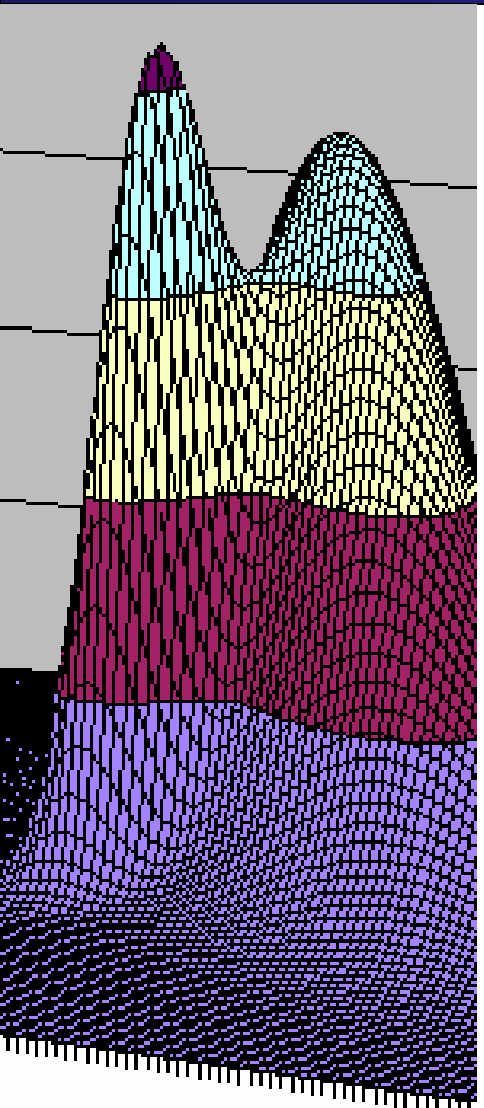
The School of Pharmacy
University of London

3rd SETAC Special Science Symposium



Setting the scene: Is mixtures risk assessment necessary and feasible?

Andreas Kortenkamp

The School of Pharmacy, University of London

2-3 February 2011, Brussels

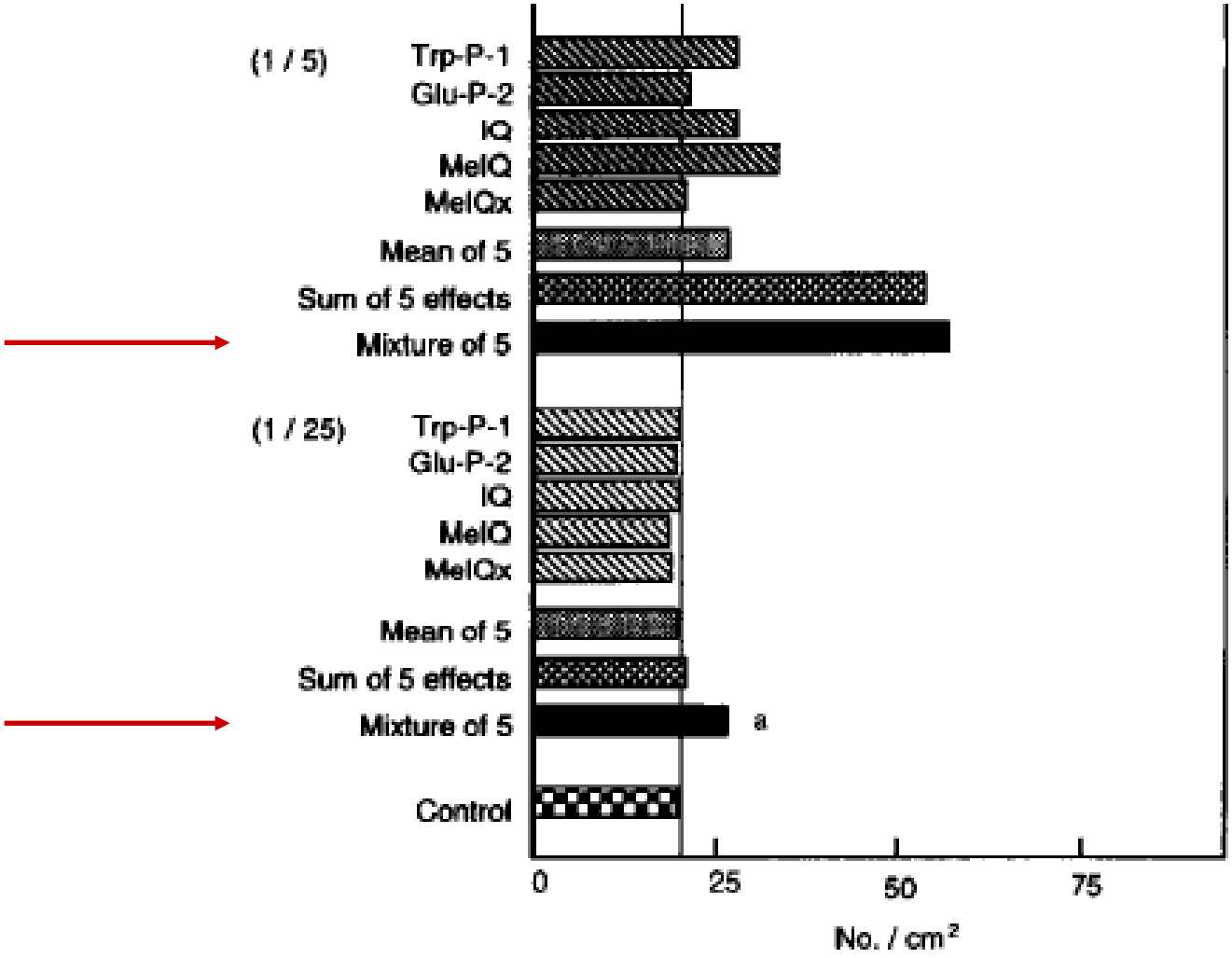
The state of affairs anno 1989

What is synergy?
Berenbaum 1989, Pharmacol Rev **41**, 93

- Summation of effects
- Isobole method (= *dose addition*)
- Multiplication of effects
- Compare effect of mixture with those of components

Example: GST-positive foci in livers

Hasegawa *et al.* (1996) Food Chem Toxicol **34**, 1097

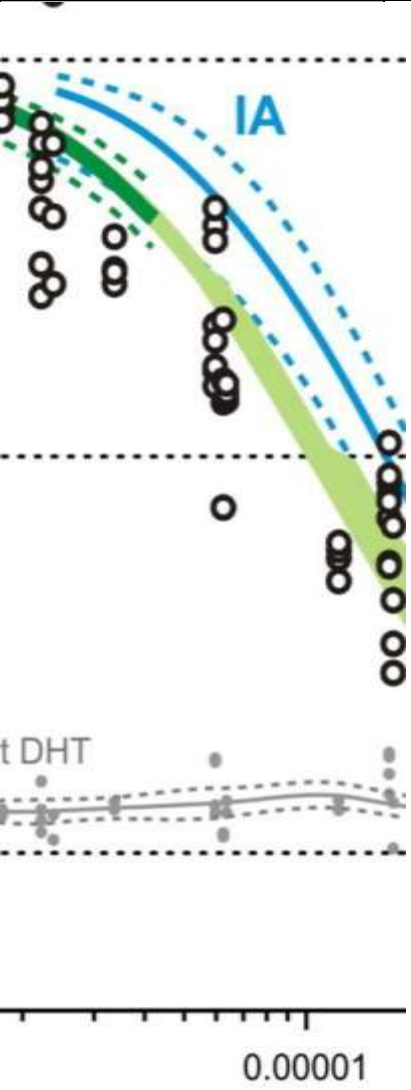


The state of affairs anno 1989

What is synergy?
Berenbaum 1989, Pharmacol Rev **41**, 93

- “The last approach (…), and the one that is by far the most often used, is that in which explicit criteria are conspicuous by their absence. Here, authors claim to have demonstrated synergy without specifying any method or criterion at all, apparently assuming that the conclusion is self-evident…”

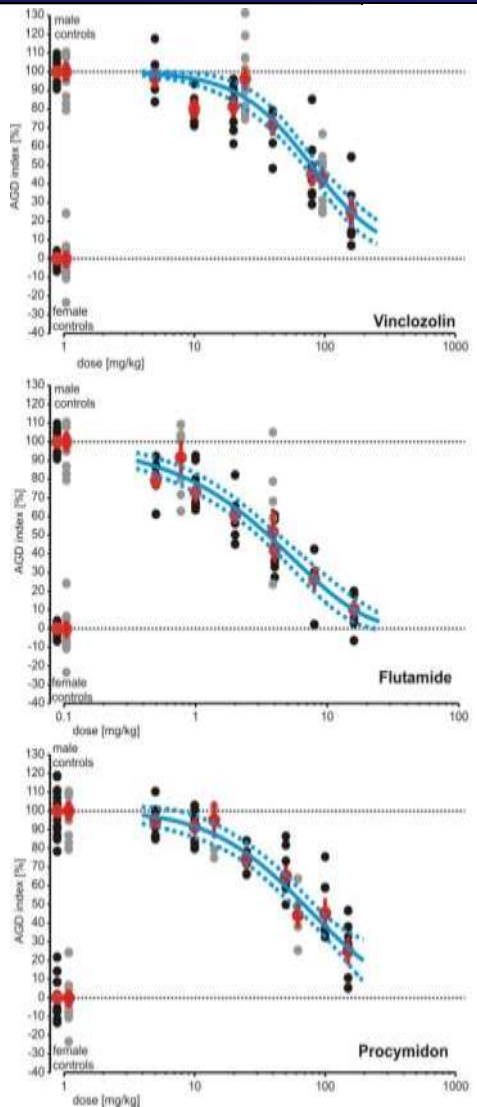
Prediction of mixture effects?

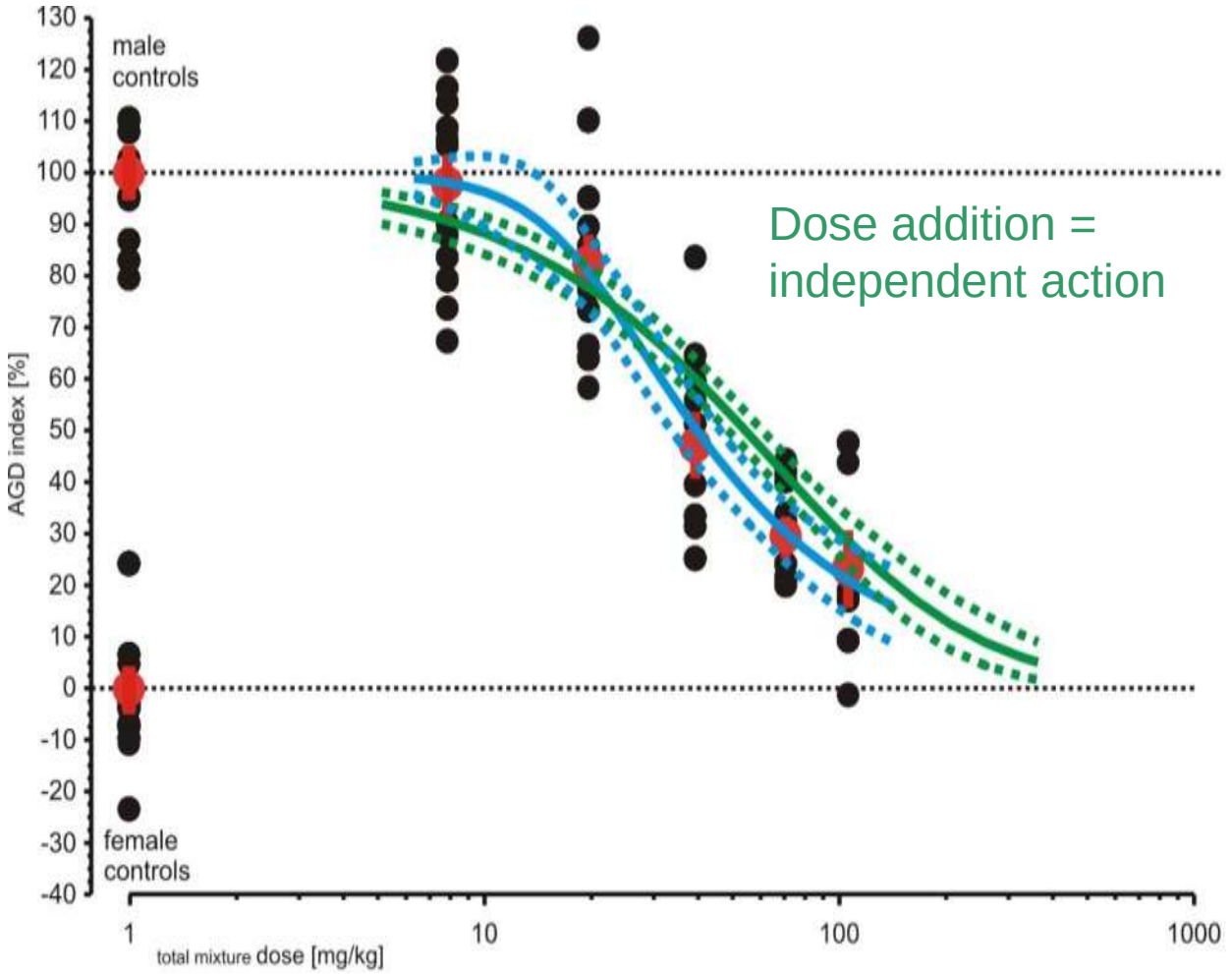


- Assumption: chemicals act without interfering with each other
- Effects can be predicted by using *dose (concentration) addition* or *independent action*
- Deviations from predicted additivity rare

Assessment and prediction (1)

Hass *et al.* 2007 EHP **115** Suppl 1, 122





Assessment and prediction (2)

Brian *et al.* 2005 EHP **113**, 721

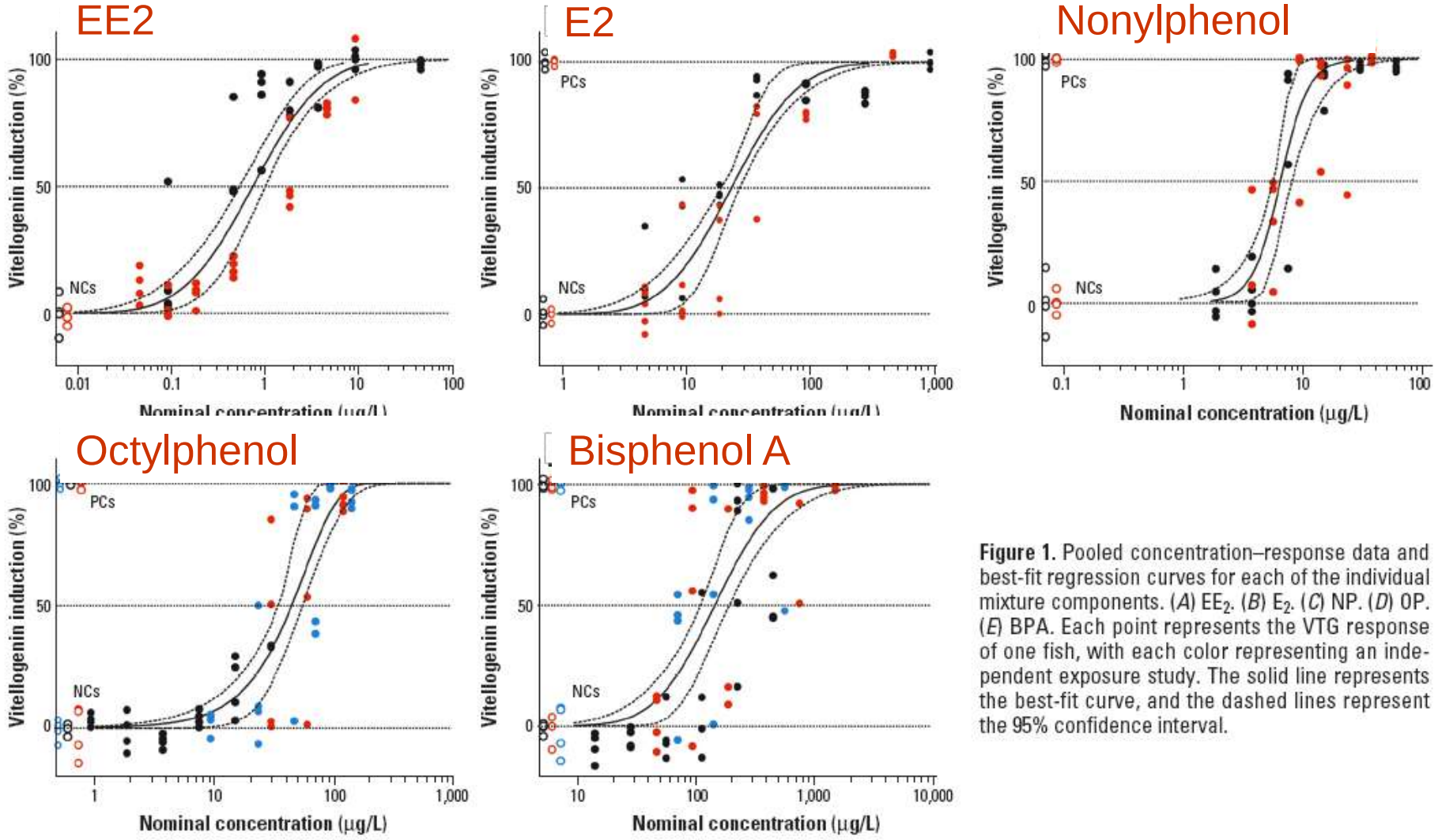


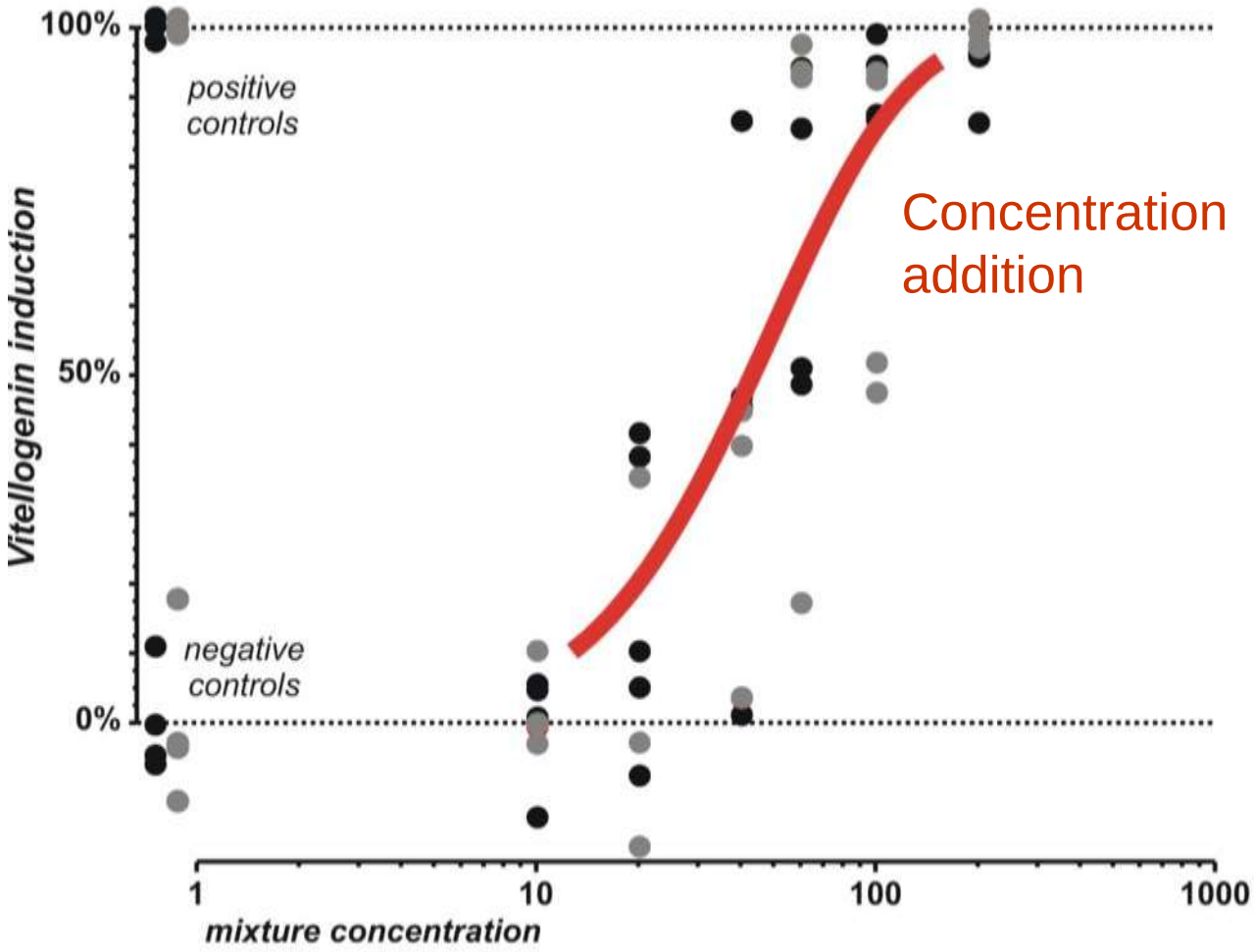
Figure 1. Pooled concentration–response data and best-fit regression curves for each of the individual mixture components. (*A*) EE_2. (*B*) E_2. (*C*) NP. (*D*) OP. (*E*) BPA. Each point represents the VTG response of one fish, with each color representing an independent exposure study. The solid line represents the best-fit curve, and the dashed lines represent the 95% confidence interval.

Assessment and prediction (2)

Brian *et al.* 2005 EHP **113**, 721

Vitellogenin induction in fish

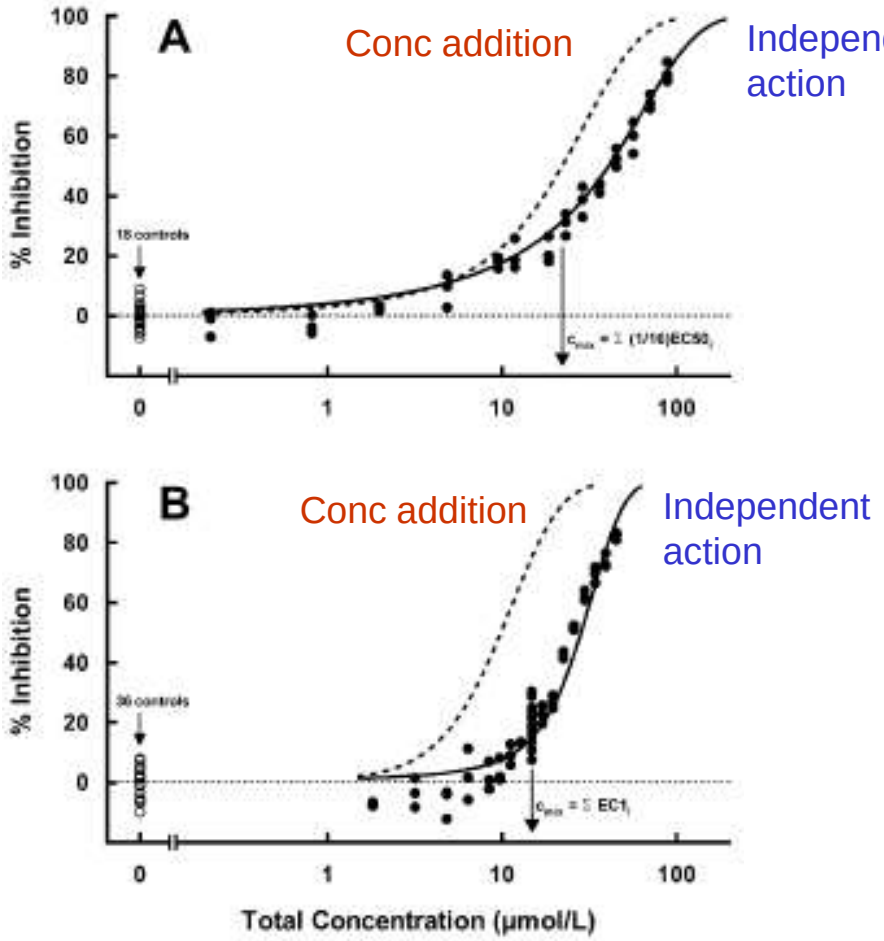
5 components



Algal toxicity of 16 dissimilarly acting toxicants

Faust *et al.* (2003) Aquat Toxicol **63**, 43

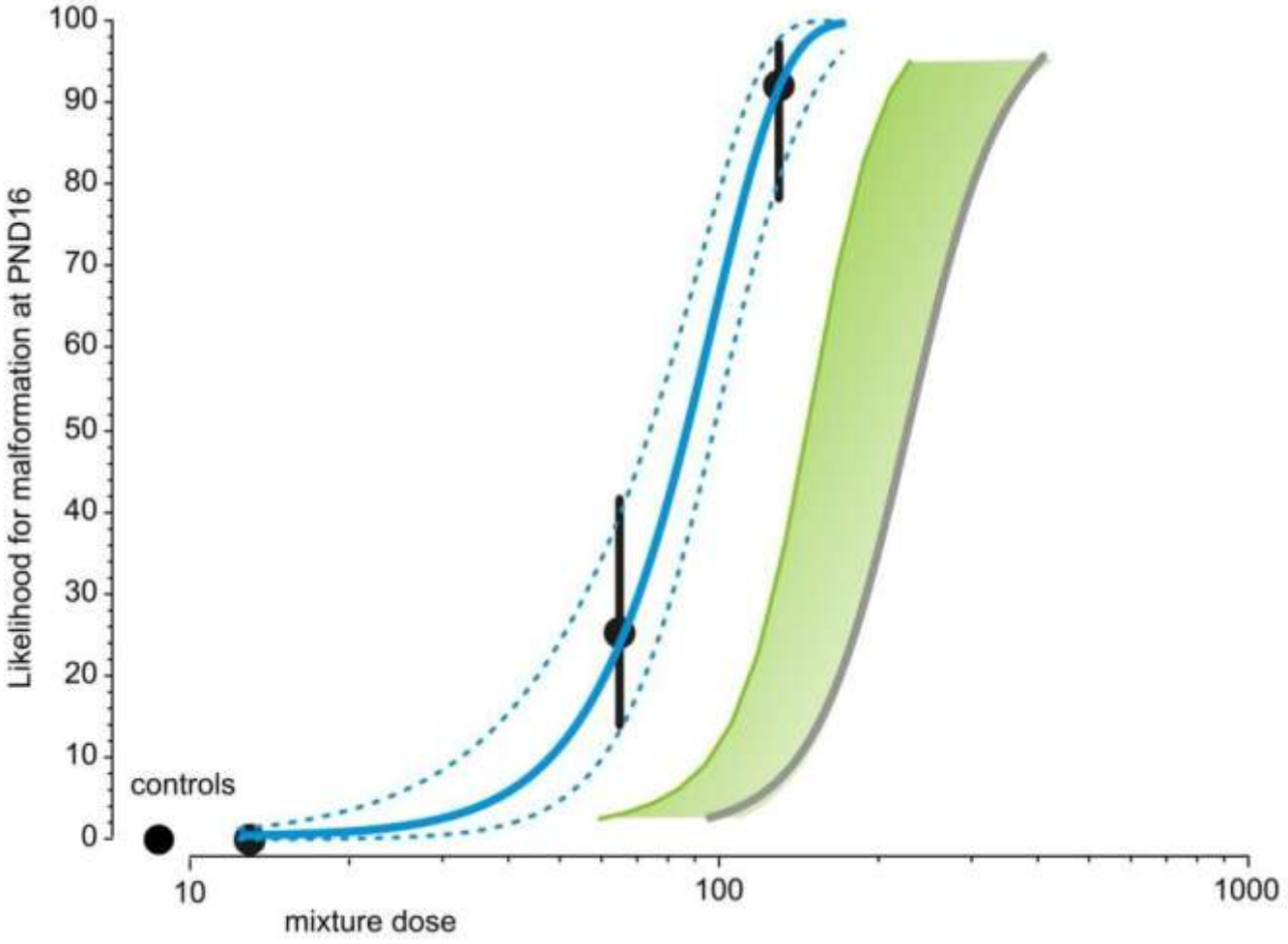
Aclonifen
8-Azaguanine
Azaserine
CCCP
Chloramphenicol
DTMAC
Fenfuram
Kresoxim-methyl
Metalaxyl
Metazachlor
Metsulfuron-methyl
Nalidixic acid
Norflurazon
Paraquat
Terbutylazim
Triadimenol



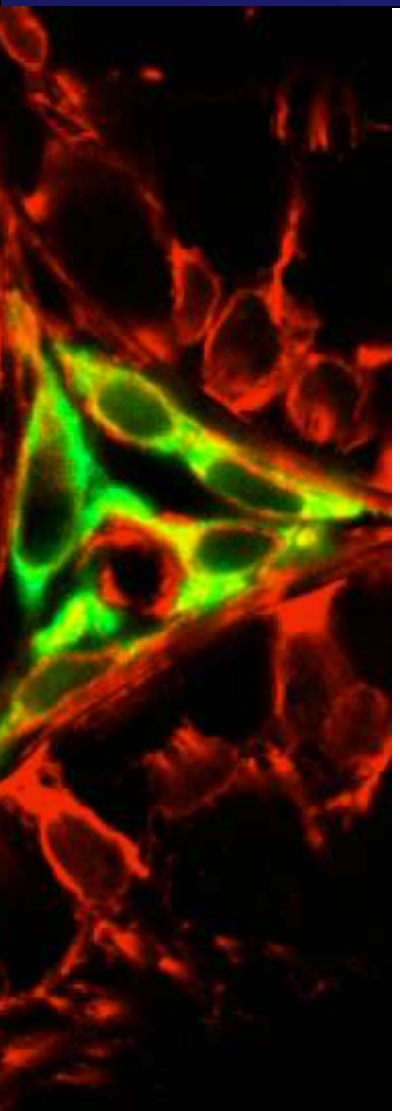
Prediction of mixture effects?

Synergism with genital malformations

Christiansen *et al.* 2009, EHP **117**, 1839



Summary: human toxicology



- *Dose addition* a valuable tool
- Basis: evidence from studies with mutagens, endocrine disrupters, dioxins
- No example where *independent action* gave predictions different from dose addition that were in agreement with observed mixture effects
- Synergisms or antagonisms rare

Summary: Ecotoxicology

- *Concentration addition* a valuable tool
- Evidence from numerous studies with a variety of pollutants
- *Concentration addition* predicts stronger combination effects than *independent action*
- Synergisms or antagonisms rare

Is a consideration of mixture effects necessary from a scientific viewpoint?

- Chemicals risk assessment normally ignores mixture effects
- Exposure: to several chemicals simultaneously

Is a consideration of mixture effects necessary from a scientific viewpoint?

Current practice justified if:

- Only one chemical is toxic, all others “inert”
- Joint effect of mixture not larger than effect of most toxic component

Comparing mixture effects with those of components

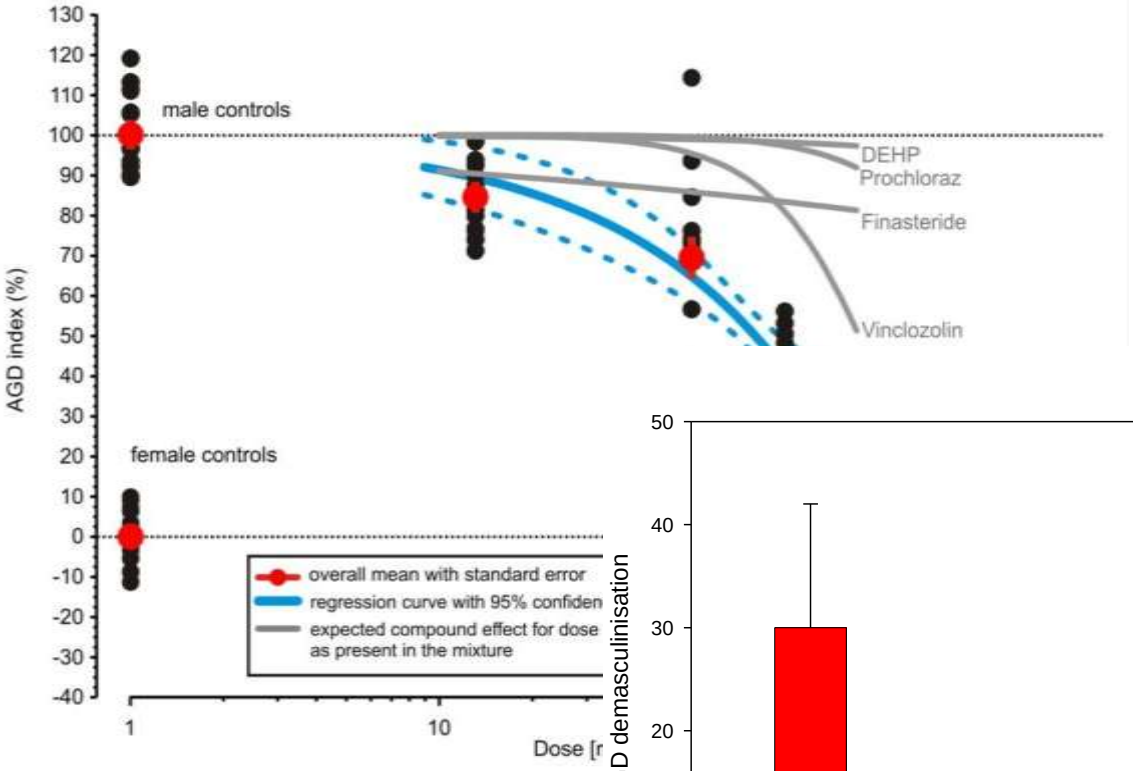
Finasteride

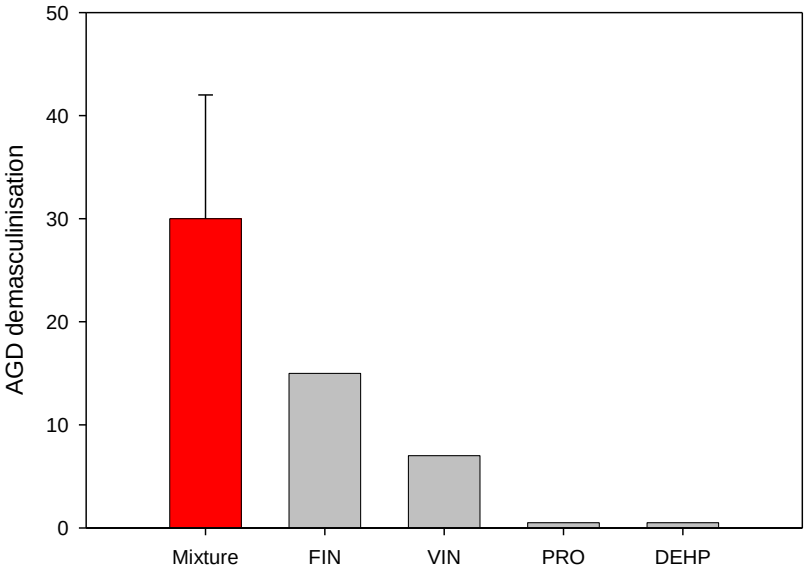
Vinclozolin

DEHP

Prochloraz

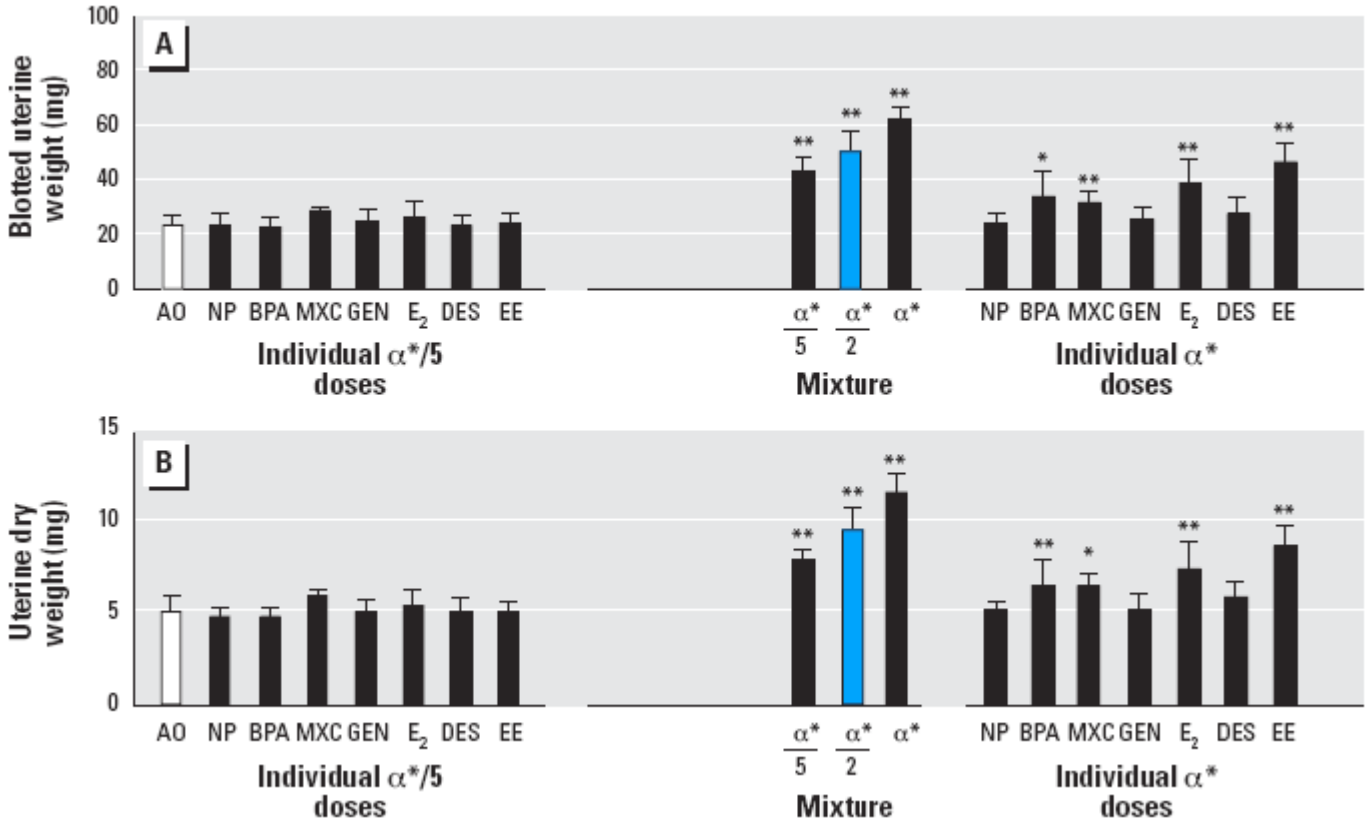
Christiansen *et al.* 2009, EHP **117**, 1839





Comparing mixture effects with those of components

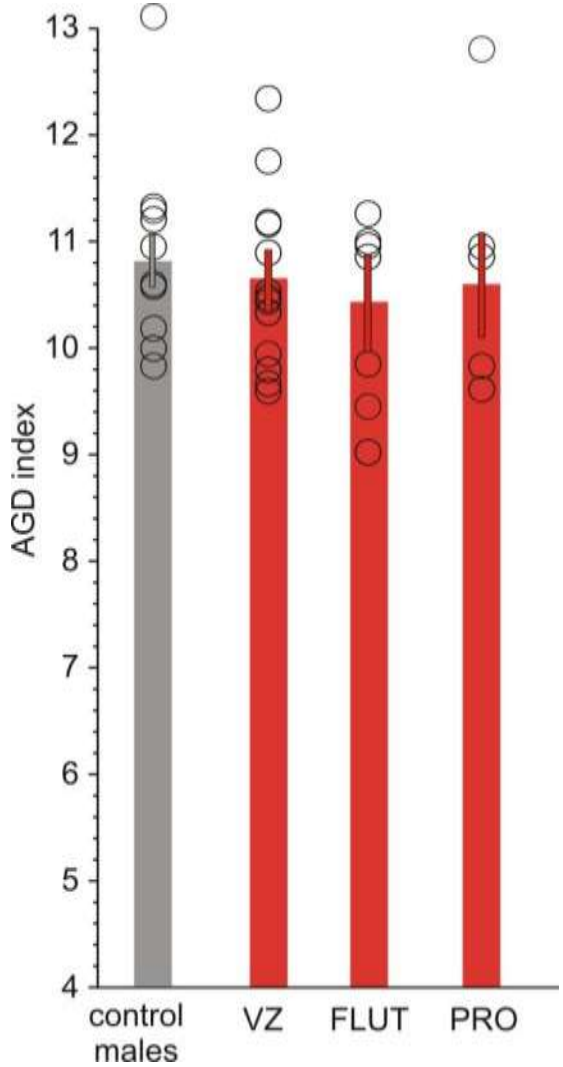
Tinwell and Ashby (2004) EHP **112**, 575



Comparing mixture effects with those of components

Similarly acting chemicals: Something from “nothing”

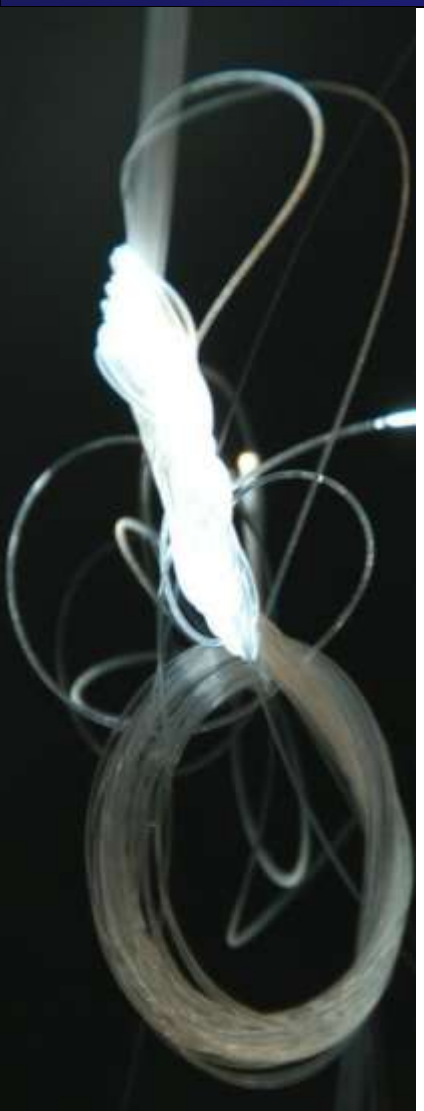
Hass *et al.* 2007, EHP **115** (Suppl 1), 122



Is there sufficient protection at exposures not exceeding ADI's or PNEC's?

- **Scientific consensus**: mixtures of similarly acting compounds require special consideration
- **Dose addition**: Every component contributes, even at doses below thresholds

When is a mixture “safe”?
The case of dose addition



$$\frac{\text{Intake}_1}{\text{Tolerable Daily Intake}_1} + \frac{\text{Intake}_2}{\text{Tolerable Daily Intake}_2} < 1$$

Mixture effect equal (no) effect at TDI if every component is present at **TDI / n**

How many mixture components are we dealing with?

Independent action – the orthodox view

- Mixtures pose no health concern as long as each component stays below NOAELs (Feron et al. 1995, COT 2002)

- ”As a matter of fact, presently available data on exposure to mixtures of chemicals at doses well below the NOAELs of the individual constituents indicate that such exposure is of no health concern“ (Carpy et al. 2000, *European Crop Protection Association*).

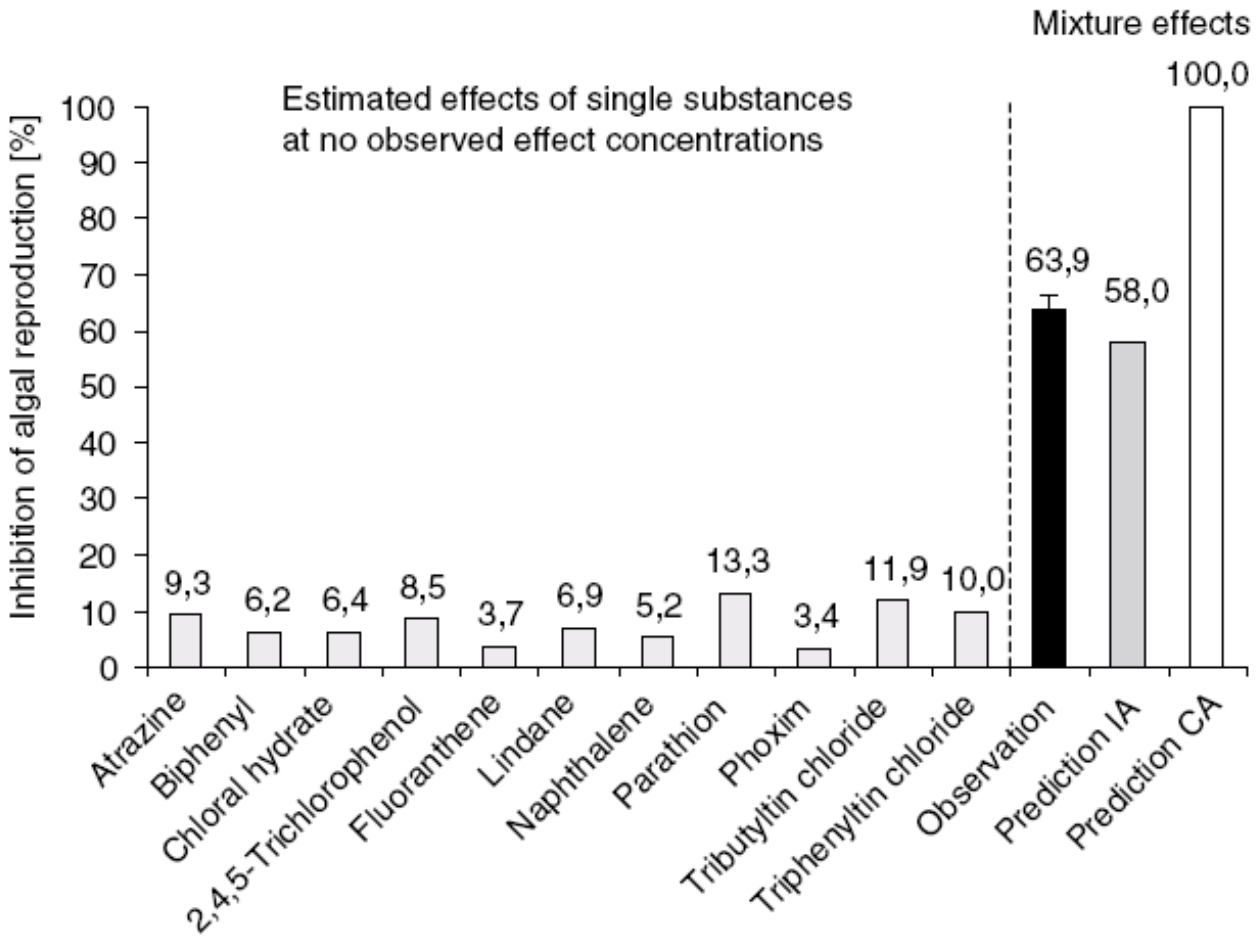
Combination effects of **dissimilarly** acting chemicals at conc < NOAEL

Reference	Mixture components	Species / Endpoint	Individual concentrations	Joint effect
Hermens et al. 1985 Ecotoxicol Environ Saf 9: 3211-326	33 aquatic pollutants from 3 groups with probably different modes of action	Fish / Acute mortality	4% of EC50 (assumed to be below NOEC)	50%
Payne et al. 2001 Environ Health Perspect 109: 391-397	4 organo-chlorine pesticides exerting effects on cell proliferation in different ways	MCF-7 cell proliferation	25-100% of NOEC	Significant proliferative effect
Walter et al. 2002 Ecotoxicology 11: 299-310	11 aquatic priority pollutants selected for structural diversity by chemometric analysis	Algae / Reproduction	NOEC	64%
Faust et al. 2003 Aquatic Toxicol 63: 43-63	16 toxicants known to interact with completely different molecular target sites in algae	Algae / Reproduction	6.6-66% of NOEC	18%

Combination effects of **dissimilarly** acting chemicals at conc < NOAEL

Mixture of dissimilar algal toxicants

Walter *et al.* (2002) Ecotoxicol **11**, 299

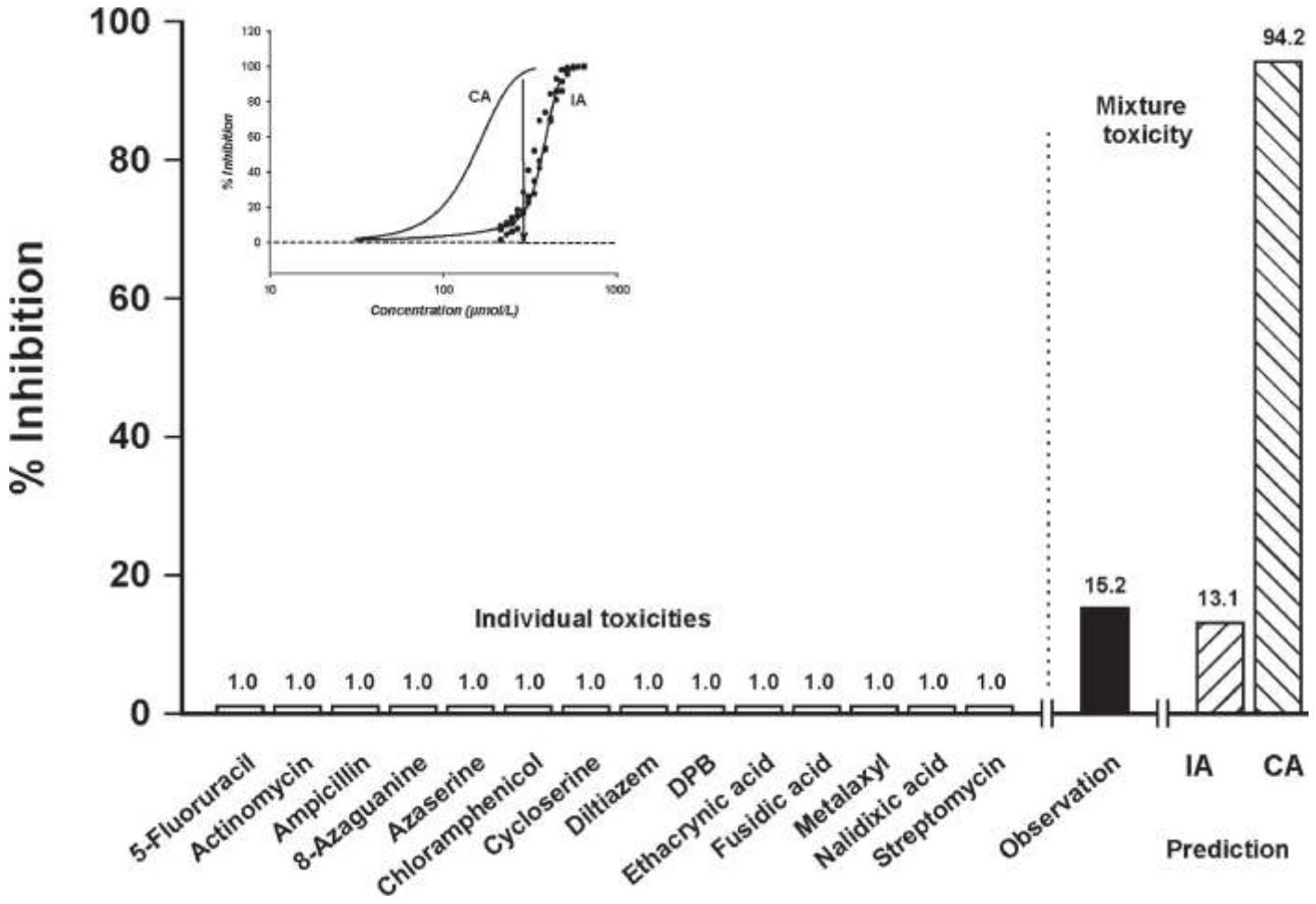


Combination effects of **dissimilarly** acting chemicals at conc < NOAEL

Mixture of dissimilarly acting bacterial toxicants and pharmaceuticals

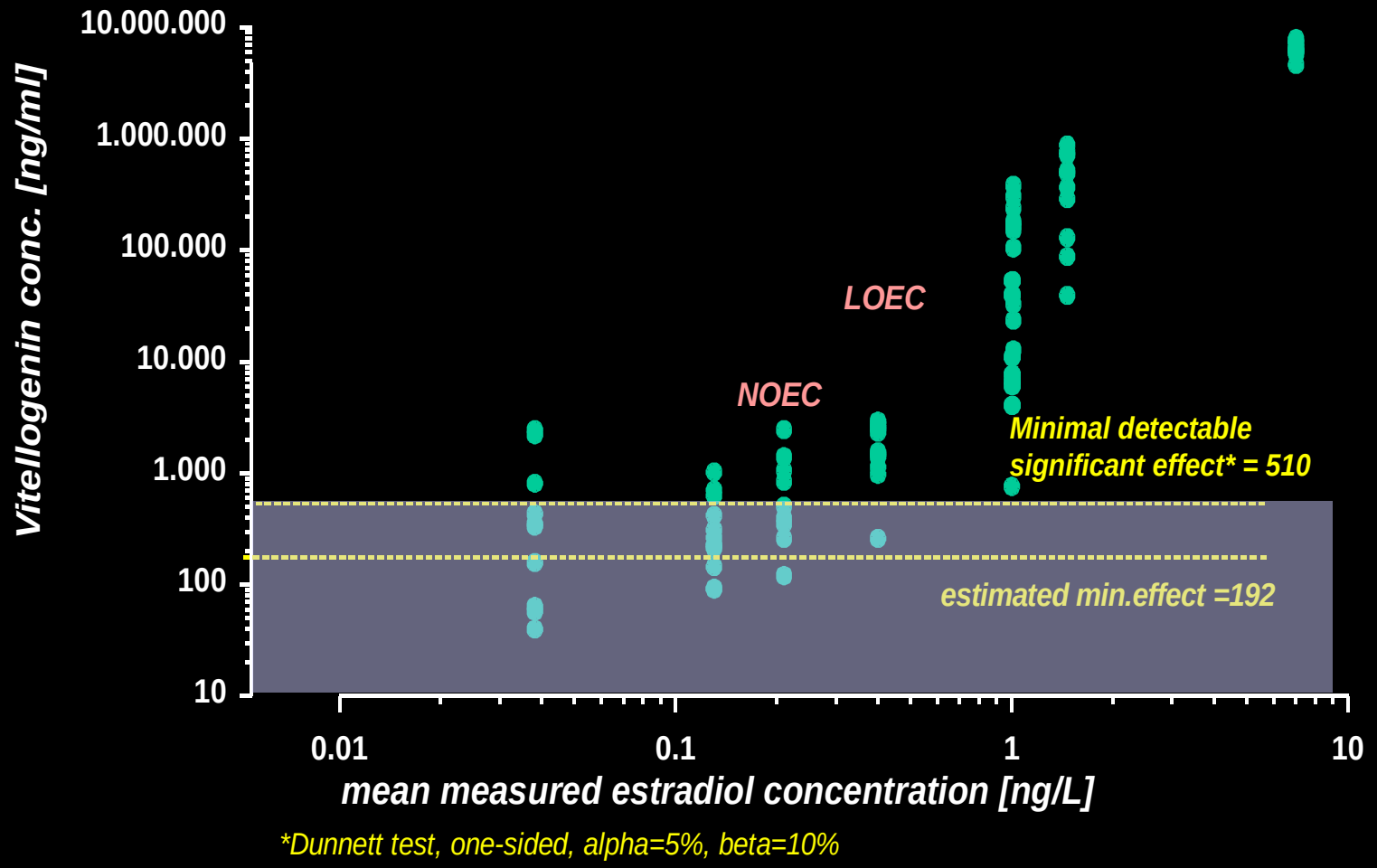
Backhaus, Sumpter and Blanck (2008)

In Kümmerer (Ed.) Pharmaceuticals in the Environment, 3rd revised ed., pp. 257-276, Springer, Berlin, Heidelberg



What is a NOAEL?

Vitellogenin induction in fish



NOAEL

“A grey zone where effects can neither be confirmed nor ruled out with certainty”

M. Faust

When is a mixture “safe”?
The case of independent action

Independent action

$$E_{1,2,..n} = 1 - [(1-e_1)(1-e_2)...(1-e_n)]$$

100 agents with zero effect: joint effect = 0

100 agents with 1% effect: joint effect = 63%

100 agents with 0.1% effect: joint effect = 9.5%

Pertinent issues

- ADI (TDI, PNEC) - zero effect levels?
- How many chemicals act together?
- Which chemicals should be considered / grouped together?

Combined exposures - a topic for risk assessment!

Regulatory implications

- Risk assessment: dose addition a good approximation
- Bottleneck I: mixtures exposure assessment – lack of data
- Is truly cumulative risk assessment covered by EU law?
- Is it within the remit of EU scientific committees?

“From research to regulation”

- Combined exposures in **risk assessment**
- Combined exposures in **standard setting** for individual chemicals

Certain chemicals…

…disrupt hormone action in foetal life by

- blocking the receptor
- suppressing sex hormone synthesis

Demasculinisation

Relevance to humans

Grouping principles

" *The developing tissue does not care by which mechanism fetal androgen action is compromised – whether by* ***suppression of androgen synthesis*** *or by* ***antagonism at the receptor*** *– the outcome is the same*"

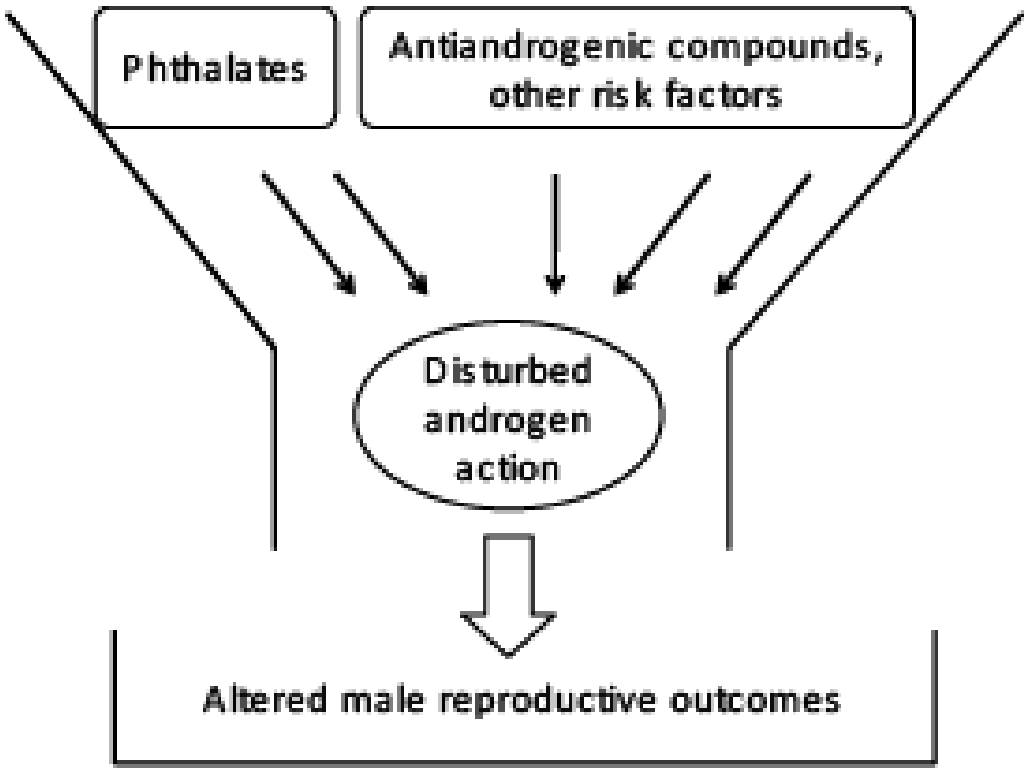
Earl Gray Jr.

Evidence of mixture effects

- Mixtures of phthalates work together (Howdeshell *et al.* 2002)
- Mixtures of androgen receptor antagonists work together (Hass *et al.* 2007, Metzdorff *et al*. 2007)
- Mixtures of phthalates and anti-androgens with mixed mode of action work together (Ryder *et al.* 2008, Christiansen *et al.* 2009)
- Dose addition provides good approximations of observed mixture effects

Cumulative risk assessment for antiandrogens

Common adverse outcomes *US National Academy of Sciences, NRC 2008, Phthalates cumulative risk assessment*



Grouping

- **Phthalates**: BBP, DBP, DEHP, DIBP, DINP, DPP
- **Pesticides**: vinclozolin, procymidone, linuron, prochloraz, ketoconazole, tebuconazole, fenitrothione
- **Env chemicals**: PBDEs, pp-DDE, certain PCBs, certain PCDD/F
- **Others**: certain parabens?, UV-filter substances?

Hazard quotient, hazard index

Hazard quotient

$$\frac{\text{Intake}}{\text{Tolerable Daily Intake}} < 1$$

Hazard index

$$\frac{\text{Intake}_1}{\text{Tolerable Daily Intake}_1} + \frac{\text{Intake}_2}{\text{Tolerable Daily Intake}_2} < 1$$

$$HI = \sum_{i=1}^{n} \frac{EL_i}{AL_i}$$

An application of dose addition

Known knowns (15 chemicals): **high intake**

Table 6 Hazard quotient and hazard index calculations for high intakes of anti-androgens

Chemical	High intake (lg/kg/day)	RfD AA (lg/kg/day)	Hazard quotient (high intake / RfD AA)	Ratio of HQ and HI %
DBP	6	100	0.06	2.98
DiBP	1.5	200	0.008	0.37
BBP	4	330	0.012	0.60
DiNP	1.7	1500	0.001	0.06
DEHP	3.6	30	0.12	5.96
Vinclozolin	9	50	0.18	8.94
Prochloraz	14	50	0.28	13.93
Procymidone	9	100	0.09	4.47
Linuron	0.6	100	0.006	0.30
Fenitrothion	5	200	0.025	1.24
p,p′-DDE	1	100	0.01	0.50
BDE 99	0.02	10	0.002	0.10
Bisphenol A	1.5	12.5	0.12	5.96
Butyl paraben	100	100	1.00	49.66
Propyl paraben	100	1000	0.1	4.97
Hazard Index			2.01	

Known knowns (15 chemicals)

- 7 chemicals explain > 90% of expected combination effect
- Poor quality data for BPA
- Dito: butyl-, propyl paraben
- **PBDE and *pp'*-DDE contribute little**

Kortenkamp and Faust (2010)
Internat J Androl **33**, 463

Standard setting for individual chemicals

Mixture effect equal (no) effect at TDI if every component is present at **TDI / n**

- An additional *mixture assessment factor*?
- How large?
- How many chemicals contribute?

Thank you