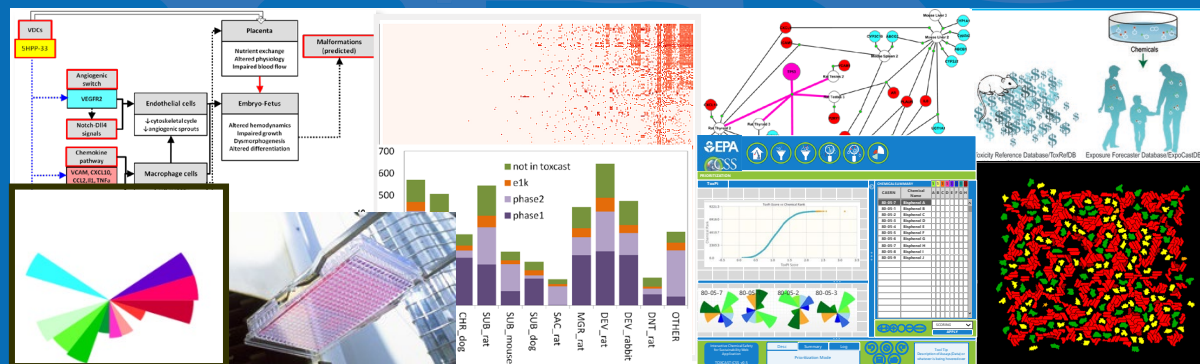


# Threshold of Toxicological Concern (TTC) a useful tool in the Computational Toxicology armory



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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.

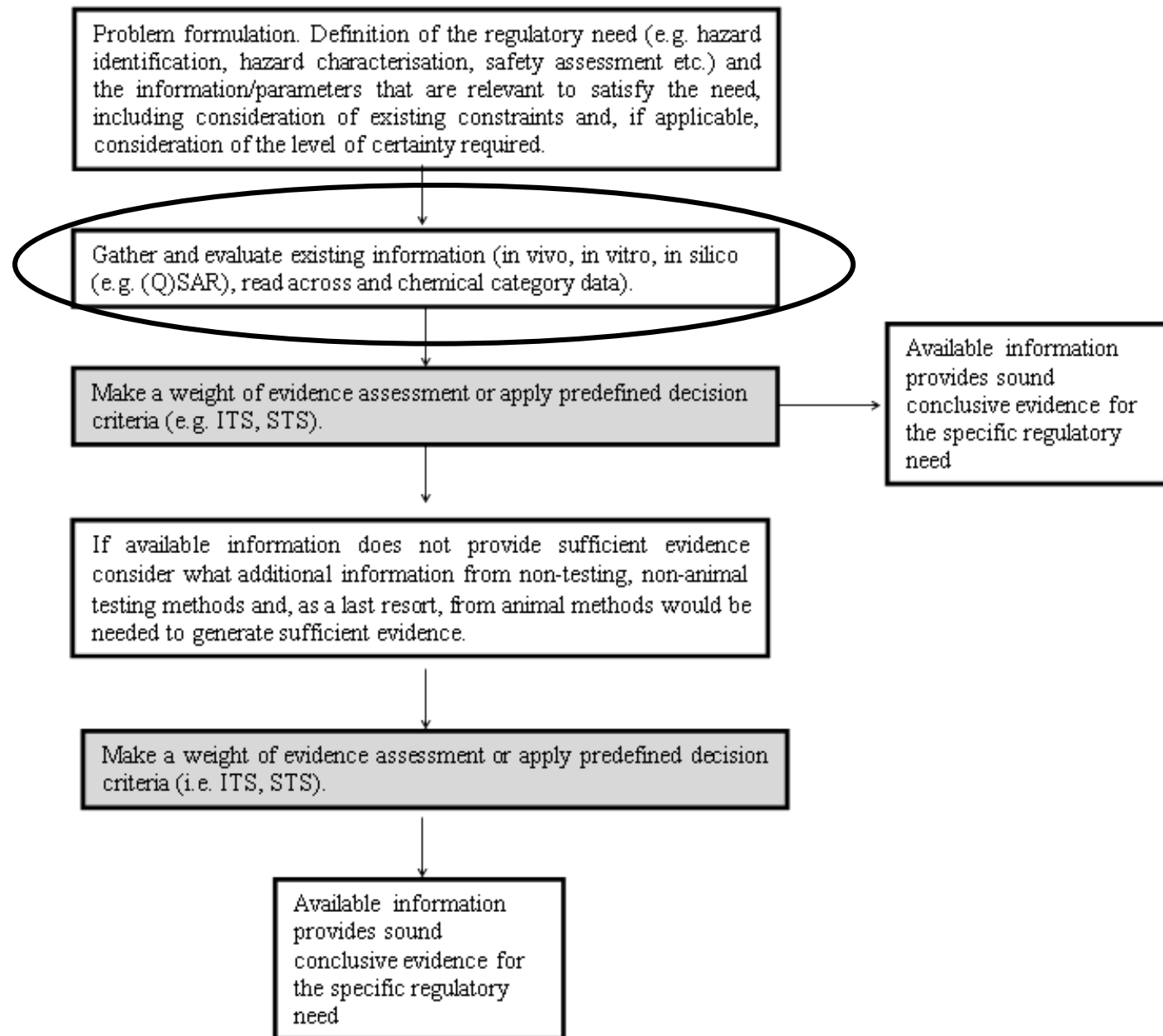
# Regulatory and Non-Regulatory drivers

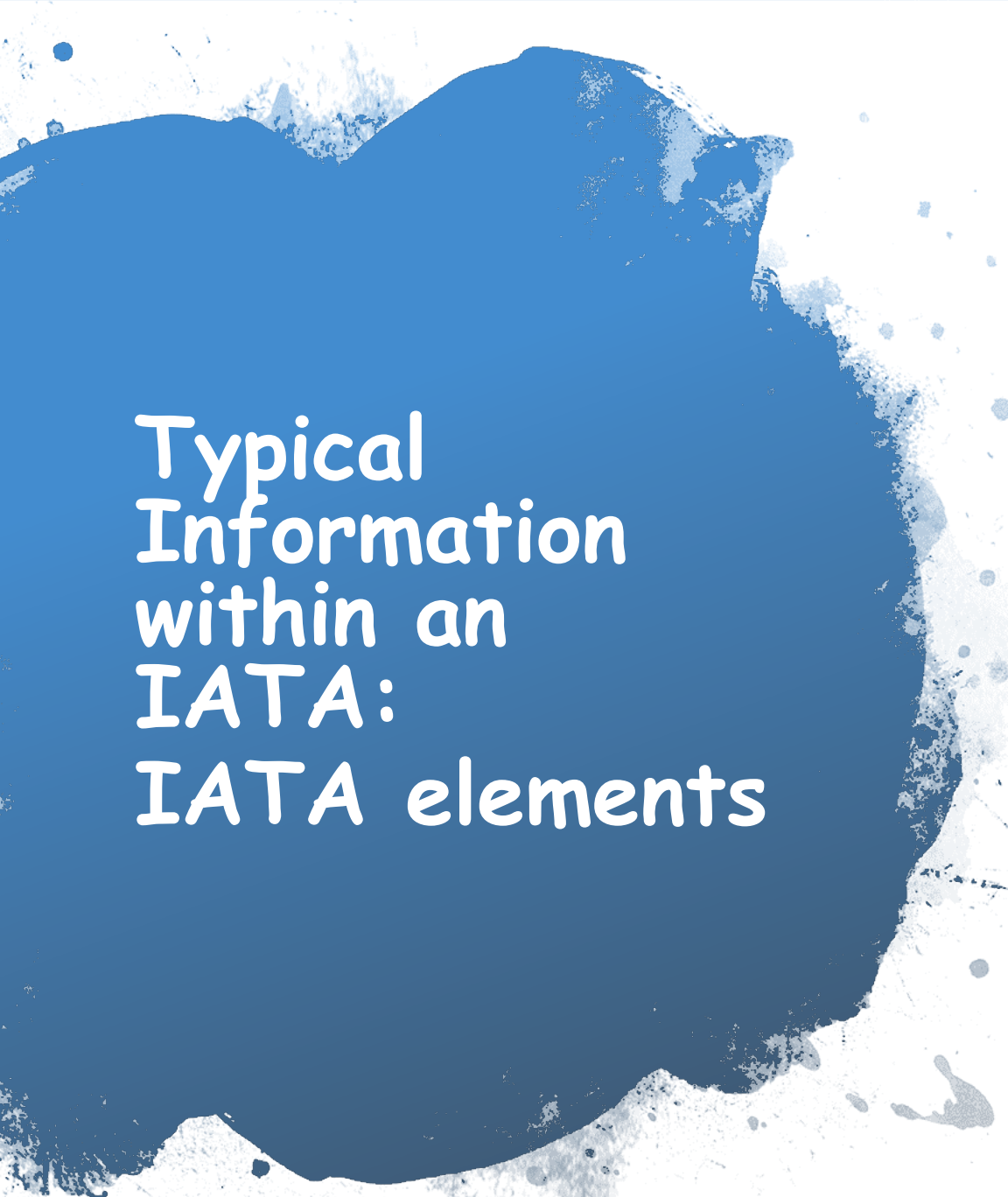
- In the US, the new Frank Lautenberg Chemical Safety for the 21<sup>st</sup> Century Act (LCSEA) requires that a risk based prioritisation is conducted for all substances in commerce, ~40,000, many of which are lacking sufficient publicly available toxicity information.
- EPA Administrator signed memo 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 Domestic Substances 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





# 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

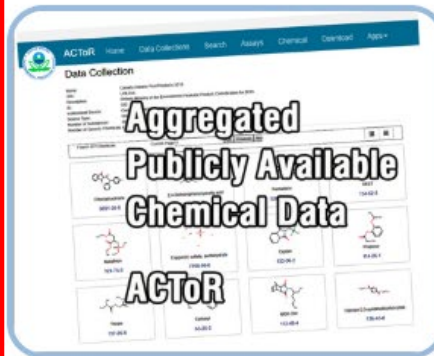
## <https://comptox.epa.gov/>

Environmental Topics

Laws & Regulations

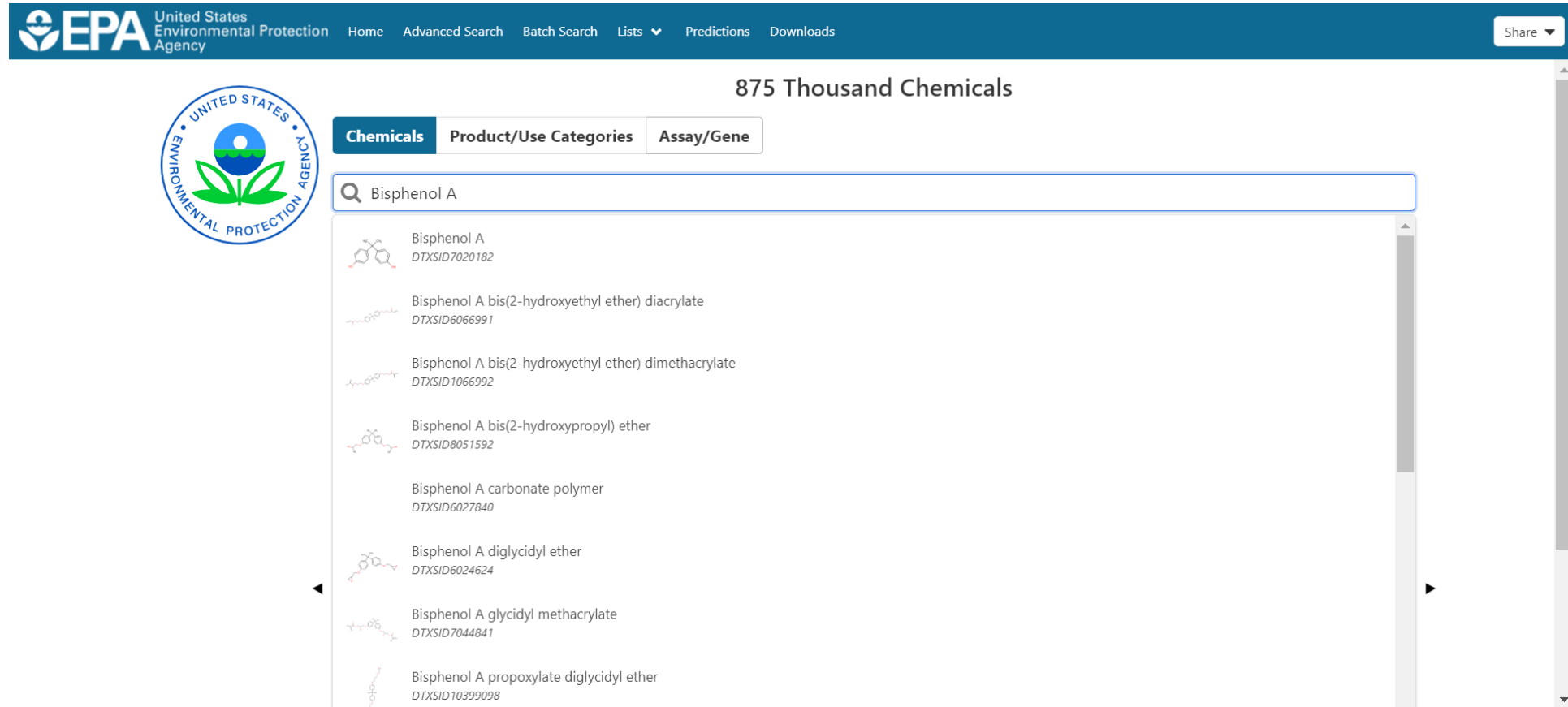
About EPA

Search EPA.gov



# CompTox Chemicals Dashboard: Landing Page

- Different entry points depending on domain of interest



The screenshot shows the CompTox Chemicals Dashboard landing page. At the top is a dark blue header with the EPA logo and navigation links: Home, Advanced Search, Batch Search, Lists (with a dropdown arrow), Predictions, and Downloads. A 'Share' button with a dropdown arrow is on the right. Below the header, the text '875 Thousand Chemicals' is displayed. On the left is a circular EPA seal. To its right are three tabs: 'Chemicals' (selected), 'Product/Use Categories', and 'Assay/Gene'. A search bar contains the text 'Bisphenol A'. Below the search bar is a list of search results, each with a chemical structure icon, the name of the chemical, and its DTXSID. The results are: Bisphenol A (DTXSID7020182), Bisphenol A bis(2-hydroxyethyl ether) diacrylate (DTXSID6066991), Bisphenol A bis(2-hydroxyethyl ether) dimethacrylate (DTXSID1066992), Bisphenol A bis(2-hydroxypropyl) ether (DTXSID8051592), Bisphenol A carbonate polymer (DTXSID6027840), Bisphenol A diglycidyl ether (DTXSID6024624), Bisphenol A glycidyl methacrylate (DTXSID7044841), and Bisphenol A propoxylate diglycidyl ether (DTXSID10399098). A vertical scrollbar is on the right side of the results list.

United States Environmental Protection Agency

Home Advanced Search Batch Search Lists Predictions Downloads

Share

875 Thousand Chemicals

Chemicals Product/Use Categories Assay/Gene

Q Bisphenol A

Bisphenol A  
DTXSID7020182

Bisphenol A bis(2-hydroxyethyl ether) diacrylate  
DTXSID6066991

Bisphenol A bis(2-hydroxyethyl ether) dimethacrylate  
DTXSID1066992

Bisphenol A bis(2-hydroxypropyl) ether  
DTXSID8051592

Bisphenol A carbonate polymer  
DTXSID6027840

Bisphenol A diglycidyl ether  
DTXSID6024624

Bisphenol A glycidyl methacrylate  
DTXSID7044841

Bisphenol A propoxylate diglycidyl ether  
DTXSID10399098

# CompTox Chemicals Dashboard: Landing Page for a specific chemical

United States  
Environmental Protection  
Agency

[Home](#)
[Advanced Search](#)
[Batch Search](#)
[Lists](#)
[Predictions](#)
[Downloads](#)

Copy
Share
Submit Comment
Search all data

## Bisphenol A

80-05-7 | DTXSID7020182

Searched by DSSTox Substance Id.

DETAILS

EXECUTIVE SUMMARY

PROPERTIES

ENV. FATE/TRANSPORT

HAZARD

▶ ADME

▶ EXPOSURE

▶ BIOACTIVITY

SIMILAR COMPOUNDS

GENRA (BETA)

RELATED SUBSTANCES

SYNONYMS

▶ LITERATURE

LINKS

COMMENTS

Wikipedia

**Bisphenol A (BPA)** is an organic synthetic compound with the chemical formula  $(\text{CH}_3)_2\text{C}(\text{C}_6\text{H}_4\text{OH})_2$  belonging to the group of diphenylmethane derivatives and bisphenols, with two hydroxyphenyl groups. It is a colorless solid that is soluble in organic solvents, but poorly soluble in water. It has been in commercial use since 1957.

BPA is a starting material for the synthesis of plastics, primarily

...  
[Read more](#)

Intrinsic Properties

Structural Identifiers


Linked Substances

Presence in Lists

Record Information


Quality Control Notes

# CompTox Chemicals Dashboard: Executive Summary of 'existing' data

 United States  
Environmental Protection  
Agency

HomeAdvanced SearchBatch SearchLists▼PredictionsDownloads

Copy▼Share▼Submit Comment

 Search all data

DETAILS

**EXECUTIVE SUMMARY**

PROPERTIES

ENV. FATE/TRANSPORT

HAZARD

▶ ADME

▶ EXPOSURE

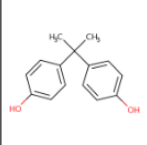
▼ BIOACTIVITY

TOXCAST: SUMMARY

EDSP21

TOXCAST/TOX21

PUBCHEM



Bisphenol A

80-05-7 | DTXSID7020182

Searched by Expert Validated Synonym.

**Quantitative Risk Assessment Values**

✓ IRIS values available [↗](#)

✗ No PPRTV values

✓ EPA RSL values available [↗](#)

✓ Minimum RfD: **0.050 mg/kg-day** (chronic, IRIS, oral, 8) [↗](#)

✗ No RfC calculated

✗ IVIVE POD not calculated

**Quantitative Hazard Values**

✓ Minimum oral POD: **3.8 mg/kg-day** (reproductive, HPVIS, oral, 6) [↗](#)

✗ No inhalation POD values

✓ Lowest Observed Bioactivity Equivalent Level: [CYP1A1](#), [CYP1A2](#), [Tpo](#), [ESR2](#), [ESR1](#), [ESR1](#), [NR1I3](#), [PPARA](#), [NR1I2](#), [Cyp2c11](#), [MMP3](#), [Esr1](#)

**Cancer Information**

✗ No cancer slope factor

✗ No inhalation unit risk value

✓ Carcinogenicity data available: University of Maryland carcinogenicity warning; [↗](#)

✗ No genotoxicity findings reported

**Reproductive Toxicology**

✓ 200 Reproductive toxicity PODs available [↗](#)

**Executive Summary**

REGIONAL SCREENING

Class	THQ	Value
risk-based SSL (mg/kg)	THQ = 0.1	5.8
GIABS (unspecified)	THQ = 1	1
GIABS (unspecified)	THQ = 0.1	1
ABS (unspecified)	THQ = 0.1	0.1
RfDo (mg/kg-day)	THQ = 0.1	0.05
screening level (residential Soil) (mg/kg)	THQ = 0.1	320
screening level (industrial soil) (mg/kg)	THQ = 0.1	4100

14

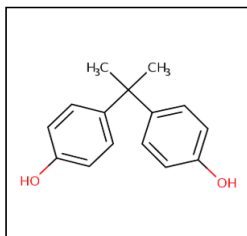
# QSAR Predictions

OPERA Models: LogP: Octanol-Water

Bisphenol A

80-05-7 | DTXSID7020182

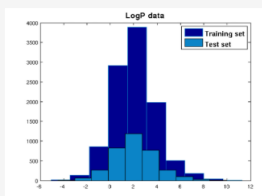
Print PDF



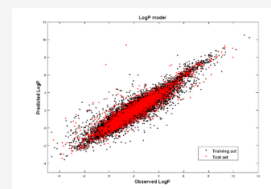
## Model Results

Predicted value: 3.35  
Global applicability domain: Inside  
Local applicability domain index: 0.877  
Confidence level: 0.813

## Model Performance



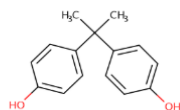
QMRF



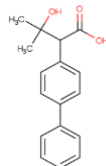
Weighted KNN model

5-fold CV (75%)		Training (75%)		Test (25%)	
Q2	RMSE	R2	RMSE	R2	RMSE
0.850	0.690	0.860	0.670	0.860	0.780

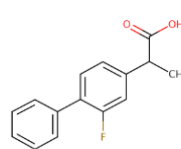
## Nearest Neighbors from the Training Set



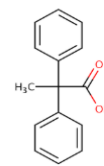
Bisphenol A  
Measured: 3.32  
Predicted: 3.35076



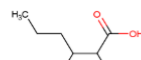
BUTANOIC ACID, 2-(4-BIPHENYL)-3-HYDROXY-3-METHYL-  
Measured: 3.25  
Predicted: 3.39062



Flurbiprofen  
Measured: 4.16  
Predicted: 3.94445



2,2-Diphenylpropionic acid  
Measured: 2.69  
Predicted: 2.84603



80-05-7 | DTXSID7020182

Searched by Expert Validated Synonym.

16



# 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

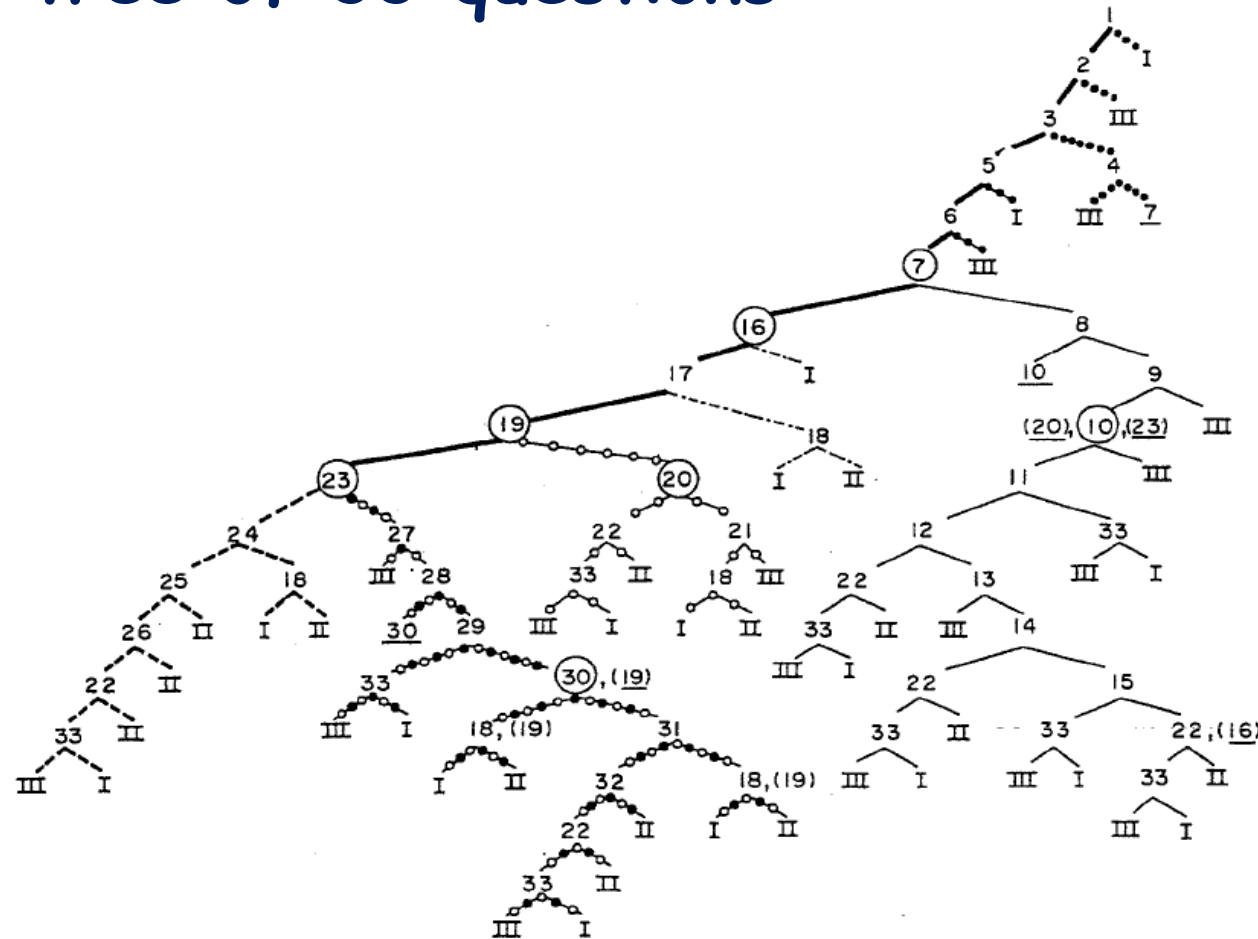
- Two types of TTCs:
- 'General' TTC is based on a predicted tumour risk of 1 in a million, derived through an analysis of cancer data
- Structural based TTCs are based on frequency distributions (5<sup>th</sup> percentile) of NO(A)ELs of non-cancer endpoints

# 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

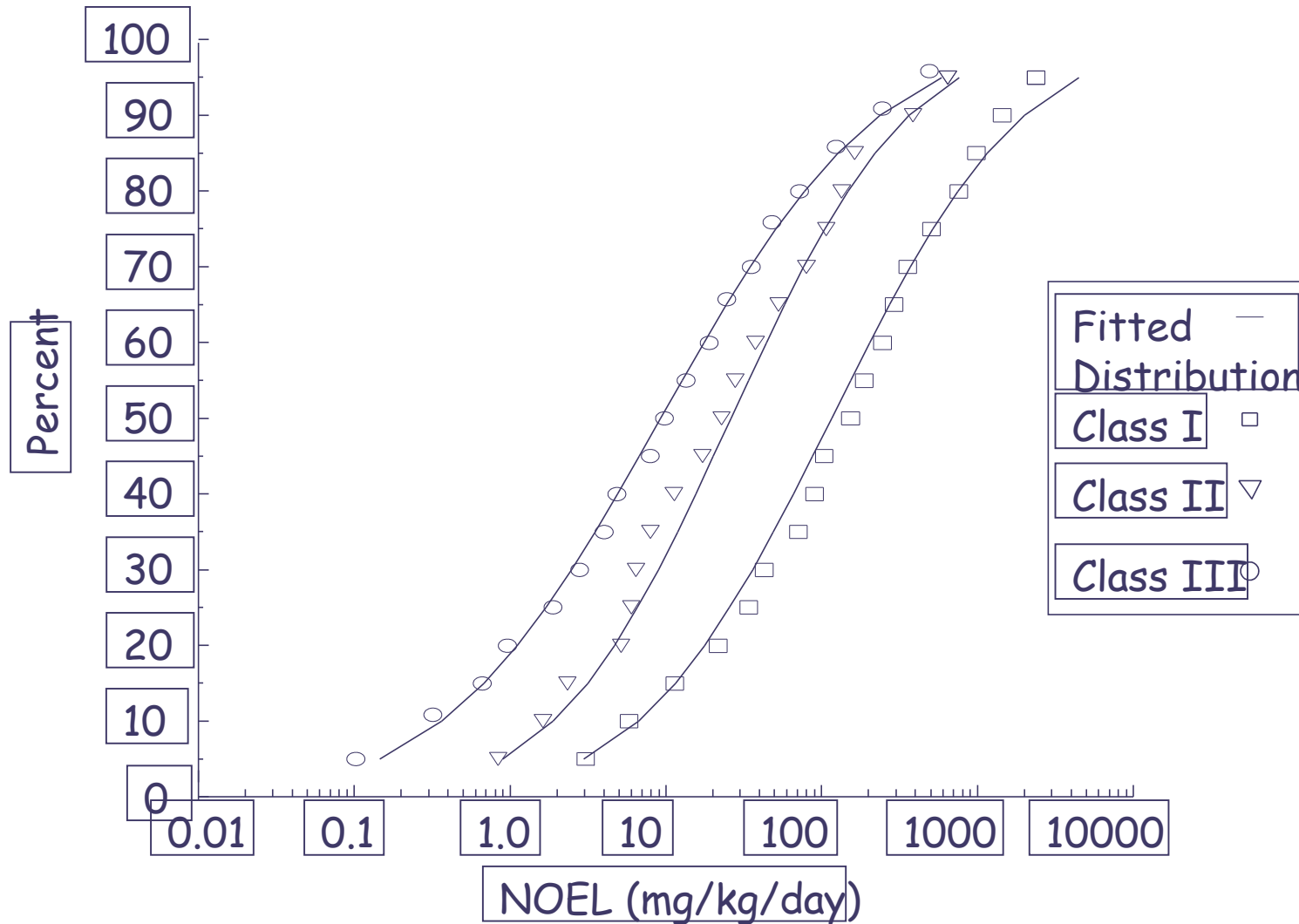
- Decision tree of 33 questions



# 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 <sup>a</sup>	No. of Chemicals	5th Percentile NOEL (µg/kg/day)	Human Exposure Threshold (µg/day) <sup>b</sup>
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)

<sup>a</sup> Cramer *et al.* (1978) structural classes

<sup>b</sup> The human exposure threshold was calculated by multiplying the 5<sup>th</sup> 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, 1979t
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 - gava		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 - gava		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
79	3	13	C.I. Disperse Red 22	6471-40-4	rat	720 chr	fod	Oral - diet		1100	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 5<sup>th</sup> percentile of the cumulative distribution function
- This 5<sup>th</sup> 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 5<sup>th</sup> percentile is 0.15 mg/kg bw/day

# 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 5<sup>th</sup> percentile of the theoretical distribution
- `10**(np.percentile(samples, 5))`

# 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

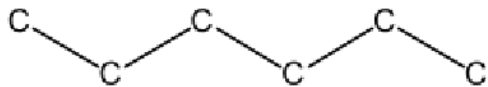
Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) v3.1.0-1851-1525...

File Edit Chemical Compounds Toxic Hazard Method Help

« » Chemical identifier Go!

Available structure attributes	
Names	Created from SMILES
SMILES	CCCCC

Structure diagram



First Prev 1/1 Next Last

**Toxic Hazard**

Estimate

Low (Class I)

Intermediate (Class II)

High (Class III)

☒ Verbose explanation

# OECD Toolbox

QSAR Toolbox 4.4 [Document 1]

**QSAR TOOLBOX**

Input Profiling Data Category definition Data Gap Filling Report

Profiling Custom profile

Apply View New Delete

**Documents**

Document 1  
[C: 608;Md: 0;P: 0] MUNRO non-cancer I

**Profiling methods**

Options 1 Selected

Select All Unselect All Invert About Options

☒ Toxic hazard classification by Cramer  
☐ Toxic hazard classification by Cramer (ex)  
☐ Ultimate biodeg  
☐ Uncouplers (MITOTOX)

**Metabolism/Transformations**

Options 0 Selected

Select All Unselect All Invert

☐ Microbial metabolism simulator  
☐ Rat liver S9 metabolism simulator  
☐ Skin metabolism simulator  
☐ Tautomerism

Filter endpoint tree...

Structure

**Structure info**  
**Parameters**  
**Physical Chemical Properties**  
**Environmental Fate and Transport**  
**Ecotoxicological Information**  
**Human Health Hazards**  
**Profiling**  
 General Mechanistic  
 Toxic hazard classification by Cramer

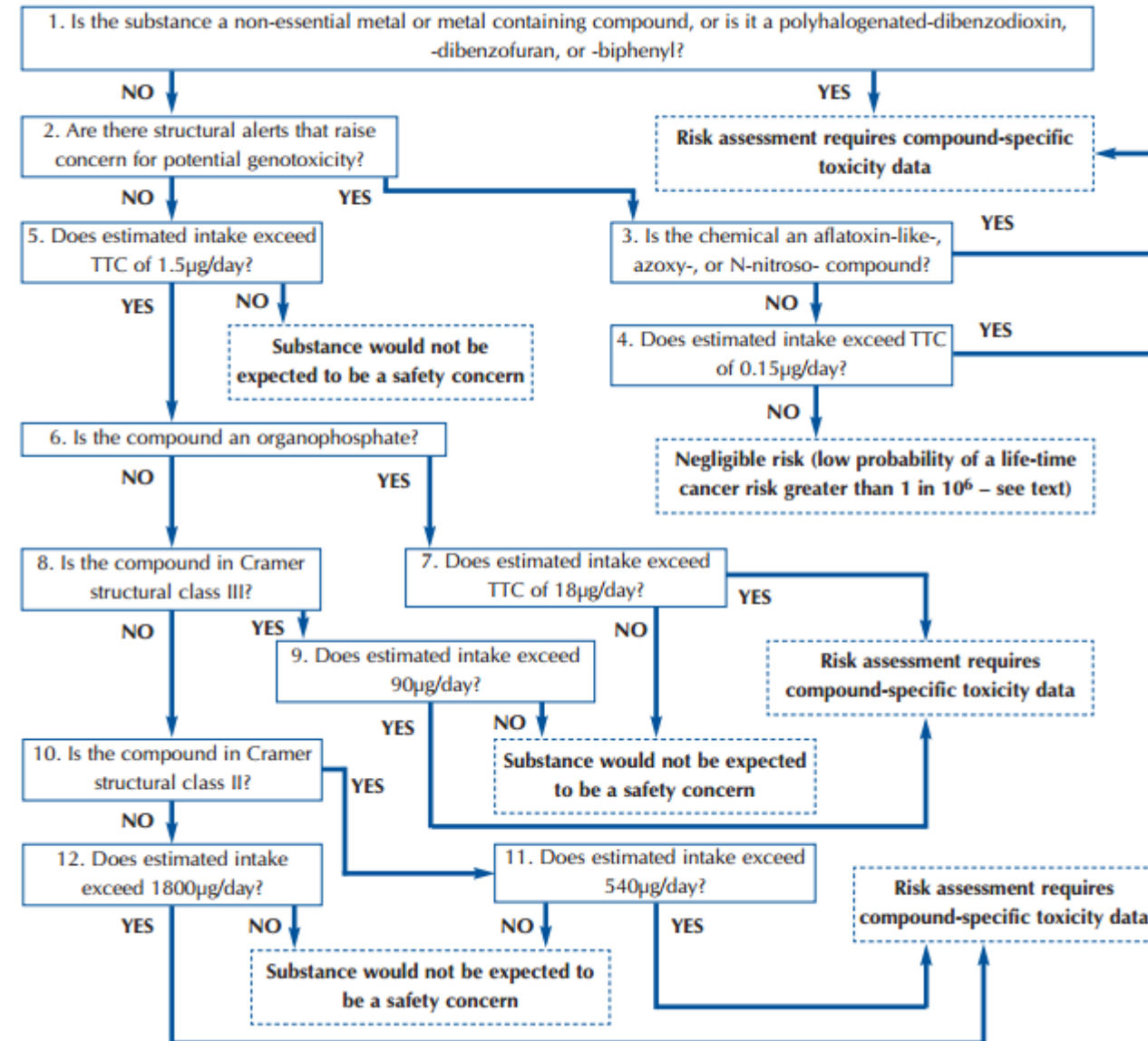
1	2	3
	Na <sup>+</sup>	
High (Class III)	High (Class III)	

Introduce chemical structure(s)

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

# Kroes et al (2004) workflow

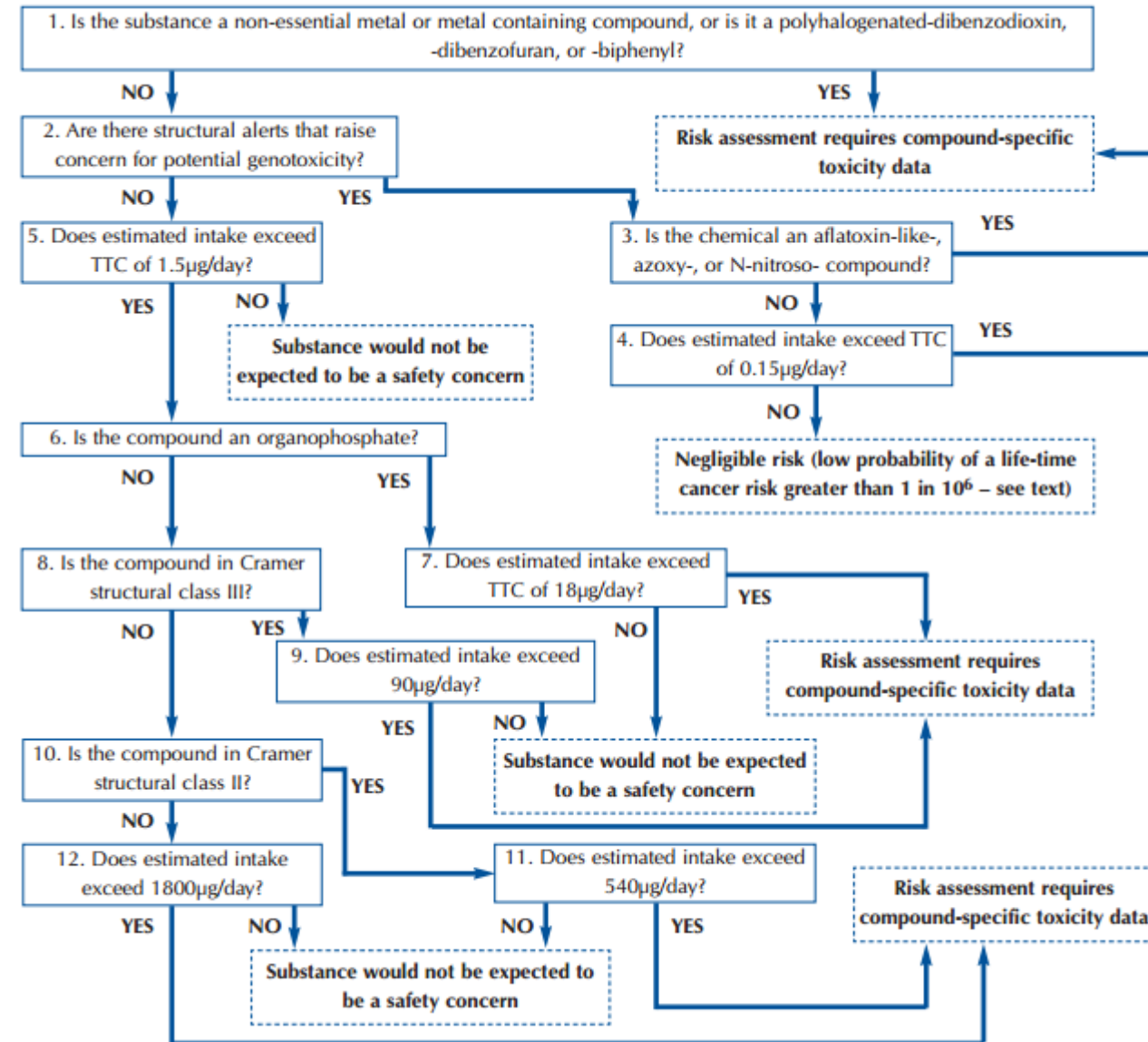
- Applying the TTC in practice
- Is the substance even applicable for TTC?
- Typical exclusions:
  - Metals and Organometallics
  - Proteins
  - Steroids
  - Substances with a potential for bioaccumulation
  - Nanomaterials
  - Radioactive substances
  - Mixtures of substances containing unknown chemical 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

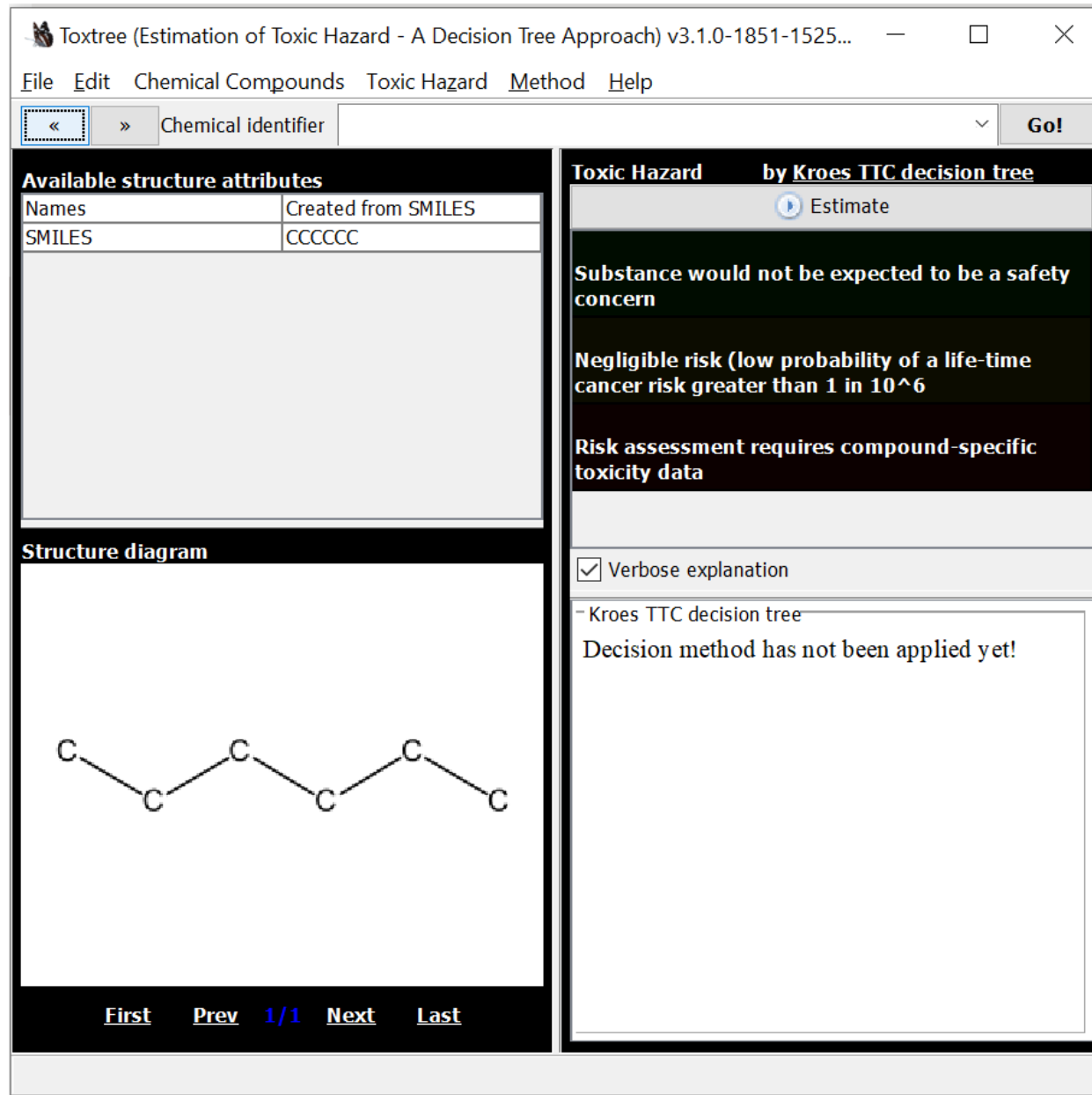
Type of substance	µg/person/day (µg/kg-day for 60 kg adult)
Alerts for potential genotoxic carcinogenicity	Kroes: 0.15 (0.0025 µg/kg-day) ICH: 1.5 (0.025 µg/kg-day)
Acetylcholinesterase inhibitors (AChEI) Organophosphate/carbamate	18 (0.3 µg/kg-day )
Cramer Class III	90 (1.5 µg/kg-day)
Cramer Class II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



The screenshot displays the Toxtree software interface, titled "Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) v3.1.0-1851-1525...". The interface includes a menu bar (File, Edit, Chemical Compounds, Toxic Hazard, Method, Help) and a toolbar with a "Go!" button. The main window is divided into several panels:

- Chemical identifier:** A text input field with a "Go!" button.
- Available structure attributes:** A table showing attributes for the chemical structure.

Available structure attributes	
Names	Created from SMILES
SMILES	CCCCC
- Structure diagram:** A visual representation of the chemical structure, showing a zigzag chain of six carbon atoms (C-C-C-C-C-C).
- Toxic Hazard by Kroes TTC decision tree:** A panel containing the decision tree results.
  - Estimate:** A button to initiate the estimation.
  - 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<sup>6</sup>)**
  - Risk assessment requires compound-specific toxicity data**
- Verbose explanation:** A checkbox that is currently checked.
- Kroes TTC decision tree:** A text area stating "Decision method has not been applied yet!".

At the bottom of the interface, there are navigation buttons: [First](#), [Prev](#), [1/1](#), [Next](#), and [Last](#).

# 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.

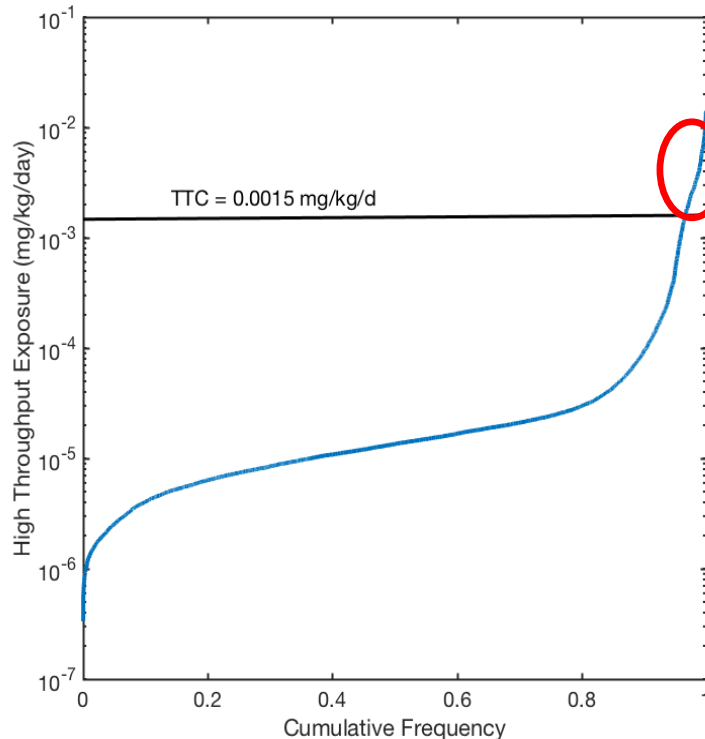
\*\*Clinical trials of up to 14 days – class 3 impurities can be treated as normal impurities

# 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  $1.5 \mu\text{g}/\text{kg}\text{-day}$  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  $1.5 \mu\text{g}/\text{kg}\text{-day}$

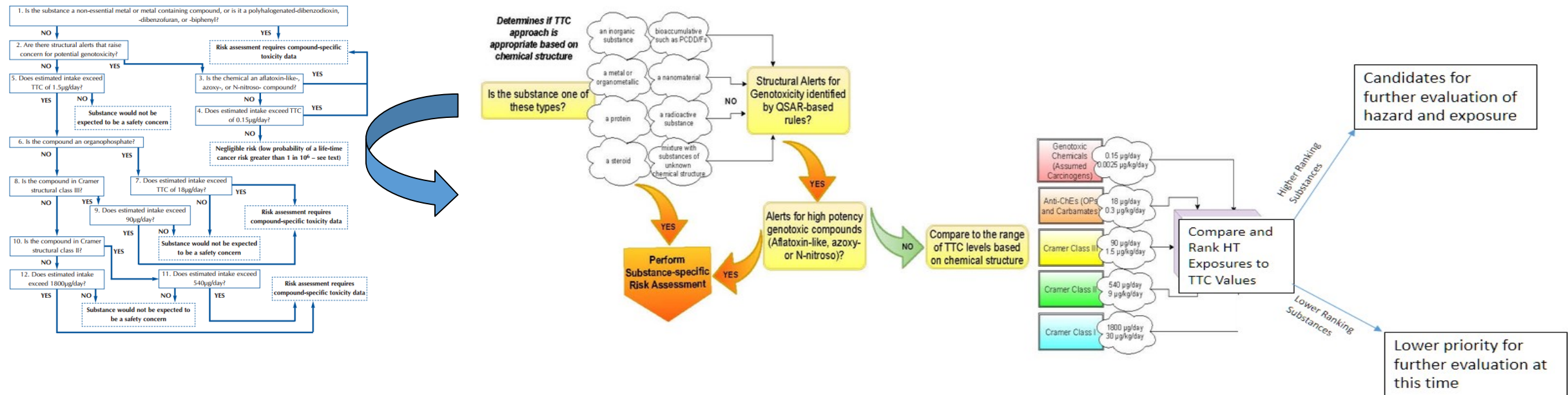


Initial evaluation showed the approach of using the ratio of exposure to TTC (HTE: TTC) appeared promising for risk-based prioritisation



# 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



# Risk-Based prioritisation

- Processed substances through the Kroes module within Toxtree but some adaptations needed to be 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)

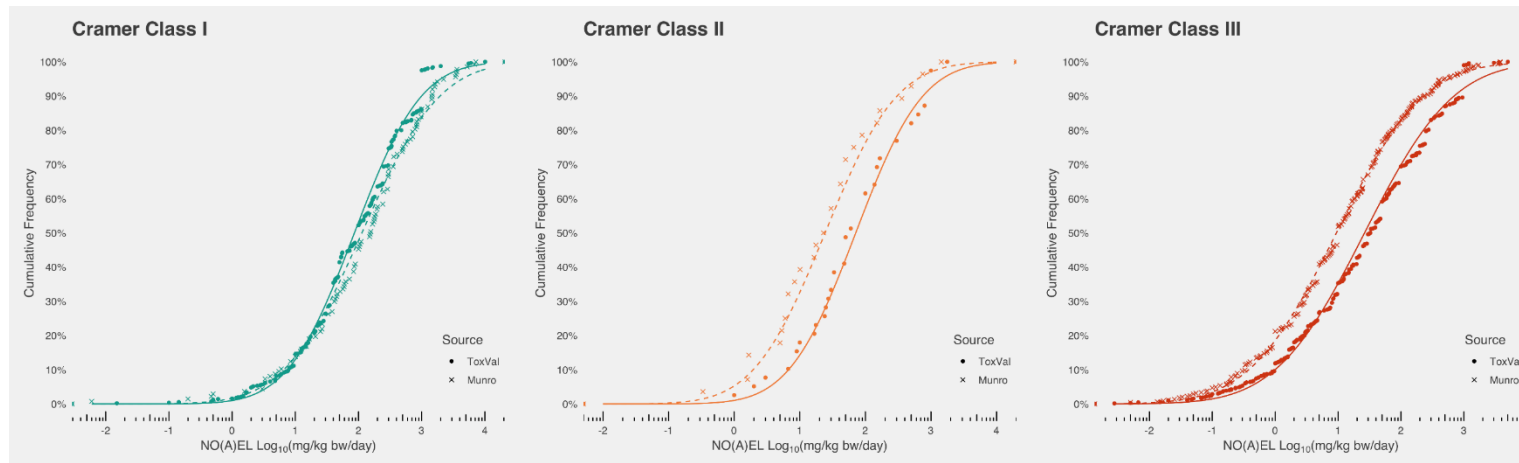
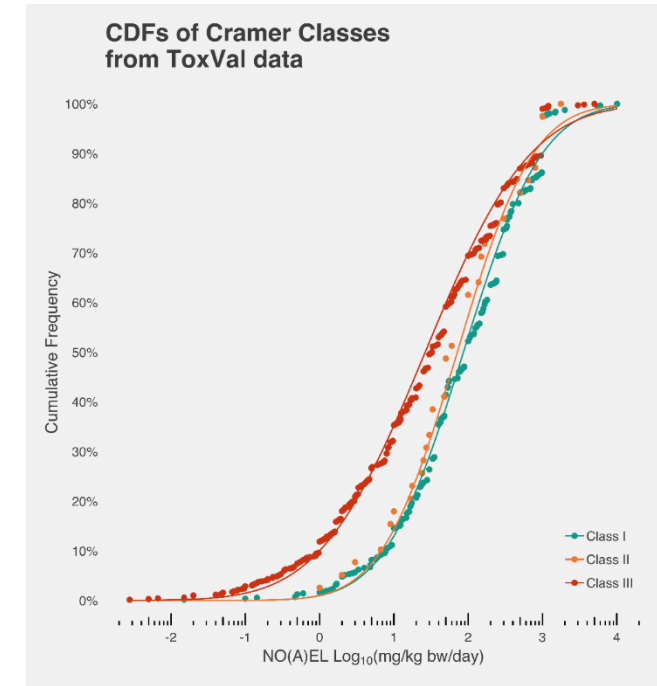
# Risk-Based prioritisation

- 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

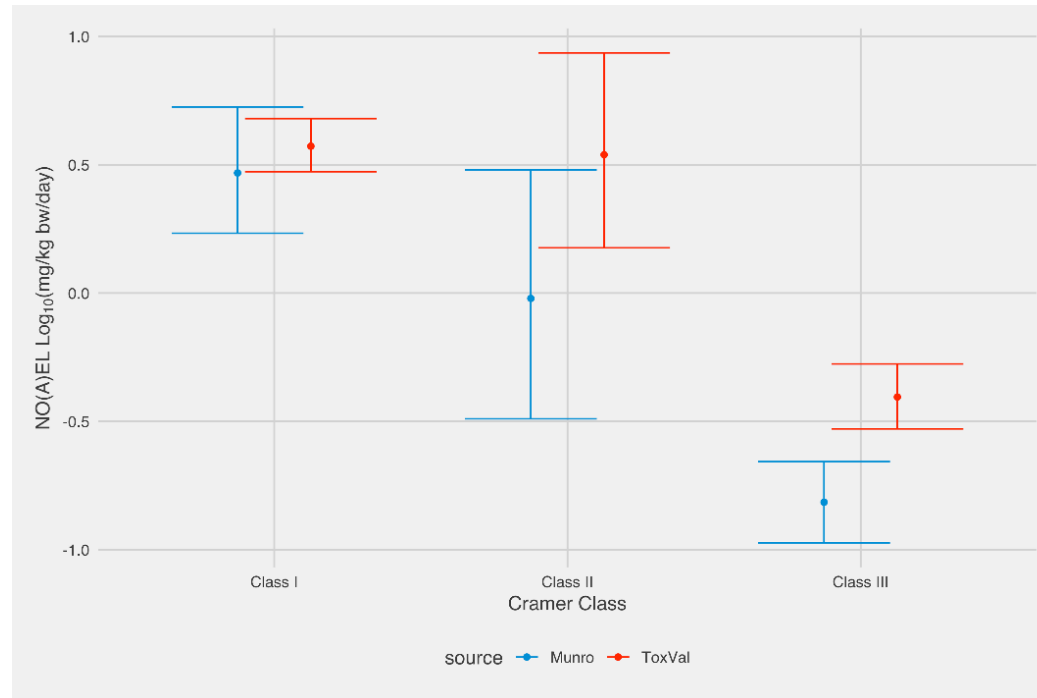
Follow the Kroes et al (2004) workflow to assign substances present in ToxVal to their respective Cramer classes and use the associated repeat dose toxicity data to derive new TTC values

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 5<sup>th</sup> 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 5<sup>th</sup> 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, there are no established TTC values 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 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