Mode of action approaches to mixtures

Joop Hermens
Institute for Risk Assessment Sciences
Utrecht University

Mixtures

Is the knowledge sufficient for implementing mixture toxicity in regulations?

- If we don’t take mixture toxicity into account, we can be sure that risks will be underestimated.
- We can simplify the problem of mixture toxicity by including information on the mode of action of chemicals.
- Tools for generic risk assessment (REACH) of mixtures may be different than the tools for site specific risk assessment (WFD).
- Presentation is focused on effects on the environment and on tools/guidance in risk assessment.

Outline

- Mixture toxicity and mode of action
- Non specific toxicity: block approach and total body residues
- Complex cases: complex similar action, synergism, antagonism and multiple mechanisms
- Tools in ecological risk assessment of mixtures

Joint effects based on mode of action (after Plackett and Hewlett)

	A and B act similar	A and B act dissimilar
no interaction between A and B	simple similar action	independent action
interaction between A and B	complex similar action	dependent action

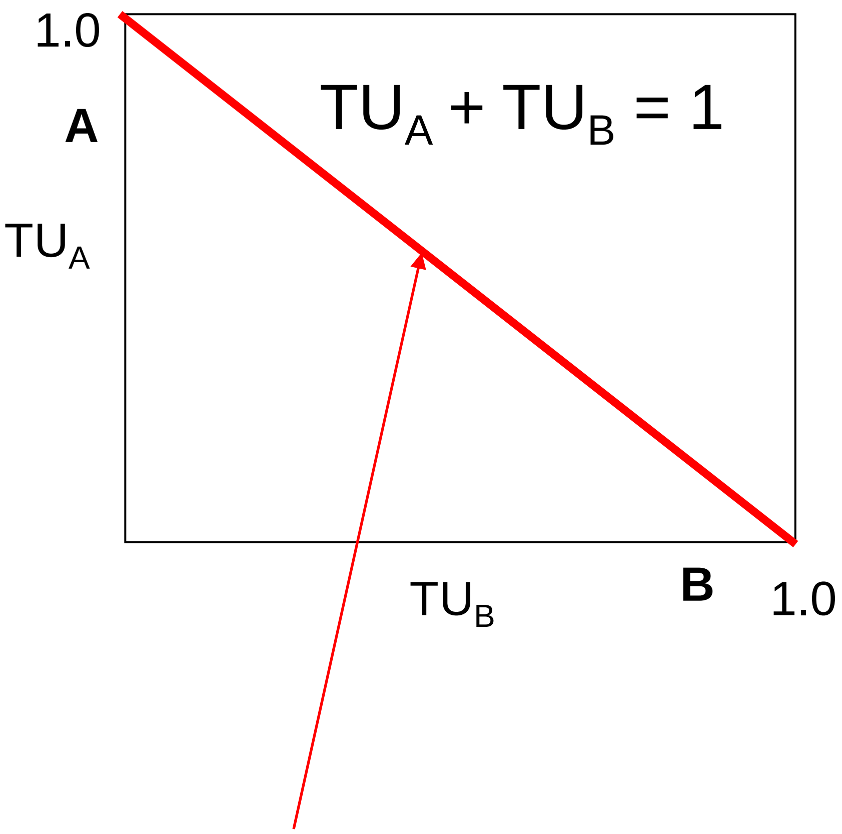
Plackett R.L. and Hewlett , P.S. 1952. Journal of the Royal Statistical Society Series B-Statistical Methodology 14:141-163.

Toxic unit concept

chemical	C	PNEC	C/PNEC
	mg/L	mg/L	TU
A	0.3	0.1	3
B	20	40	0.5

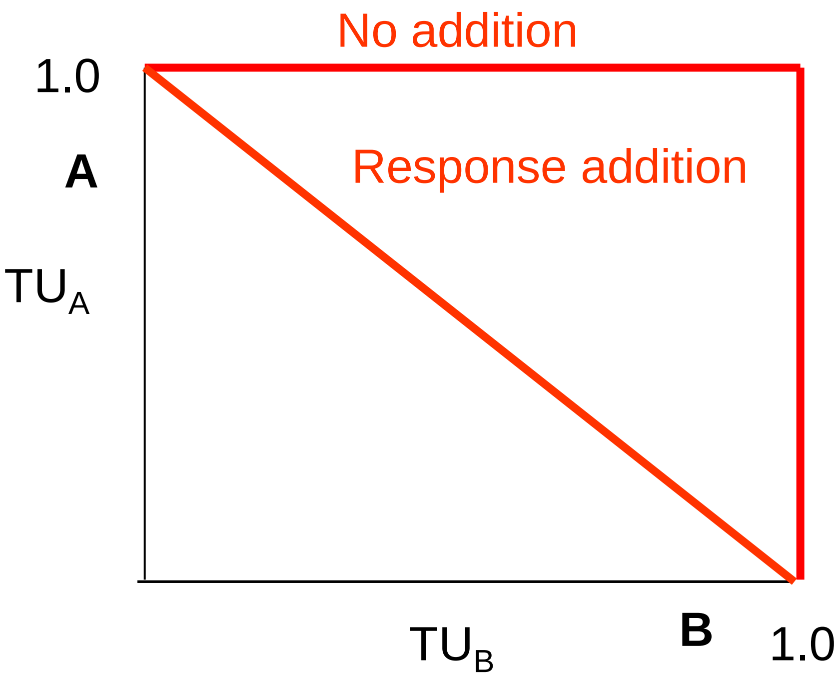
C concentration
PNEC predicted no-effect concentration
TU toxic units

Simple similar action: concentration or dose addition



Chemicals with similar action
Effect in mixture: Dose or concentration addition
Σ TU = 1

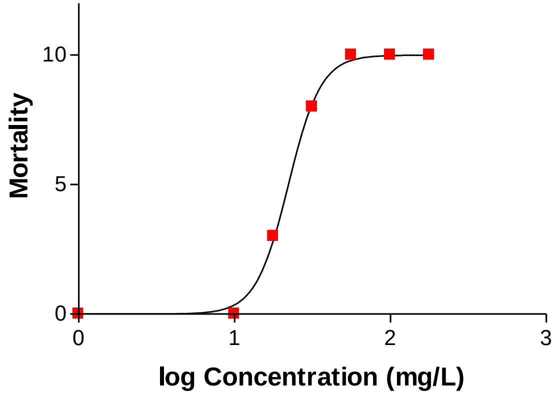
Independent action (chemicals with dissimilar action): response or no-addition



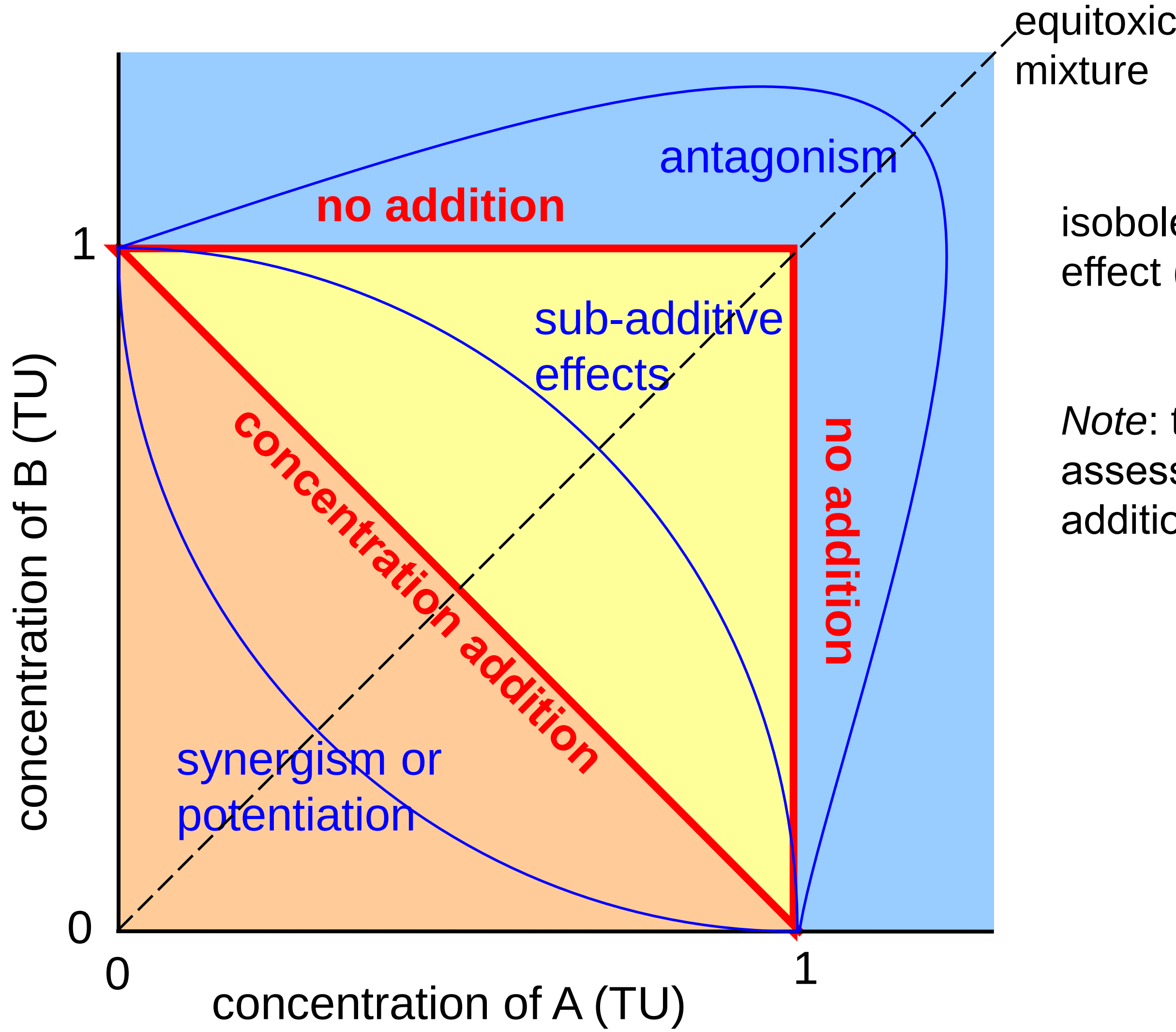
Response of mixture:

No addition:
max { P_A, P_B }

Response addition:
$1-\{(1-P_A)*(1-P_B)\}$
$P_A + P_B$



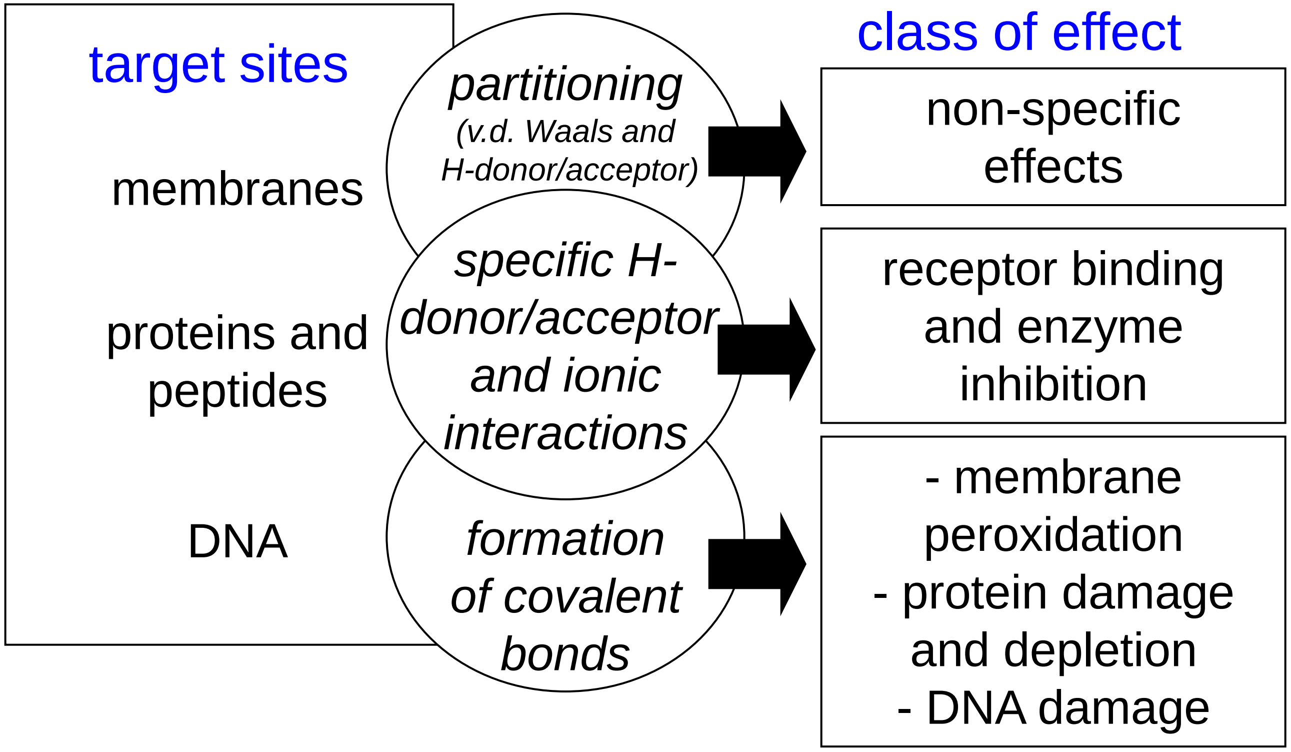
Joint effect of mixture of 2 chemicals



isobole is line of equal toxic effect (equal strength)

Note: the current policy for risk assessment is one of “no addition”

Target sites and toxic mechanisms



Escher, B.I. and Hermens, J.L.M. 2002. Environ. Sci. Technol. 36, 4201-4217.

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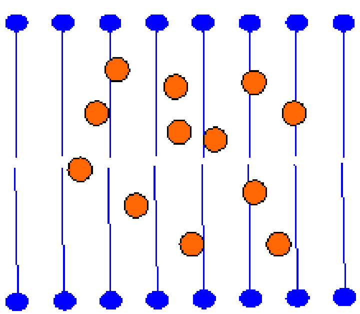
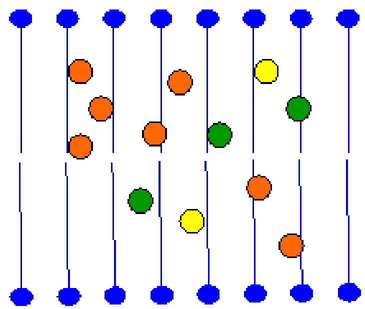
Total target (membrane) concentrations for non specific toxicity: a relevant chemical group parameter

Cell membrane



Single chemical



Mixture of chemicals

Endpoint (in vivo)	Total membrane concentration mmol/kg membrane lipid	Total body residue mmol/kg
Effects on survival	~50	~2.5
Sublethal effects	~5	~0.25
NOEC ecosystem	~0.25	~0.01

Total internal concentrations for non specific toxicity: Body residues of PAHs to an amphipod

Compound	External EC50 28-day ug/L	Internal IEC 28-day umol/g
Naphthalene	1266	5.8
Phenanthrene	95	7.6
Pyrene	79	6.1
PAH mixture		6.1

Landrum, P.F., Lotufob, G.R. Gossiauxa, D.C., Gedeonc, M.L. and. Leed, J.-H. 2003. *Chemosphere* 51, 481-489.

Mixtures of chemicals with non specific toxicity

endpoint	number of chemicals in mixture	LC50 mixture (Σ TU)	Reference
LC50 guppy	50	0.9	Könemann, H. 1981. Toxicology 19, 229-238.
LC50 fathead minnow	23	1.2	Broderius, S. 1985. Aquatic Toxicology, 6, 307-322.
$NOEC_{growth}$ Daphnia	10	1.1	Hermens, J.L.M. et al. 1985. Aquat. Toxicol. 6, 209-217.
EC50 Daphnia	50	Concentrations 500 times lower than E50 still show toxicity in a mixture	Deneer, J.W. et al. 1988. Aquatic Toxicology, 12, 33-38

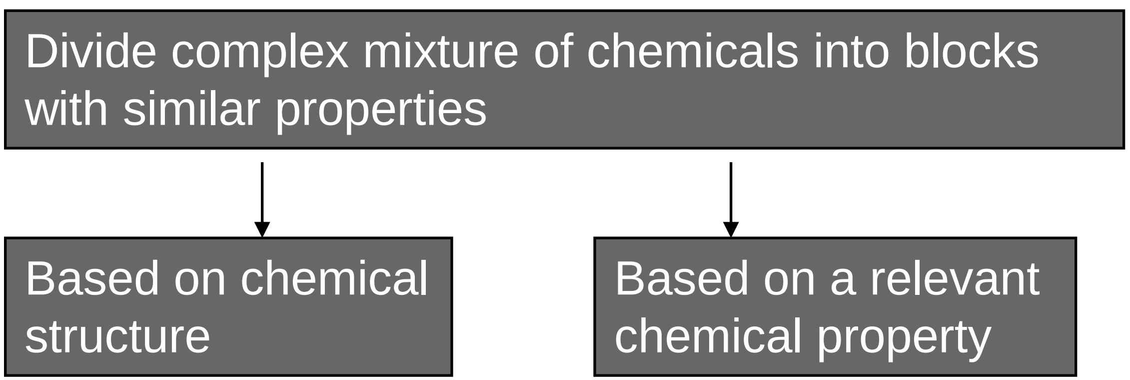
Concentration addition: (Σ TU) = 1.0

Chemicals far below NOEC may still show toxicity in mixtures

Generic risk assessment of mixtures of chemicals with non-specific toxicity: block approach

Example: Petroleum hydrocarbons

Hydrocarbon block approach



Useful approach in risk assessment - REACH

EU - Technical Guidance Document on Risk Assessment, Appendix IX; Environmental Risk Assessment for petroleum substances

Generic risk assessment of mixtures of chemicals with non-specific toxicity

Oil is an example of a UVCB substance: chemical substances of unknown or variable composition, complex reaction products and biological materials.

Other UVCBs: surfactants, ….

Generic risk assessment of mixtures: Block approach based on chemical structure

See:

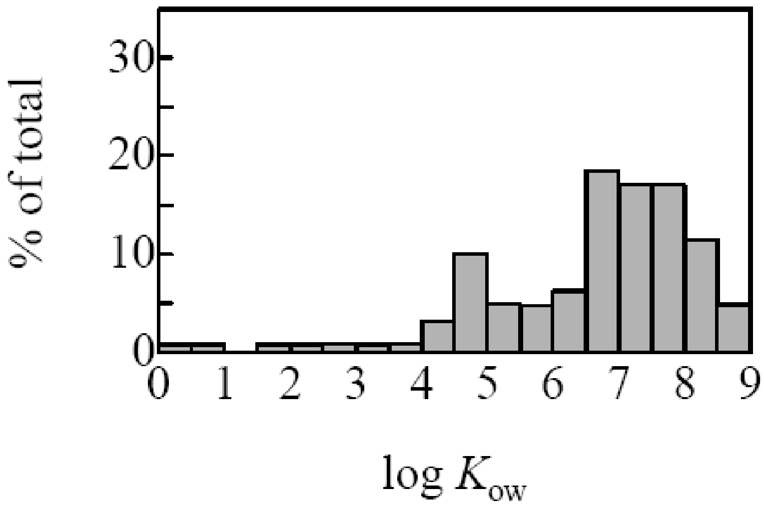
MacLeod, M. et al. 2004. Environ. Sci. Technol. 38, 6225-6233.

Block approach based on a chemical property (experimental): hydrophobicity profiles

Hydrophobicity ~ Octanol water partition coefficient

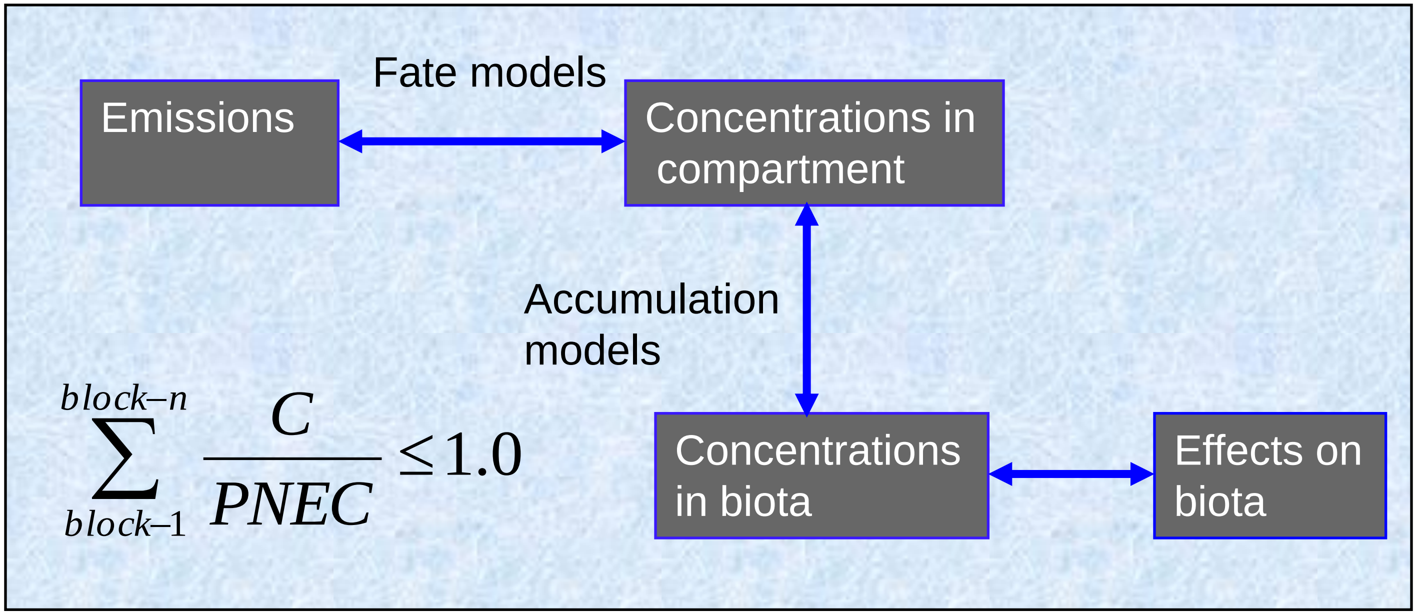
- Retention time on C18 column is related to octanol-water partition coefficients.
- Separation of mixtures on HPLC RP C18 column in fractions.
- Measure total concentrations in fractions.

<u>kerosine</u>



Verbruggen, E.M.J. PhD thesis. Predicting hydrophobicity, bioconcentration and baseline toxicity of complex organic mixtures, 1999.

Block and hydrophobicity profiles as input into models



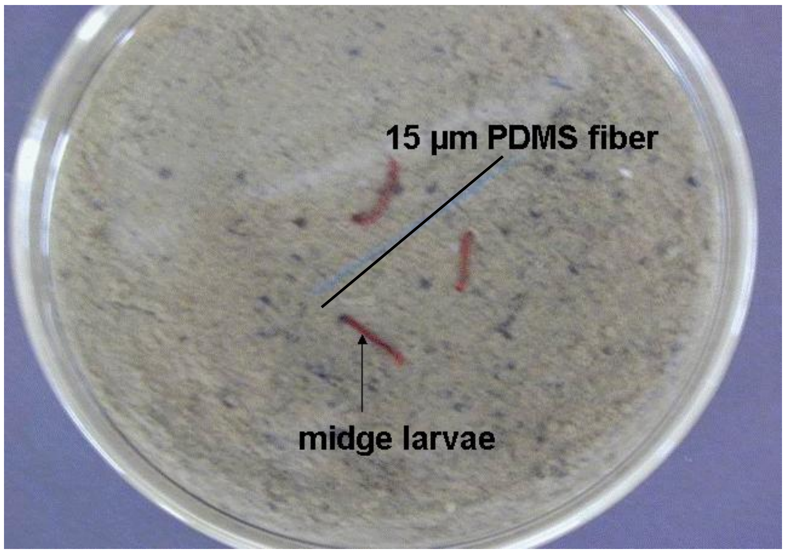
$$\sum_{block-1}^{block-n} \frac{C}{PNEC} \leq 1.0$$

NOEC ecosystem
~ 0.01 mmol/kg

Block approach is promising for UVCB: Petroleum hydrocarbons, surfactants, ….

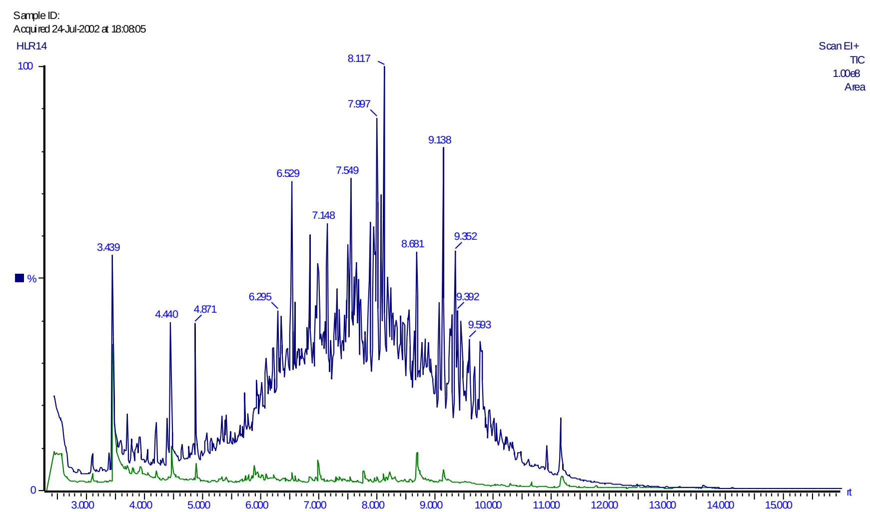
Site specific assessment of mixtures of chemicals with non-specific toxicity: total body residues

Biomimetic extraction



Measurement of

total molar concentration in extract

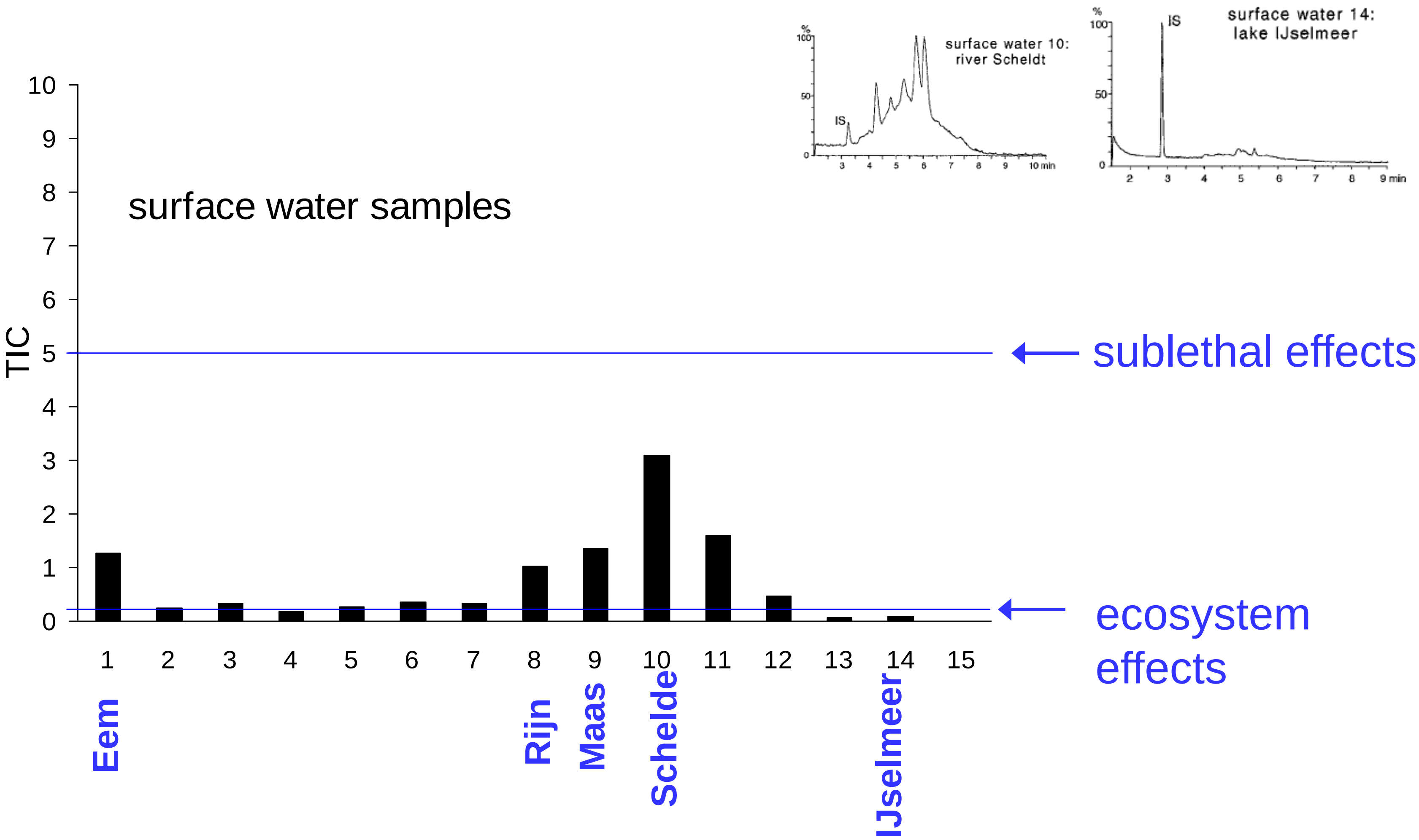


Hydrophobic phase as surrogate for organisms:
- SPME fiber (PDMS, PA)
- empore disk (C18)

Techniques for measuring total concentrations: Vapor pressure osmometry or GC-MS

Verhaar, H.J M. et al. 1995. Environ. Sci. Technol. 29, 726-734.
Parkerton, T.F. et al. 2000. Toxicol. Lett. 112, 273-282.
Van Loon, W.M.G.M.; et al. 1997. Environ. Toxicol. Chem. 16, 1358-1365.
Leslie, H.A. et al., 2002. Environ. Sci. Technol. 36, 5399 -5404.

Total body residues in river water samples (sampler: empore C18 disk)



Van Loon, W.M.G.M. et al. 1997. Environ. Toxicol. Chem. 16, 1358-1365.

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Beyond concentration addition

	A and B act similar	A and B act dissimilar
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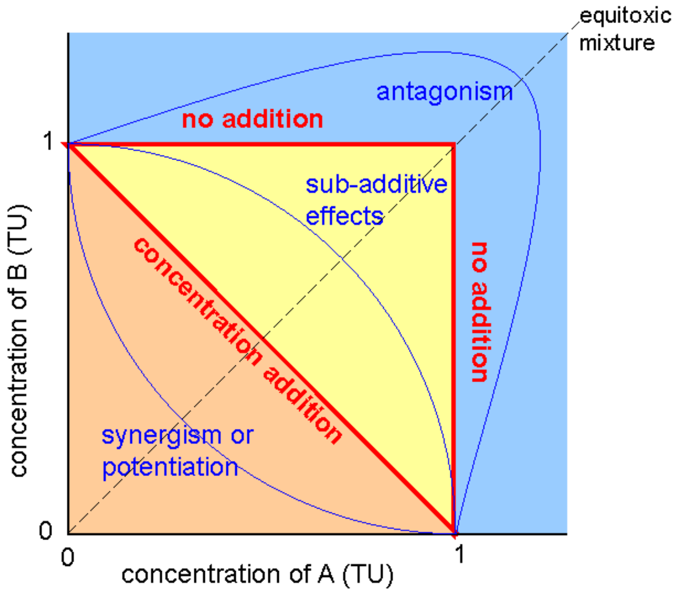
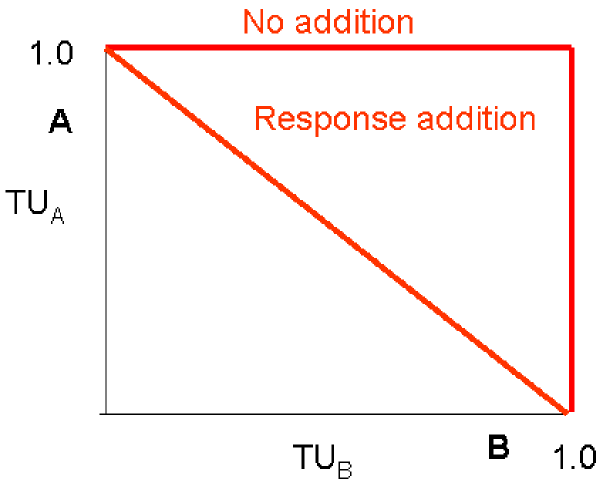


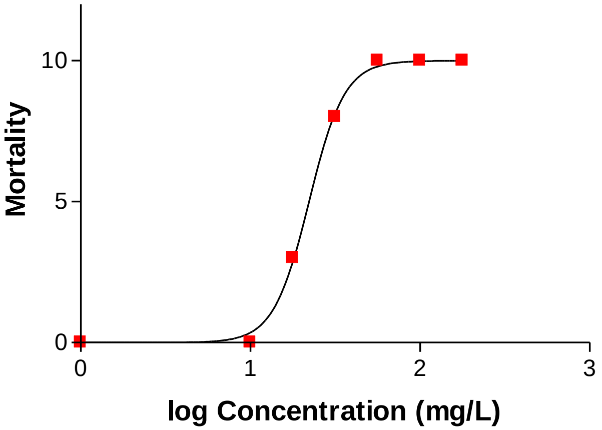
Chemicals with dissimilar modes of action: independent action



Response of mixture:

No addition:
max { P_A, P_B }

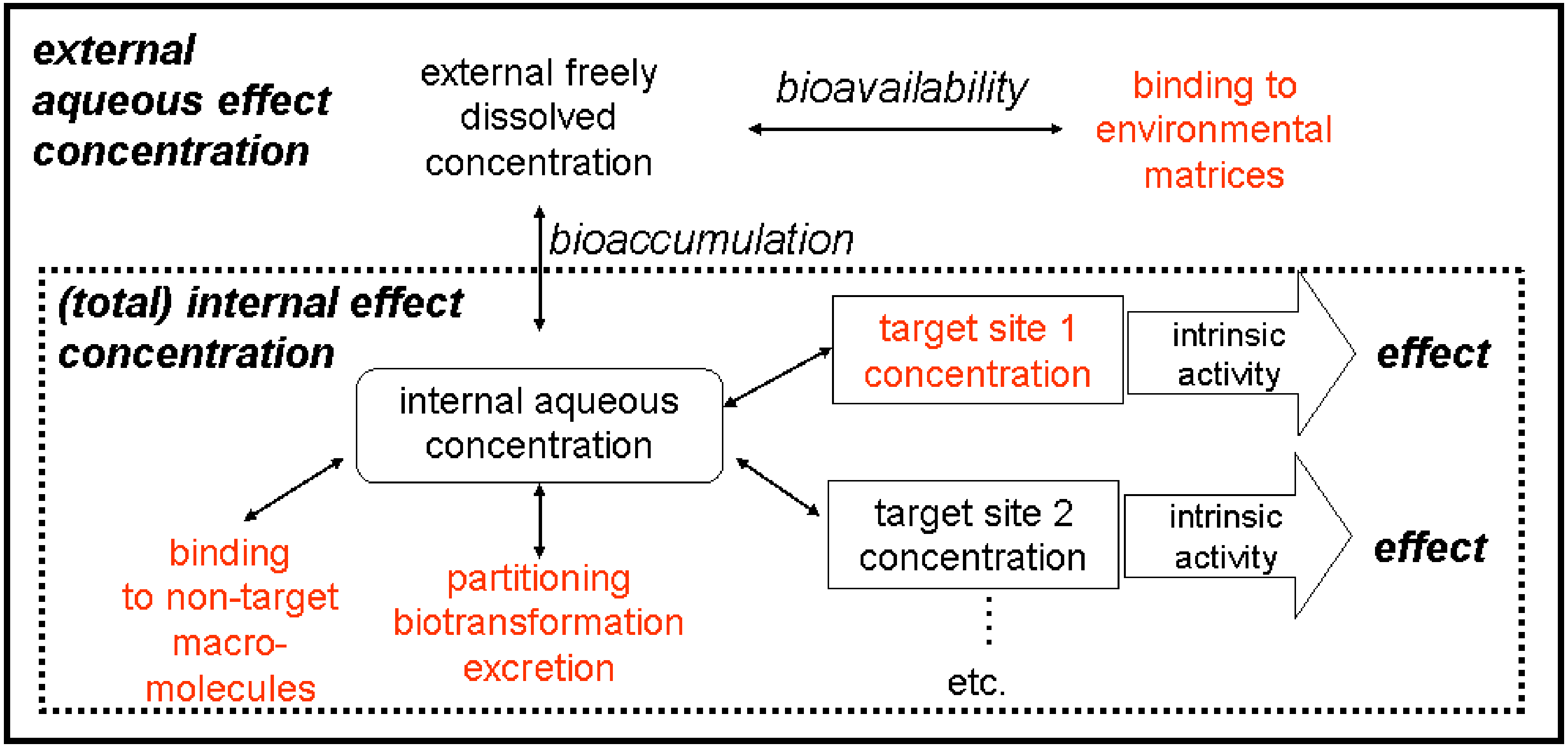
Response addition:
$1-\{(1-P_A)*(1-P_B)\}$
$P_A + P_B$



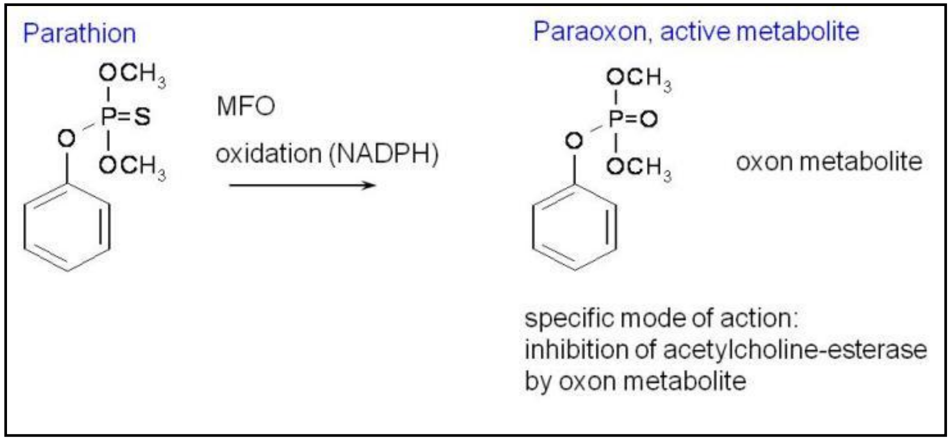
- For prediction of mixture effects dose-response curve are needed.
- If C < NOEC: no contribution to effects in mixture.

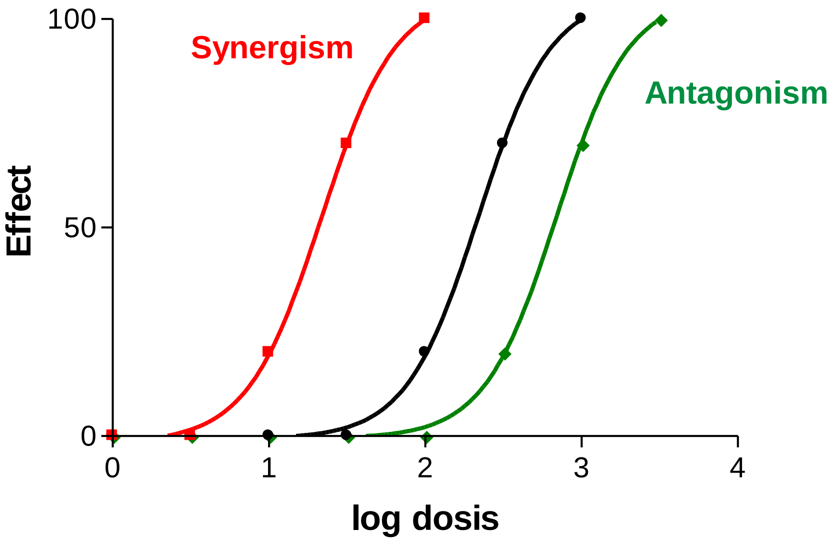
Example of independent action: mixture of 16 compounds with a different mode of action in an algal assay, see Grimme, H. Altenburger, R. Backhaus, Faust, M., Bödeker, W. Scholze, 2000, UWSF-Z. Umweltchem. Ökotox. 12(4), 226-234

Complex similar action (competition), synergism and antagonism



Synergism / antagonism at level of biotransformation: example of organophosphate insecticides

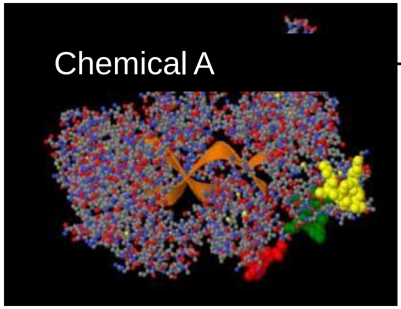




Complex similar action (competition) at level serum protein binding

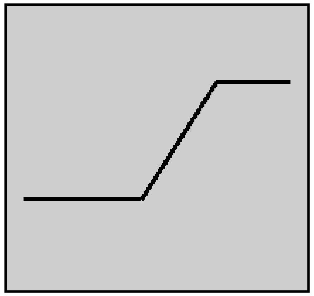
Protein binding: adsorption process - saturation

Protein bound



Chemical B

Free concentration of A in serum



Synergistic effects with drug intake (human)

In general: competition may occur in adsorption processes resulting in unexpected observations

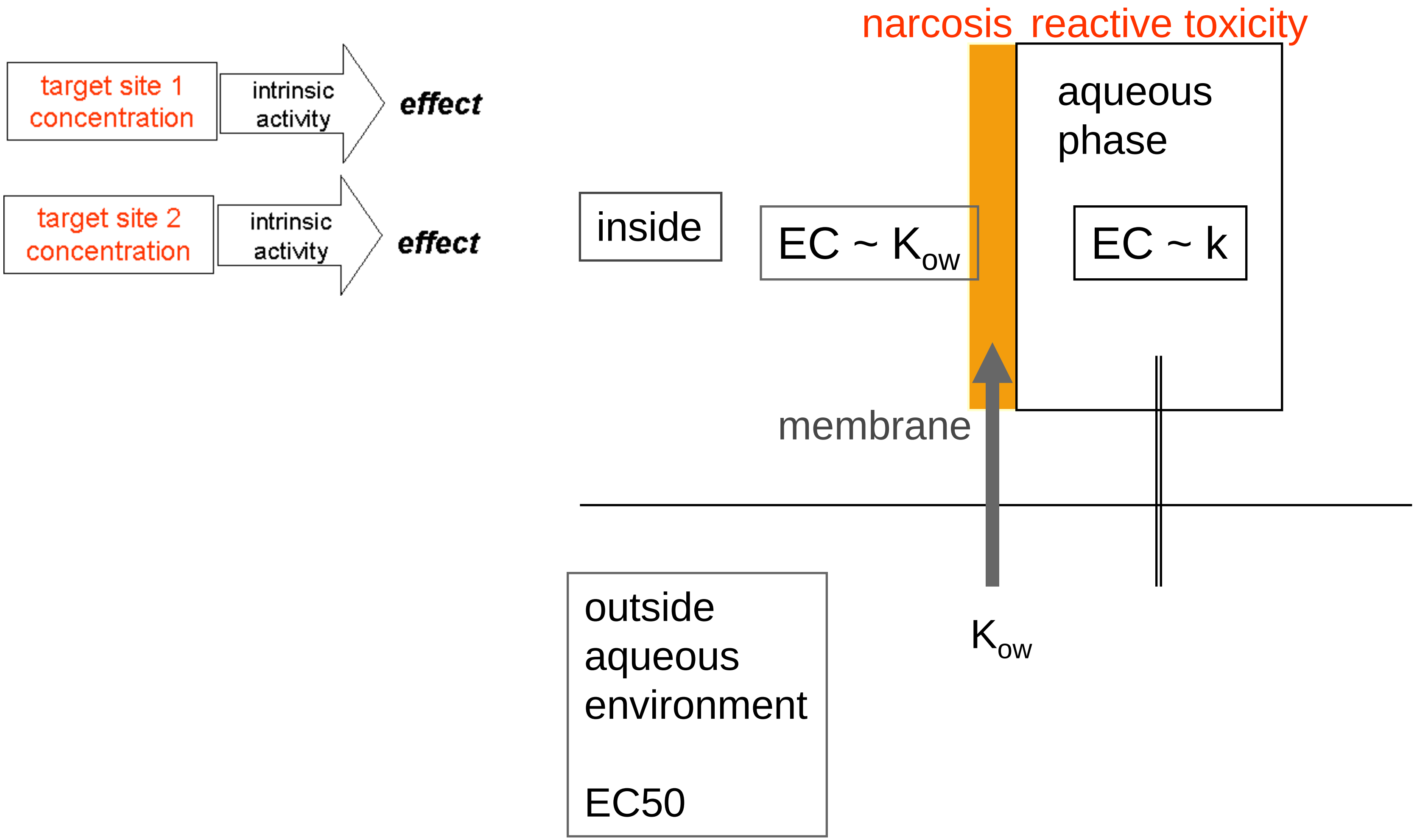
Complex similar action (competition) at level of sediment sorption

Example of competitive sorption to sediment of alcohol ethoxylates (surfactants): $C_n(EO)_m$

Droge, S.J. et al. 2009. Environ. Sci. Technol. 43, 5712-5718.

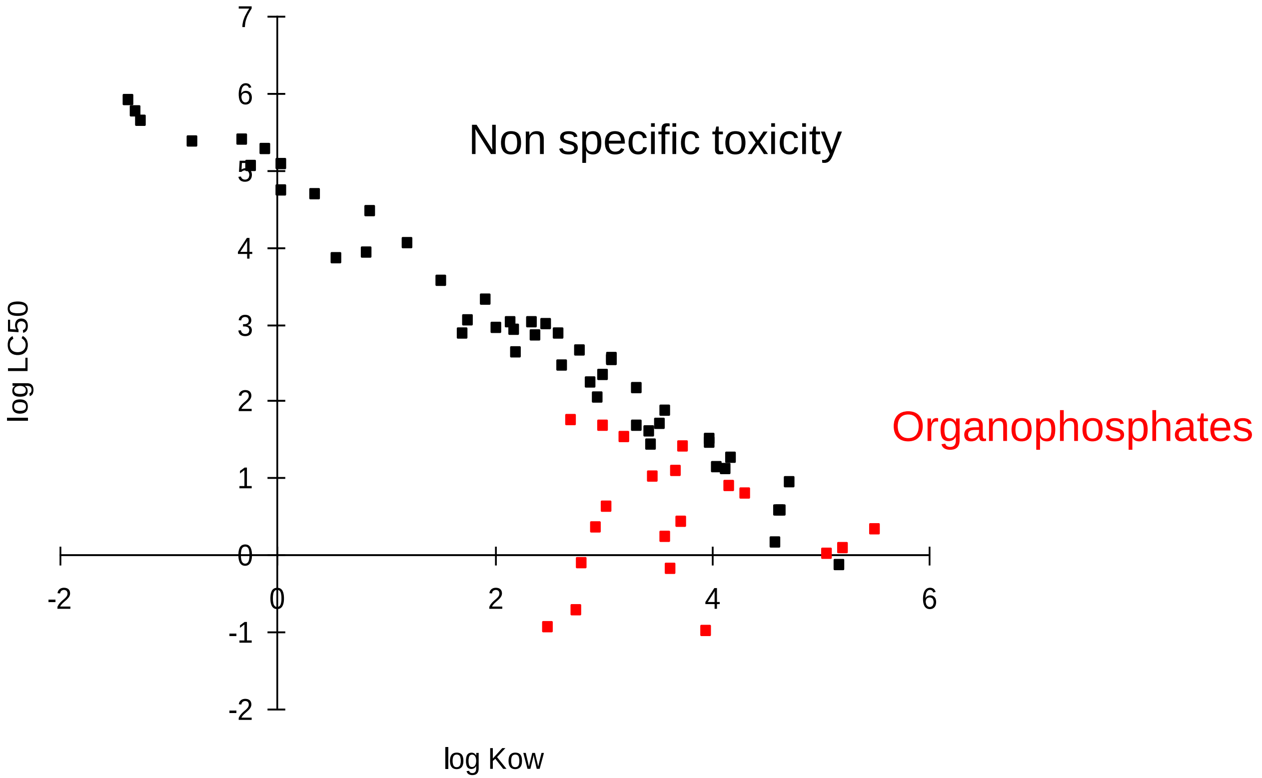
Droge, S.J. and Hermens, J.L.M. 2010. Environ. Pollut. 158, 3116-3122.

Chemicals may act via multiple mechanisms: example organophosphates



Organophosphates: specific, but also non specific toxicity

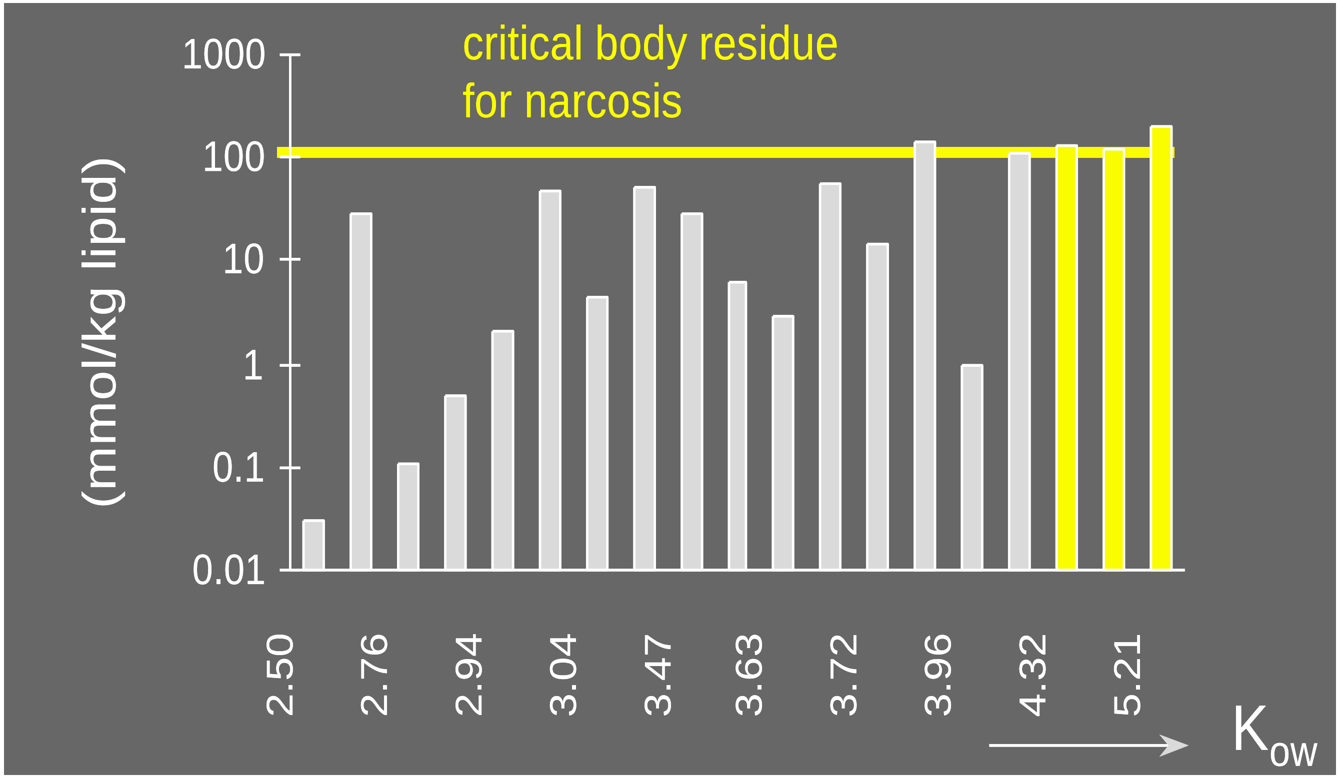
Fish LC50 versus K_{ow}



data from Hermens et al. 1987 and de Bruijn et al. 1991

Organophosphates: specific, but also non specific toxicity

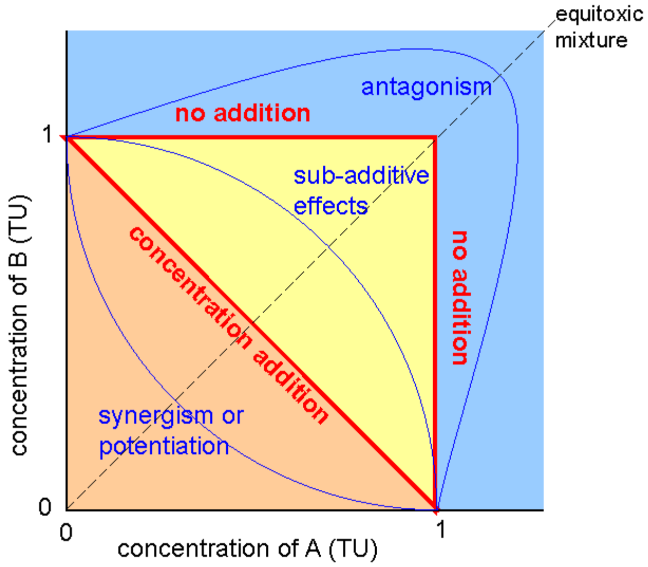
Estimated body residues at LC50



- Some organophosphates act only via non specific toxicity.
- Each organic chemical will contribute to “total” non specific toxicity.

Freidig, A.P. et al. 2000. Quant. Struct. Act. Relat. 19, 547-553.

Complex similar action (competition), synergism and antagonism: consequences for risk assessment?



- Synergism, antagonism and complex similar action do occur.
- Scientifically very interesting cases.
- Difficult to predict.
- Are these rare cases?

Outline

- Mixture toxicity and mode of action
- Non specific toxicity: block approach and total body residues
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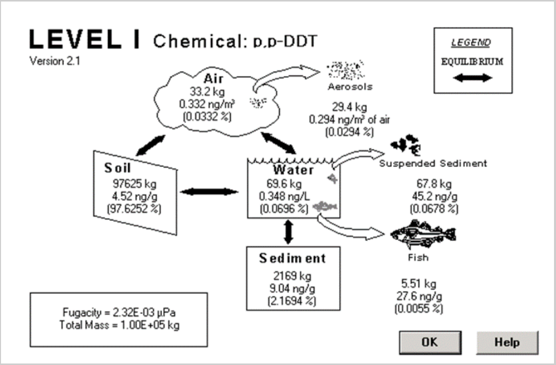
- Tools in ecological risk assessment of mixtures

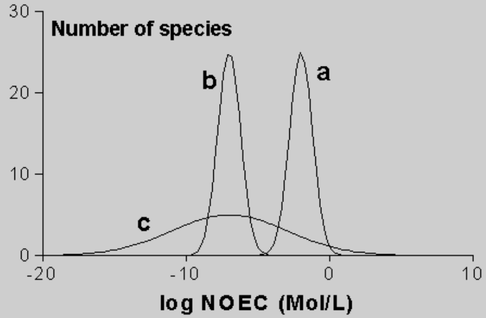
Ecological Risk Assessment: Risk = PEC/PNEC ratio (quantitative)

Exposure assessment



PEC:
Predicted
Environmental
Concentration

Effect assessment

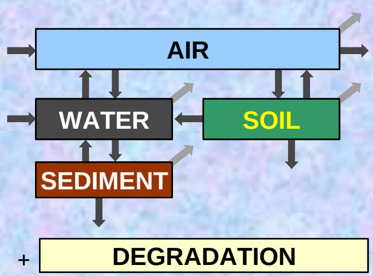


PNEC:
Predicted
No-observed Effect
Concentration

Generic risk assessment of chemical products (UVCB substance) – REACH

Generic risk assessment
of chemicals and products

PEC



PNEC

Block approaches

$$\sum_{block-1}^{block-n} \frac{C}{PNEC} \leq 1.0$$

Products of mixtures of chemicals with similar action

Guidance:

concentration addition: $\sum \frac{C}{PNEC} \leq 1.0$

Tools:

- Total body residues
- Block approach

Site specific ecological risk assessment of mixtures - WFD

WFD: Water Framework Directive

Site specific risk assessment

- PEC-PNEC ratio for individual contaminants (priority chemicals)
- TEF concept

$$\sum \frac{C}{PNEC} \leq 1.0$$

- Effect studies, incl. field studies

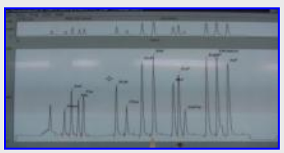
- Chemical group parameters
- Biomarkers
- Effect parameters at cellular level

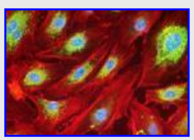
- Toxicity identification evaluation
- Effect directed analysis

Group parameters for estimating effects of mixtures based on mode of action

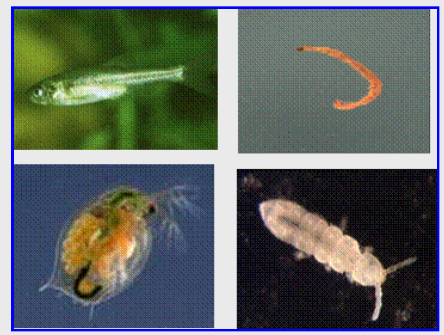
Mode of action

Chemical group parameters
Biomarkers (simple biochemical parameters)
Effect parameters *in vitro*





In vivo effect parameters in risk assessment (whole organism)



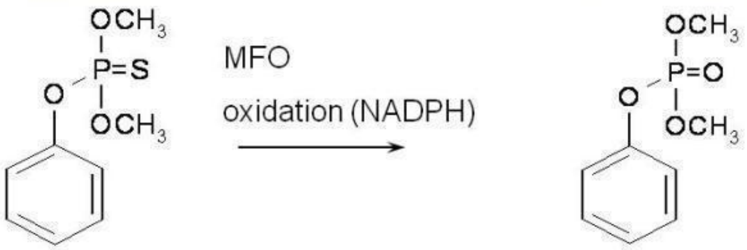
Non specific toxicity
Effects on endocrine system
Enzyme inhibition or induction
Specific receptor interactions

etc.

Specific toxicity of organophosphates

Parathion

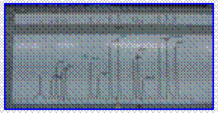
Paraoxon, active metabolite

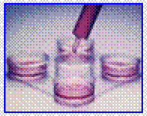


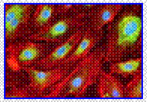
oxon metabolite

specific mode of action:
inhibition of acetylcholine-esterase
by oxon metabolite

Chemical group parameters
Biomarkers (simple biochemical parameters)
Effect parameters *in vitro*

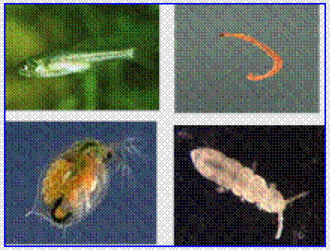






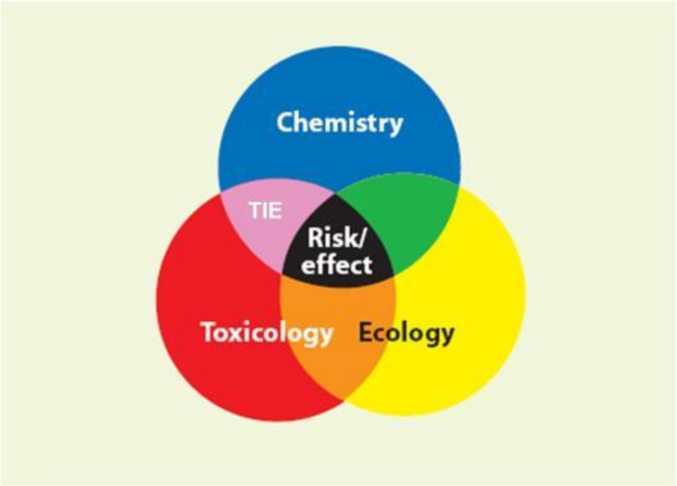
X

In vivo effect parameters
in risk assessment
(whole organism)



No simple parameter that can predict *in vivo* effects at whole organism level

Site specific risk assessment: WFD



1. If major contaminants and concentrations are known:

- Concentration addition for chemicals with similar modes of action.

$$\sum \frac{C}{PNEC} \leq 1.0$$

- Response addition for other combinations.

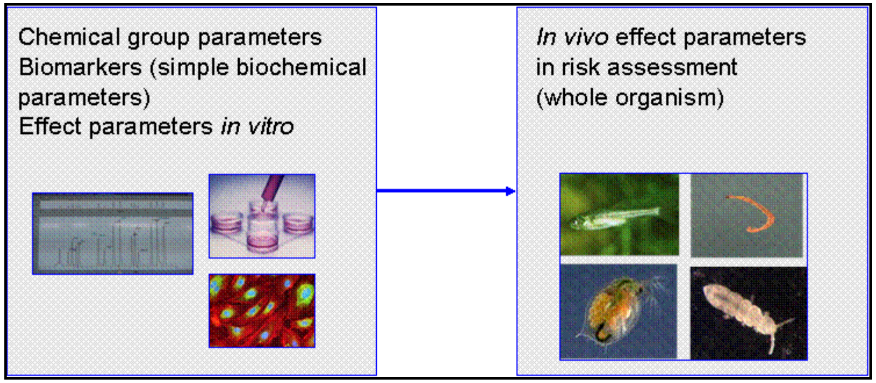
$1\text{-}\{(1\text{-}P_A)*(1\text{-}P_B)\}$

2. Unknown composition

- Bioassays (*in vivo*) and ecology as warning.
- Combination of toxicology (*in vivo, in vitro*) and chemistry.

Questions / challenges

- Further development of block approaches.
- Are there alternatives to the classical PEC/PNEC (*in vivo*) approach? Can we develop quality criteria for *in vitro* effects?
- Calibration of *in vitro* effects to *in vivo* effects.
- Mechanistic studies into complex similar action (competition, synergism and antagonism).
- Further development of effect directed analysis.
- Development of specific risk assessment procedures for mixtures.



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