NoMiracle: Novel Methods for Integrated Risk Assessment of Cumulative Stressors in Europe

Society of Toxicology Teleseminar
13th June 2008

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Pete Kille, Cardiff University, Cardiff, UK
Steve Stürzenbaum, Kings College, London, UK
Jan Baas – Vrije Universiteit, Amsterdam, The Netherlands

Outline of the presentation:

- Science and Technology Objectives
- Research Pillar on effects : Some results
  - Mixture toxicity modelling
  - Toxicogenomics and “mode of action”
  - Dynamic energy budget models

- NoMiracle events and information

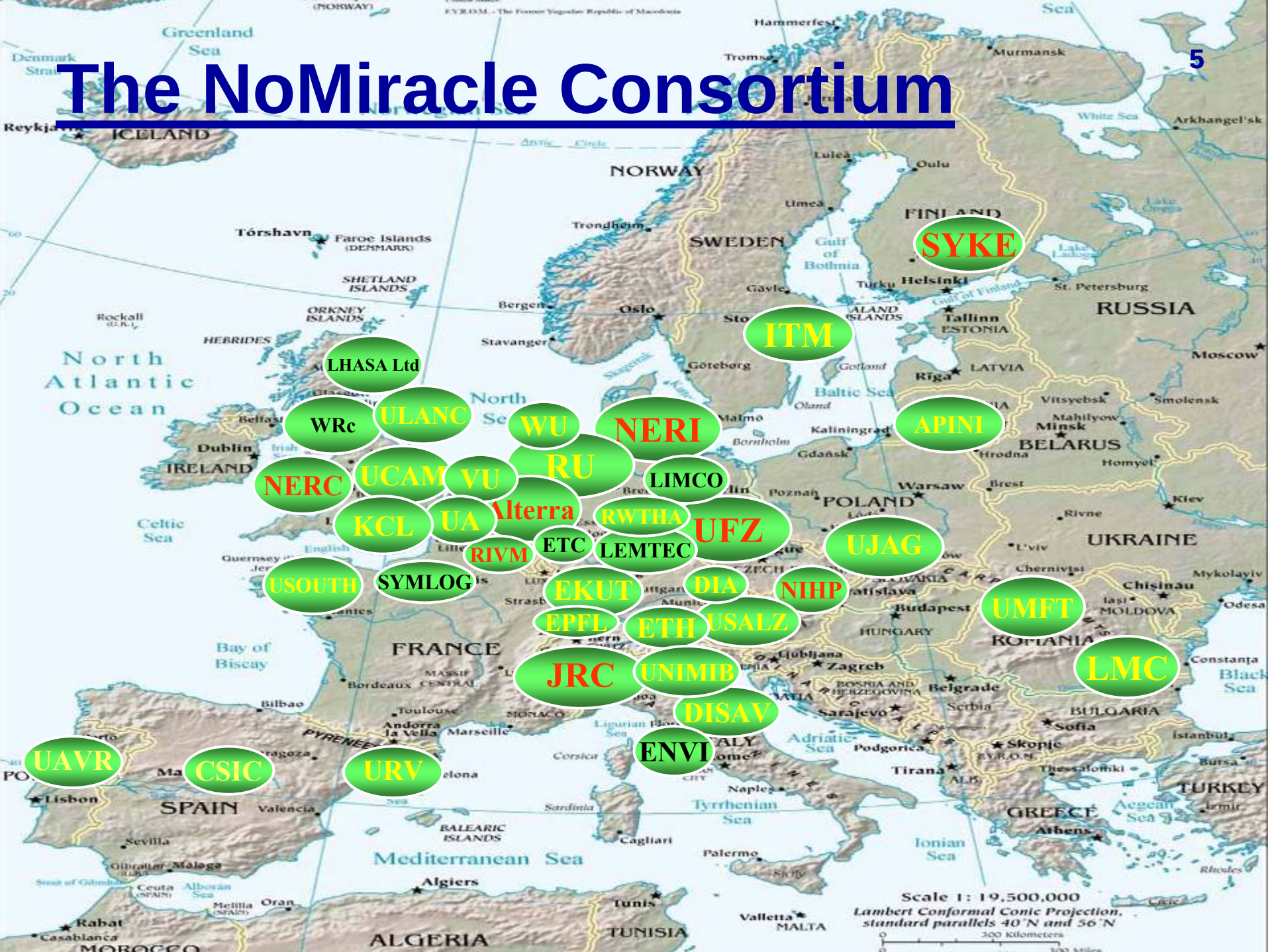
Science & technology objectives

1. Refine methods for assessing the cumulative risks from combined exposures to mixtures of chemical and physical/biological agents.
2. Integration of the risk analysis of environmental and human health effects. – Open workshop Frankfurt 8/9 Sept 2008
3. To improve understanding of and develop adequate tools for effect assessment.
4. To develop a research framework for the description and interpretation of cumulative exposure and effect.

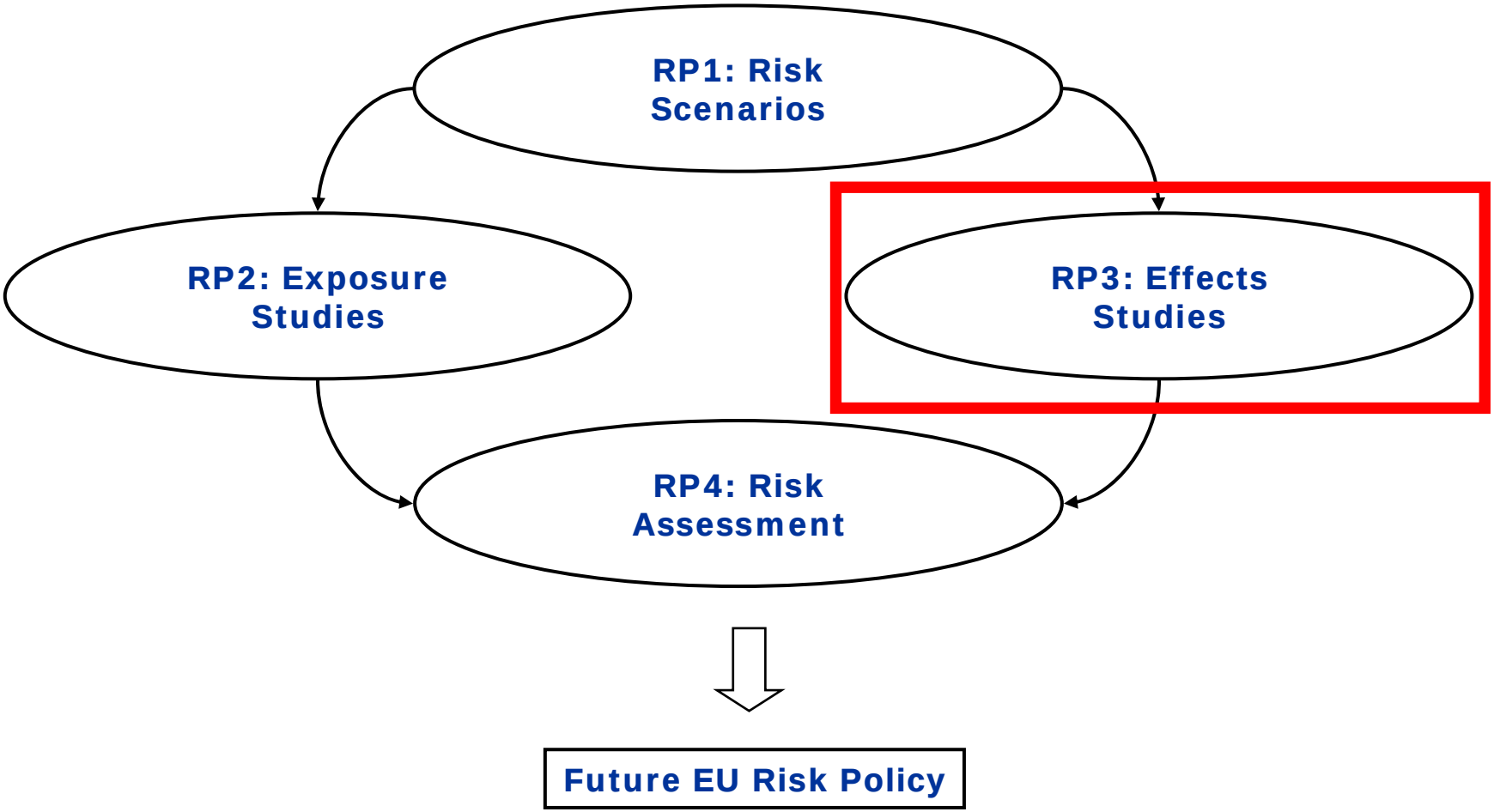
Science & technology objectives

5. Quantify, characterise and reduce uncertainty in current risk assessment methodologies, e.g. by improving scientific basis for safety factors.
6. To develop assessment methods which take into account geographical, ecological, social and cultural differences in risk concepts and risk perceptions across Europe
7. To improve the provisions for the application of the precautionary principle and to promote its operational integration with evidence-based assessment methodologies

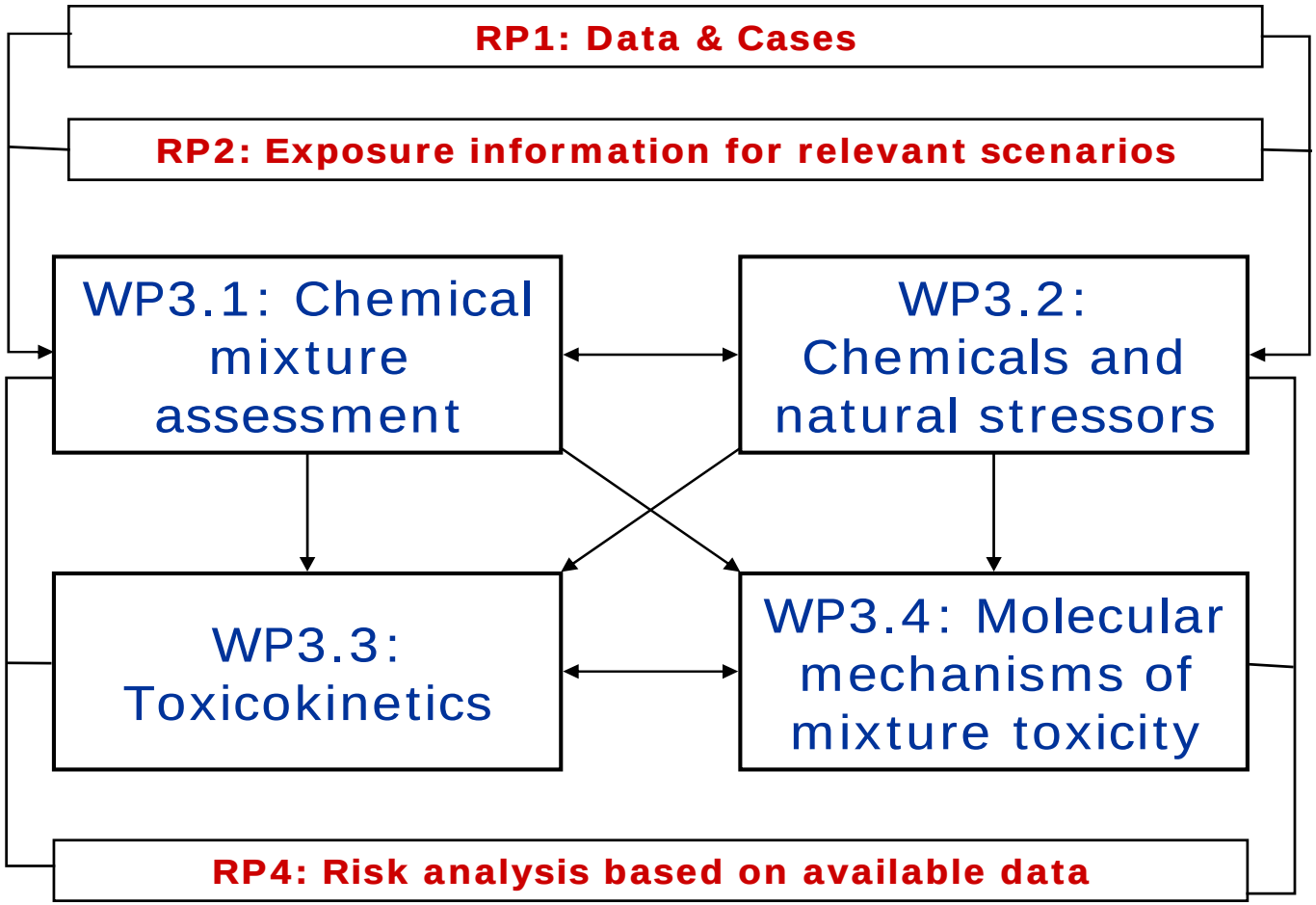
The NoMiracle Consortium



Consortium overall structure



Research Pillar 3 structure



Ecotoxicology = 75%, Human health toxicology = 25%

Science & technology objective 1

Refine methods for assessing the cumulative risks from combined exposures to mixtures of chemical and physical/biological agents.

Statistical tools for identification of interactions in mixtures

Slides from Claus Svendsen

Models for non-interacting mixtures

- Similar action model: (concentration addition)

$$M = \sum_{i=1}^{n} TU_i$$

$$TU_i = \frac{[c_i]}{[ECx_i]}$$

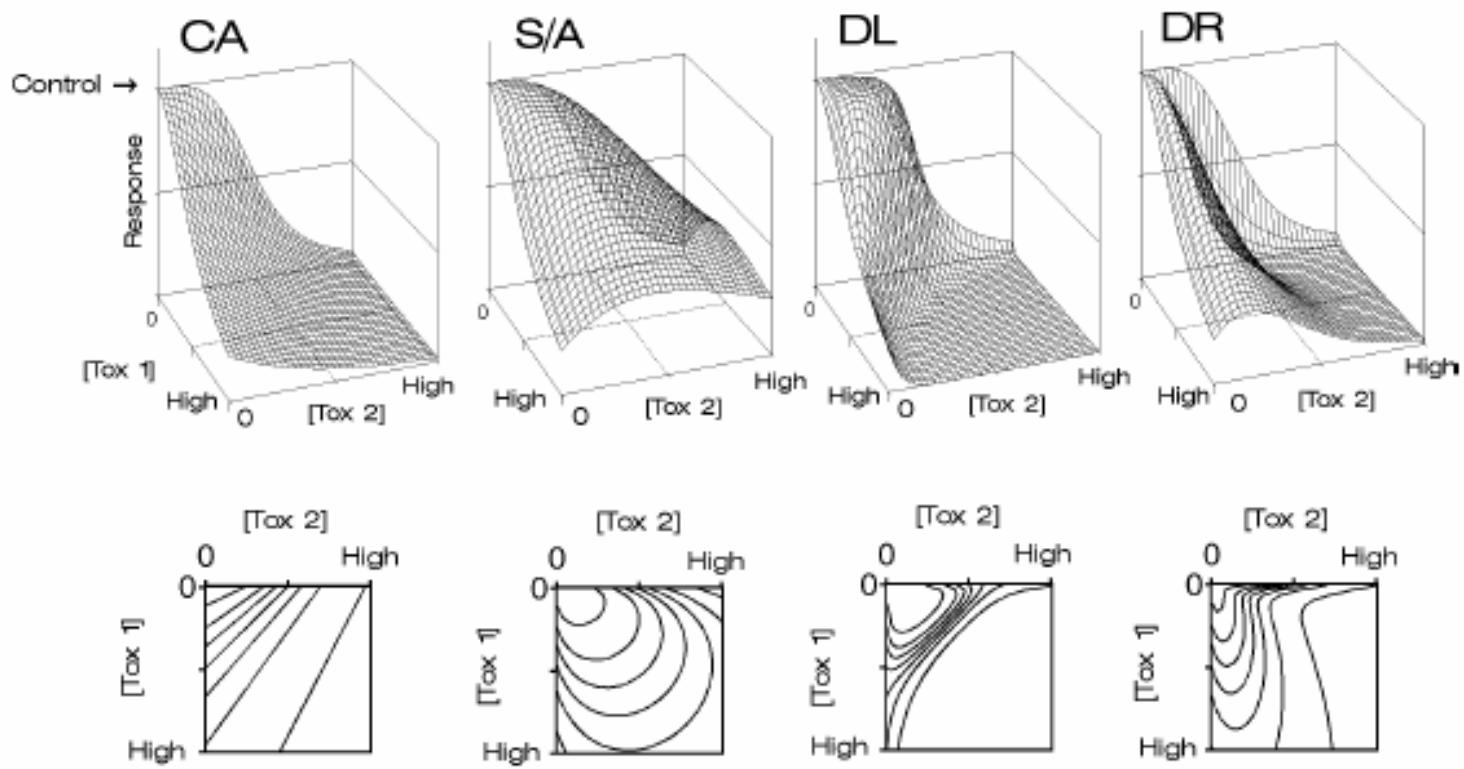
- Dissimilar action model: (independent action)

$$h(c_1, c_2, \ldots, c_n) = \prod_{i=1}^{n} h(c_i)$$

Extend the reference models by adding "deviation" parameters

Add parameter "a" Add parameter "b"

1) CA & IA → 2) S/A → 3A) DL or 3B) DR



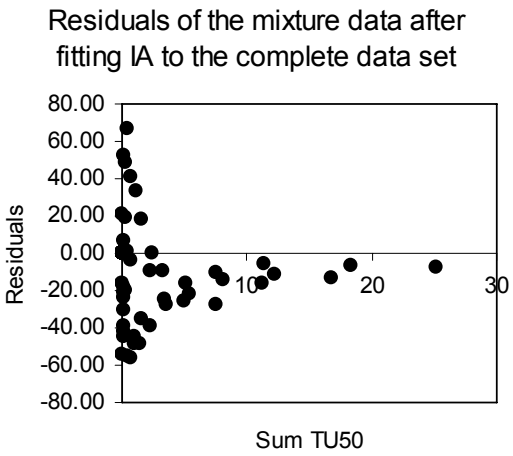
The nesting allows statistical testing for best fit (using likelihood theory)

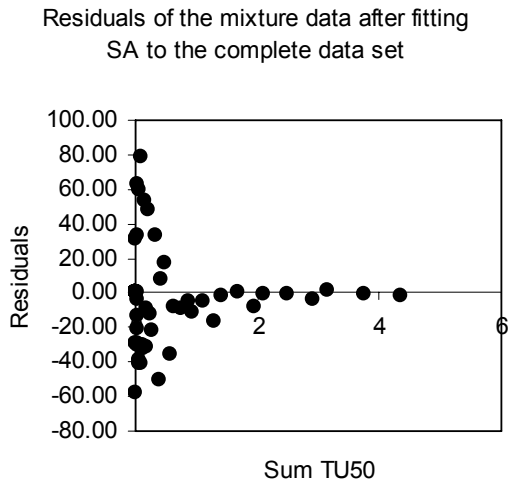
(Jonker et al ET&C 2005)

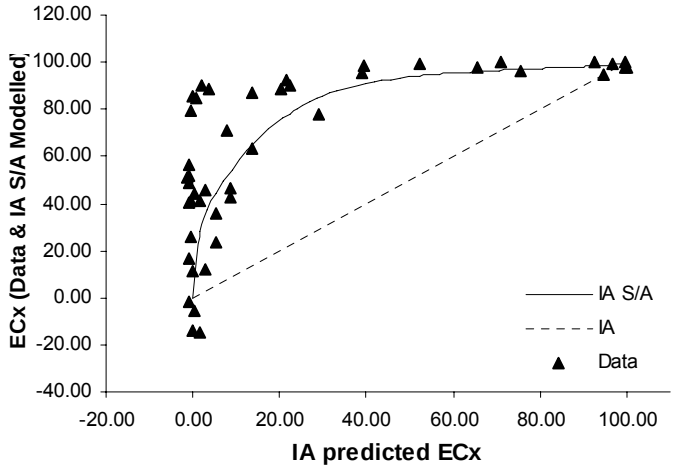
An example: Cd + diuron in *C. elegans*

Predict ECx values by IA and compare to data

IA residuals **IA + S/A residuals** **Model fits**







Building a database of binary mixture studies ~150 (collaboration with Nina Cedergreen University of Copenhagen)

Species from bacteria to (in vitro) mammalian systems

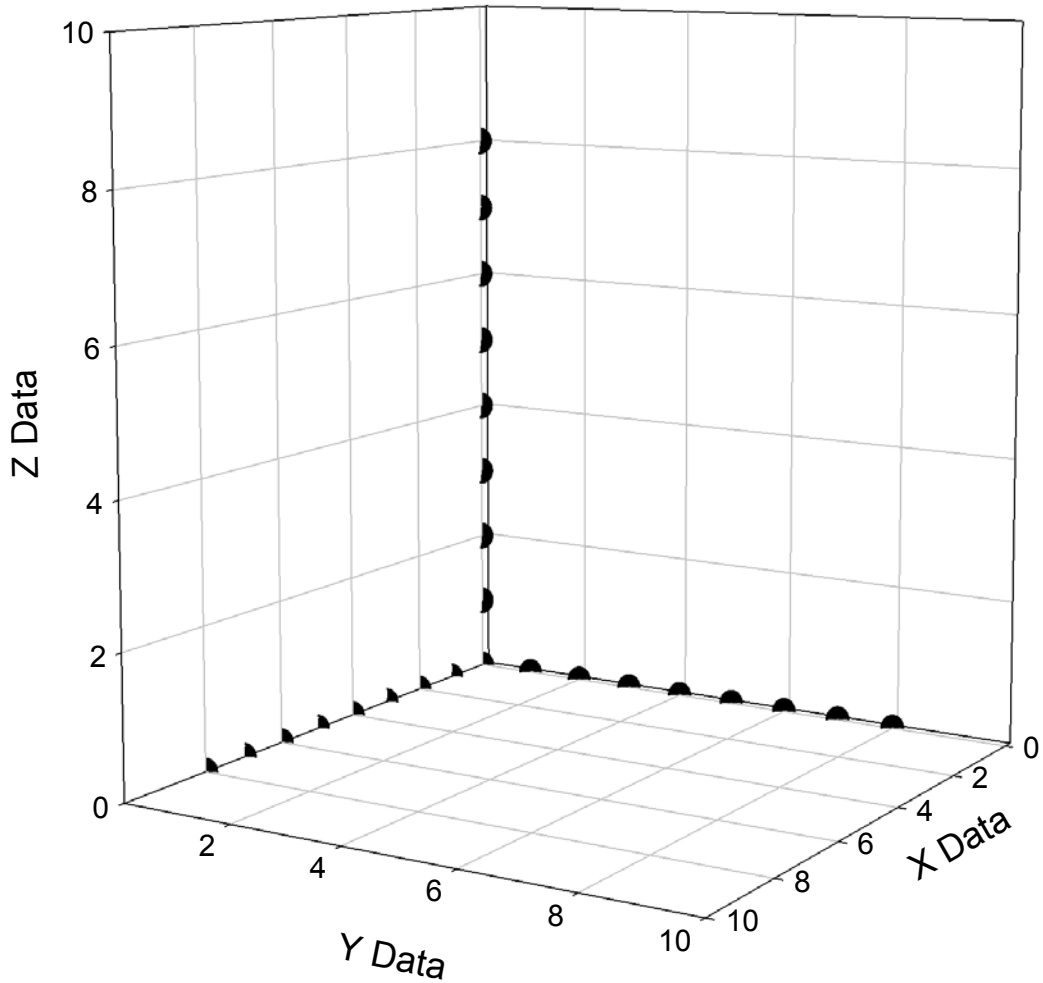
Define the distribution of maximum deviations e.g. EC_{50} gives EC_x

So far binary – what about more complex?

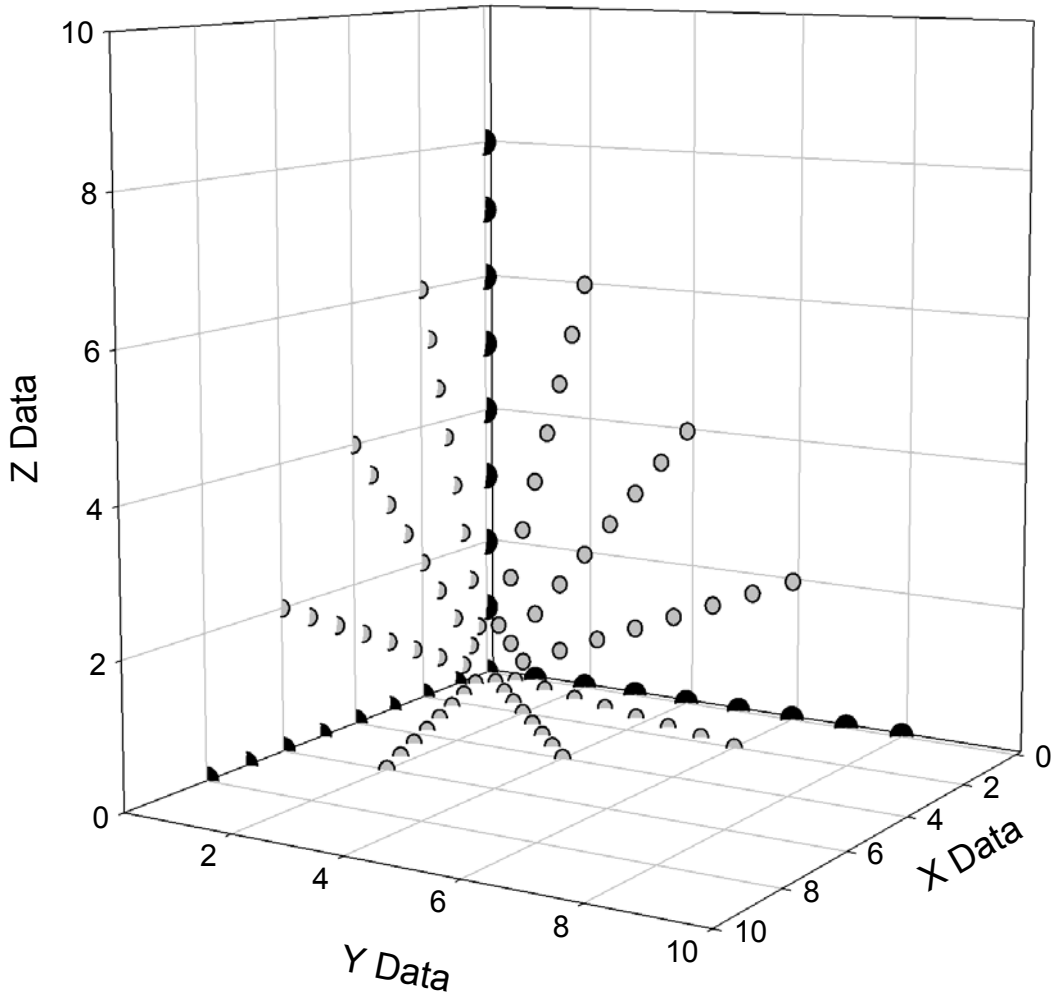
Ternary mixture modelling - approach

- Build from binary mixtures knowledge
- Predict the level of deviation when mixing 3 chemicals with known binary interactions
- So far only CA and only synergism or antagonism (not dose level or dose ratio)

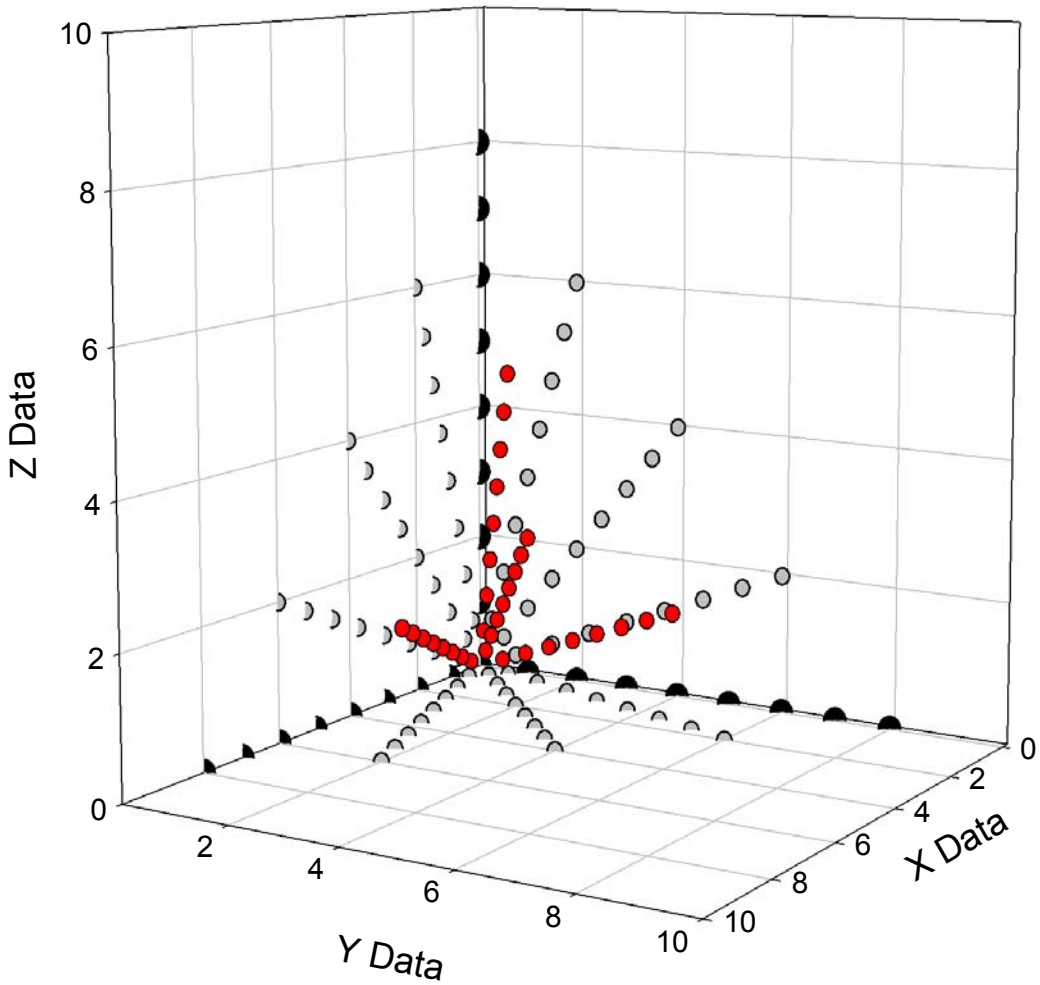
The Experimental design Singles



The Experimental design
Singles – Binary

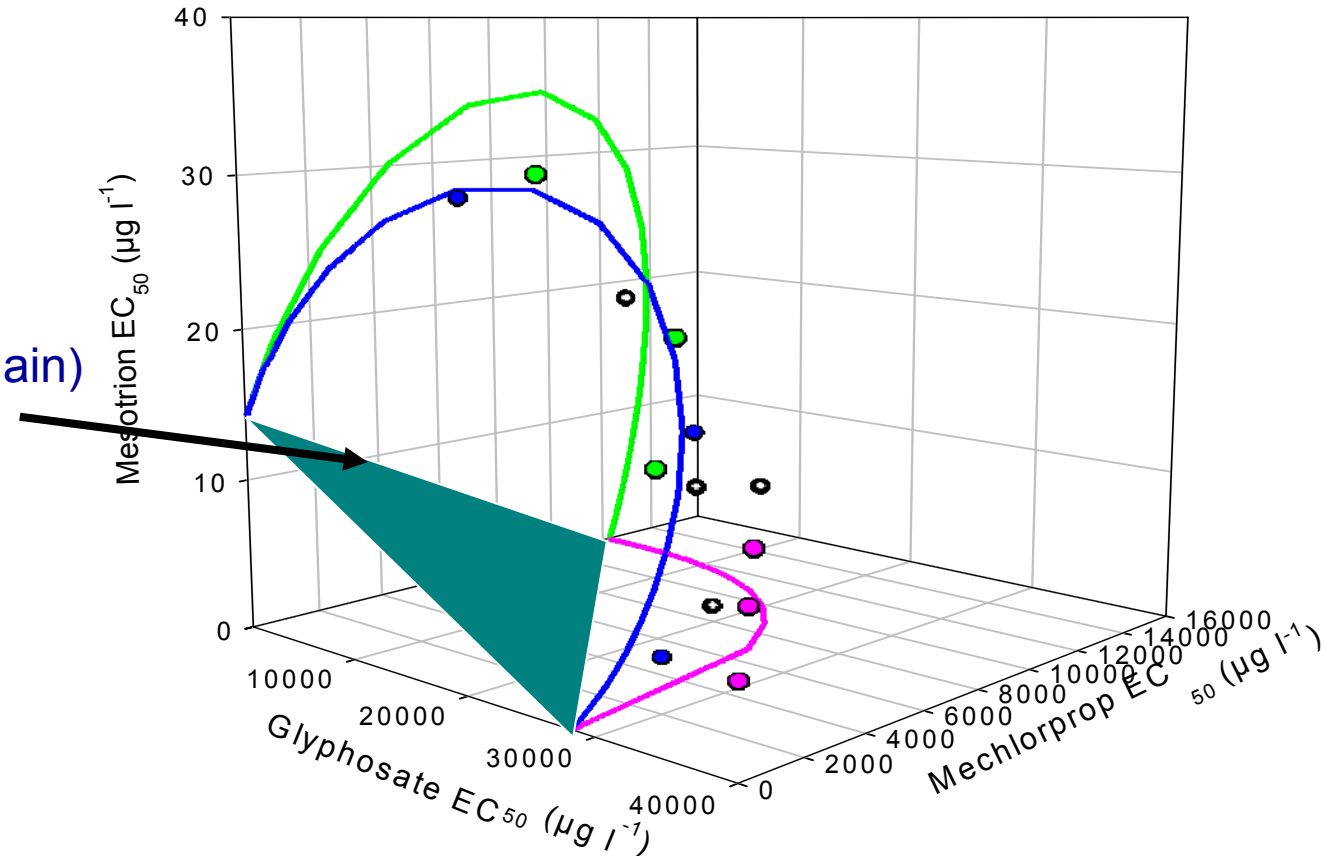


The Experimental design
Singles – Binary - Ternary

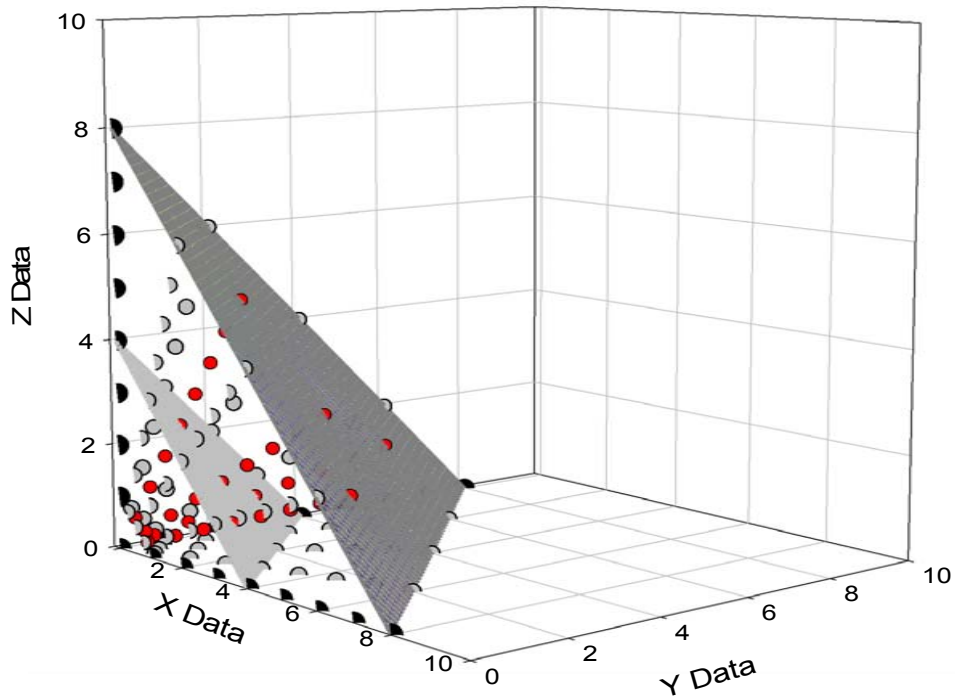


Data analysis procedure –2 ways
1) Isobols & two step regression

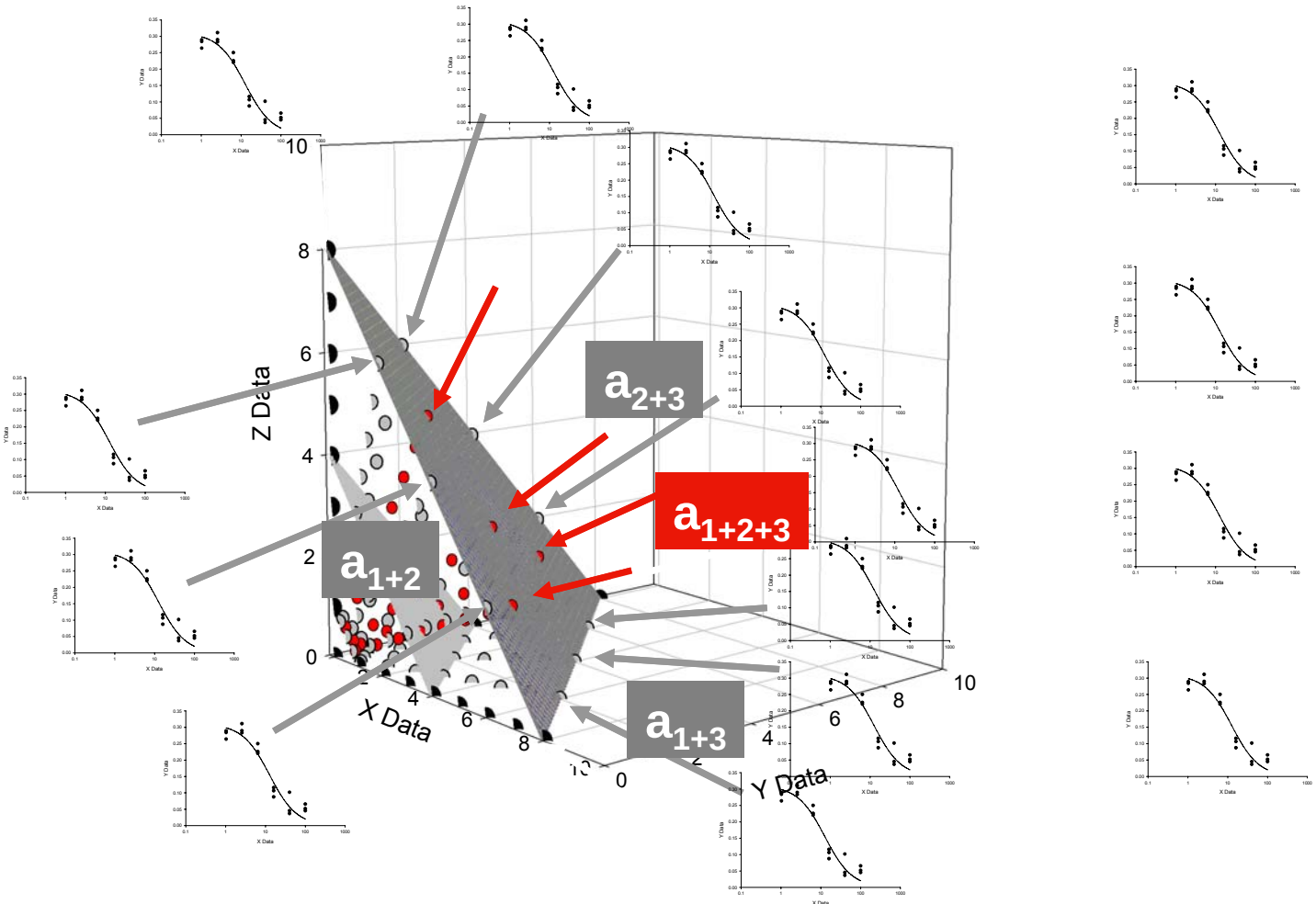
CA (reference plain)



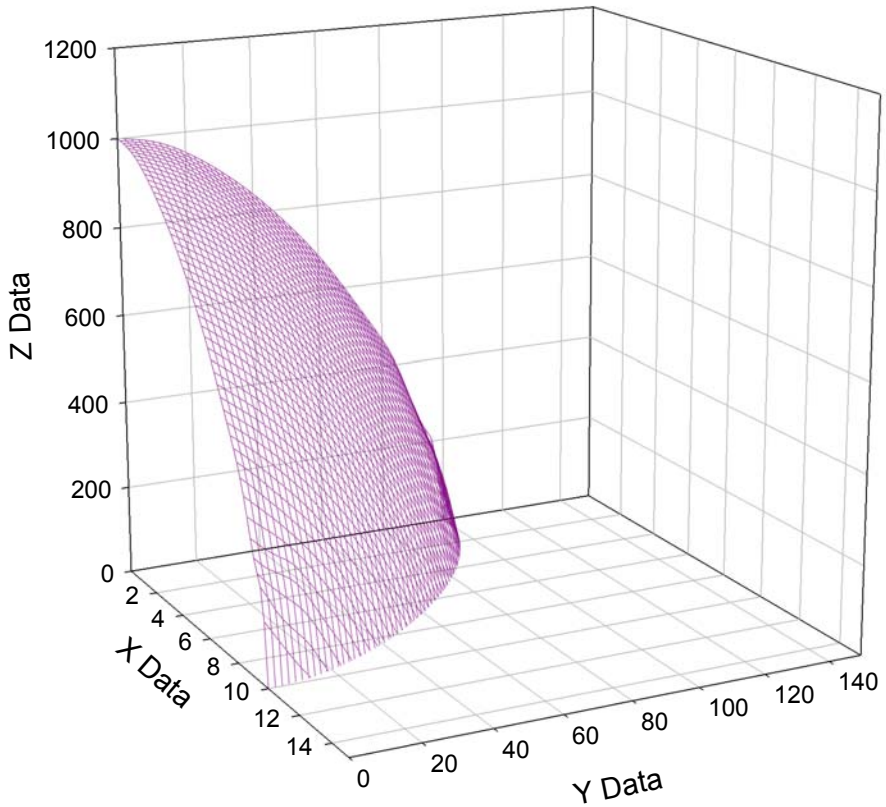
Data analysis procedure – 1) Isobols & two step regression



Data analysis procedure – 1) Isobol & two step regression

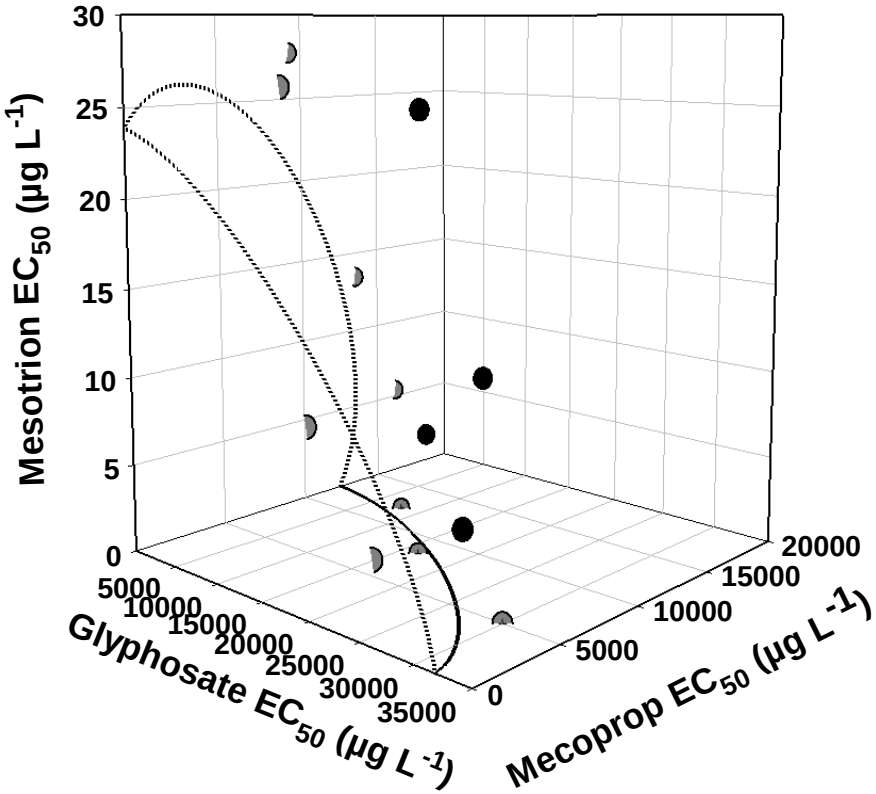


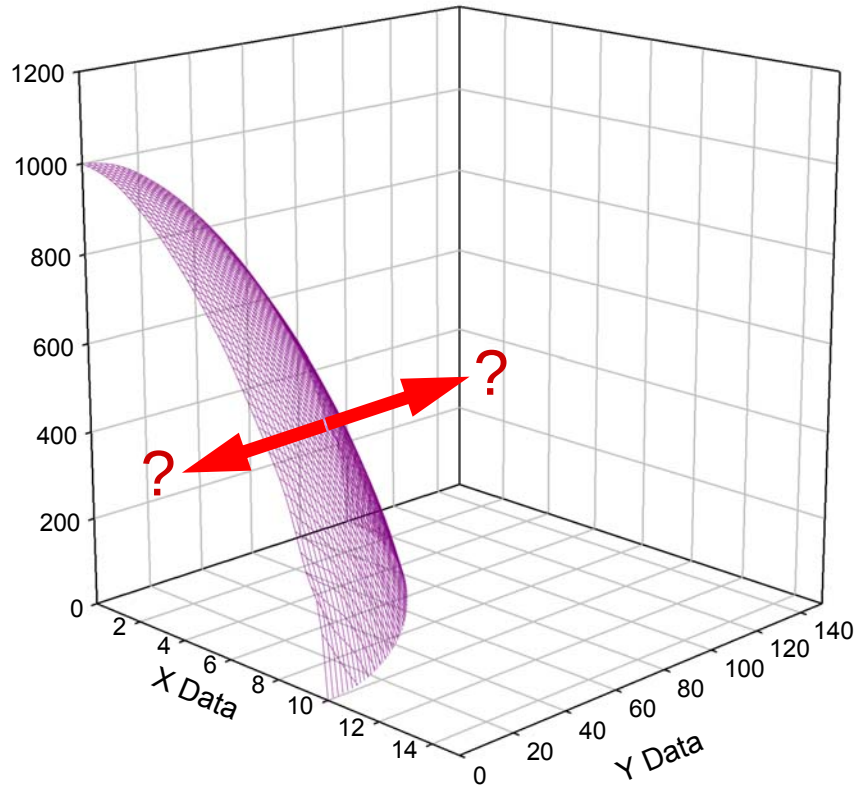
Visualisation of response surface



Antagonism

Results – *Lemna* antagonism for three herbicides



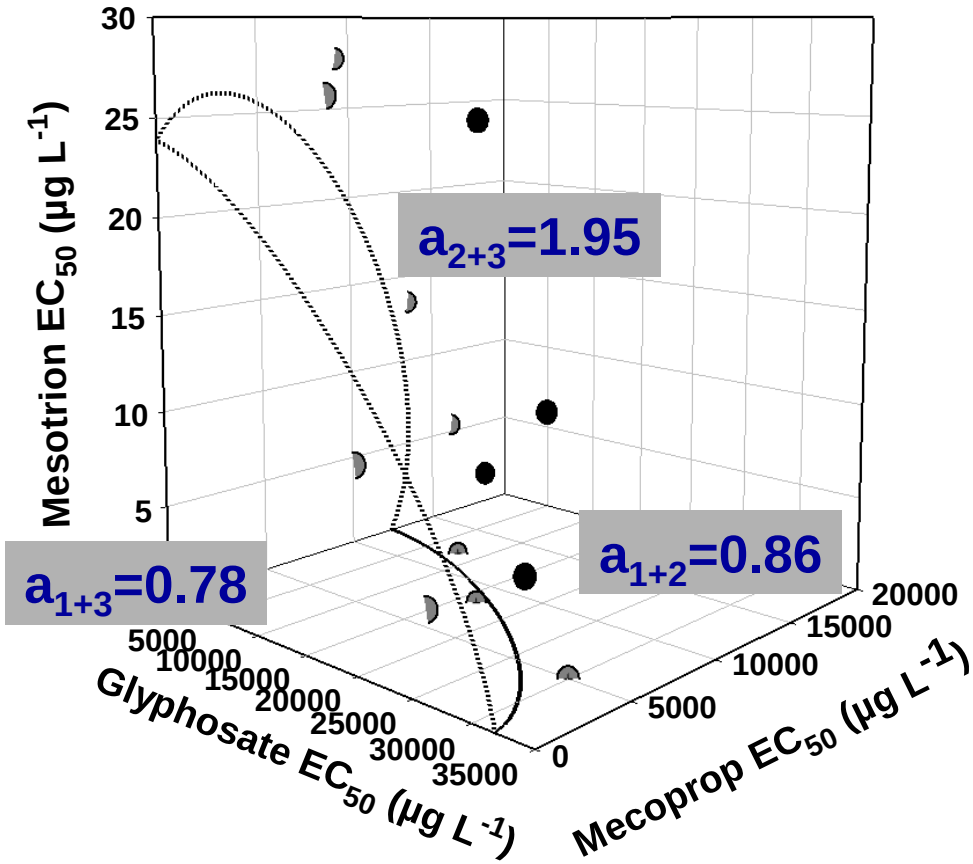


Data provided by Dr Nina Cedergreen University of Copenhagen, Denmark

Results - *Lemna* for three herbicides

Glyphosate – mecoprop - mesotrion

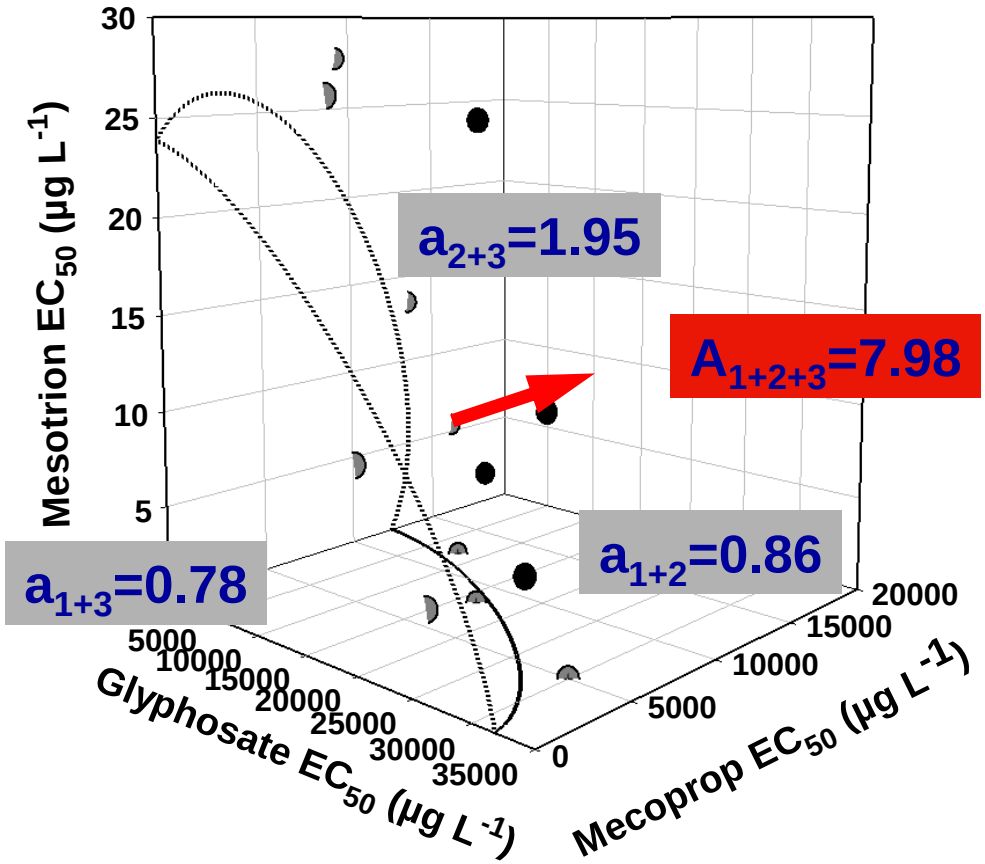
Binary interactions



Results - *Lemna*

Glyphosate – mecoprop - mesotrion

Ternary interactions

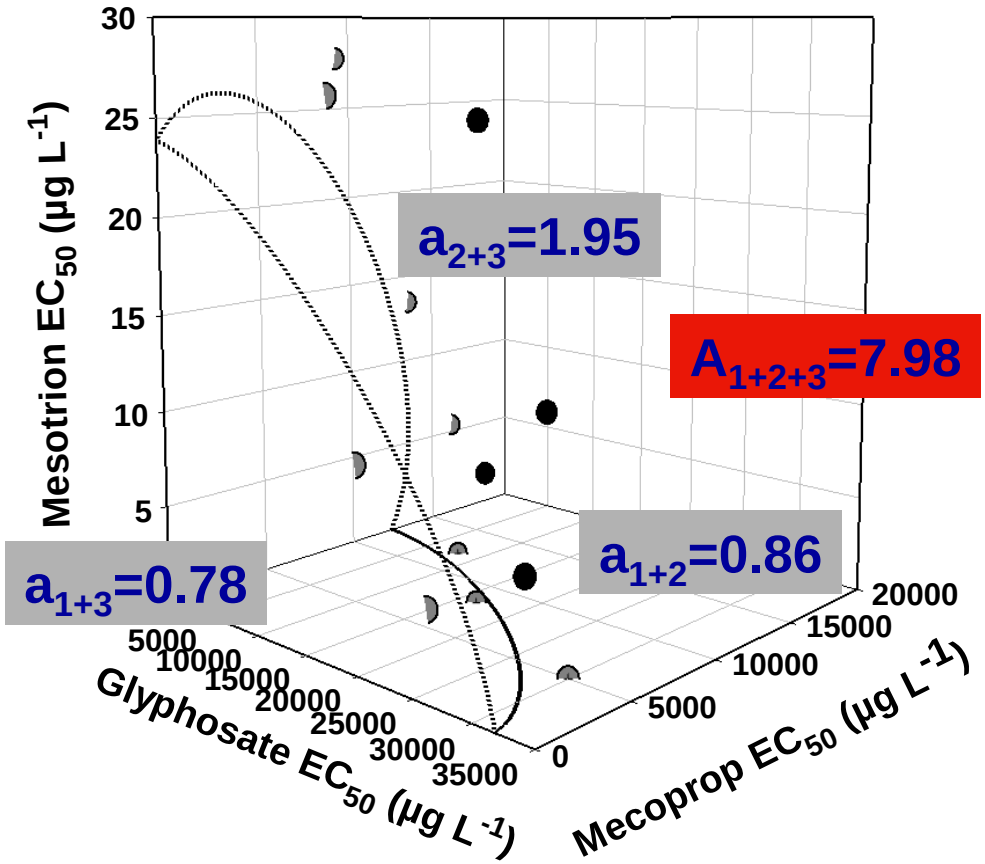


Results - *Lemna*

Glyphosate – mecoprop - mesotrion

Conclusion:
The ternary mixture is more antagonistic than the combination of binary antagonisms predict.

Question:
How big is the under prediction and what is the deviation from CA??



Results – Summary for *Lemna* studies

Chemicals			ΣTU at max. dev.		
Chemical 1	Chemical 2	Chemical 3	bin.	CA SA	CA SA+
Lemna minor:					
Glyphosate	Mecoprop	Mesotrion	1.97	2.89	2.84
Glyphosate	Mecoprop	Mesotrion	1.63	1.63	2.03
Glyphosate	Mecoprop	Terbuthylazine	1.41	1.41	1.45
Glyphosate	Mecoprop	Terbuthylazine	1.64	1.64	2.04
Terbuthylazine	Mesotrion	Mecoprop	2.19	2.19	2.19
Terbuthylazine	Mesotrion	Mecoprop	1.25	1.25	1.78
Diquat	Acifluorfen	Mesotrion	2.22	2.22	2.19
Diquat	Acifluorfen	Mesotrion	1.66	1.87	1.74

Lemna (Antagonism): For ternary if:

1) CA SA > IA = antagonism in the ternary mixture
2) ΣTU CA SA < ΣTU CA SA+ = magnitude of maximum deviation in the ternary greater than predicted based on antagonisms from the binaries.

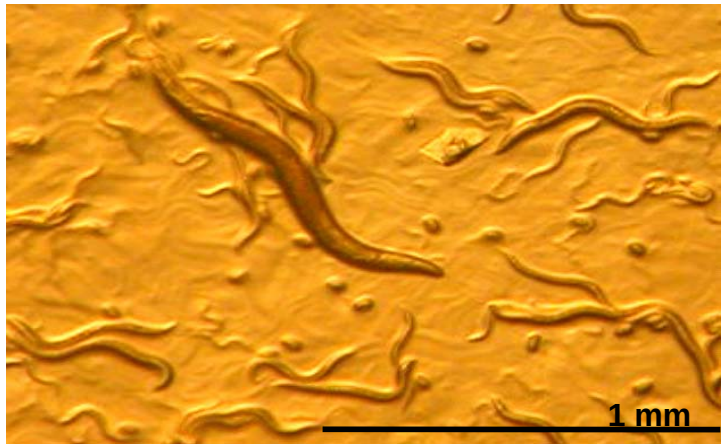
Some ternary deviations greater than predictions for antagonism (some less). Not yet found for synergism

Science & technology objective 3

To improve understanding of and develop adequate tools for sound exposure assessment.

Toxicogenomic tools in model and non-model species

...the species...



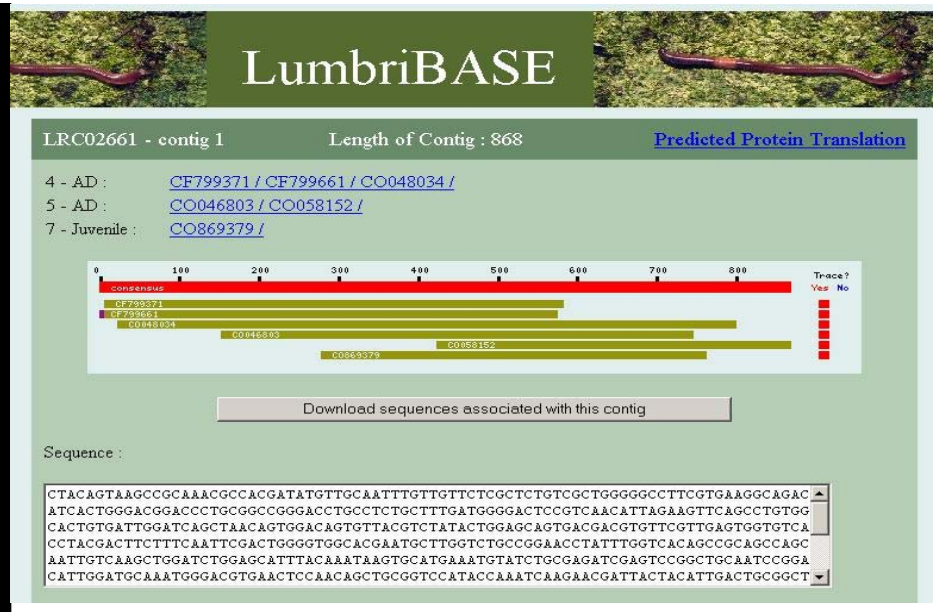
Lumbricus rubellus

- Free-living in the soil
- Detritivorous
- Non-self-fertilizing, hermaphrodite

Caenorhabditis elegans

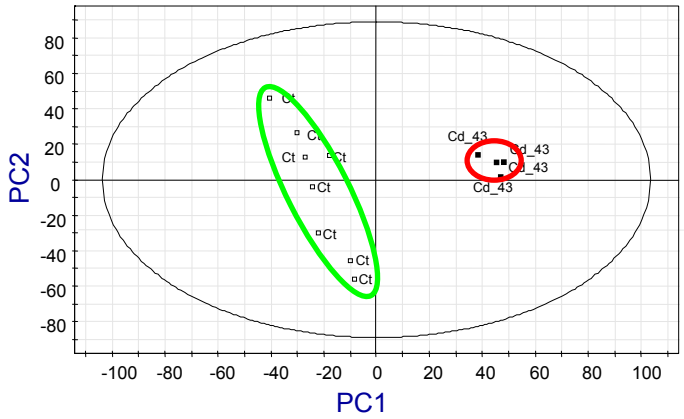
- Free-living nematode - lives in the soil
- Bacterivorous
- Self-fertilizing, hermaphrodite

Getting the bits of earthworm DNA - ESTs

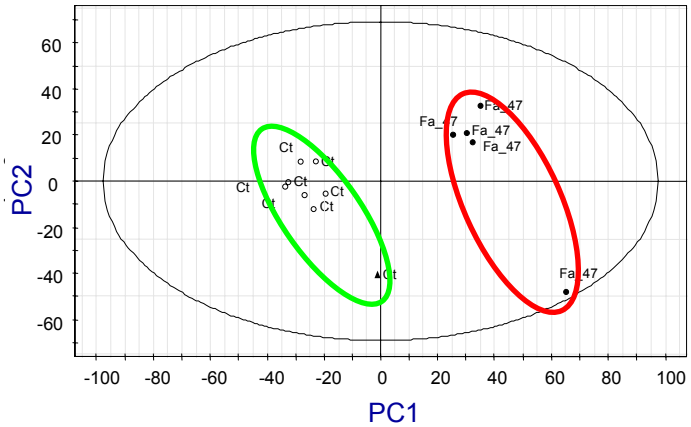


Patterns of effect

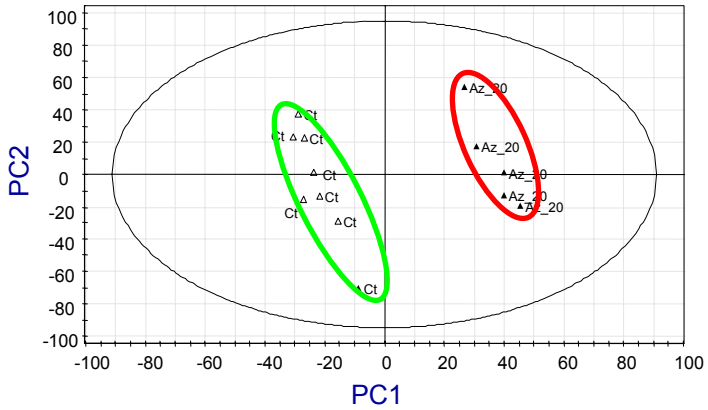
Cd



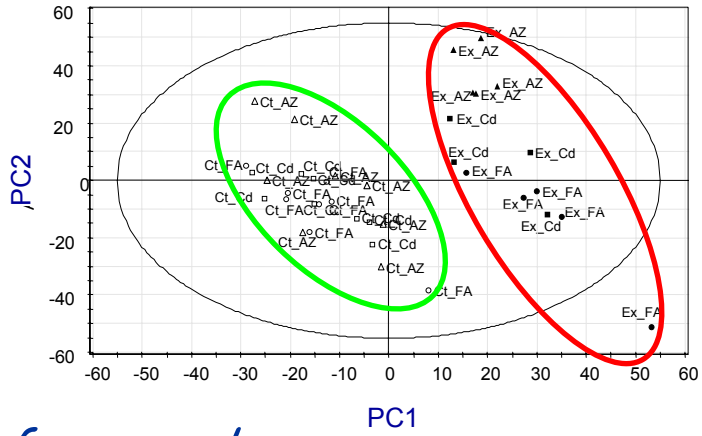
Fluoranthene



Atrazine

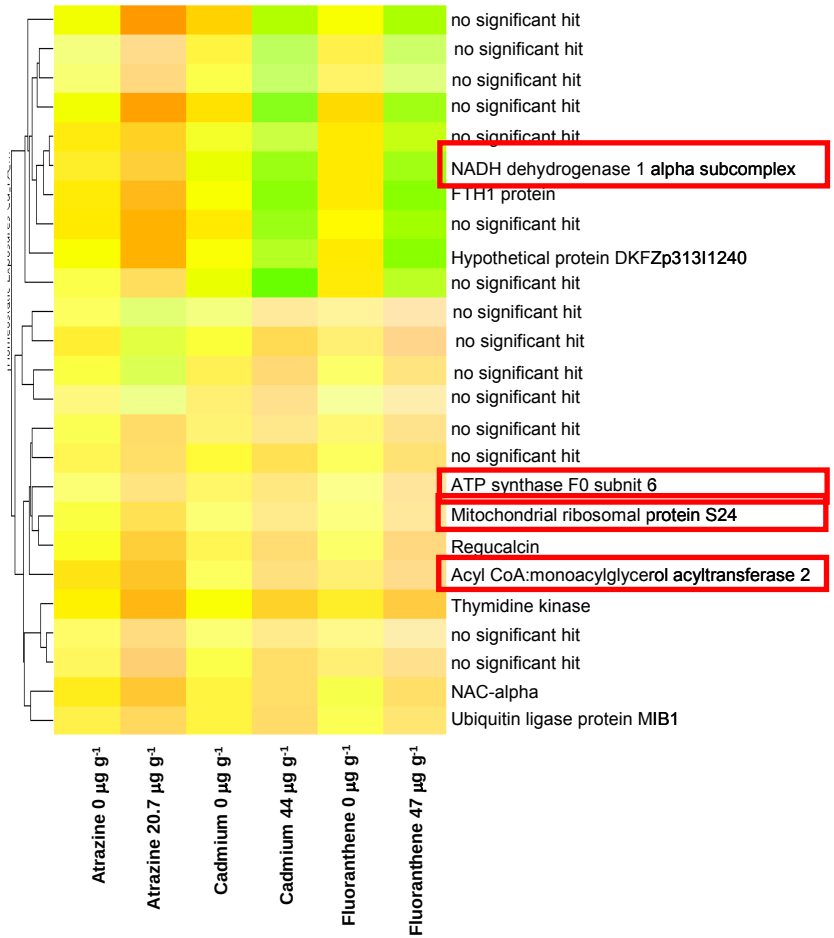


All exposed / controls



Low dose = repro. EC_{10} = different from no dose

Causes



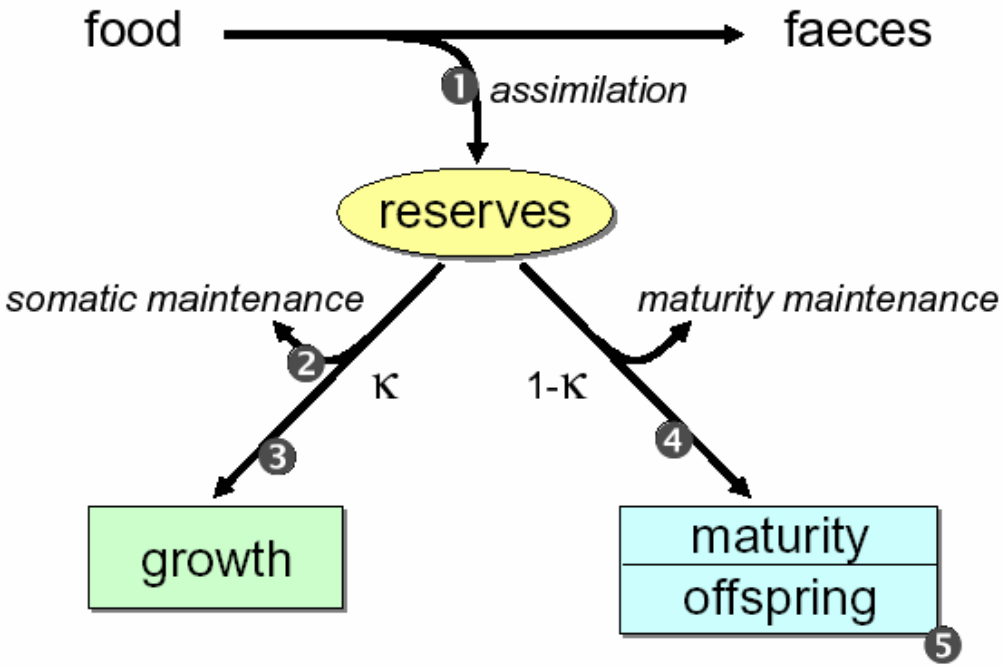
Many unknowns

Some hypotheticals

Some other pathways e.g. protein degradation….

….but strong mitochondrial signature

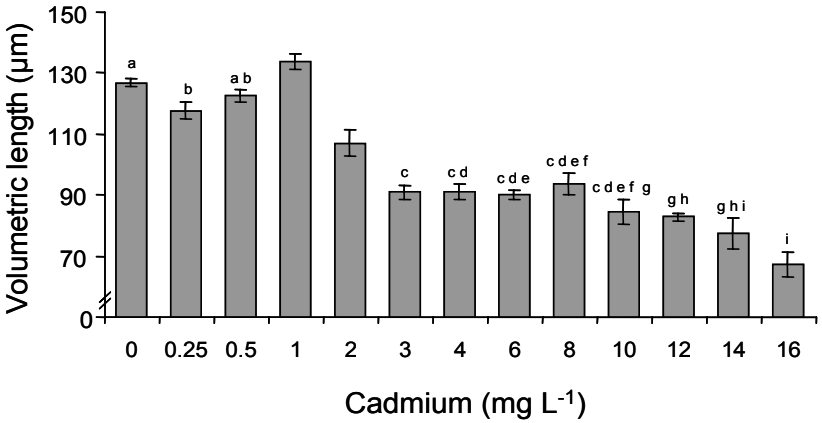
Resource allocation –dynamic energy budget



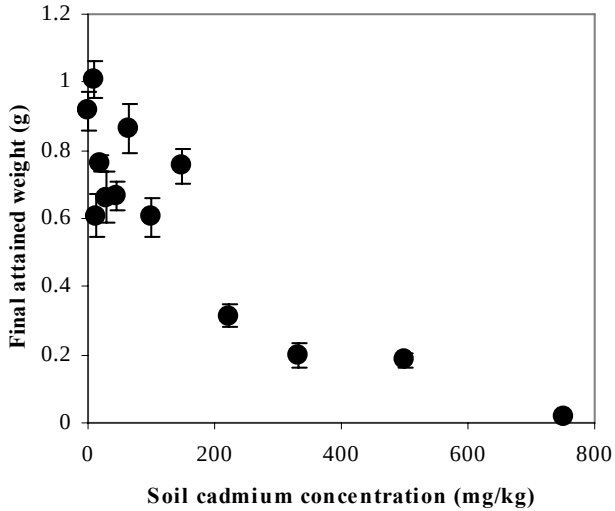
1. assimilation
2. maintenance costs
3. growth costs
4. reproduction costs
5. hazard to embryo

Cadmium DEB effect– assimilation

Nematodes



Earthworms

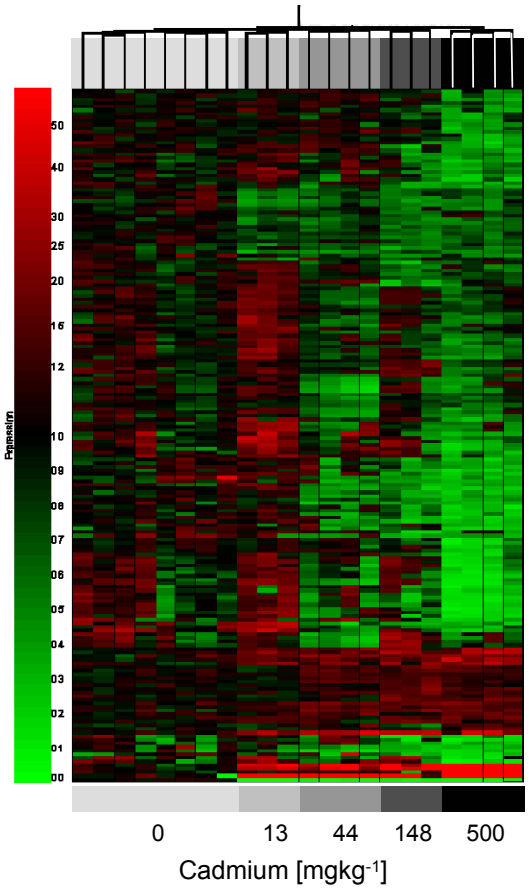


Dose effect

Dose effect

Cd changes on gene expression - nematodes

Condition tree

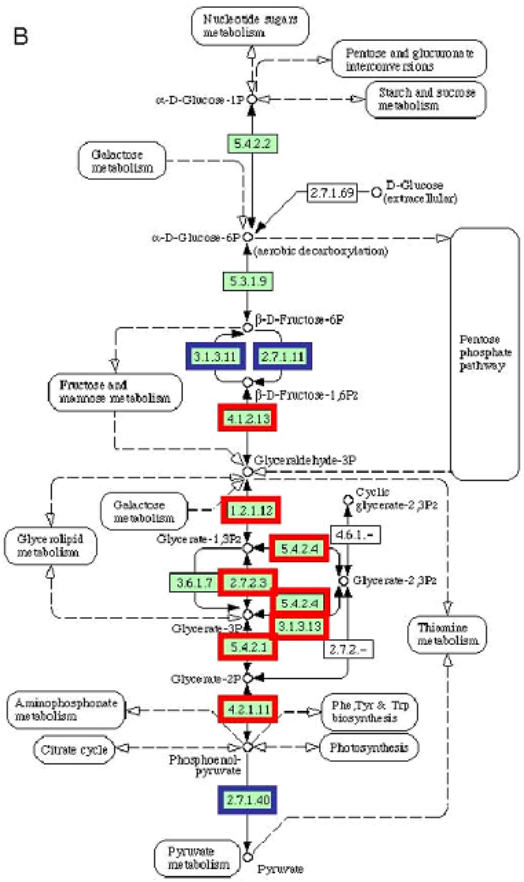


Significant pathways

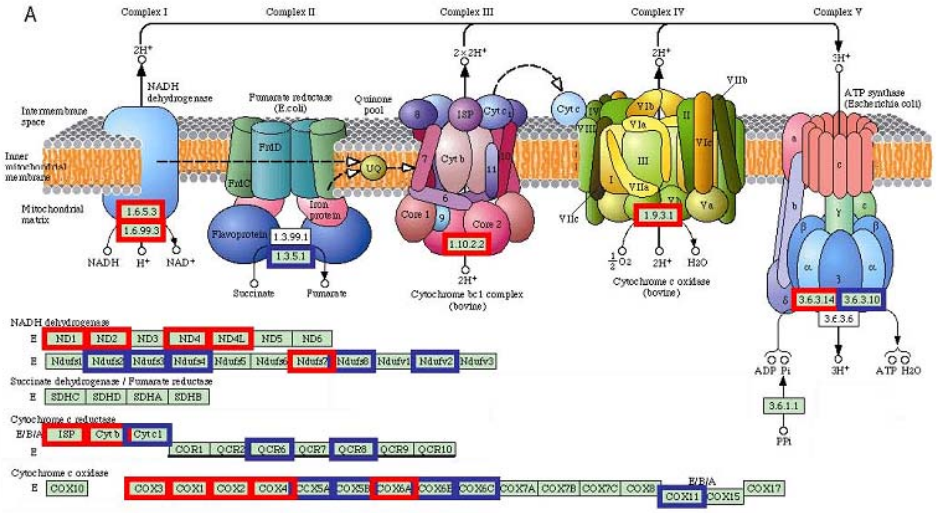
Biological process	Genes in Category	Genes in List in Category	p-Value
GO:31323: regulation of cellular metabolism	620	58	0.00211
GO:19222: regulation of metabolism	625	58	0.00254
GO:51244: regulation of cellular physiological process	641	59	0.00281
GO:50794: regulation of cellular process	641	59	0.00281
GO:50791: regulation of physiological process	642	59	0.00291
GO:19219: regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	618	57	0.00316
GO:6350: transcription	635	58	0.00361
GO:45449: regulation of transcription	615	56	0.00451
GO:6629: lipid metabolism	109	14	0.00975
GO:44238: primary metabolism	2438	181	0.0107
GO:8643: carbohydrate transport	22	5	0.0114
GO:6355: regulation of transcription, DNA-dependent	570	50	0.0141
GO:6351: transcription, DNA-dependent	581	50	0.0195
GO:6139: nucleobase, nucleoside, nucleotide and nucleic acid metabolism	1024	81	0.0253
GO:7155: cell adhesion	46	7	0.0262
GO:50789: regulation of biological process	2085	153	0.03
GO:50875: cellular physiological process	3791	265	0.0326
GO:8152: metabolism	2770	198	0.0342
GO:7582: physiological process	4588	316	0.0343
GO:44237: cellular metabolism	2558	183	0.0417
GO:6470: protein amino acid dephosphorylation	97	11	0.0461
GO:44257: cellular protein catabolism	22	4	0.049
GO:51603: proteolysis during cellular protein catabolism	22	4	0.049
GO:19941: modification-dependent protein catabolism	22	4	0.049
GO:6511: ubiquitin-dependent protein catabolism	22	4	0.049
GO:6631: fatty acid metabolism	22	4	0.049
GO:16311: dephosphorylation	98	11	0.0491

Cd changes on gene expression - earthworms

Glycolysis



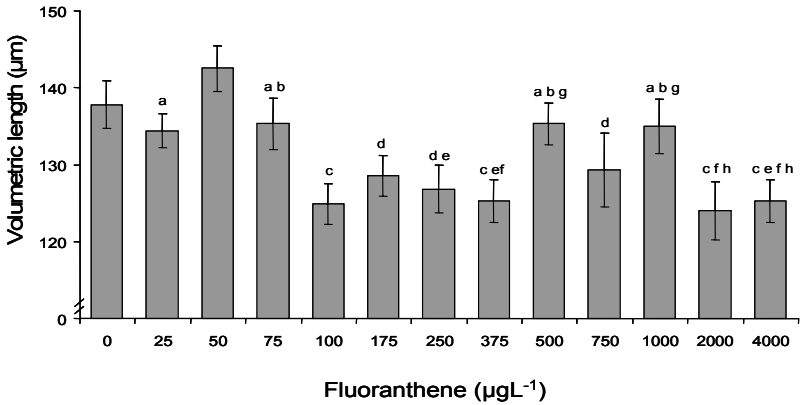
Electron transport chain



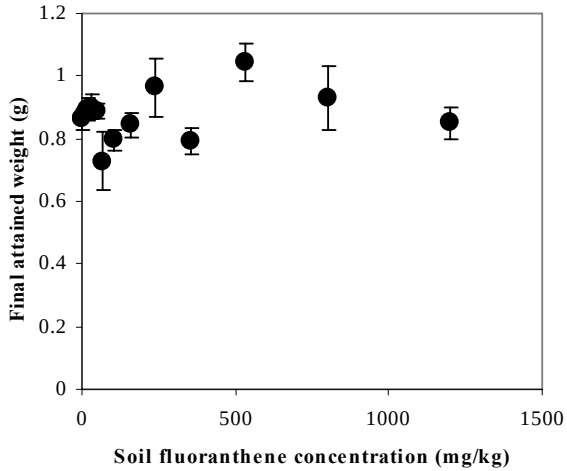
- Lots of metabolic genes represented on array
- Many change expression

Fluoranthene DEB effect – somatic and maturity maintenance

Nematodes



Earthworms



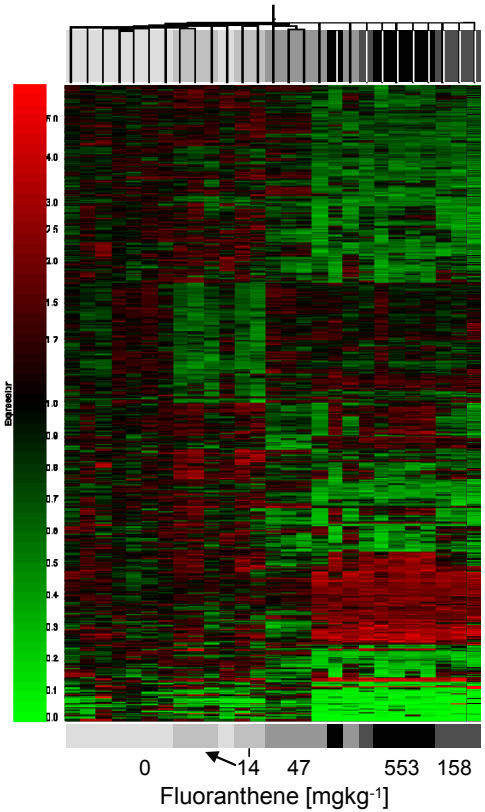
Dose effect

Dose effect

Dose effect

Fluoranthene changes on gene expression

Condition tree

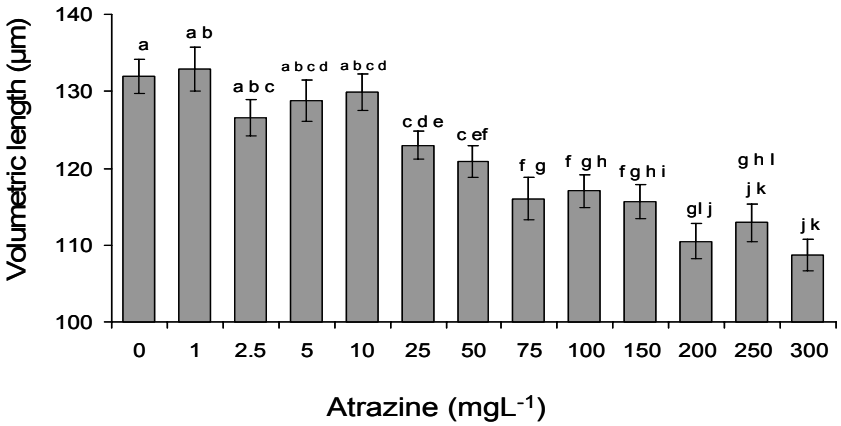


Significant pathways

Biological process	Genes in Category	Genes in List in Category	p-Value
GO:9309: amine biosynthesis	20	7	0.000291
GO:44271: nitrogen compound biosynthesis	20	7	0.000291
GO:6629: lipid metabolism	109	18	0.000573
GO:6082: organic acid metabolism	112	17	0.0021
GO:19752: carboxylic acid metabolism	112	17	0.0021
GO:6397: mRNA processing	29	7	0.00336
GO:16071: mRNA metabolism	29	7	0.00336
GO:6519: amino acid and derivative metabolism	89	13	0.00929
GO:6520: amino acid metabolism	80	12	0.00993
GO:6118: electron transport	211	24	0.0136
GO:6631: fatty acid metabolism	22	5	0.0166
GO:6605: protein targeting	30	6	0.0167
GO:6396: RNA processing	52	8	0.0282
GO:6807: nitrogen compound metabolism	106	13	0.0354
GO:9308: amine metabolism	106	13	0.0354
GO:16070: RNA metabolism	88	11	0.0448

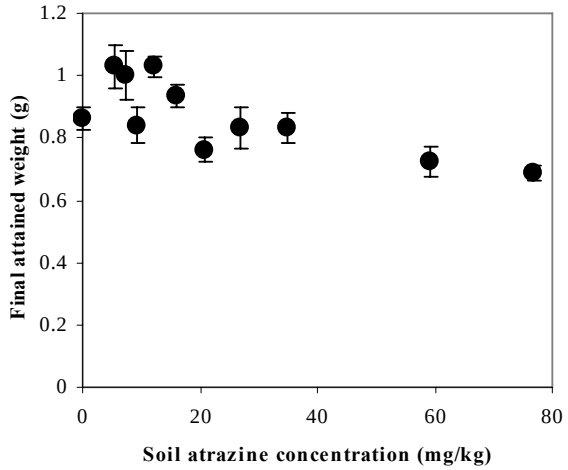
Effect on body size? - Atrazine

Nematodes



Dose effect

Earthworms



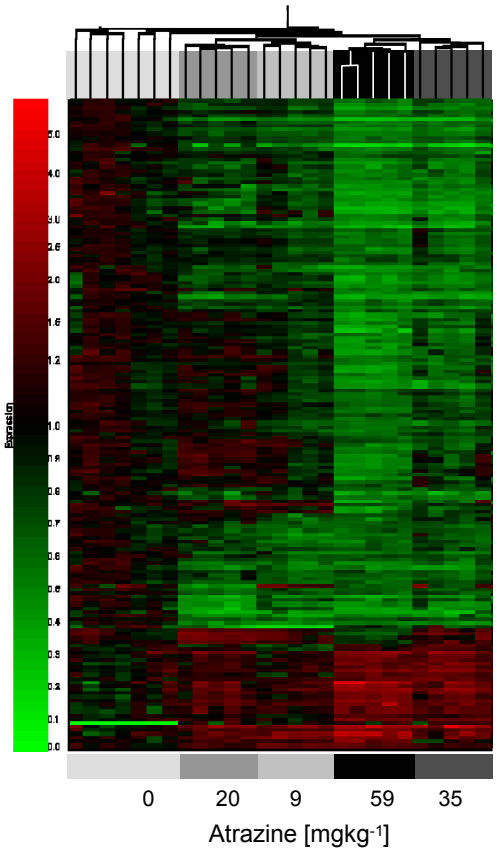
Dose effect

Atrazine DEB effect - assimilation

Dose effect

Atrazine changes on gene expression

Condition tree



Significant pathways

Biological process	Genes in Category	Genes in List in Category	p-Value
GO:7275: development	2666	186	3.13E-05
GO:9791: post-embryonic development	1491	110	0.000348
GO:9792: embryonic development (sensu Metazoa)	2072	145	0.000357
GO:9790: embryonic development	2074	145	0.000373
GO:2164: larval development	1376	102	0.000512
GO:2119: larval development (sensu Nematoda)	1376	102	0.000512
GO:3: reproduction	1220	91	0.000901
GO:42303: molting cycle	97	12	0.00637
GO:18988: molting cycle (sensu Protostomia and Nematoda)	97	12	0.00637
GO:6259: DNA metabolism	200	20	0.0064
GO:31323: regulation of cellular metabolism	620	48	0.00832
GO:50789: regulation of biological process	2085	136	0.00942
GO:19222: regulation of metabolism	625	48	0.00961
GO:45449: regulation of transcription	615	47	0.0113
GO:19219: regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabc	618	47	0.0123
GO:51244: regulation of cellular physiological process	641	48	0.0149
GO:50794: regulation of cellular process	641	48	0.0149
GO:50791: regulation of physiological process	642	48	0.0153
GO:6355: regulation of transcription, DNA-dependent	570	43	0.0184
GO:6350: transcription	635	47	0.0193
GO:6351: transcription, DNA-dependent	581	43	0.0246
GO:40007: growth	1856	119	0.0261
GO:8643: carbohydrate transport	22	4	0.0294
GO:9059: macromolecule biosynthesis	268	22	0.0364
GO:44249: cellular biosynthesis	408	31	0.0382

Conclusions

- Common effect on expression of metabolic genes (in the mitochondria)
- Toxicity through resource allocation (AKA the energy budget)
- Can use a combination of ecological models and transcript analysis to define exact link between toxicological and physiological "modes of action" for chemicals.

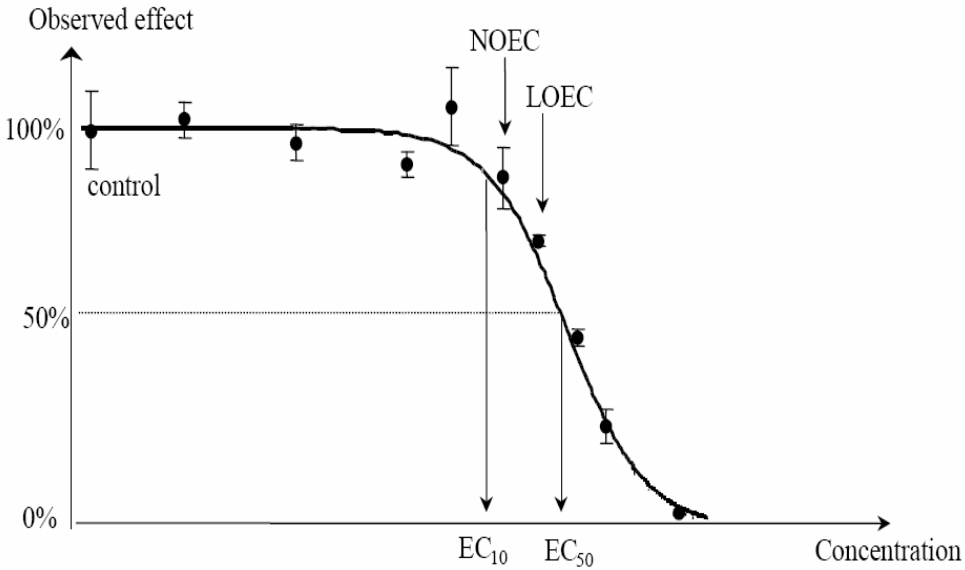
Science & technology objective 4

To develop a research framework for the description and interpretation of cumulative exposure and effect.

Dynamic energy budget toxicity (DEBtox) models for mixtures.

Slides from Jan Baas

LC_{50}, NOEC, LOEC



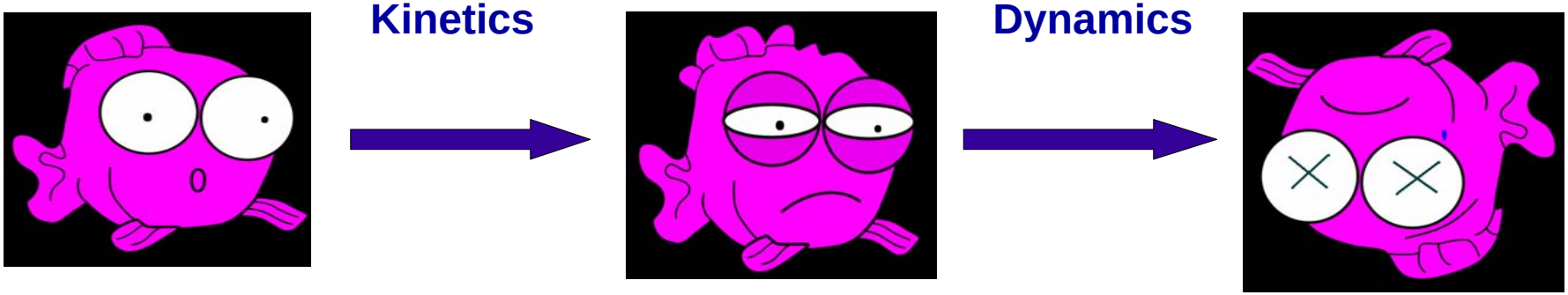
No Observed Effect Concentration is a result from a statistical test not an indication of no effect.

High variation in the control means: NOEC>EC_{50}

LC_{50}, NOEC, LOEC all depend on the exposure time.

If no effect is desired why not focus on real no effect??

Alternatives to single time point empirical approaches

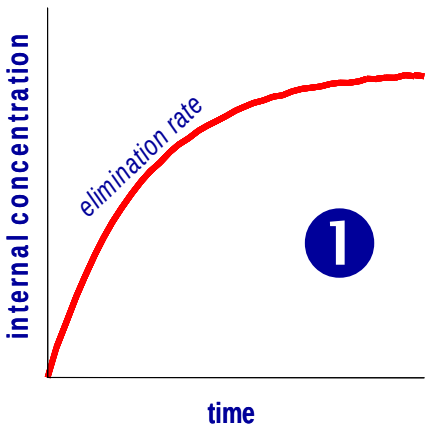


Consider toxicity as a process in time using biological process based modeling to predict (no) effects

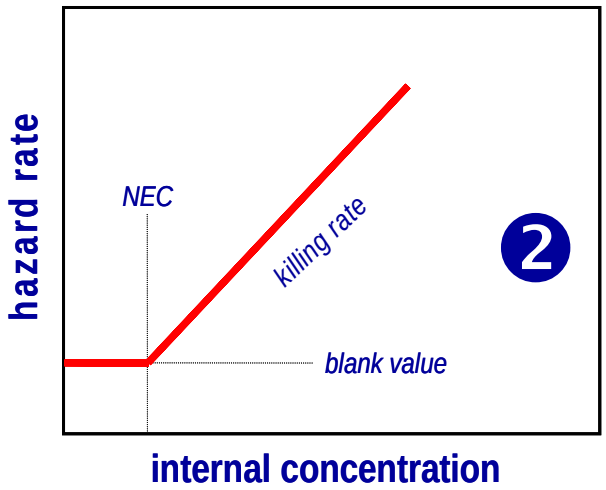
Biological based models

Effect depends on combination of *internal* concentration and hazard rate based on that internal concentration

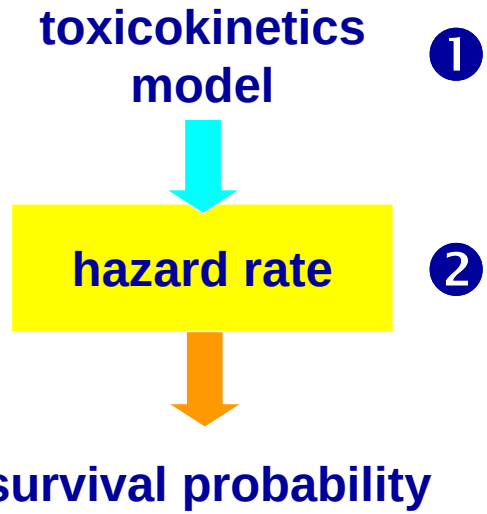
Toxicokinetic model

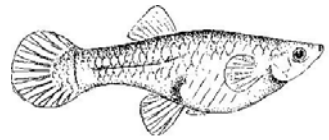


Hazard model



Process model (DEBtox)

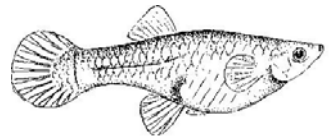




Poecilia reticulata

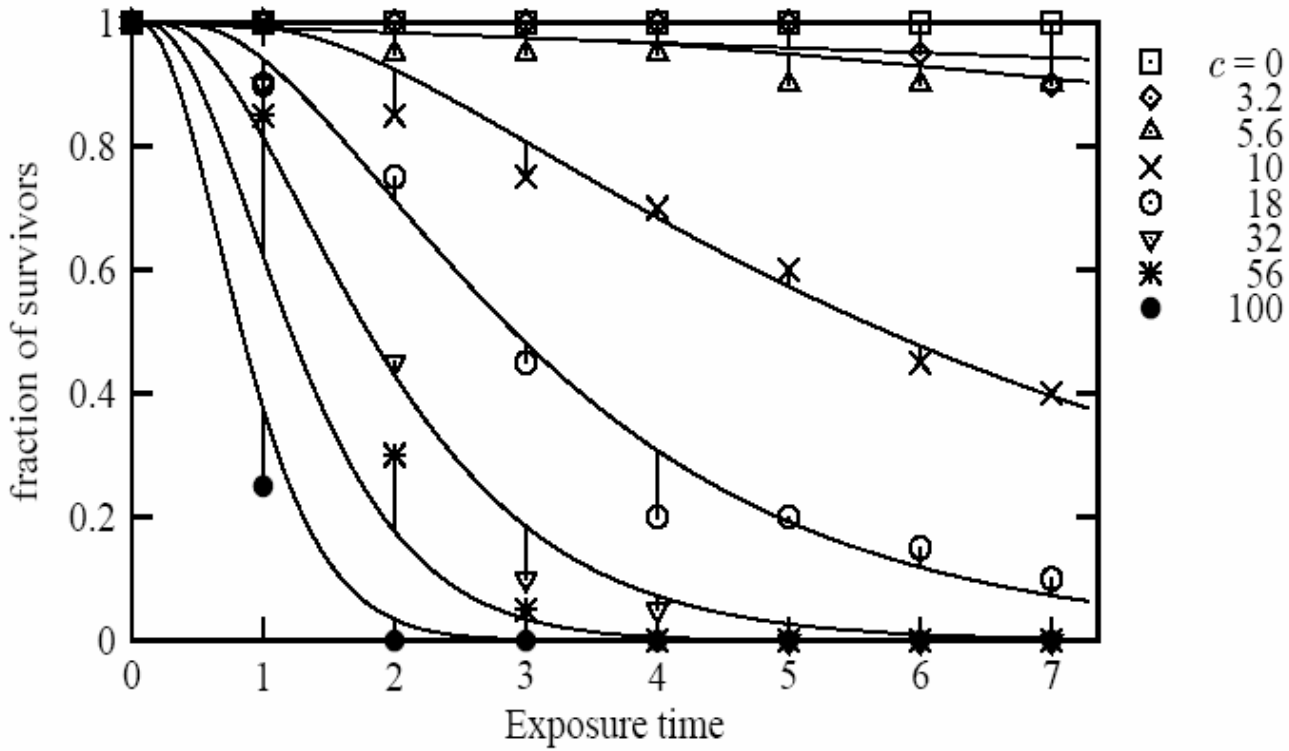
Example for dieldrin survival

	concentration *dieldrin* (µg l^{-1})							
time (d)	0	3.2	5.6	10	18	32	56	100
0	20	20	20	20	20	20	20	20
1	20	20	20	20	18	18	17	5
2	20	20	19	17	15	9	6	0
3	20	20	19	15	9	2	1	0
4	20	20	19	14	4	1	0	0
5	20	20	18	12	4	0	0	0
6	20	19	18	9	3	0	0	0
7	20	18	18	8	2	0	0	0



Poecilia reticulata

Example for dieldrin survival



Elimination rate	0.73	d^{-1}
Blank hazard rate	0.0064	d^{-1}
NEC	2.8 (2.1-3.1)	µg/L
Killing rate	0.031	L/(µg d)

Applying process models for mixtures

Requirements for experiments for process modelling:

- Design that allows measurements at intermediate times
- Design experimental setup that can find interactions

- Experiment with *Folsomia candida* held on compacted soil.
- Exposure to 6 binary mixtures of Cd, Cu, Zn and Pb
- 6 concentrations of each metal (36 combinations) in a factorial design

Results using single time point models

Results for the mixture of Copper and Cadmium

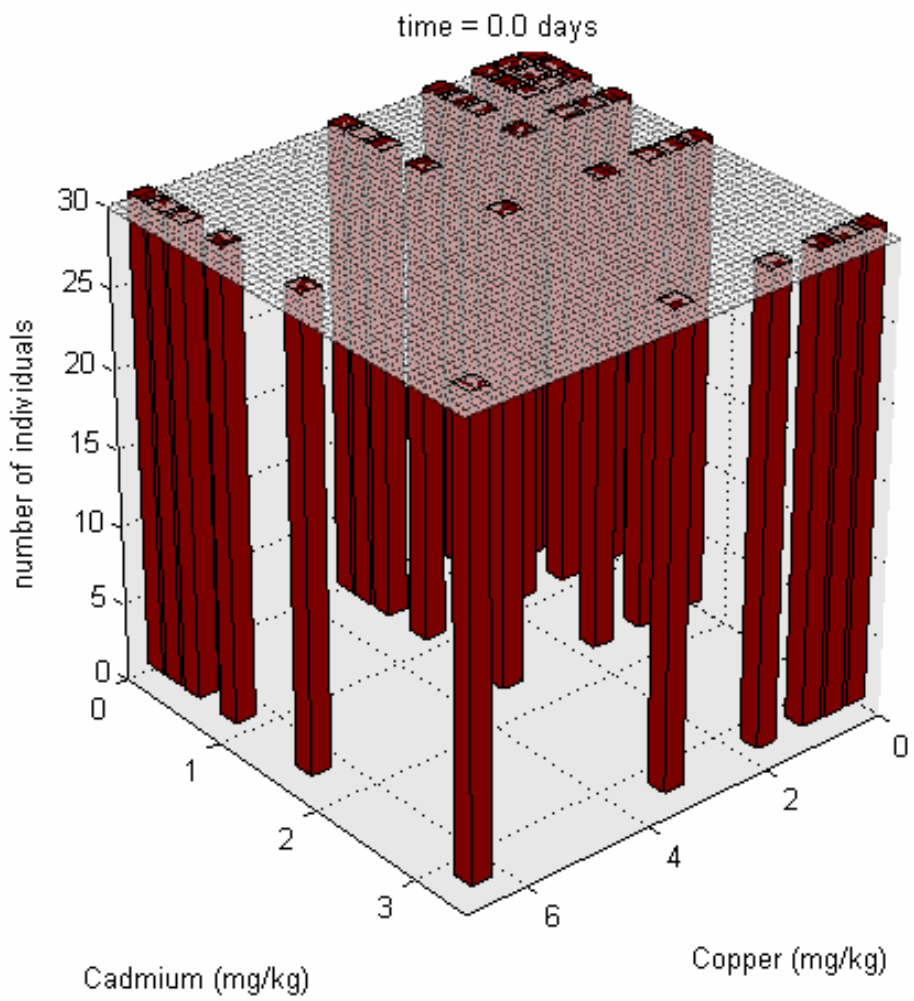
Time point (days)	CA	IA
2 – 3	No interaction	Synergism
4 – 5	Synergism	Synergism
6	Synergism	DL Synergism
7 – 9	DL Synergism	DL Synergism
10 – 15	No interaction	DL Synergism
16	DR Interaction	DL Synergism
17	No interaction	DR Synergism
18 – 21	Syn Id Antag hd	DL Synergism

Comparison to Concentration Addition / Independent Action using analysis model of Jonker et al ET&C 2005

Survival for the Cu/Cd mixture

Actually no interaction in this mixture.

Apparent interactions due to different kinetics



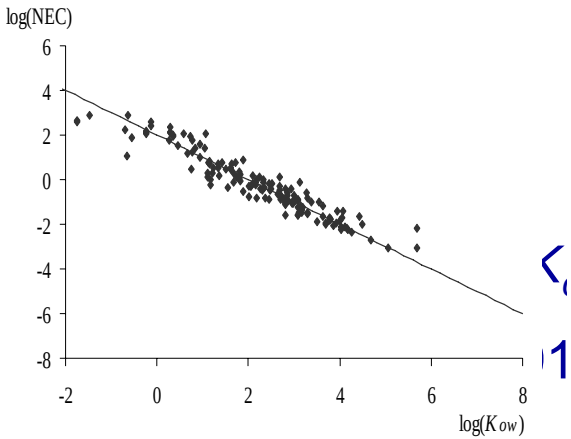
DEB for complex mixture

Developing relations between parameters

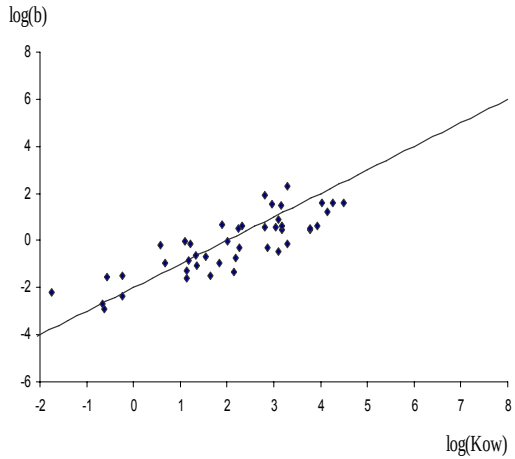
Narcotics hazard model predicts relation between K_{ow} and DEBtox parameters:

Log NEC	=	**- log K_{ow} + constant**
Log b (kill rate)	=	**+ log K_{ow} + constant**
Log Ke (elimination rate)	=	**-0.5 log K_{ow} + constant**

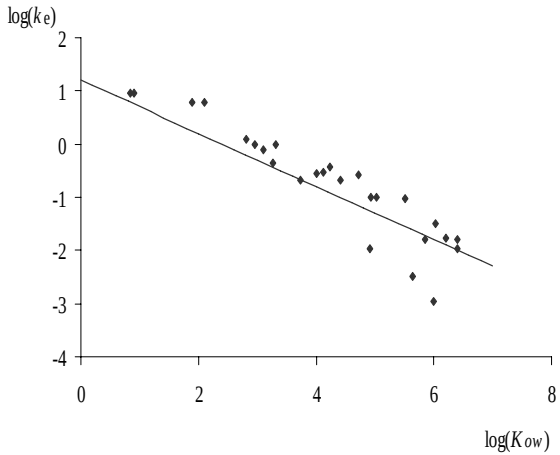
Log NEC



Log KR



Log ER

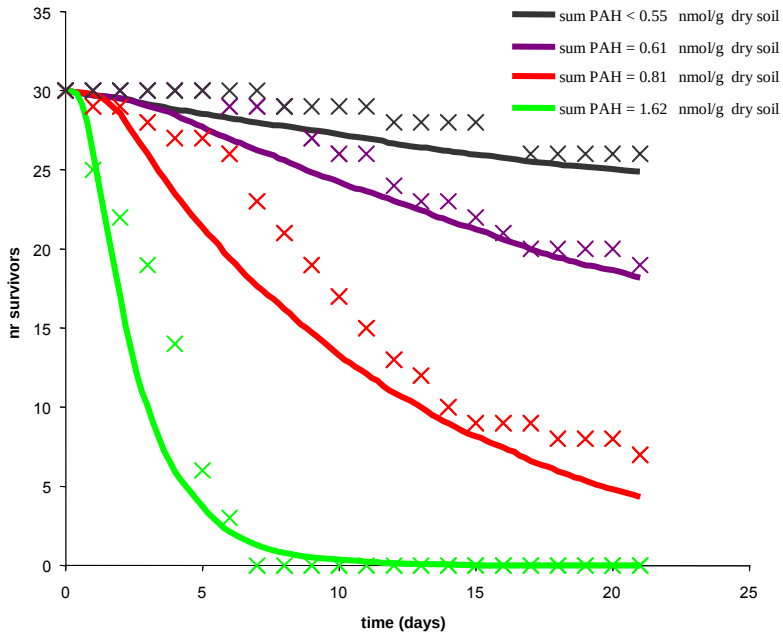


All this seems to check out pretty much OK

Based on Pymephalus promelas data from Center for Lake Superior Environmental Studies 1985–1990

Experimental validation

- **Experiment with fully grown *Folsomia candida***
- **Test with phenantherene only**
- **Test with mixture of phenanthrene, fluoranthene and pyrene**
- **Measured survival over 28 days including intermediate times**



Predicted mixture effect, based on measurements for phenantherene only (line) and use of DEBtox parameter QSARs to predict measured mixture effect (x).

Conclusions DEBtox models

- Process based models such as DEBtox can describe the progression of toxicity in time
- DEBtox can be applied to mixtures and can explain why in some cases interactions are seen for particular timepoints
- For narcotic compounds they can be combined with QSAR to allow predictions of the long-term effects of complex mixtures.

May 2005

March 2006

May 2006

June 2006

Oct. 2006

Feb. 2007

May 2007

June 2007

Sept. 2007

NoMiracle Newsletter No. 9

NOvel Methods for Integrated Risk Assessment of CumuLative stressors in Europe

Communicating Chemical Risks

The role of risk perception and communication for characterizing and managing cumulative stressors

Christina Benighaus, Guest Editor-Dialog
Hans Lekkle Coordinator – HERI

Meeting the Challenge of Communicating Chemical Risks
2nd NoMiracle Open Workshop,
Stuttgart, April 2007

Communicating about chemicals is a challenging task for risk managers in companies and regulatory authorities. The Second Open Workshop in Stuttgart was organised by NoMiracle partner DIALOGIK. It focused on good practice in risk communication and "How can we do better?". Case studies examining the perception and management of risks, from the chemical as well as from other sectors like food safety and public health governance, gave insights into how the wide range of risks can be handled and communicated.

The workshop invited a broad audience of stakeholders from companies, NGOs, regulators, EU representatives, academia (especially young researchers and Ph.D. students from different disciplines) and project partners from similar EU projects. Individual invitations were sent to experts of target group and the announcements were disseminated among associations, industries and regulatory authorities. As well, the workshop was offered as a student seminar at the University of Stuttgart. Around 50 participants accepted the invitation and a wide, interdisciplinary audience

brainstorming exercise, but unfolding along the following classic sequence: what (perception and communication), how (methods), to what purpose (for assessment and management) with which results.

In this issue

1. Communicating Chemical Risks
2. NoMiracle Second Open Workshop on "Communicating Chemical Risks"
   Stakeholder Feedback Descriptions of courses
4. Risk Governance Framework
6. Lessons for Good Risk Communication
7. Variation and Meaning of Expert Views on Multiple Risks and Uncertainties: Insights from NoMiracle Work
9. Use and Disuse of Risk Information – The Significance of Information Frameworks in Integrated Risk Assessment and Manageme
10. Panel Discussion: Addressing the Challenge

Dr. Tuomo Karjalainen

Open workshops and conferences

- Workshop: ”Integrated Assessment of Environmental and Human Health”, Frankfurt: Cambridge, September 2008

- 5th NoMiracle Workshop: ”Cumulative Risk Assessment”, the Netherlands, spring 2009

- Final NoMiracle Workshop: September 2009, Aarhus, Denmark

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