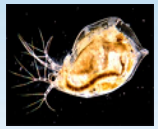

Modes of action in ecotoxicology

What can we learn for toxicant detection?

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National Hydraulics and
Environment Laboratory – EDF R&D

AquaLife 2012

EDF R&D

Content

Introduction – Why should we care about modes of action?

Classification of modes of action:

- Narcosis / Baseline Toxicity
- Reactivity
- Specific modes of action

Joint action of several compounds (mixtures)

- Is mixture toxicity predictable?
- Are mixture effects relevant in surface waters?
- Implications for toxicant detection in toximeter

Introduction

Compounds differ with respect to detectable effects in the toximeter

- ratios between alarm thresholds and EC50 values
- alarm onset times
- response patterns
  - immobilisation
  - excitation

➔**This is not surprising as toxic compounds can interact with the organism in various ways .**

The mode of action describes the interaction of the compounds with the organism.

Introduction – Why care for modes of action?

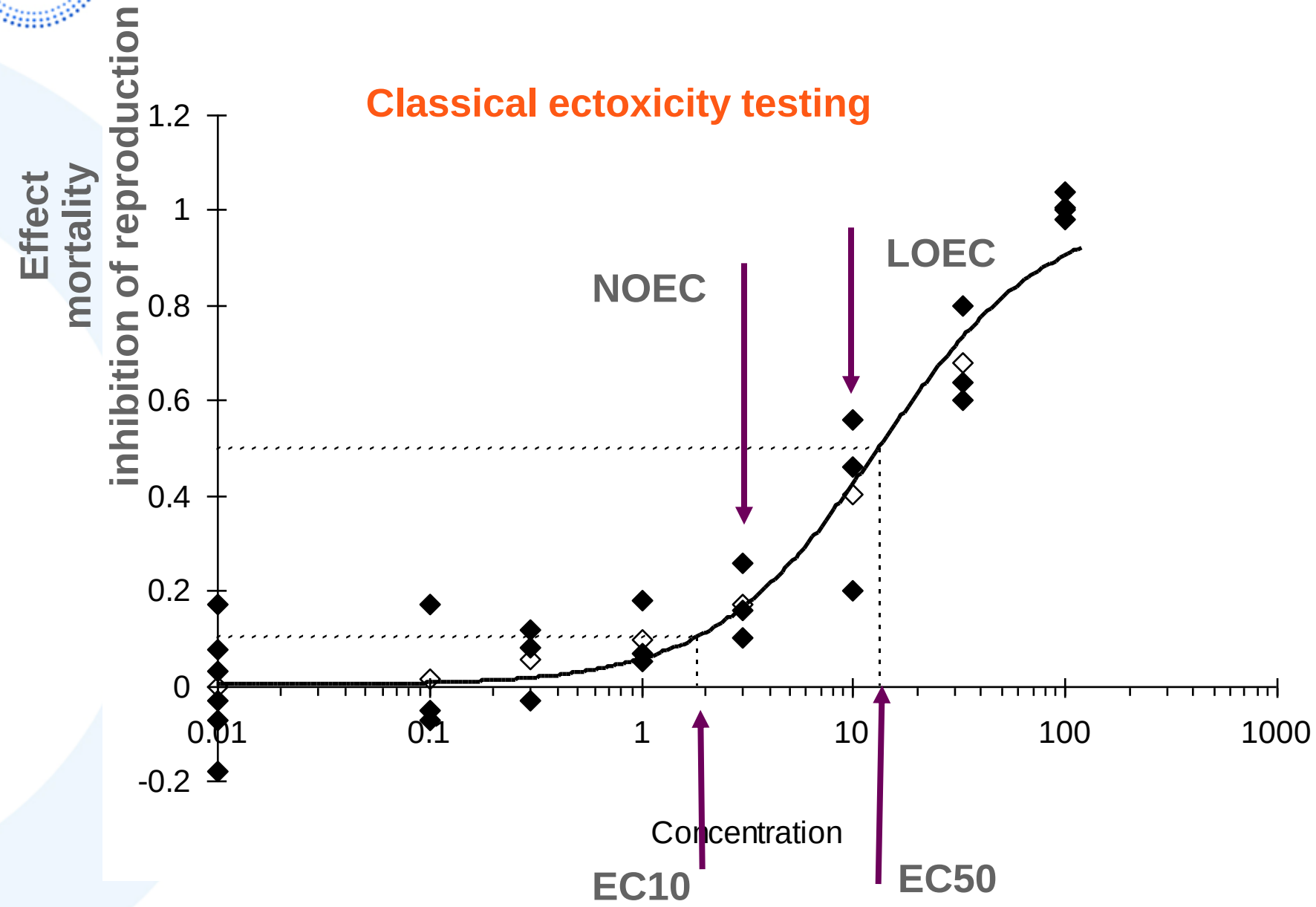
- Understanding why a substance is toxic.
- The mode of action is essentiel for
  - inter-species extrapolation
  - toxicity prediction from chemical structure
    - Quantitative (Qualitative) Structure Activity Relationship (QSAR)
  - mixture toxicity prediction
    - Concentration Addition (CA) for compounds with similar modes of action
    - Independent Action (IA) for compounds with dissimilar modes of action
  - detectabilty of compounds in the toximeter?

Introduction

Everything is a poison. It is the dose that makes things non toxic.

Paracelsus (1520?)

From exposure to effect



What type of effects can be measured?

Every compound can cause death (depending on the dose c.f. Paracelsus)

But does every compound induce behavioural changes?

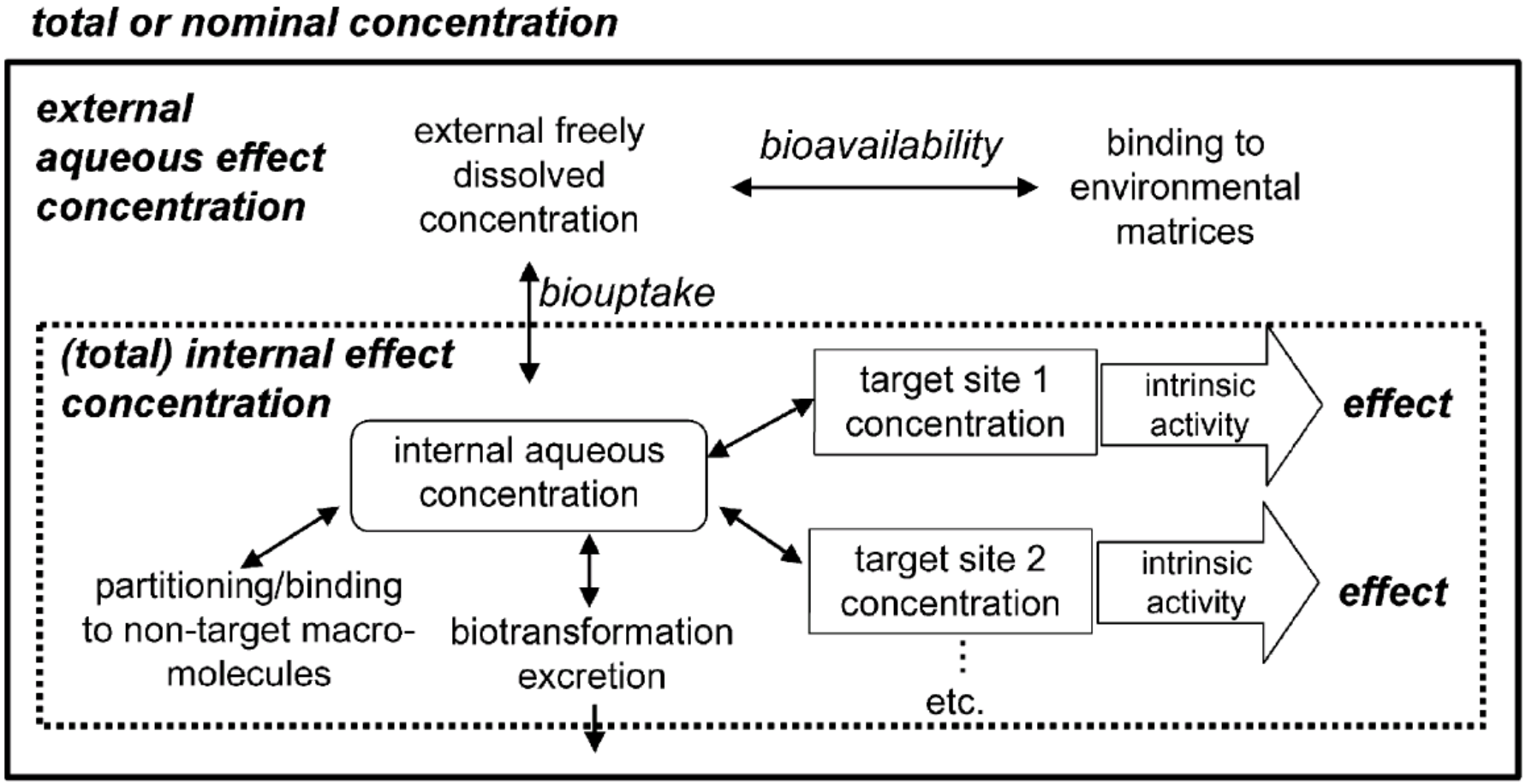
Aim: quantify the sensitivity of the toximeter

- understand how sensitivity can be generalised
- analyse and potentially predict sensitivity of toximeter towards specific compounds
  - alarm threshold << EC50
  - alarm threshold ≈ EC50

Sensitivity is not a universal feature -

anecdotal evidence ➔ mechanistic understanding

From exposure to effect



Introduction – Terminologie

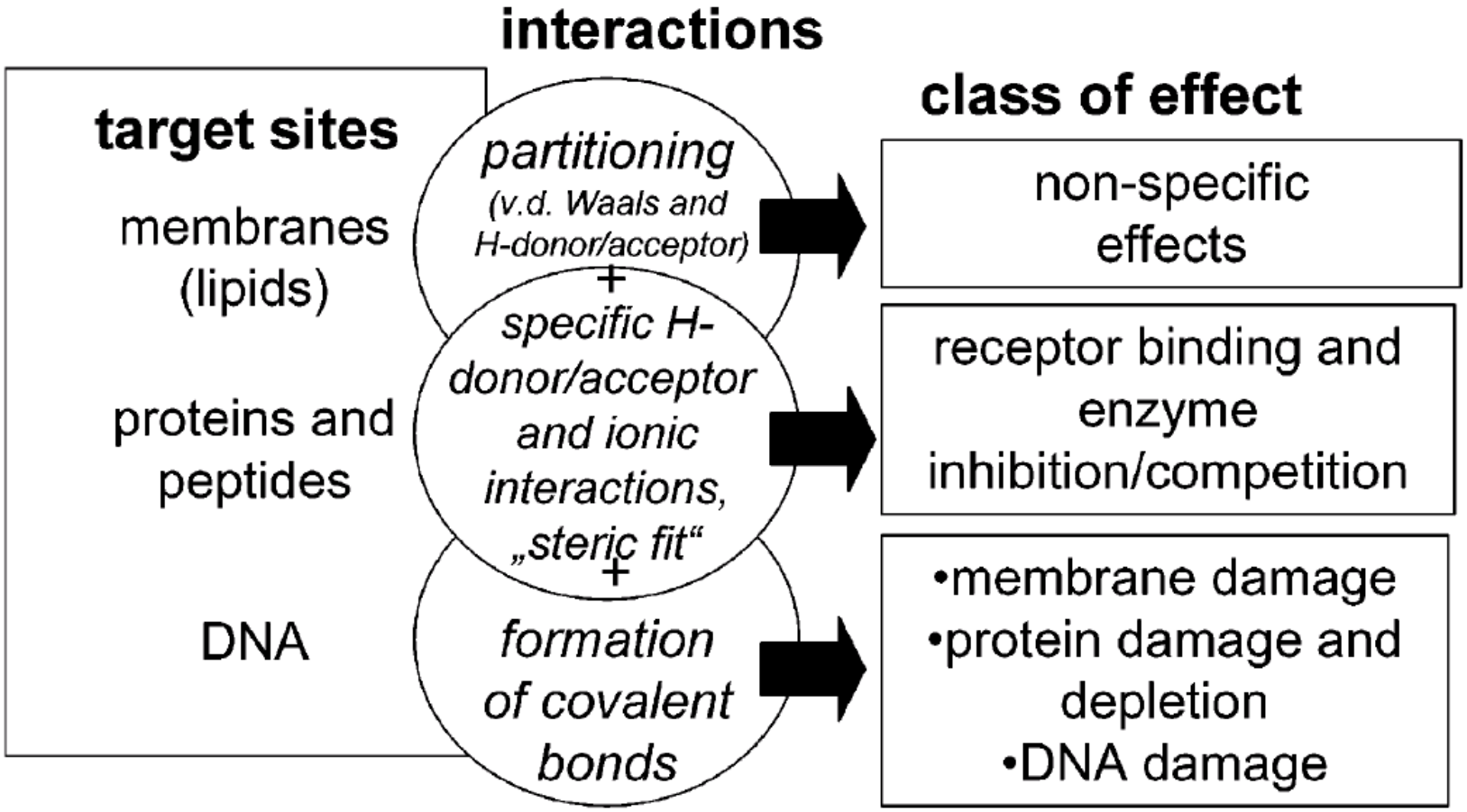


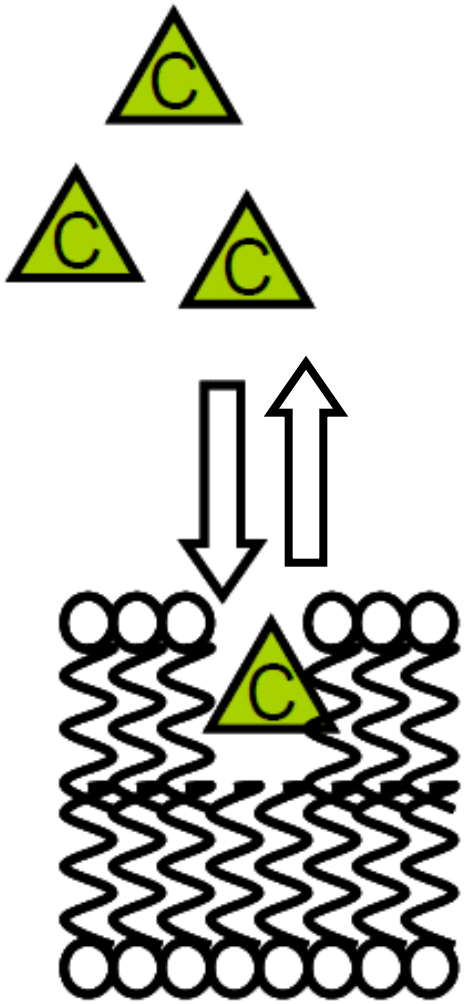
FIGURE 2. Rationale behind the classification of chemicals according to mechanism: target sites and type of interaction.

Non-Specific Effects - Baseline Toxicity

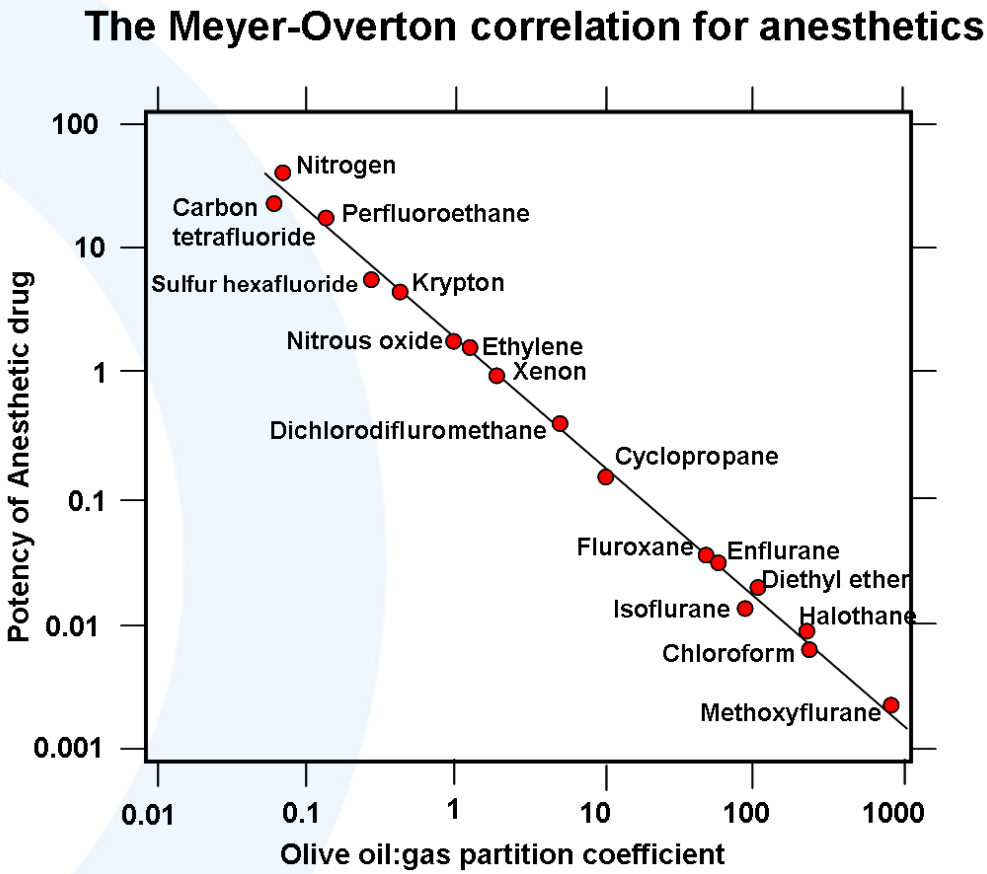
Mode of action:

- partioning of compounds in biological membranes

➔ non-specific perturbation of integrety and function of membranes



Narcosis in Pharmacology



Narcosis theory, postulated by Meyer (1899) and Overton (1902) based on the observation:

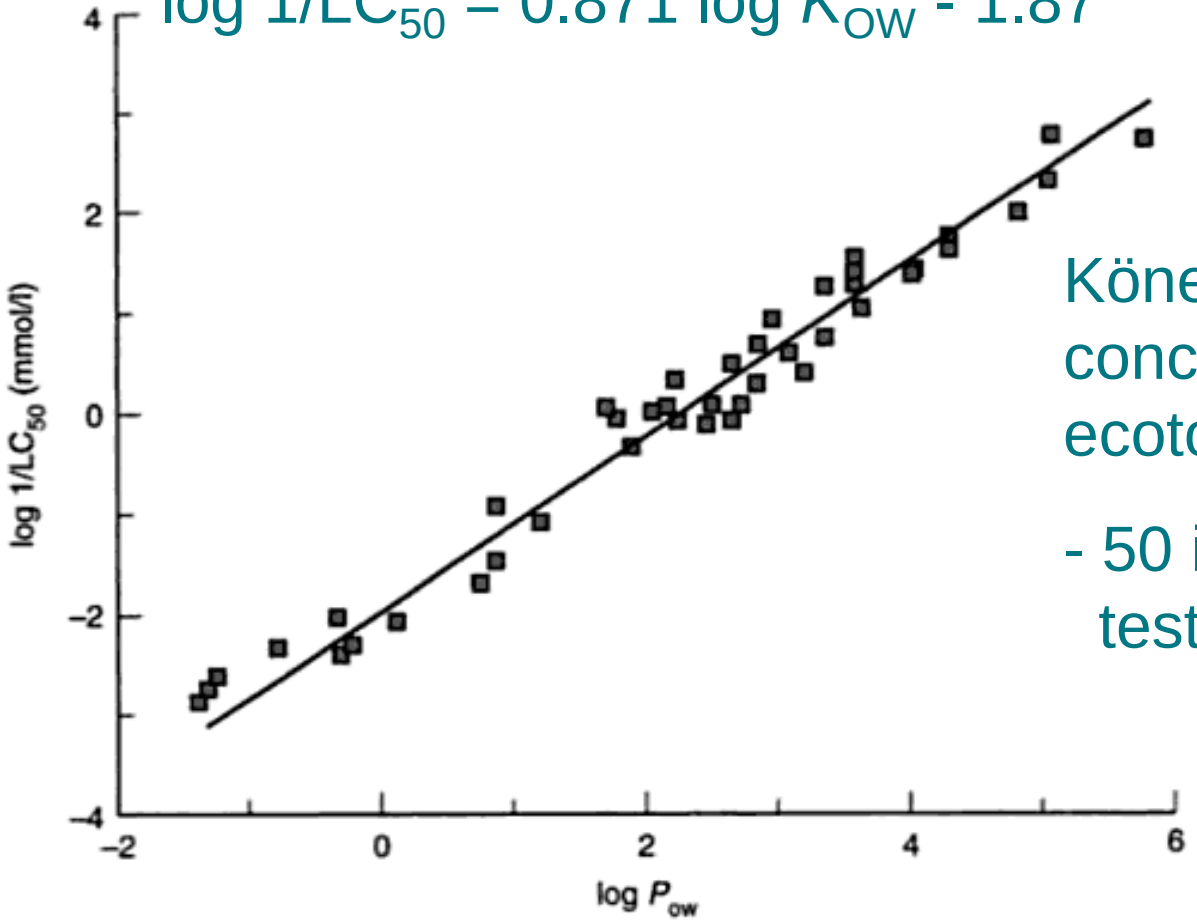
« The aesthetic potential of a compound is proportional to its lipophilicity. »

Meyer (1899). "Zur Theorie der Alkoholnarkose". *Arch. Exp. Pathol. Pharmacol.* **42**

Overton (1901). "Studien über die Narkose zugleich ein Beitrag zur allgemeinen Pharmakologie". *Gustav Fischer, Jena*

Narcosis in Ecotoxicology

$\log 1/LC_{50} = 0.871 \log K_{OW} - 1.87$



Könemann adopted the concept of narcosis to ecotoxicology

- 50 industrial compounds tested for fish toxicity

Figure I.2 Representation of the Könemann equation: the relationship between the toxicity to fish (log $1/LC_{50}$ in mmol/l) of 50 industrial pollutants and their 1–octanol/water partition coefficient (log P_{OW}). The regression line is given by log $1/LC_{50}$ = 0.871 log P_{OW} – 1.87 (Könemann, 1981b).

Könemann, H. (1981), "Quantitative Structure-Activity Relationships in fish toxicity studies. Part 1: relationship for 50 industrial pollutants", Toxicology, 19, 209-221.

Baseline Toxicity

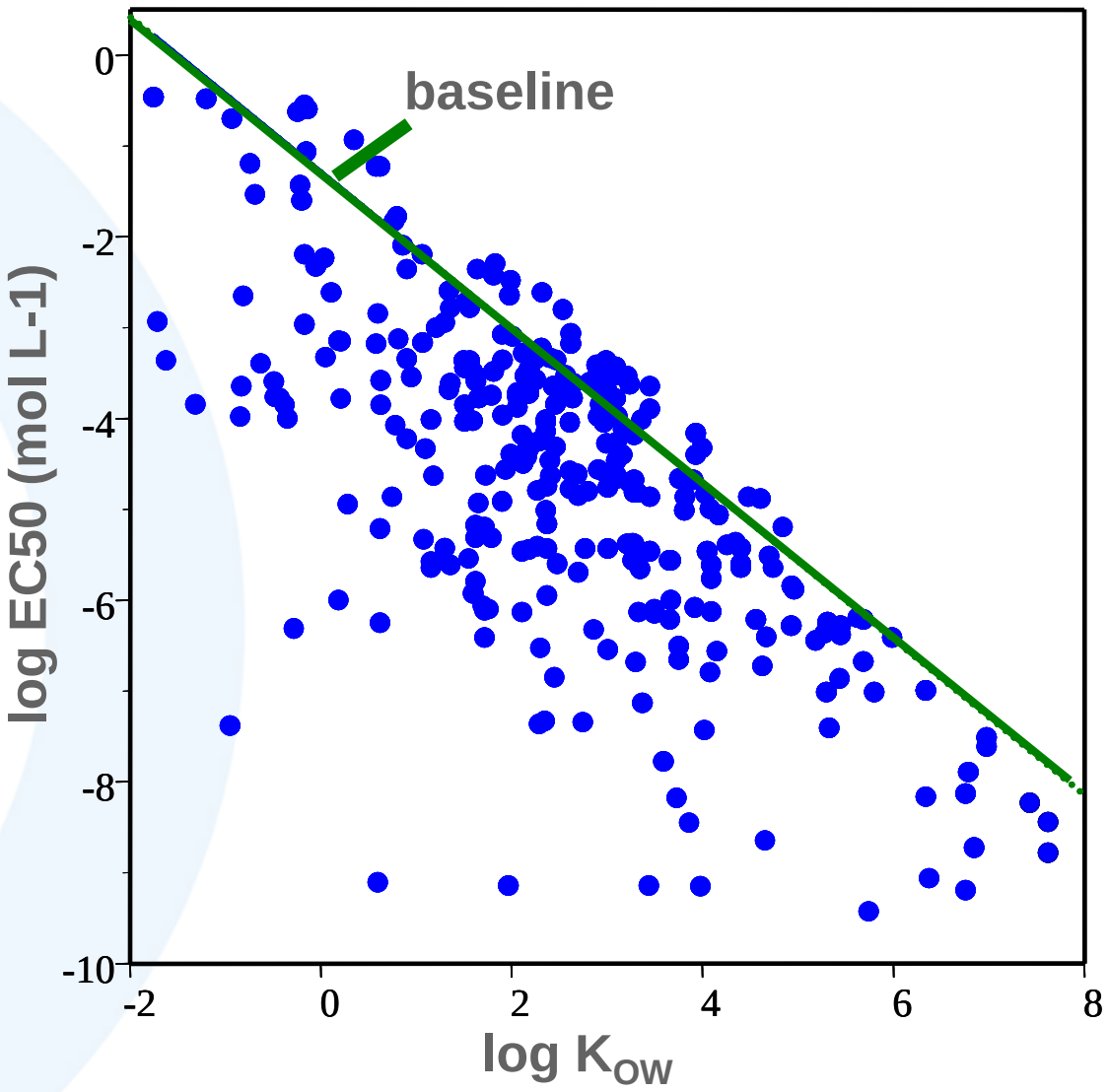
$$\log EC_{50} = a * \log K_{OW} + b$$

Taxa	Dauer	Endpunkt	a	b
Algen				
Skeletonema costatum [e]	96-h	Populationswachstum	-0.72	-0.94
Scenedesmus subspicatus [e]	48-h	Zell-Vermehrung	-0.86	-0.93
Selenastrum capricornutum [e]	72/96-h	Populationswachstum	-1.00	-1.23
Scenedesmus vacuolatus [b]	24-h	Zell-Vermehrung	-0.863	-0.897
Grünalgen [c,d]	96-h	Populationswachstum	-0.885	-1.4
Arthropoda				
Daphnia magna [a,e]	48-h	LC_{50}	-0.94	-1.32
Daphnia magna [a]	48-h	LC_{50}	-0.95	-1.19
Daphnia magna [a,f]	48-h	LC_{50}	-0.91	-1.28
Fische				
Alburnus alburnus [e]	96-h	LC_{50}	-0.75	-1.12
Pimephales promelas [e]	96-h	LC_{50}	-0.85	-1.41
Pimephales promelas [g,d]	96-h	LC_{50}	-0.94	-1.25
Poecilia reticulata [h]	96-h	LC_{50}	-0.85	-1.41

Quelle: [a] Van Leeuwen et al. 1992; [b] Altenburger et al. 2004, [c] Calamari et al. 1983; [d] ECOSAR; [e] Verhaar et al. 1995, [f] Hermens et al. 1984; [g] Veith et al. 1983; [h] Könemann 1981

Same type of relationship can be observed for all species

Baseline Toxicity



Algal toxicity as a function of log Kow (EC50 of 300 substances)

➔Baseline toxicity is the minimal toxicity

Baseline Toxicity - Conclusion

- universal effect
  - all species
  - all organic compounds
- reversible effect

➔minimal toxicity (no compounds can be less toxic than acting via partitioning)

ca. 70% of all compounds exert toxicity in the narcosis range (observed toxicity within a factor of 100 of prediction based on lipophilicity)

Reactive and Specific Modes of Action

- reactive and specific compounds are more toxic than predicted from their lipophilicity
- reactive and specific compounds interact directly with target sites
  - formation of chemical bonds
  - receptor affinity

➔ Their toxicity depends not only on target site concentration but also on the intrinsic potential to react or to bind.

Reactivity

- reactive molecules (i.g. electrophiles) can form covalent bonds with biomolecules
  - alkylation and oxydation of
    - membranes
    - proteines
    - glutathion
    - DNA
- non-reversibles effects

Specific Modes of Action - Inhibition or Competition

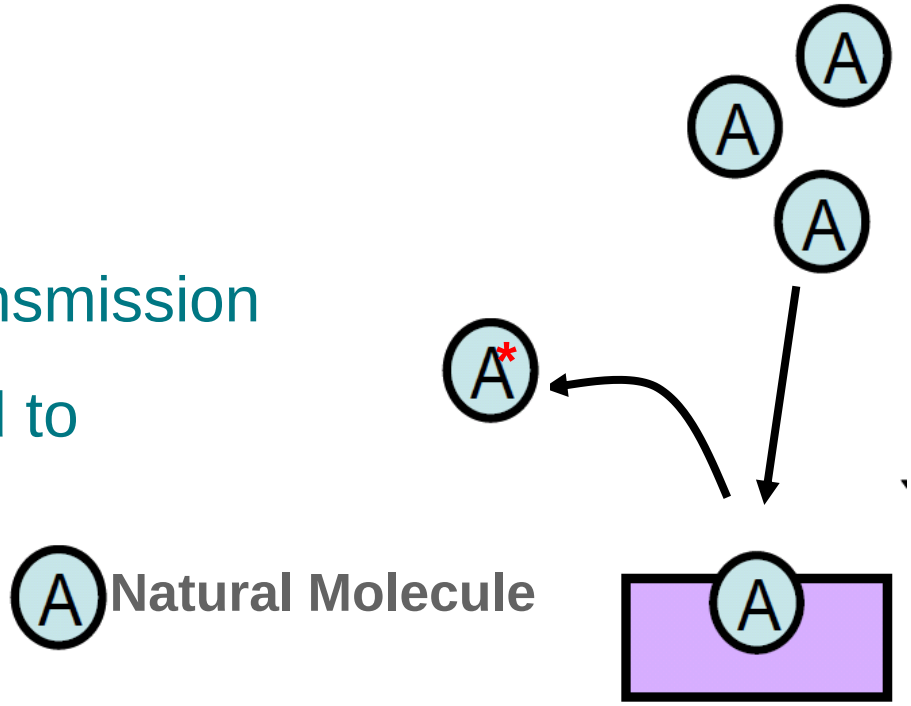
- compounds with similar structure to a biomolecule:

- blocking or competition to molecular receptor.

  - Acetylcholinesterase
  - Estrogene receptor
  - Ah-receptor

→perturbation signal transmission

→Activity typically limited to a group of species



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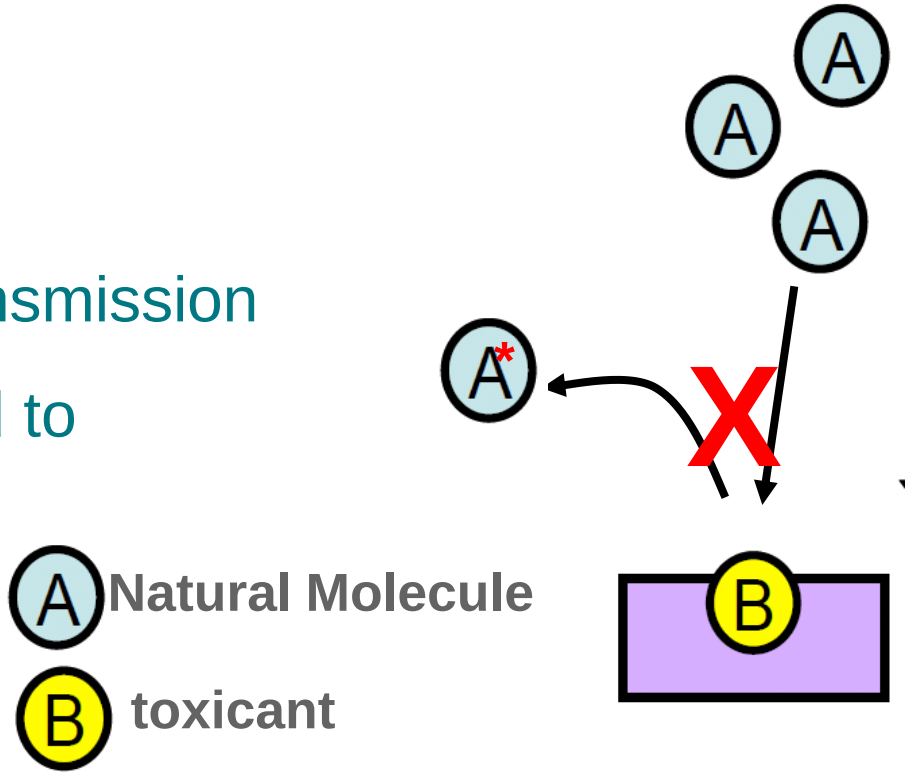
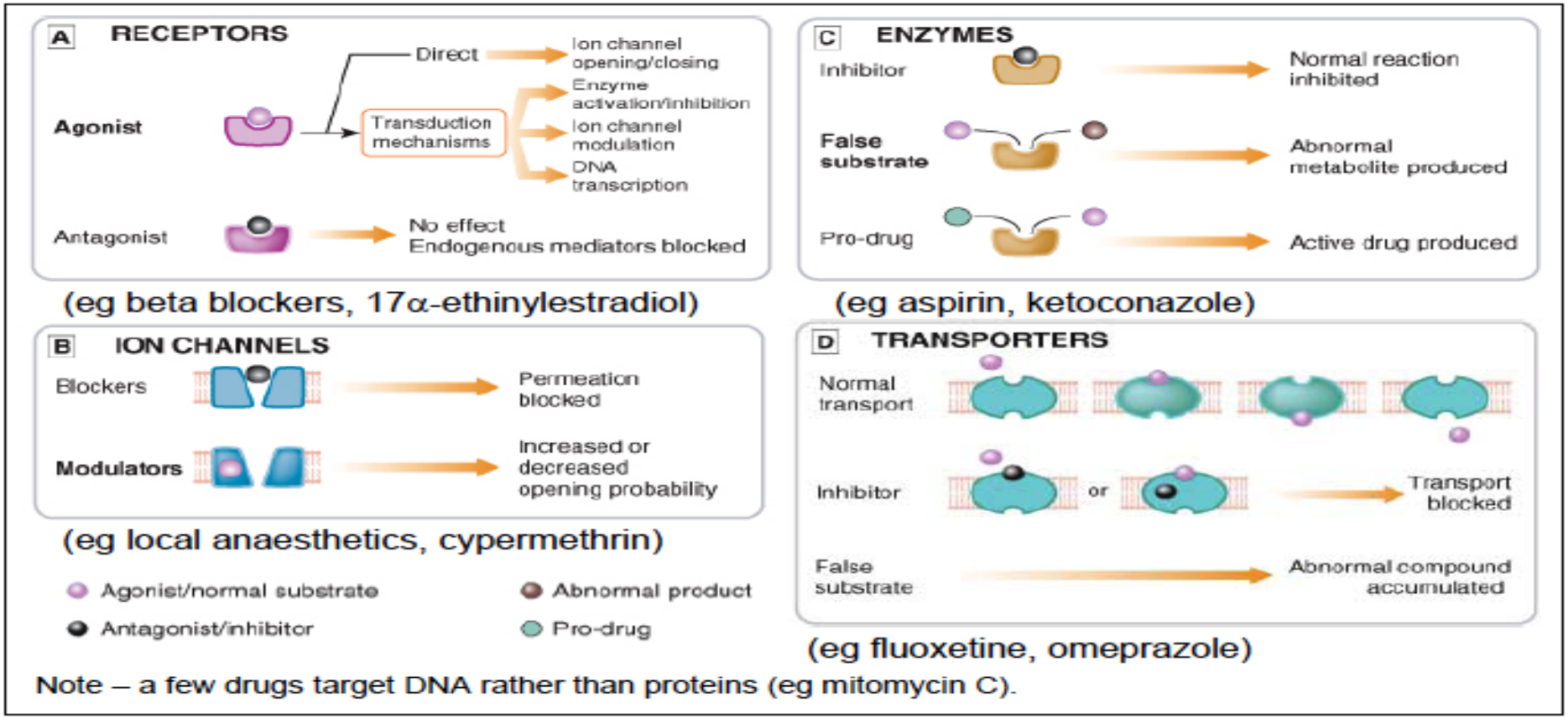


Specific Target Sites



Interference with these sites are specially targeted for development of pesticides (or pharmaceutiques)

Specific Modes of Action of Insecticides

- Neurotoxics

  Perturbation of neurotransmission

- Endocrine disruptors

  Interaction with mecanisms regulating growth and development

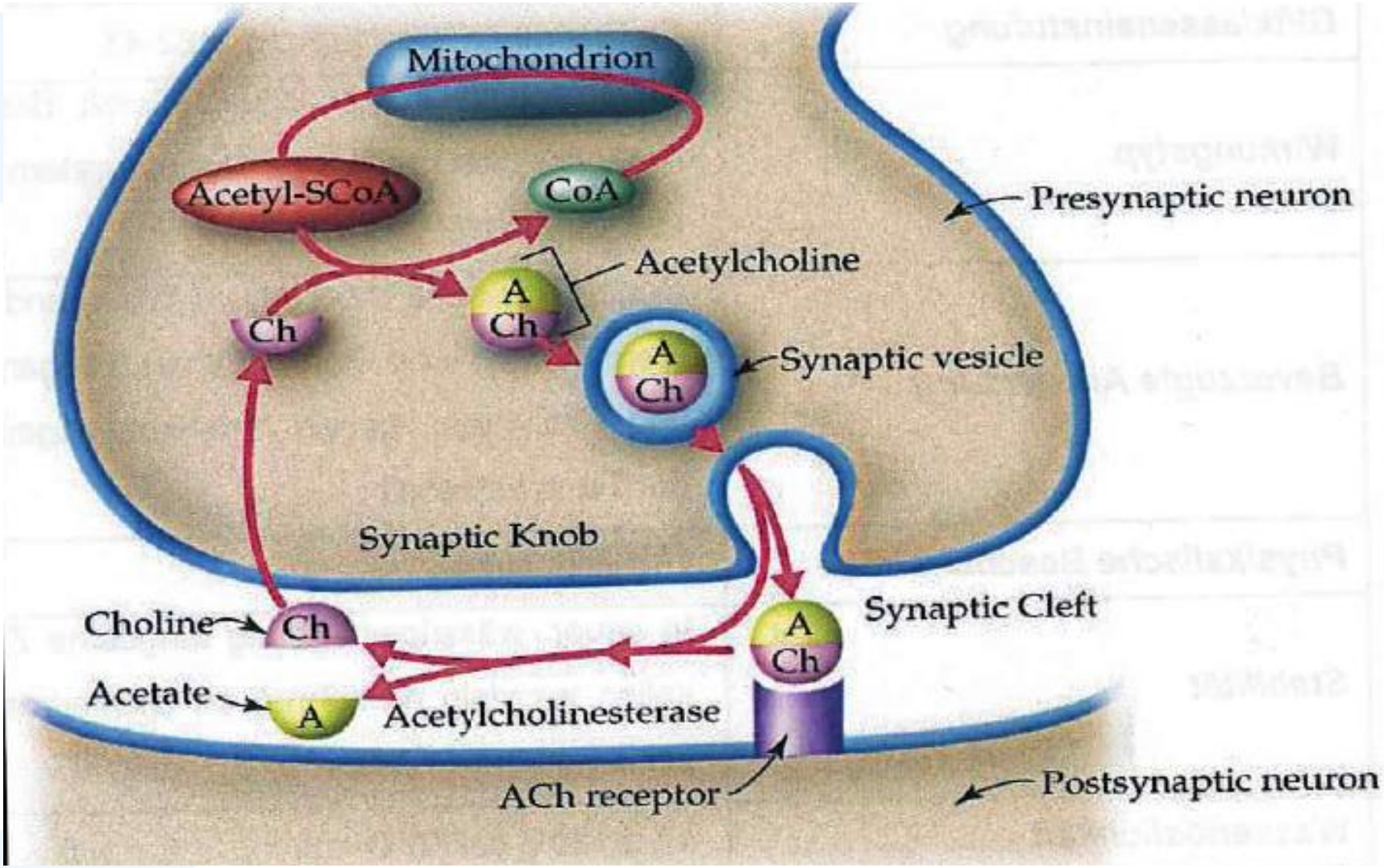
- Action on cellular respiration

Neurotoxics

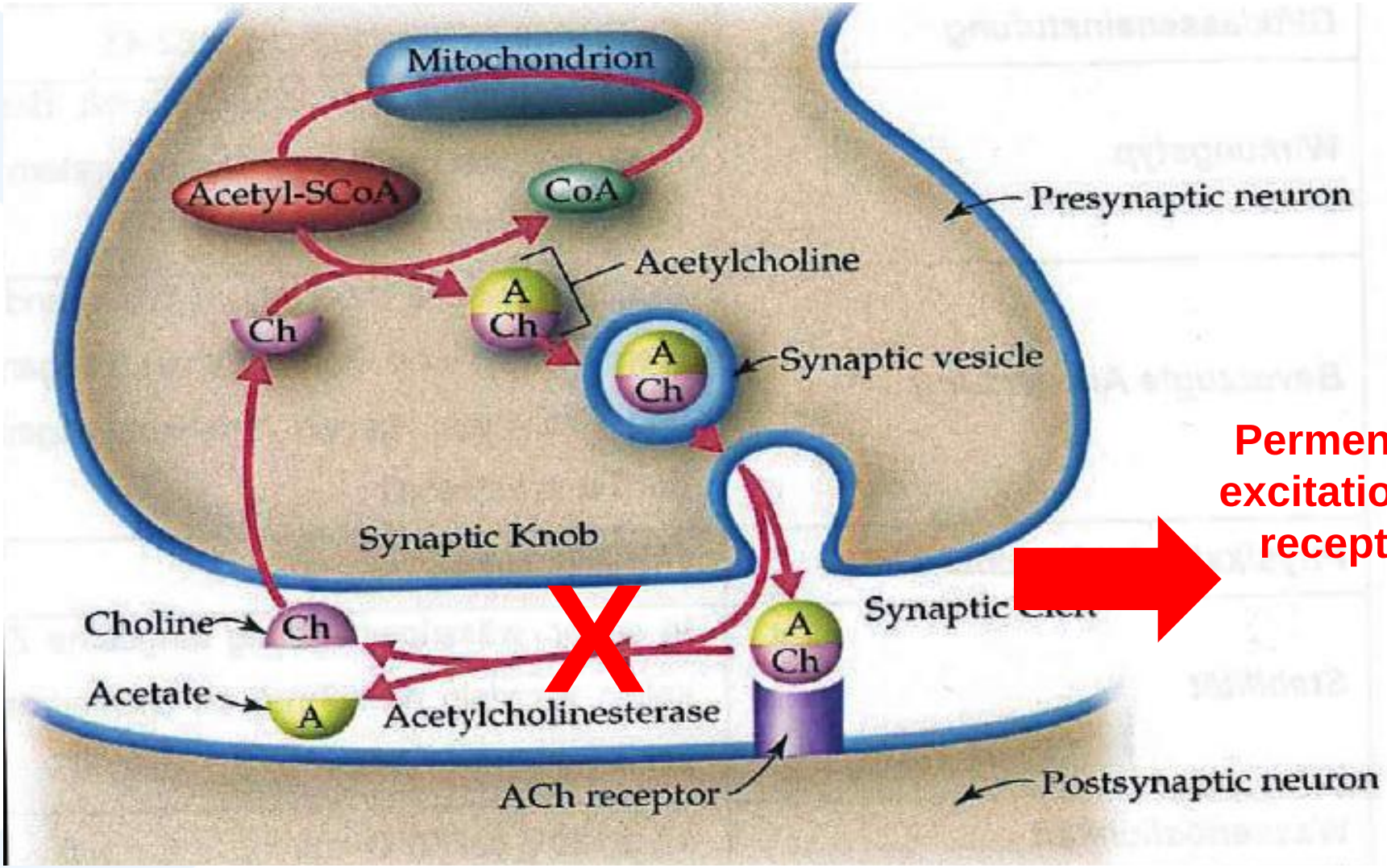
Perturbation of neurotransmission

- Inhibition of Acetylcholinesterase (AChE)
- Agonists of the nicotinic cholinergic receptor (nAChR)
- Sodium channel modulateur
- Muscular ryanodine receptor modulators

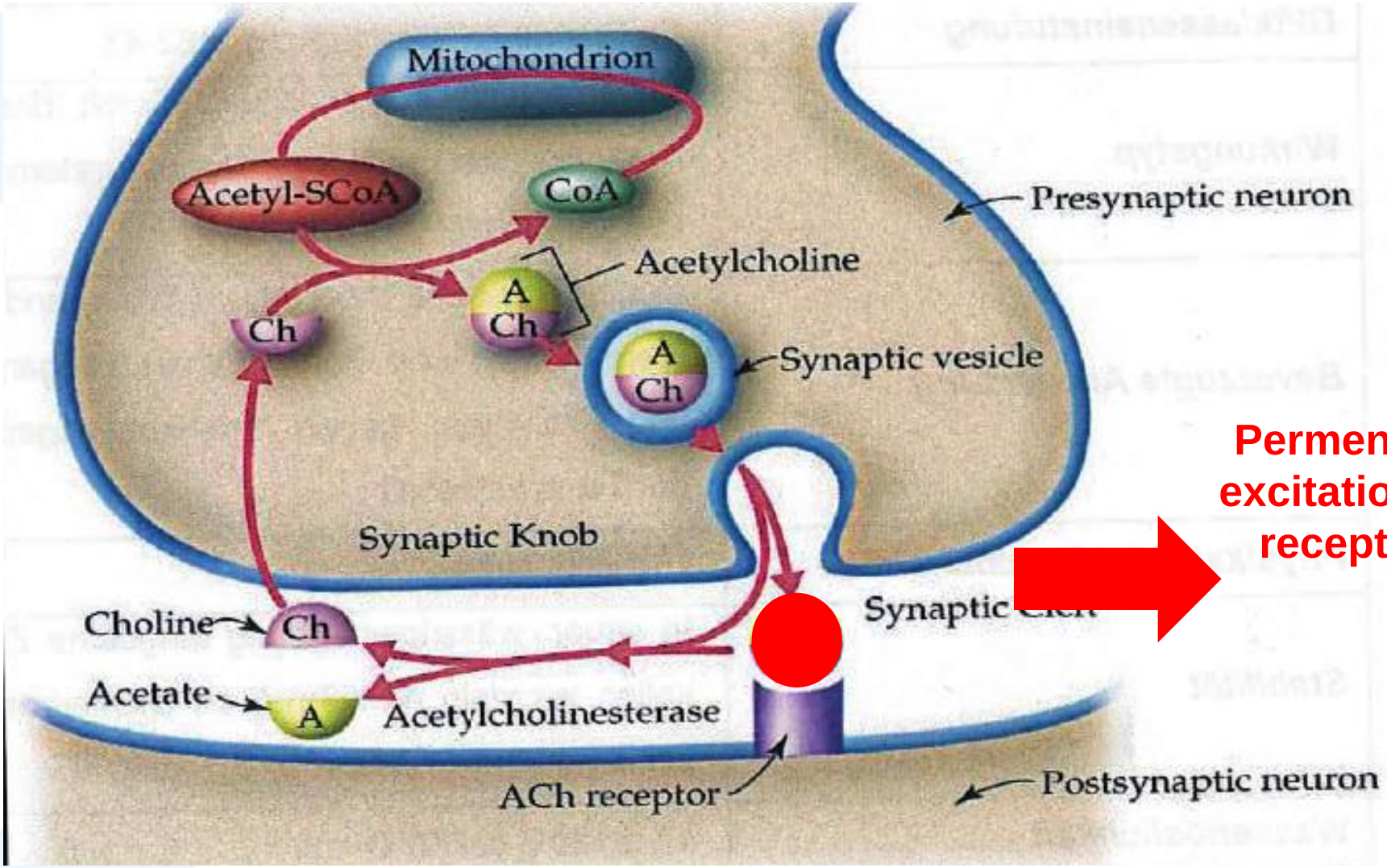
Neurotoxic: Inhibition of AChE



Neurotoxic: Inhibition of AChE

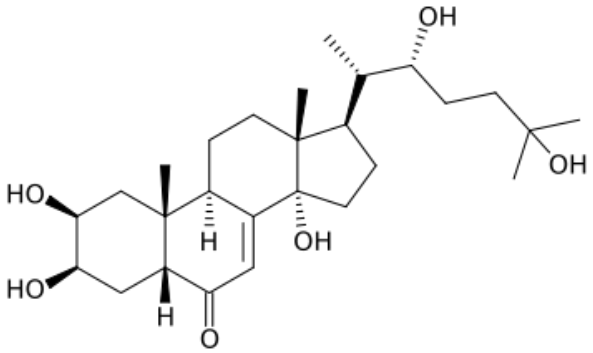


Agonists of the Nicotinic Cholinergic Receptor (nAChR)



Permenent excitation of receptor

Interaction with Mechanisms Influencing Growth and Development



Agonistes of ecdysone

L'ecdysone : - steroid hormone
- interferes with moulting of arthropodes

agonist of ecdysone : - imitation of hormone
- provokes premature moulting

Summary

Modes of action may contribute to understanding of detectablity of compounds in the toximeter

- several modes of action are potentially relevant for behavioural changes
  - neurotoxins
  - endocrine disruptors
- compouns with other modes of action may only be detectable at EC50 concentration

Mixture – Terminology

augmentation

coalism

enhancement

potentiation

sensitation

superadditivity

supraadditivism

synergism

synergy

additivity

additivism

independence

indifference

non-interaction

summation

zero-interaction

antagonism

antergism

depotentiation

desensitation

infraadditivity

negative synergism

non-interaction

potentiation

subadditivity

zero-interaction

no addition

Einführung

Für die Vorhersage eines Mischungseffektes muss man die Konzentrations/Dosis-Wirkungsbeziehungen aller Stoffe der Mischung kennen.

Für die Beurteilung einer potentiellen Interaktion zwischen den Stoffen (Synergie oder Antagonismus) braucht man eine Vorstellung (Konzept oder Modell) für Mischungseffekt ohne Interaktion:

Formulierung einer Nullhypothese.

aber:

Was ist ein vernünftige Hypothese für einen Mischungseffekt?

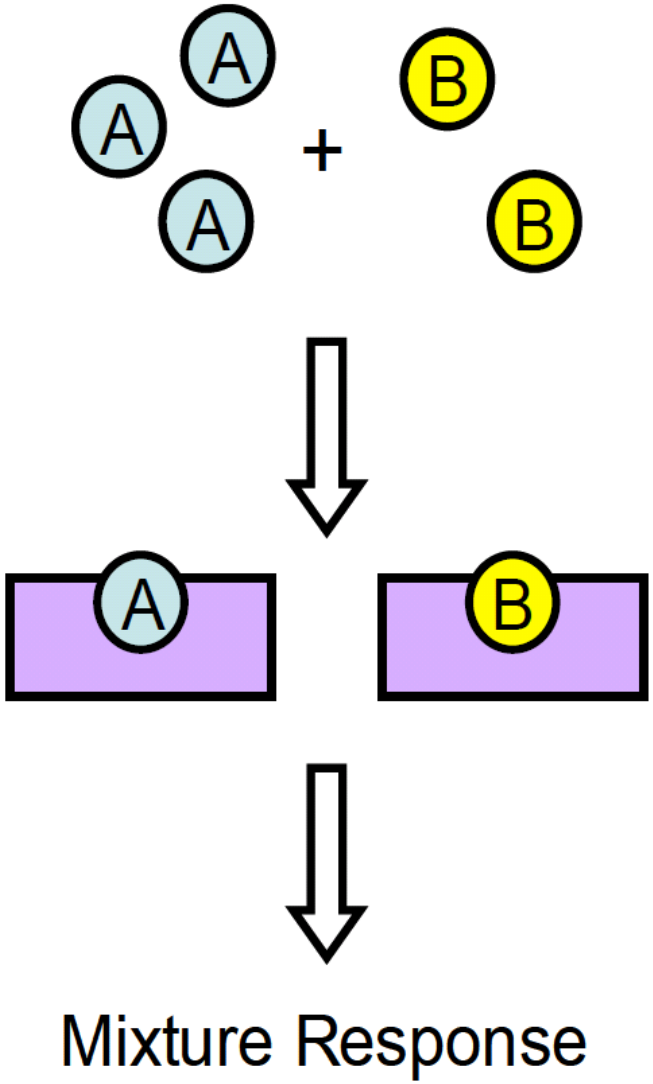
Vorhersagemodelle

Concentration Addition (CA)

Hypothese:

- Ähnliche Wirkmechanismen
- identischer Wirkort
- (eventuell unterschiedliche Wirksamkeit)

➔ Eine Substanz kann als Verdünnung der anderen betrachtet werden.



Vorhersagemodelle

Concentration Addition (CA)

Universelle Formel

$$\sum_{i=1}^{n} \frac{c_i}{ECx_i} = 1$$

n = Anzahl Substanzen
c_i = Konz von Substanz i
EC_X = Effektkonz. X%
X = Effektlevel in %

Formel für binäre Mischungen

$$\frac{c_1}{ECx_1} + \frac{c_2}{ECx_2} = 1$$

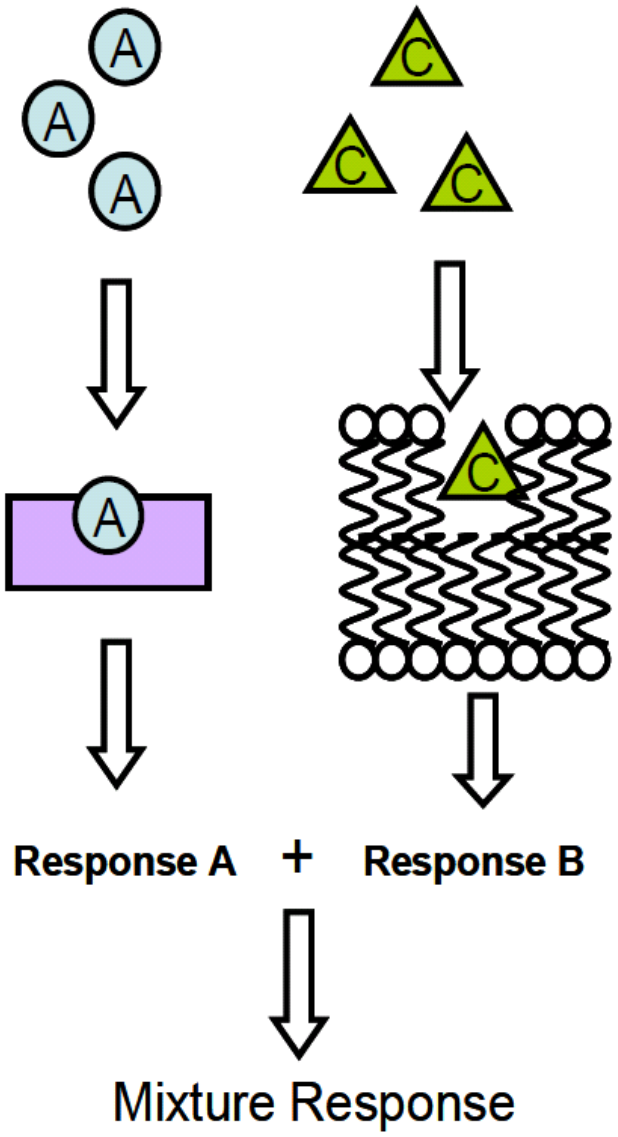
Vorhersagemodelle

Independent Action (IA)
Response Addition (RA)

Hypothese:

- Unähnliche Wirkmechanismen
- Unterschiedliche Wirkorte

➔ Der Effekt einer Substanz ist völlig unabhängig von der anderen (auch im statistischen Sinne).



Vorhersagemodelle

Independent Action (IA)
Response Addition (RA)

Universelle Formel

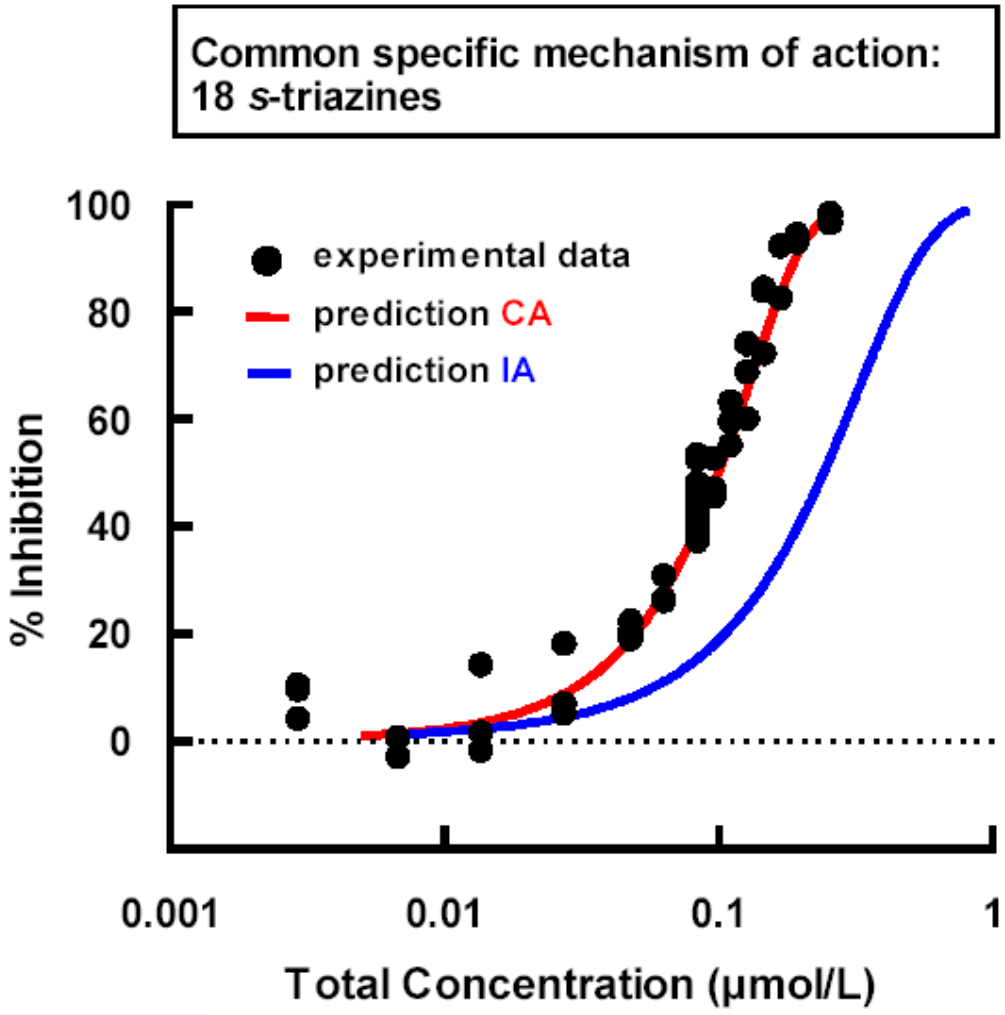
$$E(c_{mix}) = 1 - \prod_{i=1}^{n} \left[1 - E(c_i)\right]$$

Formel für binäre Mischungen

$$E(c_{mix}) = E(c_1) + E(c_2) - E(c_1) \bullet E(c_2)$$

n = Anzahl von Substanzen
c_i = Konz der Substanz i
$E(c_i)$ = Effekt der Konz c_i von Substanz i

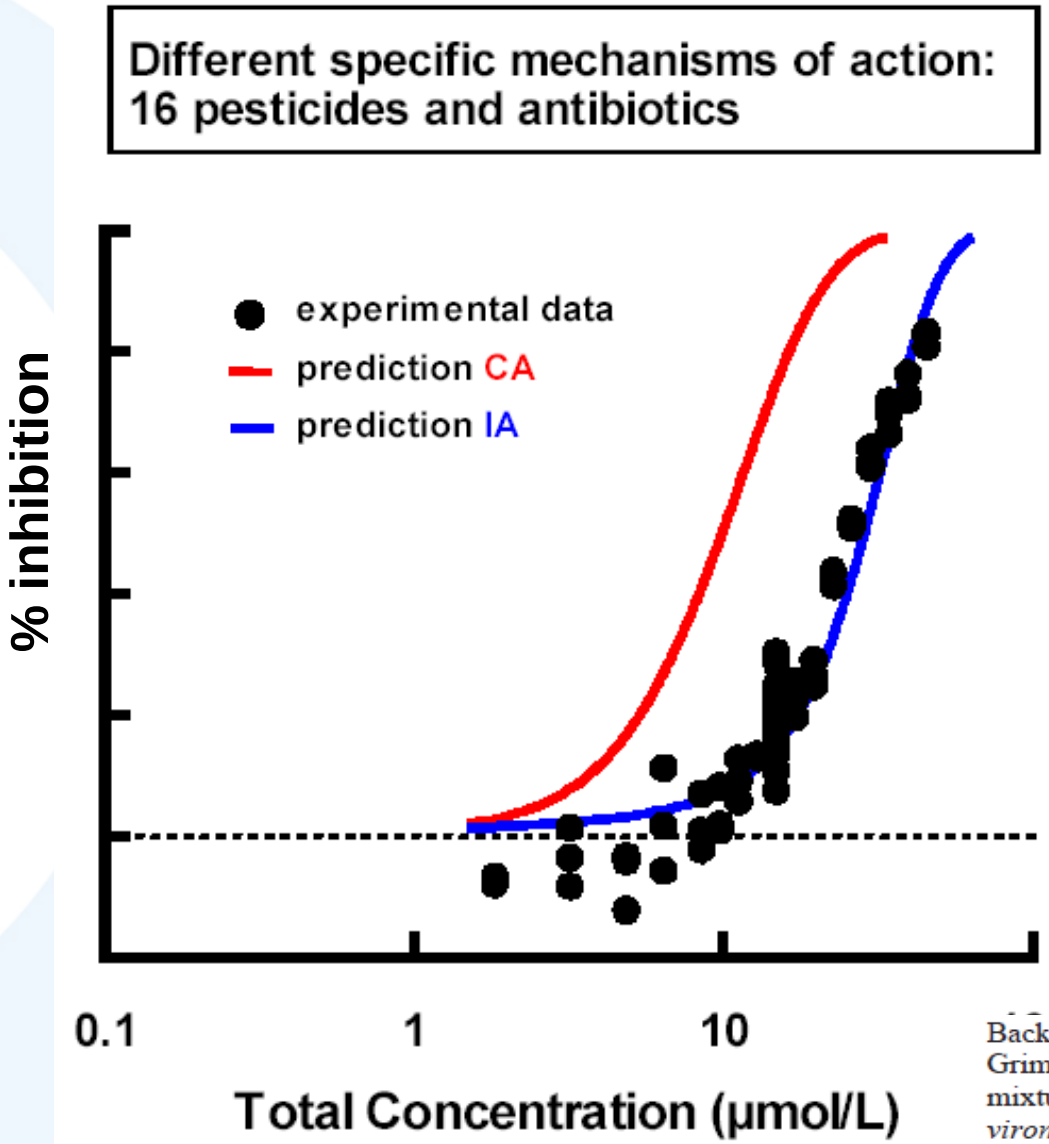
Referenzfälle



Das CA-Modell sagt die Toxizität von Mischungen ähnlich wirkende Stoffe korrekt vorher in verschiedenen Testsystemen (Algen und Bakterien) und in verschiedenen Mischungs-verhältnissen (EC50 et EC01)

Altenburger R, Backhaus T, Boedeker W, Faust M, Scholze M, Grimme LH. 2000. Predictability of the toxicity of multiple chemical mixtures to *Vibrio fischeri*: Mixtures composed of similarly acting chemicals. *Environ Toxicol Chem* 19:2341–2347.

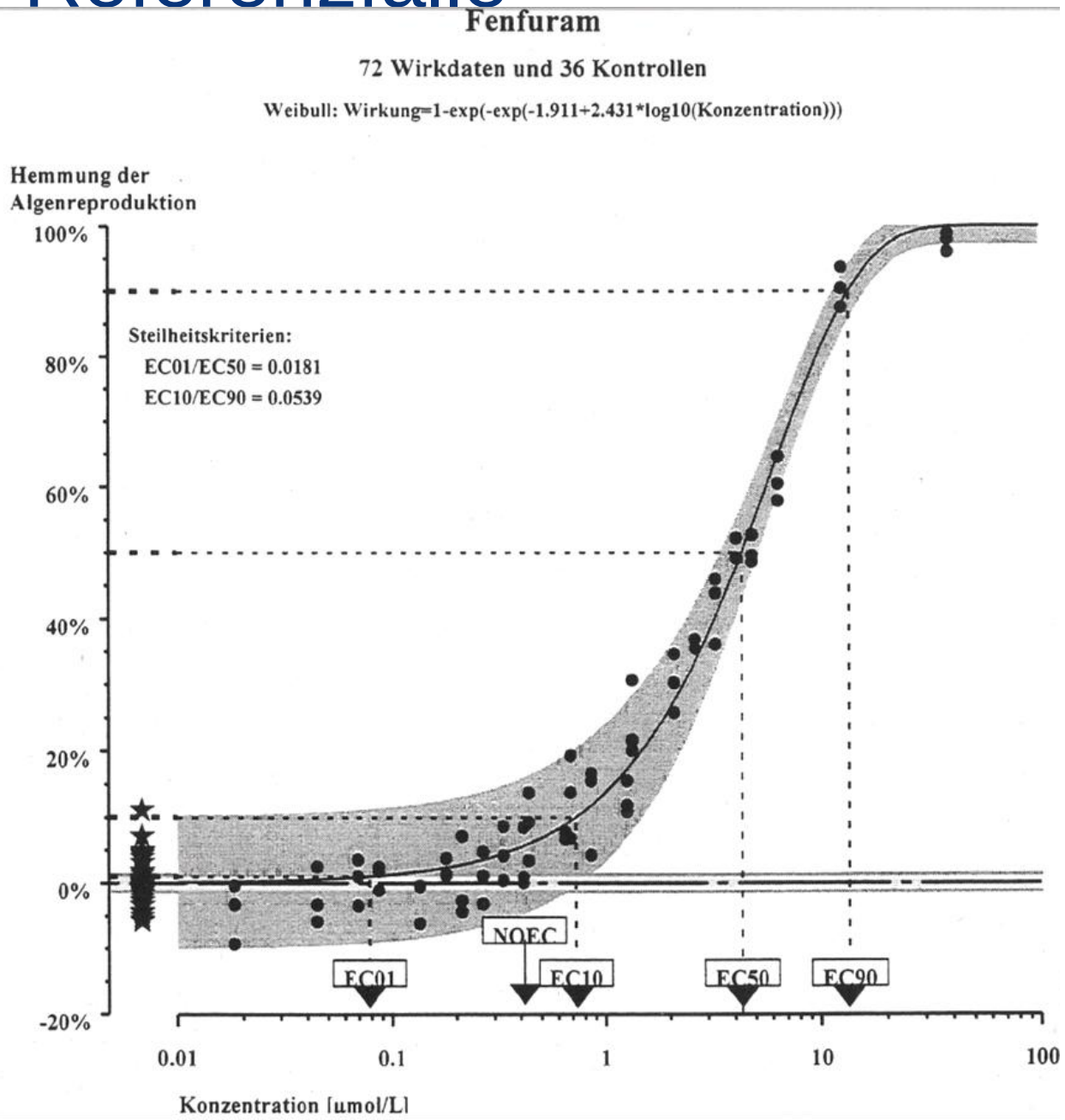
Referenzfälle



Das IA-Modell sagt die Toxizität von Mischungen unähnlich wirkender Stoffe korrekt vorher in verschiedenen Testsystemen (Algen und Bakterien) und in verschiedenen Mischungs-verhältnissen (EC50 et EC01)

Backhaus T, Altenburger R, Boedeker W, Faust M, Scholze M, Grimme LH. 2000. Predictability of the toxicity of a multiple mixture of dissimilarily acting chemicals to *Vibrio fischeri*. *Environ Toxicol Chem* 19:2348–2356.

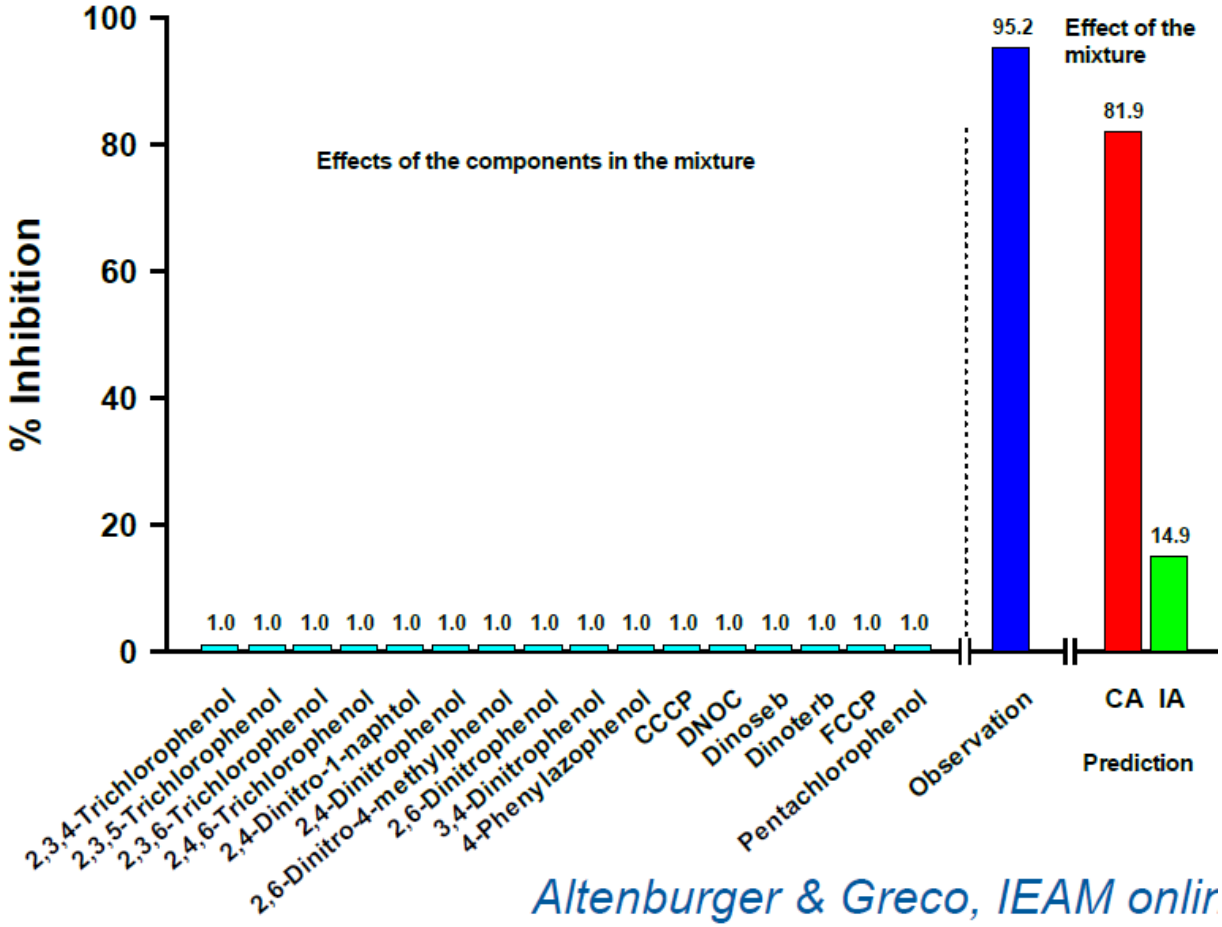
Referenzfälle



Konsequenzen aus CA

Something from nothing

Silva et al. ES&T 36:1751



Altenburger & Greco, IEAM onlir

Implikationen der Modelle

CA

- Jeder Stoff der Mischung trägt zum Kombinationseffekt bei (selbst wenn er einzeln keinen Effekt hervorruft (low dose)
  - Für Umweltmischungen können sich daraus Problemen ergeben (Organismen können hunderten oder tausenden Stoffen gleichzeitig exponiert sein)
- Berechung auf Basis von EC50-Werten möglich (nicht aus NOEC, LOEC, etc.) für das gleiche Effektlevel (z.B. Toxic Units).

IA

- Wenn eine Substanz allein keinen Effekt erzeugt, trägt sie auch nicht zum Kombinationseffekt bei.
- Für die Berechunge müssen kleine Effekte der Einzelstoffe quantifiziert werden.
  - Das ist in der Regel schwierig.
  - Entsprechende Informationen sind nicht verfügbar.
- Berechnung aus EC50, NOEC oder LOEC ist nicht möglich.

Conclusion

Mixture toxicity is reasonably well predictable

Syngistic effects are rather rare in environmental samples

Typically toxicity of environmetal samples is driven by few main contributors to toxicity (despite hundreds of compounds present at low dose).

All evidence from classic toxicity tests

Aim:

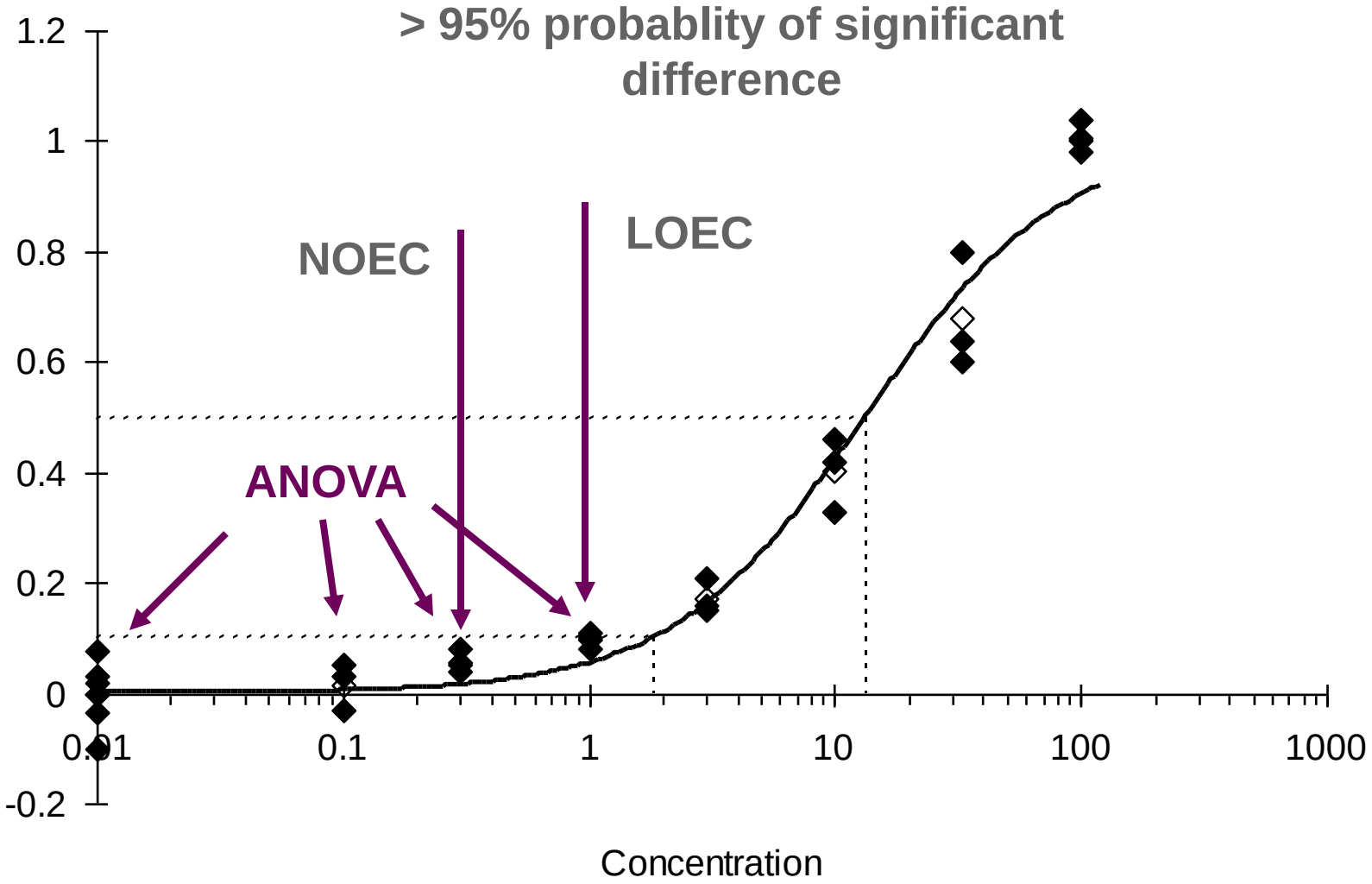
Is mixture toxicity predictable when using non-classical endpoints (behaviour)?

Does the presence of multiple low-dose compound mixtures increase the sensitivity of the toximeter?

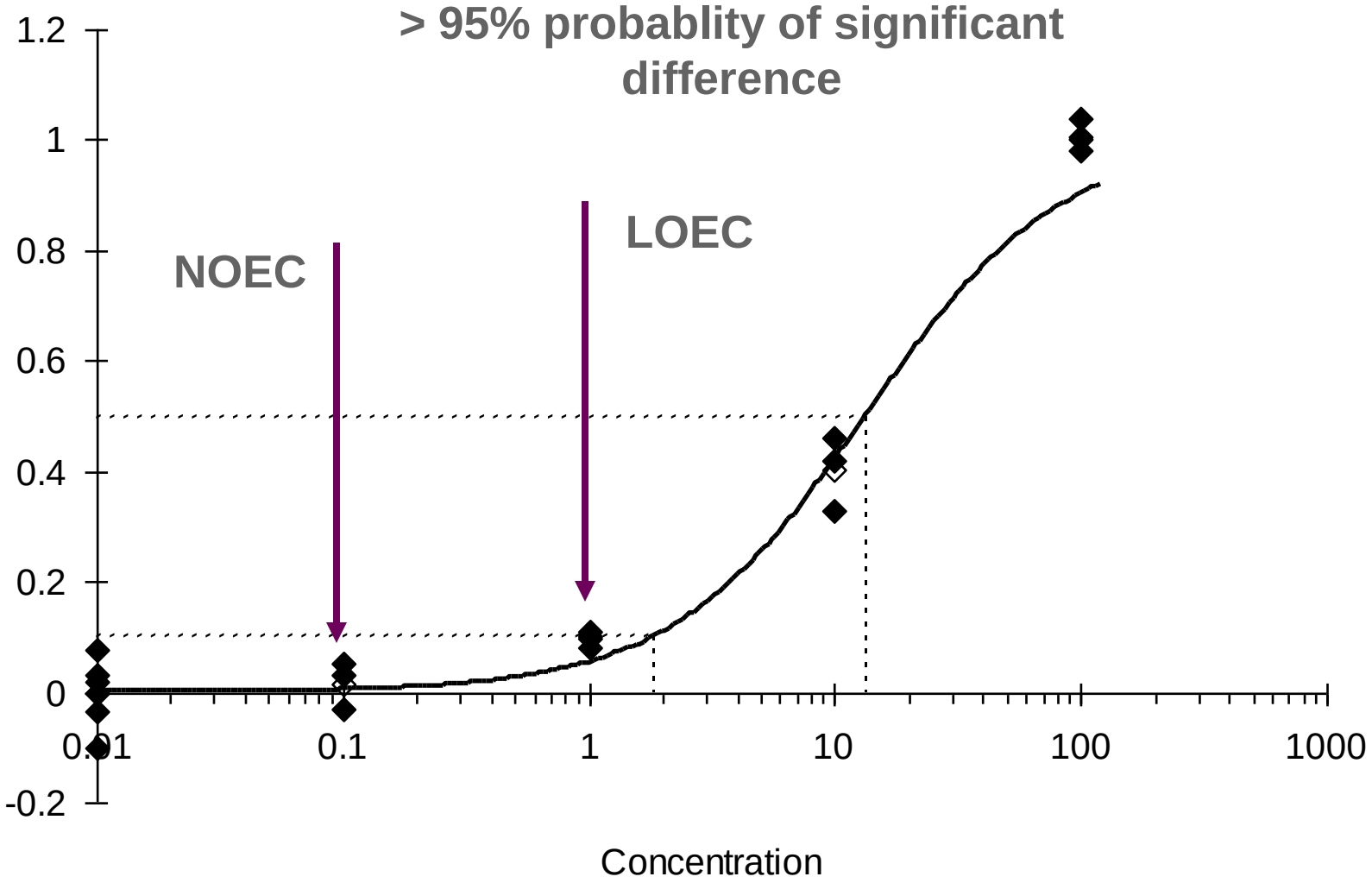
Thank you for your attention

Recommanded reading:
Escher et Hermens (2002) Modes of action in ecotoxicology: Their role in body burdens, species sensiticity, QSARs, and mixture effects, Environ Sci Tech 36, 4201

Problems of NOECs



Problms of NOECs



Problemes of NOECs