The threshold of toxicological concern (TTC)

-

a suitable tool for mixture risk assessment?

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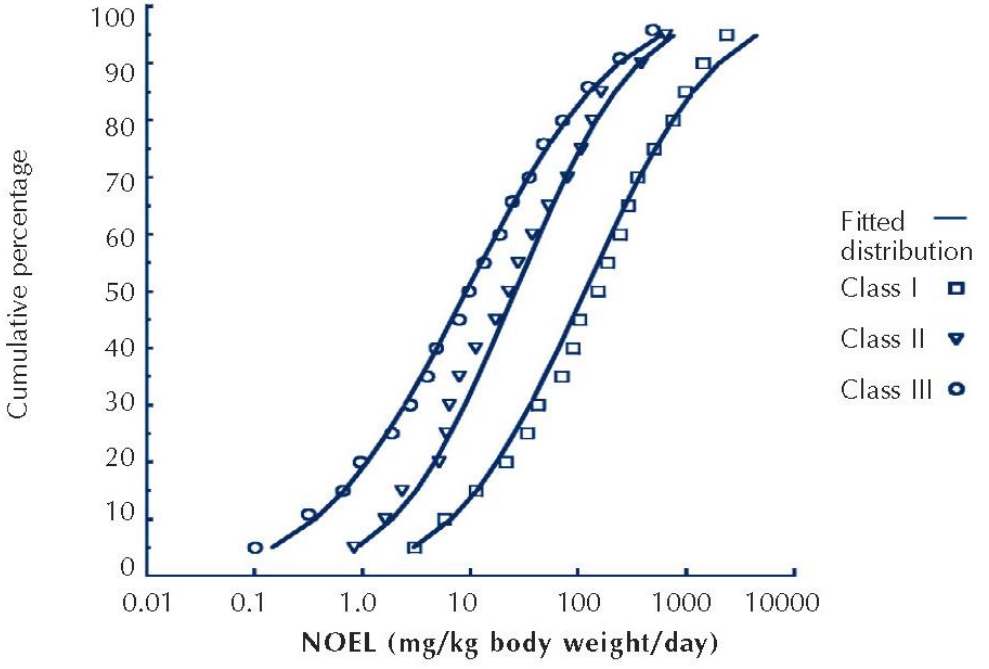
The Threshold of Toxicological Concern (TTC)

Generic human exposure threshold value, below which

“the probability of adverse effects on human health is considered to be very low. “

The Threshold of Toxicological Concern (TTC)

The TTC is the lower 5% percentile of a pool of log-normally distributed NOELs, divided by an AF of 100.



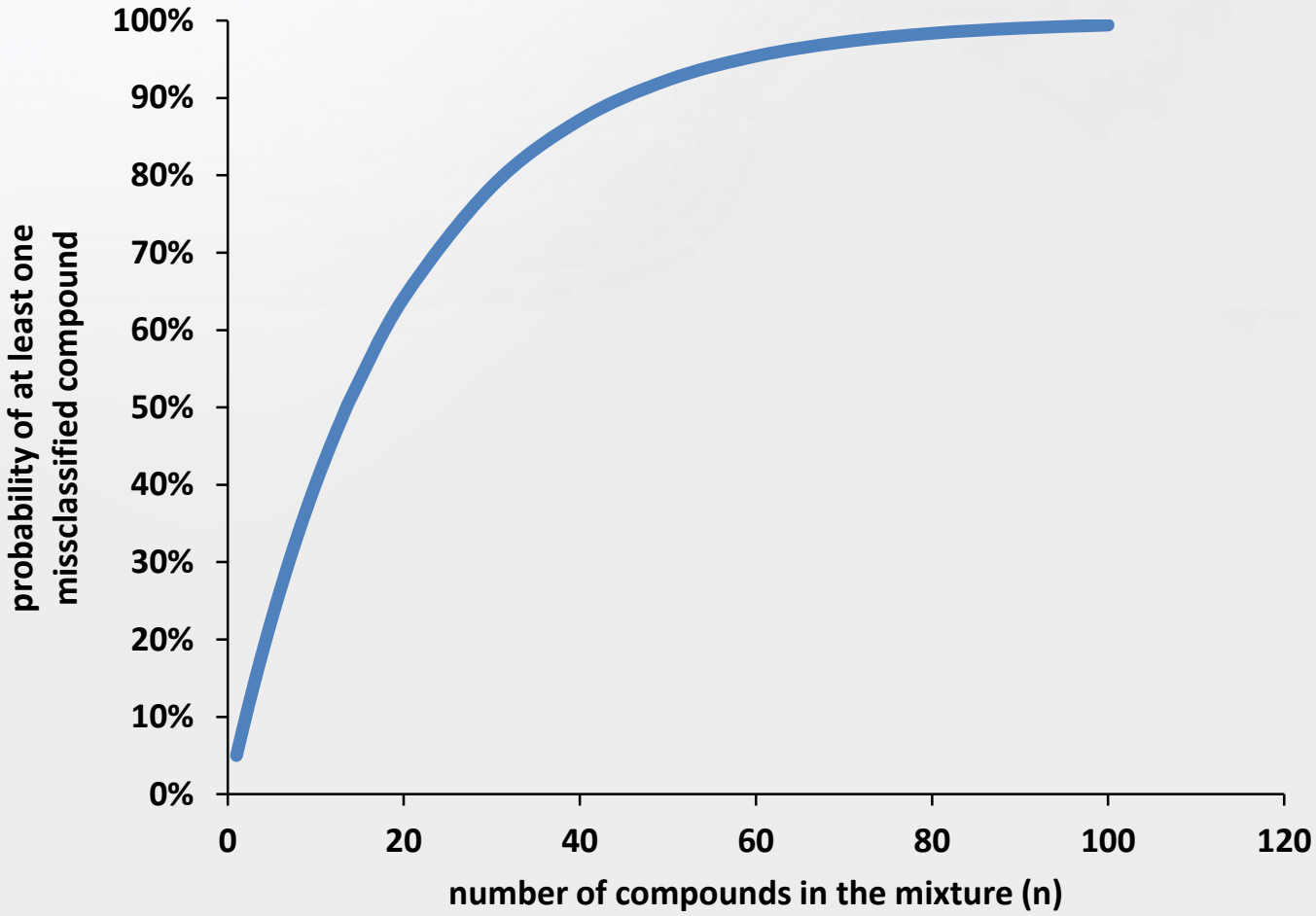
S. Barlow, Threshold of Toxicological Concern, ISLI Monograph Series, 2005. After: Munro IC, et al.; Food Chem Tox , 34. 829-867, 1996

Consequences of using the lower 5% percentile

- 5% risk that any ***one*** given compound has a NOEL/100 < TTC (risk for misclassification, risk of a false-negative)
- Obviously: the more compounds, the higher the risk of misclassification
- Mathematically speaking:

$$1-0.95^{n}$$

Consequences of using the lower 5% percentile



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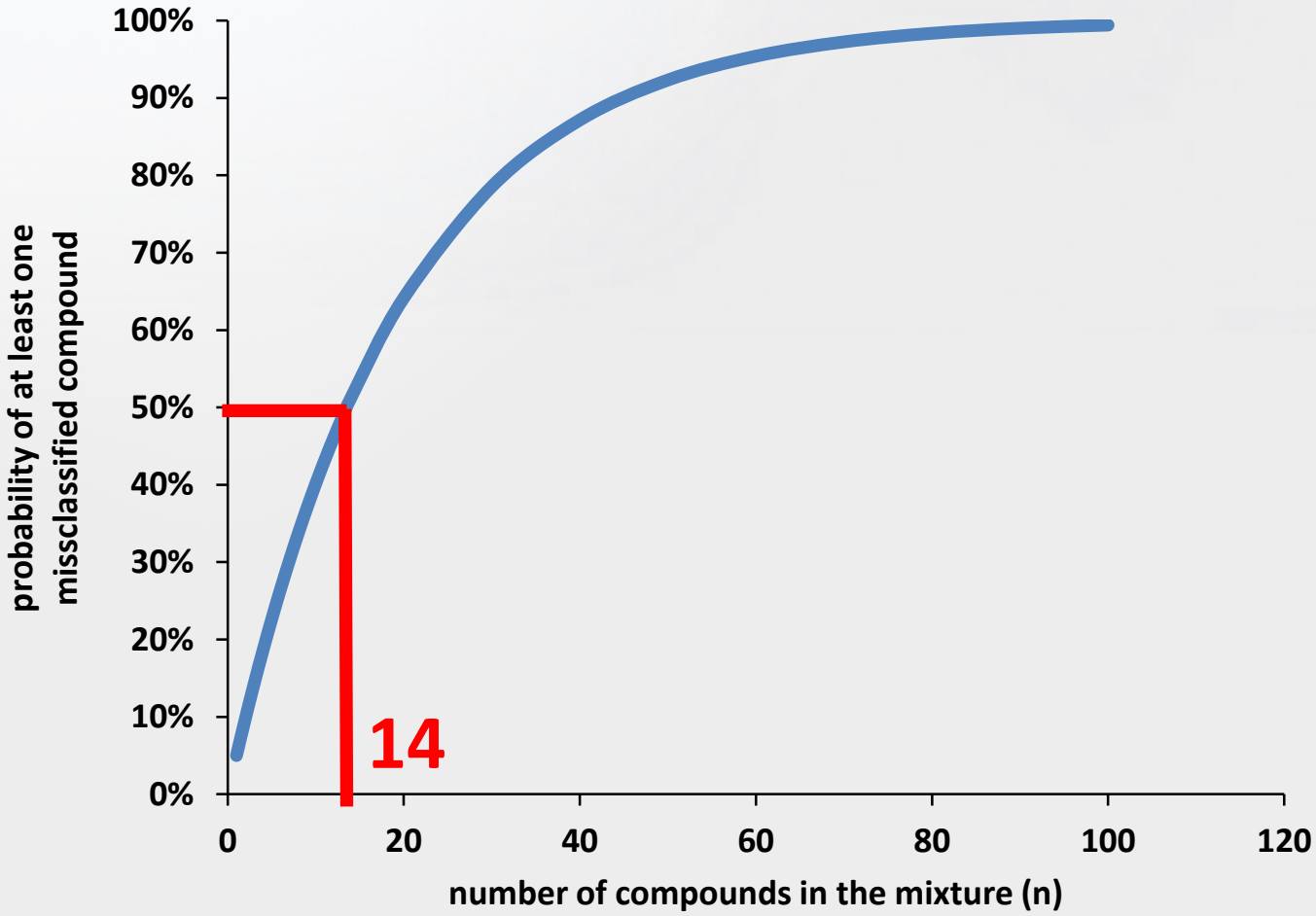


Consequences of using the lower 5% percentile

- 5% risk that a given compound has a NOEL/100 < TTC
- Obviously: the more compounds, the higher the risk of misclassification
- Mathematically speaking:

$$1-0.95^n$$

- In a 14 compound mixture there is a 50% risk that at least one misclassified compound is present.

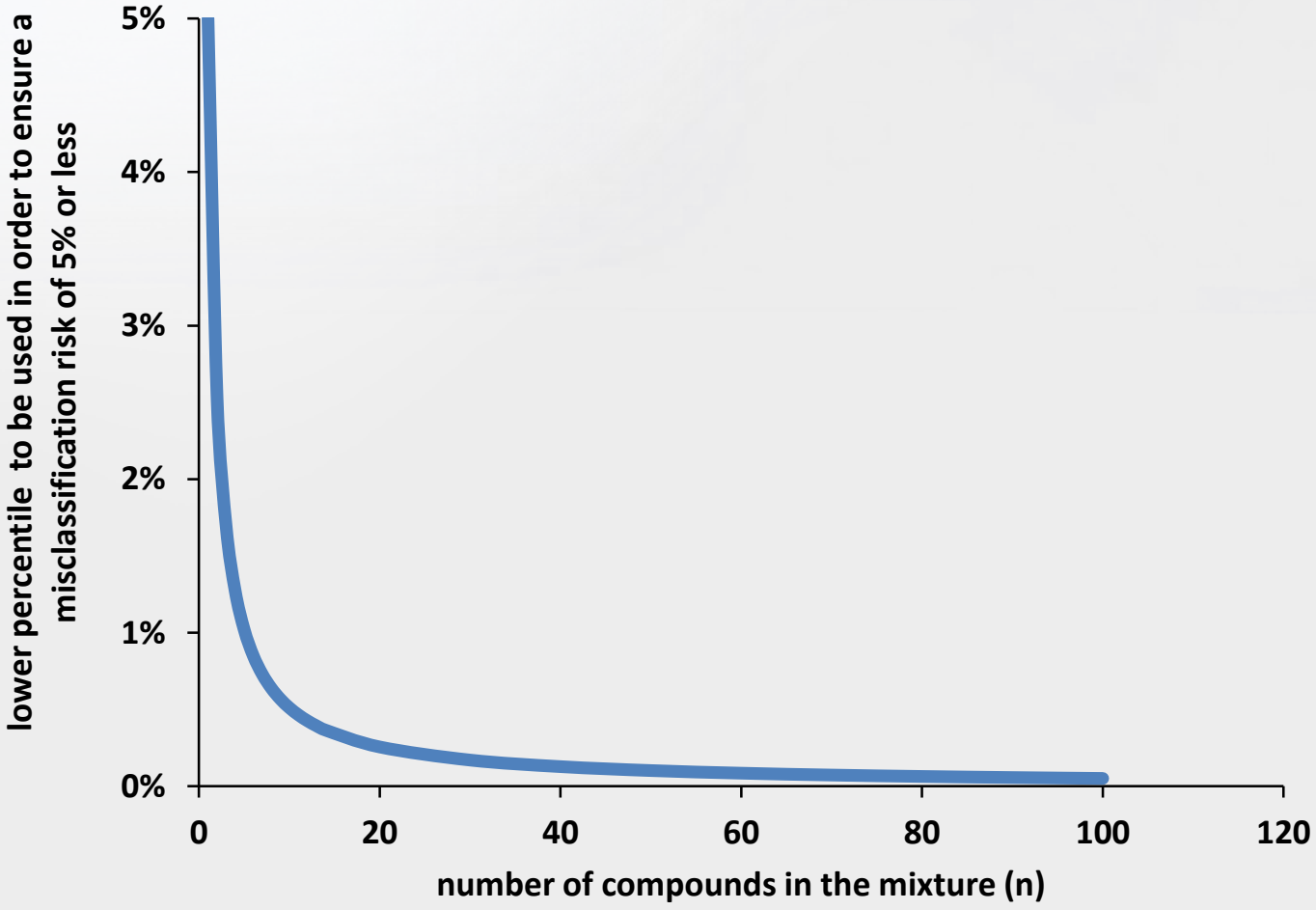
n	Risk of misclassification
10	40%
20	64%
30	79%
40	87%
50	92%

Which percentile to achieve an overall misclassification risk of ≤ 5%?

- Depends on the number of compounds
- Mathematically speaking:

$$1-0.95^{1/n}$$

Which percentile to achieve an overall misclassification risk of ≤ 5%?



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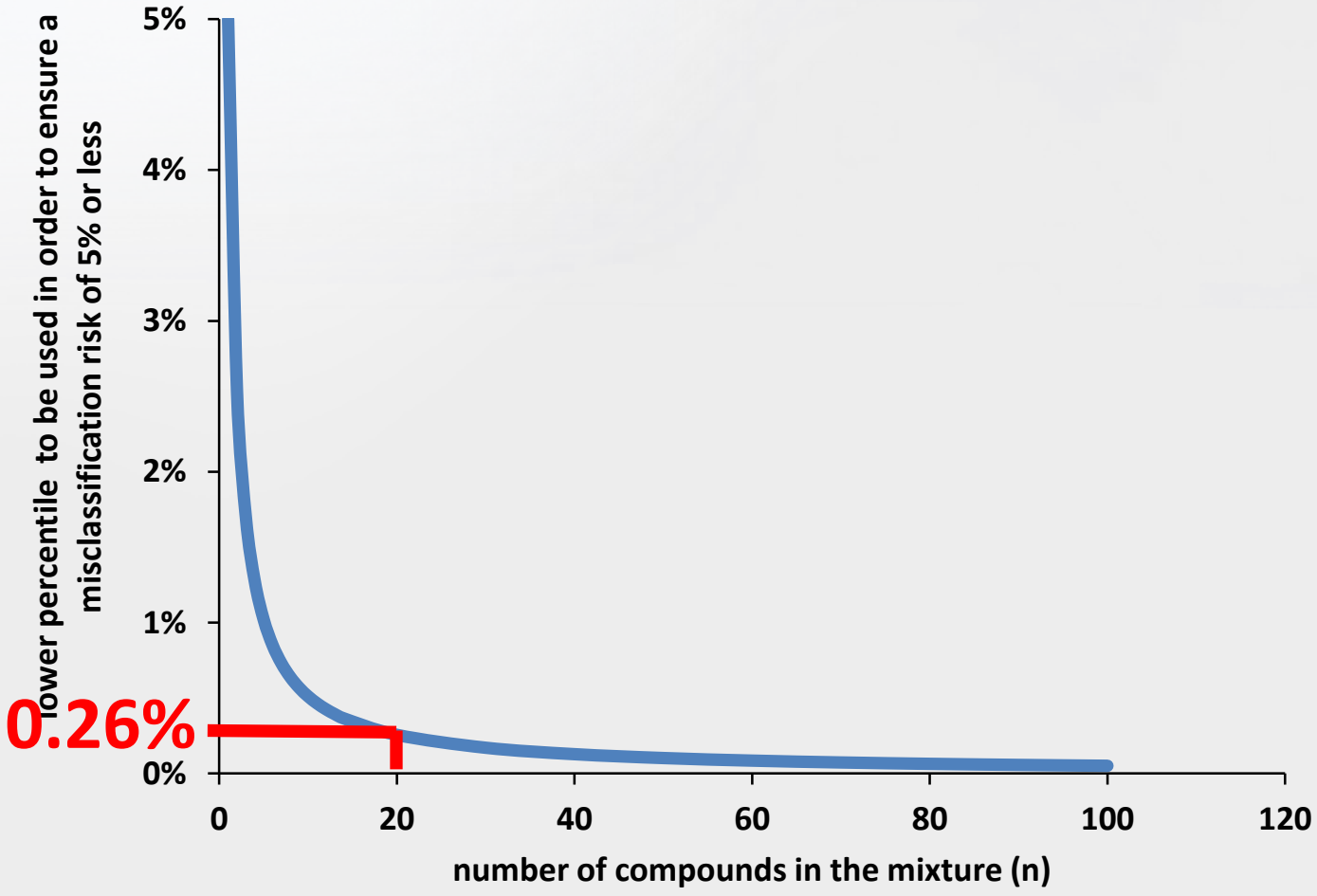


Which percentile to achieve an overall misclassification risk of ≤ 5%?

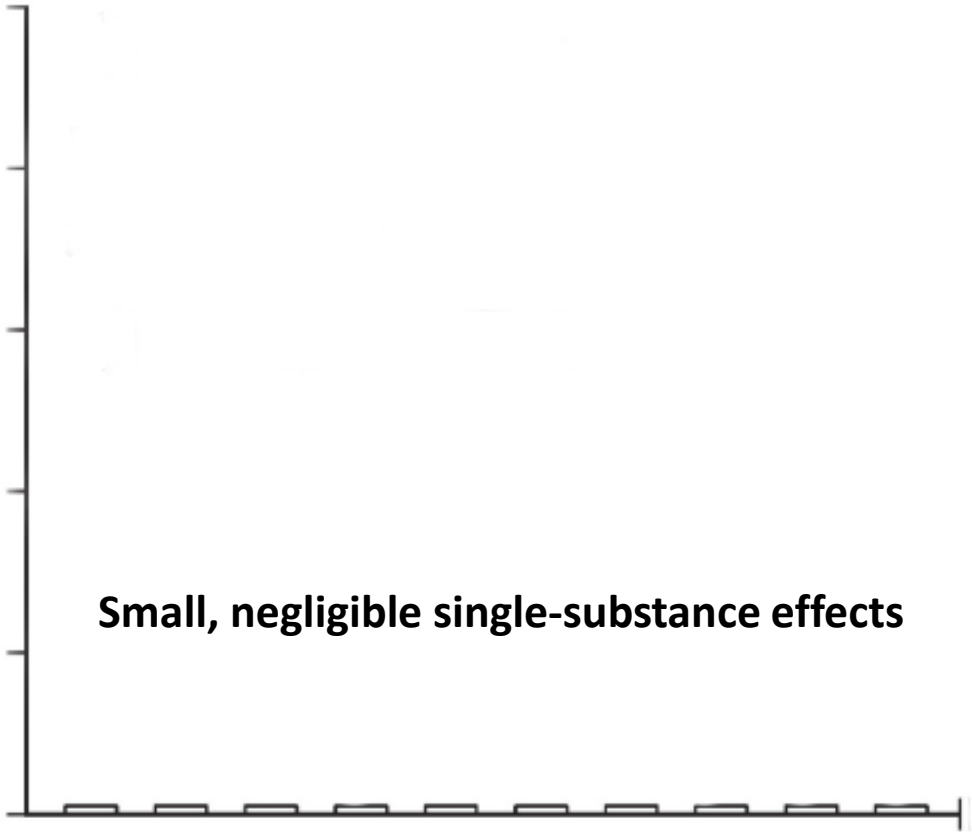
- Depends on the number of compounds
- Mathematically speaking:

$$1-0.95^{1/n}$$

- In a 20 compound mixture the lower 0.26% percentile needs to be used in order to ensure an overall misclassification risk of 5%.

n	percentile
10	0.51%
20	0.26%
30	0.17%
40	0.13%
50	0.10%

Why are mixtures of specific concern?



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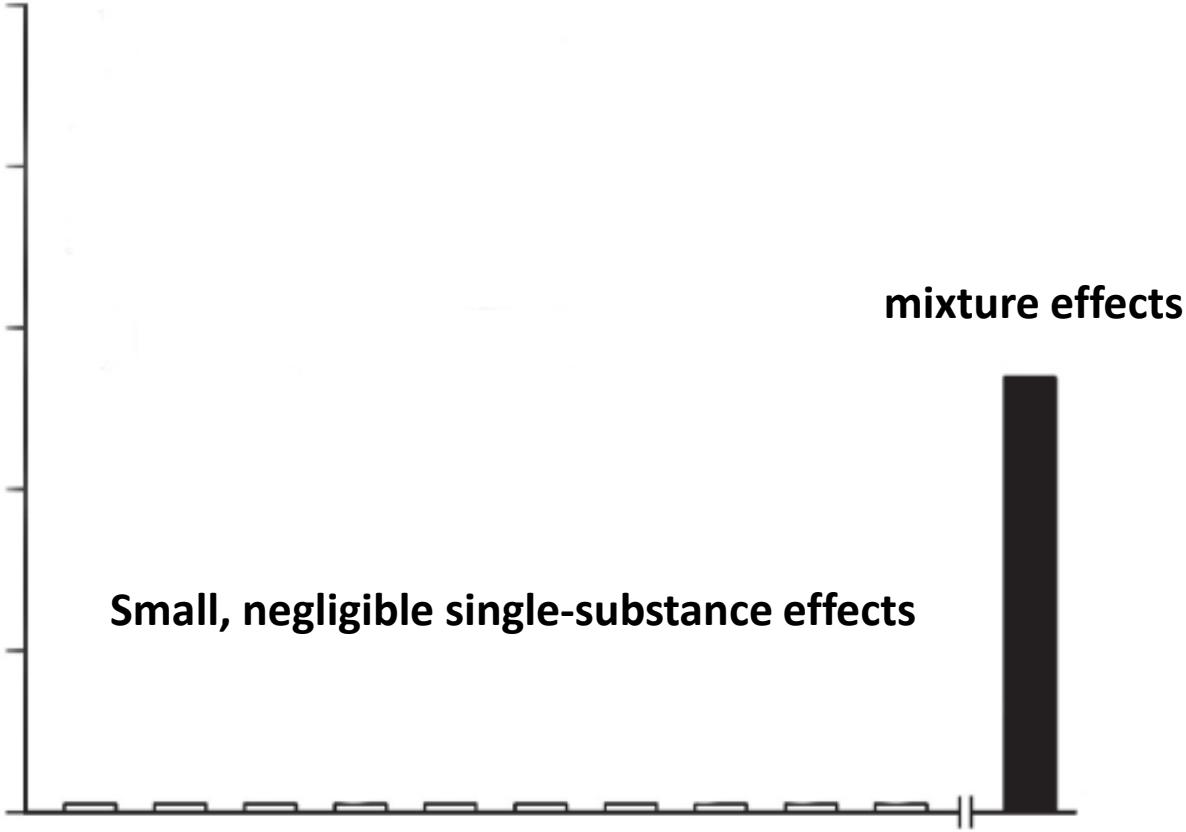

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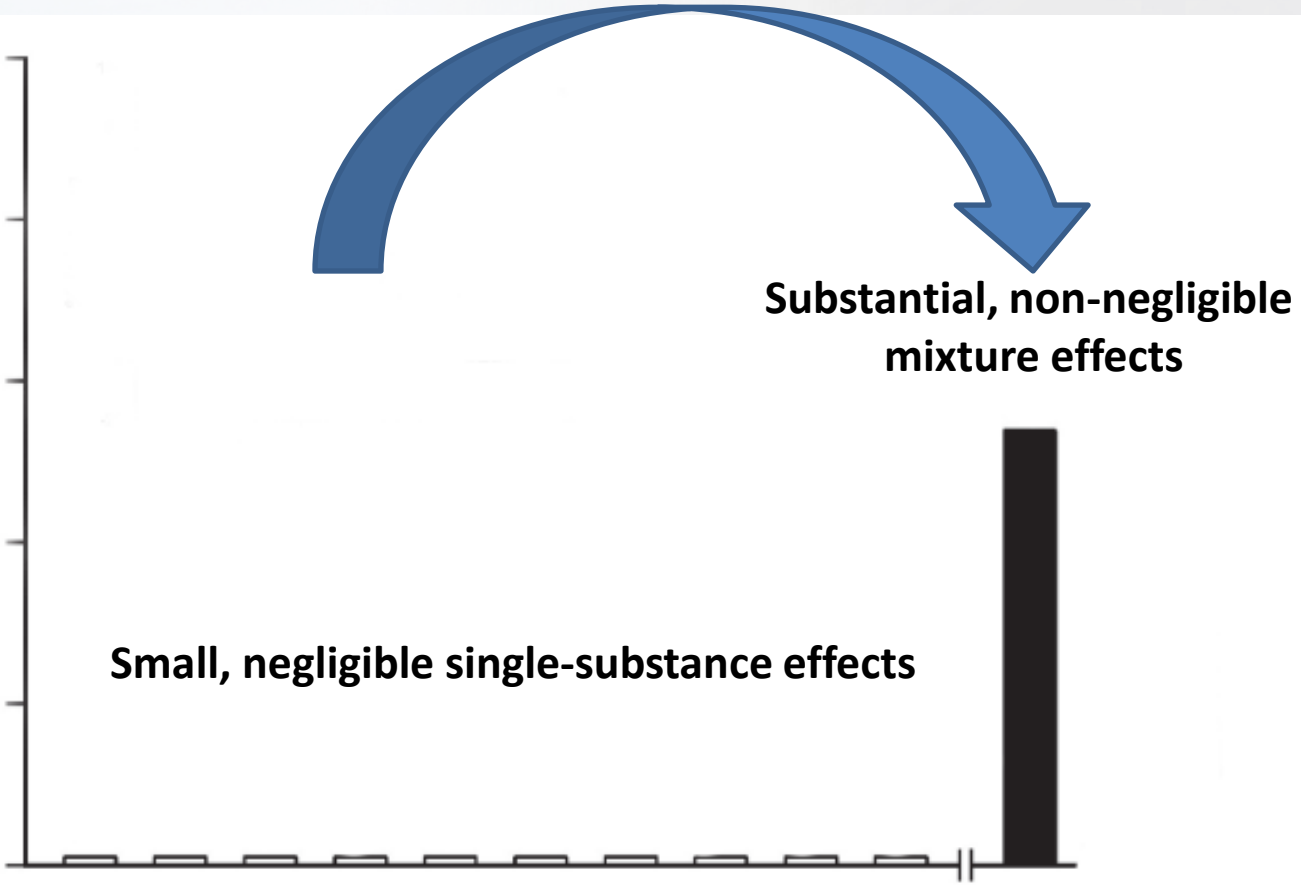

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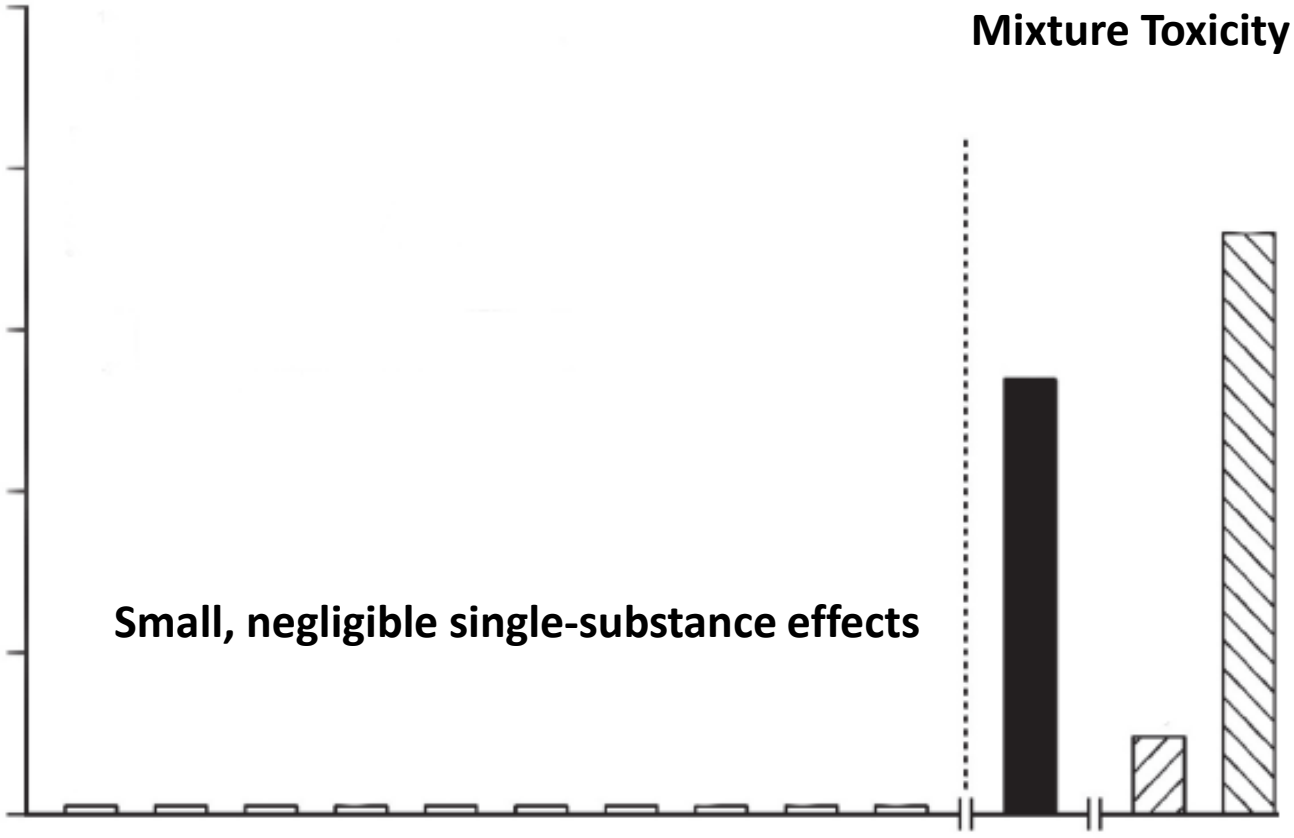
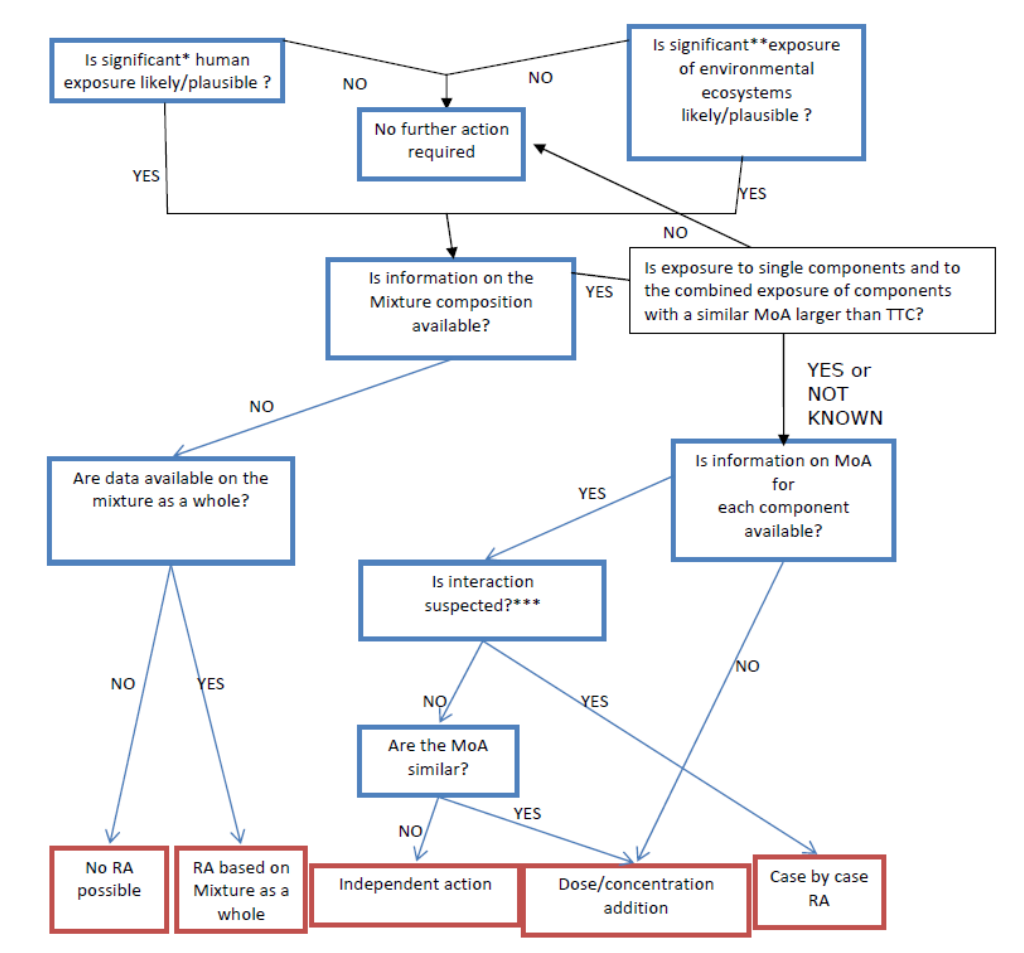


Why are mixtures of specific concern?

- Mixture toxicity is higher than the toxicity of each compound at the concentration present in the mixture

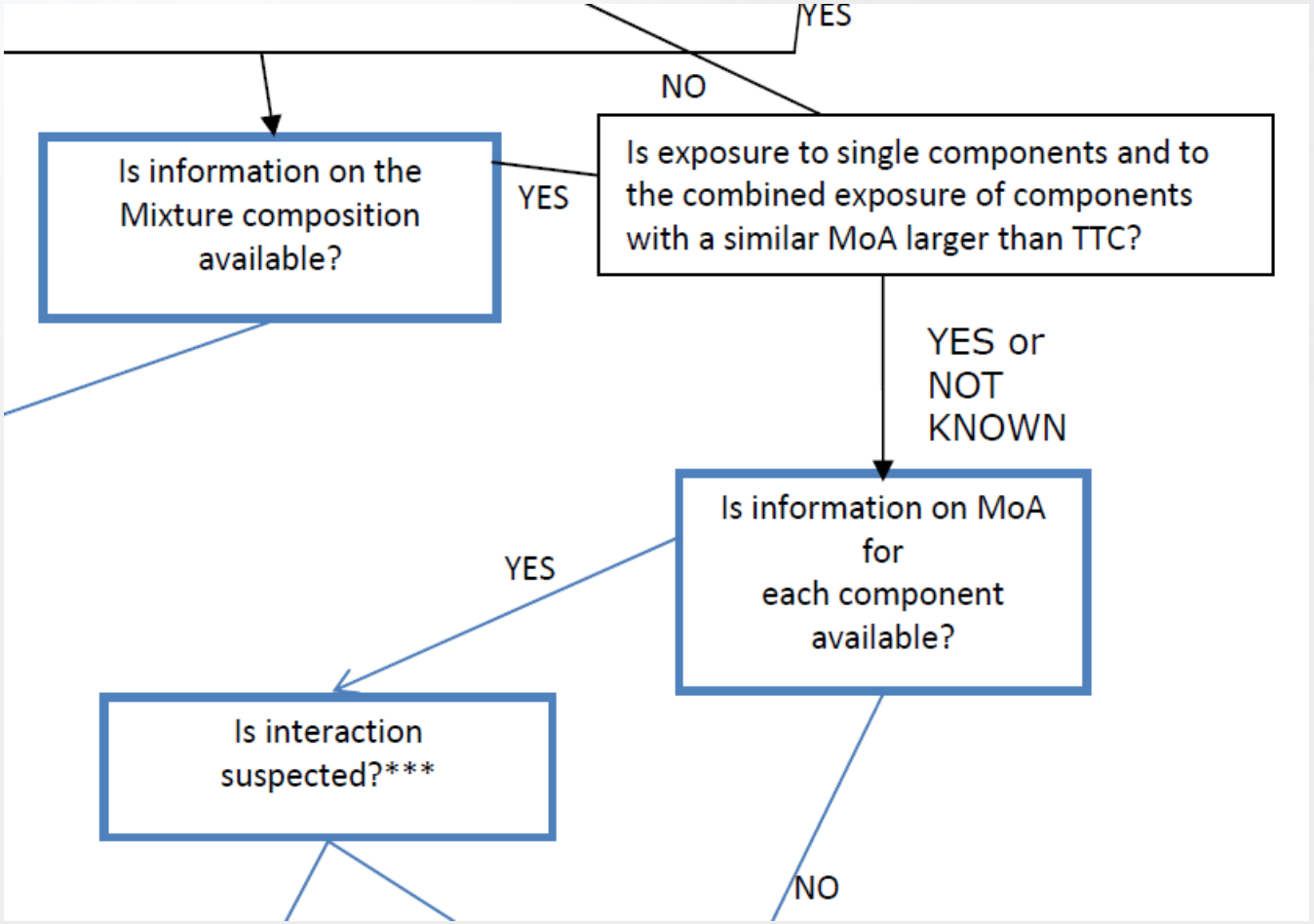
- Compliance with individual threshold values (ADI, TDI, TTC) does not necessarily safeguard against unwanted mixture effects

Suggested use of the TTCs in mixture risk assessment



(Scientific Opinion on Mixture Toxicity Assessment, SCHER, SCENIHR, SCCS)

Suggested use of the TTCs in mixture risk assessment



(Scientific Opinion on Mixture Toxicity Assessment, SCHER, SCENIHR, SCCS)

Suggested use of the TTCs in mixture risk assessment

- Compounds with dissimilar modes of action do not contribute to the mixture toxicity if present below their individual TTC
- If exposure to compounds with dissimilar modes of action is below their individual TTC, there is no mixture risk > TTC

(Final Scientific Opinion on Mixture Toxicity Assessment, SCHER, SCENIHR, SCCS)

Mixture toxicity concepts

Similarly acting substances: Concentration Addition

$$ECx_{(Mix)} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

c_i = Concentration of component *i* in the mixture (*i = 1...n*)

ECx_i = Concentration of substance *i* provoking a certain effect *x* when applied alone

$ECx_{(Mix)}$ = Predicted total concentration of the mixture, that provokes x% effect.

pi = relative fraction of component *i* in the mixture

Dissimilarly acting substances: Independent Action

$$E_{Mix} = 1 - \prod_{i=1}^{n} (1 - E_i)$$

E_{Mix} = Effect of the mixture of *n* compounds

E_i = Effect of substance *i*, when applied singly

TTCs and Independent Action

$$E_{Mix} = 1 - \prod_{i=1}^{n} (1 - E_i)$$

$$E_{Mix} = E_1 + E_2 - E_1 \times E_2$$

If $E_1=0$ AND $E_2=0$ then $E_{Mix}=0$,

Ergo: $$TTC_{Mix} = \min\left(\frac{TTC_i}{p_i}\right)$$

Price et al. Risk Analysis, 2009

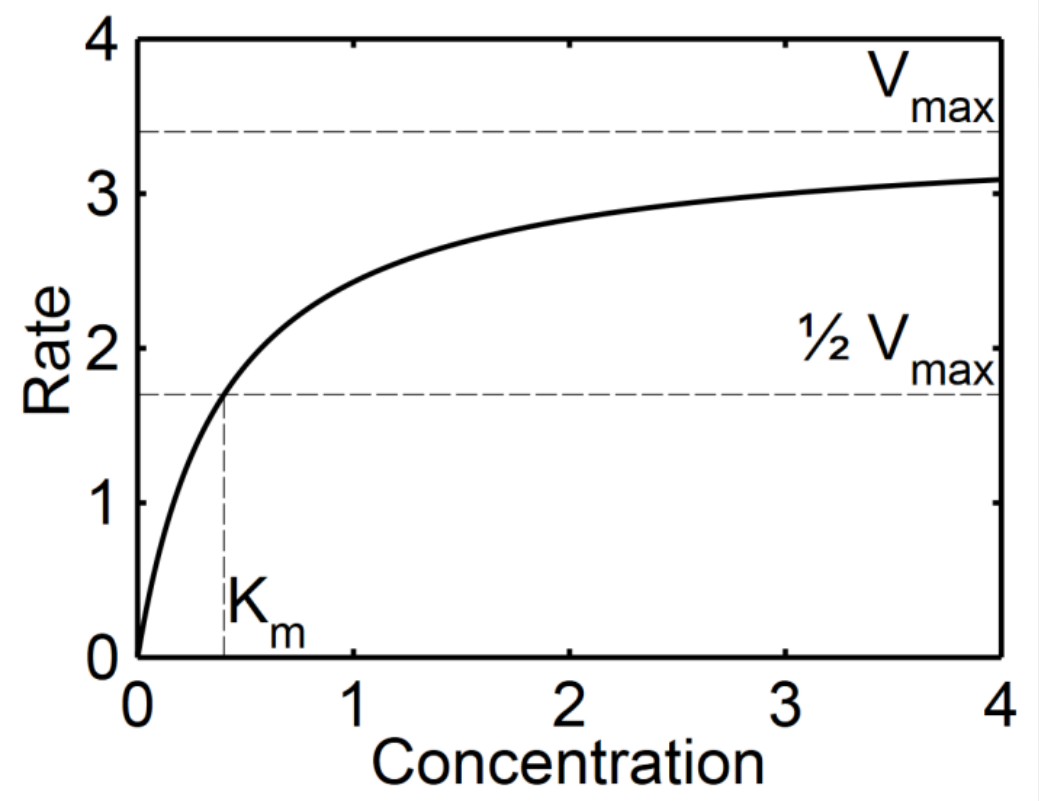
TTC and IA and some pitfalls

- TTC must actually describe a true zero-effect concentration if IA is supposed to safeguard against unwanted mixture effects
- We know that NOELs are no true zero-effect concentrations
- How about the lower 5% percentile of a log-normal distributed collection of NOELs divided by an Assessment Factor of 100?

Are TTCs true zero effect levels?

Truly Zero = no effect on a molecular level

$$v = \frac{V_{\max} \times [c]}{K_M + [c]}$$



TTC and IA and some pitfalls

- TTC must actually describe a true zero-effect concentration,
- AND the compounds must be completely independently acting

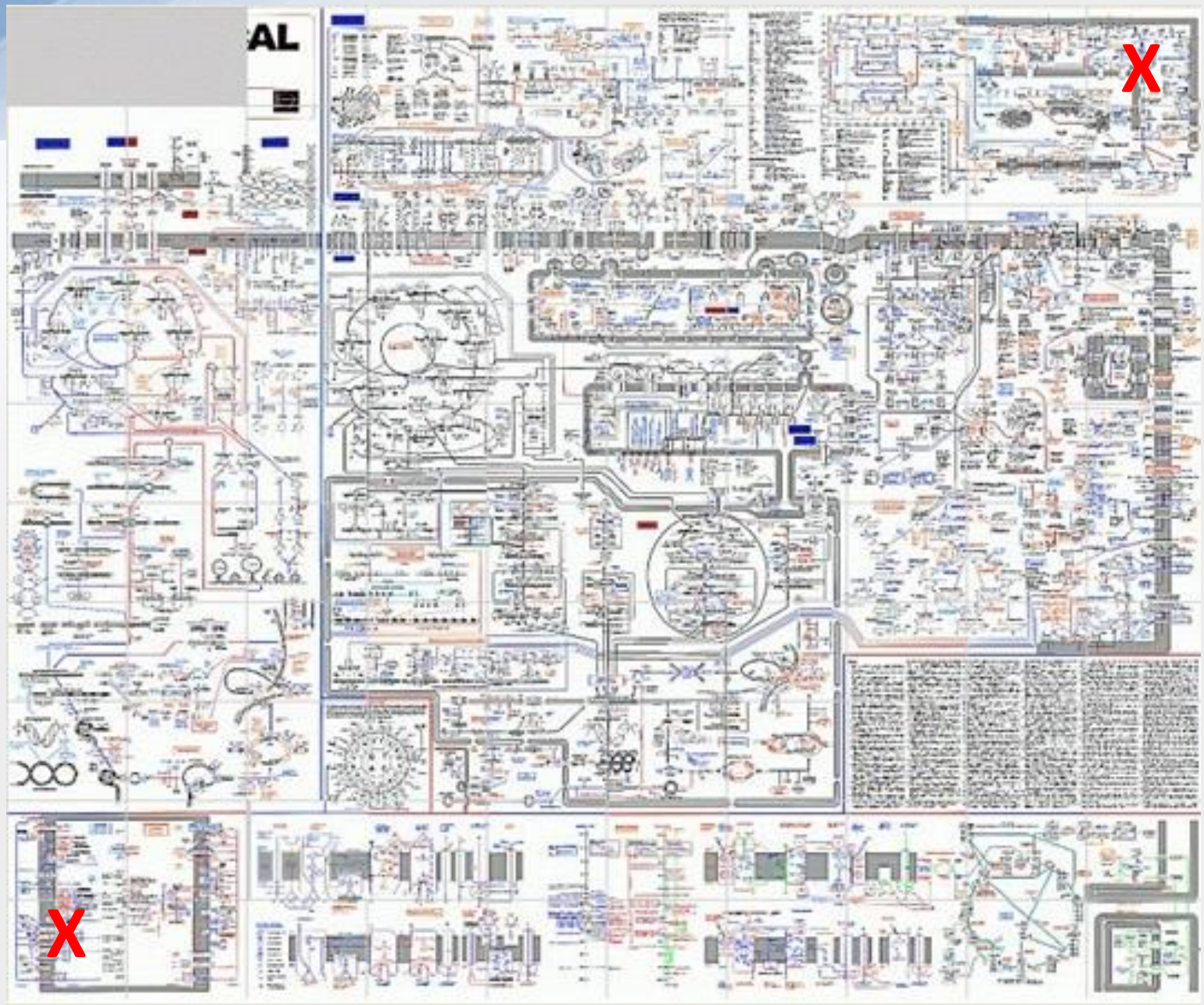
… if IA is supposed to safeguard against unwanted mixture effects at individual concentrations < TTC

Independent Action

Bliss, 1939:

Independent Action

One toxicant does not influence the toxicity of another one.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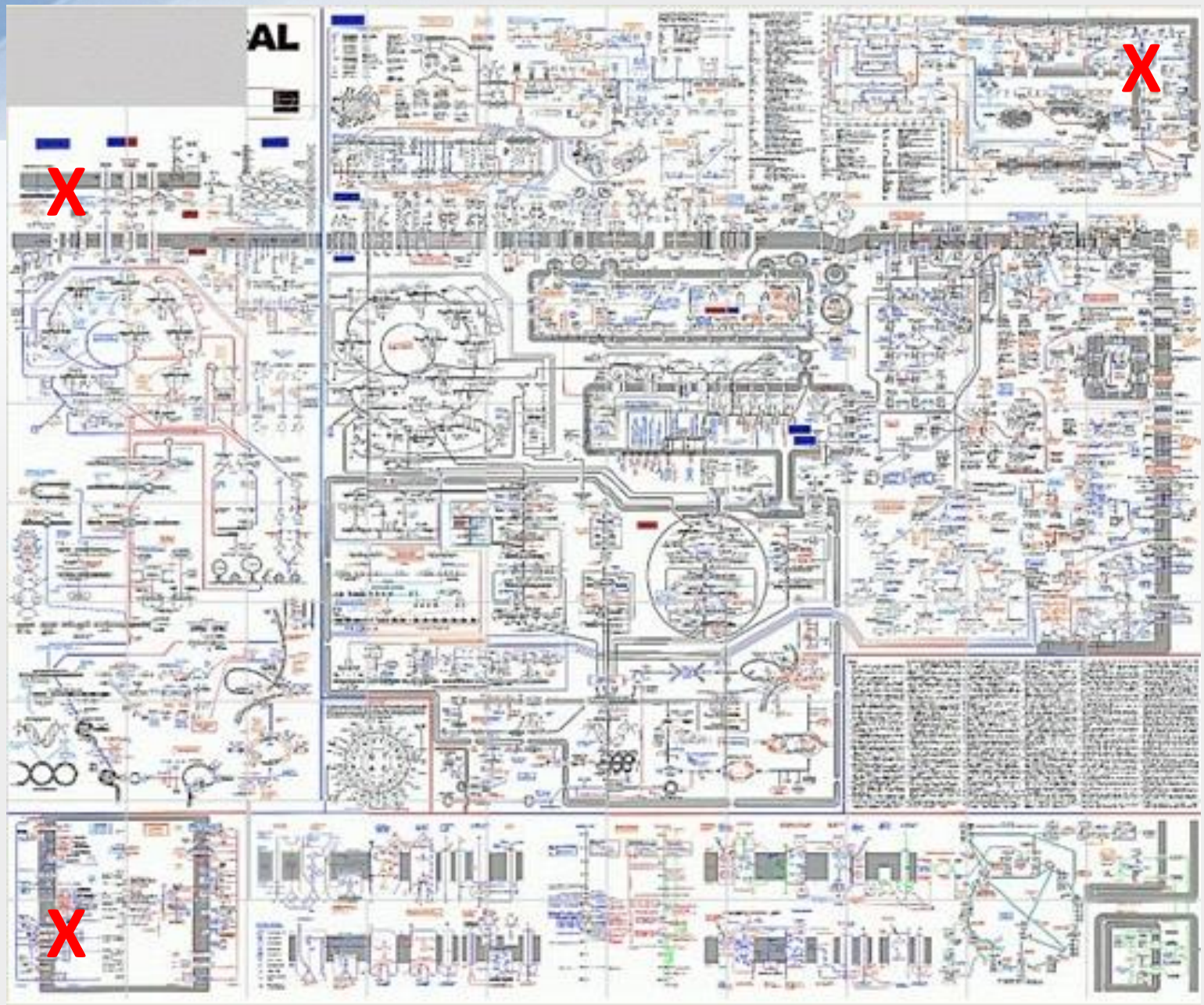
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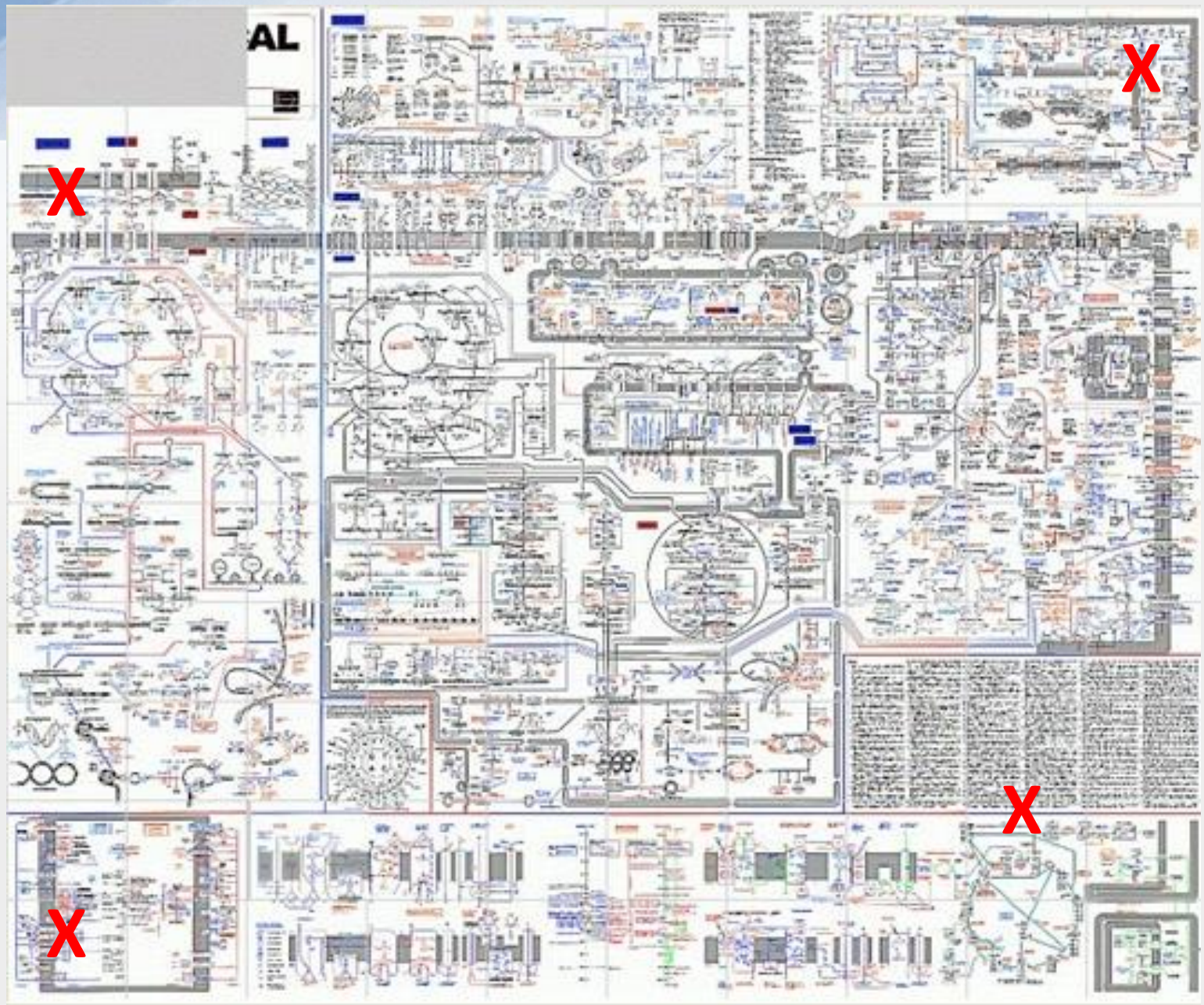
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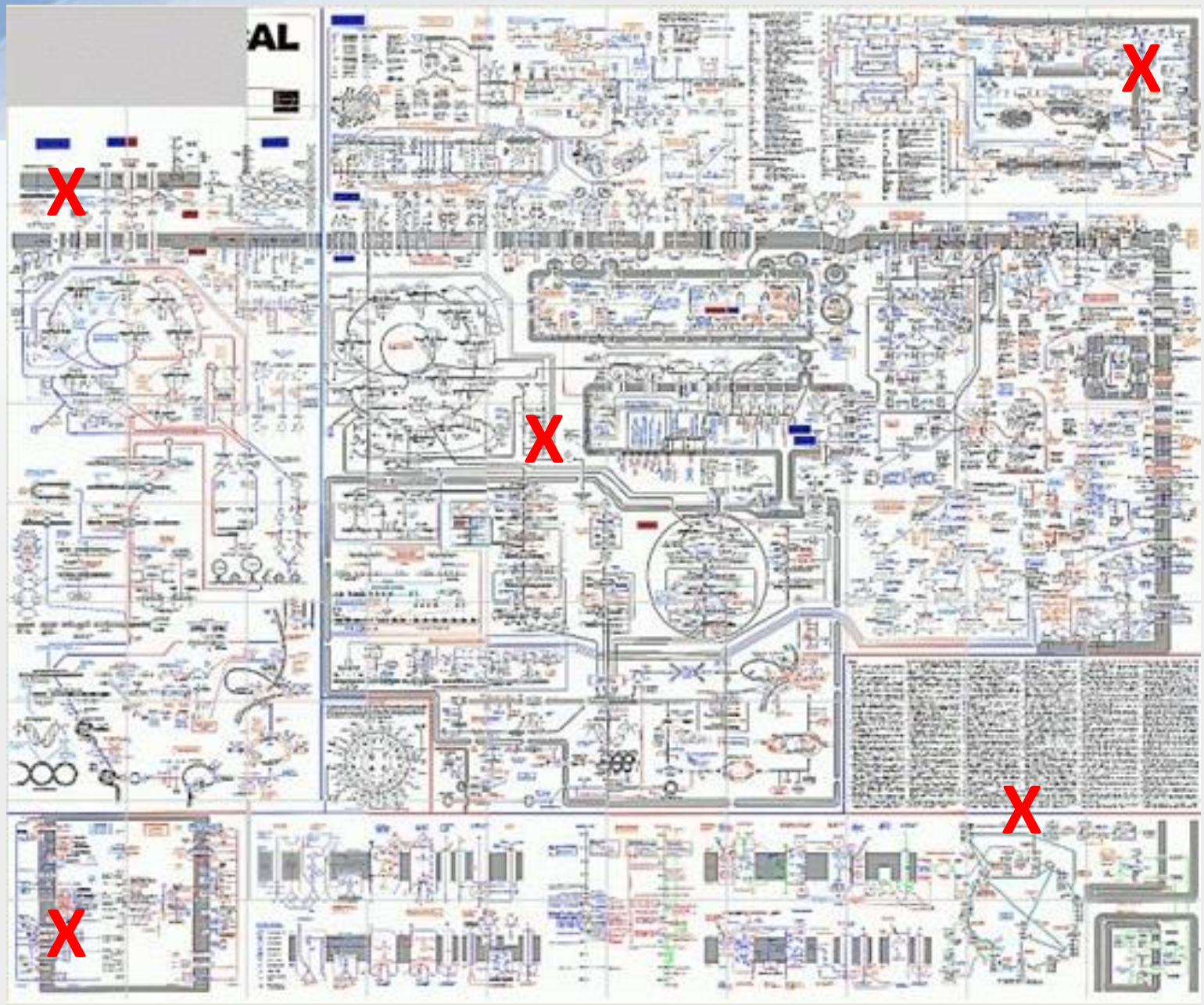
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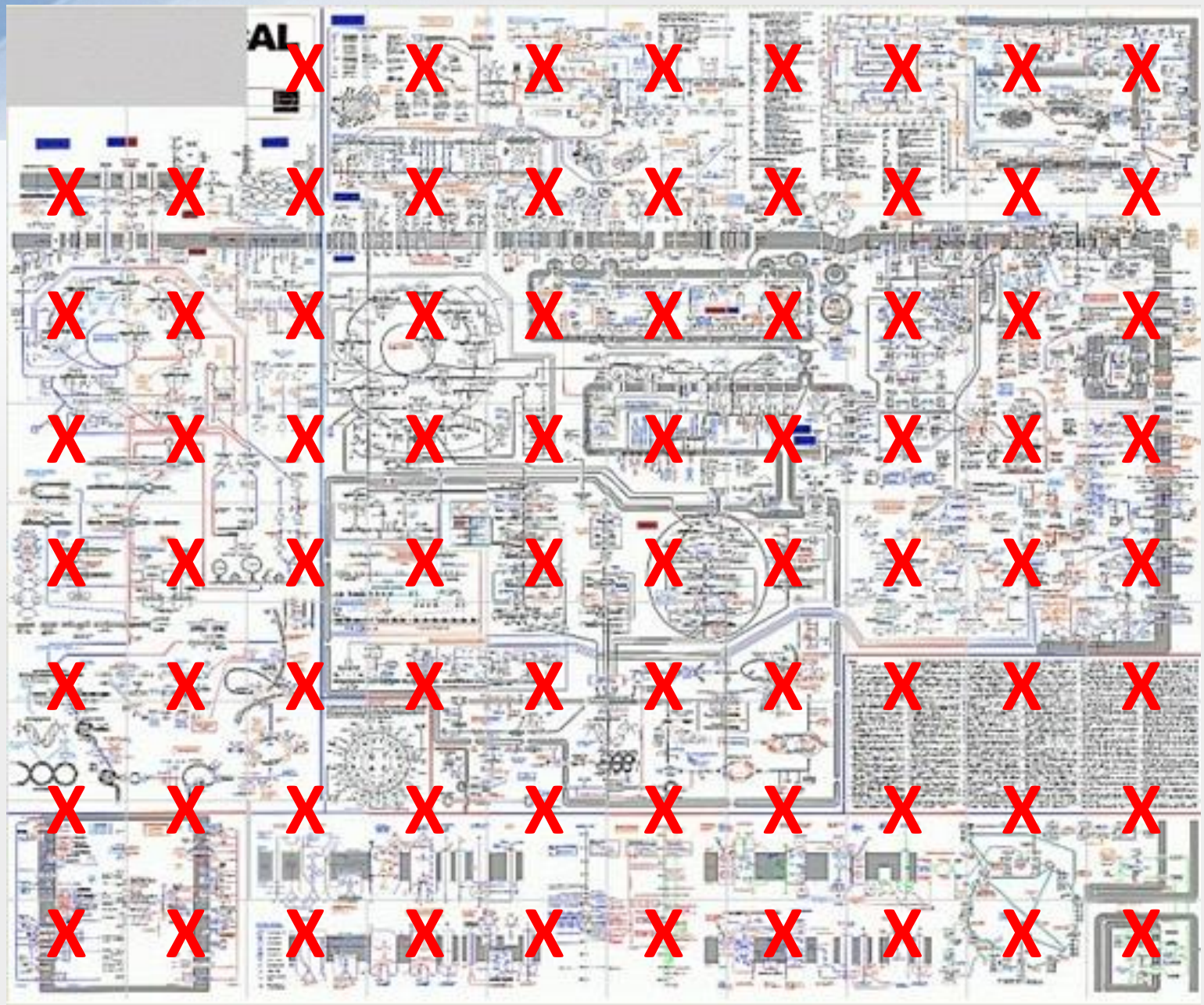
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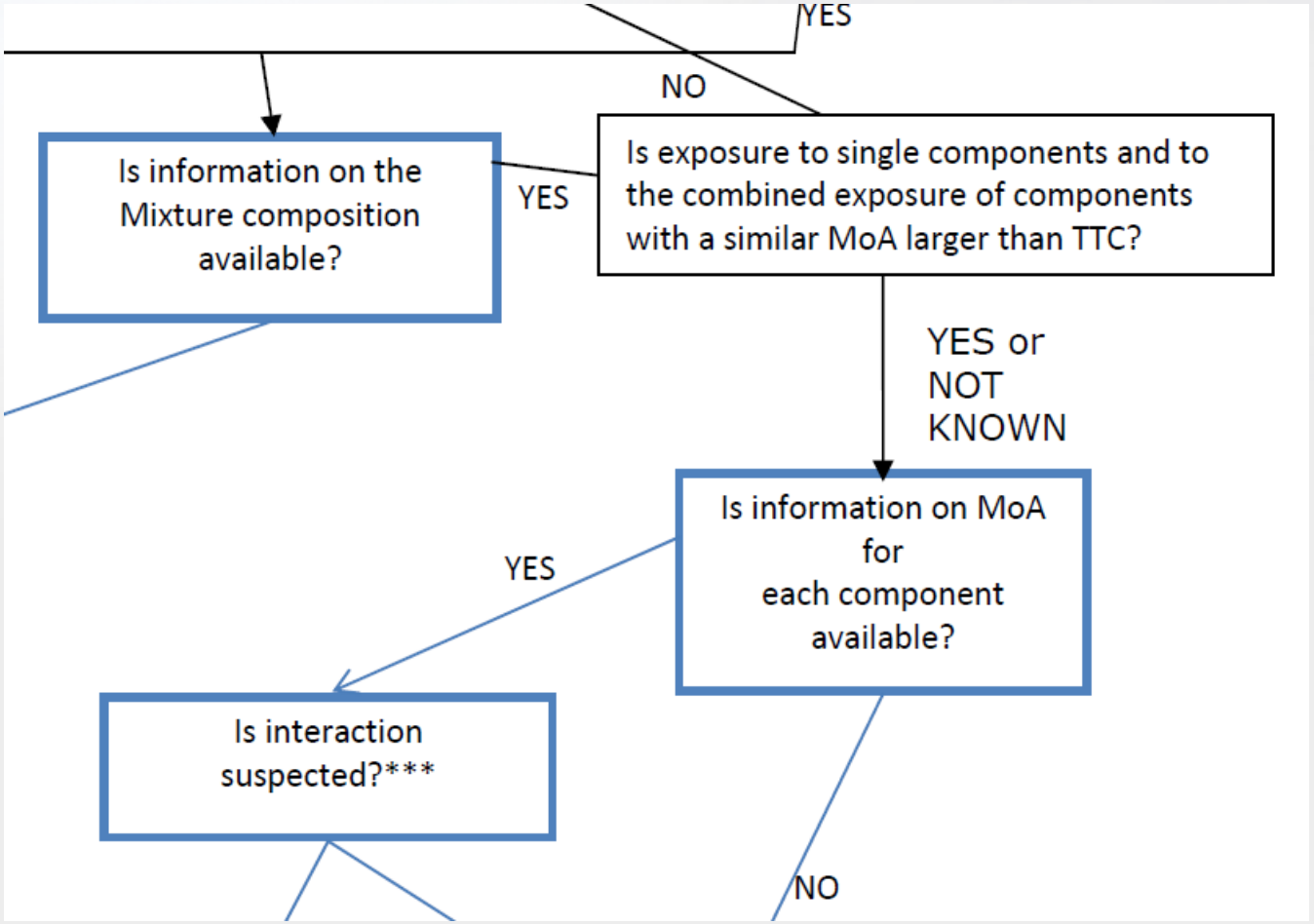
Suggested use of the TTC concept in mixture risk assessment



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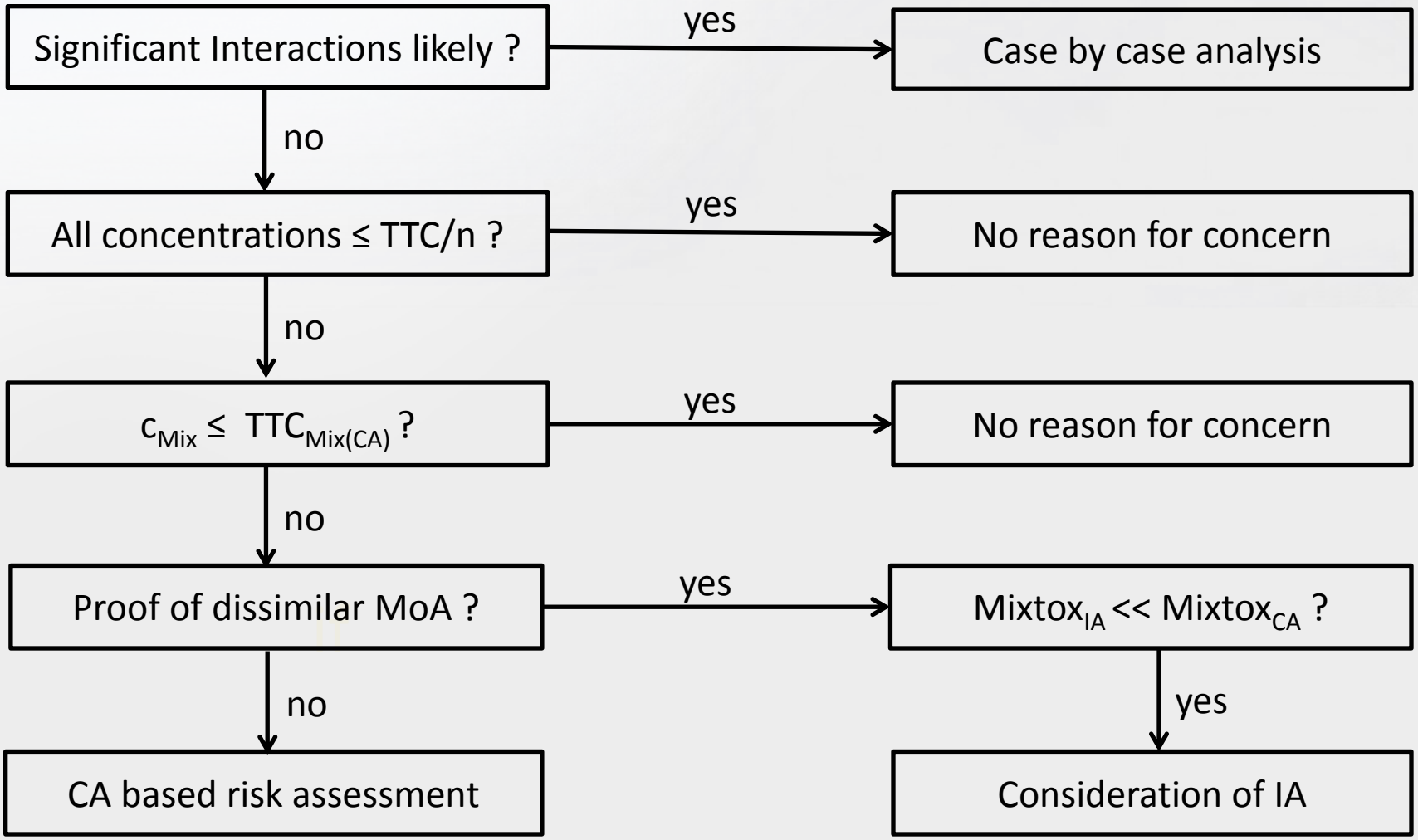
TTC and Concentration Addition

$$TTC_{Mix} = \left(\frac{p_1}{TTC_1} + \frac{p_2}{TTC_2} \right)^{-1}$$

$$E_{TTC} \geq E(Mix)$$

$$\text{if} \quad c_i \leq \left(\frac{TTC_i}{n} \right)$$

Suggested use of TTC for mixture risk assessment

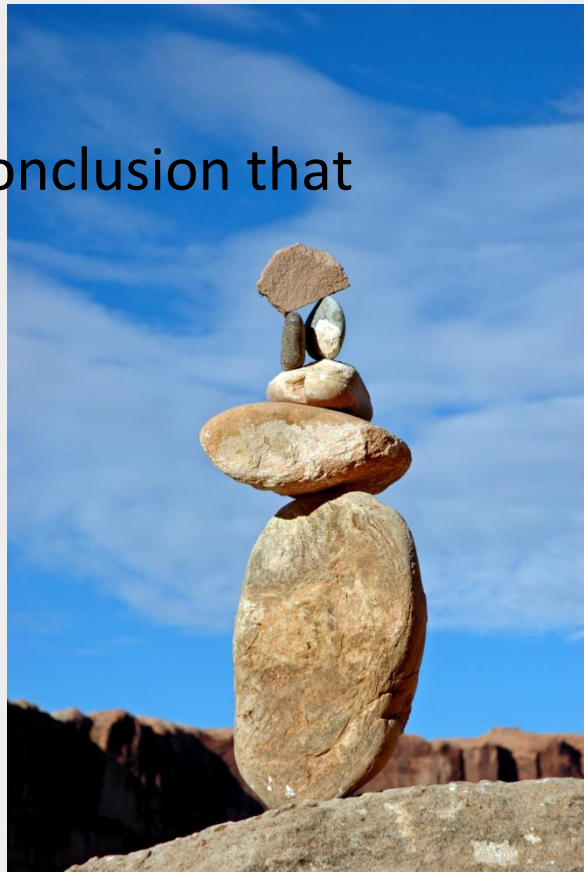


Summary and conclusions

- The use of the lower 5% percentile leads to an inflated misclassification risk for mixtures (misclassification risk = risk that at least one of the compounds has a NOEL/100 < TTC).
- In order to keep the misclassification risk at 5%, the percentile needs to be adjusted accordingly (roughly divided by *n*).

Summary and conclusions

- Mode of Action driven mixture assessments are very data demanding.
- IA is of very limited use
- The application of IA does **not** allow the conclusion that mixture effects are absent at low doses
- Use CA for estimating the mixture TTC (~ TTC/n)
- Incorporation into a tiered approach



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