Review

IMPLEMENTATION OF BIOAVAILABILITY IN STANDARD SETTING AND RISK ASSESSMENT: SUGGESTIONS BASED ON A WORKSHOP WITH EMPHASIS ON METALS

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Bioavailability is increasingly recognised as the key issue linking increased levels of toxicants with actually occurring adverse effects in ecosystems, whilst taking the modifying effects of the abiotic components of the environment into account. Various factors may affect bioavailability in the field, and often these factors are time- and space-dependent. This is one of the main reasons why legislators have been reluctant in implementing bioavailability in risk assessment procedures. Over the last few years, however, considerable scientific progress has been made with regard to better understanding of chemical and ecological mechanisms responsible for rendering chemicals available for uptake and toxicity. As a consequence, legislators face the challenge to anticipate the scientific progress and to implement bioavailability in legislation. This paper discusses the possibilities of implementing various methodologies within a maximum period of time of three years.

KEY WORDS: *background levels, bioaccumulation, biomimetic simulation techniques, dissolved organic carbon, organic compounds, sediment, soil, water*

Biological availability is a key word that is often encountered in current publications on the fate of pollutants in ecosystems and their effects on individual species, populations, and the ecosystem. Despite several years of research within the area of bioavailability, its implementation in standard setting and (site specific) risk assessment is still hampered by the lack of knowledge of the fundamental processes constituting bioavailability, and the lack of generalised procedures for translating the results of bioavailability research into procedures suited for risk assessment and standard setting (1).

Bioavailability implies that, within a given time frame, only a (small) fraction of the total amount of a chemical substance present in, for instance, the water column or the soil can actually be taken up (or made available for uptake) by living organisms and micro-organisms, and can subsequently induce adverse effects (2).

In sediments, metals may be present:

1. In an inert form: strongly bound to the solid material and not available for interaction with the biosphere,
2. Sorbed to exchange complexes and available to biota capable of releasing the metal from the complexes,
3. Dissolved in the pore water and bound to either dissolved organic matter (DOC), loosely bound as inorganic metal species like $MeCl^+$, $MeOH^+$, $MeCO_3^+$, etc.
4. Dissolved in the pore water and present as the free metal ion.

It is often assumed that only the free metal ion is the species that can actually be taken up by biota and it is therefore suggested that instead of the total metal load present in the sediment (or in the water column), risk assessment procedures should increasingly be based on the activity of the free metal ion in solution (3, and references cited herein).

The ultimate means of assessing the biological availability of substances is the actual measurement of the amount of chemicals accumulated within organisms, preferably at the site of toxic action. This would either require dispatching of individual species to determine the total amount of chemicals present within an organism, or measuring internal contents by other means such as blood or fat sampling. From an ethical point of view, this is not desirable. Furthermore, there may be physiological mechanisms that may affect either the uptake or the level of chemicals in the (micro)organism, which could limit the interpretation of the concentration within a (micro)organism. From a pragmatic point of view, it is not always feasible to determine the levels of contaminants in organisms on a regular basis. There is no infinite pool of fish in our rivers and streams, or an infinite pool of earthworms in soil. Therefore, the assessment of internal concentrations and effects is costly and time-consuming, and no unified methodologies are as yet available to do so. Also, many factors that differ considerably in time and space may affect bioavailability in the field. This is why measurements in the field often do not provide a good insight in the bioavailable fraction. In view of these limitations, there is a desire to develop chemical methodologies that can be used to mimic the biological availability of substances. Considerable progress has been made over the last decade in this area.

Legislators on the other hand have been challenged to adequately anticipate the scientific progress made within the area of bioavailability. To rise to this challenge, a small group of Dutch researchers at the Dutch Environmental Research Institutes RIZA and RIVM prepared a workshop on this theme in collaboration with experts from other research institutes, industry and universities. The workshop was organised under the umbrella of the Dutch Steering Group that is responsible for setting the Dutch Environmental Quality Standards for chemicals. The main aim of the workshop was to assess the various methodologies that are currently in use to measure or estimate biological availability, in terms of usefulness for risk assessment and standard setting. The first requirement for possible implementation was that the methodology proposed had to be judged by the experts as being sound from a scientific viewpoint. Thereafter, the proposed methodologies had to be useful for risk assessment and standard setting from a pragmatic viewpoint, and the costs and benefits of implementation (as compared with the current situation) had to be clear. Methodologies passing the criteria set were subdivided in three categories on the basis of the time period in which the experts expect that the methodology could be implemented. The following categories were distinguished:

1. Immediate implementation possible (i.e., within <1 year).
2. Implementation possible within a period of 1-3 years (e.g., scientific research completed, field validation still needed).
3. Implementation only after a period of time exceeding 3 years (promising approach, but more research needed).

For each pre-selected methodology for assessing bioavailability, all relevant information was included in a so-called fact sheet. During the workshop, an analysis of each fact sheet was prepared, containing the Strengths, Weaknesses, Opportunities and Threats (SWOT analysis, see Appendix for an illustration). On the basis of the SWOT analysis, a final judgement was made whether the method is suited for risk assessment and standard setting, whereas the timeframe for implementation was assessed in the case of a positive outcome.

RESULTS

One method was considered to be suited for direct implementation (normalisation for elementary carbon). Normalisation for elementary carbon may be used for adjusting standards and for generic risk assessment, and may substitute for the current practise of normalisation on the basis of organic matter. As such, the implementation of the method would result in a (slight) modification of current standards. Instead of organic matter, organic carbon is the constituent of the solid soil or sediment matrix that actually binds organic and inorganic contaminants. Measurement of the organic carbon content of sediments or arable soils produces a univocal measure of the capability of the solid matrix to modify contaminant uptake and toxicity. The method is being used internationally.

Table 1 *Overview of the methods considered suited for implementation in risk assessment and standard setting within a time frame of 1–3 years.*

Method	Suited for either adjustment of standards, or implementation in generic or site-specific risk assessment	Provisions
Passive sampling of hydrophobic organic micropollutants (with log K_{ow} 4–8) in the aquatic environment	Site-specific risk assessment	– Methods need to be properly validated. – The link with uptake and ecotoxicological effects needs to be clarified.
Tenax extraction	Site-specific risk assessment	– Methods need to be properly validated. – The link with uptake and ecotoxicological effects needs to be clarified.
0.43 mol/L HNO_3 extractable metal content	Generic standard setting	– More insight is to be obtained regarding differences between available background levels and available metal levels following metal addition to soils using this method – The link with uptake by biota and ecotoxicological effects needs to be clarified.
0.01 mol/L $CaCl_2$ extractable metal fraction	Site-specific risk assessment	– The underlying basic assumptions need to be validated (different organisms and plants, various soil types and various soil properties, as well as different emission sources). Application in combination with other extraction methods (like 0.43 mol/L HNO_3).
Measuring actual dissolved and total concentrations of pollutants in water	Generic risk assessment	– Only for highly hydrophobic organic micropollutants.
DOC correction for copper in water	Site-specific risk assessment	– Dependent on collection of chronic toxicity data. This approach can also be used for other metals.

Six methods were scaled for implementation within 1-3 years. The characteristics of these methods are given in Table 1. For all six topics, most of the fundamental research efforts have been completed, but the actual consequences of implementation cannot yet be foreseen. It is recommended that the methods of "Passive sampling of hydrophobic organic micropollutants (with log K_{ow} 4–8) in the aquatic environment" and "Tenax extraction" should be further processed jointly. With regard to DOC-correction, it is judged necessary by the Dutch experts to consider international developments, such as the application of Biotic Ligand Models within the framework of the European Risk Assessment of metals. It was also emphasised that new methods should not only be restricted to copper, but should be applicable to "all" metals. The two extraction methods for metals are linked to each other as well as to three of the topics for which a time frame of >3 years is foreseen. It is therefore recommended not to limit further activities within the area of metal extraction to the two methods with a short time frame, but to include the latter topics as well. The framework for subsequent or parallel extractions is to be used as a guide for further work in this area. A time frame for the development of an overall framework for implementation of bioavailability of >3 years is foreseen.

Seven methods were judged to require further research. Two of the methods with a time scale of 1-3 years deal with biomimetic simulation techniques for organic compounds and one is related to directly measuring dissolved and total concentrations of organic pollutants in water. The other methods within this category deal with the strong interaction of copper with DOC, and include simulation methods for bioavailable metal fractions in soils and sediments. The latter methods are related and it is proposed to consider both methods simultaneously when further investigating their applicability. It is further recommended that these methods be linked to two of the methods requiring research for >3 years before implementation (the method of Diffuse Gradients in Thin films (DGT) and subsequent or parallel extractions). The advantage of the proposed methodologies over the methods currently applied within risk assessment and standard setting is related to the fact that the proposed methodologies have the potential of avoiding unnecessary overestimation of

Table 2 *Overview of the methods considered suited for implementation in risk assessment and standard setting within a time frame of > 3 years.*

Method	Suited for either adjustment of standards, or implementation in generic or site-specific risk assessment	Provisions
Passive sampling for other micropollutants in the aquatic environment and for all compounds in soil.	Site-specific risk assessment	- >3 years for other compounds in the aquatic environment and for all compounds in soil. - The link with uptake and ecotoxicological effects needs to be laid.
Distinction between soft and hard bodied species.	Site-specific risk assessment	- (Extensive) additional research is to be carried out, probably even >5 years are needed.
Site-specific risk assessment and transfer functions	Site-specific risk assessment	- Transfer functions need to be linked to uptake and actually occurring effects. Otherwise application for assessing remediation priorities of polluted sites.
Use of DGT (diffusive gradients in thin films) to assess bioavailable fractions	Site-specific risk assessment	- Only for water and sediment and provided that the link with ecotoxicological effects is clarified (this is a difficulty for most simulation techniques for determining bioavailability).
Subsequent or parallel extractions	Generic risk assessment	- The link with ecotoxicological effects needs further support.
Correction for local background concentration only	Derived criteria	- No, unless a trajectory for incorporation of bioavailability in risk assessment is started (temporary solution). - Implementation of the correction for local background levels in itself possible within <1 year.
Two-step risk assessment of zinc in anaerobic sediments, incorporating AVS corrections.	Site-specific risk assessment	- No, unless the benefits of the assessment are shown. Amongst others, relationships with ecotoxicity need to be substantiated. - >3 years, mainly because of the need to substantiate the relationship with ecotoxicity.

the risk associated with the presence of contaminants in ecosystems. Further details on the methods judged to require further research are given in Table 2.

CONCLUSIONS

On the basis of the discussion of the fact sheets during the workshop, the following is recommended:

- Implement the proposed method for the normalisation of the levels of organic compounds on the basis of elementary carbon present in the substrate towards the levels in standard sediment and standard soil.
- Modify the existing standards for local natural background levels as a short-term solution to substitute for the current practices in the Netherlands of normalisation on the basis of so-called standard soil or sediment. This means the initiation of a trajectory for incorporation of bioavailability.
- Establish clusters of research groups that are commissioned to prepare proposals for the implementation of the six methods for which it is foreseen that implementation is possible within 1–3 years, such as DOC correction for copper in water (Box I). This includes an investigation into the means of financing necessary research activities.
- Look for broader (international) support for further research activities and means of implementation within an international framework (EU) for all options with a timeframe >3 years. The recommendations of the workshop may be one of the criteria for the selection of research proposals within the area of biological availability.
- Stay alert on new developments within the broad area of bioavailability and continue looking for means of implementing the most promising new insights.

The fact sheets and the main findings and recommendations of the workshop are reported in a joint report by RIZA and RIVM, which is available upon request (4).

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Appendix:

SUMMARY SWOT TABLE "DOC-correction for copper in water"

Contents

Standards (such as maximum permissible concentration, target value) for copper in surface water are deduced from laboratory tests in which the metal has been added as a highly soluble copper salt. Due to very low dissolved organic carbon (DOC) concentrations in the test medium, copper ions in general have a maximum availability for uptake by organisms. Standards that are deduced from laboratory tests in which exposure is expressed on the basis of actually measured total copper concentrations in the aqueous phase therefore reflect truly bioavailable metal fractions. Under field conditions, it is evident that a significant fraction of the copper present in surface water is bound to DOC. Hence copper toxicity in surface waters will be less than that predicted on the basis of laboratory testing, and current standards will not take this effect into account.

For copper and other metals for which binding to DOC is relevant, the following is proposed :

- Standards for dissolved metal: to either correct monitoring data for the fraction of metal bound to DOC before comparing the data to the current risk limits or to adjust the current standards for dissolved metal by expressing the standards in terms of the DOC levels of the surface water.
- Standards for total metal: to modify the current risk limits by taking the modifying effects of binding to DOC into account.

Framework

Site-specific risk assessment of copper in surface water.

Assumptions

- Uptake of copper by biota proceeds via the aqueous phase, and the biological availability of the metal fraction bound to DOC is negligible for all aquatic organisms.
- Binding of metals to DOC is independent of other water parameters (e.g., pH and alkalinity). Should this basic assumption not be met, then the ranges of pH and alkalinity should be given such that the DOC correction proposed here is applicable. The recently developed Biotic Ligand Models may be useful for this purpose.
- The (average) complexation constant K ("average" because we are dealing with many Cu complexes) for binding of Cu to DOC is constant over the years and independent of the composition of the DOC.

Evaluation

The Maximum Permissible Concentrations (MPCs) for copper and zinc in Dutch surface waters are systematically exceeded: in many cases the measured concentrations exceed these standard limits by more than a factor of 3. Binding to DOC appears to be relevant at least for copper. It is expected that the standards will hardly be exceeded after correction for binding to DOC. It is expected that the inclusion of binding to DOC will result in an MPC for copper in the rivers Rhine and Meuse (3 mg DOC per liter) of 3.2 μg/L instead of the current value of 1.5 μg/L.

Limitations

- DOC levels in surface waters are not routinely measured.
- Binding to DOC is relevant for a limited number of metals.
- The metal-specific (average) complexation constant K for the binding of metal to DOC has not been determined for most metals. Data

from empirical field surveys are available only for copper.

- Lack of knowledge of the dependence of K on other water parameters like pH and alkalinity may be a limiting factor.
- The impact of complexation to DOC on toxicity has only been shown for copper during acute toxicity tests with Daphnids. More data for other trophic levels are needed.
- A basic assumption is that copper uptake occurs only via the aqueous phase. More data are needed to substantiate this assumption.
- Empirical data collected in the field show that binding to DOC is substantially "different" in acid surface waters (pH <6), although it should be noted that alkalinity in these "acid" waters is low as well.

Applicability

For copper, it seems possible to take binding to DOC into account. Currently, research aimed at assessing the effect of DOC binding on copper toxicity for other water organisms than Daphnids (fish and algae) is about to be completed. Implementation will probably take 1-3 years. Insufficient information is available for other metals.

Sažetak

PRIMJENA BIORASPOLOŽIVOSTI U POSTAVLJANJU STANDARDA ZA PROCJENU RIZIKA: SUGESTIJE TEMELJENE NA SEMINARU S NAGLASKOM NA METALE

Biološka dostupnost sve se više smatra ključnim problemom vezanim s povećanom razinom toksičnih tvari što izazivaju neželjene učinke u ekosustavima pri čemu se uzimaju u obzir promjenljivi učinci abiotičkih sustava okoliša. Mnogi čimbenici mogu utjecati na biološku dostupnost u prirodi. Ovi su čimbenici i vremenski i prostorno ovisni. To je i glavni razlog zašto su zakonodavci oklijevali primijeniti biološku dostupnost u procese procjene rizika. Proteklih je godina, međutim, napravljen značajan napredak s obzirom na bolje razumijevanje kemijskih i ekoloških mehanizama odgovornih za dostupnost kemikalija apsorpciji i za njihovu toksičnost. Kao posljedica toga zakonodavci se suočavaju s izazovom da prihvate znanstveni napredak i primijene biološku dostupnost u zakonima. Ovaj članak raspravlja mogućnosti raznih metodologija u razdoblju od tri godine.

KLJUČNE RIJEČI: *bioakumulacija, organski spojevi, otopljeni organski ugljik, sediment, tehnike biomimetičke simulacije, temeljne razine, tlo, voda*

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