Measuring bioavailability: from a scientific approach to standard methods

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What do we want (1)

- Improve the present system based on total concentration
- Prospective and retrospective use

Is regulation waiting for this improvement?
- Decisions can be made within existing system
- Decisions are accepted
- Hampering of spatial developments is not the responsibility of decision maker

We need a real improvement

Taking decisions is risk full
What do we want (2)

- Bioavailability as a tool in regulation and risk assessment
- We also want
  - Scientific success (papers)
    - Focus on methods
  - Straight forward and defendable decisions
    - Based on reliable and accepted data
  - Affordable investigations
    - Simple and cheap set of methods
  - Realistic regulation for producers and users of chemicals
    - Methods to show ‘no risk’
Linking science to risk assessment and regulation

- From science to regulation
  - Scientific Research → Framework → Management tool → Decision making

- Regulation and Risk assessment
  - Decision making → General accepted toolbox
  - Already filled based on total concentration
  - Filled by science
  - Opening for new bioavailability tools
Connecting worlds

Science:
• Definitions
• Methods
• Concepts/models
• Uncertainty

Regulation:
Real world
• Decision making
• Risks (no risks)
• Responsibility
• (economic) feasibility
• Yes or no
• Equal treatment

ALTETRA
WAGENINGEN UR
Explainable concept

- Explainable for Science
- Not explainable for the public and large part of decision makers
Keep it simple

- Agreement on the concept

- A good definition needs at least one A4

ISO 17402
What does science offer?

- Total concentration
- Detectable concentration
- Bioaccessible concentration
- Bioavailable concentration
- Internal effect concentration

For regulation: Result of standard method = Total concentration

Methods available

Unknown

Methods available

Frische et al, 2003
What is measurable?

- ‘Total’ concentration. Many standards for specific compounds
- Potential available fraction (bioaccessible)
- Actual available fraction (passive sampling)
- Results of bioassays
How to communicate?

- Limit discussion with regulation to measurable items:
  - Total concentration
  - Bioavailable concentration
  - Non-bioavailable concentration

- Keep other terminology (bioaccessable, NER, bound residue, etc.) for scientific discussions.

- Do not include things we cannot measure within regulation. Justify if necessary

FIG. 9. Illustration of differences in availability between natural background and anthropogenic concentrations.
Methods in regulation

- National level
  1. Standard method (NEN, DIN, BS, etc)
  2. National accepted method
  3. National Expert judgement (Your opinion)

- European level
  1. Standard method (CEN, OECD)
  2. Method accepted in Europe (= standard)
  3. European expert judgement (Our opinion?)

If we like it or not, we need standardized procedures (CEN, OECD)
European regulation

  - Values for organic contaminants in water
  - Based on non-filtrated samples
    - Bioavailability is not taken into consideration
    - More prominent role for sediment

- European project to deliver standard CEN-methods to measure organic contaminants in water (2014)

Start with regulation, followed by standardized methods
Who do make standards?

- National standardization bodies
- ISO (world)
  - Using national experts
  - Voting by national standardization bodies
- CEN (Europe)
  - Using ISO working groups
  - Voting by national standardization bodies
- OECD
- Not by
  - Individual scientists
  - This meeting
Standardization/consensus

- International standardization ISO/CEN, OECD
  - No national standardization
- Input in International standardization by National experts
- No consensus with large list of national methods, based on (slightly) different frameworks
- ISO/TC190 Soil Quality, Working group Bioavailability
  - Start with having consensus on the framework
  - Make a guideline
Soil quality — Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials

Qualité du sol — Lignes directrices pour la sélection et l'application des méthodes d'évaluation de la biodisponibilité des contaminants dans le sol et les matériaux du sol
Result of Guideline

- **Chemical methods**
  - Concentration in water phase (actual)
  - Concentration in equilibrium with water phase
  - Make methods for the future

- **Biological**
  - Large number of effect standards are available

- **Validation**
  - Link measurement with effect, Bioavailable for

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1990th Martin Alexander group Essential for knowledge development. Methods, weak extraction, are old fashioned
Chemical standards for trace elements

- Amount in water phase (mg/l)
  - 0.001 M CaCl$_2$. ISO 21268-1
  - 0.1 M NH$_4$NO$_3$. ISO19730

- Potential available (mg/kg)
  - 0.43 M HNO$_3$

- ISO/CD 14858. Soil quality — Environmental availability in soil — Use of soil extracts for the assessment of trace element bioavailability
Chemical standards for organic chemicals

- Amount in water phase (mg/l)
  - Solved as compound (activity) + complexed with DOC
  - Direct measurement (well soluble, low $K_{ow}$, Ionic)
    - Easily in water. Water standards are available
    - How to obtain the water phase in soil
    - Effect of filtration
  - Passive sampling (broader spectrum of compounds)
Chemical standards for organic chemicals

- Potential available
  - Amount in equilibrium with water phase (mg/kg)
- Use TENAX and cyclodextrine
- Sink for organic chemicals. Extracts potential available fraction in approx. 20 hours
  - Established method

ISO/CD 16751. Soil quality — Environmental availability of non-polar organic compounds — Part 1: Determination of potential availability using a strong adsorbent or complexing agent
Methods to measure water phase

Equilibrium sampling to measure $C_{\text{free}}$

1. Equilibrate SPME fiber with sample ($a_{\text{ESD}}=a_{\text{Sample}}$)

2. Measure concentration in the polymer ($C_{\text{PDMS}}$)

3. Apply conversion factor to determine dissolved concentration

Desorption kinetics using Tenax® /Cyclodextrine extraction

- Sediment or soil suspension
- Liquid medium
- Tenax/cyclodextrine®
- Biocide (formaldehyde)

\[ \frac{C_t}{C_0} = F_{\text{rap}} \exp (-K_{\text{rap}} t) + F_{\text{slow}} \exp (-K_{\text{slow}} t) \]

$C_t$ and $C_0$ are the sorbed amounts at time $t$ (h) and at the start of the experiment, respectively.

$F_{\text{rap}}$ and $F_{\text{slow}}$ are the rapidly and slowly desorbing fractions.

$K_{\text{rap}}$ and $K_{\text{slow}}$ (h$^{-1}$) are the rate constants of rapid and slow desorption.

Reid et al, 2003
Prediction biodegradation of PAHs

\[
\frac{C_t}{C_0} = F_{\text{fast}} \cdot e^{k_{\text{fast}} \cdot t} + F_{\text{slow}} \cdot e^{-k_{\text{slow}} \cdot t} + F_{\text{very slow}} \cdot e^{-k_{\text{very slow}} \cdot t}
\]

Tenax 60 °C

Tena 20 °C

1994

2010 passive landfarm
Retrospective versus prospective

- **Retrospective**
  - Investigated intensively
  - Bioavailable part is responsible for risks
  - Measurement of bioavailable part
  - Next step: including in regulation

- **Prospective**
  - Existing assessment system
  - Focus Industry on non-bioavailable part
  - Focus regulation on available part
- Non-bioavailable part
  - NER, Non Extractable Residue (Unknown)
  - Total – Bioavailable part (Unknown)
  - Extractable – Bioavailable part (Defined = measurable)

- Bioavailable part = potential available
  - Time frame? Weeks, months, years, decades
  - Soil life. Weeks to months
    - Earthworm test takes 1 month
Methods in Science and regulation

**Fit for purpose**

- **Science:** Suitable for investigated soil and investigated chemical. Correct answer.
  - Standardization not necessary

- **Regulation:** Suitable for all soils and a large list of chemicals. Correct in 95% of cases
  - Standardized method
Conclusions

- We need a limited number of standard methods
- Chemical
  - Bioavailable part
    - Actual, passive sampling (Chiel Jonker)
    - Potential (Tenax/cyclodextrine)
  - Non-bioavailable part
    - Extractable – Bioavailable? (Kirk Semple)
- Biological
  - Relevant organisms (Jörg Römke)
  - Validation
- Suitable for retrospective and prospective assessment
Suitable for developing countries

- Dieldrin contamination
  - Middle of nowhere
  - Partly covered with sand dunes
  - Removal not an option ($1,500,000)

- Risks
  - Direct contact
  - Transport to groundwater

- Cover
  - Stabilization
  - Evaporation of rainfall
  - Fence vegetation, non consumable, Vetiver, Jatropha

Define use

Mauretania, Ledfatar
Isolation, prevent contact and decrease leaching

Ledfatar, 2009

Non-permeable layer

Clean soil

Vegetation to stabilize the cover

Non-degradable pesticides

Original soil