II	2.975E-03	59.5
	261	Cramer III	2.14E-04	4.28
Escher et al (2010)	58	Cramer I	3.6E-03	71
	7	Cramer II	4.8E-04	10
	138	Cramer III	1.8E-04	4
Carthew et al (2009)	38	Cramer I	0.049	980
	50	Cramer III	8.5E-03	170

Step 6: Reproducing published values: Escher et al (2010)

Structural category	# chemicals in Escher	Reported TTC mgm3	Reported TTC ug/person /d	#reproduced values	TTC mgm3	TTC ug/person /d
Cramer I	58	3.6E-03	71	58	4.57E-03	91.44
Cramer III	138	1.8E-04	4	137*	2.78E-04	5.56

Step 6: Reasons why TTC values were so different

- **Differences between chemistry coverage?**

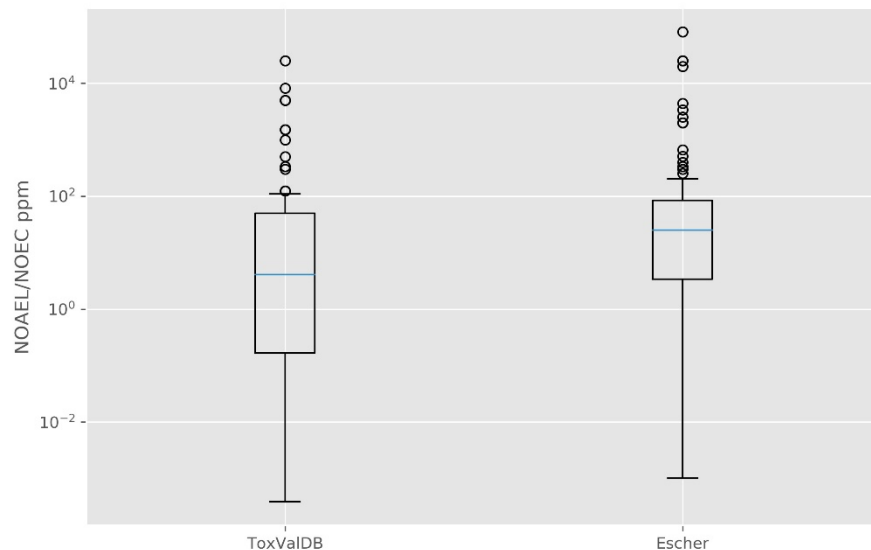2D Visualization using ToxPrint fingerprints
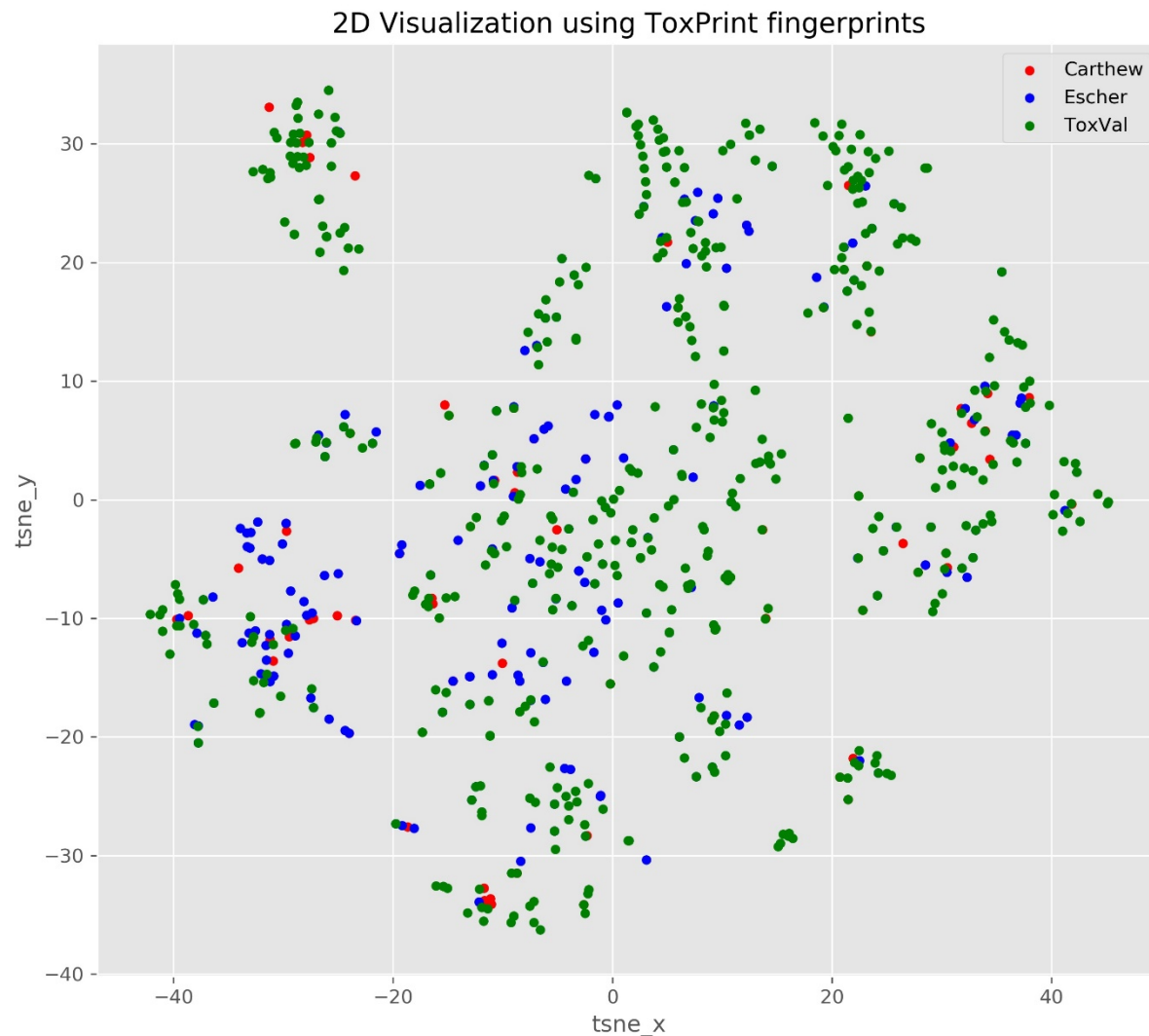
Step 6: Reasons why TTC values were so different

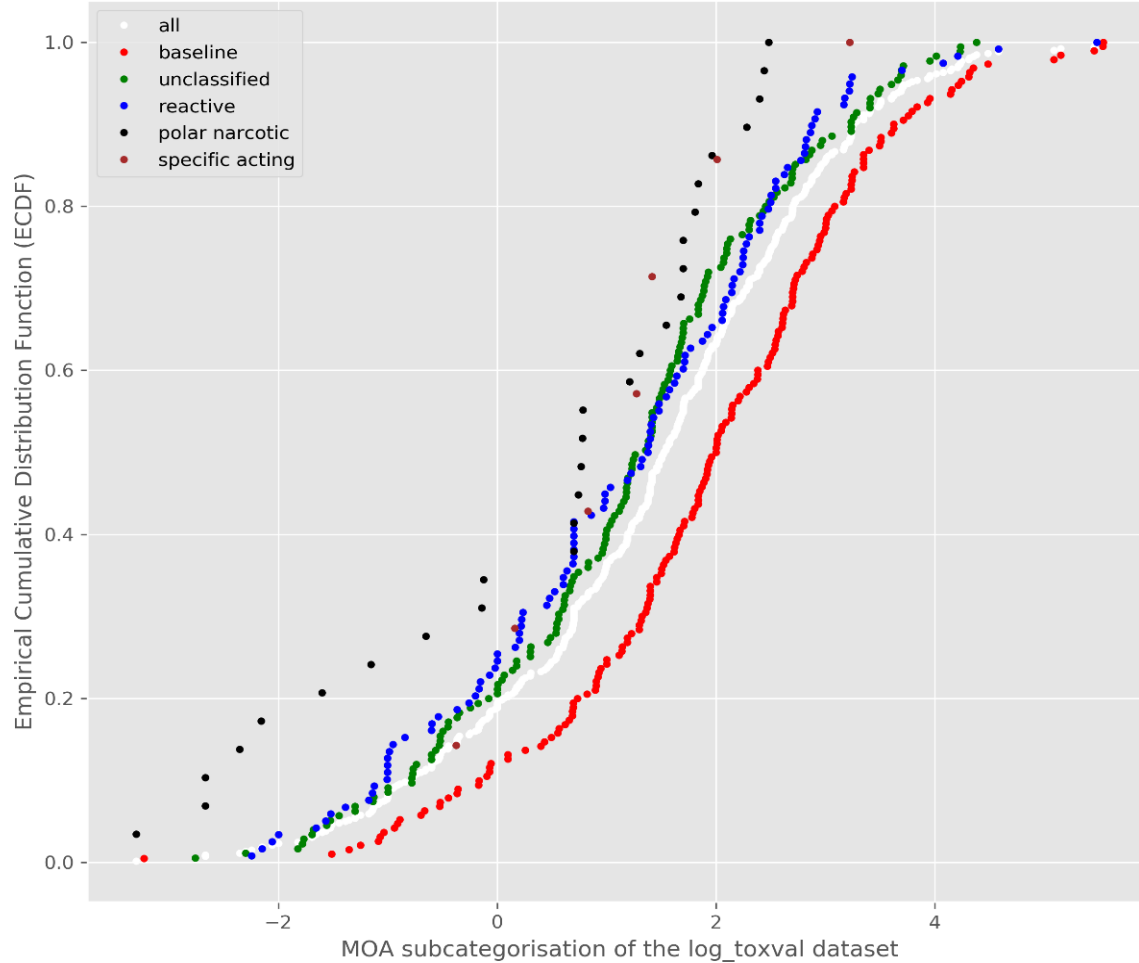- **Differences in the underlying toxicity data?**
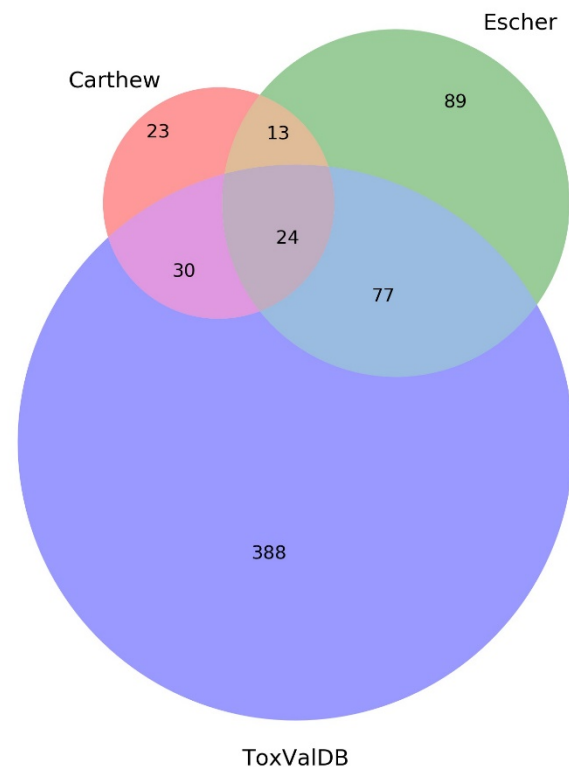
Step 6: Reasons why TTC values were so different

- **Explored the overlapping chemicals**
  - **Low correlation between the 2 datasets – can not reject the null hypothesis that the means are the same on the basis of the t-test**
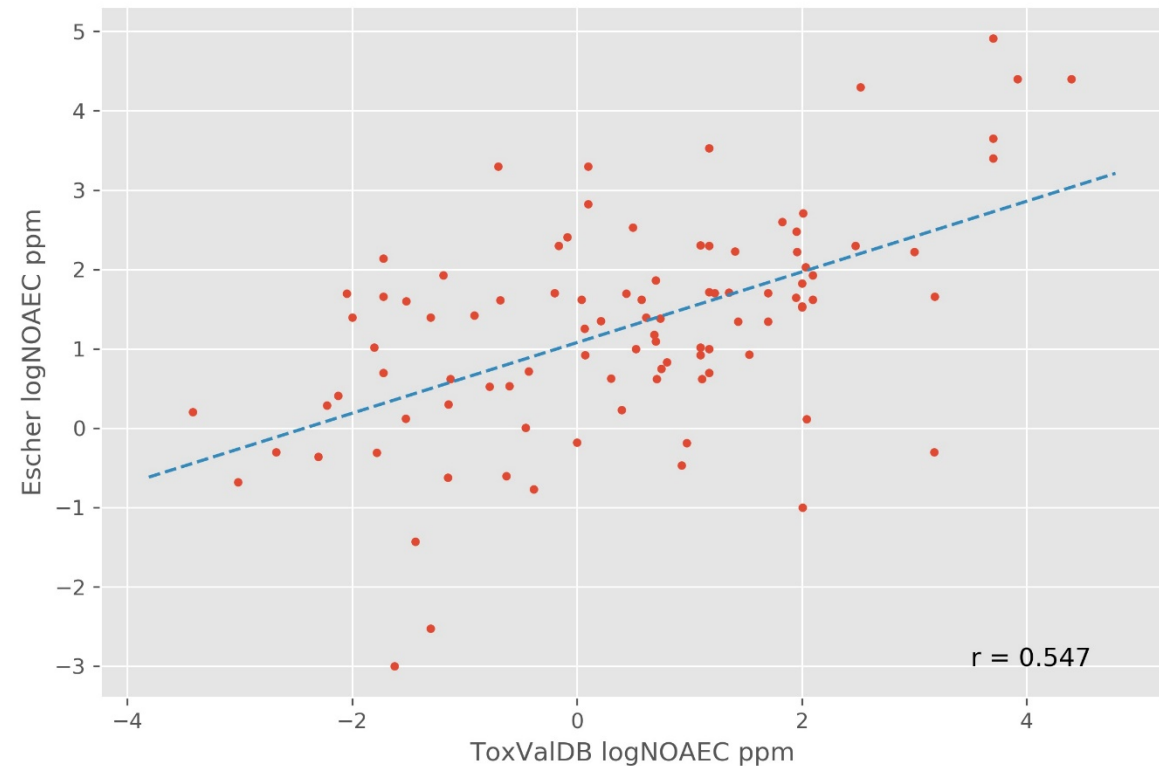
Step 7: Re-categorising substances

- **Profiled substances by Aquatic MOA profilers based on work by Veith et al (2009) who explored a relationship between acute inhalation and acute fish toxicity**

- **Better separation between the baseline and reactive MOA classes to facilitate derivation of new TTC values**

Step 7: Deriving TTC values

MOA class	#Chemicals	5th percentile median bootstrapped	TTC mg/m3	TTC ug/person/d
Baseline	190	0.1567	1.11E-03	22.39
Reactive	118	0.0299	2.14E-04	4.286

See Nelms and Patlewicz, 2020
https://www.frontiersin.org/research-topics/13793/advances-and-refinements-in-the-development-and-application-of-threshold-of-toxicological-concern-tt

Summary remarks – Next steps

- Using Cramer structural classes did not appear to discriminate the chemicals and data identified within the ToxValDB
- Further QC of the underlying data is still merited and ongoing – to resolve the differences between existing datasets and ToxValDB
  - Merits an evaluation of the variability of the underlying data itself
- Use of predefined alert schemes to subcategorise e.g. Verhaar type schemes were promising but machine learning approaches to subset and group substances is worth exploring further in the context of a read-across type approach
- Combining and enhancing the underlying dataset
- Local vs systemic effects – had explored use of alert schemes to identify respiratory sensitisers, irritants/corrosives as well as physicochemical properties such as Vapour Pressure, pKa (inconclusive findings)