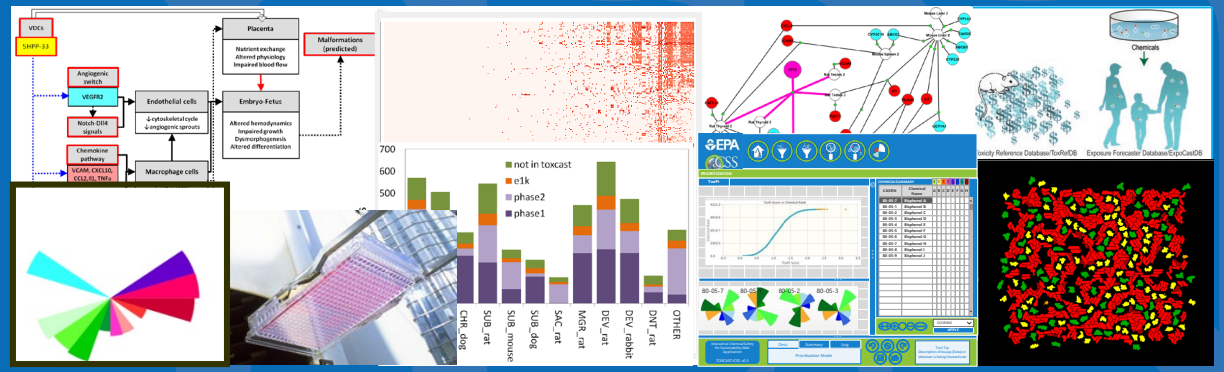
Threshold of Toxicological Concern (TTC) a useful tool in the Computational Toxicology armory



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University of Louisville : Human Health Risk Assessment
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Conflict of Interest Statement

- No conflict of interest to declare.

Disclaimer:

- The views expressed herein are those of the presenter and do not necessarily reflect the views or policies of the U.S. EPA

Outline

- Regulatory and Non Regulatory drivers
- Computational Toxicology approaches
- Integrated Approaches to Testing and Assessment (IATA)
- Decision contexts
- Threshold for Toxicological Concern (TTC)
- Summary remarks
- Acknowledgements

Regulatory and Non-Regulatory drivers

- Societal demands for safer and sustainable chemical products are stimulating changes in toxicity testing and assessment frameworks
- Chemical safety assessments are expected to be conducted faster and with fewer animals, yet the number of chemicals that require assessment is also rising with the number of different regulatory programmes worldwide.
- In the EU, the use of alternatives to animal testing is promoted.
- Animal testing is prohibited in some sectors e.g. EU Cosmetics regulation
- The European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) legislation lays out specific information requirements, based on tonnage level triggers.
- REACH-like schemes also have been established in China, South Korea, and Turkey.

Regulatory and Non-Regulatory drivers

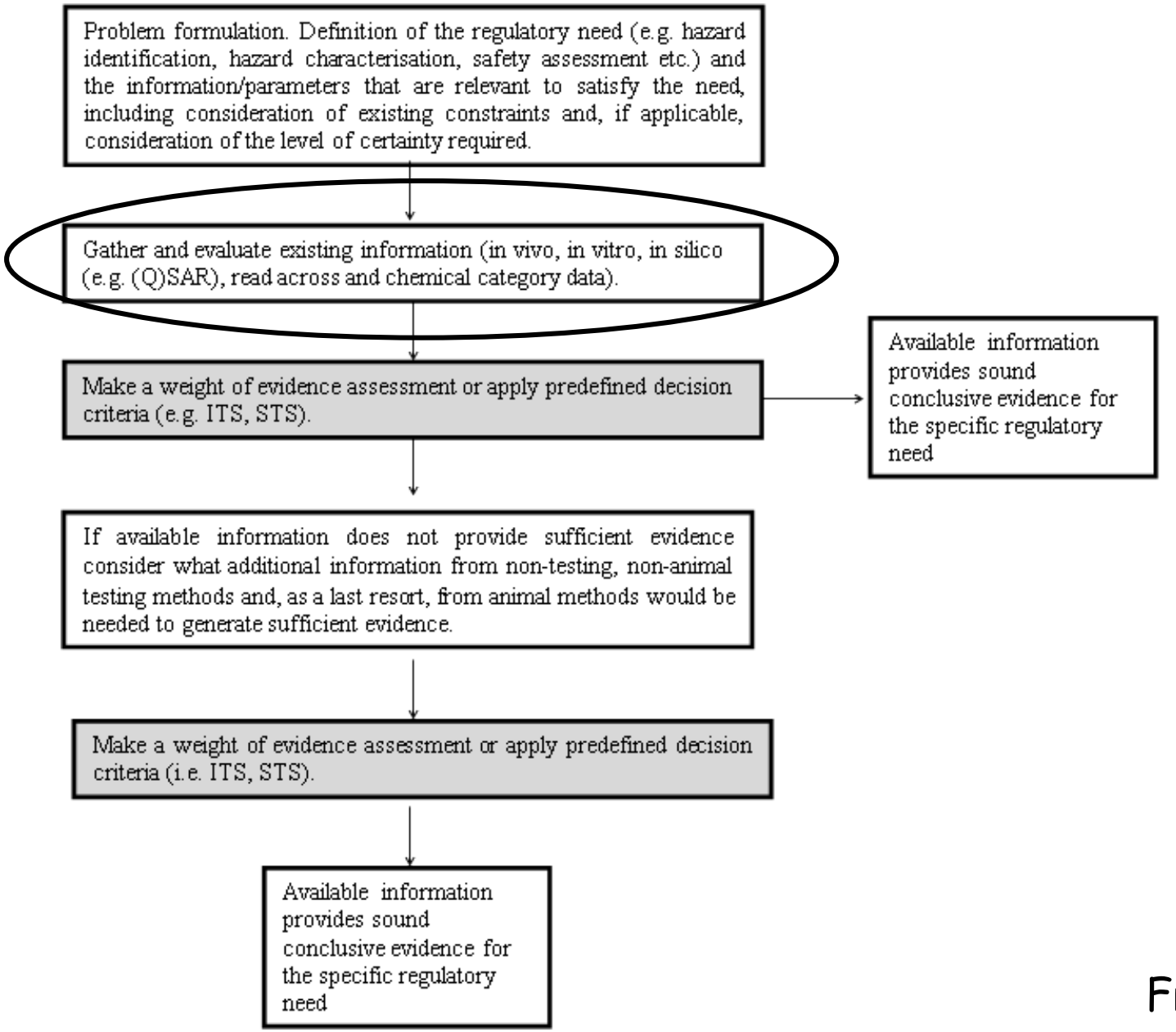
- In the US, the new Frank Lautenberg Chemical Safety for the 21st Century Act (LCSA) requires that a risk based prioritisation is conducted for all substances in commerce, ~40,000, many of which are lacking sufficient publicly available toxicity information.
- EPA Administrator signed memo 10/9/19 to "direct the agency to aggressively reduce animal testing, including reducing mammal study requests and funding 30% by 2025 and completely eliminating them by 2035"
- Risk based prioritisation is also an important aspect of regulatory frameworks in Canada (the Domestics Substance List), Australia and the EU.
- New Approach Methods (NAMs) offer a means of facilitating the regulatory challenges in chemical safety assessment

Integrated Approaches to Testing and Assessment (IATA)

- "Integrated Testing Strategies (ITS) are …. approaches that integrate different types of data and information into the decision-making process. …"

- "*A* tiered approach to data gathering, testing, and assessment that integrates different types of data (including physicochemical and other chemical properties as well as *in vitro* and *in vivo* toxicity data). When combined with estimates of exposure in an appropriate manner, the IATA provides predictions of risk."

General framework of an IATA



From OECD

Typical Information within an IATA: IATA elements

- Historical information on the chemical of interest
- Non-standard *in vivo* tests
- Information from "similar" chemicals
- Predictions from other 'non-testing' approaches such as (Q)SAR, TTC
- *In chemico* tests
- *In vitro* tests
- Molecular biology, -omics
- Exposure, (bio-)kinetics

Computational toxicology tools add value to most regulatory decisions

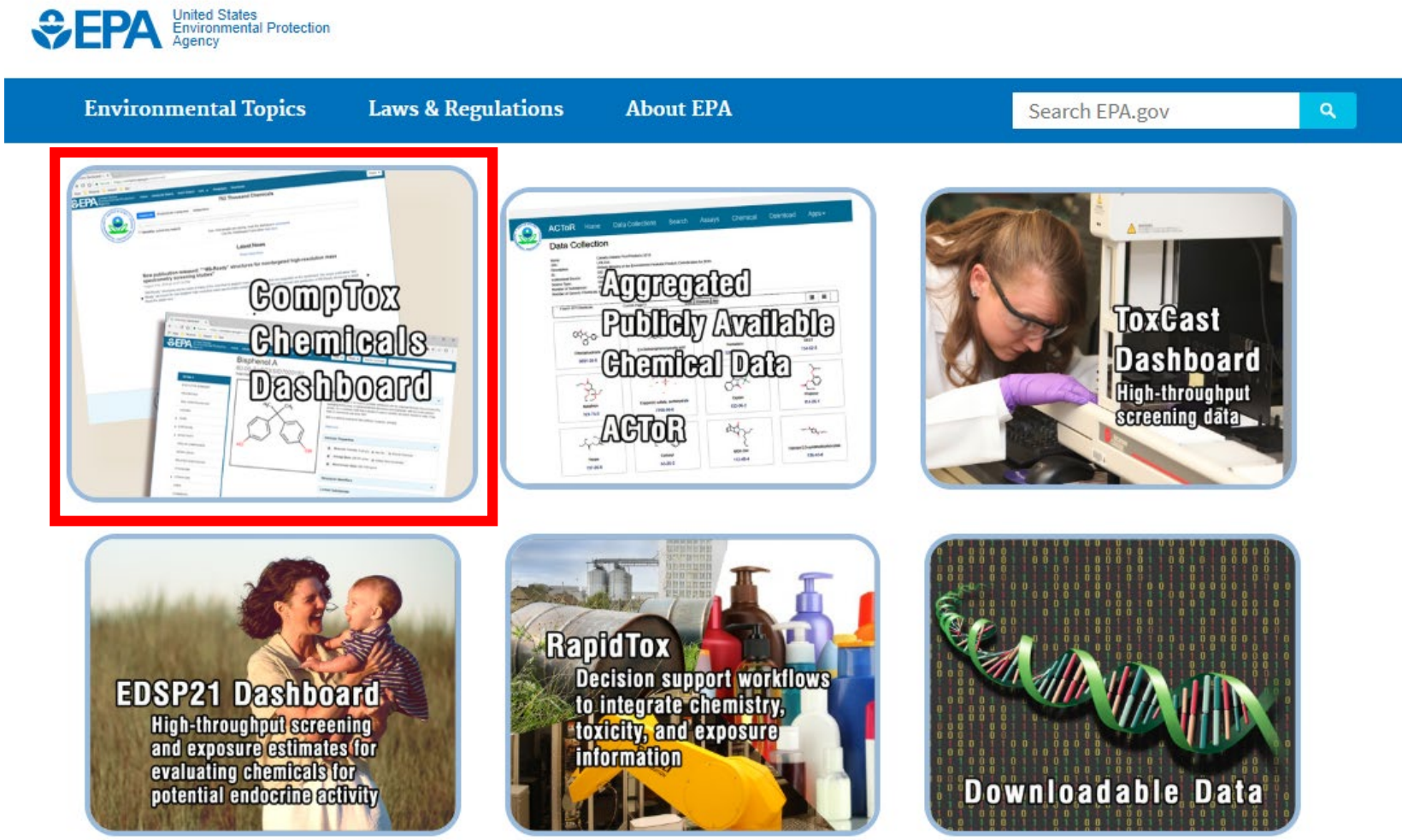
- Screening level hazard assessment
- Risk-based Prioritisation
- Risk Assessment
- Exposure Assessment

EPA CompTox Chemicals Dashboard

- A publicly accessible website delivering access:
  - ~875,000 chemicals with related property data
  - Experimental and predicted physicochemical property data
  - Integration to "biological assay data" for 1000s of chemicals
  - Information regarding consumer products containing chemicals
  - Links to other agency websites and public data resources
  - "Literature" searches for chemicals using public resources
  - "Batch searching" for thousands of chemicals
  - DOWNLOADABLE Open Data for reuse and repurposing

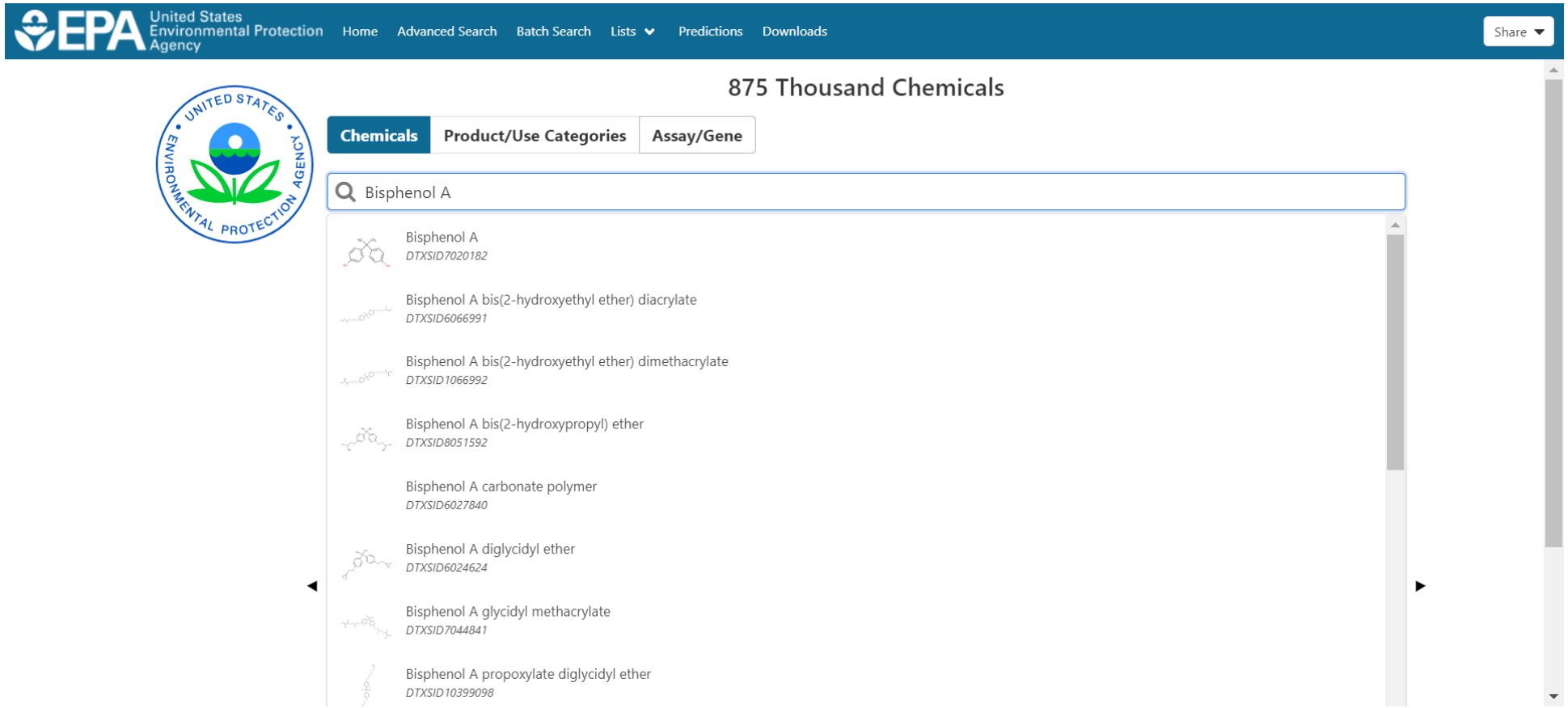
https://comptox.epa.gov/

The CompTox Portal https://comptox.epa.gov/

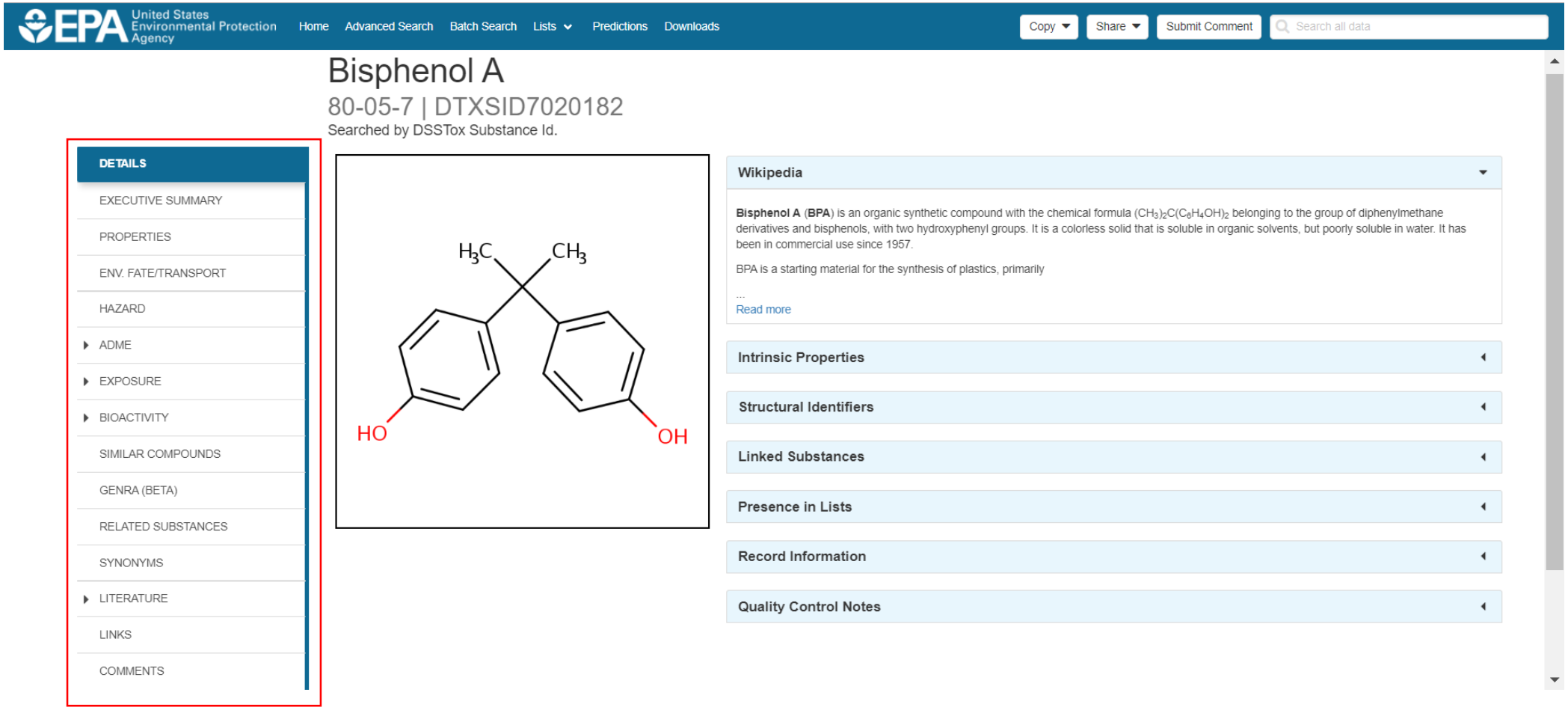


CompTox Chemicals Dashboard: Landing Page

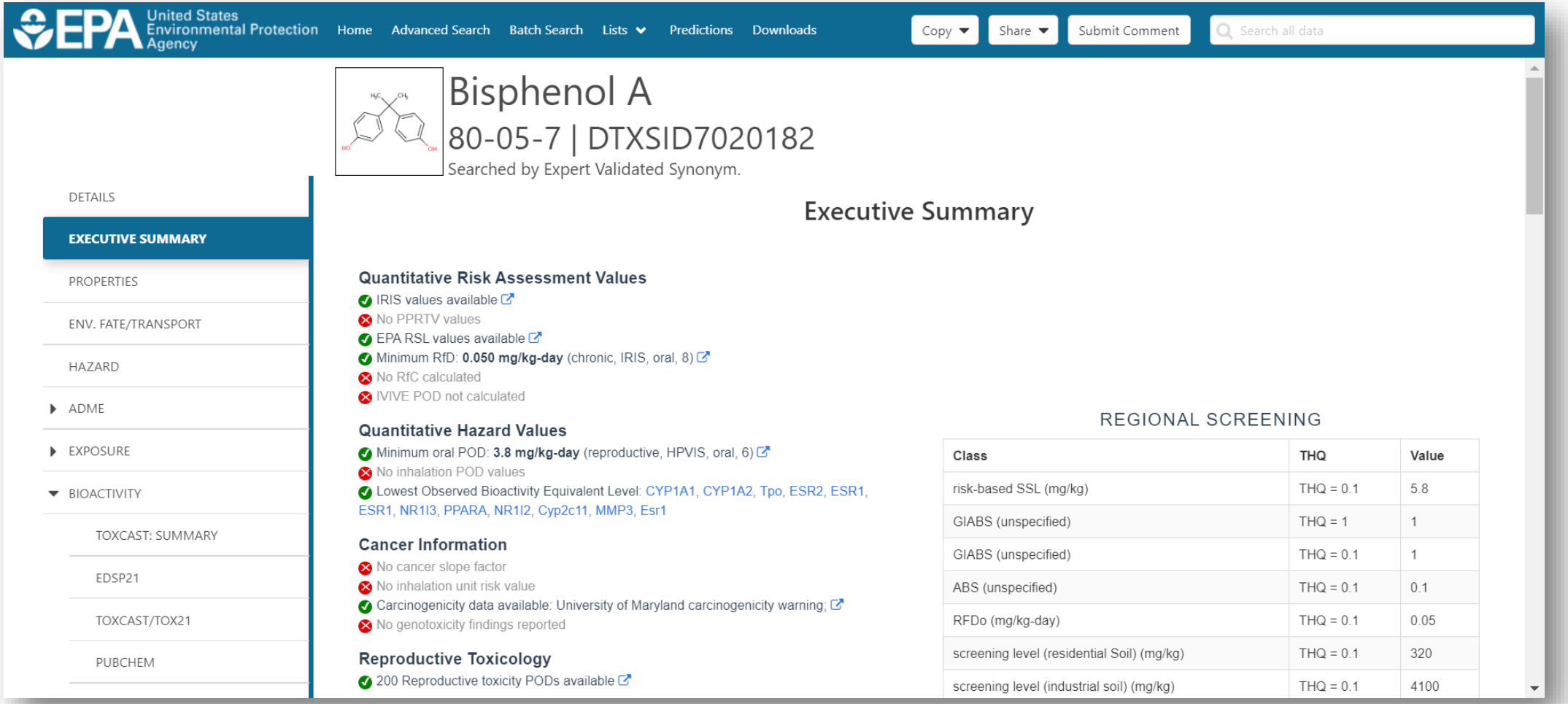
- Different entry points depending on domain of interest



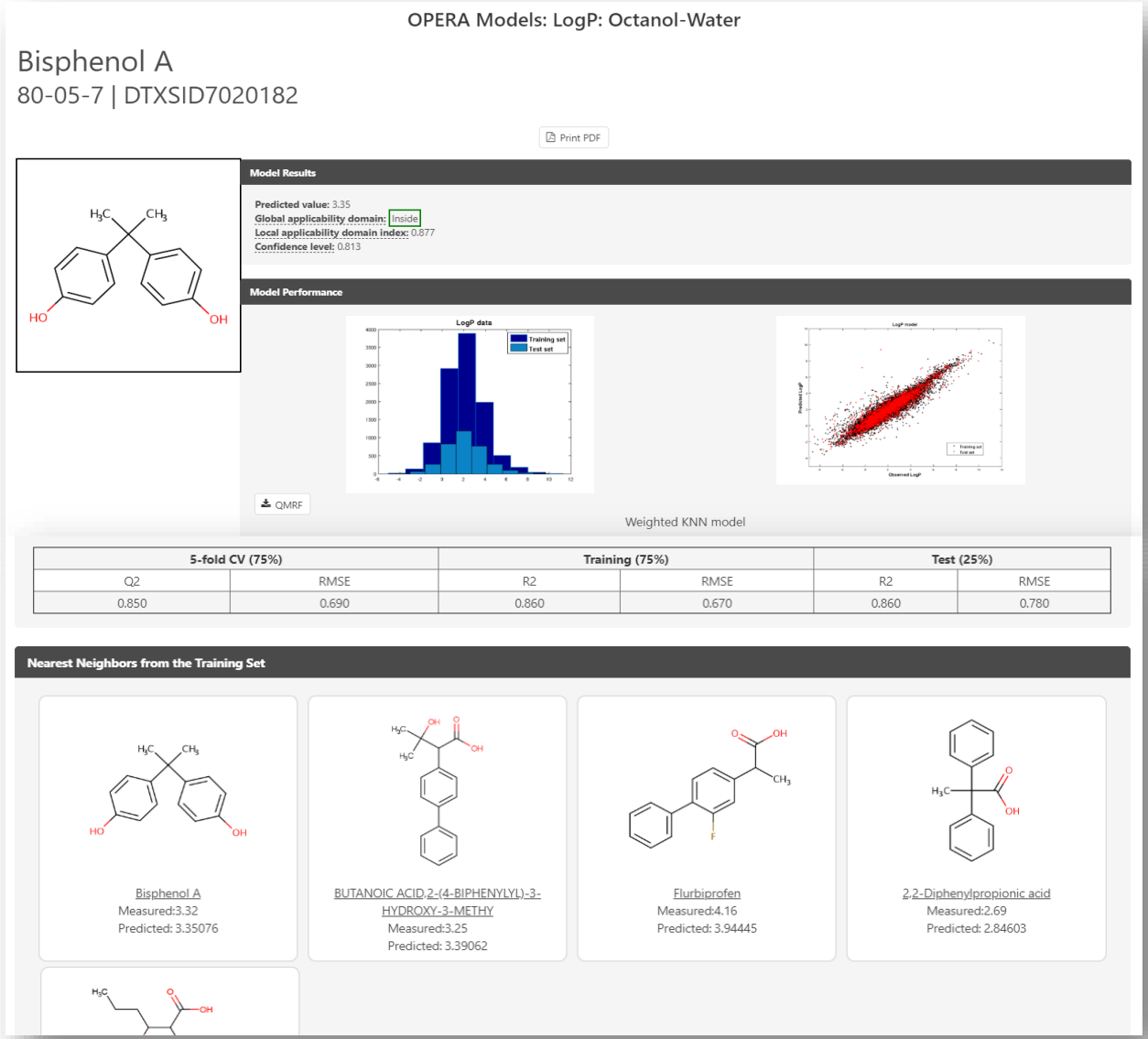
CompTox Chemicals Dashboard:
Landing Page for a specific chemical



CompTox Chemicals Dashboard:
Executive Summary of 'existing' data



QSAR Predictions



Generalised Read-Across (GenRA)

Screening level hazard assessment

- Another approach to consider is 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

TTC – Threshold of Toxicological Concern

- Based on this knowledge, an estimate of the probability of no adverse effects occurring for a substance of unknown toxicity at a specified daily intake is made

- 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

- Helpful for prioritising substances for risk assessment e.g. food flavouring substances, food contact materials, pesticide metabolites in groundwater, impurities in pharmaceutical manufacturing operations.

- The TTC concept is not intended to be applied to chemicals which are regulated and for which specific requirements exist regarding their hazard assessment

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

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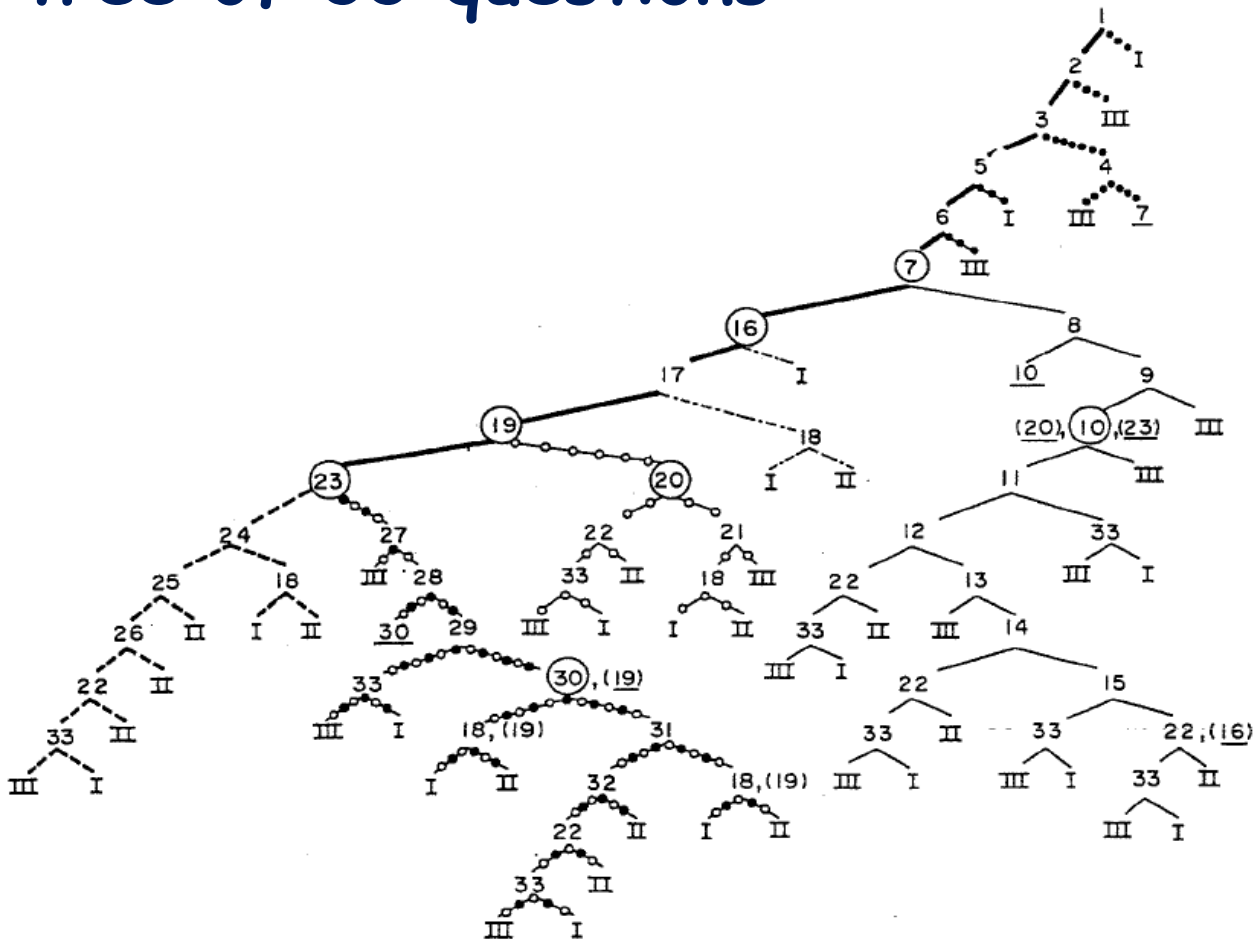
A bit of history..

- Efforts to derive structural based TTCs on endpoints other than carcinogenicity have typically made use of the structural decision rules defined by Cramer et al. (1978)
- Munro et al. (1996) explored the relationship between structure and toxicity by compiling a large database of ~600 substances that had been tested for a variety of non-cancer endpoints (chronic effects from repeated dose, repro, developmental etc studies)
- The resulting dataset contained 2941 NOELs for a total of 613 organic substances
- The substances were then assigned to one of three structural classes as defined by Cramer et al (1978)

Cramer decision tree

- Decision tree of 33 questions

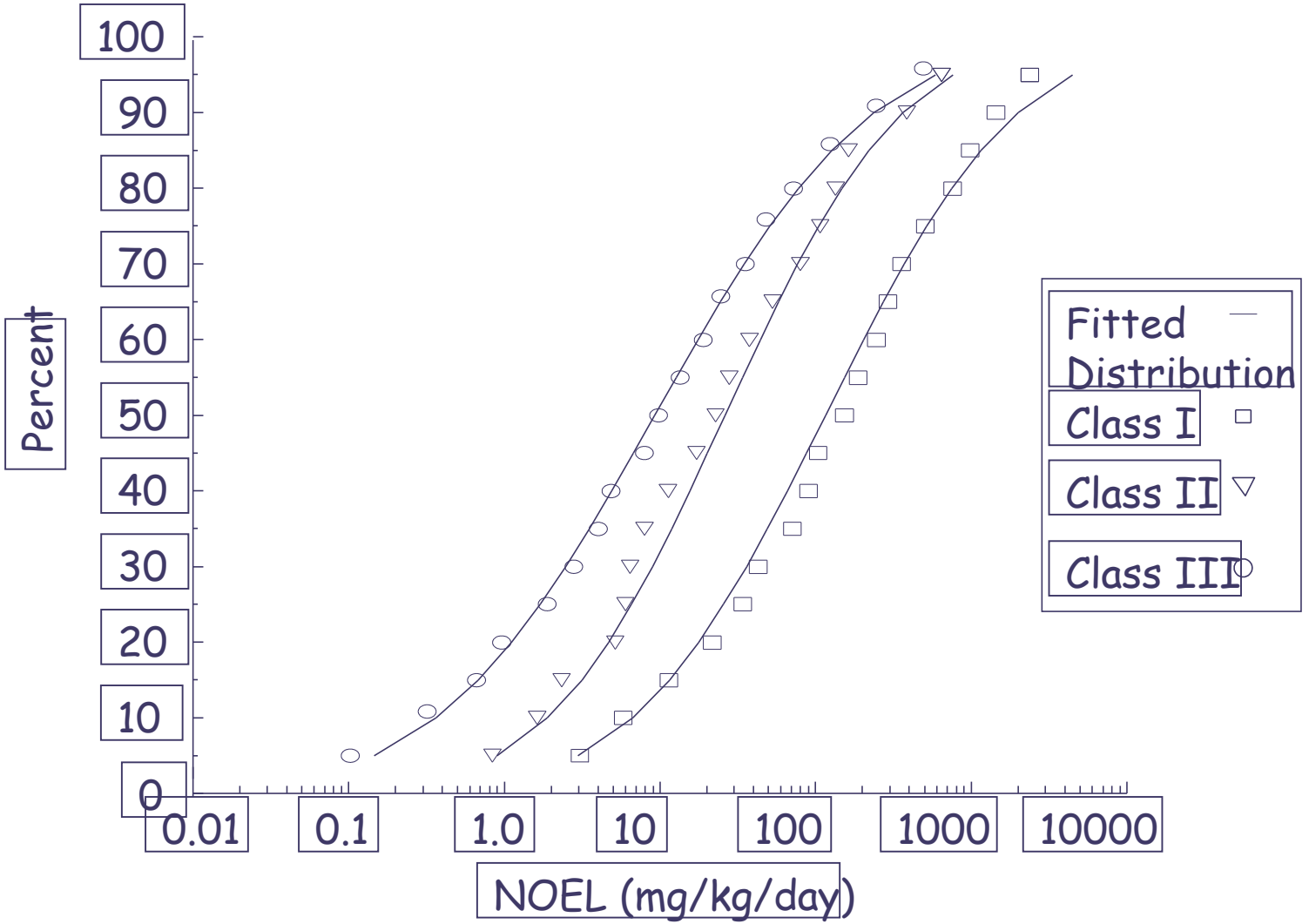


Cramer decision tree

- Decision tree of 33 questions
- CLASS I = simple structures efficiently metabolised to innocuous products; anticipated low order of oral toxicity
- CLASS II = intermediate structures (less innocuous than substances in Class I, but no positive indication of toxic potential)
- CLASS III = complex structures; metabolism to reactive products suggestive of potential toxicity

- The distributions of NOELs were found to differ for the three classes of chemicals revealing how structural class has an important bearing on toxicity

Cumulative Distributions of Structural Class NOELs



TTC values based on Cramer structural classes

Structural Class[a]	No. of Chemicals	5th Percentile NOEL (µg/kg/day)	Human Exposure Threshold (µg/day)[b]
I	137	2,993	1,800 (30 µg/kg bw/d)
II	28	906	540 (9 µg/kg bw/d)
III	447	147	90 (1.5 µg/kg bw/d)

[a] Cramer *et al.* (1978) structural classes

[b] The human exposure threshold was calculated by multiplying the 5th percentile NOEL by 60 (assuming an individual weighs 60 kg) and dividing by a safety factor of 100.

Replicating Munro's TTC values in practice

- EFSA has published the Munro dataset in electronic format
- See supporting information https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2011.EN-159
- Download the Munro original dataset as a csv file.

	A	B	C	D	E	F	G	H	I	P	
1	Structu	ID_Munro	NAME_Munro_1996	CAS_original	Species tested	Exposure	Study type	Exposure	Exposure	NOEL_calculated_Munro_mg/kg/day	Reference
67	3	1	(1 -naphthyl)ethylene-diamine dihydro chloride, N-	1465-25-4	rat	728	chr	fod	Oral - diet	39	NCI, 1979b
68	3	2	(2-chloroethyl)trimethyl-ammonium chloride	999-81-5	rat	756	chr	fod	Oral - diet	138	NCI 1979c
69	3	3	(chloroacetyl)-acetanilide, 4'-	140-49-8	rat	609	chr	fod	Oral - diet	790	NCI, 1979c
70	3	4	1,1'-(2,2,2-trichloroethylidene) bis(4-chloro)-benzene	50-29-3	rat	546	chr	fod	Oral - diet	16	NCI, 1978c
71	3	5	11-oxo-11H-pyrido(2,1-b) quinazoline-2-carboxylic acid		rat	11	terat	gav	Oral - gav	90	Nishimura
72	3	6	2(2,4,5-trichlorophenoxy) propionic acid	93-72-1	rat	730	chr	fod	Oral - diet	2.6	Mullison,
73	3	7	2-(2-methyl-4-chlorophenoxy) propionic acid	93-65-2	rat	90	sub	rod	Oral - diet	2.5	Verschuur
74	3	8	4-(2-methyl-4-chlorophenoxy) butyric acid	94-81-5	rat	91	sub	rod	Oral - diet	12	Rhodia Inc
75	3	9	C.I. Disperse Blue 1	2475-45-8	rat	91	sub	rod	Oral - diet	62	NTP, 1986
76	3	10	C.I. Orange 3	6373-74-6	mus	91	sub	gav	Oral - gav	500	NTP, 1988
77	3	11	C.I. Acid Red 14	3567-69-9	mus	91	sub	fod	Oral - diet	1171	NTP, 1982
78	3	12	C.I. Disperse Yellow	2832-40-8	rat	91	sub	fod	Oral - diet	250	NTP, 1982

Replicating Munro's TTC values in practice

- Munro et al (1996) found that the data fitted a log normal distribution well. They derived the 5th percentile of the cumulative distribution function
- This 5th percentile was multiplied by 60kg and divided by a safety factor of 100 to derive the associated TTC value
- In R, the easiest way to do this is as follows:
- Library(dplyr)
- Library(fitdistrplus)
- Munro <- read.csv('munro_original_dataset.csv') [make sure to adjust the NOELs reported depending on whether they are chronic or subchronic]
- Fln = fitdist(Munro$NOEL, 'lnorm')
- Quantile(Fln, probs = 0.05)
- *Estimate = 0.153*
- Reported Munro 5th percentile is 0.15 mg/kg bw/day

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Replicating Munro's TTC values in practice

- In python
- Import numpy, pandas and scipy libraries
- Calculate mean, std of the Munro dataset for a specific structural class but having converted the Munro NOELs to their Log10 equivalents
- mean = np.mean(munro['LogNOEL'])
- std = np.std(munro['LogNOEL'])
- Use the mean, std to create a sample normal distribution
- samples = np.random.normal(mean, std, size = 1000)
- Take the 5th percentile of the theoretical distribution
- 10**(np.percentile(samples, 5)

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Applying TTC in practice

- Assign substance based on the Cramer structural rules into one of the 3 class using of the software tools (Toxtree, OECD Toolbox)
- Requires a structure format such as SMILES representation or a mol file
- The structural class designation will permit the selection of the most appropriate TTC value to use..

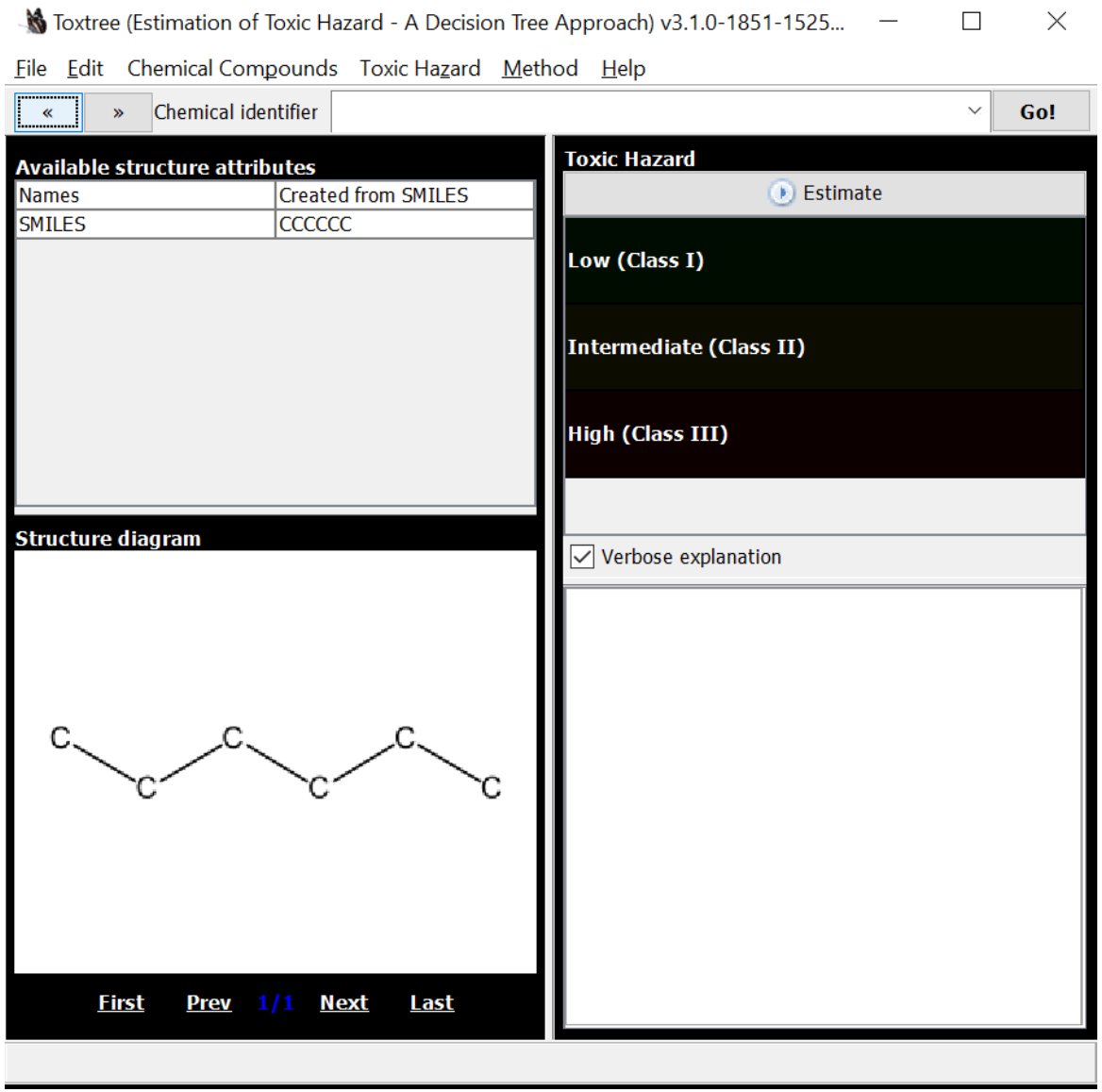
- BUT……it is not quite that simple!

Toxtree

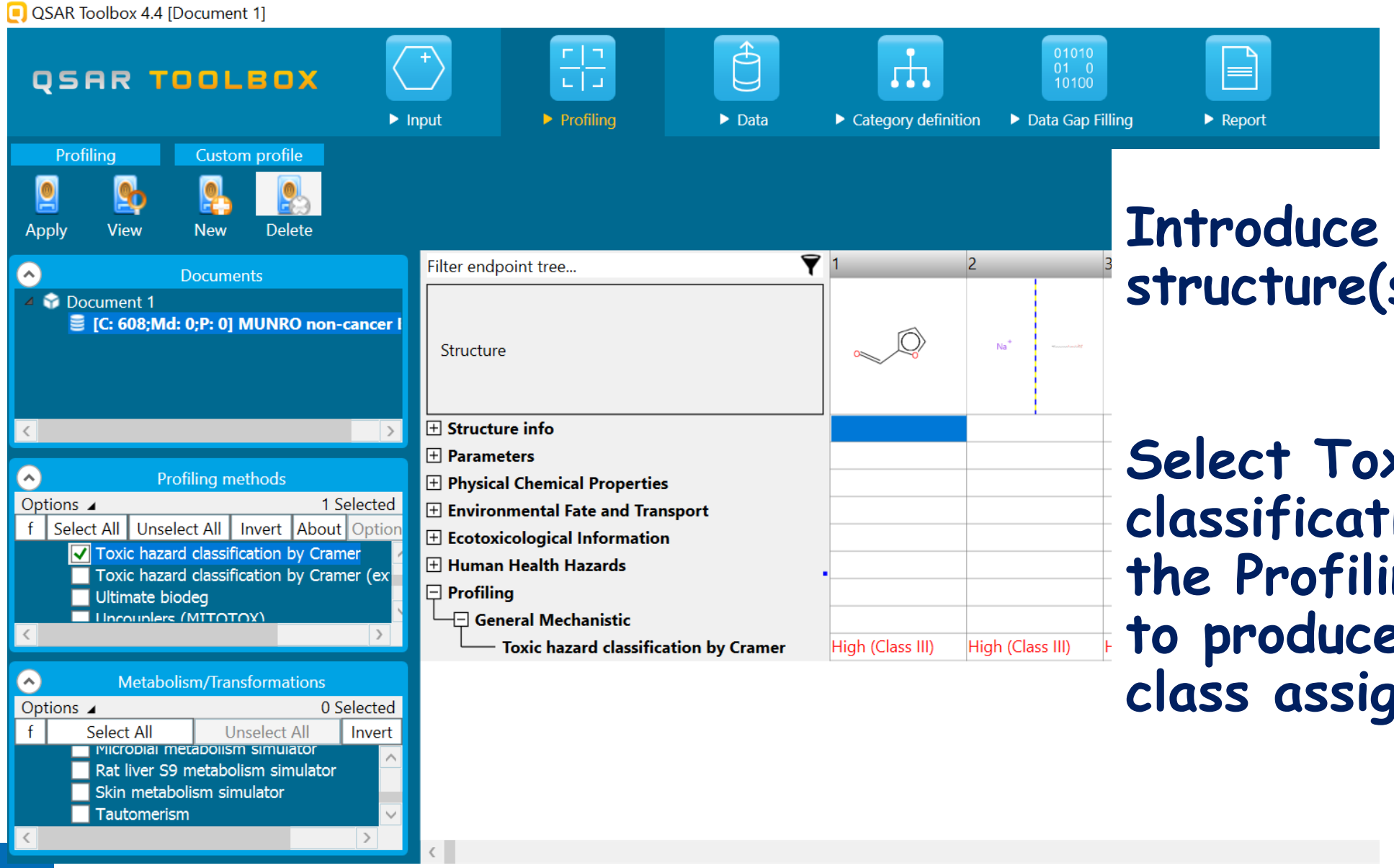
Toxtree – select Cramer rules

Introduce chemical structure

Click Estimate to produce the Cramer class assignment



OECD Toolbox



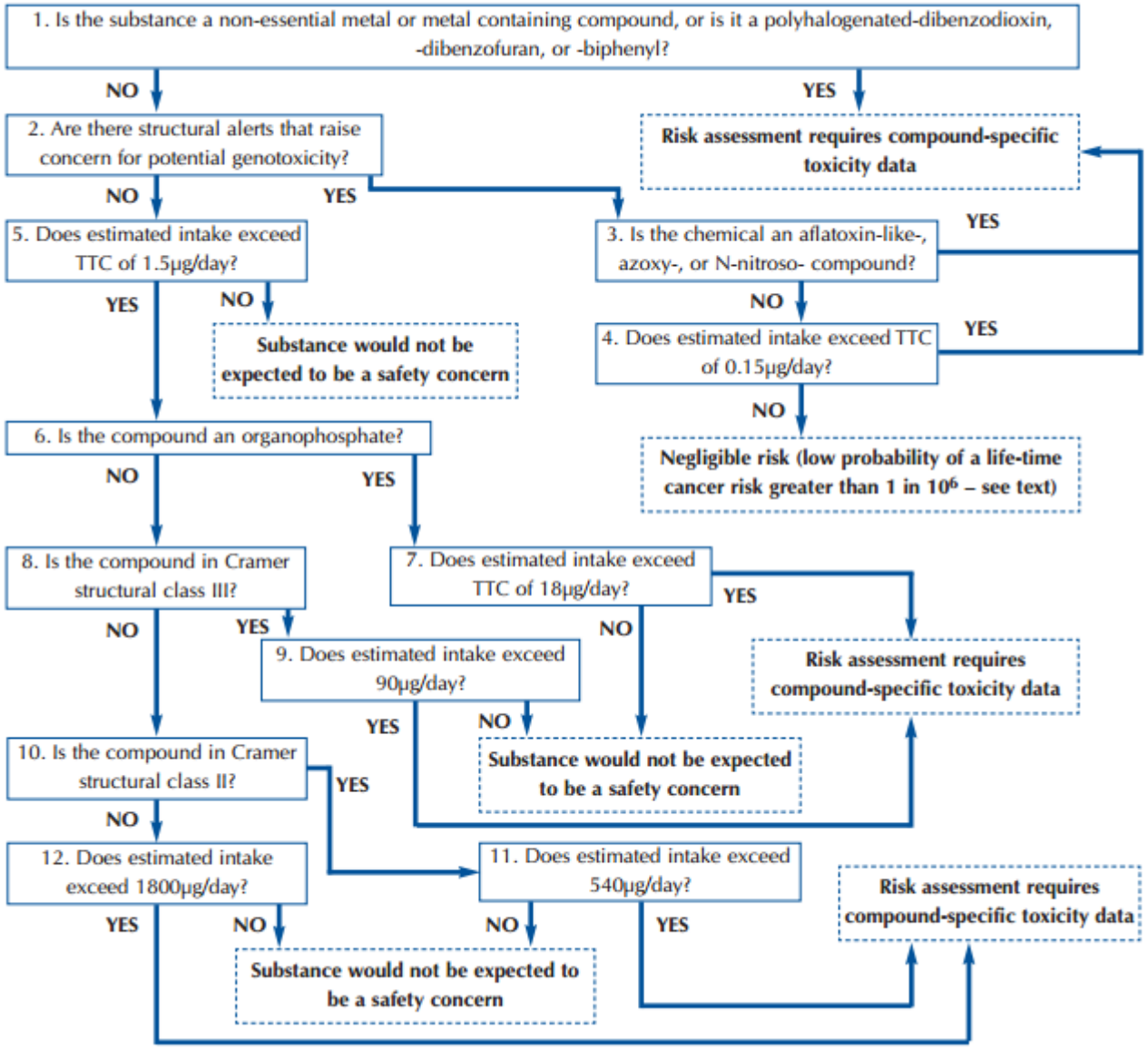
Introduce chemical structure(s)

Select Toxic hazard classification under the Profiling methods to produce the Cramer class assignment

Kroes et al (2004) workflow

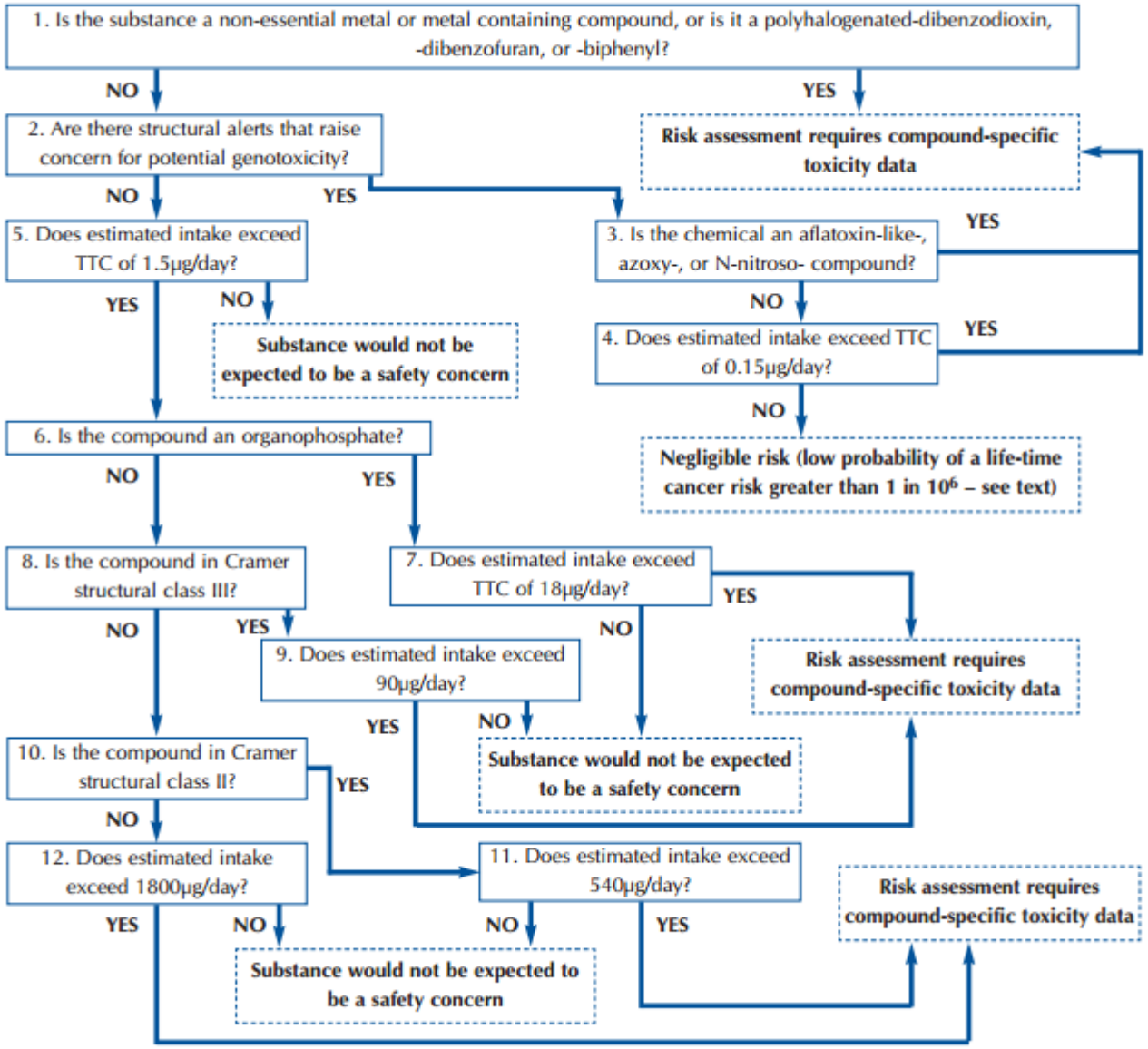
- Applying the TTC in practice
- Is the substance even applicable for TTC?

- Typical exclusions:
  - Metals and Organometallics
  - Proteins
  - Steroids
  - Substances with a potential for bioaccumulation
  - Nanomaterials
  - Radioactive substances
  - Mixtures of substances containing unknown chemical structures



Kroes et al (2004) workflow

- Does the substance present any structural alerts for genotoxicity?
- If yes – is it one of the high potency carcinogens classes? – stop or assign most conservative TTC value
- If no alerts, consider whether the substance is an organophosphate (OP) or carbamate – which are associated with a specific TTC value
- If not an OP or carbamate – progress to consideration of the Cramer classes



Oral TTC values

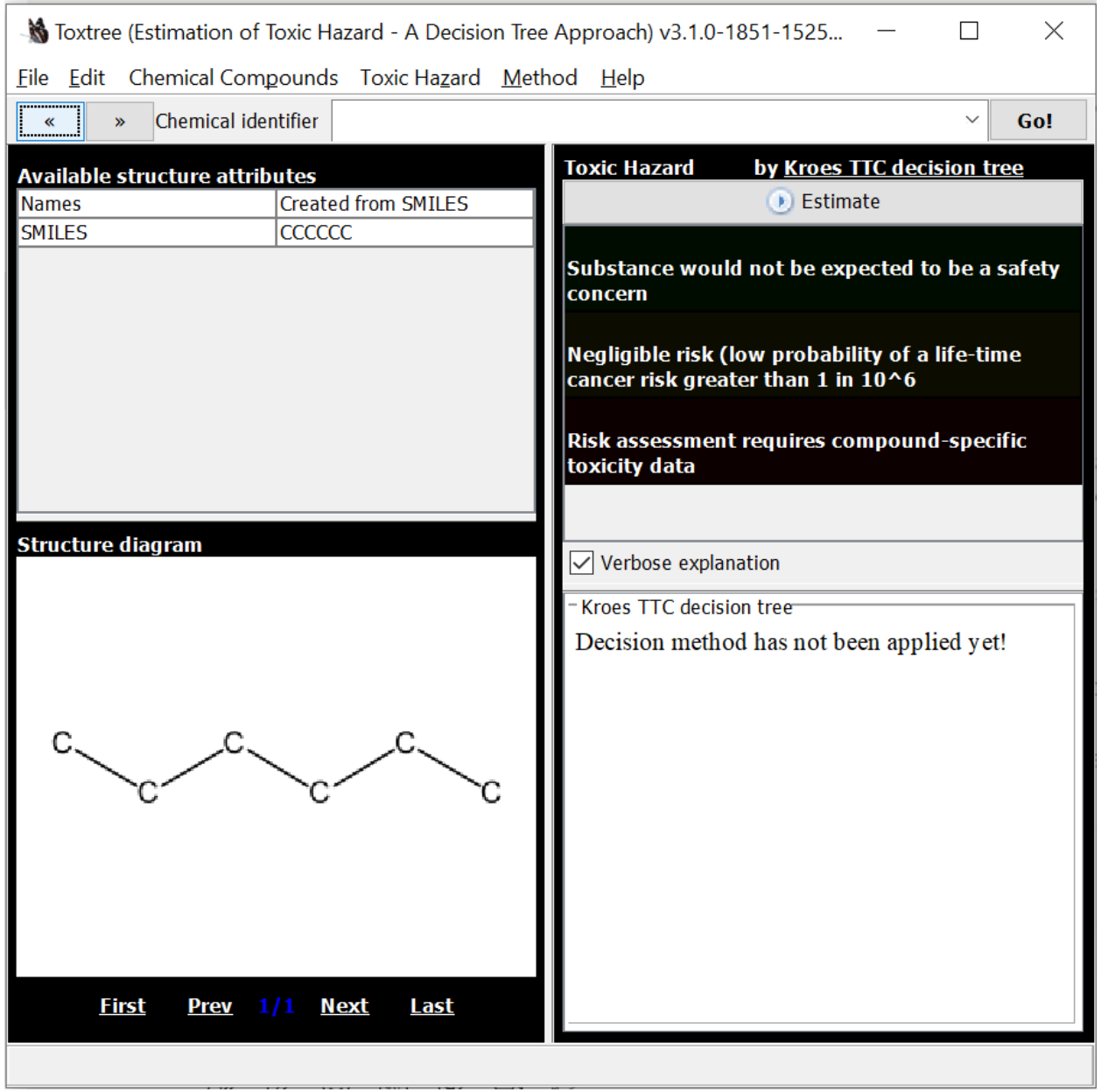
Type of substance	µg/person/day (µg/kg-day for 60 kg adult)
Alerts for potential genotoxic carcinogenicity	Kroes: 0.15 (0.0025 µg/kg-day) ICH: 1.5 (0.025 µg/kg-day)
Acetylcholinesterase inhibitors (AChEI) Organophosphate/carbamate	18 (0.3 µg/kg-day)
Cramer Class III	90 (1.5 µg/kg-day)
Cramer Class II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

Toxtree - Kroes

Introduce chemical of interest

Introduce exposure level

Process substance through the Kroes workflow to determine TTC value that is most applicable or whether a substance specific risk assessment is required



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Assumptions

- TTC assumes a lifetime exposure (every day for ~70 years)
- TTC values that are established are for the ORAL route of entry
- Are there situations when higher TTC values could be proposed when exposure duration is likely to be more shorter term <1 year
- Proposals have been made in the pharma sector to evaluate genotoxic impurities (can a higher TTC value be set to accommodate the risk/benefit of a particular pharmaceutical, proposals for higher TTC values when accounting for occupational vs consumer exposures – can a 1 in 10^5 risk be tolerated instead of a 1 in 10^6

Staged TTC values

Acceptable Daily Intakes* for an Individual Impurity, μg/day Clinical trials or marketed product								
	Single Dose	< 14 days	≤ 1 mo.	≤ 3 mo.	≤ 6 mo.	≤ 12 mo.	>1 – 10 years	>10 years to lifetime
M7	**	**	120	20	20	20	10	1.5
EMA	120	60	60	30	10	5	1.5 (marketed)	1.5

*Compound-specific risk assessments to derive acceptable intakes should be applied instead of the TTC-based acceptable intakes where sufficient carcinogenicity data exist.

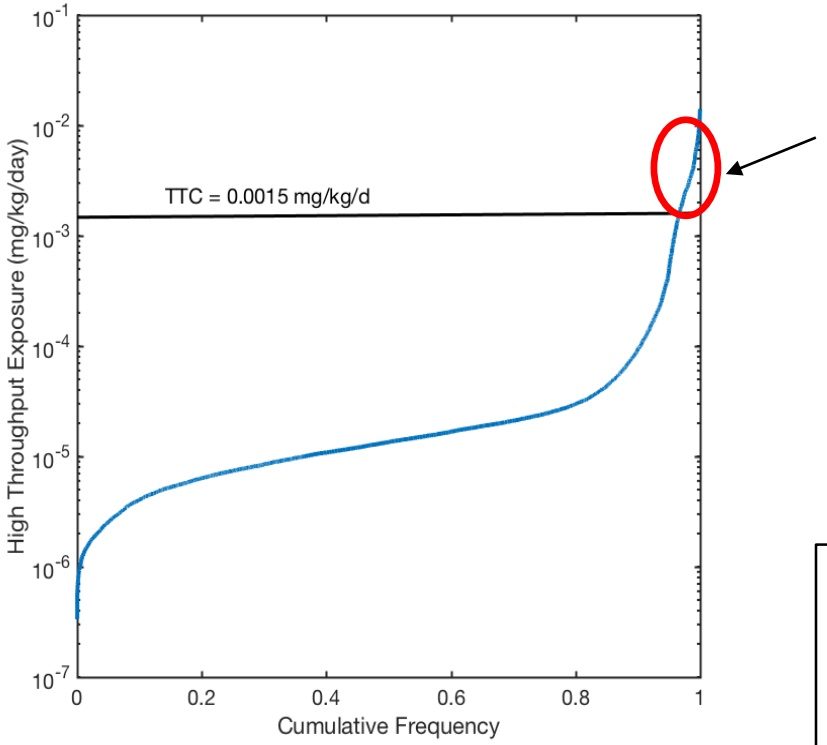
**Clinical trials of up to 14 days – class 3 impurities can be treated as normal impurities

Risk-Based prioritisation

- Rank ordering large numbers of chemicals at the same time that are data poor and for which no exposure information might be known apriori
- One approach considered involved coupling Threshold of Toxicological Concern (TTC) with High Throughput Exposure (HTE) modelling (predicted exposure values) to rank order substances for further evaluation
- Wambaugh and colleagues (2014) developed a rapid heuristic high throughput exposure (HTE) model that enables prediction of potential human exposure to thousands of substances for which little or no empirical exposure data are available.
- The HTE model was calibrated by comparison to NHANES urinary data that reflects total exposure (all routes/sources)

Integrating TTC with predicted HT exposures

- Compared the conservative Cramer Class III TTC value of 1.5 µg/kg-day to the previously calculated median and upper 95% credible interval (UCI) of total daily median exposure rates for 7968 chemicals

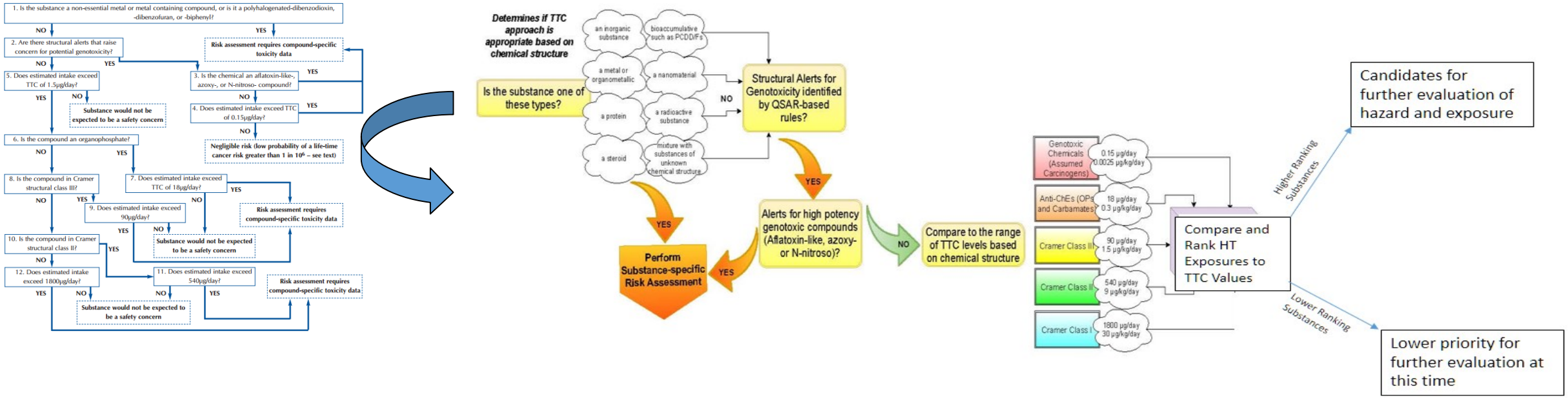


only 273 (fewer than 5%) were found to have UCI daily exposures estimates that exceeded the Cramer Class III TTC value of 1.5 µg/kg-day

Initial evaluation showed the approach of using the ratio of exposure to TTC (HTE: TTC) appeared promising for risk-based prioritisation

Risk-Based prioritisation

- Refined the approach using the Kroes et al structure-based workflow for TTC



- None of the substances categorised as Cramer Class I or Cramer Class II exceeded their respective TTC values.
- No more than 2% of substances categorised as Cramer Class III or acetylcholinesterase inhibitors exceeded their respective TTC values.
- Majority of chemicals with genotoxicity structural alerts did exceed the relevant TTC – recommendations were proposed for next steps

Patlewicz et al, 2018

Risk-Based prioritisation

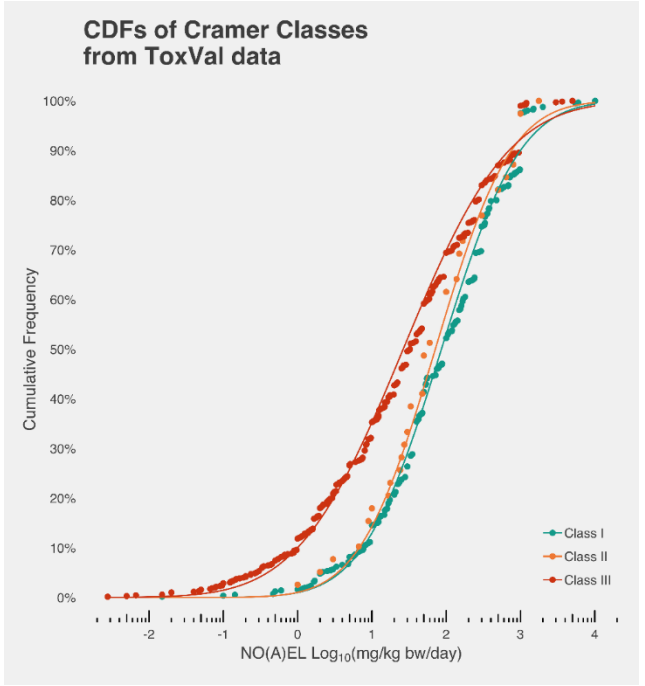
- Processed substances through the Kroes module within Toxtree but some adaptations needed to made since the batch process required exposure information upfront
- Deconstructed the Kroes workflow into different steps to mirror the published workflow
- Created ad hoc modules to identify steroids, organophosphates, carbamates and scripts were written to parse out relevant outputs from an initial batch profiling of the substances through the Kroes workflow
- R scripts are provided as supplementary information in Patlewicz et al (2018)

Risk-Based prioritisation

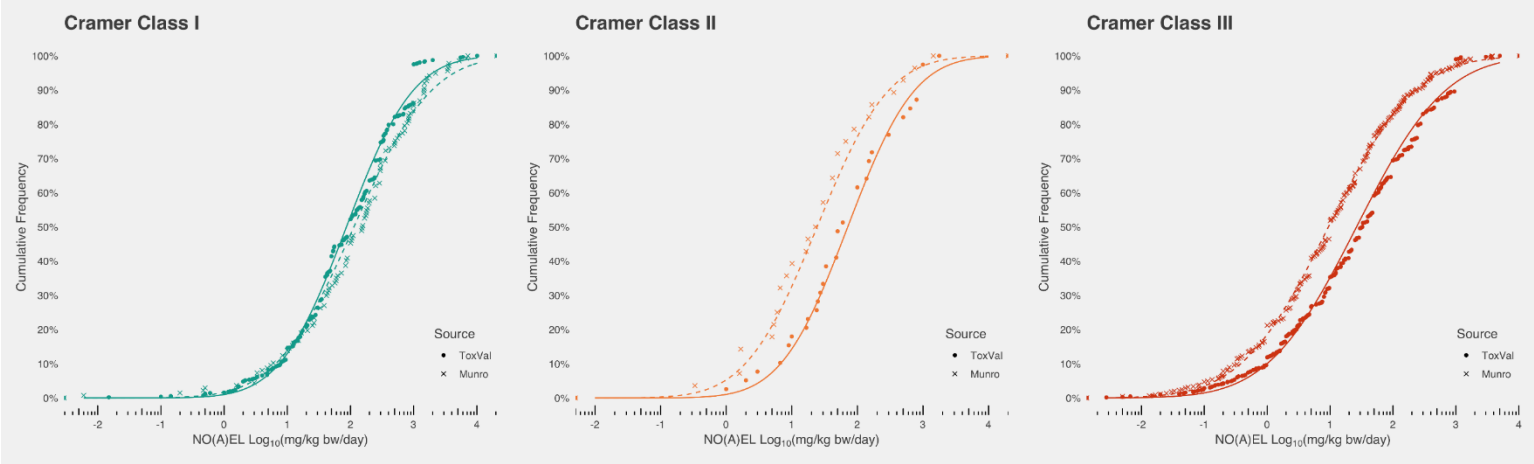
- Investigate relevance of existing TTC values for substances of interest to EPA
- Extracted data from EPA's ToxValDB, which aggregates *in vivo* testing data from over 40 sources including US federal and state agencies, as well as international agencies such as the European Chemicals Agency and the World Health Organisation
- 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

Risk-Based prioritisation

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

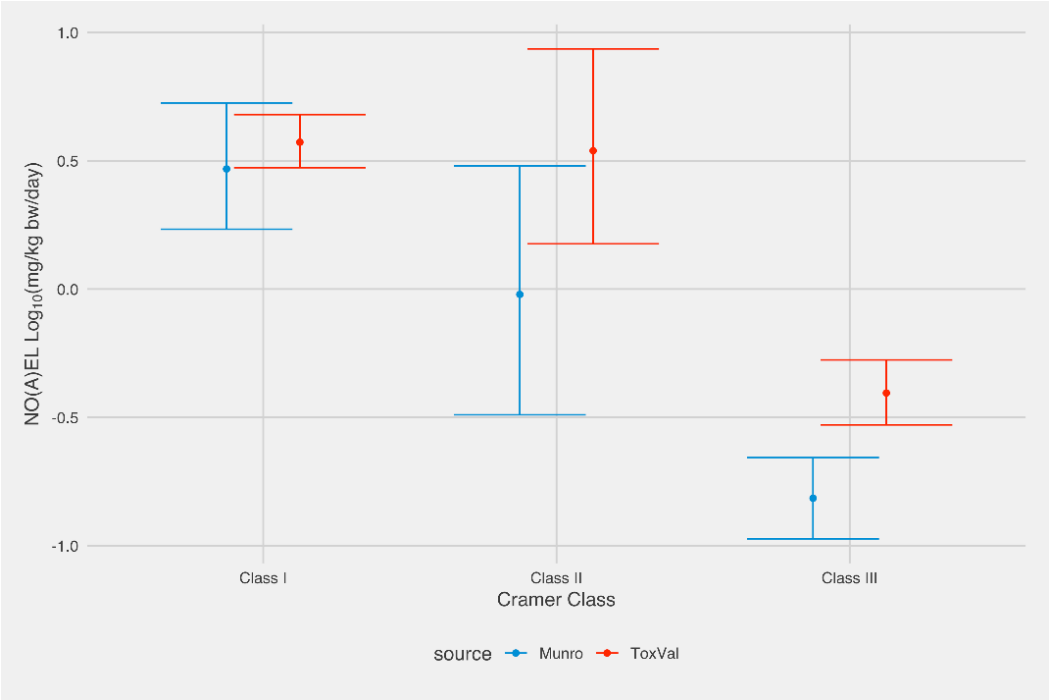


Evaluate whether the TTC values from ToxVal and Munro are statistically equivalent & derive confidence intervals for the new TTC values



Risk-Based prioritisation

- 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 set largely explained the difference in 5th percentile values
- Derived new modules for OPs

Nelms et al, 2019

Risk-Based prioritisation: inhalation route of entry

- Whilst TTC values for oral route of exposure are well established, there are no established TTC valued for inhalation
- Current focus is investigating the feasibility of deriving new TTC values using the ToxValDB
- Processing the substances with NO(A)EL/NO(A)EC values through the Kroes workflow –replicates other similar efforts published by Carthew et al (2009) and Escher et al (2010)

Risk-Based prioritisation: inhalation route of entry

- For substances assigned into the Cramer structural classes, have found that the Cramer classes are not effective at discriminating the potency – other approaches to subcategorise the substances are being explored
- Furthermore the distribution of toxicity values do not fit a log normal distribution – bootstrapping the percentile of the empirical data to derive a value for TTC purposes is an alternative approach

Disclaimers – only scratched the surface

- TTCs for other endpoints – e.g. skin sensitisation
- eco TTCs
- Other routes of exposure…beyond oral routes of entry
- Other chemical/substances of interest e.g. cosmetics, medical devices
- Augmenting Cramer structural class II with more chemicals e.g. work by RIFM
- Internal TTCs vs external TTCs e.g. work led by P&G
- Cancer endpoints – work is ongoing to augment and curate the original Carcinogenicity Potency Database that was used to derive the cancer TTC threshold originally used by the FDA and the conservative threshold used in Kroes et al (2004)

Take home messages

- Computational toxicology approaches impact many aspects of regulatory contexts
- Outlined how computational approaches fit within an IATA
- Described the TTC approach and how is it evolved and how it is used in practice in screening level hazard assessment decision contexts
- Illustrated how coupling HTE and TTC can be used as part of risk-based prioritisation application
- Discussed ongoing research efforts in this field
- TTC – Threshold of Toxicological Concern is a pragmatic means of waiving testing when exposures are v low and when little or no toxicity data exists.
- BUT it does not overrule traditional risk assessment practices