Ecotoxicology & ERA

Planning an experiment
Partial life tables
Mixture toxicity and interactions
Experimental design
QSAR

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Problems for discussion

- How to estimate the risk to a population of long-lived iteroparic organisms?
  - We are unable to study the effects of a toxic substance throughout the life of the organism
- How do toxic substances work when mixed with other substances?
  - Are the effects of the toxic substances simply additive or are there other effects?
- Do environmental conditions affect the toxicity of pollutants?
  - Do pH, temperature, humidity, etc. modify the effects of toxic substances on organisms?

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How to check which elements of the life history of a species are crucial for its sensitivity to adverse environmental factors?

λ sensitivity to perturbations in matrix elements

$$s_{ij} = \frac{\partial \lambda}{\partial a_{ij}}$$

λ elasticity to perturbations of matrix elements

$$e_{ij} = \frac{a_{ij}}{\lambda} \frac{\partial \lambda}{\partial a_{ij}}$$

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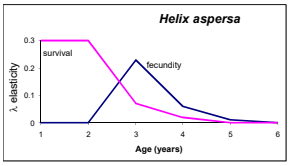
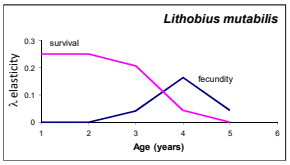
λ elasticity in snails and centipedes





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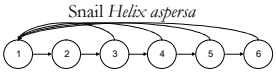
λ Lambda elasticity comparison for two species

Snail *Helix aspersa*



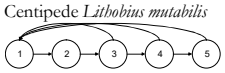
Projection matrix for *H. aspersa*

1	2	3	4	5	6
0	0	75	75	75	75
0,052	0	0	0	0	0
0	0,2	0	0	0	0
0	0	0,25	0	0	0
0	0	0	0,25	0	0
0	0	0	0	0,15	0

Elasticity

0	0	0.23	0.057	0.014	0.002
0.303	0	0	0	0	0
0	0.303	0	0	0	0
0	0	0.073	0	0	0
0	0	0	0.018	0	0
0	0	0	0	0.002	0

Centipede *Lithobius mutabilis*



Projection matrix for *L. mutabilis*

1	2	3	4	5
0	0	5	22	13
0,049	0	0	0	0
0	0,69	0	0	0
0	0	88	0	0
0	0	0	0,457	0

Elasticity

0	0	0.042	0.163	0.044
0.25	0	0	0	0
0	0.25	0	0	0
0	0	0.207	0	0
0	0	0	0.044	0

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Toxicity of mixtures of toxic chemicals and interactions with environmental factors

- Toxic substances rarely occur singly:
  - pollution from smelters → e.g. Zn + Cd + Pb + SO_2 + NO_x + ...
  - pollution from chemical industry → PCB + WWA + SO_2 + NO_x + ...
- Different groups of toxic chemicals act on different biochemical and physiological mechanisms:
  - e.g. pesticides vs. metals
- Some toxic chemicals can react with each other, resulting in products that are either more or less toxic than the substrates
- Environmental conditions can influence toxicity

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Toxicity of mixtures

Effect(a)
Effect(b)
Effect(c)
Effect(d)
.
.
.
Effect(n)

Effect(a+b+c+d+...+n) = ?

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Two models of mixture toxicity:

1. CA = *Concentration Addition*

(SA = *Similar Action,* Loewe model)

- Toxicants 1 and 2 have the same mechanism of toxicity
- Let c_1 and c_2 be concentrations of toxicants 1 and 2 producing in combination an effect *E*.
- Let $cE1$ and $cE2$ are the concentrations of toxicants 1 and 2 needed to produce effect *E* alone

$$\frac{c_1}{cE1} + \frac{c_2}{cE2} = 1$$

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How to add concentrations of toxicants?
Toxicity Units (TU)

$$E_{mix}[TU] = \sum_{i=1}^{n} \frac{c_i}{EC_{50_i}}$$

E_{mix} – the toxicity of the mixture

c_i – the concentration of the substance *i* in the mixture

EC_{50_i} – EC_{50} for the substance *i*

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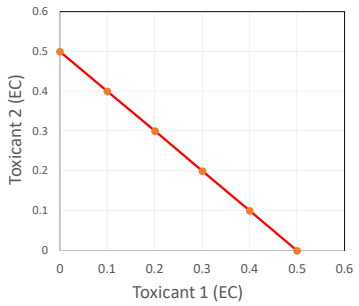
Toxicity of mixtures - additive model



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Two models of mixture toxicity:

1. EA = *Effect Addition*

IA = *Independent Action,* Bliss model)

- **Toxicants have different mechanisms of toxicity**
- → independent probability p of the effect of individual substances (1, 2,...n):

$p_{mix} = 1-(1-p_1)(1-p_2)...(1-p_n)$

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

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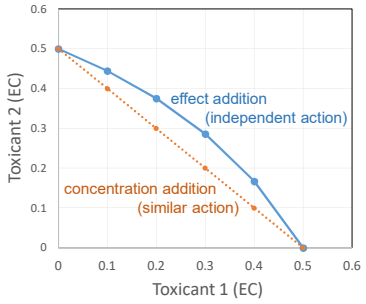
Toxicity of mixtures: concentration addition *vs.* effect addition



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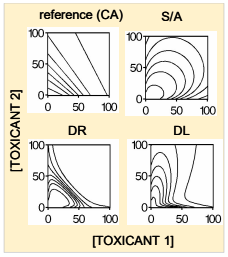
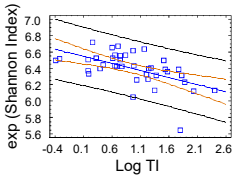
Usefulness of toxicity units and toxicity indices

In laboratory experiments on the toxicity of mixtures



In field studies in areas contaminated with many substances



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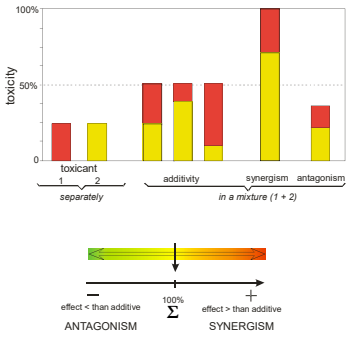
Interactions



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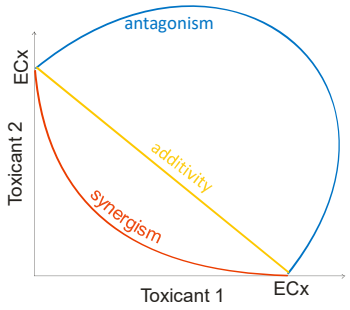
Interactions between two substances



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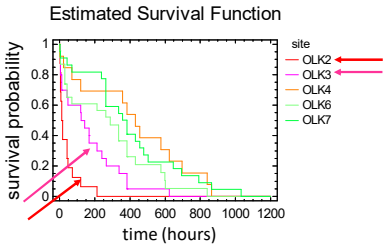
Interactions between toxicants in reality:

ground beetles (*Poecilus cupreus*) living in highly contaminated areas (OLK2, OLK3) are more sensitive to the pesticide (dimethoate)



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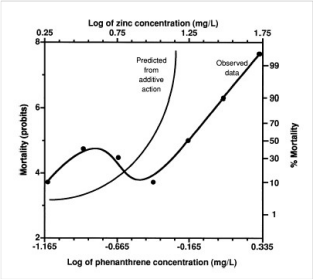
Interactions between toxicants:

effect of the interaction of Zn and phenanthrene on their toxicity to fish *Cyprinodon variegatus*



C. J. Moreau, P. L. Klerks, C. N. Haas. 1999. Interaction Between Phenanthrene and Zinc in Their Toxicity to the Sheepshead Minnow (*Cyprinodon variegatus*). Arch. Environ. Contam. Toxicol. 37: 251–257.

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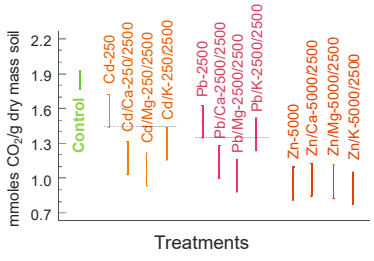
Interactions with non-toxic substances can also be important:

the influence of base metals – macronutrients on the toxicity of Cd, Pb and Zn for soil microorganisms



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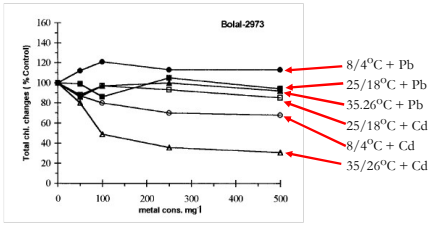
Interactions of toxicants with other environmental factors:

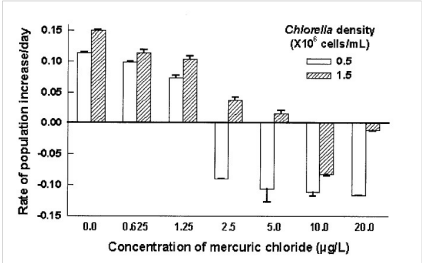
influence of temperature on the toxicity of Cd and Pb to plants



I. Oncel, Y. Keles, A.S. Ustun. 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environmental Pollution 107: 315-320. 19 /36

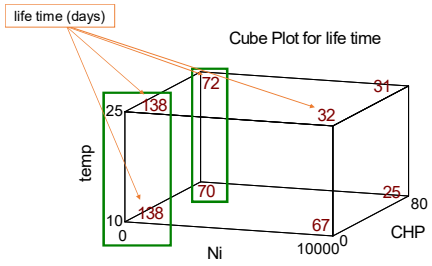
Interactions of toxicants with other environmental factors:

influence of food availability (algae - Chlorella) on the dynamics of rotifers population



S. S. S. Sarma, S. Nandini, T. Ramirez Perez. 2001. Combined Effects of Mercury and Algal Food Density on the Population Dynamics of *Brachionus patulus* (Rotifera). ull. Environ. Contam. Toxicol. 67: 841–847. 20 /36

Interaction Ni*CHP*T in carabids



Bednarska et al. (2008) Combined effect of environmental pollutants (nickel, chlorpyrifos) and temperature on the ground beetle, *Pterostichus oblongopunctatus* (Coleoptera: Carabidae). Ecotox. Environ. Sci., MS08-286. 21 /36

Experimental plans to study the effects of several toxic substances simultaneously

- Complete plan $n^{(k)}$: the ability to evaluate all *k* main effects and the effects of all interactions, but very laborious :
  - e.g.: 3 concentrations for 4 toxicants → 3^4 = 81 treatments x 3 replicated = 243 units
  - for 5 toxicants → 729 units
  - for 6 toxicants → 2187 units
- Partial (fractional) plans $n^{(k-p)}$: no possibility of assessing the effects of higher-order interactions, but assessing the main effects and effects of lower-order interactions possible with much less work
  - Often used in industry for economic reasons

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Experimental plans useful in ecotoxicology

- **A complete multi-level plan (full factorial)**
  - all combinations of all concentrations are tested: $n^{(k)}$
  - by far the best, but the most labor-intensive: min. 3 concentrations → for 3 toxicants N=3^3 = 27 (with no replicates!)
- **Partial (fractional) plans**
  - only some combinations are tested: $n^{(k-p)}$
    - **Box-Behnken design:** $3^{(k-p)}$ → for 3 toxicants N=15
    - **central composite designs:** no assumptions about the number of concentrations → any set of continuous values of input data can be analyzed (*n*)

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A complete multi-level plan $n^{(k)}$

Design for 3 substances, each at 3 concentrations → $3^{(3)}$

Factors	*Low*	*High*	*Levels*
Factor_A	0.0	1.0	3
Factor_B	0.0	1.0	3
Factor_C	0.0	1.0	3

→ in a complete plan, all main effects and the effects of all interactions can be interpreted

run	*Factor_A*	*Factor_B*	*Factor_C*
1	0.0	0.0	0.0
2	0.5	0.0	0.0
3	1.0	0.0	0.0
4	0.0	0.5	0.0
5	0.5	0.5	0.0
6	1.0	0.5	0.0
7	0.0	1.0	0.0
8	0.5	1.0	0.0
9	1.0	1.0	0.0
10	0.0	0.0	0.5
...	...	...	...
27	1.0	1.0	1.0

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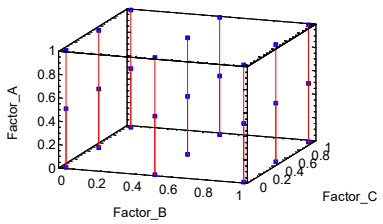
Complete plan for 3 substances and 3 concentrations



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Fractional plan $3^{(k-p)}$ – Box-Behnken design

Design for 3 substances, each at 3 concentrations

Factors	Low	High	Continuous
Factor_A	0	1.0	Yes
Factor_B	0	1.0	Yes
Factor_C	0	1.0	Yes

run	Factor_A	Factor_B	Factor_C
1	0.0	0.0	0.5
2	1.0	0.0	0.5
3	0.0	1.0	0.5
4	1.0	1.0	0.5
5	0.0	0.5	0.0
6	1.0	0.5	0.0
7	0.0	0.5	1.0
8	1.0	0.5	1.0
9	0.5	0.0	0.0
10	0.5	1.0	0.0
11	0.5	0.0	1.0
12	0.5	1.0	1.0
13	0.5	0.5	0.5
14	0.5	0.5	0.5
15	0.5	0.5	0.5

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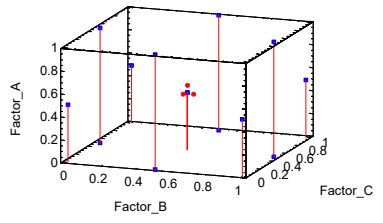
Box-Behnken design for 3 chemicals



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In fractional designs, it is necessary to check the correlation matrix between the effects studied

Correlation matrix between effects for Box-Behnken design

	A	B	C	AA	AB	AC	BB	BC	CC
A	--								
B	0.0000	--							
C	0.0000	0.0000	--						
AA	0.0000	0.0000	0.0000	--					
AB	0.0000	0.0000	0.0000	0.0000	--				
AC	0.0000	0.0000	0.0000	0.0000	0.0000	--			
BB	0.0000	0.0000	0.0000	-0.0714	0.0000	0.0000	--		
BC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	--	
CC	0.0000	0.0000	0.0000	-0.0714	0.0000	0.0000	-0.0714	0.0000	--

→ no correlations >0.5 → interpretation of results should be easy

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Is it possible to do without experiments in ecotoxicology?

- Short answer: NO
- More complex answer: to some extent YES
  - the effects of individual toxicants can be roughly predicted from their chemical structure
    - → **QSAR** – *Quantitative Structure-Activity Relationships*
  - the effects of mixtures can be estimated using appropriate interaction model: additive, antagonistic or synergistic

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QSAR – Quantitative Structure-Activity Relationships

- Used in the medical industry to develop new drugs
- Relies on combining a limited number of empirical data and chemical knowledge to assess the toxicity of analogues of substances with known toxicity
- One of the easiest ways is to predict toxicity based on the hydrophobicity of a substance
  - the hydrophobicity of the substance affects:
    - availability for organisms (bioavailability) and the tendency for accumulation
    - metabolism
    - toxicity

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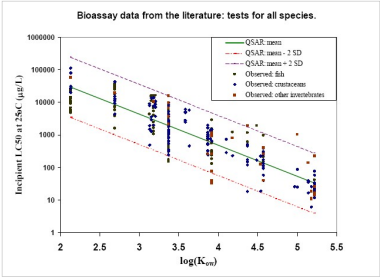
K_{ow} – Octanol/Water partitioning coefficient as a measure of the hydrophobicity of chemicals: toxicity of various hydrocarbons occurring in crude oil - assessment of the toxicity of oil spills into the sea



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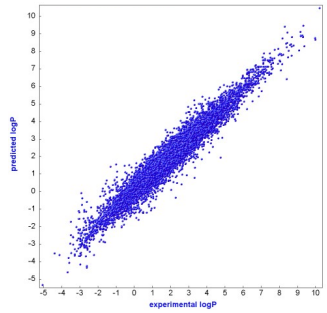
K_{ow} – Octanol/Water partitioning coefficient as a measure of the hydrophobicity of chemicals: K_{ow} is very easy to measure and predict



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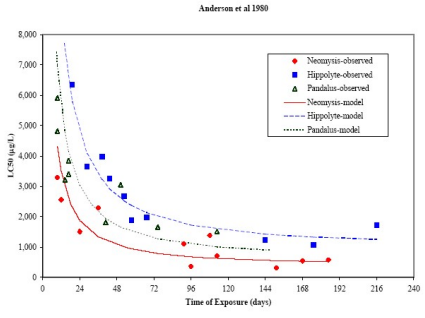
Don't forget about the exposure time...



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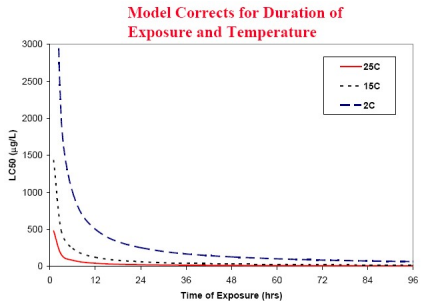
...and temperature in the environment



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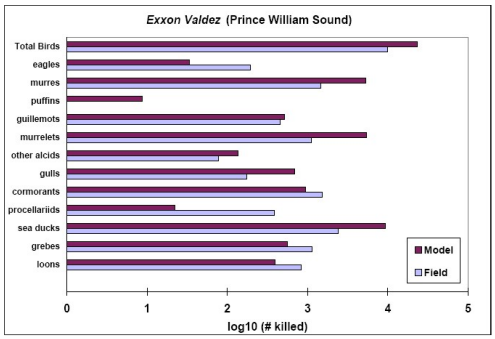
How does it work in reality?



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Summary

- Long-lived animals → λ elasticity analysis → **focus on the life story elements with the greatest impact on λ**
- In nature, toxic substances are rarely found individually → **many chemicals affect organisms at the same time**
- Toxic substances may interact → **antagonism or synergism possible**
- Studying multi-factor effects can be very costly → **complete *vs.* fractional experimental designs**
- Toxicity can sometimes be predicted with a satisfactory accuracy by means of modelling-based methods → ***K_{ow}*, QSAR, mixture toxicity models**

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