SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

State of the Art in the Cramer Classification Scheme and Threshold of Toxicological Concern

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Threshold of Toxicological Concern Approach in Regulatory Decision-Making: The Past, Present, and Future

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The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

Conflict of Interest Statement

- Former employee of Unilever plc and DuPont
- Spouse holds shares in Unilever plc as a legacy from being a former employee

Outline

- Threshold of Toxicology Concern
  - Background and Methodology
  - Present
  - Future
- References
- Acknowledgements

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

- Used to evaluate food flavouring substances, food contact materials, pesticide metabolites in groundwater, genotoxic impurities in pharmaceutical manufacturing operations.

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

Methodology

- Two types of TTCs:
- TTC is based on a predicted tumour risk of 1 in a million, derived through an analysis of genotoxic chemicals
- TTC is based on frequency distributions (5th percentile) of NOAELs of non-genotoxic chemicals

History of TTC

- Frawley (1967) proposed a threshold of toxicological concern of 0.1 mg/kg for packaging materials on the basis of NOELs from 220, 2-year chronic toxicity studies on food additives, industrial,& consumer chemicals and pesticides
- Chemicals were grouped into 5 categories on the basis of their NOELs: >1, >10, >100, >1000, >10000 mg/kg of diet
- Most chemicals (180/220) had NOELs greater than 100 mg/kg of diet, 19 had NOELs below 10 mg/kg of diet but all of these were pesticides or heavy metals and 5 chemicals had NOELs below 1 mg/kg of diet but these were pesticides with known toxicity

History of TTC

- Frawley suggested a level of 10 mg/kg of diet for food packaging materials. Applying an additional safety factor of 100 gave a level of 0.1 mg/kg in the human diet.

History of TTC

- Rulis conducted a similar analysis using the FDA's Priority Based Assessment of Food Additives (PAFA) database containing 159 compounds with subchronic and chronic studies
- Determined that an intake of between 1-10 μg/kg bw/day of various chemicals might not pose a risk to humans

FDA – Threshold of Regulation (ToR)

- In 1995, the FDA adopted the threshold of regulation for food contact substances
- These were substances that would result in minimal migration into food but which would be exempted from regulation as food additives
- The threshold was set at 0.5 ppb or less for substances used in food contact articles i.e. an intake of 1.5 μg/person/day (0.025 μg/bw/day)
- Below this level FDA required no specific toxicity testing and performs an abbreviated safety assessment mainly focussed on intake assessment

FDA – Threshold of Regulation

- The value of 0.5ppb was derived from a distribution plot of chronic dose rates based on the dose descriptor TD50, the daily dose rate required to induce a calculated 50% tumour incidence based on analysis of the CPDB and linear extrapolation to a 1 in a million risk

FDA – Threshold of Regulation

However several conditions had to be met:

- The substance must not have been shown to be carcinogenic
- The structure of the substance does not provide reason to suspect it might be carcinogenic
- The substance is free of carcinogenic impurities of specified potency

A Tiered ToR?

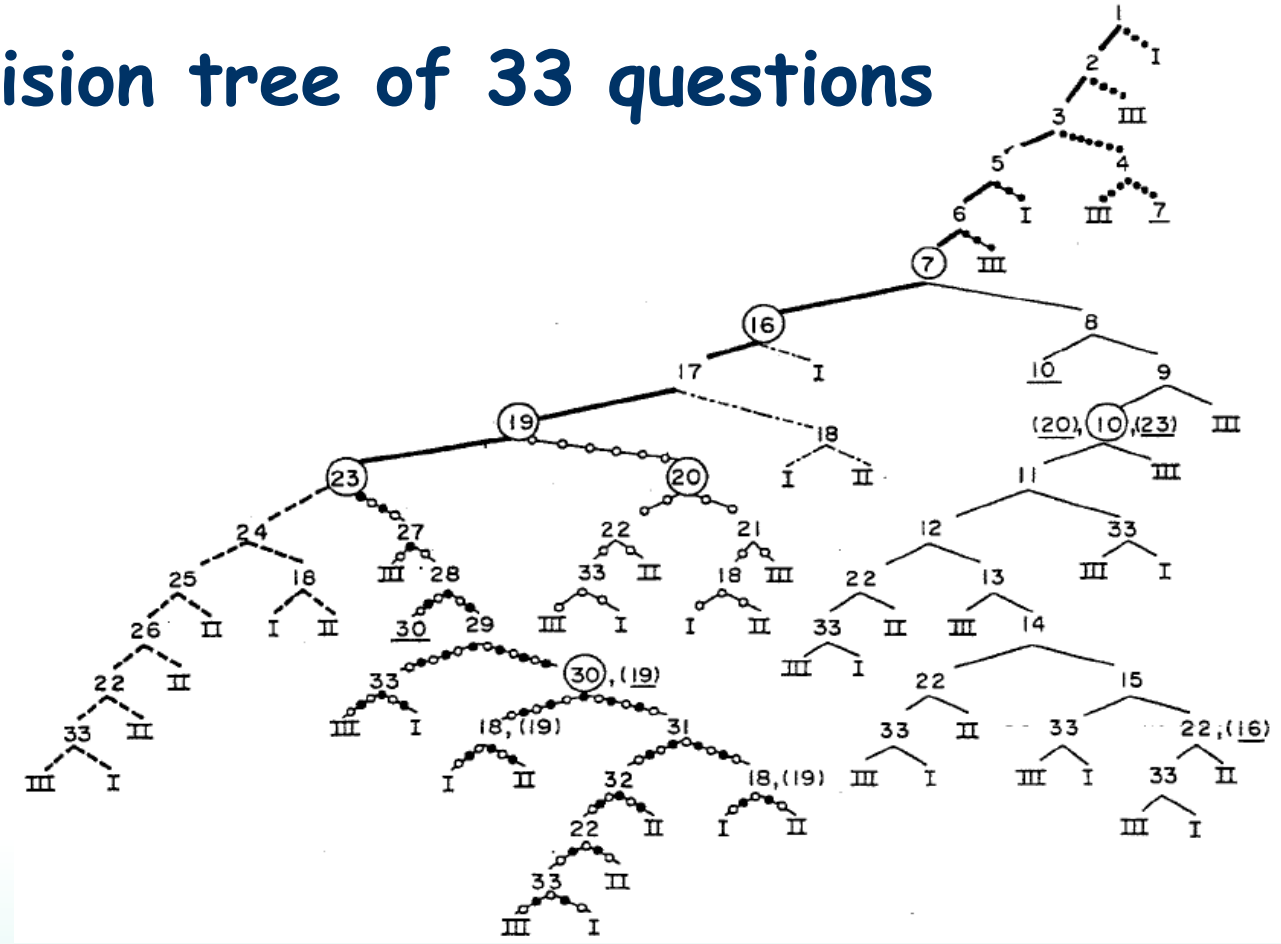
- Further work by the FDA (Cheeseman et al, 1999) has provided support for the use of higher thresholds:
- A threshold of 15μg/person/day was proposed for substances without carcinogenicity structural alerts or with an Ames negative assay
- Substances with a negative Ames test, no structural alerts and a LD50 greater than 1000 mg/kg had a proposed threshold of 45μg/person/day
- This tiered approach has not been adopted by the FDA

Structural based TTCs

- 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 over 600 substances that had been tested for a variety of non-cancer endpoints by the oral route
- The resulting database 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

Cramer Structural Classes

- Decision tree of 33 questions



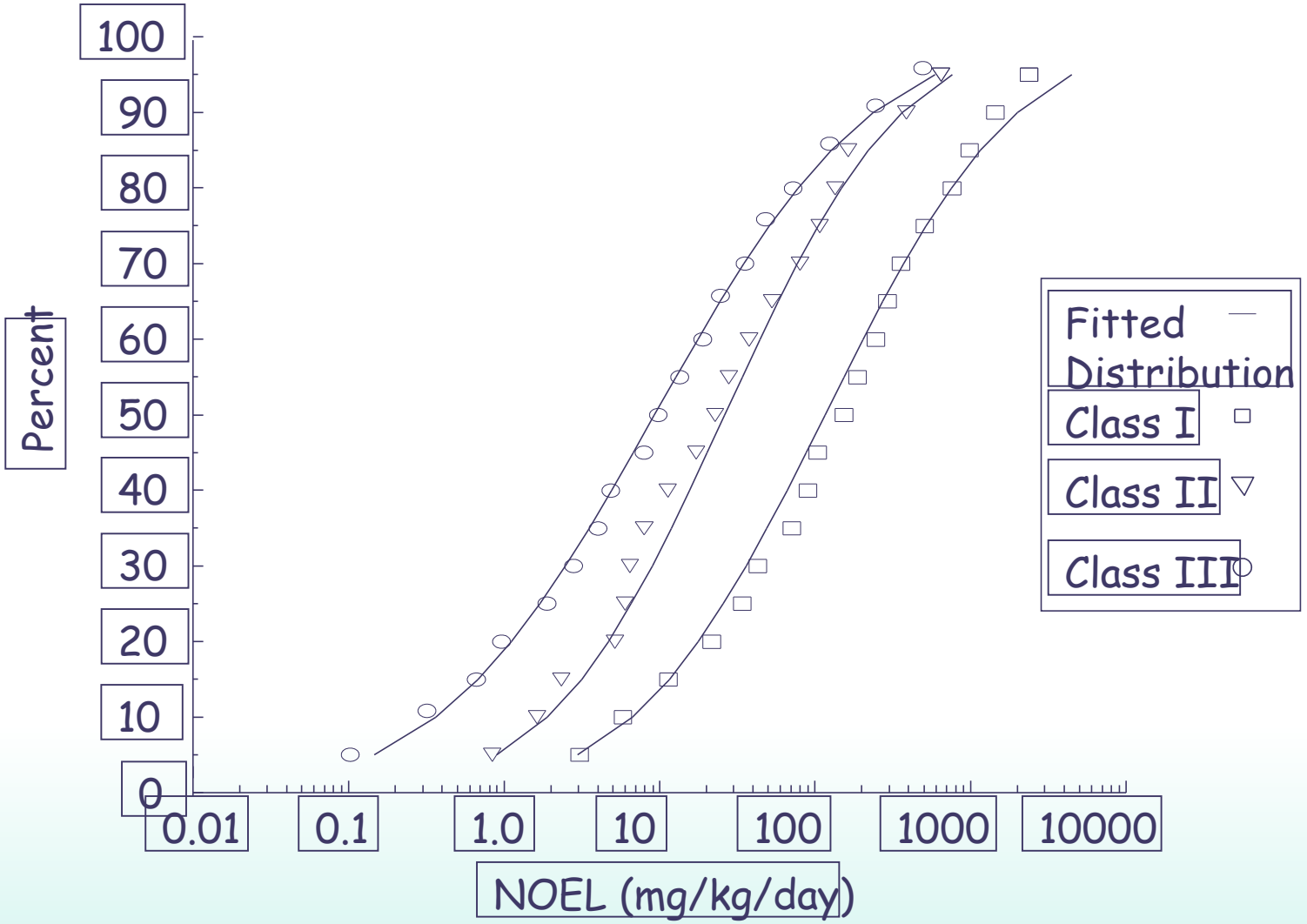
Cramer Structural Classes

- 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

Munro et al (1996)

- Plotted Cramer Class versus NOELs
- Estimated 5th percentile
- Assumed 100 fold safety factor
- Defined Human Exposure Threshold

Cumulative Distributions of Structural Class NOELs



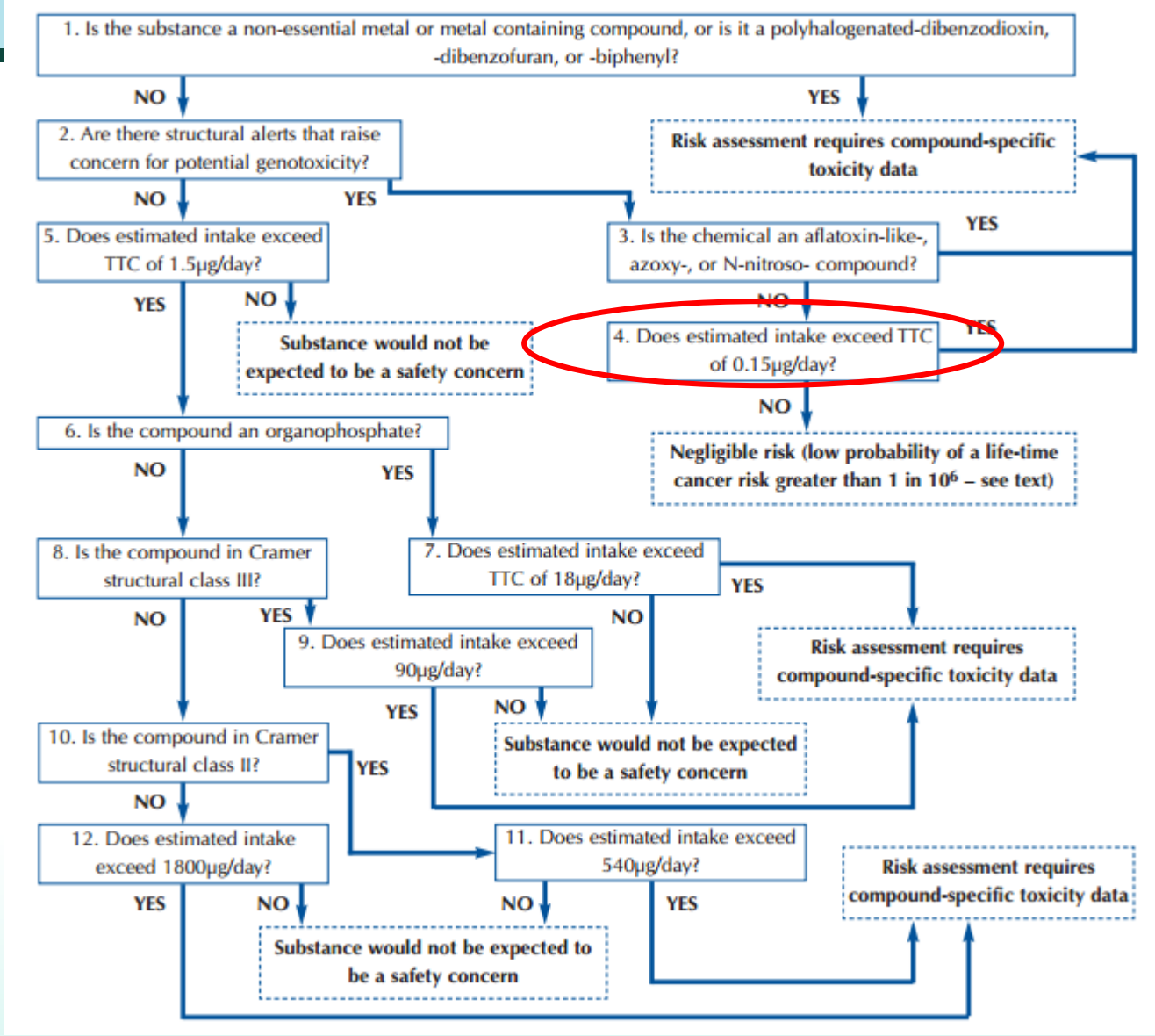
Cramer TTC values

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.

ILSI Europe Structure Based Tiered TTC



Felter et al (2009) Proposal for a 1.5 ug/day if negative Ames data

Kroes et al (2000,2004); Barlow, 2005

Applying the TTC in practice

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

Applying the TTC in practice

- Do we need a Class II?
- OPs and carbamates TTC – carbamates can be folded in Class III. OPs can be maintained in the existing specific TTC
- Routes for exposure other than oral
  - Escher et al (2010) and Carthew et al (2009) established TTC based on inhalation data
  - The EU COSMOS project explored oral to dermal extrapolation
- TTC for other endpoints – prenatal developmental toxicity van Ravenzwaay, 2010; skin sensitisation, Safford, 2008

TTC and shorter durations of exposure

- TTC assumes a lifetime exposure
- 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 - Mueller et al, 2006

	Duration of exposure				
	⩽1 month	>1–3 month	>3–6 month	>6–12 month	>12 month
Allowable Daily Intake (μg/day) for different duration of exposure (as normally used in clinical development)	120[a]	40[a]	20[a]	10[a]	1.5[b]
	or	or	or	or	
	0.5%[c]	0.5%[c]	0.5%[c]	0.5%[c]	[c]
	whichever is lower	whichever is lower	whichever is lower	whichever is lower	

Known carcinogens should have compound-specific risk calculated (see text and Fig. 1).

a Probability of not exceeding a 10^{-6} risk is 93%.

b Probability of not exceeding a 10^{-5} risk is 93%, which considers a 70-year exposure.

c Other limits (higher or lower) may be appropriate and the approaches used to identify, qualify, and control ordinary impurities during developed should be applied. In particular, approaches that foresee a very low dose of the API ("microdoses") may facilitate higher limits than 0.5%.

Staged TTC

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

TTC in skin sensitisation

- TTC values are derived based on systemic toxicity endpoints
- Could a TTC approach be established for skin sensitisation
- Safford (2008) investigated the feasibility of establishing a Dermal Sensitisation Threshold (DST) below which there would be no appreciate risk of sensitisation
- Followed the same principles as used in deriving the ToR

TTC in skin sensitisation

- Approach involved:
- Estimating the proportion of skin sensitisers in the world of chemicals (ELINCs was used as a convenient dataset for which C&L information was available, 20% incidence of sensitisers was used)
- Investigating the distribution of sensitisation potencies for known skin sensitisers (The EC3 values taken from Gerberick et al (2005) for a set of 211 chemicals was used)
- Calculating the risk of sensitisation in humans based on potency estimated/ EC3 values were converted to predicted human sensitisation potency (EC3 ->NESIL)
- NESILs converted to AELs by applying appropriate assessment factors depending on product type

TTC in skin sensitisation

- No acceptable risk was defined as such – a probability of 95% at which the DST should be selected was proposed
- In Safford et al (2011), a refinement was made to incorporate more sensitisation data and an evaluation of the reaction chemistry domains
- For substances assumed to be non-reactive based on their mechanistic domain assessment, a DST of 900 ug/cm2 could be established. An untested chemical would have a probability of 0.26% of presenting a skin sensitisation risk at a skin exposure level of 900 ug/cm2

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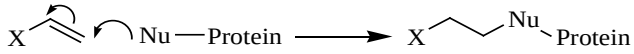
- In Safford et al (2015), the DST was extended for protein reactive chemicals - 64ug/cm2

Reaction chemistry mechanistic domains

Aptula AO, Roberts DW, Patlewicz GY, Schultz TW. Non-Enzymatic Glutathione Reactivity and In Vitro Toxicity: A Non-Animal Approach to Skin Sensitization. *Toxicol. in Vitro* 2006, 20(2): 239-247.

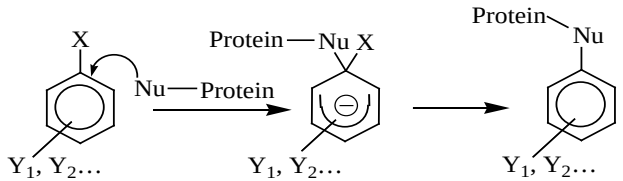
Mechanistic domain	Protein binding reaction	Modified protein

Michael acceptors



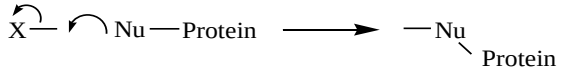
Identification characteristics. Double or triple bond with electron-withdrawing substituent X, such as -CHO, -COR, -CO_2R , -CN, -SO_2R, -NO_2...Includes para quinones and ortho quinones, often formed by oxidation of para and ortho di-hydroxy aromatics acting as pro-Michael acceptors. X can also be a heterocyclic group such as 2-pyridino or 4-pyridino.

S_NAr electrophiles



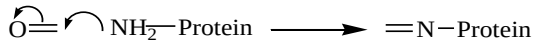
Identification characteristics. X = halogen or pseudohalogen, Y's are electron withdrawing groups (at least two) such as -NO_2, -CN, -CHO, -CF_3, -SOMe, -SO_2Me, ring fused nitrogen...One halogen is too weak to act as an X, but several halogens together can activate.

S_N2 electrophiles



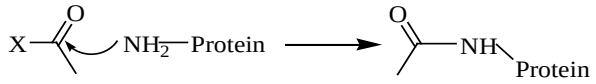
Identification characteristics. X = halogen or other leaving group, e.g. OSO_2(R or Ar), OSO_2O(R or Ar) bonded to primary alkyl, benzylic, or allylic carbon. OR and NHR or NR_2 do not usually act as leaving groups, but can do so if part of a strained 3-membered ring (e.g. epoxides, ethylenimine and substituted derivatives).

Schiff base formers



Identification characteristics. Reactive carbonyl compounds such as aliphatic aldehydes, some α,β- and α,γ-diketones, α-ketoesters. Not simple monoketones and aromatic aldehydes. Other hetero-unsaturated systems can behave analogously, e.g. C-nitroso compounds, thiocarbonyl compounds (C=S), cyanates and isocyanates, thiocyanates and isothiocyanates.

Acylating agents



Identification characteristics. X = halogen, or other group (e.g. -OC_6H_5) such that XH is acidic enough for X^- to act as a good leaving group. Includes anhydrides, cyclic or non-cyclic. X = -Oalkyl does not qualify, except when part of a strained lactone ring, e.g. β-propiolactone (but not γ-butyrolactone). Analogous reactions can occur with attack at sulfonyl S, phosforyl P and thioacyl C.

TTC in skin sensitisation

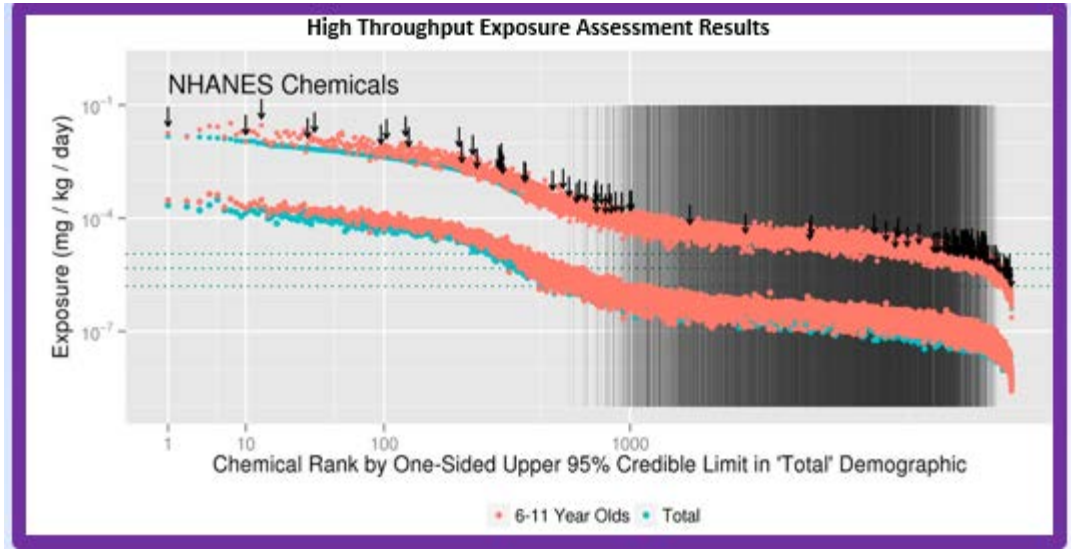
- Parallel work was also conducted to identify high reactive substance for which a DST should not be used
- This was akin to the High Potency carcinogens excluded from the TTC
- Examples of chemicals excluded include Michael acceptors with more than 1 activating group on the double bond, Quinones, di-imines and quinone-imines, isocyanates and isothiocycanates

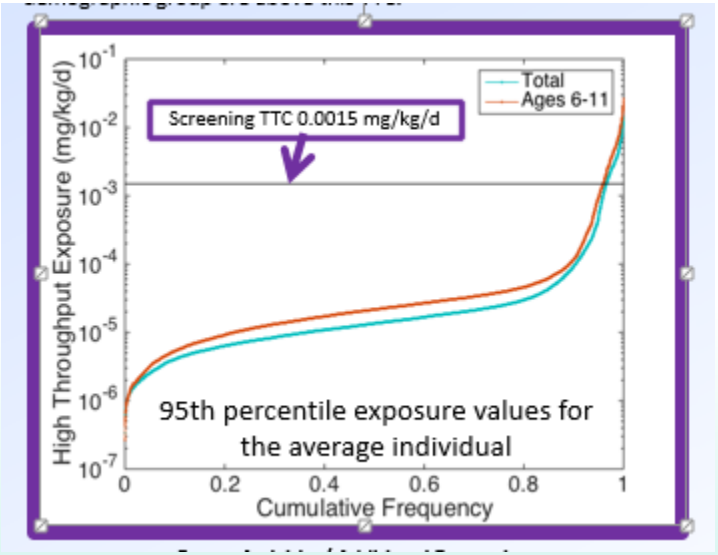
TTC in skin sensitisation

- DST for skin sensitisation proposed by Safford (2008)
- Refined based on reaction mechanistic domains Safford et al (2011)
- DST extended to address skin sensitisers (Safford et al (2015)
- High potency skin sensitisers that should be excluded from the DST approach were proposed by Roberts et al (2015)

Integrating TTC with predicted HT exposures

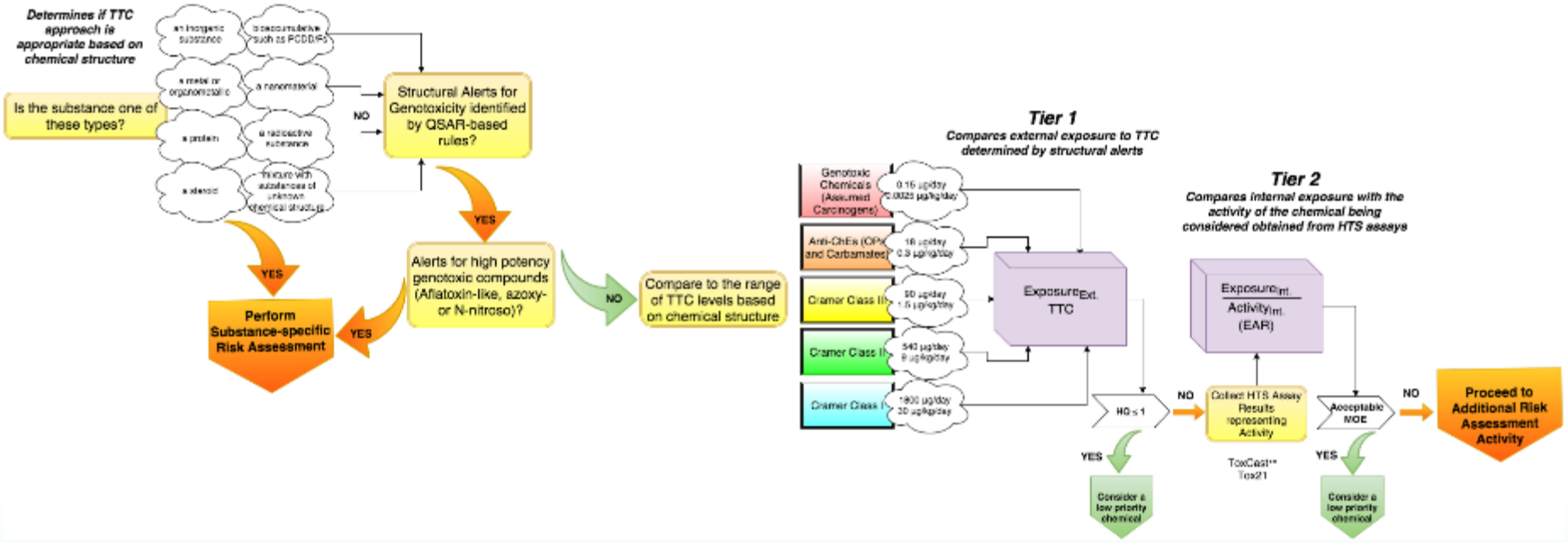
- Can TTC values be integrated with predicted HT exposure information





Integrating TTC with predicted HT exposure data

- Can TTC values be integrated with predicted HT exposures



Take home messages

- TTC – Threshold of Toxicological Concern is a pragmatic means of ~~waiving~~ prioritizing testing when exposures are v low and when little or no toxicity data exists.
- Does not overrule traditional risk assessment practices
- Well established for oral exposures

References - TTC

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