From Chemical Exposure to Ecosystem Effects: A critical overview of the risk assessment process

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Outline

Part I
• Introduction
• The risk assessment process
• Limitations and drawbacks of current approach
• Challenges

Part II
• Suggested new approaches
  Case Study: *Llobregat river*
• Conclusions
Growing use of chemicals by our technological society:

- **CAS:** \(\sim 8,400,000\) registered compounds (\(\sim 240,000\) requiring evaluation)
- **European Union:** \(\sim 100,000\) compounds available [EINECS, 2011].
- **REACH:** \(\sim 30,000\) compounds (10,000 already registered)

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**A. Breakdown of the Chemicals in commerce – USA**

- Progress achieved on analytical methods (LC-MS, GC-MS) allowed to quantify many compounds at their environmental occurrence levels.

- These chemicals (+ their transformation products) can potentially reach the environment, being their environmental and health effects difficult to predict.

- Pollution in surface waters is considered one of the main causes of impairment of aquatic ecosystems and biodiversity loss

What is risk?

General:

\[ \text{Risk} = \text{Occurrence probability} \times \text{Adverse Effects} \]

Chemical:

**Environmental Exposure**
- Measured concentration (MEC)
- Predicted concentration (PEC)

**Adverse Effects**
- Ecosystem effects
- Health effects

Adverse Effects Characterization:

Chemical Exposure \[\rightarrow\] \textbf{DIRECT EFFECTS ASSESSMENT} \[\rightarrow\] Ecosystem (Structural & Functional)

Persistence Bioaccumulation Toxicity

\[\rightarrow\] \textbf{INDIRECT EFFECTS ASSESSMENT}
Classical approach ERA method for ecotoxicology based chemical risk

Ecotoxicological Risk associated to a single compound:

- Hazard Quotients HQ (or Toxic Units, TU):

\[
HQ_i = \frac{c_i}{C(ref)_i}
\]

- \( C_i \): concentration of compound \( i \)
- \( C(ref)_i \): reference concentration of compound \( i \)
- \( PNECi \): Predicted No Effect Concentration of compound \( i \)
- \( EC50 \) or NOEC/Assessment Factor (10 to 1000)
- \( EQS \) (regulatory level)
- Etc...
RISK AGGREGATION MODELS:

1) Concentration Addition model (CA):

- All components are assumed to share the same mode of action mechanisms

\[ HQ_{mixture} = \sum_{i} HQ_i \]

(Loewe and Muinschnek, 1926)

2) Independent Action (IA):

- All components are assumed to act by dissimilar mechanisms
  - Response (i.e., effects) addition

\[ HQ_{mixture} = 1 - \prod_{i=1}^{n} \left[ 1 - HQ_i \right] \]

(Bliss, 1939)
• **IA** predicts lower mixture toxicity than **CA**.

• On experimental samples experimental and calculated toxicities do not always coincide.

• When compared to experimental values, **IA** tends to underestimate whereas **CA** tends to overestimate toxicity.

• Even though **IA** and **CA** models are conceptually very different, results are no so much.

• Modes of action are unknown for many contaminants.

• **CA** *(expressed as HQ or TU)* is often recommended as **first tier**.

Limitations and drawbacks of the current methodological approach

Exposure:

- Compounds occur in the environment as complex mixtures
- We actually ignore a large part of them

The spectrum of contaminants identified in a sample is just a portion of those present, and their significance in terms of risk is essentially unknown!
Limitations and drawbacks of the current methodological approach

• Direct dependence of HQ on ecotoxicity data can be a limitation:
  – Ecotoxicity data are not available for all compounds (QSAR modeling methods can partially solve it).
  – Need to use HQ at different trophic levels in order to have a meaningful ecological interpretation
  – Ecotoxicity depends on the organism, time exposure, end point etc. There is a dispersion of data in the literature (data available are not always consistent!).

• On real samples calculated and experimental toxicities do not always coincide

• Due to the additive aggregation, the more compounds we analyze the higher is HQ.

• HQ values are only comparable for the same analytical profiles.

• The extrapolation from ecotoxicology (experimental or calculated) to ecosystem effects is not straightforward.
...Three key issues to be answered by an Environmental Risk Assessment process:

1) Is there any relationship between *chemical pollution* exposure and *ecosystem impairment*?

2) Exposure to *multiple chemicals* may result on any mixture effect ("cocktail effect")?

3) What to analyze? (*prioritization* of target compounds)
   
   "*not all measurable compounds are worth to be measured*"

ERA method of choice will be largely dictated by which of the above questions we want to focus
...Our task ahead:

In short, and quoting A.J. Hendriks (2013):

“How to deal with > 100,000 Substances, Sites and Species: Overarching Principles in Environmental Risk Assessment”.

A. J. Hendriks, Environ. Sci. & Technol. 2013, 47, 3546-3547
New proposed methodological approaches

1. Statistical characterization of multichemical environmental mixtures risk
2. Use of network theory to characterize the interaction between chemicals and taxons
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- 7 sites in 3-4 campaigns

- **51 organic microcontaminants:**
  - 22 pharmaceuticals
  - 29 pesticides

- **Biological variables:**
  - Macroinvertebrate community
    - Biomass
    - Biodiversity (Shannon-Wiener)
  - Diatom quality index
  - Photosynthesis efficiency
  - Chlorophyll-a

MODELKEY-GOCE- 511237
Coping with the “hidden part of the Iceberg”: Statistical characterization of multichemical environmental mixtures risk

**Hypothesis:**
- We assume that the “known part” is a representative statistical sample of the whole system (the usual process in statistical inference).

**Process:**
- The probability density function of HQ of each sample is obtained
- Parameters characterizing the pdf provide information about the whole sample

**Comments:**
- We argue that the inclusion of more compounds eventually analyzed would not alter the statistics to a great extent.
- The assumption seems reasonable at least for those unknown compounds showing environmental levels and structural features similar to those analyzed, such as metabolites and transformation products.
- Using the pdf and some statistical criteria, it is possible to prioritize the compounds with highest risk (HQ) contribution
HQs of sample constituents fit a Log-Normal distribution

(Ln HQ fit a Normal distribution)

Log-Normal distribution (pdf)

Normal distribution
(CUMMULATED PROBABILITY)

μ (mean) informs about toxic load
σ (std. Dev.) informs about how toxic load HQ is allocated among the different compounds
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Some examples of Log-normal distributions for TU(Daphnia)

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Assessment of pollution risk vs. *Daphnia* in the different sites as a function of the statistical parameters $\mu$ and $\sigma$

**COMPOUND PRIORITIZATION (vs. *Daphnia*)**
- Diazinon, Fenitrothion, Linuron
- Diclofenac, Gemfibrozil, Ibuprofen, Erythromycin, Clofibric
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Correlations between statistical parameters $\mu$ and $\sigma$ characterizing $\text{TU}$ (or $\text{HQ}$) distributions and some biological variables

<table>
<thead>
<tr>
<th>Bioassay</th>
<th>Biological variable</th>
<th>$Z_{\text{bio}} = a \cdot \mu + b \cdot \sigma + c \cdot \mu \cdot \sigma + d$</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
<th>$d$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td>Chl-a</td>
<td></td>
<td>2.00E+02</td>
<td>-6.79E+02</td>
<td>-5.55E+01</td>
<td>2.46E+03</td>
<td>0.568</td>
</tr>
<tr>
<td></td>
<td>Diatom_PC1</td>
<td></td>
<td>3.11E+01</td>
<td>-1.11E+02</td>
<td>-8.96E+00</td>
<td>3.86E+02</td>
<td>0.566</td>
</tr>
<tr>
<td></td>
<td>Ymax</td>
<td></td>
<td>-7.41E-01</td>
<td>2.56E+00</td>
<td>2.18E-01</td>
<td>-8.32E+00</td>
<td>0.671</td>
</tr>
<tr>
<td>Daphnia</td>
<td>Macroinv. Biodiv. (Shannon-Wiener)</td>
<td></td>
<td>5.37E-01</td>
<td>-3.48E+00</td>
<td>-1.73E-01</td>
<td>1.34E+01</td>
<td>0.875</td>
</tr>
<tr>
<td></td>
<td>Macroinv. Biomass</td>
<td></td>
<td>8.28E-01</td>
<td>-1.73E+00</td>
<td>-2.55E-01</td>
<td>4.97E+00</td>
<td>0.818</td>
</tr>
</tbody>
</table>
Network theory as a possible tool for the characterisation of pollutants interactions with biota
Introduction(1)

• **Binary Network**: a collection of *nodes* and *links*

• **Adjacency matrix**: a binary network with $n$ vertices is represented by an $n \times n$ adjacency matrix $A$ with elements

\[ A_{ij} = \begin{cases} 1 & \text{if } i \text{ and } j \text{ are connected}, \\ 0 & \text{otherwise}. \end{cases} \]

Examples: communication networks, internet, electric, transport....and many others
• **Weighted Network**: a collection of ‘nodes’ and ‘links’
• **Weight matrix**: a weighted network with \( n \) vertices is represented by an \( n \times n \) weight matrix \( W \) with elements
  \[
  W_{ij} = \text{weight of connection between } i \text{ and } j 
  \]

**Examples**: same as before, but introducing additional information
Introduction (3)

- **Bipartite Network**: a collection of ‘nodes’ belonging to two classes and ‘links’. Only nodes belonging to different classes are connected.

(Examples: Mutualistic networks in ecology: plant-pollinator, seed-disperser, host-parasit)

Case Study: Llobregat River
Macroinvertebrates vs. Pesticides+ Pharmaceuticals

- Interaction between pollutants and ecosystem organisms can be conveniently represented by a Weighted Bipartite Graph

### Pollutants:
- Pesticides
- Pharmaceuticals

### Organisms
- Macroinvertebrates

#### Pollutants:
- Ketoprofen
- Naproxen
- Ibuprofen
- Indomethacin
- Diclofenac
- Acetaminophen
- Propyphenazone
- Clofibric acid
- Gemfibrozil
- Bezafibrate
- Carbamazepine
- Ranitidine
- Erythromycin
- Sulfamethoxazole
- Trimethoprim
- Ofloxacin
- Atenolol
- Metoprolol
- Bentazon
- 2,4-D
- MCPA
- Mecoprop
- Propanil
- Fenitrothion
- Isoproturon
- Atrazine
- Diuron
- Alachlor
- Chlorotoluuron
- Diazinon
- Dimethoate
- Linuron
- Metolachlor
- Molinate
- Simazine
- Terbutylazine

#### Organisms
- Tubificidae
- Enchytraeidae
- Naidae
- Ephemeroptera
- Baetis sp.
- Caenis sp.
- Alcalcarella sp.
- Chironomus spp
- Cricotopus sp.
- Cryptochironomus
- Nanocladius bicol
- Orthocladiinae
- Paracadius sp.
- Paratanytarsus sp
- Polypedilum spp.
- Pottastia sp.
- Prodiamesa oliva
- Rheocricotopus sp
- Stictochironomus
- Tanytarsus sp.
- Corynoneura sp.
- Tanypodinae

Data source: Modelkey
Adjacency and/or Weight Matrices allow to obtain important quantitative information regarding both types of nodes (pollutants or organisms) and links (interactions).

Questions like the following ones can be conveniently addressed:

- What are the compounds having more influence on the organisms?
- What are the most sensitive organisms to pollutants?
- How are distributed the interactions?
- Compound or organism relevance comes from few pair specific interactions or because some nodes (compounds or organisms) act as ‘hubs’ (they exhibit high connectivity)?
- Etc....
<table>
<thead>
<tr>
<th>Macroinvertebrates</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight interaction Matrix</td>
<td></td>
</tr>
<tr>
<td>$w_{ij}$: Spearman correl.coefficient between species $i$ and compound $j$ (n=7 sites)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>WEIGHT MATRIX</th>
<th>Macroinvertebrates</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanytarsus sp.</td>
<td>0.70</td>
<td>0.90</td>
</tr>
<tr>
<td>P. pacificus sp.</td>
<td>0.60</td>
<td>0.80</td>
</tr>
<tr>
<td>Rh. montanus sp.</td>
<td>0.50</td>
<td>0.70</td>
</tr>
<tr>
<td>Enchytraeidae</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>Naididae</td>
<td>0.30</td>
<td>0.50</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>0.20</td>
<td>0.40</td>
</tr>
<tr>
<td>Chironomus spp.</td>
<td>0.10</td>
<td>0.30</td>
</tr>
<tr>
<td>Bubflies</td>
<td>0.00</td>
<td>0.20</td>
</tr>
<tr>
<td>Ephebicidae</td>
<td>0.90</td>
<td>0.70</td>
</tr>
<tr>
<td>Abr. spp.</td>
<td>0.80</td>
<td>0.60</td>
</tr>
<tr>
<td>T. paludal</td>
<td>0.70</td>
<td>0.50</td>
</tr>
<tr>
<td>P. pacificus sp.</td>
<td>0.60</td>
<td>0.40</td>
</tr>
<tr>
<td>C. montanus sp.</td>
<td>0.50</td>
<td>0.30</td>
</tr>
<tr>
<td>Oth. chironomus</td>
<td>0.40</td>
<td>0.20</td>
</tr>
<tr>
<td>Polychaeta spp.</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>N. clad.</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>Crystididae</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>R. montanus sp.</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Strength of node $k$ (Compound or Taxon) = \sum w_{ik}**
What are the compounds having more influence on the organisms?

Negative interactions in general predominate over positive: pollutants tend to exert a negative influence on macroinvertebrate taxons.
What are the most sensitive organisms to pollutants? How is the interaction?

Negative interactions predominate (pollution decreases abundance)

Negative interactions predominate (pollution decreases abundance)

Positive interactions predominate (pollution increases abundance)
How are distributed the interactions?

Does compound or organism relevance come from few pair specific interactions or because some nodes (compounds or organisms) act as ‘hubs’ (they exhibit high connectivity)?

Strength tends to grow with degree (number of links): both compounds and macroinvertebrates show a generalistic interaction.
Conclusions

Classical Environmental Risk Assessment (ERA) based on HQ (or TU) shows some limitations due to:

1. Our ignorance gaps on both exposure (exact sample composition) and effects (ecotoxicological data)
2. Inadequacy of the compound aggregation process utilized in the prediction of Mixture Toxicity of unknown mixtures
3. Extrapolation from ecotoxicology to real ecosystem effects

There is a need of new overarching principles in Environmental Risk Assessment, able to deal with high numbers of different compounds, environmental effects and geographic diversity, as well as, with an unavoidable and intrinsic degree of uncertainty

Two new ERA models which partially overcome the above limitations are proposed:

1. Use of statistical methods (Probability Distributions)
2. Use of Network Theory
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