

Cocktail Effects: Mathematical Modeling; and Low Dose Effects

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*Is dose addition better than independent action?
Is there any effect at all at human relevant doses?*



Chemical risk assessment

The testing of every chemical combination is impossible.....



- >200,000 chemicals in the EU
- different combinations
- different ratios in combination

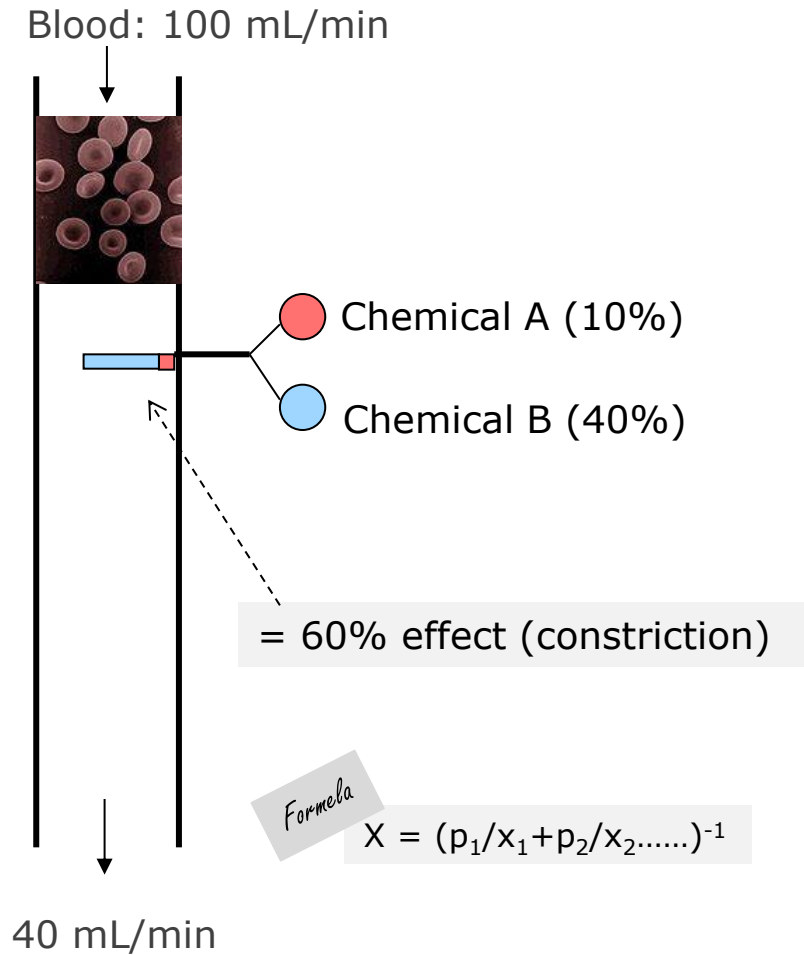
Therefore:

We need to be able to calculate cocktail effects from knowledge on single chemicals

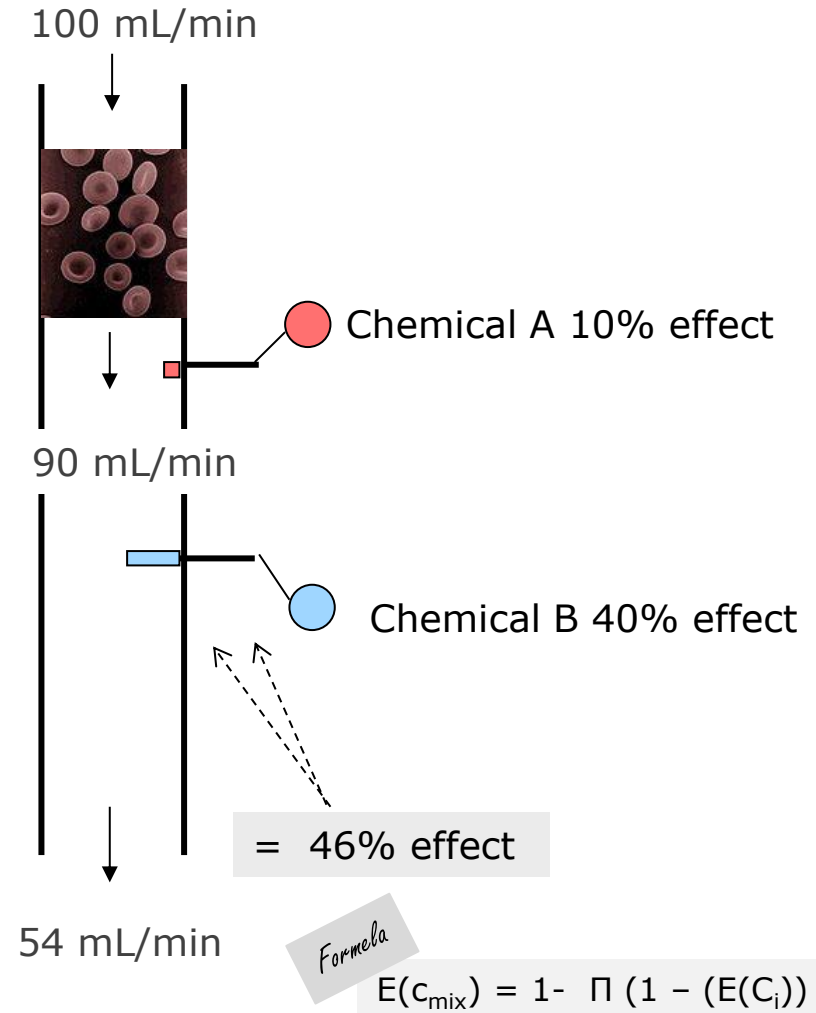
-> Mathematical models

Mathematical models:

Dose addition (DA)

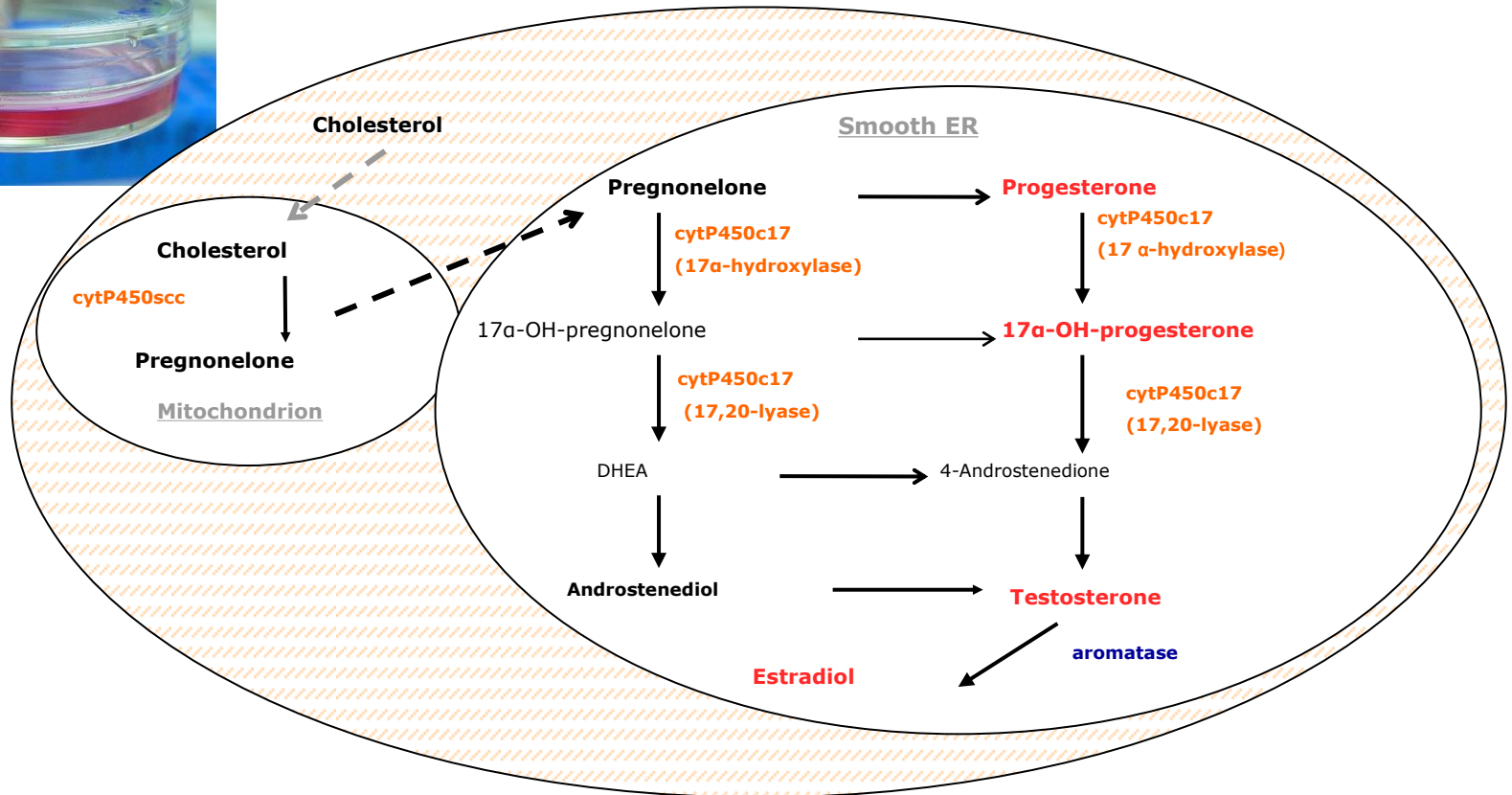
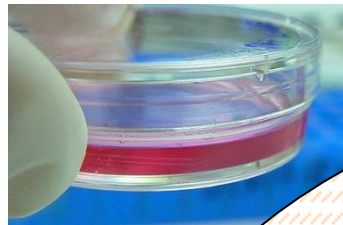


Independent action (IA)

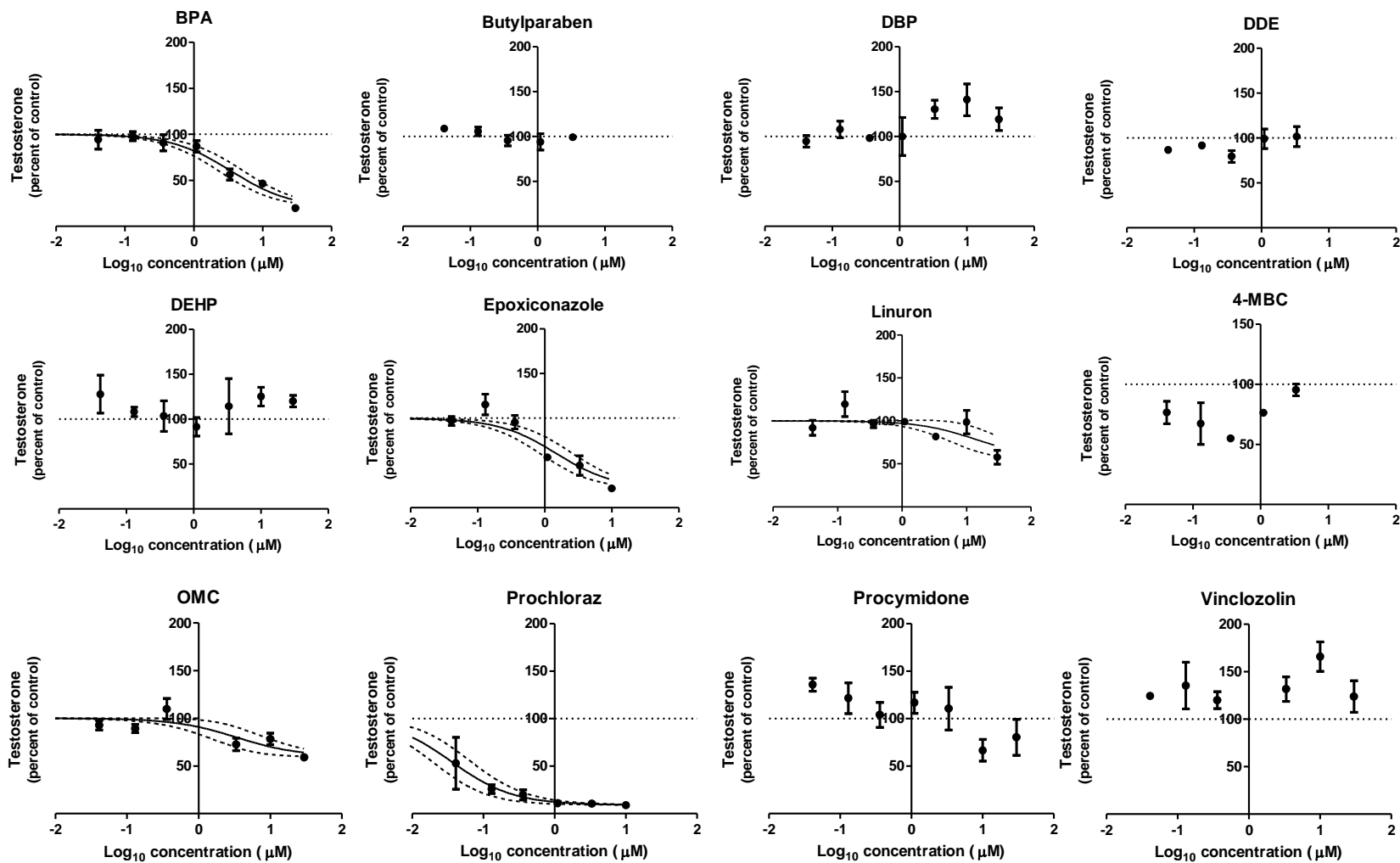


The test system...

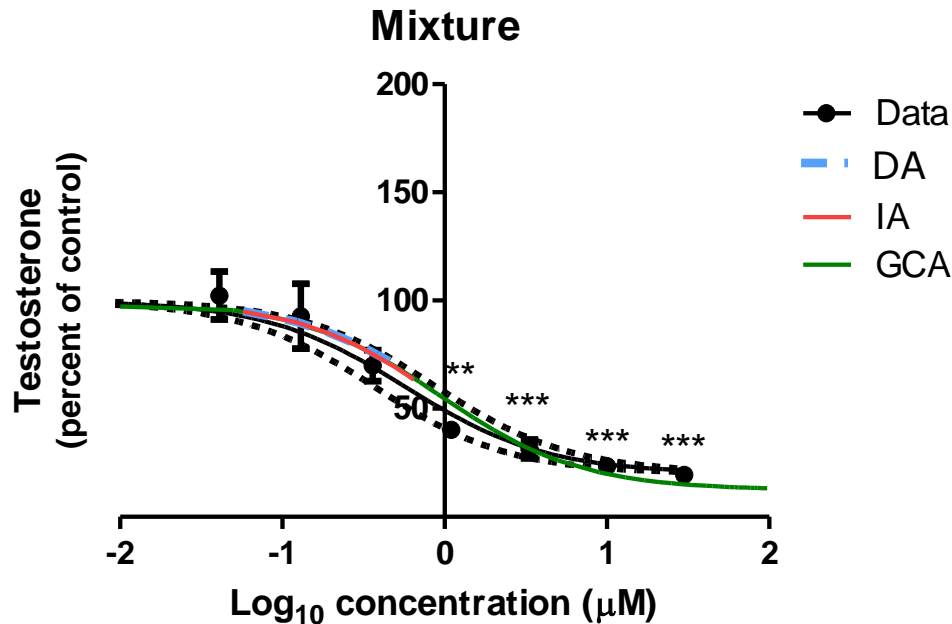
The *in vitro* H295R cell line steroid synthesis test



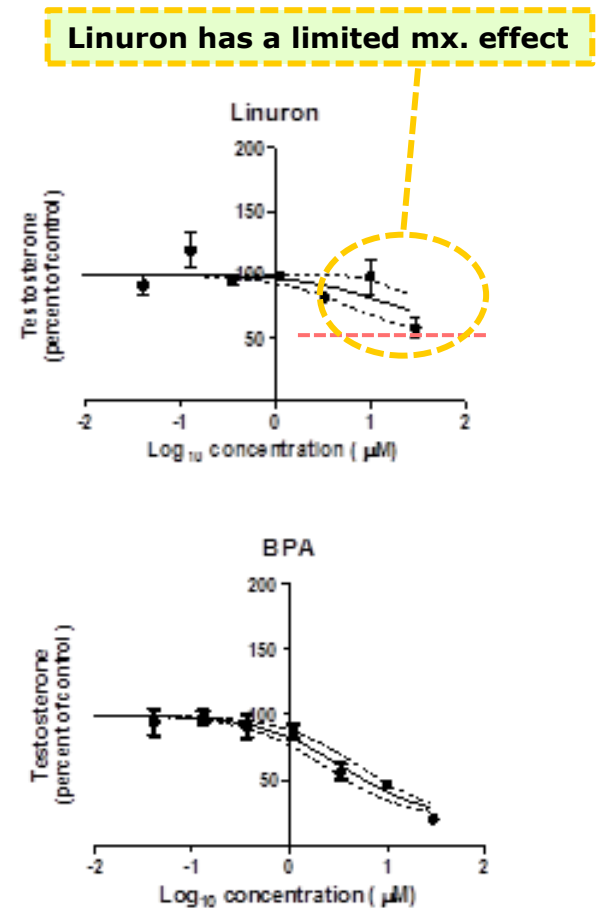
The effects of 12 chemicals on testosterone



Testosterone was predicted for a cocktail of the 12 chemicals



Generalized concentration addition (GCA) is a variant of DA developed by Gregory Howard and Thomas Webster (Boston University School of Public Health)



Conclusion part 1:



Dose addition (DA) and its extrapolations are reasonable choices for the assessment of cocktail effects

Independent action (IA) does not seem to add extra value

Part 2: Low dose cocktail effects

Are cocktail effects of environmental chemicals relevant at human doses?



- We tested low doses relevant to the general population

Low dose effects of perfluorononanoic acid in combination with a cocktail of chemicals

Perfluorononanoic acid (PFNA)

**0
0.0125
0.25 mg/kg bw/day**

with or without a chemical cocktail

14 days, oral,



)

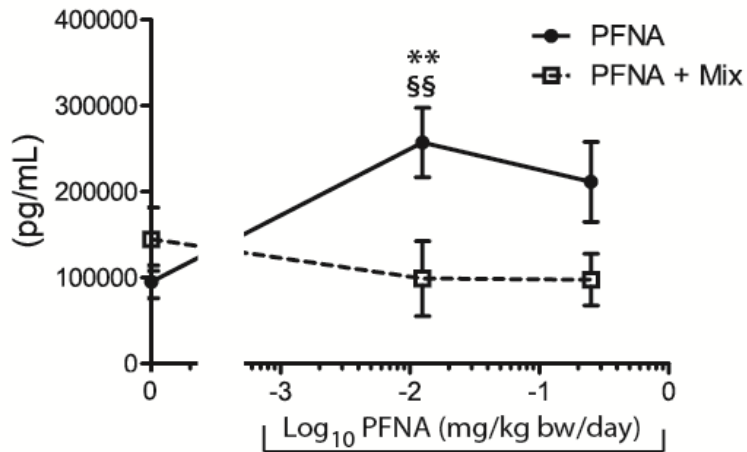
Cocktail:

| Chemical | dose (mg/kg bw/day) |
|-----------------|---------------------|
| • | |
| • Bisphenol A | 0.01 |
| • Butyl paraben | 0.51 |
| • DBP | 0.06 |
| • DDE | 0.01 |
| • DEHP | 0.09 |
| • Epoxiconazole | 0.05 |
| • Linuron | 0.004 |
| • MBC | 0.39 |
| • OMC | 0.68 |
| • Prochloraz | 0.06 |
| • Procymidone | 0.09 |
| • Vinclozolin | 0.05 |
| • Bergamottin | 0.20 |
| • Glabridin | 0.30 |

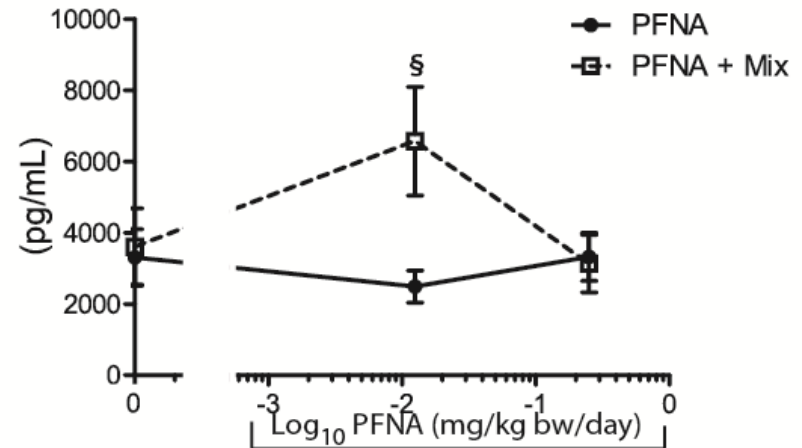
Hadrup et al. Archives of Toxicology 2015

Effects on hormones:

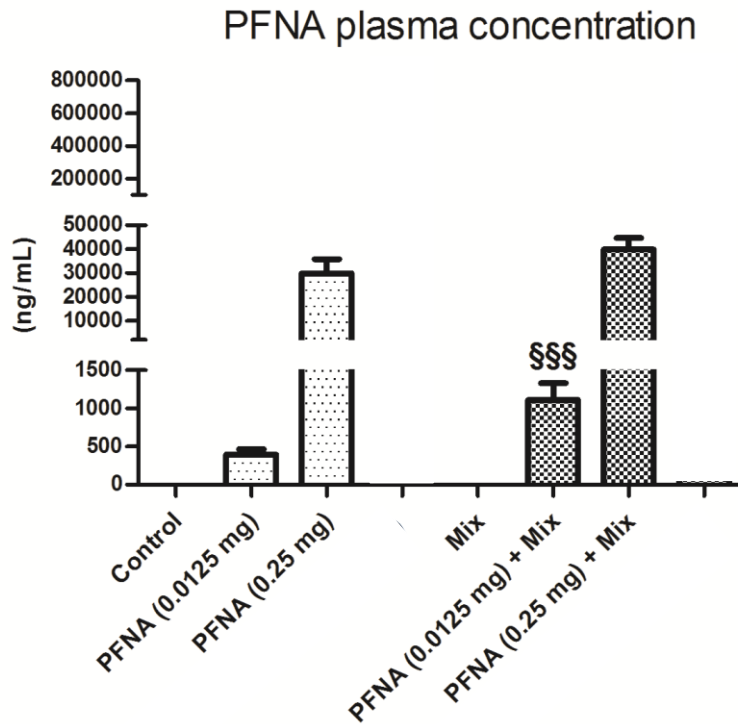
Corticosterone



Testosterone



And a toxicokinetic interaction was discovered



Toxicokinetic effect →

The cocktail affects what the body does to PFNA...

Most humans have a set of chemicals in their body...



February 2015

Fourth National Report on Human Exposure to Environmental Chemicals



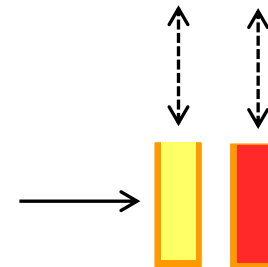
Do chemicals, at blood levels that humans have in their body, induce a toxicological footprint?

We used the geometric mean (blood/urine)
age 0-99 years, males and females in the USA

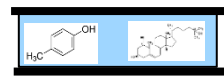
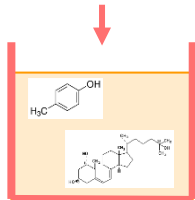
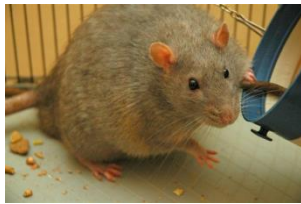
Of  chemicals



? Dose to rats?



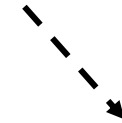
Metabolomics



Column separation



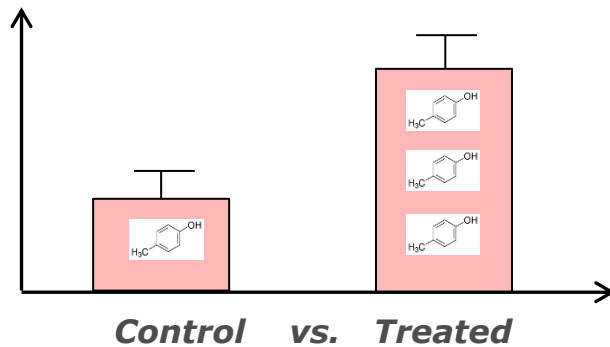
Mass detection



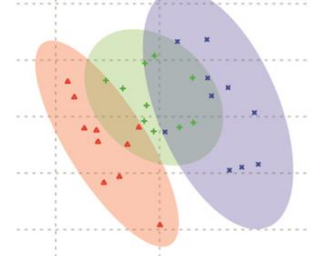
8400 data points



Identification of metabolites



PLS DA analysis





Major findings

Dose addition and its extrapolations are reasonable choices for the assessment of cocktail effects

Independent action (IA) does not seem to add extra value

We observed cocktail effects at doses approaching human levels

Including

- Imbalances in hormones

- Toxicokinetic interaction

- Liver toxicity

- Changes in the metabolome

For these data we used a mixture of the following employees....

Birgitte Møller Plesning, Dorte Lykkegaard Korsbech, Heidi Letting, Lene Sofie Dahl Svensson, Anne Ørngreen, Maja Danielsen, Eva Ferdinansen, Elise E. Navntoft, Eigil V. Frank, Kenneth R. Worm, Sarah G. Simonsen, Liljana Petrevska, Lis Abildgaard Andersen, Niels Hadrup, Kristine Kongsbak, Annette Petersen, Terje Svingen, Kasper Skov, Line Olrik Berthelsen, Camilla Taxvig, Karen Mandrup, Niels Lund Hansen, Marianne Dybdahl, Mikael Pedersen, Julie Boberg, Ulla Hass Henrik L. Frandsen, and Anne Marie Vinggaard

Tak til Fødevareministeriet og Fødevarestyrelsen