Mixture identification from co-exposure modeling as a first steps towards hazard characterization

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Outlines

- Risk assessment of mixtures: brief overview

- Modeling co-exposures to define mixtures: statistical methods

- Definition of mixtures from exposure or food consumption: comparison

This is about mixture identification and not about mixture risk assessment
Some definitions related to mixtures

- **Aggregate Exposure** is the exposure to a single/multiple chemicals associated with multiple pathways (diet, air, dust, etc.) and routes (ingestion, inhalation, dermal contact, etc.).

- **Co-Exposure / Combined Exposure** is the exposure to multiple chemicals associated with a single/multiple pathways and routes.

- **Cumulative Risk Assessment** is the risk of a common toxic effect associated with concurrent exposure by a single/multiple relevant pathways and routes of exposure to a group of chemicals that share a common mechanism of toxicity.
Risk assessment of mixtures based on additivity

Concentration/Dose addition (DA)

- Similar mode of action and biological site
- Cumulative Risk Assessment: chemicals are expressed in a reference chemical using toxicity equivalence factors (TEF) and then summed
- Same family of chemicals: dioxins, furans, dioxins-like PCB, organophosphorus pesticides

Response addition/Independent Action (IA)

- Mode of action: dissimilar but contribute to a common result
- Biological site: possibly, but not necessarily, different
- Statistical concept of independent events (Bliss, 1939)
- No risk when doses of all chemicals < threshold effects
Interactions ?

Cytotoxicity of two pesticide mixtures on colon cells (Lehegarat et al. Poster Eurotox 2011)

Some mixtures do not comply with DA or IA: Their components can interact and the mixture effects can be smaller (antagonistic) or larger (synergistic) than the sum of individual effects.
Identification of mixtures

In the diet, the following can be found: pesticides (>300), inorganic contaminants and minerals (>30), additives (>10), phyto-oestrogens (10), dioxins, PCB, furans, brominated flame retardants (>50), mycotoxins (>20), etc...

Individuals are exposed to a large number of various substances

Multiplicity of possible combinations of chemicals for which testing toxicological combined effects is unrealistic

Which mixtures must be studied for their combined effects?

Some criteria:
- High exposure to mixtures components
- High correlations between mixture components
- Large number of individuals impacted
Dietary co-exposure assessment

Combine, for each chemical $p$ and each individual $i$
- the quantity $Q_{i,a}$ of food $a$ consumed
- the chemical concentration $C_{p,a}$ in food $a$

Sum all the commodities $A_p$

Then divide the total by the body weight $bw_i$

$$E_{p,i} = \frac{\sum_{a=1}^{A_p} Q_{i,a} C_{p,a}}{bw_i}$$

For acute exposure:
- $Q_{i,a}$: consumed quantities during a day
- $C_{p,a}$: a high percentile of the concentration distribution

For chronic exposure:
- $Q_{i,a}$: mean consumed quantities during a day, a week...
- $C_{p,a}$: mean concentration
Co-exposures to two pesticides

Individuals highly exposed to both pesticides

Individuals weakly exposed to both pesticides

In real life: more than 2 chemicals
Define combinations from a large number of chemicals
Objectives

Due to particular combinations of consumed food and chemical concentrations, individuals are highly exposed to different mixtures

- Cluster individuals with similar profiles in subgroups
- Extract mixtures by studying correlations/latent variables
Statistical methods to identify mixtures

- Methods based on classification/clustering
  Ex: Bayesian nonparametric model applied to acute exposure

- Methods based on reduction of dimension
  Ex: Non Negative Matrix applied on
    - chronic exposure
    - food consumption
Methods based on classification/clustering

Applied to acute co-exposures:
1. Cluster individuals with similar co-exposure profiles in subgroups
Methods based on classification/clustering

Applied to acute co-exposures:

1. Cluster individuals with similar co-exposure profiles in subgroups
2. Study exposure correlations: chemicals with high correlations are gathered together in mixtures

Correlations matrix of group light blue
Methods based on classification/clustering

Applied to acute co-exposures:

1. Cluster individuals with similar co-exposure profiles in subgroups
2. Study exposure correlations: chemicals with high correlations are gathered together in mixtures

Statistical methods

- K-means
- Hierarchical clustering
- Mixtures of distributions
  (Baysian nonparametric model)
Mixtures extracted from 79 pesticides acute co-exposures

PERICLES French research programme (2009-2012)

<table>
<thead>
<tr>
<th>Mixture 1</th>
<th>Mixture 2</th>
<th>Mixture 3</th>
<th>Mixture 4</th>
<th>Mixture 5</th>
<th>Mixture 6</th>
<th>Mixture 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphenylamine</td>
<td>Linuron</td>
<td>Fenitrothion</td>
<td>Lambda-Cyhalothrin</td>
<td>DDT</td>
<td>Maleic hydrazide</td>
<td>Imazalil</td>
</tr>
<tr>
<td>Tolyfluanid</td>
<td>Chlorfenvinphos</td>
<td>Quinoxyfen</td>
<td>Iprodione</td>
<td>Dieldrin</td>
<td>Chlorpropham</td>
<td>Methidathion</td>
</tr>
<tr>
<td>Phosalone</td>
<td>Ethion</td>
<td>Fenhexamid</td>
<td>Procymidone</td>
<td></td>
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</tr>
<tr>
<td>Propargite</td>
<td></td>
<td>Triadimenol</td>
<td>Cyprodinil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captan</td>
<td></td>
<td>Pyrimethanil</td>
<td>Fludioxonil</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Penconazole</td>
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</tbody>
</table>

25 pesticides gathered together in 7 mixtures
Mixture made up of 2 to 6 pesticides
Methods based on reduction of dimension

Applied to chronic co-exposures:
1. Reduce the dimension of co-exposure to $P$ chemicals in a set of $K$ latent variables ($K<P$)
2. Define mixture using the new weights on latent variables
3. Identify corresponding subgroups of individuals using classification/clustering methods

Statistical methods
- Principal component analysis
- Factor analysis
- Non-negative matrix factorization
  - Deal with null values
  - Deal with positive values
Non-negative matrix factorization

\[ E = W \times H + \epsilon \]

- **K latent variables = K mixtures of P chemicals**
- **\( W_{p,k} \):** weight of each chemical in the latent variable \( k \)
  - High weights provide mixture components
- **\( H_{k,i} \):** weight of each latent variable \( k \) in the whole co-exposure of the individual \( i \)
  - Classification applied to \( H \) provides subgroups of individuals
NMF applied to chronic co-exposure to 79 pesticides

Which foods are involved in the exposure to the mixtures?
Difficult to revert to food intakes

<table>
<thead>
<tr>
<th>K</th>
<th>Mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Chlorthal-dimethyl</td>
</tr>
<tr>
<td></td>
<td>Difenoconazole</td>
</tr>
<tr>
<td></td>
<td>Ethion, Chlorthalvinphos, Linuron</td>
</tr>
<tr>
<td>10</td>
<td>Dieldrin, DDT</td>
</tr>
<tr>
<td></td>
<td>Chlordane, Heptachlor</td>
</tr>
<tr>
<td></td>
<td>Hexachlorobenzene</td>
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</tbody>
</table>
Methods based on reduction of dimension

Applied to food consumption:

1. Identify principal food combinations (latent variables) in reducing dimension
2. Combine consumption systems with chemical concentrations
3. Extract principal chemicals combinations based on consumption systems

<table>
<thead>
<tr>
<th>k</th>
<th>Mixtures</th>
<th>Food vectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Dieldrin, DDT, Chlordane, Chlorpropham, Pirimiphos-methyl, Malathion</td>
<td>Pork, beef, wheat, barley potatoes, wine grapes</td>
</tr>
<tr>
<td>8</td>
<td>Chlorthal-dimethyl, Difenconazole, Ethion, Chlorfenvinphos, Linuron, Iprodione, Maleic-hydradize, Dithiocarbamate</td>
<td>Potatoes, onions, carrots, turnips, cabbages, leeks</td>
</tr>
</tbody>
</table>

Same pesticides found with the method based on exposure
## Work from exposure or consumption?

<table>
<thead>
<tr>
<th></th>
<th>From exposure</th>
<th>From food</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td>- No zero value when a large number of foods are studied</td>
<td>- Characterize population from their food habits</td>
</tr>
<tr>
<td></td>
<td>- Only way to deal with aggregated exposure (various sources)</td>
<td>- Provide foods involved</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Easy to make recommendations</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>- Difficult to identify foods involved</td>
<td>- Require specific method to deal with a large number of zeroes due to null consumptions</td>
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<td></td>
<td></td>
<td>- Limited in case of aggregated exposure (various sources)</td>
</tr>
</tbody>
</table>
What could be the use of mixture definition?

Prioritization of mixtures to study for toxicological effects

- Only 4 (among 42) components of the 10 mixtures obtained with chronic co-exposures modeling, presented a risk regarding acceptable daily intake (French TDS 2, Nougadere et al. Environmental Research 2012): and in combinations?

- Plan toxicological tests for mixtures, (PERICLES program)
- The 7 mixtures obtained with acute co-exposures were tested
  - Different human cell strains (colon, liver, kidney, brain, intestine)
  - Different tests
    - Cytotoxicity (MTT, NRU, ICW and XCELLigence)
    - Genotoxicity (Comet and H2AX assays)
    - Activation HepG2-hPXR
  - Mixtures/ Individual components
  - Equimolar concentrations/ exposures proportions
Conclusions and limitations

PERICLES French research project: conclusions

- The 7 mixtures tested on human cells produce additive but also synergistic and antagonistic effects
- Similar mixtures for both statistical methods
- Dissimilar mixtures from exposure/consumption
- Dissimilar mixtures from acute/chronic exposure

Co-exposure calculations

- Importance of chemical concentration in a single sample of food, especially for acute exposure
- Treatment of censored data impacts the mixtures
Thanks to all the PERICLES participants

Articles from PERICLES program (exposure part)

- A. Crépet and J. Tressou (2011). *Bayesian nonparametric model for clustering individual co-exposure to pesticides found in the French diet*. Bayesian Analysis. 6(1), 127:144.

- A. Crépet *et al.* *Identification of the main pesticide residue cocktails to which the French population is exposed.* (Accepted in Environmental Research).

- A. Crépet *et al.* *The PERICLES research program: an integrated approach to characterize the combined effects of mixtures of pesticides residues to which the French population is exposed.* (Accepted in the special issue on mixtures of Toxicology).