

Matti Leppänen
Finnish Environment Institute
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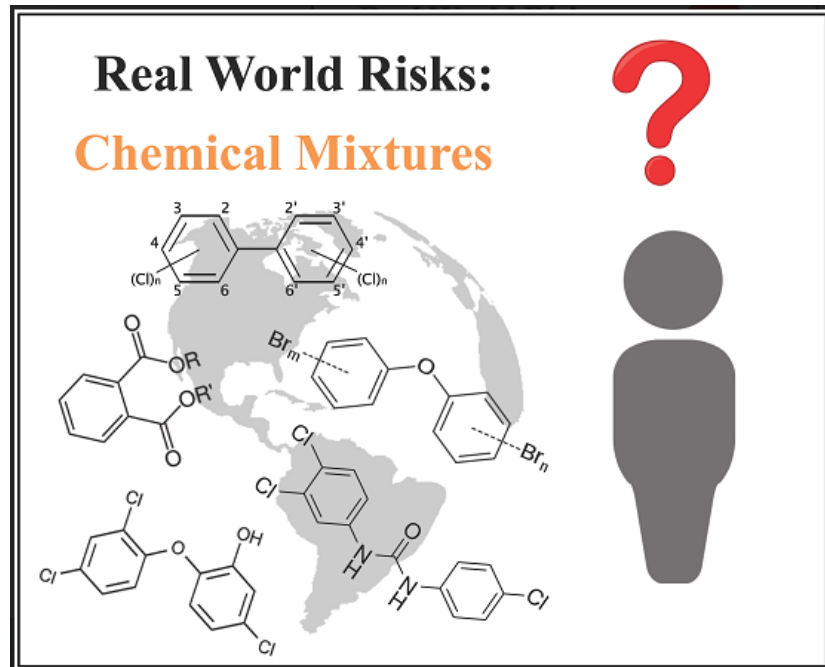


Challenges in the environmental samples

- Assessment based on single chemical threshold values

However,

- Unknown contaminants
 - Typically mixtures
 - Bioavailability
- > Risk?



Challenges with the environmental samples

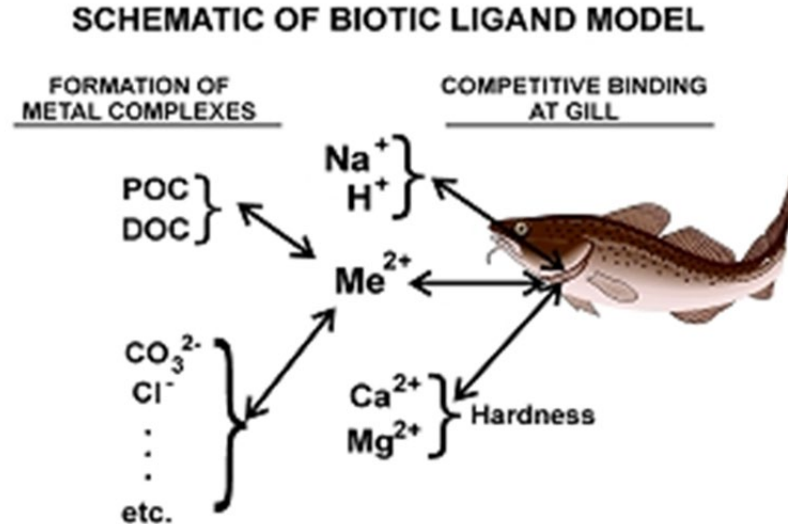
- **Mixture** toxicity
- Concentrations below thresholds, but still toxic samples
 - "Something from nothing"
- Act together?
 - Concentration addition
- Are independent?
 - Synergism/antagonism
- **Bioavailability** of chemicals
- Only freely dissolved can pass cell membranes
- Total \neq Dissolved (filtered) \neq Bioavailable
- Usually not considered in the water quality criteria thresholds



How to account for bioavailability?


Theory & practise for metals

- Cationic metals (Me^{2+}) interact mainly as ions
- Form complexes
- Compete with other ions
- Only a fraction in water is bioavailable
 - Uptake by biota



Metal bioavailability models in practise (1)

Freshwater only



"User-friendly" Biotic Ligand Model Version 2.3 - December 2013

Please register at www.bio-met.net to ensure you're using the most recent version of the tool

Start

Help

Glossary

Generic EQS Bioavailable

Login

About this tool

This software tool estimates the potential risk to the aquatic environment posed by **copper**, **nickel** and **zinc** after considering **bioavailability**. The tool will calculate Local EQS values and Bioavailable Metal Concentrations based on information on local water physicochemistry. This tool has been developed as part of the *bio-met project* and has been designed to operate in Microsoft Excel 2007 and 2010. A **web-based** version of this tool, together with a fuller description of the science underpinning the tool, a description of the tool's operation and validation, case studies and comprehensive guidance on its use are available at www.bio-met.net.

This software tool is based on calculations from Biotic Ligand Models. It is currently only applicable for use in European freshwaters and is intended to be used as part of tiered risk assessment or as an early tier in compliance assessment.

Hints and Tips

You can enter data for up to 2000 samples. Make sure that each sample is entered on a separate row. You can paste data in from another spreadsheet, so long as it is **laid out in the same order** as in the bio-met tool.

How to use this tool

Please read these instructions carefully before you start. Further guidance on using this tool can be obtained by visiting www.bio-met.net

1. To use this software tool, you must ensure that macros are "enabled" in this workbook. Either click the **"options button"** in the security warning that may have appeared above this worksheet and select **"enable this content"**, or click the **"Microsoft Office Button"** in the top left of the screen and select the following options: >>Excel Options, >>Trust Centre, >>Trust Centre Settings, >>Macro Settings, >>Enable all macros
2. Now click the green **Start** button. This will open the main Date Entry and Results sheet.
3. This sheet contains an empty table (if it isn't empty, click the **Clear Data** button to empty it).

Metal bioavailability models in practise (2)

Freshwater only

- www.Bio-met.net is for Ni, Cu, Zn, Pb

Optional Measured Nickel Conc (dissolved) [µg/L]	Optional Measured Zinc Conc (dissolved) [µg/L]	Required pH	Required DOC [mg/L]	Required Ca [mg/L]
22		6,5	20	1

RESULTS (Nickel)				
Local EQS (dissolved) [µg/L]	BioF	Bioavailable Nickel Conc (µg/L)	RCR	Notes
28,93	0,14	3,04	0,76	Y

- www.PNEC-Pro.com is a Dutch version
- EU has bioavailable Environmental Quality Standards for Ni, Pb.
Some member states apply also Zn and Cu EQSs

Component-based mixture risk assessment

Concentration addition model

- A case where concentrations of chemicals are analysed
- Chemicals have either threshold values (PNECs) or Effective Concentrations (EC_{xx})
- Risk ratios can be summed up
- ≥ 1 indicates risk

When based on water quality criteria (thresholds):

$$\sum_i \frac{MEC_i}{EQS_i} \quad \sum_i \frac{MEC_i}{PNEC_i}$$

When based on effective concentrations (EC50s, NOECs, EC10s etc.):

$$\frac{MEC_i}{ECx_i} = TU \quad \text{SUM } TU = \sum_{i=1}^n TU_i.$$

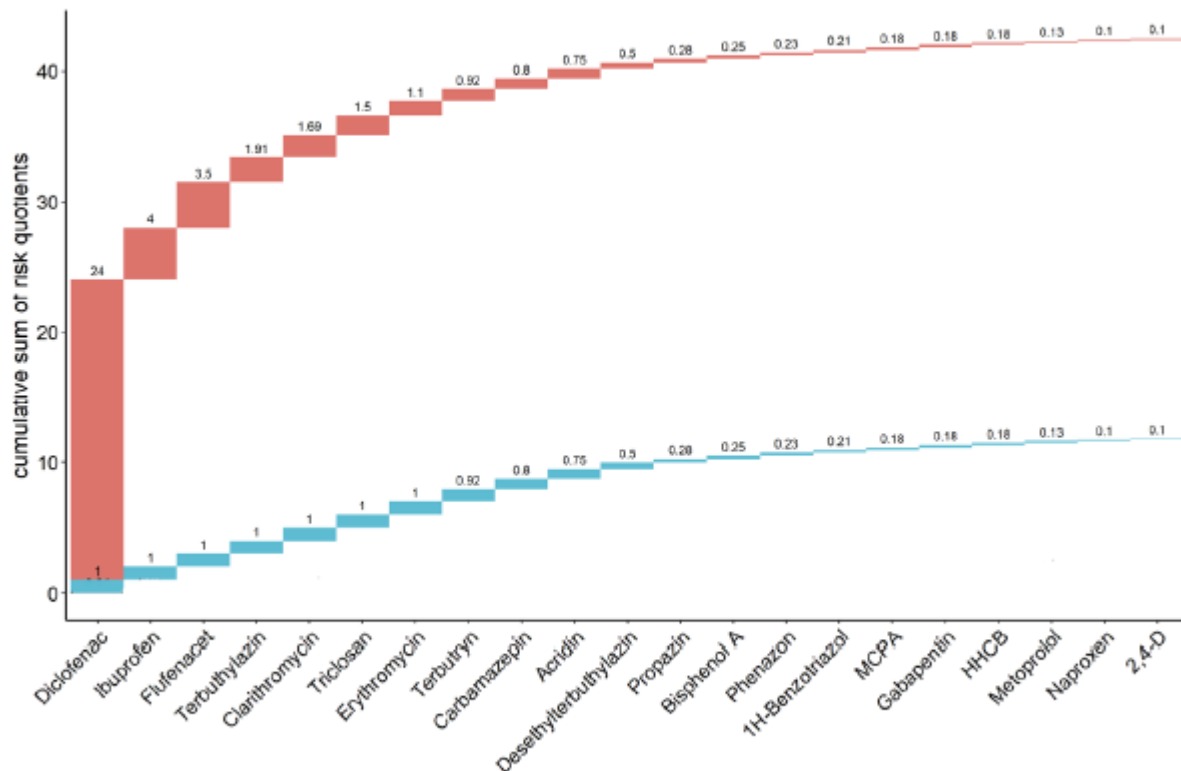
TU's separately for e.g. algae, invertebrates, fish



Example, Risk ratio (Risk quotient; Hazard quotient)



A typical mixture



Risk Quotient = Environmental Concentration / Environmental Threshold

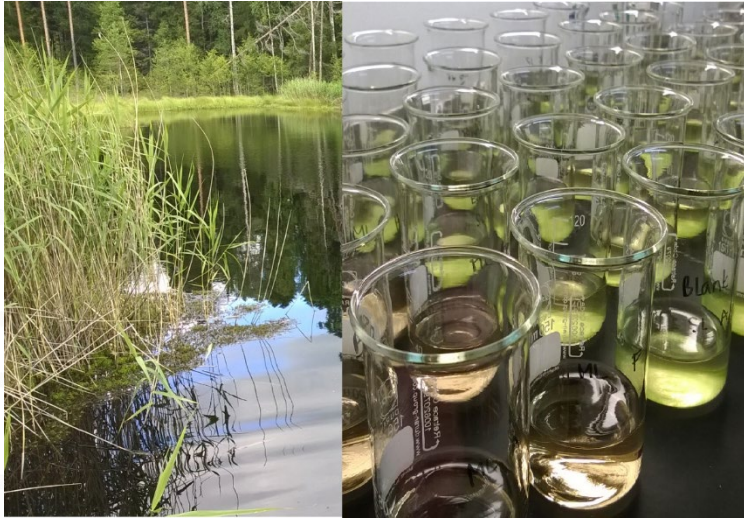
Data from: Markert, N., Rhiem, S., Trimbom, M. and Guhl, B., 2020. Mixture toxicity in the Ertf River: assessment of ecological risks and toxicity drivers. Environmental Sciences Europe, 32, pp.1-13.



SYKE

Whole mixture assessment

Effect-based methods (EBMs)



- *Biotests*
 - *In vivo/in vitro* lab tests
- *Biomarkers*
 - *In situ* exposed biota
- *Ecological indicators*
 - Quality indices; species, abundance



EBMs are gaining support in the EU administration

“In the WFD review, a more holistic approach, taking into account the presence of mixtures of chemicals acting together (for example through the use of effect-based tools in addition to group EQSs), could be considered, to provide a more accurate assessment of risks and a more appropriate targeting of monitoring and measures”

(from discussion document later endorsed by the water directors)

Wernersson AS, Maggi C, Carere M. Technical report on aquatic effect-based monitoring tools. Technical Report 2014–077.

In vivo biotests

Individual level effects, either acute or chronic

- Can be related to population level responses
- Acute; mortality, immobility, Chronic; growth, reproduction

Respond to all stress factors (also other than contaminants)

Can be chemical group specific (algae; herbicides)

Typical standard species; green algae, water flea, fish

- Many standards (ISO, OECD, ASTM, CEN)
- Baltic sea specific; brackish water species

Test matrices; water, sediment, extracts



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In vitro biotests

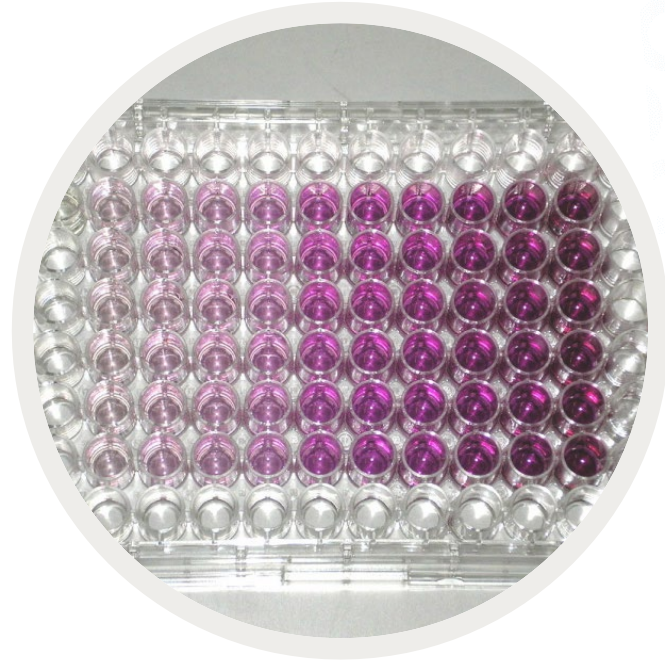
Typically cell line or single cell species tests

Measure molecular level responses, receptor bindings etc.

Usually fast, high through-put tests, e.g. apply well plate/light emission

Chemical group specific responses

- Mutagenicity, endocrine disrupters, neurotoxicity, many receptor binding tests etc.



Using “water quality criteria” of a specific *in vitro* response (1)

Trigger values for environmental samples

- Effect-based trigger value = “Environmental quality standard”
 - Test specific!
 - For screening and risk assessment (pass/fail)
- Available for several response types
 - Escher ym. 2018. Sci. Tot. Env. 628-629:748-
- Most advanced is endocrine disruption (ISO standards)
 - Receptor binding tests ER-CALUX, YES, A-YES

Using “water quality criteria” of a specific *in vitro* response (2)

Trigger values for environmental samples

- Assumptions:
 - Reference chemical and unknowns bind to the same receptor
 - Reference chemical (E2 hormone) effect level concentration is known = trigger value (e.g. 0,4 ng E2/L)
 - Sample response is normalized to reference chemical response (x ng E2 equivalents/L)
 - Exceedance indicates risk of unknowns endocrine disrupters in a sample

Recommendations for a monitoring or environmental risk assessment program



Comprehensive risk assessment is made of combination of chemical analysis and effect-based methods

- Perform chemical analysis (suspect screening)
- Find the most abundant chemicals \neq hazard
- Do the component-based risk assessment \approx hazards
- Complement chemical analyses with EBM
 - Select chemical matching biotests if specific chemical group is in interest
 - At least use basic *in vivo* tests to see mixture effects
 - A battery of different EBM approaches is recommended
 - Possibly study local biota for biomarkers

An example of environmental risk assessement

Case gypsum (CaSO_4) on fields

- Effect of sulfate on riverine biota
- Chemical analysis
 - On site online sensor
 - Lab tests
 - Mussel behaviour
 - Mussel glochidia survival
 - Moss growth
 - Field *in situ* exposures
 - Periphyton colonization & growth
 - Trout egg incubation
 - Field surveys
 - Mussel abundance before-after
 - Fish abundance before-after



Thank you!

Contact:
matti.t.leppanen@syke.fi

