U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

Updates to the Cramer et al. Decision Tree and the Threshold of Toxicological Concern: the Expanded Decision Tree and Its Use in Safety Assessments

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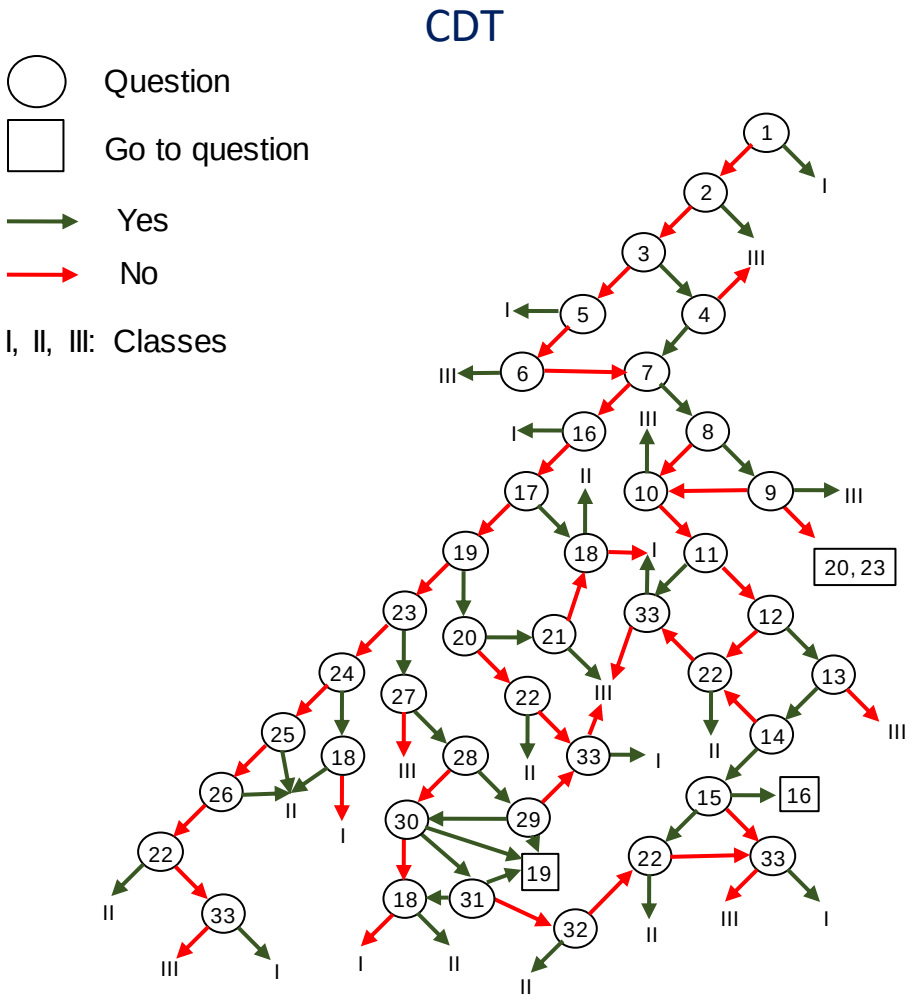
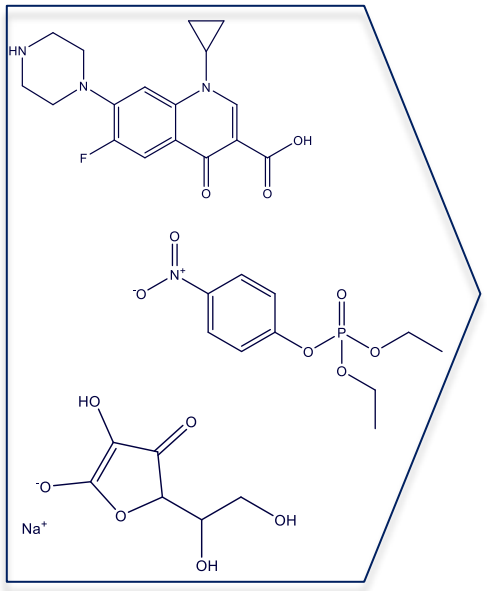
The Cramer at al. Decision Tree (CDT) (1978)

The CDT is a sequence of yes/no questions that either leads to another question, or the assignment of the substance to one of three classes of relative chronic oral toxicity.

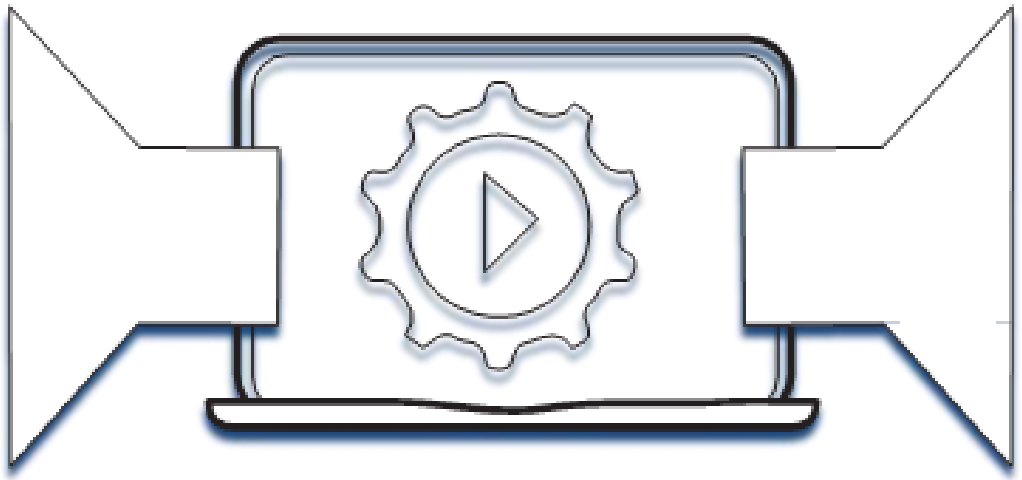
- 33 main questions
- 11 sub-questions



Three classes of relative chronic oral toxic potential



CDT



State-of-the-art at the time of its publication (1978)

Relative Chronic Oral Toxicity

- Class I: Low order of oral toxicity
- Class II: Intermediate Toxicity
- Class III: Substances having structural features that suggest either “significant toxicity” or “permit no strong initial presumptions of safety”

The Threshold of Toxicological Concern (TTC)

- 1996: a database of 613 substances was compiled and each substance was assigned to its CDT class. For each CDT class, a Threshold of Toxicological Concern (TTC) value was calculated (Munro *et al*., 1996).

$$\text{Class TTC [µg/p/day]} = \frac{5^{th} \text{ percentile NEL [mg/kg bw/day] x Avg. bw (60 kg bw/p) x 1000 µg/mg}}{\text{100 (safety factor)}}$$

	Class I	Class II	Class III
TTC (µg/p/day)	1,800	540	90

- If intake ≤ class TTC: reasonably expected to be safe.

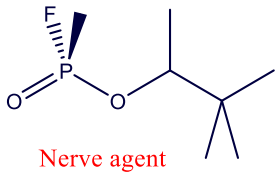
Why and how were the CDT and its associated TTCs updated?

Limitations of the CDT, Munro DB & TTC

- CDT was designed with mostly flavors in mind: limited scope
- Munro Database (DB) is relatively small (613)
- Class II TTC is based on NELs for only 28 compounds

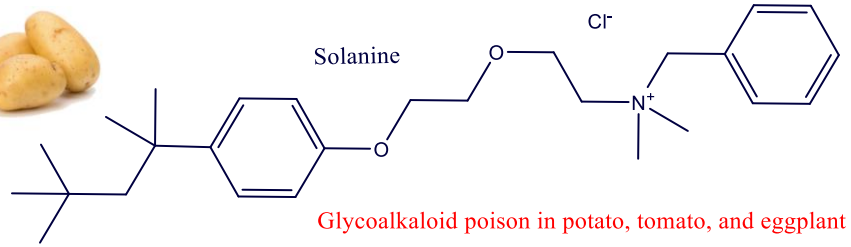
Update: Expanding the scope of the DB & EDT

Soman



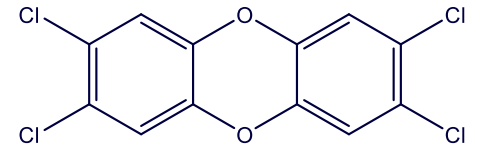
Nerve agent

Solanine

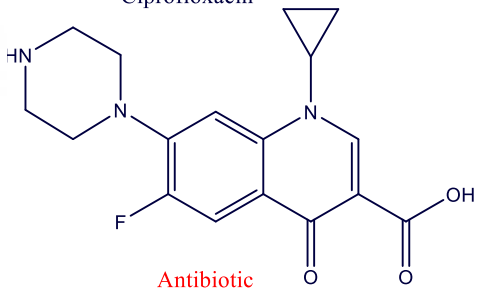


Glycoalkaloid poison in potato, tomato, and eggplant

2,3,7,8-tetrachlorodibenzo[b,e][1,4]dioxine

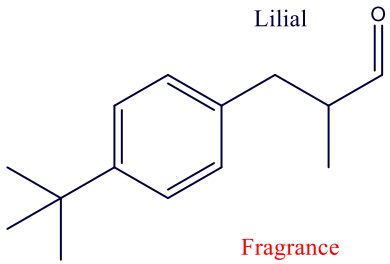


Ciprofloxacin



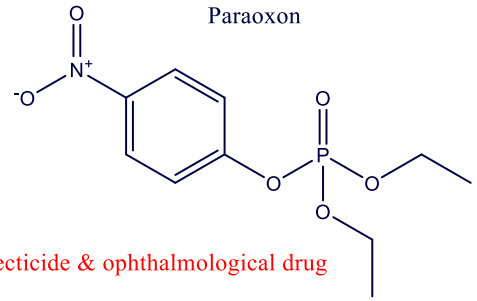
Antibiotic

Lilial



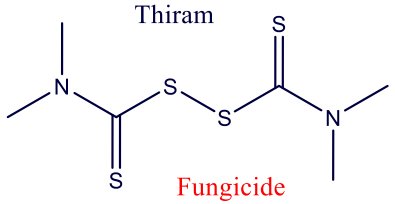
Fragrance

Paraoxon



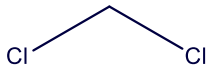
Insecticide & ophthalmological drug

Thiram



Fungicide

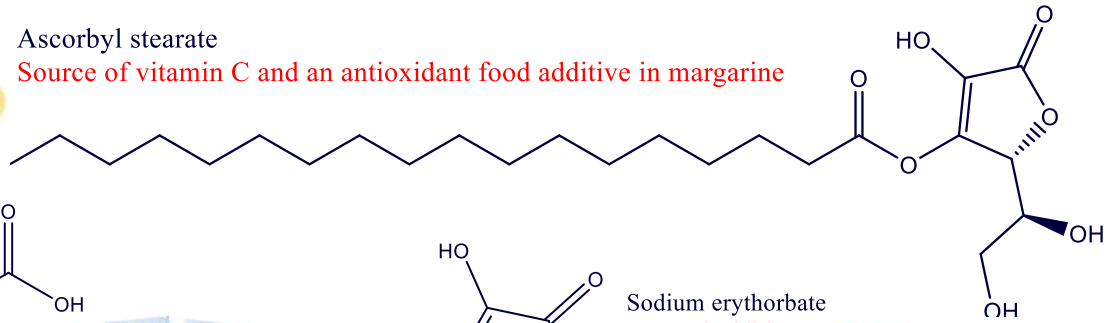
Dichloromethane



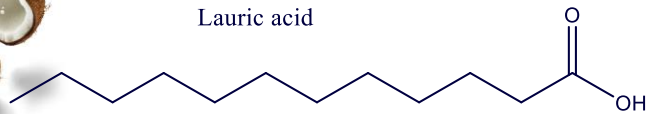
Solvent

Ascorbyl stearate

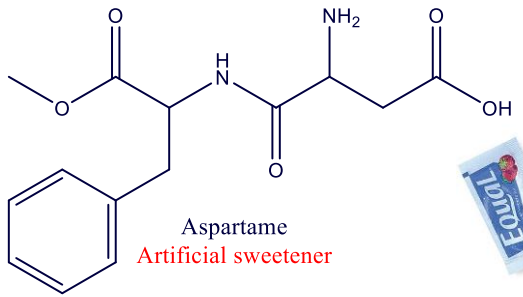
Source of vitamin C and an antioxidant food additive in margarine



Lauric acid

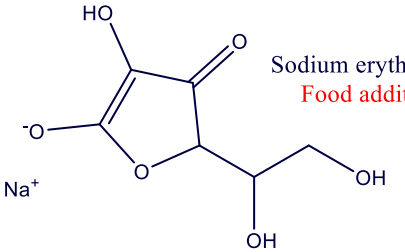


In coconut milk and oil, breast milk, macadamia nut, pumpkin seed



Aspartame

Artificial sweetener



Sodium erythorbate

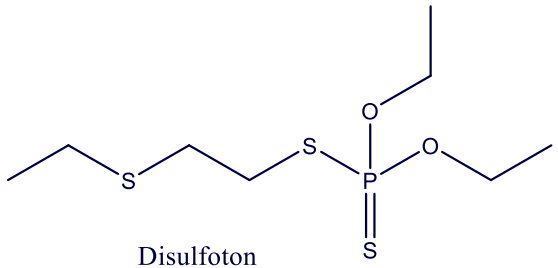
Food additive

Update: Expanding the size of the database

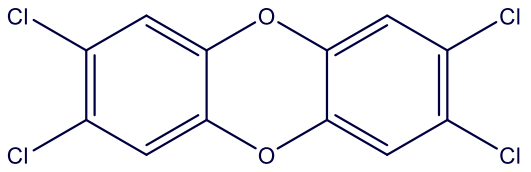
		Non-toxic	Low toxicity	Medium toxicity	High toxicity	Very high toxicity	Extreme toxicity
	Classes	**I**	**II**	**III**	**IV**	**V**	**VI**
No. of compounds	**EDT DB (used for calc.)**	174	240	242	393	173	41
	EDT DB (total)*	219	349	344	613	334	50
	Classes	**I**		**II**	**III**		
	Munro, 1996, DB	137		28	448		

* Some substances in the EDT have only a LEL, hence they were not used to calculate class TTCs, but were used to help formulate EDT questions.

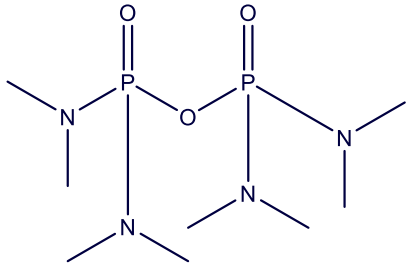
NELs are adjusted for duration



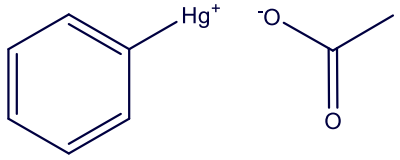
Disulfoton
NEL: 0.0000456 mmol/kg bw/day



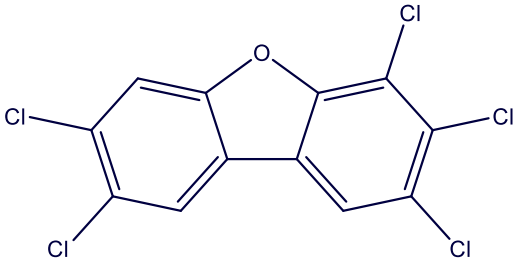
2,3,7,8-Tetrachlorodibenzo-p-dioxin (Dioxin)
NEL: 0.0000000039 mmol/kg bw/day



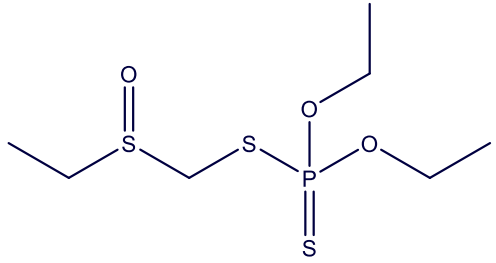
Systophos/Schradan/Octamethyl
NEL: 0.0000087 mmol/kg bw/day



Phenylmercuric acetate
NEL: 0.000025 mmol/kg bw/day



2,3,4,7,8-Pentachlorodibenzofuran
NEL: 0.0000005 mmol/kg bw/day



Phorate sulfoxide
NEL: 0.000019 mmol/kg bw/day

Additional limitations of the CDT

- CDT is over 4 decades old: questions are outdated
-
-
-
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Solutions for the limitations of the CDT

- CDT questions were updated to reflect current state of science

Additional limitations of the CDT

- CDT is over 4 decades old: questions are outdated
- Limited number of elements, moieties, functional groups, and congeneric groups
-
-
-

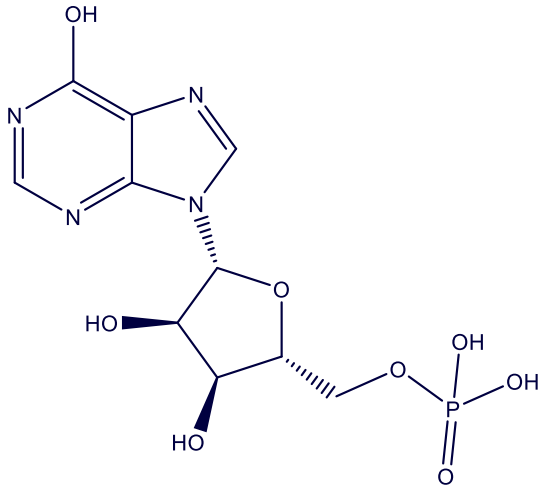
Solutions for the limitations of the CDT

- CDT questions were updated to reflect current state of science
- Created new questions
-
-
-

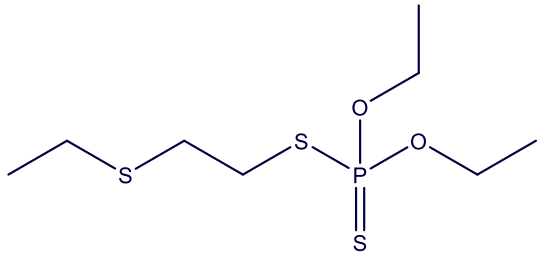
Examples of new questions created to address additional elements, functional groups, reactive moieties, and congeneric groups of compounds

Example 1: Covalently bound phosphorus (new element)

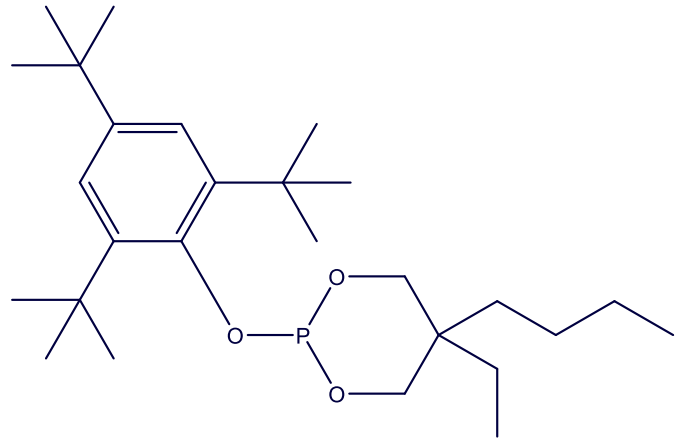
- P is found in a wide variety of compounds used in food contact materials, plastics in general, pharmaceuticals, solvents, herbicides, rodenticides, insecticides, some natural and synthetic toxins.
- EDT DB has 82 organophosphorus compounds and 20 compounds containing both P and one or more halogens. They exhibit a very broad range of toxicities.
- CDT defaults substances containing covalently bound P into CDT Class III unless they are a “normal constituent of the body”.



Inosine monophosphate NEL:
2000 mg/kg bw/day or
5.744 mmol/kg bw/day
Widely used as a flavor enhancer



Disulfoton NEL:
0.0125 mg/kg bw/day or
0.0000456 mmol/kg bw/day
Insecticide



Ultranox 641 NEL:
2.9 mg/kg bw/day or 0.015 mg/kg bw/day
Antioxidant/stabiliser in polymers

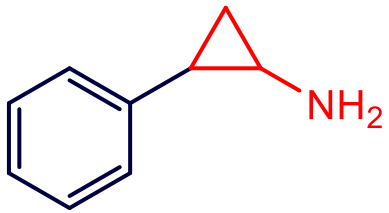
Example 1: Phosphorus (new element)

- Q1i: Nucleotides, nucleosides, phospholipids, monophosphates of amino acids, or their hydrolysis products (Class I)
- Q2a,b,c,d: classify a wide variety of P-containing substances into Classes III, V, and VI
- Q6b,c,d: capture very highly toxic compounds that may (or may not) contain P (Class V)

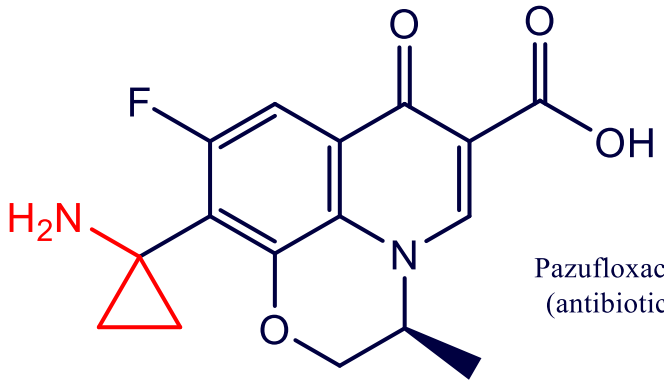
Compounds containing covalently bound P can be sorted into EDT Classes I, III, V, and VI to better differentiate their true toxic potentials.

Example 2: Aminocyclopropyl moiety

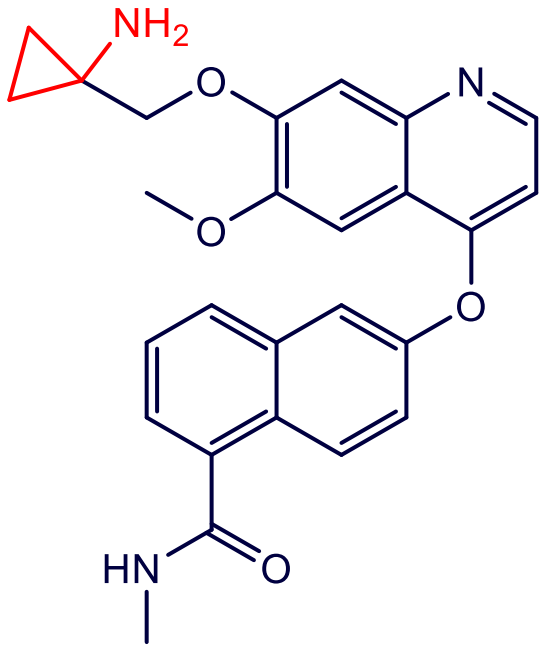
Cyclopropylamines are prevalent motifs in pharmaceuticals and agrochemicals:



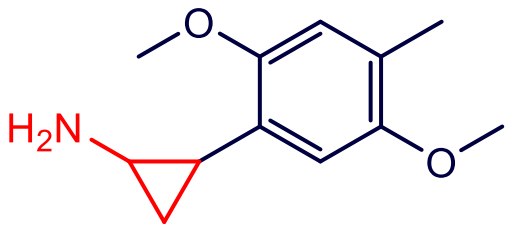
Tranylcypromine (antidepressant, monoamine oxidase inhibitor)



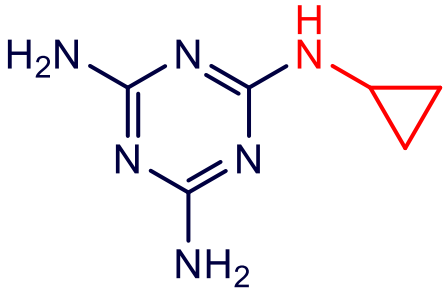
Pazufloxacin (antibiotic)



Lucitanib (investigational angiogenesis inhibitor)

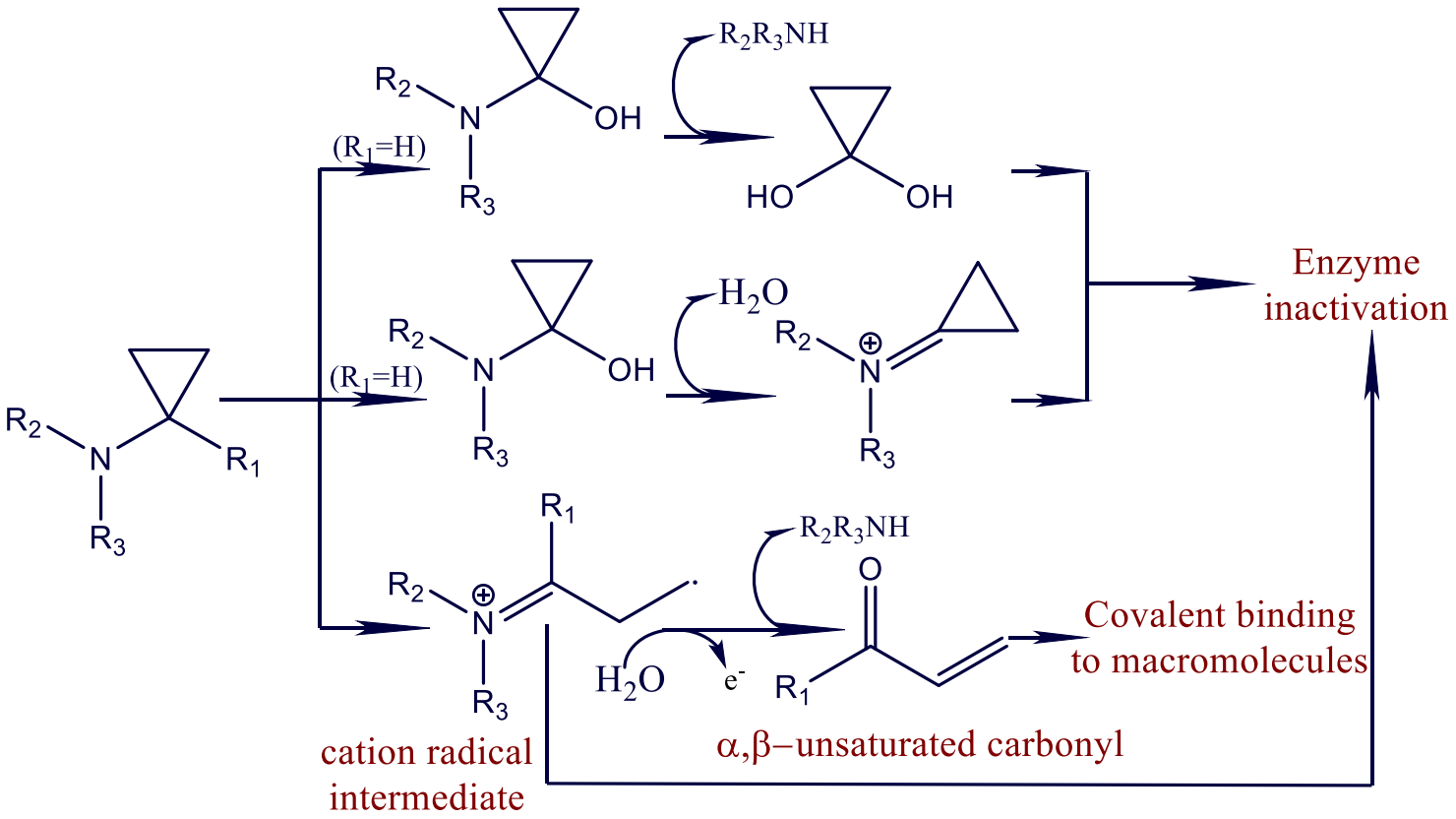


2,5-Dimethoxy-4-methylphenylcyclopropylamine (DMCPA, psychedelic drug)



Cyromazine (insecticide and an acaricide)

Example 2: Aminocyclopropyl moiety



The cyclopropylamine moiety is a known inactivator of CYP450 and other enzymes such as MAO and ADH.

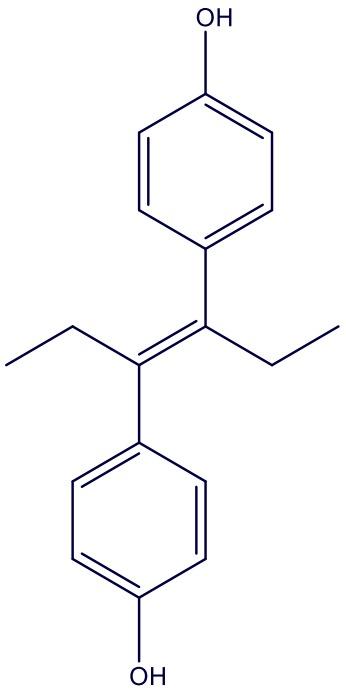
Q28r: Does the substance contain at least one aminocyclopropyl moiety?
If yes, assign to Class V.

Reference: Kalgutkar, A. S., Gardner, I., Obach, R. S., Shaffer, C. L., Callegari, E., Henne, K. R., ... & O'Donnell, J. P. (2005). A comprehensive listing of bioactivation pathways of organic functional groups. Current drug metabolism, 6(3), 161-225.

Example 3: Selected nonsteroidal estrogens (new congeneric groups)

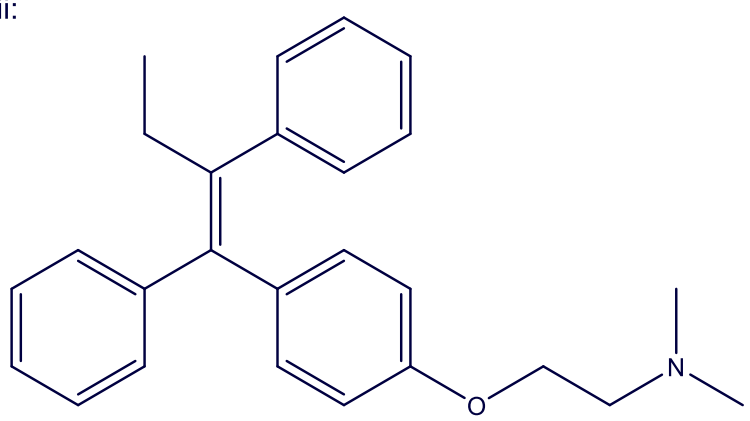
Q6g aims to capture nonsteroidal estrogens, namely stilbestrols (Q6gi) & triphenylethylenes (Q6gii). As their name suggest, they are selective estrogen receptor modulators.

i:



diethylstilbestrol

ii:



tamoxifen

Example 3: Selected nonsteroidal estrogens (new congeneric groups)

Q6g i) two benzene rings connected by a 2- or 3-carbon chain (connector, with or without unsaturation) and a hydroxy, corresponding ester, methoxy, and/or ether in the para position on each ring with or without methyl, ethyl, and/or ethylidene substitution on one or more connector carbons (not more than one per carbon). One or more halogen(s) are allowed anywhere on the molecule along with methyl group(s) in the meta position on the benzene ring(s) or ii) two benzene rings connected by a -C=C- (connector) and one connector carbon is substituted by a benzene ring (a total of three benzene rings) and the other connector carbon is either unsubstituted or substituted by a methyl or ethyl group or a halogen. Any or all of the benzene rings may be substituted by a hydroxy, corresponding ester, methoxy, and/or ether in the para position, but this is not required.

Additional limitations of the CDT

- CDT is over 4 decades old: questions are outdated
- Limited number of elements, moieties, & functional groups
- Ambiguous phrases and definitions

Elimination of ambiguous phrases & definitions

Common terpene: an isoprenoid compound reported in the literature as a more than trace constituent of two or more generally consumed foods, either raw or as ordinarily prepared for consumption, without added ingredients.

Sterically hindered: means posing steric hindrance to a functional group equivalent to or greater than that exhibited by *o*-tert-butyl or a 2,6-disubstitution on an aromatic ring.

Normal constituent of the body:….

Common component of food:….

Solutions for the limitations of the CDT

- CDT questions were updated to reflect current state of science
- Created new questions
- Eliminated ambiguous phrases and definitions

Additional limitations of the CDT

- CDT is over 4 decades old: questions are outdated
- Limited number of elements, moieties, & functional groups
- Ambiguous phrases and definitions
- Non-structure-based questions

Elimination of non-structure-based and/or ambiguous questions and phrases

Q1: Is the substance a *normal constituent of the body* or an optical isomer of such? (Class I)

Q22: Is the substance a *common component of food* or *structurally closely related* to a common component of food? (Class II)

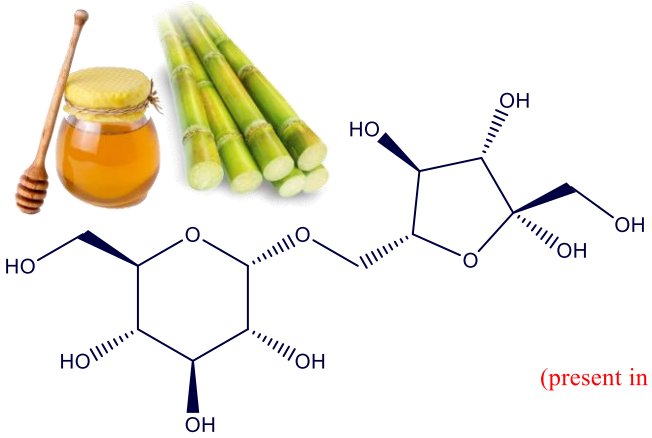
Solutions for the limitations of the CDT

- CDT questions were updated to reflect current state of science
- Created new questions
- Eliminated ambiguous phrases and definitions
- Eliminated non-structure-based questions

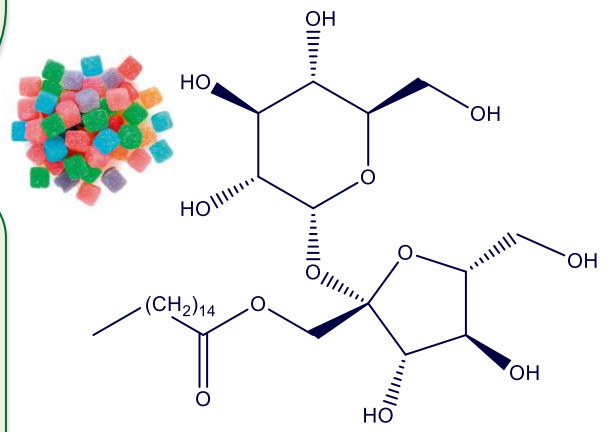
Replacing non-structure-based questions with structure-based questions (e.g. Q1a, b, c, d, e, f, g, h, i, j, & k)

Q1e) Monosaccharides and hydrolysable oligosaccharides and polysaccharides in addition to simple monosaccharide derivatives (phosphate esters, deoxy sugars, amino sugars, mono- and poly-methylated, sulfated, and sulfonic acid derivatives of monosaccharides…..)

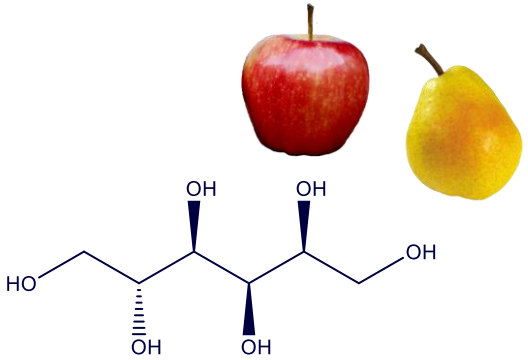
Q1f) Sugar alcohols or sugar acids and their corresponding esters in addition to derivatives of sugar alcohols that are both alkoxylated and esterified…



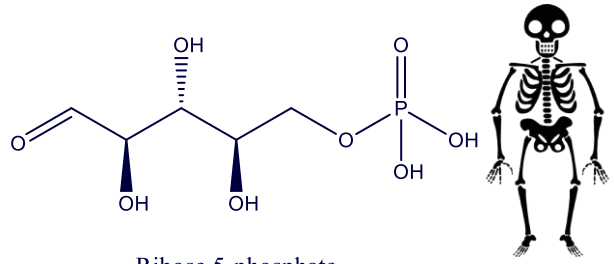
Isomaltulose
(present in honey and sugarcane extracts,used to produce isomalt (a sugar alcohol))



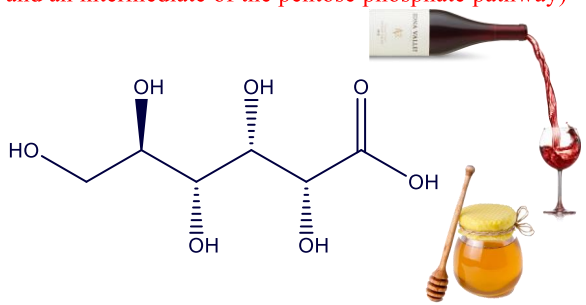
Sucrose monopalmitate (sucrose esters: emulsifiers)



Sorbitol
(present in apples, pears, peaches, and prunes or made from potato starch)



Ribose 5-phosphate
(both a product and an intermediate of the pentose phosphate pathway)



Gluconic acid (sugar acid)
(found in fruit, honey, and wine; food additive (acidity regulator))

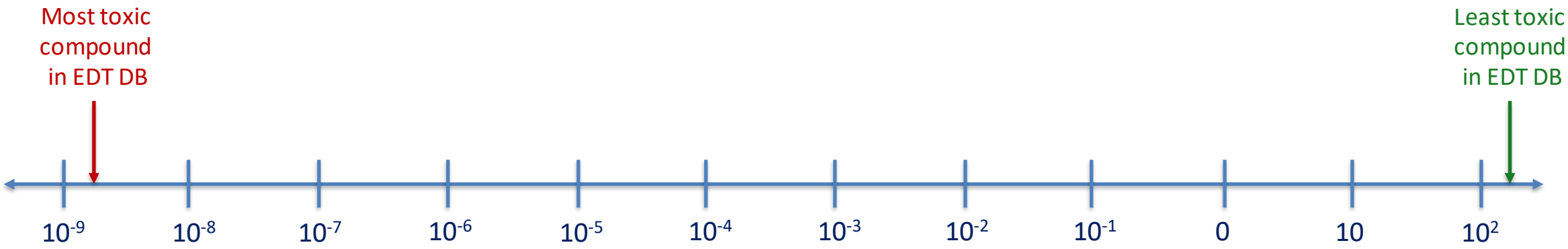
Additional limitations of the CDT

- CDT is over 4 decades old: questions are outdated
- Limited number of elements, moieties, & functional groups
- Ambiguous phrases and definitions
- Non-structure-based questions
- Large overlap between classes of different toxic potentials

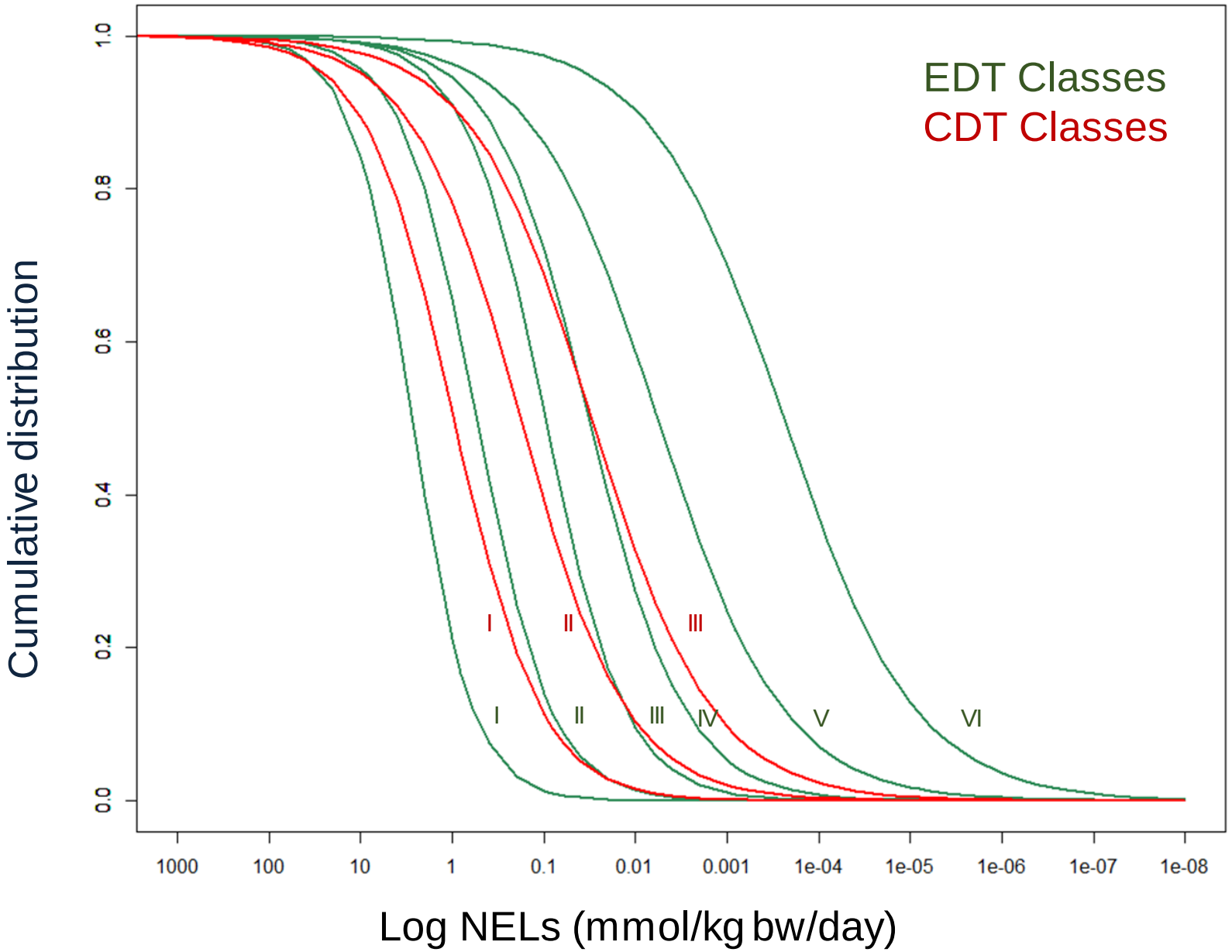
Solutions for the limitations of the CDT

- CDT questions were updated to reflect current state of science
- Created new questions
- Eliminated ambiguous phrases and definitions
- Eliminated non-structure-based questions
- Mitigated overlap between classes of different toxic potentials

Why were the number of classes doubled?



NEL range [mmol/kg bw/day] in the EDT DB used for TTC calculations



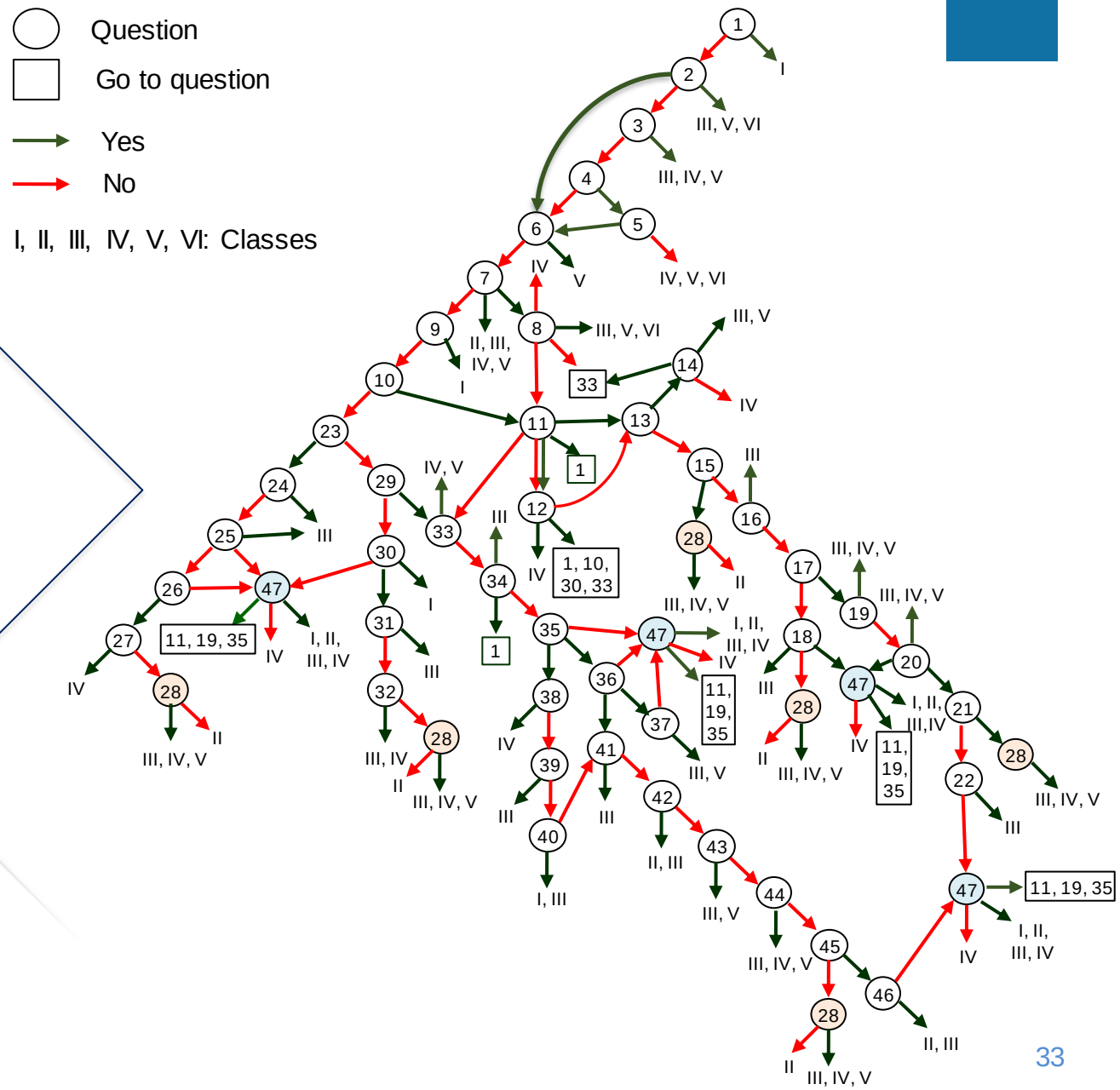
Comparison of NEL distributions for compounds in the EDT DB grouped into their CDT and EDT classes: significantly decreased overlap with the EDT

Results: the EDT and the updated TTCs

The Expanded Decision Tree (EDT)

The EDT is a sequence of (chemical) structure-based yes/no questions that either leads to another question, or the assignment of the substance to one of six classes of relative chronic oral toxicity.

- 47 main questions
- 134 sub-questions
- Numerous sub-sub-questions

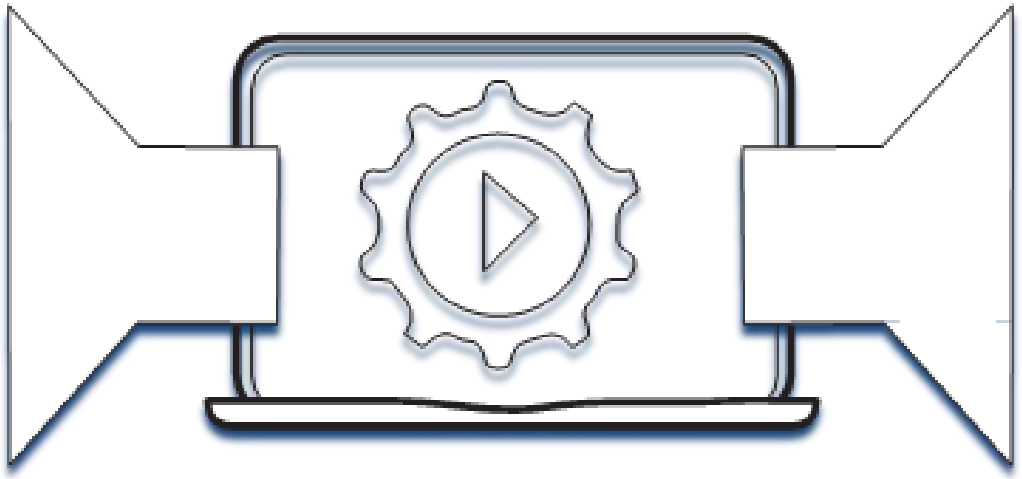


Applicability domain of the EDT

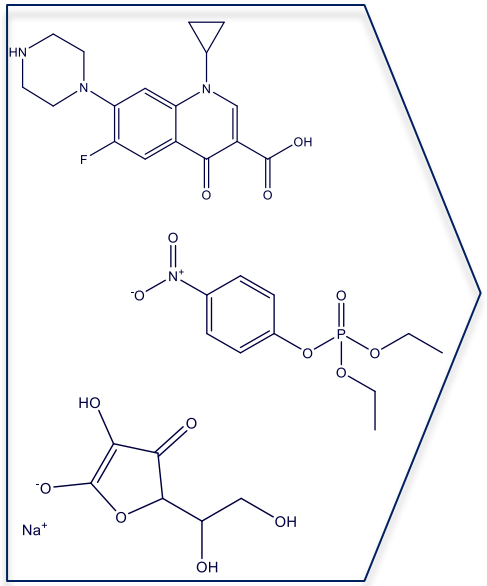
All chemicals except:

- Elements and inorganic substances
- Proteins
- Unhydrolyzable polymers

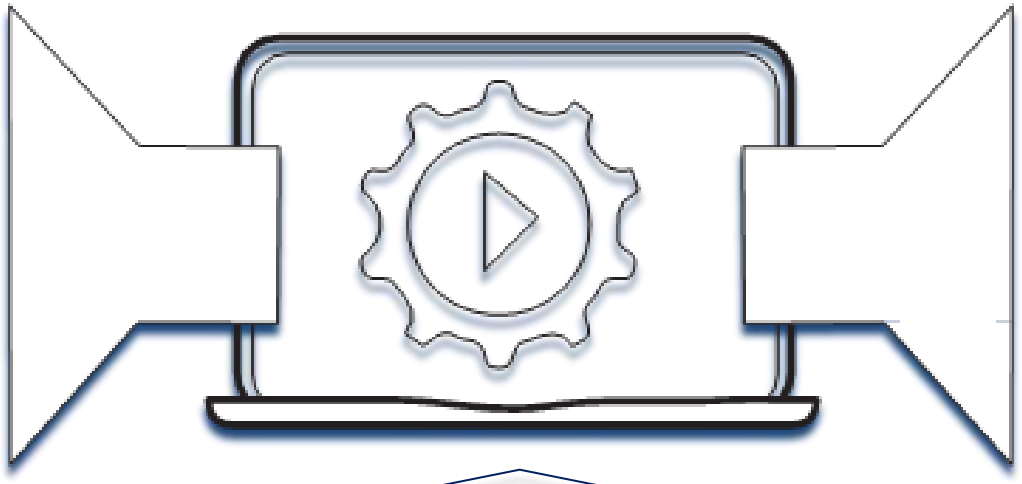
EDT



Six classes of relative chronic oral toxic potential



EDT



Basis:
the Cramer et al. (1978) DT, a collection of toxicological & ADME* data and info on structure-activity relationships on thousands of compounds

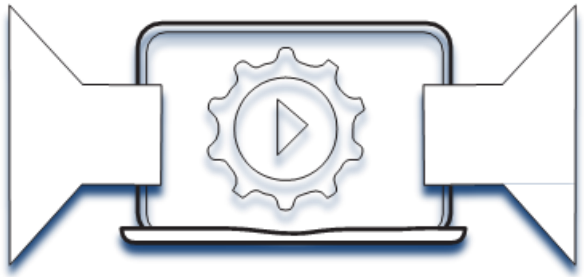
Relative Chronic Oral Toxicity

- Class I
- Class II
- Class III
- Class IV
- Class V
- Class VI

*Absorption, distribution, metabolism, and excretion

EDT TTCs

EDT

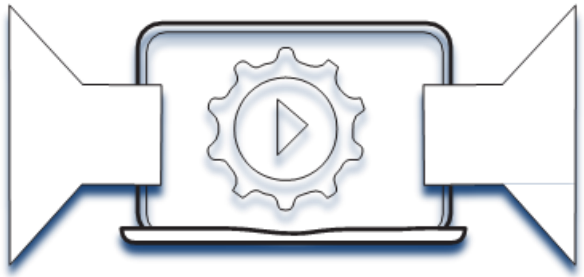


Relative Toxicity	TTC Level*
Class I	28,400 µg/p/d
Class II	4,240 µg/p/d
Class III	1,010 µg/p/d
Class IV	196 µg/p/d
Class V	4.79 µg/p/d
Class VI	0.108 µg/p/d

* Preliminary values, pending QC

EDT TTCs vs CDT TTCs

EDT



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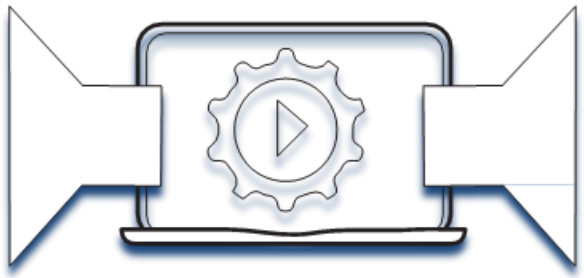
vs

CDT TTC range of 1,800 to 90 µg/p/d

* Preliminary values, pending QC

Applications of the EDT

EDT



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Combined with the TTC levels, the EDT may be used for

- ✓ Safety evaluation (tool in the toolbox; acute *vs* chronic toxic potential)
- ✓ Screening and prioritization of chemicals for further testing; identify compounds of concern
- ✓ Post-market: ensure that compounds at current intake levels are still safe
- ✓ Read-across
- ✓ Mixture evaluation and determine sufficient similarity of mixtures
- ✓ May be used to support cumulative toxicity assessment

* Preliminary values, pending QC

Our goals

01

Predictive tox tool

To develop a fast and efficient tool capable of predicting the relative chronic oral toxic potential of chemicals.

02

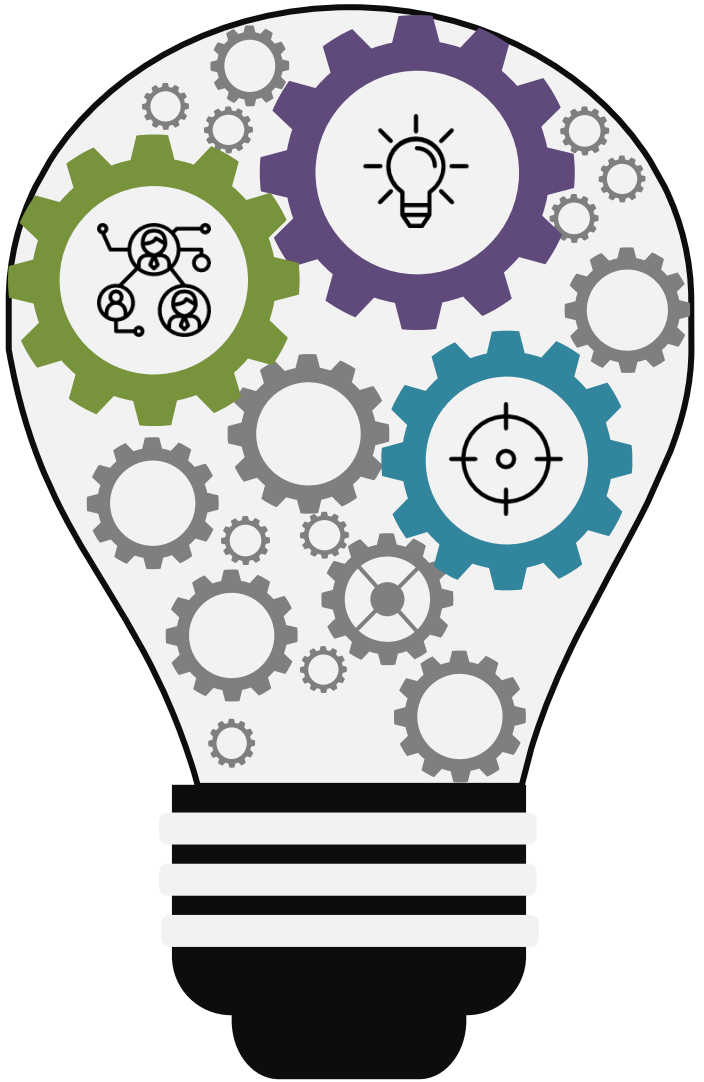
3R

To provide scientists with a tool that can help replace, reduce, and/or refine animal testing.

03

Gain and spread knowledge

To advance our understanding of structure-toxicity relationships and, in general, toxicology.



Other EDT Team Members:

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EDT Software Team:

- Patra Volarath, Ph.D.
- Kirk Arvidson, Ph.D.

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- All those reviewing the EDT publications
- Drs. Antony Williams and Richard Judson from US EPA

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