Using mixture toxicity approaches to identify vulnerable species, drivers of mixture toxicity and priority pesticide mixtures.

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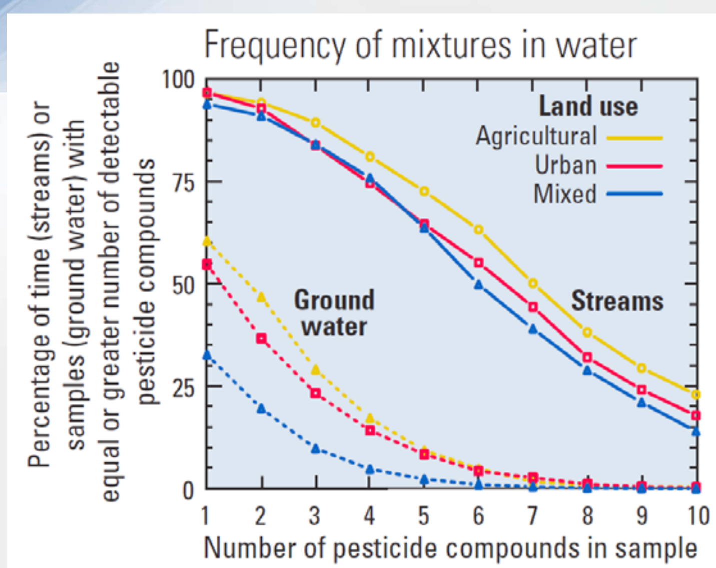

US Department of the Interior, US Geological Survey (2006) Pesticides in the Nation's Streams and Ground Water, 1992–2001, Circular 1291

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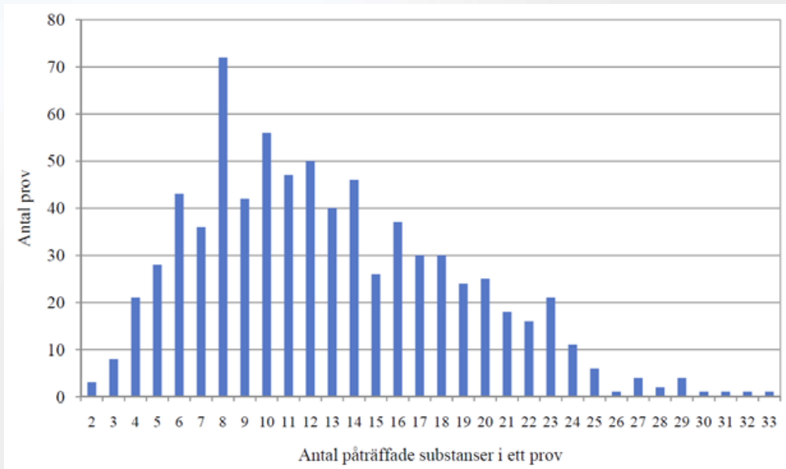
Pesticide Monitoring Data Sweden



Stina Adielsson, Sarah Graaf, Melle Andersson & Jenny Kreuger (2009) Resultat från miljöövervakningen av bekämpningsmedel (växtskyddsmedel). ISSN 0347-9307. Swedish University of Agricultural Sciences, Uppsala.

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EFSA Guidance Documents

- EFSA guidance for edge-of-field scenarios
- EFSA guidance for birds & mammals
- EFSA guidance for bees
- EFSA guidance for non-target terrestrial plants

<u>ALL</u> consider mixture effects in an environmental context

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Mixture toxicity concepts

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

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 ($i = 1 \ldots n$)
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

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Reviews on the predictive power of CA

- ❑ Rodney SI, Teed RS, Moore DRJ. Estimating the Toxicity of Pesticide Mixtures to Aquatic Organisms: A Review. Hum Ecol Risk Assess, 2013
- ❑ Coors, A, Frische, T. Predicting the aquatic toxicity of commercial pesticide mixtures. Env Sci Europe, 2011
- ❑ Verbruggen E, Brink P. Review of recent literature concerning mixture toxicity of pesticides to aquatic organisms, 2010
- ❑ Belden JB, Gillom RJ, and Lydy MJ. How well can we predict the toxicity of pesticide mixtures to aquatic life? Int Env Ass Man, 2007
- ❑ Deneer JW. Toxicity of mixtures of pesticides in aquatic systems. Pest Manage Sci, 2000

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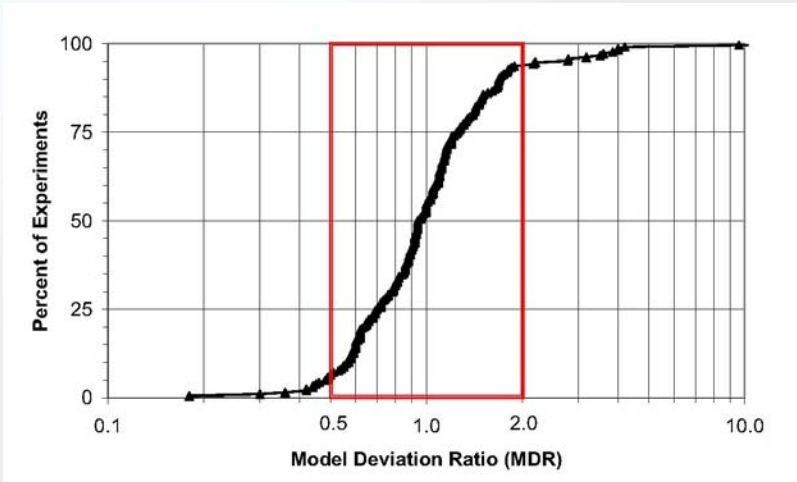
Average predictability of pesticide mixtox by CA



From : Belden et al. IEAM, 2007

MDR= Prediction/Observation

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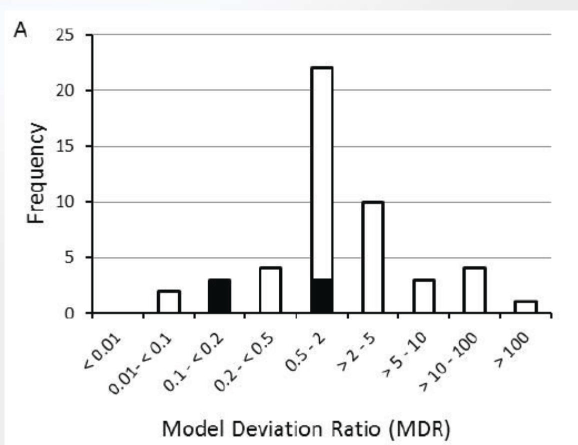
Predictability of pesticide product toxicity by CA



From : Coors & Frische, Env Sci Europe, 2011

MDR= Prediction/Observation
White boxes: Dissimilar MoA
Black boxes: Similar MoA

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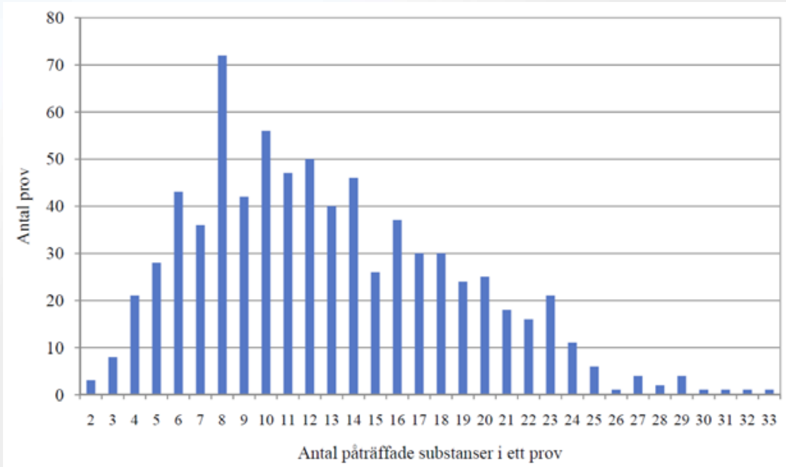
Pesticide Monitoring Data Sweden



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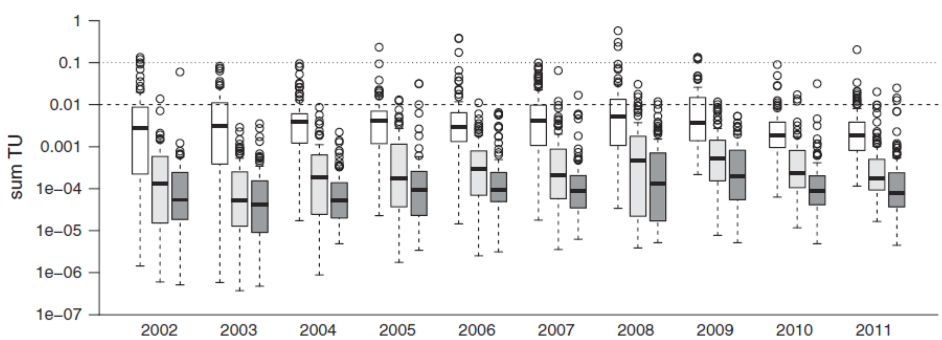
CA-expected toxicity of pesticide mixtures in Swedish Streams



Bundschuh, Mirco, Willem Goedkoop, and Jenny Kreuger. "Evaluation of pesticide monitoring strategies in agricultural streams based on the toxic-unit concept—Experiences from long-term measurements." Science of The Total Environment 484 (2014): 84-91.

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A closer look at some details

- One sample from one site
- M42
- 42 compounds detected simultaneously
- "trace" amounts ignored
- Analysis based on Hazard Index, sums of toxic units
  - Hazard Index: Sum of conc/EQS
  - Toxic Unit: Sum of conc/EC50 for algae, invertebrates, fish

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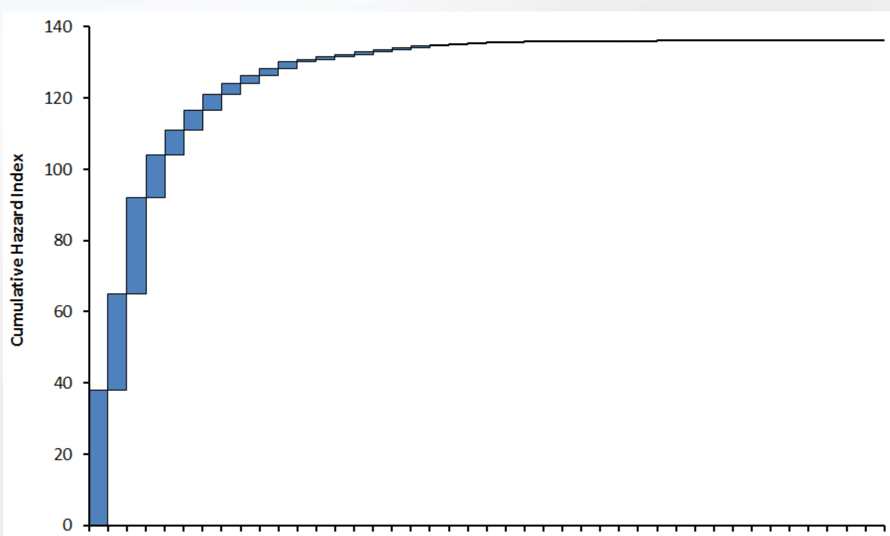
Cumulative HI-Distribution (EQS)



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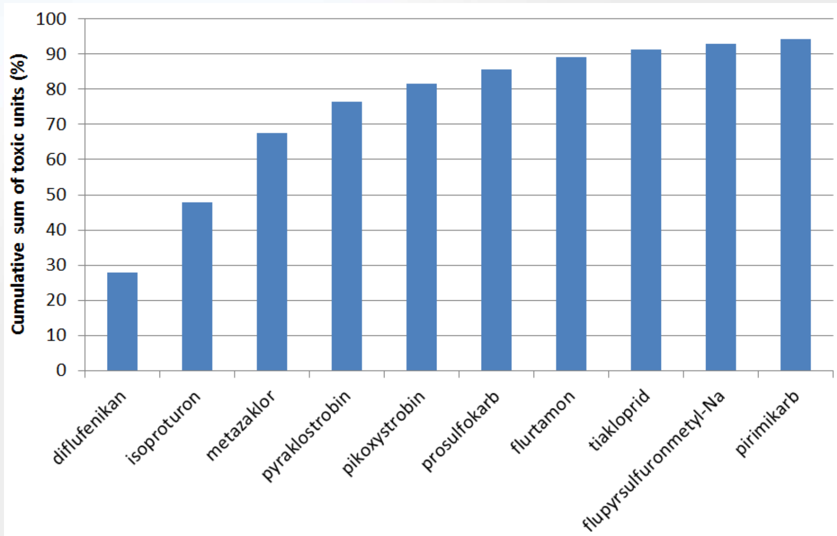
Cumulative HI-Distribution (EQS)



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Top 10 – EQS, Algae, Invertebrates, Fish

EQS	Algae	Invertebrates	Fish
diflufenikan	diflufenikan	isoproturon	pyraklostrobin
isoproturon	isoproturon	pirimikarb	prosulfokarb
metazaklor	metazaklor	pyraklostrobin	pikoxystrobin
pyraklostrobin	prosulfokarb	pikoxystrobin	glyfosat
pikoxystrobin	flupyrsulfuronmetyl	prosulfokarb	metazaklor
prosulfokarb	flurtamon	glyfosat	isoproturon
flurtamon	diuron	azoxystrobin	azoxystrobin
tiakloprid	klomazon	cyprodinil	protiokonazol-destio
flupyrsulfuronmetyl	glyfosat	Metazaklor	bitertanol
SUM: 136	**SUM: 1.3**	**SUM: 0.04**	**SUM: 0.03**

BUT…!

- Those pesticides don't act similarly! How do you justify the use of CA?
- Summing up risk quotients (conc/EQS) violates fundamental assumptions of CA!
- You are ignoring synergisms!

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The maximum possible difference between IA- and CA-predicted toxicities

$$\frac{EC50_{IA}}{EC50_{CA}} \leq \frac{\sum_{n}^{i=1} TU_i}{\max_{i \in (1,\ldots,n)}(TU_i)}$$

TU: Toxic Unit, conc / EC50

- the maximum possible ratio between the CA- and the IA-predicted EC50 is *n* (the number of mixture components).
- the more "imbalanced" the mixture – in terms of TU contributions to the mixture - the smaller the maximum possible error.

Junghans , et al. (2006) Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. Aquatic Toxicology 76, 2006

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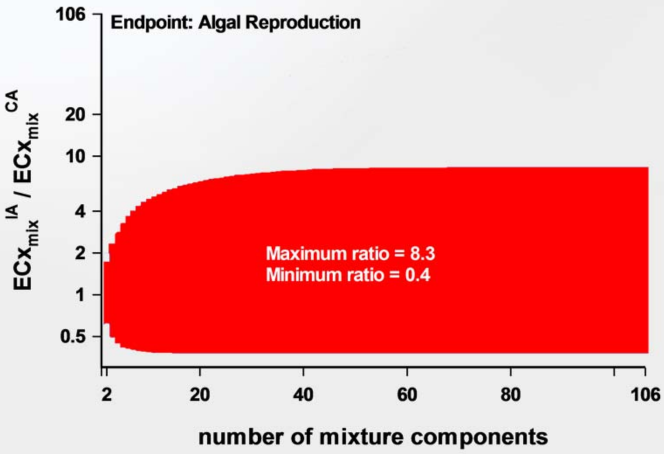
Ratios EC50(IA) / EC50(CA) for algal toxdata



Faust et al., (2000) Competing Concepts for the Prediction of Mixture Toxicity: Do the Differences Matter for Regulatory Purposes? Public Report of the BEAM Project

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Quantitative consequence of ignoring IA

Maximum possible ratio between the CA- and IA-predicted toxicities is

3.6

for the presented mixture

Applying Concentration Addition

$$Hazard\ Index = \frac{MEC_{MIX}}{EQS_{MIX}} = \sum_{i=1}^{n} \frac{MEC_i}{EQS_i}$$

$$Sum\ of\ Toxic\ Units = \frac{c_{MIX}}{EC50_{MIX}} = \sum_{i=1}^{n} \frac{c_i}{EC50_i}$$

Vighi et al (2003) Water quality objectives for mixtures of toxic chemicals: problems and perspectives, Ecotox. Env. Safety, 54, 139-150

Compound 1:

MEC_1=0.4*10^{-4}	$EC50_{Algae}$:	1.0	
AF = 1000	$EC50_{Daphnids}$:	0.1	EQS = 10^{-4}
	$EC50_{Fish}$:	1.0	

Compound 2:

MEC_2= 0.8*10^{-4}	$EC50_{Algae}$:	0.1	EQS = 10^{-4}
AF = 1000	$EC50_{Daphnids}$:	1.0	
	$EC50_{Fish}$:	1.0	

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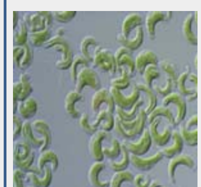
Compound 1	Compound 2	
0.4	0.8	?

$$HI = 0.4 + 0.8$$



?

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Single Components Laboratory → Concentration Addition → **Mixture Laboratory**

Single Components Laboratory ↓ EQS (PNEC) ↓ **Single Components Field**

Mixture Laboratory ↓ EQS (PNEC) ↓ **Mixture Field**

Single Components Field → $\sum_{i=1}^{n} \frac{MEC}{EQS(PNEC)}$ → **Mixture Field**

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Sum of Toxic Units = 0.8

$$HI = \sum_{i=1}^{n} \frac{MEC}{EQS} = 1.2$$

- Ratio between the sum of toxic units and the hazard index can never exceed the number of considered (groups of) species.
- That is, the ratio is usually at maximum a factor of 3.

Backhaus T, Faust M. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ Sci Technol , 2012

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Top 10 – EQS, Algae, Invertebrates, Fish

EQS	Algae	Invertebrates	Fish
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isoproturon	isoproturon	pirimikarb	prosulfokarb
metazaklor	metazaklor	pyraklostrobin	pikoxystrobin
pyraklostrobin	prosulfokarb	pikoxystrobin	glyfosat
pikoxystrobin	flupyrsulfuronmetyl	prosulfokarb	metazaklor
prosulfokarb	flurtamon	glyfosat	isoproturon
flurtamon	diuron	azoxystrobin	azoxystrobin
tiakloprid	klomazon	cyprodinil	protiokonazol-destio
flupyrsulfuronmetyl	glyfosat	Metazaklor	bitertanol
SUM: 136	**SUM: 1.3**	**SUM: 0.04**	**SUM: 0.03**

Interactions and Synergisms

Toxicokinetic

- increased or decreased uptake or elimination
- changes in the time-concentration profile of affected compounds
- changes in concentration-dependent effects

$$\frac{c_{mix}}{EC50_{mix}} = \sum_{i=1}^{n} \frac{c_i}{EC50_i}$$

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Interactions

Toxicodynamic

- Interaction on the receptor level
- changes in toxicity without changes in concentration

$$\frac{c_{mix}}{EC50_{mix}} = \sum_{i=1}^{n} \frac{c_i}{EC50_i}$$

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Interactions

Pharmakokinetic and Pharmakodynamic interactions lead to an apparent increase in the TU of the affected compound

$$\frac{c_{mix}}{EC50_{mix}} = \sum_{i=1}^{n} \frac{c_i}{EC50_i}$$

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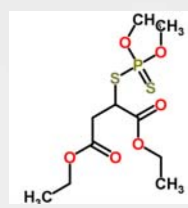
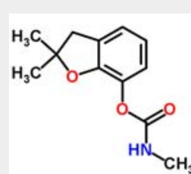
Example of a synergistic interaction

Organophosphates,
(e.g. malathion)



Carbamates,
(e.g. carbofuran)



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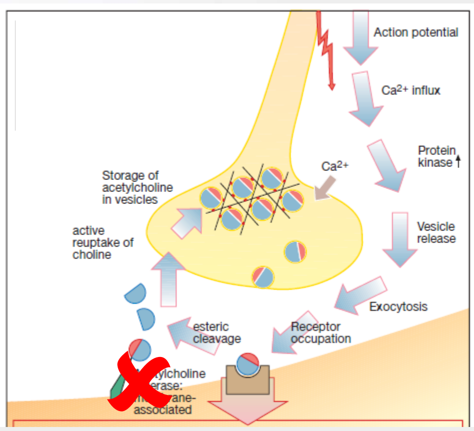
Example of an interaction

Organophosphates and carbamates share the same mechanism of action, the inhibition of acetylcholine esterase.



Lüllmann, Color Atlas of Pharmacology, 2000

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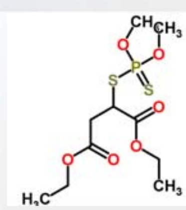
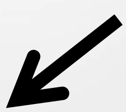
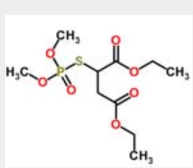
Example of an interaction



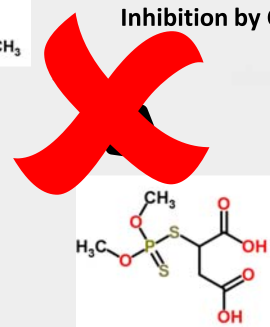
Malathion

Inhibition by Carbamates





Malaoxon (active)



Malathioin dicarboxylic acid (inactive)

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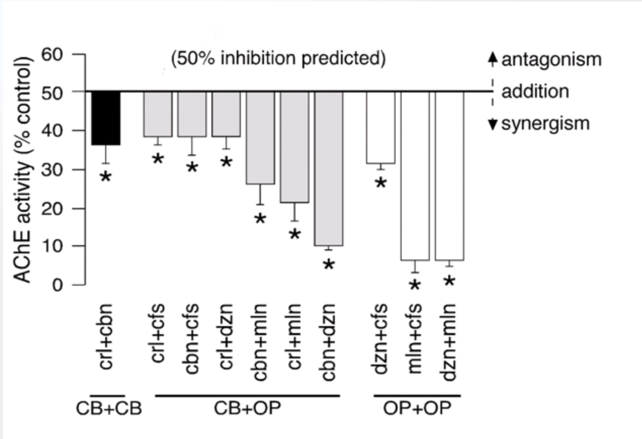
Synergisms between Carbamates and Organophosphates



DZN: Diazinon; MLN: Malathion; CRL: Carbaryl

CBN: Carbofuran; CFS: Clopyrifos

Laetz et al., EHP, 2008

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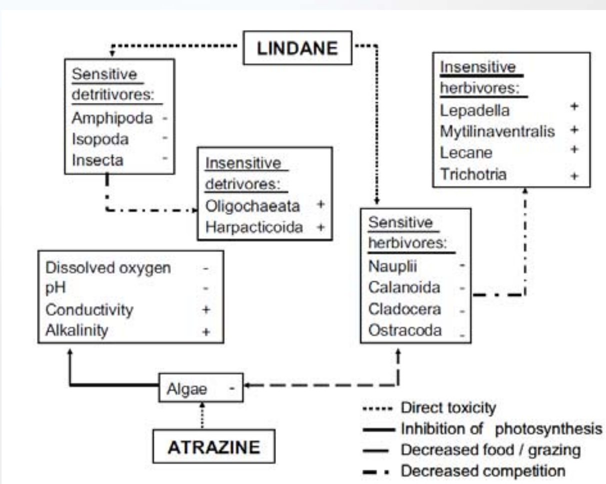
Interactions in an aquatic foodweb



Van den Brink, Paul J., et al. "Effects of a herbicide–insecticide mixture in freshwater microcosms: risk assessment and ecological effect chain." Environmental Pollution 157.1 (2009): 237-249.

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Interactions

Pharmakokinetic and Pharmakodynamic interactions lead to an apparent increase in the TU of the affected compound

$$\frac{c_{mix}}{EC50_{mix}} = \sum_{i=1}^{n} \frac{c_i}{EC50_i}$$

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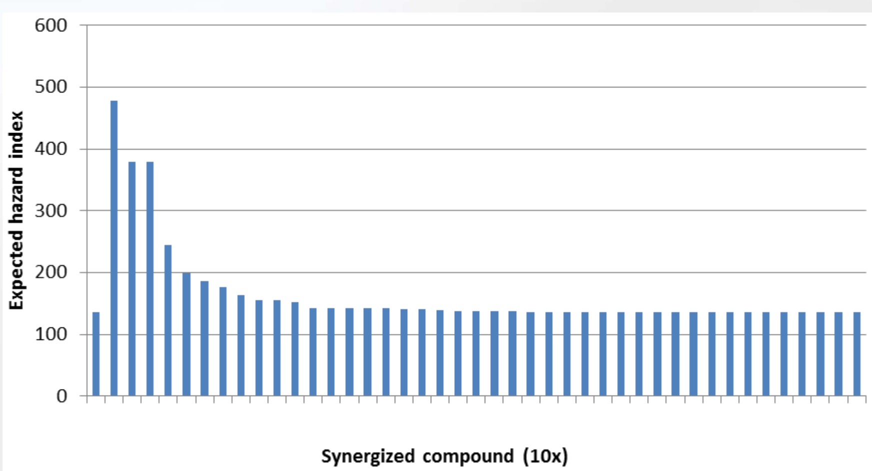
Increase in the HI if a compound is 10x more toxic than expected



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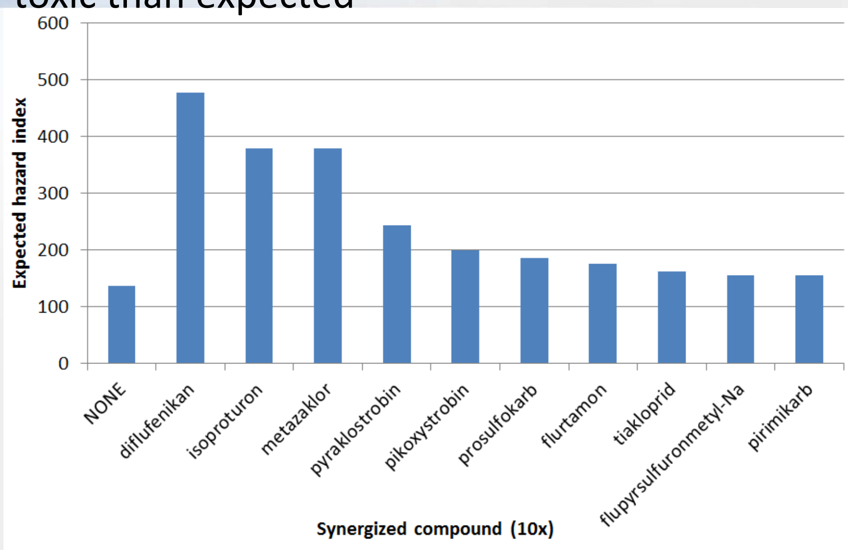
Increase in the HI if a compound is 10x more toxic than expected



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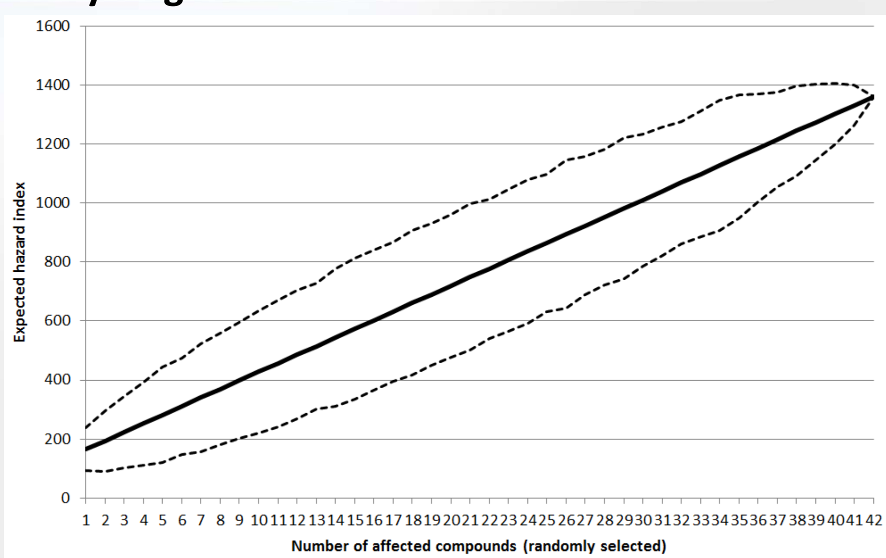
Increase of HI if a certain number of pesticides is synergized



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Quantitative consequences of synergistic interactions

If the compound with the highest risk quotient is 10x more toxic than expected: HI increases by a factor of **3.5.**

If the 10 random compounds are synergized: HI increases by a factor of **3.1 (1.6 – 4.6).**

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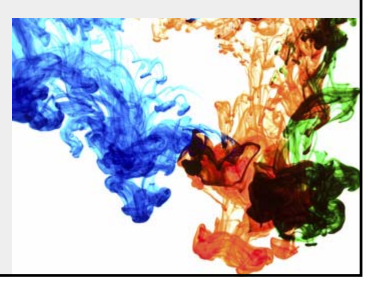
Summary & Conclusions

Concentration Addition for

- ❑ Estimating the joint ecotoxicity of a pesticide mixture
- ❑ Identifying vulnerable (groups of) species
- ❑ Identifying drivers of mixture toxicity



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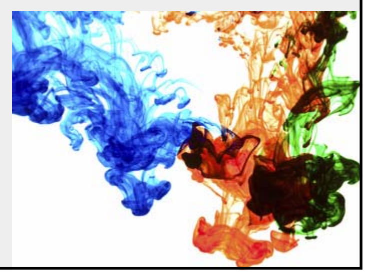
Summary & Conclusions

- ❑ Hazard Index based on EQS values or Sums of Toxic Units based on actual ecotox data for algae, invertebrates, fish
- ❑ Hazard Index is at maximum x times more conservative than the sum of toxic units (x = number of species groups considered)
- ❑ Error by using only CA can be estimated
- ❑ Mixtures buffer against synergistic interactions



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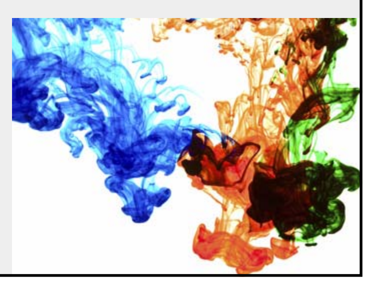
Summary & Conclusions

- ❑ The hazard index for the presented sample was 136 (different assessment factors for the different pesticides)
- ❑ Sum of toxic units for algae – the most sensitive group of organisms - was 1.3 (no assessment factor)



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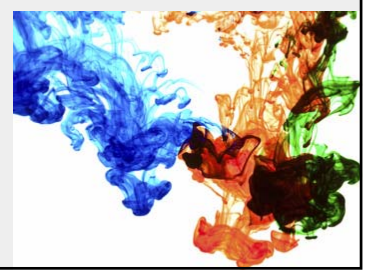
THE main challenge

Integration and harmonization between prospective and retrospective risk assessments



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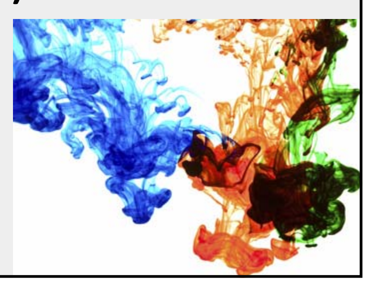
THE main challenge

Prospective (Emission based):

In the context of an application for market approval

Retrospective (Immission based)

In the context of the Water Framework Directive or the Marine Strategy Framework Directive



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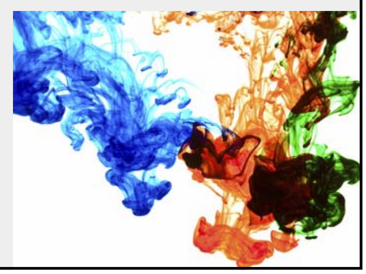
THE main challenge

Legal mandate

Ecosystem perspective: the environmental compartment should have “good ecological status”

Scientific approaches

- ❑ Interlinked assessment of chemical and biological status
- ❑ Quality standards for individual compounds
- ❑ Inadequate, see e.g. Carvalho et. al, Tox Sci 2014



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THE main challenge

Specific issues

- ❑ Which mixtures occur under which conditions in which waters?
- ❑ Means for simplification ? Prioritization?
- ❑ How to act on it?
- ❑ How to amend the current regulatory system?

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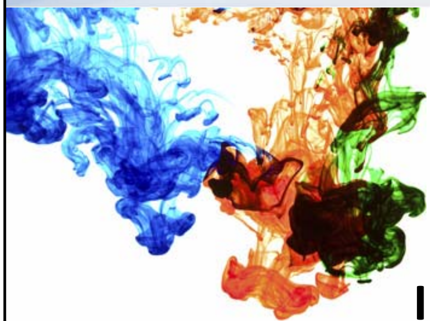
THE main challenge



Integration and harmonization between prospective and retrospective risk assessments