Chemical mixtures: challenges for research and risk assessment?
State of the art on chemical mixtures research

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Talk overview

- Mixture assessment 20 years ago
- Developments in mixture assessment
- Prediction of mixture effects / designer mixtures
- Mixtures at "environmentally relevant" doses
- Outlook
Mixture assessment –
State of science >20 years ago

- Summation of effects
- Isobole method (= dose addition)
- Multiplication of effects
- Comparison of mixture effects with those of individual components

What is synergy?
Berenbaum 1989, Pharmacol Rev 41, 93
Example: GST-positive foci in livers

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

Mixture of 5 heterocyclic amines

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Mixture assessment – State of science >20 years ago

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

Dose addition = Independent action
Figure 1. Pooled concentration–response data and best-fit regression curves for each of the individual mixture components. (A) EE2, (B) E2, (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.
Vitellogenin induction in fish

5 components

Assessment and prediction (2)
Brian et al. (2005) EHP 113, 721

Concentration addition

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Assessment and prediction (3)
Ermler et al. (2013a) Mutagenesis **28**(4):417

Micronucleus induction *in vitro*

7 similarly acting components
Assessment and prediction (3)
Ermler et al. (2013b) Arch Toxicol

Micronucleus induction *in vitro*

dissimilarly acting components

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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

Conc addition
Independent action

Conc addition
Independent action

Chemical mixtures: challenges for research and risk assessment?
Prediction of mixture effects?

Synergism with genital malformations

Christiansen et al. 2009, EHP 117, 1839

4 antiandrogens with differing mechanisms

Dose addition
Independent action
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
From a scientific viewpoint: Is a consideration of mixture effects necessary?

- Chemicals risk assessment normally ignores mixture effects
- Exposure: to several chemicals simultaneously
- “Environmentally relevant” mixtures:
  - Low levels
  - Complex multi-component mixtures of unknown composition
From a scientific viewpoint: Is a consideration of mixture effects necessary?

Current practice justified if:

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

7 estrogenic compounds

Tinwell and Ashby (2004) EHP 112, 575

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Comparison of mixture effects with those of components

Similarly acting chemicals: Something from “nothing”

Hass et al. 2007, EHP 115 (Suppl 1), 122
When is a mixture “safe”? 
Is there sufficient protection at low enough exposure levels?

- **Scientific consensus**: mixtures of similarly acting compounds require special consideration
- **Dose addition**: Every component contributes, even at doses below thresholds
- **Independent action**: No mixture effect is expected when each compound is present at a concentration that causes no effect

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

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

Mixture of 11 dissimilar algal toxicants

What is a NOAEL?

Vitellogenin induction in fish

LOEC

NOEC

Minimal detectable significant effect* = 510

Estimated min. effect = 192

*Dunnett test, one-sided, alpha=5%, beta=10%

What is a NOAEL?
When is a mixture “safe”?

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%
Questions arising

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

Dose addition as a good approximation to consider combined exposures in risk assessment.
What is similar action?

Certain chemicals disrupt hormone action in foetal life by

- blocking the androgen receptor
- suppressing sex hormone synthesis

Demasculinisation

Relevance to humans
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 anti-androgens

Common adverse outcomes


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Grouping

- **Phthalates**: BBP, DBP, DEHP, DIBP, DINP, DPP
- **Pesticides**: vinclozolin, procymidone, linuron, prochloraz, ketoconazole, tebuconazole, fenitrothione
- **Environmental chemicals**: PBDEs, pp-DDE, certain PCBs, certain PCDD/F
- **Others**: certain parabens?, UV-filter substances?
Is there a case for cumulative risk assessment? Hazard quotient, hazard index

<table>
<thead>
<tr>
<th>Hazard quotient</th>
<th>Hazard index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td>Tolerable Daily Intake &lt; 1</td>
</tr>
<tr>
<td>$\frac{\text{Intake}_1}{\text{Tolerable Daily Intake}_1}$ + $\frac{\text{Intake}_2}{\text{Tolerable Daily Intake}_2}$ &lt; 1</td>
<td></td>
</tr>
</tbody>
</table>

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

An application of dose addition

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15 anti-androgens (high intake)


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

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15 anti-androgens (high intake)

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

Anti-androgens – human tissue levels

Hazard Index

Kortenkamp et al. (2013)
Reproduction (under revision)

21 AR-antagonists
(in vitro)

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Anti-androgens – human tissue levels

Mixture effect predictions

Kortenkamp et al. (2013) Reproduction (under revision)

21 AR-antagonists (in vitro)

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Pareto Principle

- “80 % of the land is owned by 20 % of the people” (V. Pareto, 1906)

- Is this principle generally applicable to environmentally relevant mixtures?
- Prioritisation of chemicals?
Data gaps

- Knowledge of relevant human and environmental exposures
- Sufficient effect data for relevant compounds
- Grouping criteria (MOA vs CAO)
Research needs

- **Systematic compilation of information on typical exposure scenarios**
- **Identification of priority chemical mixtures which might impact on human health and the environment**
- **Scientific understanding of determinants of synergisms to anticipate synergistic effects**
Work in progress and the future...

- Identification of effective compounds in complex mixtures (Effect directed analysis)
- Identification of environmentally relevant mixtures and priority constituents
- Exposome
- Systems toxicology approaches
- Other “omics” approaches
Acknowledgements

- Andreas Kortenkamp
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  - European Union
  - UK food standards agency

- ANSES
Thank you!