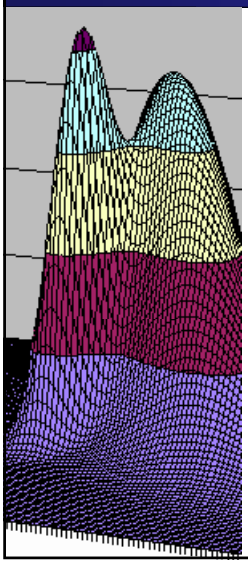
## Swedish Society of Toxicology



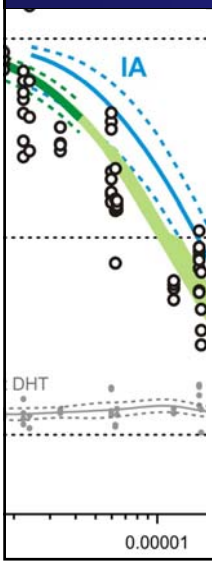
# Is mixtures risk assessment necessary and what are obstacles to making progress?

Andreas Kortenkamp

*Institue for the Environment, Brunel University London*

23 March 2012, Stockholm, Nobel Forum

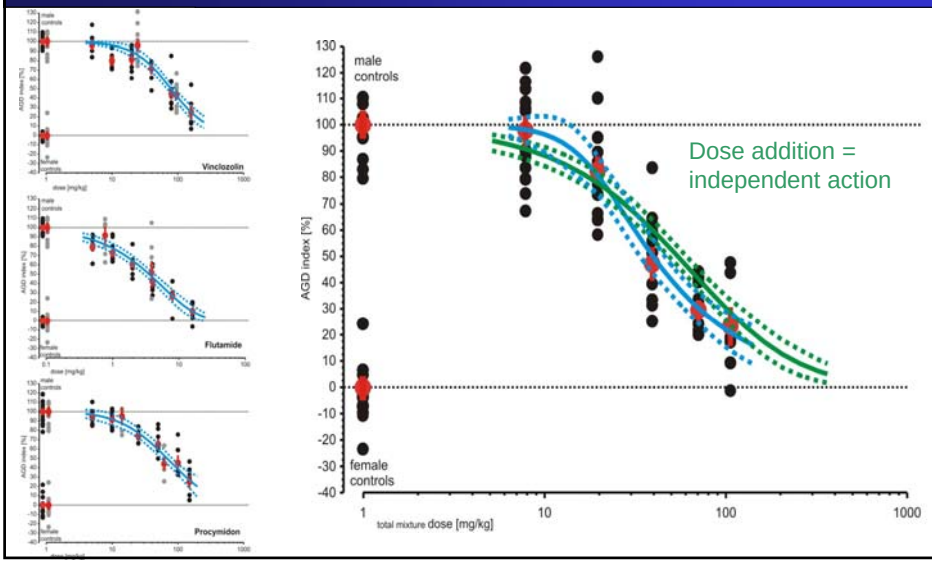
## Prediction of mixture effects?




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

# Assessment and prediction (1)

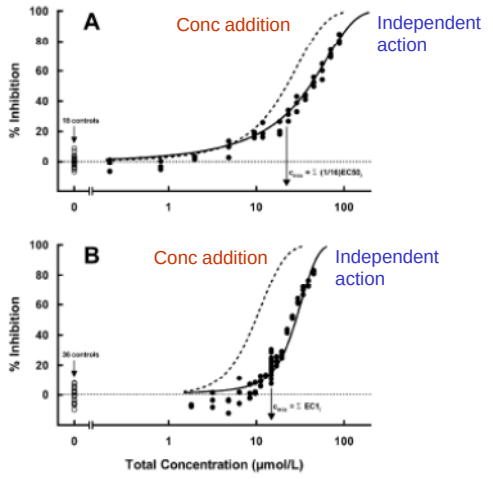
Hass *et al.* 2007 EHP **115** Suppl 1, 122




# Algal toxicity of 16 dissimilarly acting toxicants

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

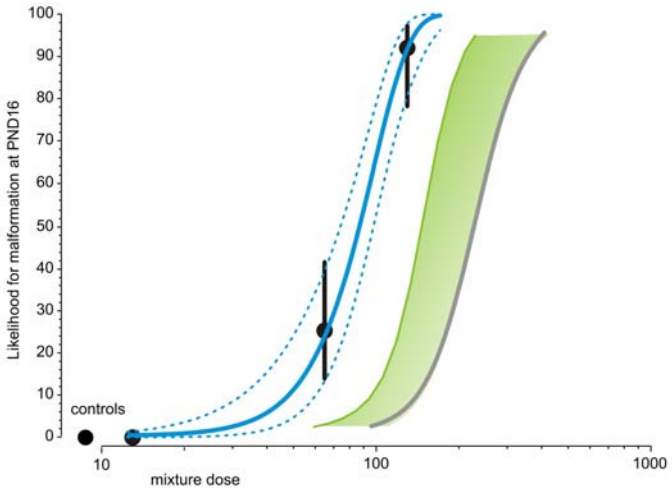
Aclonifen
8-Azaguanine
Azaserine
CCCP
Chloramphenicol
DTMAC
Fenfuram
Kresoxim-methyl
Metalaxyl
Metazachlor
Metsulfuron-methyl
Nalidixic acid
Norflurazon
Paraquat
Terbutylazim
Triadimenol



# Prediction of mixture effects?

Synergism with genital malformations

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




# Characteristics of "environmentally relevant" mixtures

- **Low levels**
- **Multi-component mixtures**
- **Unknown composition,** but not every permutation relevant

**What is the point?** - Sufficient protection when exposures are at or below ADI or TDI

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

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

If every component is present at **TDI / n** the mixture effect is equal to an effect associated with TDI (the hope: 0)

**How many mixture components are we dealing with?**

**How many are present at TDI / n?**

# 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*).

# Independent action – the orthodox view (contd.)

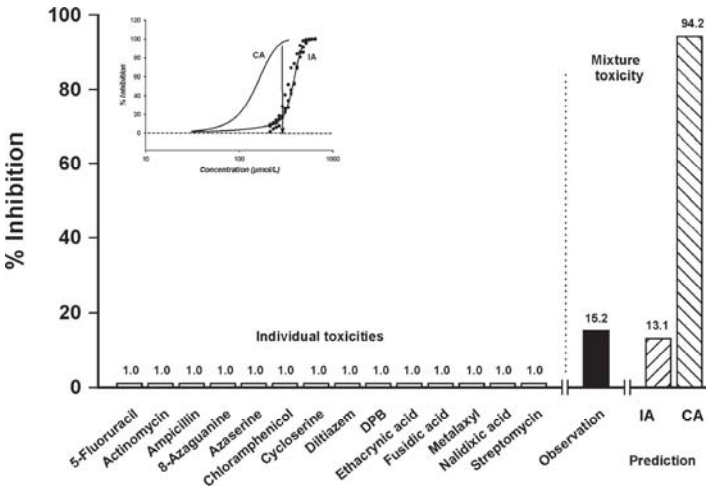
- **VKM (2009)** "*When combined exposure to plant protection products with simple dissimilar action … are below their respective effect threshold levels (NOAELs, BMDs), it is assumed that combined action of all plant protection products will be zero (see section 2.1).*"
- **VKM (2009)** "*For substances exhibiting dissimilar modes of action …, adverse effects from multiple exposures are not expected when the exposures to the individual components of the mixture are below their respective ADIs/TDIs.*"
- **COT (2002)** "*Thus, where exposure is to multiple pesticides or other chemicals at doses less than the NOAEL, adverse reactions to such exposure is unlikely.*"

# 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



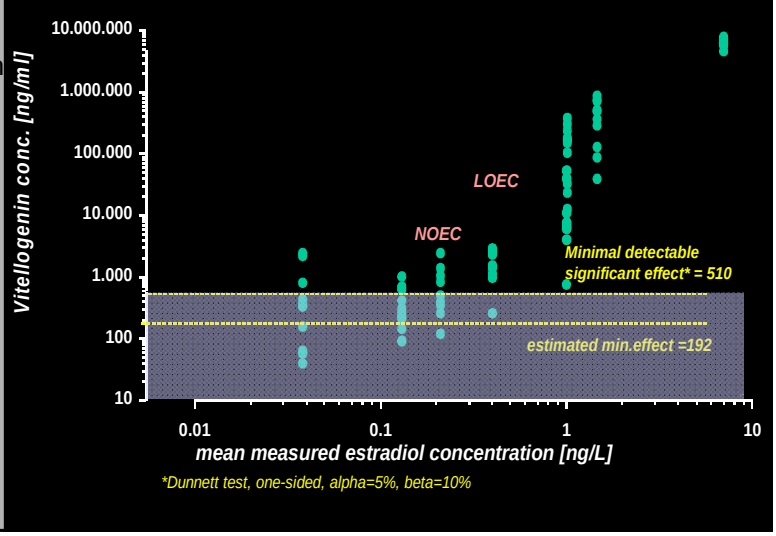
## Combination effects of **dissimilarly** acting chemicals at conc < NOAEL

| 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% |

Kortenkamp et al. (2007) EHP **115** Suppl 1, 106-114

## 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% |

**"NOAEL not a zero effect level"**
*Opinion of EU Scientific Committees on Mixture Toxicology, 2011*

## When is a mixture “safe”?

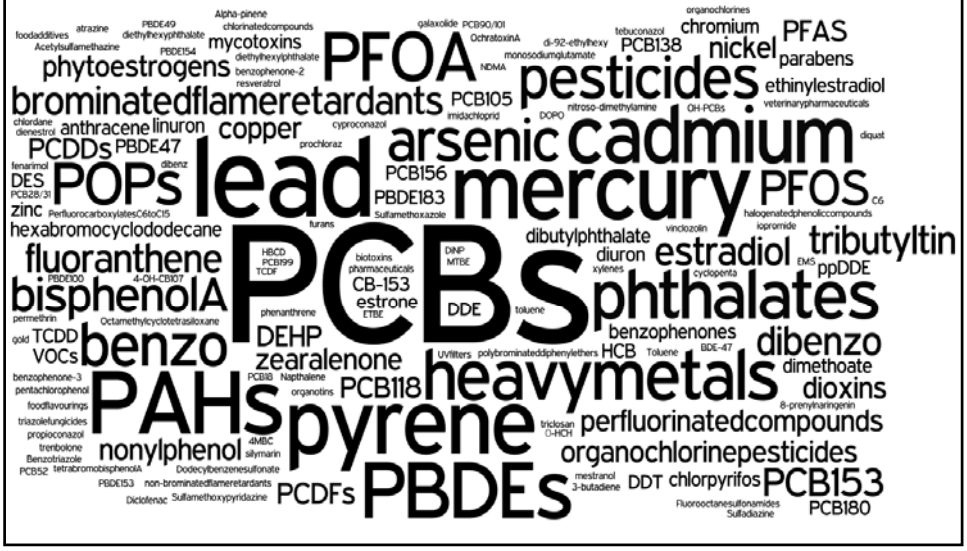
- ***Dose addition:*** dependent on number of components and sum of toxic units
- ***Independent action:*** Are ADI always zero effect levels?
- **A matter for risk assessment**

- “…for Human Health effects, if the intended level of protection is achieved for each individual substance, the level of concern for mixtures of dissimilarly acting substances should be assumed as negligible.”
- (*Opinion of EU Scientific Committees on Mixture Toxicology, 2011)*

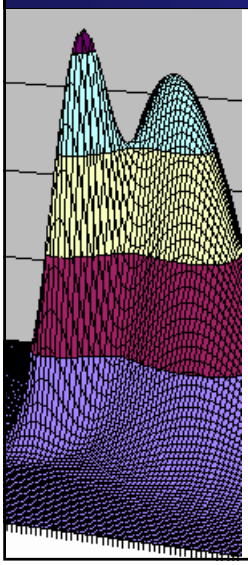
## But how much is known about exposures?

- 100,000 chemicals in commercial use in the European Union
- Unknown, but large numbers likely to be present in humans
- Vast majority of chemicals not tested – ADIs or TDIs not available
- The **“Matthew effect”**: 20 chemicals
- *Publication bias for certain substances, not because they require attention as priority pollutants, but for the very reason that **they are well researched.*** (Grandjean et al. 2011, in press)

# Chemicals considered in EU projects




# Assessing environmentally relevant mixtures: restrictions



**Data needs:**

**Effects**

**Exposure information**

**Grouping criteria**

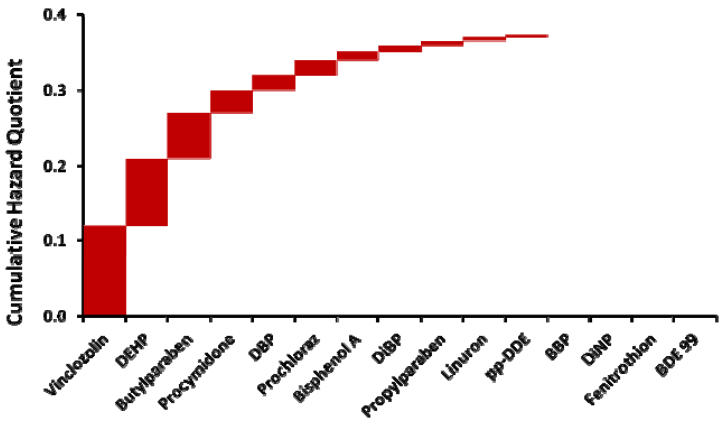
Reconstituted mixtures of anti-androgens using reported tissue levels

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

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

| Chemical | High intake (Ig/kg/ day) | RfD AA (Ig/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 | |

# Order into complexity?



# Pareto principle

**Vilfredo Pareto (1906)**

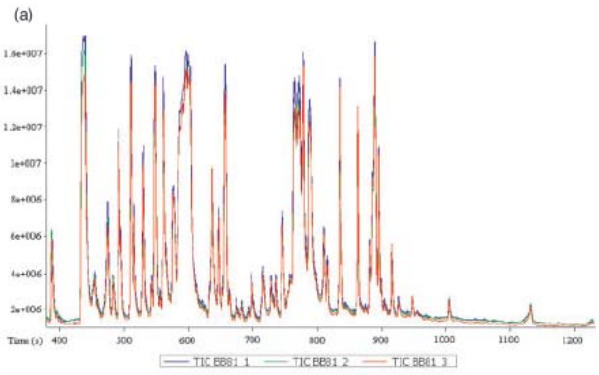
*"80% of the land in Italy is owned by 20% of the people."*

*A minority of the causes explains the majority of effects.*

**Generally applicable to environmentally relevant mixtures? Could this revolutionise risk assessment?**

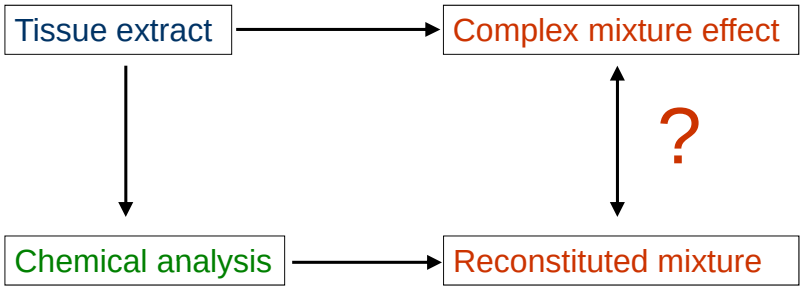
# The complexity of the human exposome

Dunn *et al.* (2008) Int J Epidem **37**, s1, i37




GC-TOF-MS total ion chromatogramme for serum

## De-constructing complex mixtures

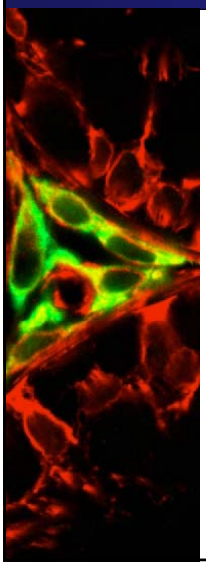



**Validation of biomarkers for use in epidemiology**

## Defining mixtures of environmental relevance

- Grouping criteria
  - Chemical similarity alone not sufficient
  - Grouping according to common effects
- Exposure information
  - Dose metric?
- Toxicity data

# Perspectives



- Move away from “Matthew effect”
- Explore applicability of Pareto principle
- Pursue unbiased exposure assessment strategies
- Risk assessment and regulation that considers mixture effects

# 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?

# 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?

# Acknowledgements

- Financial support from European Union - *contamed*
- UK Food Standards Agency
- www.nectarcluster.info

# Thank you