National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

RIWA

Screening and prioritising PMT substances: development of a robust T-score

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Wrap-up PMT workshop 2020: continuous P and M scores, initial T score

- Continuous P and M-score 0 to 1, based on QSAR estimates
  - 0.33 is P or M, 0.5 is vP, vM
  - P score based on BIOWIN 3:
    - Half-life in surface water:
    - P-score centered at 60 d:

$$t_{0.5} = 5377 * e^{-1.95*BIOWIN3}$$

$$P - score = \frac{1}{1 + 10^{\log 2\left(\frac{\log 60 - \log t_{0.5}}{\log 60 - \log 40}\right)}}$$

  - M score based on KOCWIN (Kow Method):
    - M-score centered at log Koc 3:

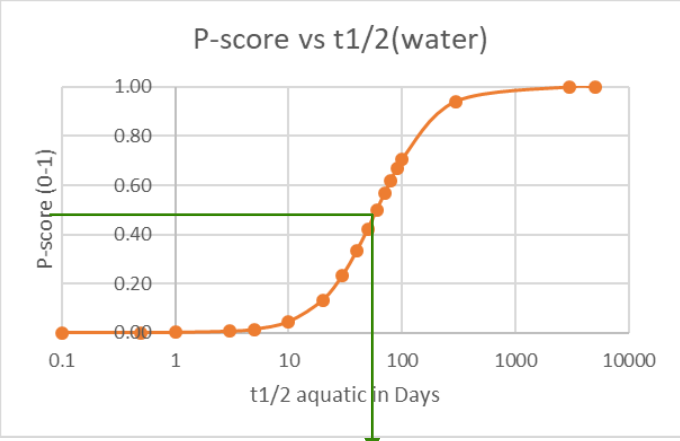
$$M - score = \frac{1}{1 + 10^{\log 2\left(\frac{3 - \log K_{oc}}{3-2}\right)}}$$

- T score prelimary based on Cramers classes
  - Five distinctive classes
  - Risk-based rather than hazard-based
  - Low discriminative power

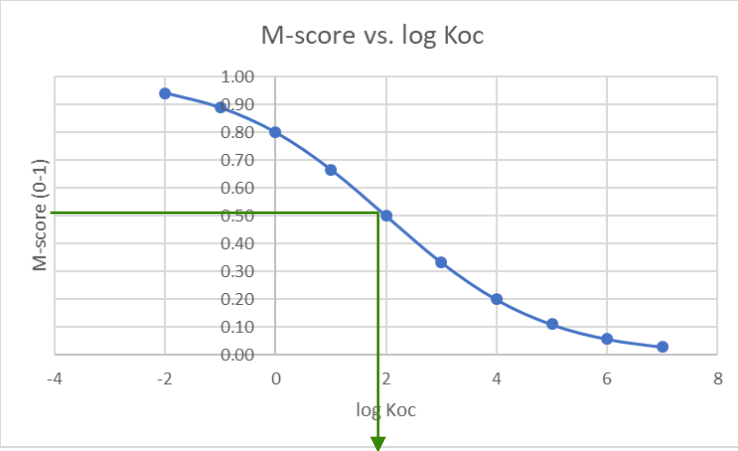
A robust T-score?

- Using different profilers for the same endpoint increases robustness

- A continuous T-score (0-1) increases distinctive power
  - Similar to P and M-scores



vP=0.5, $t_{1/2}$=60 days



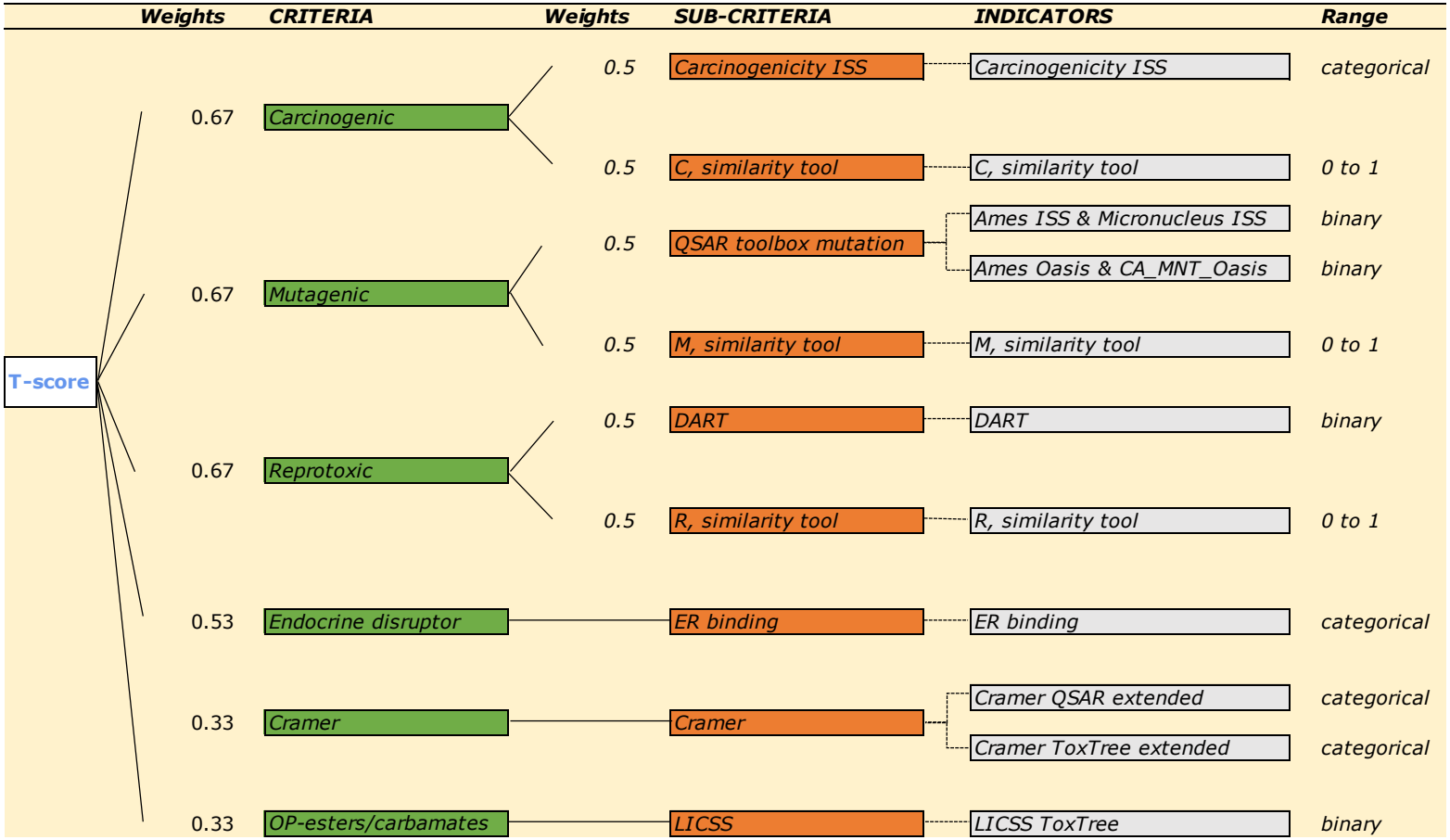
vM=0.5, logK_{oc}= 2

Continuous T-score: the components

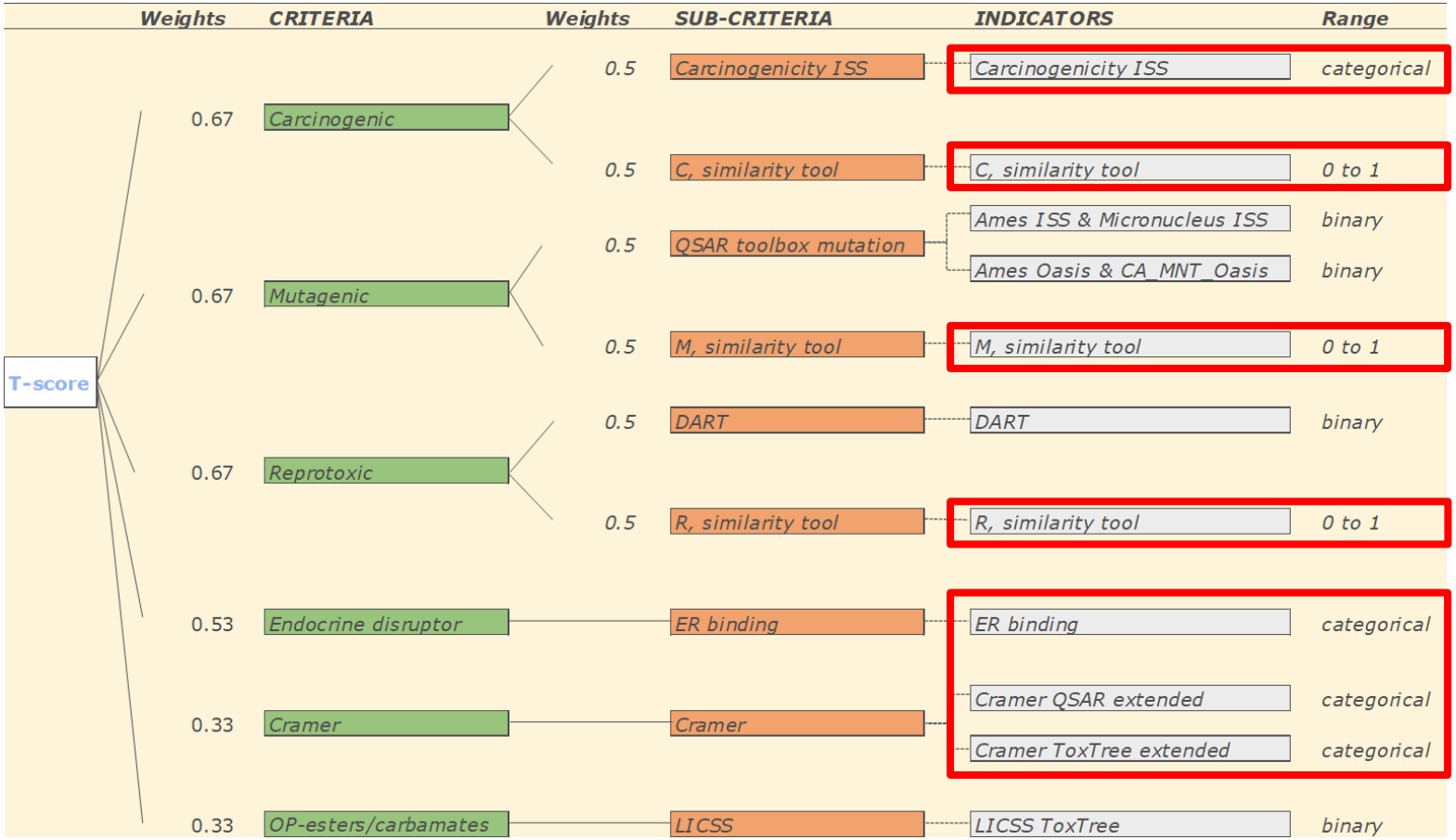
Endpoints	QSAR profilers	SVHC Similarity[1]
Carcinogenicity	ISS Carcinogenicity	Similarity to Carc (cat.1a/b) SVHC
Mutagenicity	ISS or OASIS Mut.	Similarity to Mut (cat.1a/b) SVHC
Reprotoxicity	DART scheme	Similarity to Repro (cat.1/2) SVHC
Endocrine Disruption	ER binding	
General tox	Cramer classification, OP-esters, carbamates	

[1] Wassenaar PN, Rorije E, Janssen NM, Peijnenburg WJ, Vijver MG. Chemical similarity to identify potential Substances of Very High Concern–An effective screening method. Journal of Computational Toxicology. 2019;12:100110.

T-score calculation: overview

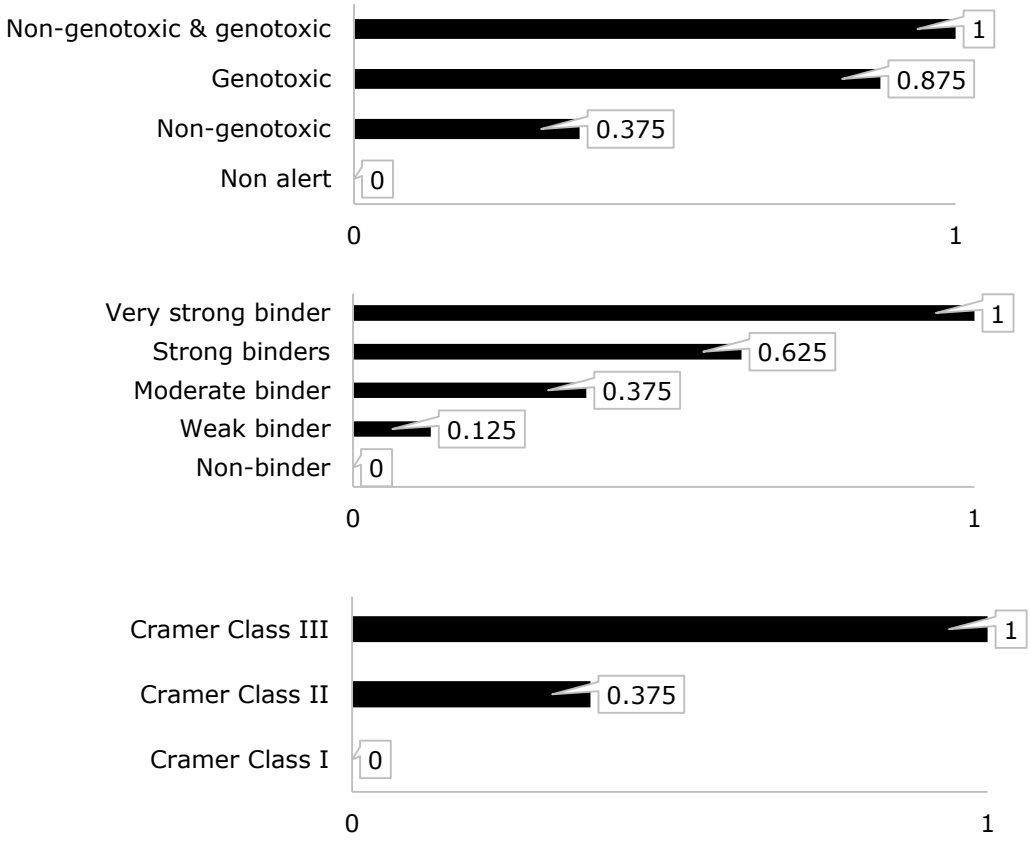


Categorical & continuous indicators: nonlinear scoring



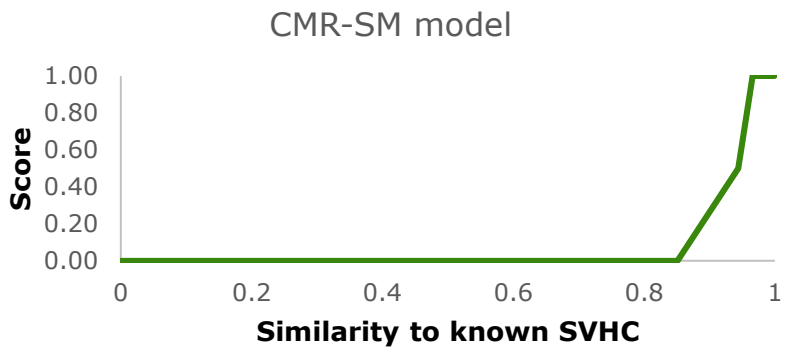
Based on pairwise comparisons using https://www.1000minds.com/about/paprika

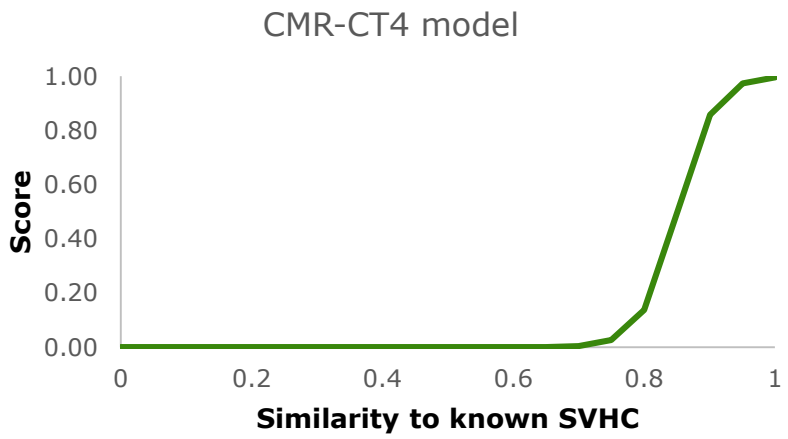
Transform functions used for QSAR toolbox profilers



INDICATORS	Range
Carcinogenicity ISS	categorical
C, similarity tool	0 to 1
Ames ISS & Micronucleus ISS	binary
Ames Oasis & CA_MNT_Oasis	binary
M, similarity tool	0 to 1
DART	binary
R, similarity tool	0 to 1
ER binding	categorical
Cramer QSAR extended	categorical
Cramer ToxTree extended	categorical
LICSS ToxTree	binary

Transform functions: SVHC similarity based on optimized cut-off points by Wassenaar et al. (2019)





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Calculating scores on each endpoint: C, M, R, ED or general tox

Endpoint	Score
Carcinogenicity	$C - score = \sum_{carcinogenicity\ ISS,\ C-similarity} 0.5score$
Mutagenicity	$M - score = \sum_{QSAR\ profilers\ mutagenicity,\ M-similarity} 0.5score$
Reprotoxicity	$R - score = \sum_{DART,\ R-similarity} 0.5score$
Endocrine Disruption	$ED - score = score_{ER\ binding}$
General tox	$Cramer - score = score_{Cramer\ classes}$ $OPester - score = score_{LICSS\ ToxTree}$

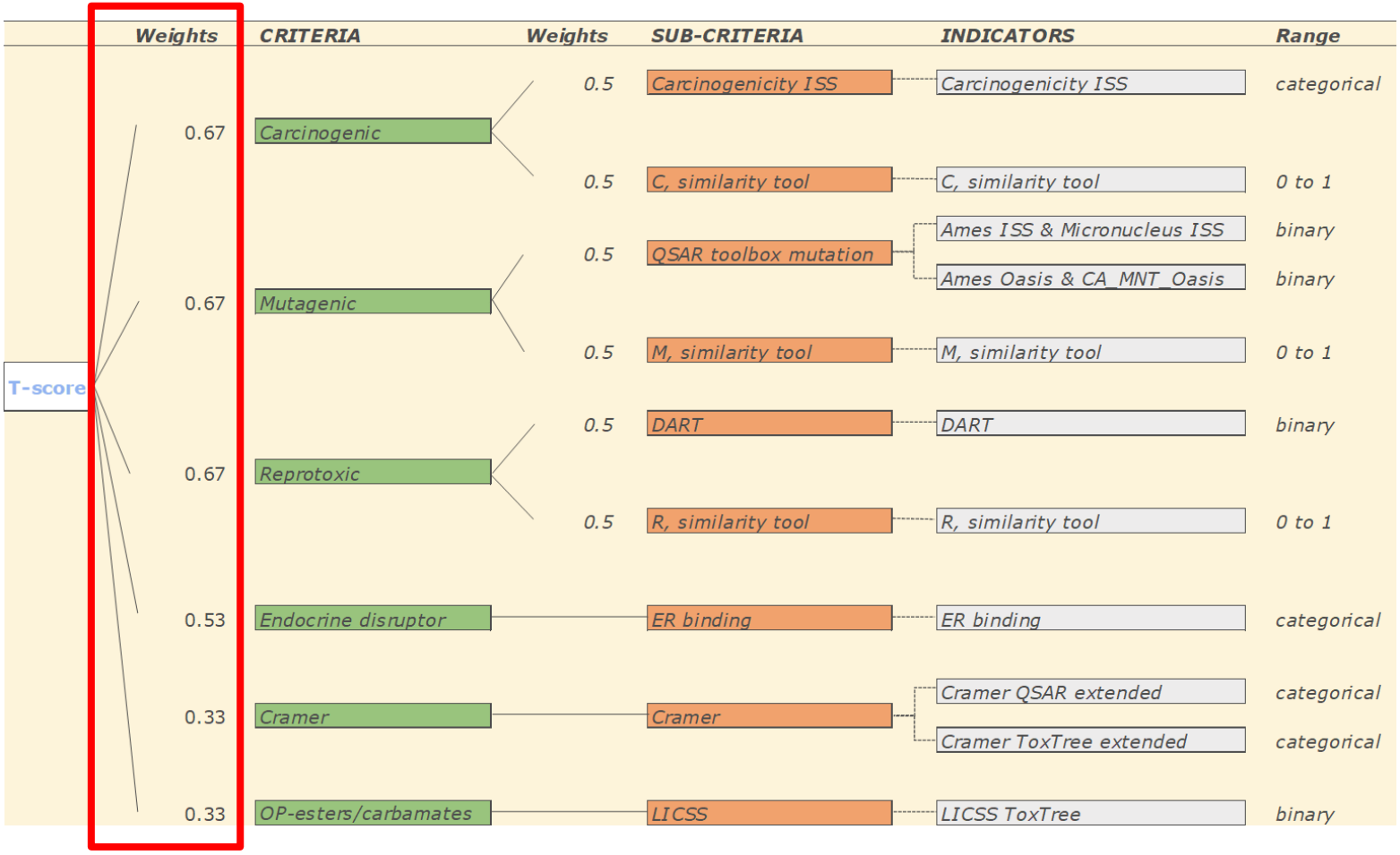
T-score calculation: summing different endpoints

- Starting points
  - Either one of the endpoints C, M, R, ED or general tox sufficient for T
    - › T-score 0.33 or higher
  - Two endpoints fulfilled is worse than one

- Solution: Response addition equation
  - › Score not filled by one endpoint can be filled proportionally by second

$$T - score = 1 - \prod\left(1 - weight * score_{C,M,R,ED,Cramer,OP-esters,carbamates}\right)$$

Weights for combining all endpoints to one score



Combining continuous P, M and T-scores

$$PMT - score = P - score^{a} * M - score^{b} * \ T - score^{c}$$

- Sum of weights a, b, and c should be one
  - For now: a & b = 0.4, c = 0.2
  - Weights can be adjusted, for example more weight to P
- PMT-score of PMT substances should be at least 0.33
- Score not filled by one endpoint can be filled by another
  - Comparable to way of thinking in GUS score

Illustrating PMT-criteria

Association of river water works (RIWA) database:
→ 1161 compounds, target screening by drinking water companies

- 628 compounds detected above LOD, including:
  - 248 industrial chemicals
  - 206 ppp and biocides
  - 108 pharmaceuticals (both human & veterinary)

Results T scoring RIWA database

- 414 substances: T > 0.67
- 550 substances: 0.66 > T > 0.33
- 136 substances: T = 0.33 (C,M,R,ED or general tox)
- 61 substances: 0.33 > T

- Highest T-scores (59 subst. >0.9)

5-chloor-2-nitro-toluene
4-methyl-3-nitro-aniline
1,2-dichloro-propane
1,2 dibromo-3-chloro-propane (DBCP)
dibenzo(a,h)antracene + other PAHs
.....

Lowest T-scores (31 subst. <0.1)

.....
Octacosane
Hexacosane
Tetracosane
Acetylsalicylic acid (Aspirine)
Aspartame

T-scores for some “marker substances”

• 1,2-diBr-3-Cl-propane (DBCP)	- 0.96	(C, M, R + SVHC similarity)
• Dibenz(a,h)anthracene	- 0.96	(C, M, R + SVHC similarity)
• Bisphenol A	- 0.90	(ED, Repro)
• 1,4-dioxane	- 0.84	(C, M, R)
• 17-b-Estradiol	- 0.77	(ED, C, M)
• Warfarin	- 0.75	(C, M, R, Cramer III)
• Morphine	- 0.69	(ED, Cramer Class III)
• Melamine	- 0.61	(C, Cramer Class III)
• Tetrabromo Bisphenol A	- 0.56	(not ED, Repro)
• Butyl Benzyl Phthalate (BBP)	- 0.42	(Repro, Carc.)
• Nitrilo Tri Acetic Acid (NTA)	- 0.18	(Repro, Carc.)
• Salicylic acid	- 0.07	(weak ER binder)
• Acetyl salicylic acid	- 0.00	(no alerts)
• Aspartame	- 0.00	(no alerts)

Results PMT-scoring RIWA database

- 258 / 1161 (P, M and T > 0.33)
- 221 / 1161 (P, M > 0.33, T>0.5)
- **48 / 1161 (P, M, T > 0.5)**
  - Most of the pharmaceuticals (tetracycline, etoposide, cortison, doxycycline, …), and Melamine also scores high

- NOT PMT → low persistence

pyrazole	**not P** (0.07), M (0.59), T (0.33), PMT (0.23)
glyphosate	**not P** (0.05), M (0.96), T (0.34), PMT (0.23)
glufosinate	**not P** (0.05), M (0.95), T (0.67), PMT (0.27)
1,4-dioxane	**not P** (0.09), M (0.73), T (0.84), **PMT (0.33)**

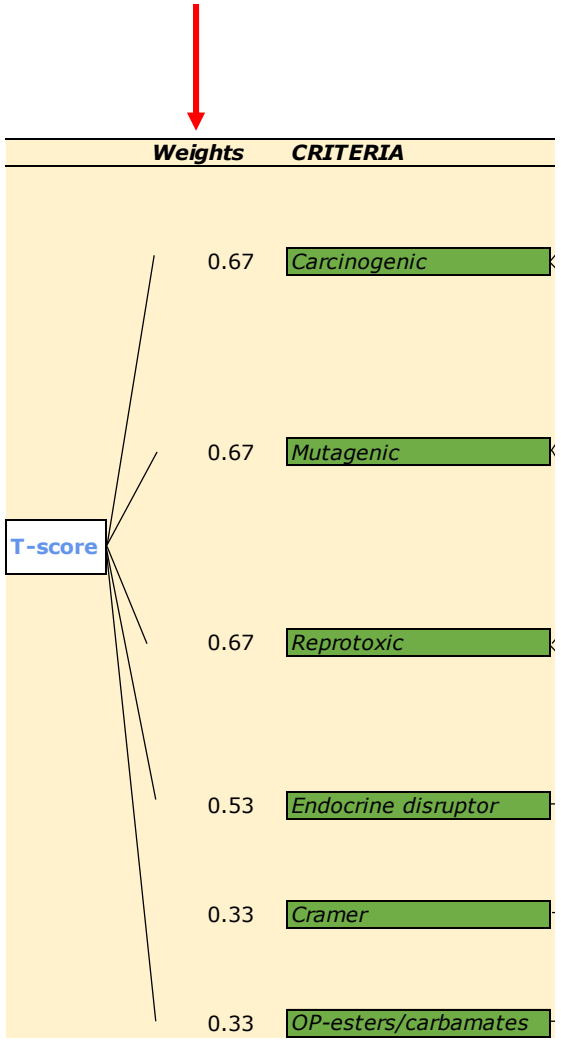
Discussion (1): extension database needed

- High T is overrepresented (?) in the RIWA database → target screening
  - RIWA dataset contains **125 (11%)** CMR SVHC substances!
    - Known carcinogen, mutagen and/or reprotoxic
  - Almost all subst. (96.5%) have at least 1 alert OR Cramer Class III
  - Most (82%) are Cramer Class III
    - This already gives a T≥0.33 score

- A 'proper' distribution of T-scores should come from an unbiased dataset (e.g., 65.000 structures EINECS + PPP + Pharma)

Discussion (2)

- Is the T-score intrinsically too 'conservative'?
  - Cramer class III (highly toxic, TTC = 90 µg/person/day) → T?
    - Weight of Cramer Class too high?
  - Strong estrogen receptor binder → T?
    - Weight of Endocrine disruption too high?



Discussion (3)

- Is the T-score intrinsically too 'conservative'?

- Not all compounds with PMT-score > 0.33 fulfill individual P, M and T criteria
  - Filtering or adjusting weights

$$PMT - score = P - score^{0.4} * M - score^{0.4} * \ T - score^{0.2}$$

Discussion (4)

- Is the T-score intrinsically too 'conservative'?

- Not all compounds with PMT > 0.33 are PMT
  - Filtering or adjusting weights

- Semi-continuous, more robust T-score
  - Additional endpoints needed?
    - E.g. ecotox

$$T - score = 1 - \prod\left(1 - weight * score_{C,M,R,ED,Cramer,OP-esters,carbamates}\right)$$

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Thank you for your attention!

Questions or remarks?

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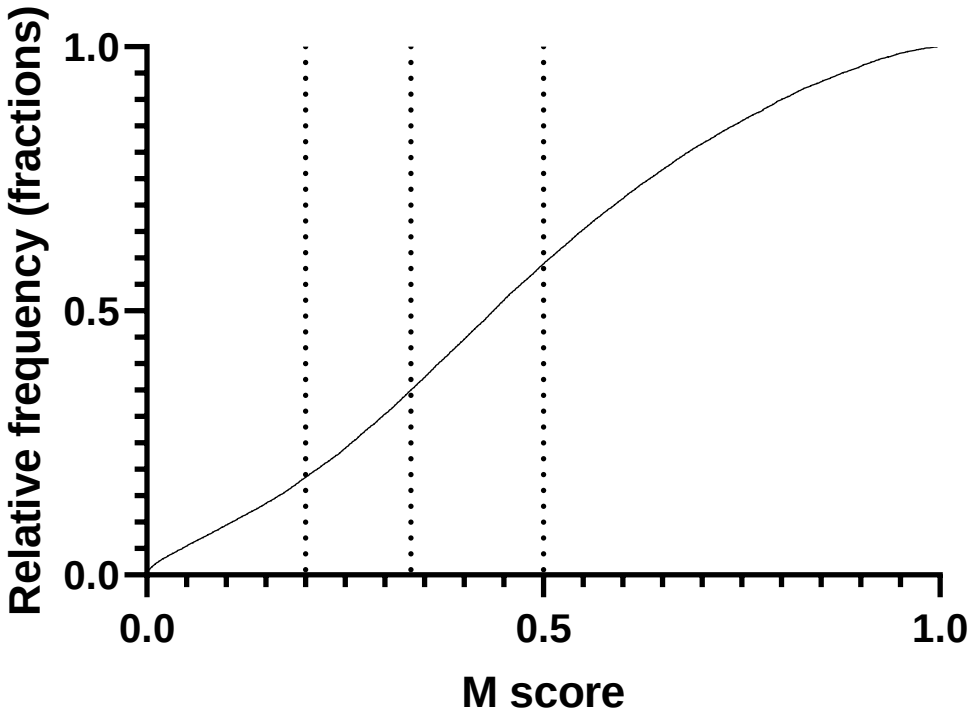
Extra slides

Histogram for Mobility (based on 65,000 compounds)



- Drawn lines represent log K_{oc} 4, 3 and 2
- log K_{oc} <4: 81%; log K_{oc} <3: 65%; log K_{oc} <2: 41%
- For more distinctive power log K_{oc} 3 and 2 used as M and vM criteria
  - Centered around log K_{oc} 2

Combination of Persistence and Mobility (65,000)

- All combinations possible
  - P 25405
  - vP 17350
  - M 42324
  - vM 26627
  - PM 12949
  - vPM 7680
  - PvM 6334
  - vPvM 3566

CMR substances:

CMR substances are substances that are **carcinogenic**, **mutagenic** or **toxic to reproduction** (**CMR**). They are of specific concern due to the long term and serious effects that they may exert on human health. Under GHS, CMR substances can be classified into 3 categories depending on the severity of hazards:

- **Category 1A**: Known human carcinogen (H340), mutagen (H350) or reproductive toxicant (H360) based on human evidence ;
- **Category 1B**: Presumed human carcinogen (H340), mutagen (H350) or reproductive toxicant (H360) based on animal studies;
- **Category 2**: Suspected carcinogen (H341), mutagen (H351) or reproductive toxicant (H361) based on limited evidence from animal studies or/and human.