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Underlying issues including approaches and information needs in risk assessment

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Abstract

Risk assessment requires a delicate consideration of the factors modifying exposure and effects. In this contribution a review is given of the qualitative and quantitative information needs that are essential for a proper risk assessment. The focus is on the details of metal exposure and exposure assessment, following the themes of environmental, physicochemical, and biological components that define exposure. Apart from a description of the principle processes and pathways, exposure assessment is placed in the context of risk assessment and its use in policy and regulatory decision making.

Keywords: Bioavailability; Bioaccessibility; Metal; Organic; Uptake; Toxicity

1. Principal exposure pathways and processes

1.1. General

Exposure of an organism to a metal refers to contact with the metal. *Assessment* of the exposure has environmental, physicochemical, and biological components that are described both qualitatively and quantitatively, as illustrated in Fig. 1. Qualitatively, the *environmental* components describe the medium in which the metal is contained (air, soil, water, food, or other substrate), the pathway through which the metal is transported from its source to the receptor organism (e.g., from source to soil, soil to air, air to water, water to receptor), and the fate of the metal (e.g., binding to soil, suspension and dispersion in air as a soil-bound particle, deposition in surface water, leaching into ground water, biomagnification in predator–prey interactions) along the pathway through which it is transported. Environmental components also include many properties of the media, such as dissolved oxygen content, pH, oxidation-reduction potential, temperature, clay content, and cation exchange capacity. The *physicochemical* components describe the inherent qualities of the metal (e.g., boiling point, solubility, chemical form, valency state). The *biological* components consist of the point of contact (e.g., gill, mouth, nose, skin) relevant to the route of entry (diffusion, ingestion, inhalation, dermal absorption) into the organism and the pathway followed by the metal inside the organism from its absorption through the gill, gut, lung, or skin to sites of action or bioaccumulation. Quantitatively, each of these components is measured and assigned the appropriate units of measure (e.g., mg/kg, $\mu g/m^3$, mg/L) and according to the most appropriate analytical method. All of these components, taken together, describe where the metal goes, from the initial source of the metal to its receptor (externally at the point of contact with the organisms of interest, and internally at the cellular/molecular target sites of action within the organism).

An important component of a metal's exposure is its *bioavailability*—the fraction of total metal that is available to exert action and effect within the receptor organism. The concept of bioavailability and the bioavailable fraction of metal in exposure assessment will be discussed throughout this book.

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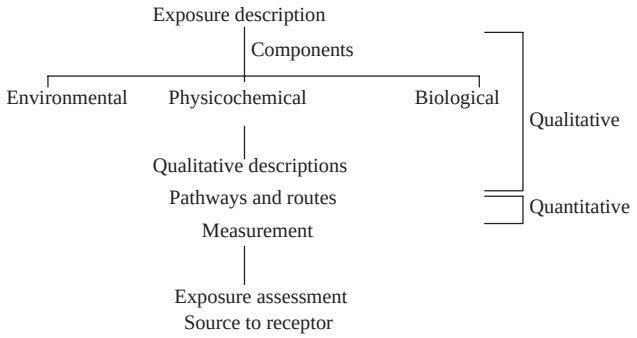

Fig. 1. Schematic overview of the components of exposure assessment.

The sections of this chapter elaborate on the details of metal exposure and exposure assessment, following the themes of environmental, physicochemical, and biological components that define exposure. Section 1 describes the principal processes and pathways involved in exposure considerations. Section 2 places exposure assessment in the context of risk assessment and its use in policy and regulatory decision making. Section 3 introduces the factors involved in assessing bioavailability, including physicochemical and biological factors.

1.2. Processes determining bioavailable fractions and organism uptake

A comprehensive and reliable risk assessment with the aim of occupational environmental and consumer health protection should consider all possible pathways of exposure and provide a quantitative estimate for humans and biota. This should relate external exposure to internal dose and both to adverse health effects. An important variable to at least roughly assess the internal dose phenomenon is bioavailability. Thus, bioavailability is a "bridge" between the external total exposure and exposure routes on one side and the adverse effects on the other.

The master scheme in Fig. 2 summarizes major pathways, describes uptake and transport processes, and identifies focus points where bioavailability plays a key role. As a "key player" in risk assessment, determining bioavailability enables a more precise and reliable description of internal exposure.

Metals and metal compounds can enter the environment at all stages of their life cycle, i.e., production, processing, transport, storage, industrial and private use of end product, recycling, and disposal, which can be by both incineration and dumping. Additionally, the naturally occurring metals also can be introduced into the environment by volcanic activities, erosion, or seasonal forest burning, etc. The probability of a release into the environment varies along the life cycle and can generally be divided into intended releases for use and unintended emissions. All environmental compartments,

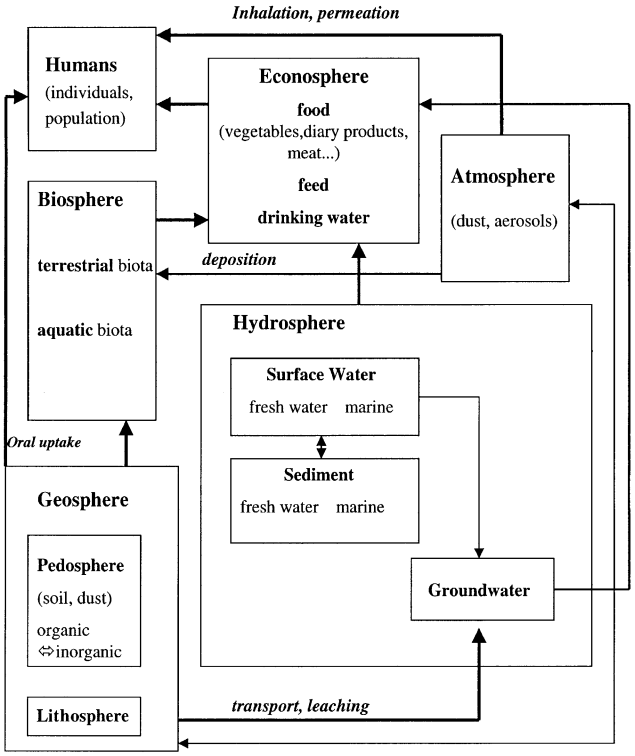

Fig. 2. Important exposure pathways and processes, including bioavailability, to consider in environmental and human health risk assessment.

biota, and the consumer may be exposed indirectly or directly.

The *geosphere* can be divided into the *pedosphere* and *lithosphere* and is both a *source* for metal compound emission (1)[1] (Table 1) and a sink due to atmospheric deposition (2), spills, run-off, erosion, and intended, direct application (3) such as fertilizer, sewage sludge, and ash application.

In respect to the geosphere's role as a source for metal compounds, bioavailability comes into play. Several exposure pathways play an important role. Their consideration in environmental and human health risk assessment requires the consideration of many parameters and processes that determine the bioavailable fraction of the metal compound. These processes are influenced by sets of chemical and environmental parameters, many of which are discussed below. (Table 2).

The *hydrosphere* (Table 3) also can be seen both as a sink and as a source of metal compounds. With respect to the latter, uptake by aquatic organisms, including sediment-dwelling organisms, is the predominant pathway (5). In particular, fish serve as food (4), and thus the path is of interest for environmental and human health protection. In addition, surface water or groundwater is used for the drinking water supply (6).

[1] Numbers refer to the points in Table 1.

Table 1
Overview of the impact of environmental parameters on the processes that determine bioavailable metal fractions

Parameter	Processes affected	Type of impact
pH	1. Sorption to biotic and abiotic solid phases	1. Decreasing pH lowers sorption, thus increasing (pore) water concentrations.
	2. Leaching	2. Similarly, decreasing pH will lower sorption, increase (pore) water concentrations, and thus increase the potential for leaching.
	3. Uptake of metals by biota	3. Apart from directly affecting metal levels in the aqueous phase, and thus directly influencing metal uptake via the (pore) water, pH may have a direct impact on biota and thus indirectly modify uptake and effects.
Ionic strength, presence of competing cations (often expressed as hardness)	4. Sorption to biotic and abiotic solid phases	4. Increasing levels of cations competing for the same sorption sites (be it on biotic or abiotic surfaces) will decrease sorption or uptake.
	5. Uptake of metals by biota	5. Increasing the ionic strength of solution will generally increase the concentrations of complexing species, decrease the free metal activity, and thus decrease metal uptake via the aqueous phase. In addition, increasing ionic strength will affect the well being of organisms.
Dissolved organic carbon (DOC)	6. Complexation in the aqueous phase	6. Increasing levels of DOC will increase complexation of metals and hence reduce the free metal activity.
	7. Uptake	7. Increases of DOC will reduce metal availability for organisms for which the free metal ion is the bioavailable species, but will at the same time increase the bioavailable fraction for biota capable of exploiting organic metal species.
	8. Leaching	8. Increases of DOC will increase (pore) water concentrations, thus increasing the potential for leaching.
Temperature	9. Direct and indirect on all exchange and equilibration processes and on biotic species	9. As a rule of thumb, increases of temperature will decrease sorption to interfaces: increased (pore) water concentrations at higher temperatures will to a varying degree be compensated by decreased sorption to biotic surfaces, and the net effect of temperature changes is difficult to predict. Organisms are sensitive to changes in temperature and any temperature change will affect the well being of biotic species.
Organic chelating agents (like humic and fuliv acids)	10. Complexation in the aqueous phase	10. Increasing levels of chelating agents will increase complexation of metals and hence reduce the free metal activity
	11. Uptake	11. Reduction of the free metal activity will reduce metal availability for organisms for which the free metal ion is the bioavailable species, but will at the same time increase the bioavailable fraction for biota capable of exploiting organic metal species.
	12. Leaching	12. Increases of chelating agents will increase (pore) water concentrations, thus increasing the potential for leaching.
Inorganic ligands	13. Complexation in the aqueous phase	13. Increasing levels of inorganic ligands will increase complexation of metals and reduce free metal activities.
	14. Uptake	14. Reduced free metal activities reduce metal availability for organisms for which the free metal ion is the bioavailable species, but at the same time increase the bioavailable fraction for biota capable of exploiting inorganic metal species.
	15. Leaching	15. Increases of inorganic ligands will increase (pore) water concentrations, thus increasing the potential for leaching.
Methylating agents	16. All processes	16. Methylating agents will affect chemical speciation: the fate of the methylated species is dependent on their properties. In some cases, species are formed that are more volatile than the nonmethylated species, and in these cases methylating will increase the impact of uptake via the air.
Redox conditions—Redox potential	17. All processes	17. Changing redox conditions will drastically affect the chemical nature of sorbents present in soil or sediment; reduction will lead to formation of stable reduced precipitates (like sulfides) that will strongly bind metals. In addition, there will be direct and indirect effects on organisms resulting in shifts in species composition. The effects will be most apparent in sediments. In a general sense, due to an increase of strongly binding ligands-precipitates, reduced conditions will reduce the external dose

Table 1 (*continued*)

Parameter	Processes affected	Type of impact
		and uptake via the pore water. On the other hand, metal bioavailablility of sediment and soil-dwelling organisms that are capable of digesting reduced materials might increase.
Sulfides	18. Sorption + uptake (indirectly)	18. Presence of sulfides will reduce metal availability for organisms exposed via the pore water. However, the formation of metal sulfides might increase the effective external dose for organisms that are capable of digesting reduced materials.
	19. Leaching	19. The presence of sulfides will decrease pore water concentrations and hence reduce the potential for leaching.
Organic carbon content	20. Sorption + uptake by biota	20. Increasing levels of organic carbon will increase the sorption capacity of solid materials, thus reducing metal concentrations in the aqueous phase. Hence, metal availability for organisms exposed via the pore water will be reduced. However, increasing levels of organic matter in the solid phase might increase the effective external dose for organisms that are capable of exploiting solid material as a food source.
	21. Leaching	21. Increasing amounts of solid-phase organic carbon will reduce (pore) water levels, thus reducing the potential for leaching (assuming that DOC levels are similar).
Suspended material	22. Sorption + uptake by biota	22. Suspended material will act as an additional sorption phase. The impact of increasing amounts of suspended material is similar to the impact of increasing amounts of organic matter.
	23. Leaching	23. Impact is similar to the impact of organic matter
Inorganic oxides	24. Sorption + uptake by biota	24. Inorganic oxides act as (effective) sorption phases: increasing levels of inorganic oxides will have effects similar to those of organic carbon or suspended material.
	25. Leaching	25. Impact is similar to the impact of organic matter or suspended material.
Clay	26. Sorption + uptake by biota	26. Clay is a complex material made up of several constituents, including oxides and organic carbon. The impact of increasing amounts of clay in general is similar to the impact of increasing amounts of organic matter.
	27. Leaching	27. Impact is similar to the impact of organic matter.
Cation exchange capacity (CEC)	28. Sorption	28. CEC is an expression of the capacity of solid materials to adsorb metals. Increasing the CEC will increase the amount of metal sorbed, and will reduce the external dose for organisms exposed via the aqueous phase. The effect of varying the CEC on the uptake of metal by species that are exposed via the solid phase depends on their efficiency at competing for metal with sorption sites on the solid material Increasing the CEC of the solid material will, as a rule of thumb, decrease metal uptake by these organisms, although exceptions to this rule have been reported.
	29. Leaching	29. Increasing CEC will reduce the potential for leaching.
Vapor pressure	30. Uptake by organisms via air	30. Increasing vapor pressure will increase the fraction that is available for inhalation.
	31. All additional processes	31. Increasing vapor pressure will reduce available concentrations in all other compartments and therefore reduce uptake, sorption, leaching, etc.
Aqueous solubility	32. Uptake	32. Increased aqueous solubility in principle allows for higher concentrations in water. When solubility is the limiting factor controlling levels in (pore) water, then increased solubility will lead to increased uptake (usually this effect is relevant only for hydrophobic organics).
	33. Leaching	33. Increased solubility will increase the leaching potential.
Henry's law constant	34. Uptake via air	34. The higher the Henry's law constant, the higher the exposure levels and the higher the effective uptake.
Aerosols	35. Uptake via air	35. Increased concentrations of aerosols will on the one hand result in increased levels of contaminants in the air, while on the other hand aerosols might limit bioavailability. Usually, the net effect is an increase of uptake with increasing levels of aerosols.

Table 2

Pathway	Selected processes determining the bioavailable fraction and organism uptake
Geosphere—groundwater	Sorption, desorption, leaching, complexation
Geosphere—terrestrial biosphere	Sorption, desorption; compartmentalization of metals Complexation Metabolization Mobilization by exudates (plants, microorganisms, etc.) Feeding behavior, habitat Morphology and physiology
Geosphere—terrestrial biosphere—econosphere	Food and feed processing (4)
Geosphere—atmosphere	Erosion, volcanism, particle sizes, evaporation for mercury
Geosphere—humans	Soil ingestion, particle size, uptake processes

Table 3

Pathway	Selected processes determining the bioavailable fraction and organism uptake
Hydrosphere—aquatic biota (including sediment-dwelling organisms)	Sorption, desorption; compartmentalization of metals Feeding behavior, habitat Morphology and physiology Metabolism Biomagnification
Hydrosphere—aquatic biosphere—econosphere	Food processing
Hydrosphere—drinking water	Complexation
Geosphere—drinking water—humans	Oral uptake, uptake processes from the gut

The most important source for direct human exposure is the *econosphere*, which contains food and drinking water. Vegetable feed and food not only are affected directly by atmospheric deposition (7) or application but also can be exposed via soil–sewage sludge/fertilizers–plant transfer. The uptake can be via the soil–root system (1,4), by sorption of evaporated chemicals in the leaf wax layer, or simply by a physical adhesion of splashed soil particles. Animals and human food of animal origin are exposed via feedstuff but also via inhalation of air (7). Final consumer exposure can be by different routes, which are usually of different relevance for the individual human being. Exposure can be by an oral, inhalation, or dermal route. In particular, contamination of food itself arises from different sources. As a consequence of complex pathways from soil to terminal products, additional sources come from, among others, industrial processing of food. Subgroups of the population, depending on nutritional and social behavior, are exposed to different amounts and possibly through different pathways. In addition to social and nutritional behavior vulnerability, subgroups such as infants and children or groups with certain diseases (e.g., diabetics) would need specified exposure assessment.

2. Use of exposure information in risk assessment

Estimating the health risk of a metal to an organism requires assessments of exposure and toxicity. Two major components of exposure assessment are (a) the *external* concentration of a metal, over a specified period of time, with which an organism is in contact, and (b) the *internal* metal concentration that, once inside the organism, reaches target sites of action. A further refinement of these exposure concentrations—*bioavailable* concentrations—will be discussed below. A major component of toxicity assessment is the dose-response relationship, characterized by the nature and severity of an adverse health response with increasing concentrations (doses) of a metal and the specific dose at which the response becomes unacceptably adverse. Risk assessment serves to estimate the likelihood that an exposure will lead to an unacceptable adverse health response. As such, exposure measurements and an understanding of the influences on the potential for exposure are important elements in the management of risk, such as for the setting of safe limits of tolerance in freshwater and near-coastal marine ecosystems to protect aquatic wildlife and in foods and drinking water to protect humans.

At high exposures and bioavailability, all metals can cause toxicity and unacceptable risk. However, at lower concentrations or low bioavailability a subclass of metals is essential for the normal biological function of organisms. These "essential trace elements" (ETEs), including tin, chromium, cobalt, copper, iron, manganese, molybdenum, nickel, selenium, vanadium, and zinc, are required to varying degrees for normal growth and development and the maintenance of good health

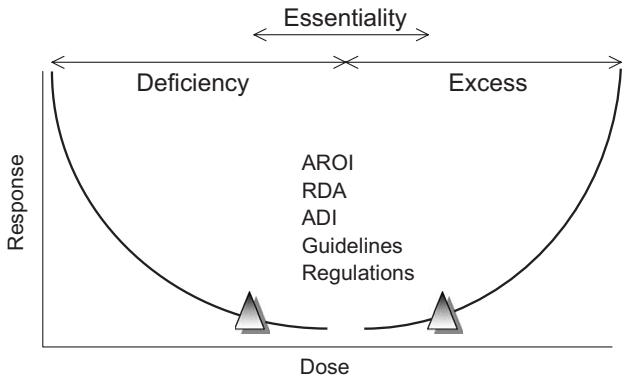

Fig. 3. Dose-response construct for essential trace elements. The zones defining the limits of essentiality, deficiency, and excess overlap. The Acceptable Range of Intake (AROI) of the World Health Organization and the Recommended Dietary allowance (RDA) of Canada and the United States, water quality criteria (WQC), and other exposure-related guidelines and regulations are based on these zone limits. Nonessential trace elements do not involve essentiality or deficiency, but follow a similar course of analysis and interpretation for the excess portion of this construct. Triangles represent the most critical dose areas for which exposure and bioavailability assessments are important.

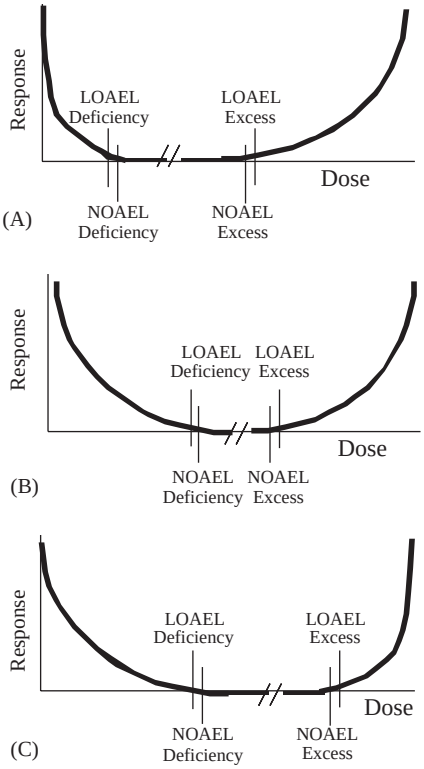

Fig. 4. Possible shapes of the U-shaped dose-response curve for copper. The breadth of the physiological balance portion (bottom) of the curve may be one of the shapes as presented, or may be broader, narrower, or crossed. Furthermore, the breadth of the curve bottom of the curve and characteristics of the excess portion (right-hand side) of the curve are different for acute and chronic dose regimens and responses.

throughout life. Because they are essential, it follows that too little exposure to ETEs can cause health and viability problems of *deficiency* and that too much exposure can cause problems of *excess*. For ETEs, organisms seek to maintain physiologic balance (homeostasis), avoiding deficiency and excess. Other metals, like arsenic, cadmium, lead, and mercury, are *non*essential (nETEs), so that the concepts of excess and its associated toxicity apply, but less so the concept of physiologic balance and not the concept of deficiency.

The theoretical dose-response curve for ETEs is presented in Fig. 3. For nETEs, only the right-hand (excess) portion of the curve applies. Six key features related to exposure and the dose-response curve are noteworthy:

For ETEs, the dose-response curve is roughly U-shaped. For entire populations of wildlife or humans, the regions of marginal deficiency and excess overlap with the region of essentiality. This means that, while most individuals within a population are in physiologic balance for ETEs, there are some individuals that are either marginally deficient or in excess. For wildlife, this is due principally to variability within and between animal species. For humans this is due to variability within the human population. These overlaps blur the distinction of deficiency and excess from essentiality.

The regions of greatest importance in this dose-response curve are the inflection points of the curves, as represented by the two triangles for ETEs and the right-hand triangle for nETEs. For ETEs, the toxic responses of deficiency or excess in these areas are subtle and hard to detect. These areas also represent the regions of overlap among deficiency, essentiality, and excess. It is most important to characterize exposures as well as possible in these regions.

For essential metals, the U-shape of the dose-response curve will likely not be symmetrical [i.e., it does not achieve the physiological ideal of (B) in Fig. 4], but rather skewed left (A) or right (C). In (A), the left skew indicates that deficiency occurs over a narrower and more potent range of metal exposure than excess. In (C), the right skew indicates the opposite. In either case, there is a broader range of exposures for a given adverse response *away* from the direction of skew, and it is unclear at this time which dose-response profile (A or C) applies to each of the ETEs. Furthermore, for most, if not all, ETEs the U-shape is also likely to be discontinuous in at least a portion of the region of essentiality (i.e., to contain an area of no toxicity within a segment of the bottom of the U-shape). This observation applies regardless of the toxic potentials in deficiency and excess.

For ETEs, the left-hand portion and bottom of the U-shaped dose-response curve (defining deficiency and essentiality) apply only to chronic exposures, since acute (short-term, 24 h) exposures do not impact states of

Table 4

Variations among metals	Bioavailability modifier
Metal-to-metal variations	Intrinsic toxic potencies, modes of action, and control mechanisms of individual metals, according to each metal's physicochemical properties
Ecosystem-to-ecosystem variations	Habitat and stressor conditions
Site-to-site variations	Circumstances and conditions at individual sites of exposure (e.g., soil, pH)
Host factor variations	Population (wildlife or human) characteristics and behavior

deficiency or essentiality. For nETEs and the right-hand portion of the ETE dose-response curve (defining excess), toxicities involve both acute and chronic exposures. Chronic exposures to metals occur through a combination of diet, drinking water, and environmental media (air, water, soil, and dust) and through a combination of routes (ingestion, inhalation, and dermal absorption). A more detailed description of the different exposure compartments and pathways is presented in Fig. 2.

The shape of the dose-response curve, whether U-form for ETEs or lineaform for nETEs, varies from metal to metal, ecosystem to ecosystem, geographic site to geographic site, and exposure scenario to exposure scenario (Table 4).

Thus, for an individual assessment of risk, it is theoretically possible to derive a series of dose-response relationships, each of which is tailor-made to the specific metal, for a specific environmental domain (animal ecosystem or human setting), site, and scenario. Within such a framework the features of deficiency, essentiality, and excess would vary with each dose-response curve. However, for practical purposes, one dose-response relationship is derived empirically for each metal and its critical endpoint and for one optimal set of conditions: modifying factors are then applied to the relationship to account for all of these variations.

The risk assessment, based on considerations of exposure and dose response, is used to make risk management decisions in the form of

- Guidelines and recommendations for daily dietary intakes of metals (i.e., the WHO Acceptable Range of Intake or, the US and Canadian Recommended Dietary Allowance);
- Enforceable regulations for drinking water standards (e.g., EU Drinking Water Directives, USEPA Maximum Contaminant Levels) and surface water standards (water quality criteria); and
- Other environmental (soil and air) limits for metals as hazardous substances.

The guidelines, regulations, and limits represent exposures situations that must, or should not, be exceeded to avoid harm with an ample margin of safety.

There are many facets in the full characterization of exposure, including

- *External exposure* (metal concentrations at the points of ingestion, inhalation, and dermal absorption into the body per unit of time), and
- *Internal exposure* (a measure of the metal in body fluids or tissues).

As such, exposure can be referred to as the concentration at the point of: (a) external oral, inhalation, or dermal contact with the organism, (b) internal contact of the bioavailable fraction at the target organ (e.g., brain), (c) the bioavailable concentration at the cell membrane or in the cellular cytosol, or (d) the molecular concentration (e.g., in in vitro preparations to investigate enzymatic reactions to metals). Both external and internal exposure involve only those quantities of metals that are bioavailable (available to be taken into the organism then available to reach an internal target site of action (organ, cell, subcellular organelle, or biomolecule), and then available to exert influence or action.

3. Parameters determining (bio)availability

3.1. Physicochemical parameters

Differences in bioavailability are central to understanding the variable occurrence of adverse effects caused by metals. Elevated contaminant levels in themselves are not necessarily indicative of actually occurring adverse effects. Bioavailability is indicative of the dynamic processes that effectively link exposure assessment to effect assessment. With regard to exposure assessment it is important to focus on metal fractions that are potentially or actually available for uptake by organisms and hence are available for exerting adverse effects. For the water phase, this is illustrated in Fig. 5 (from Peijnenburg and Jager, 2003, this issue). The potentially available metal fractions and the inert fraction jointly make up the total metal pool. The inert fraction is not available. This fraction is composed of stable crystalline metal forms of the parent rocks or of insoluble stable forms, e.g., of oxides. It is often assumed that the free metal ion is the actually bioavailable metal species in water. The free metal ion not only is the most toxic species, but it also is the species that is assumed to be predominantly taken up by biota (however, this is not true for mercury).

Chemical processes of ion exchange, adsorption, precipitation, volatilization, complexation and redox-reactions determine the physicochemical form or

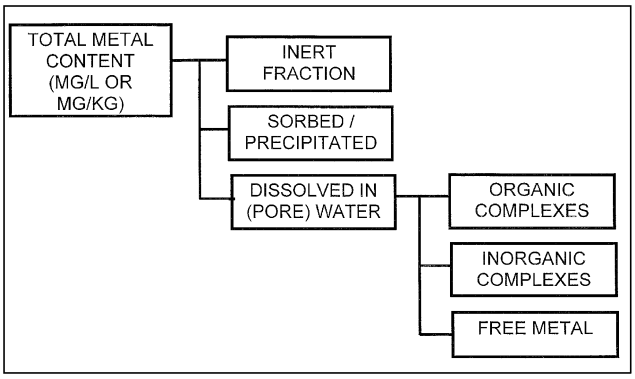

Fig. 5. The various metal fractions present in soil, sediment and water matrices. Chemical equilibria exist between the potentially and actually available metal pools indicated in the figure, and the activity of the free metal ion is modulated not only by equilibration with the dissolved metal pool, but also by the presence of organic and inorganic complexing agents. The interaction of physicochemical parameters in the system determines the equilibrium and hence, the relative magnitude of each of the metal pools.

chemical species of metals in solution and in the solid phase. These partitioning and equilibration processes determine the magnitude of potentially and actually available metal fractions for organism exposure. The different processes are described in the articles of Peijnenburg and Rensing and Maier (this issue). The most important physicochemical parameters determining the magnitude of the (potentially) dissolved metal pool are:

- pH
- Ionic strength, presence of competing cations (often expressed as hardness)
- Dissolved organic carbon (DOC, e.g., humic acids)
- Temperature
- Organic chelating agents (like humic and fuliv acids)
- Inorganic ligands
- Alkylating agents

The most important parameters affecting sorption processes to the solid matrices are

- pH
- Redox conditions—redox potential
- Sulfides
- Ionic strength/hardness
- Organic carbon content of the solid phase
- Dissolved organic carbon
- Suspended material
- Temperature
- Inorganic oxides of Fe, Mn, Al, and Si
- Organic chelating agents (like humic and fuliv acids)
- Inorganic ligands
- Clay content
- Presence of competing cations, including nutrients
- Methylating agents
- Cation exchange capacity (CEC)

Further facts might influence bioavailability, such as microorganisms with their exudates; only a selection of them can be presented.

Distribution processes between the solid matrix and solution are often expressed by means of distribution constants. The distribution of metals is controlled not only by the properties of the solid matrix, but also by the composition of the pore water. Therefore, the determination of sorption constants should consider the influence of the pore water or water column composition and of the physicochemical parameters identified above.

In addition to distribution between the solid and liquid phases, it is apparent that soils are three-compartment systems. Distribution processes between the solid or the water phase and air may be important for organisms living, for example, in the macropores of soils. In these cases, there may be direct inhalation of airborne metal species. However, for metals this exposure pathway is important only for volatile species, like metalloorganic compounds and mercury. In addition to the parameters identified above, the following parameters are important in determining the impact of metal uptake via the air compartment:

- Vapor pressure
- Aqueous solubility
- Henry's law constant (being an expression with vapor pressure and aqueous solubility combined)
- Aerosols

These parameters have a direct impact on the processes described in the schemes given, which show the most important exposure pathways. These impacts are qualitatively indicated in Table 1.

From Table 1 it is clear that there are a large number of parameters determining the effective bioavailable dose. In addition to the host factors identified in a subsequent contribution, the physicochemical factors identified here affect the bioavailable fractions to varying degrees. Also, opposite effects are notable and a number of factors covary in practice (like DOC and DOM, or redox and temperature). Although it is possible to assess the impact of each parameter semi quantitatively, it is only to a limited extent possible to truly quantify bioavailable fractions in specific ecosystems. The most notable examples of successfully determining truly bioavailable external doses are the biotic ligand models developed for a limited number of essential and nonessential elements. In these cases only a limited number of the above parameters modify the effective dose, and it was possible to quantify their impacts.

3.2. Methods for measuring physicochemical parameters

From the foregoing it is apparent that physicochemical parameters influencing bioavailability can be categorized into two classes: the potentially and actually

bioavailable metal pools and the parameters that directly or indirectly affect these metal pools. Standardized and validated methods are available for measuring most of the physicochemical parameters identified. As determination of the bioavailable metal pools is the key issues here, this chapter will concentrate on the measurement of these pools. As demonstrated in the previous scheme, total concentrations, potentially available fractions, and dissolved concentrations should be measured.

Total amounts of metals are commonly determined by the digestion of the matrix by aqua regia (heat and strong acid) or oxidizing acids. This determination does not take metal speciation or complexation into consideration; all species of a metal are quantified as "total". On the other hand, this method is standardized and validated in round robin tests in many laboratories, including in developing countries.

A number of methods for determining the potentially available fractions of metals in soils or sediments are proposed in the following sequence in order of increasing aggressiveness. Applying these methods might alter speciation.

- (Weak) salt extractions—e.g., $CaCl_2$, $Ca(NO_3)_2$, NH_4Ac, $NaNO_3$, Mg salts, $BaCl_2$, in concentrations from as low as 0.001 M to up to 1 M salt solutions.
- Reductive extractants—e.g., sodium–ascorbate, hydroxylamine–HCl, sodium dithionite.
- Weak acid extractions—e.g., acetic acid, citric acid.
- Strong complexation methods—e.g., DTPA-TEA, EDTA, NTA.
- Dilute strong acids—e.g., HNO_3, HCl, "double acid" ($HCl + H_2SO_4$).
- Combined extractants—e.g., ammonium oxalate–oxalic acid, Mehlich III (dilute acid, salt, and EDTA).

Not all methods are useful for the study of all heavy metals: reductive methods are, for example, only useful for studying metals that can be reduced, like iron and manganese. In addition, many methods have been developed with just one element in mind: the double acid extraction has been developed primarily for the study of zinc availability.

To put procedures for organic and anorganic compounds in line, the terms "potentially available metals" should include all metal species which can, under certain conditions become solubilized and partition in the aquatic phase. Thus, strong extraction solutions which do not significantly alter the solid phase, e.g., strong complexing agents with a defined pH-value, should be used.

Methods for the preparation of soil or sediment pore water are under discussion in ISO groups. The are two main objectives:

- Eluates should simulate the concentrations of constituents in pore water.
- Sufficient amounts of eluates should be easily obtainable for chemical and biological testing.

This means, e.g., that the method for the preparation of soil pore water cannot be applied, as the amount of solution obtained is too small. Therefore, shaking or column methods are proposed. The shaking method is easy to perform with a water/solid ratio of 1:2. However, the separation steps should be carefully performed to obtain clear solutions without particles. Centrifugation and/or filtration are used for this separation step. Independent of the methods used, the separation should be controlled measuring turbidity. A value of <10 FNU is proposed. A separation of particles is important for comparisons analytical data and effects data. As indicated in the previous paragraph, if particles are in the solution they will contain metals (in their matrix or sorbed) and thus significantly increase the measured concentration, while these sorbed metals are far less (if at all) available for organisms than dissolved ions. All water samples should be treated in the same manner to distinguish between dissolved metals and metals bound to suspended sediment. An example of an approach for measuring the various metal fractions in water or in extracts is given in Fig. 6.

An overview of (direct and combined) methods that are currently available for measuring elemental speciation is given in another contribution (elemental speciation chapter), whereas a limited number of additional techniques for measuring metal in dissolved phases is mentioned by Peijnenburg and Jager (2003). The reader is kindly referred to these contributions, and this topic will not be further touched upon here.

3.3. Processes at the cellular level

The occurrence of adverse, toxic effects from metals is dependent not only on the concentration of bioavailable metal in the environment but also on the organism's ability to handle metals and maintain intracellular homeostasis. Since life probably evolved in an environment rich in metals, homeostatic mechanisms developed early in evolution and many metal transporters are conserved in all forms of life. This conservation allows conclusions drawn from one organism to be applied to other organisms. In fact, many homeostatic mechanisms first discovered in yeast or *Escherichia coli* have subsequently been identified in humans. Since many metals are essential for all organisms at trace amounts but toxic in excess, cells have developed homeostatic mechanisms (Eide, 1998; Williams et al., 2000). These mechanisms include uptake, intracellular handling of

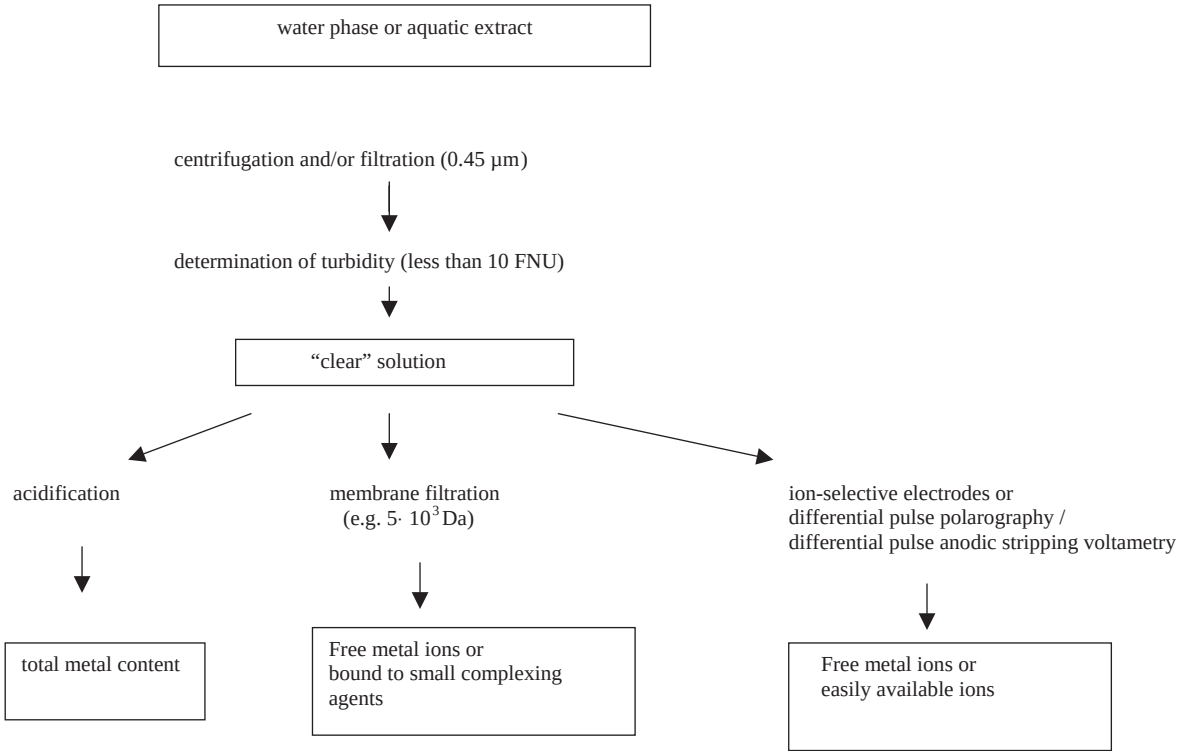

Fig. 6. Simplified methods available for distinguishing between complexed and free metal.

metals, compartmentalization, and excretion. Comprehensive studies to understand metal homeostatic mechanisms have been performed in model organisms such as *Saccharomyces cerevisiae* and *E. coli*. The advantages of these organisms are the relative ease with which molecular genetic techniques can be performed and the presence of the complete genomic sequence. These advantages allow the examination of a single transporter in contrast to a mixture of different transporters in membranes. In the past, transport of a given substance was often examined in organisms for which no genetic information was available. This approach often led to erroneous interpretation of transport characteristics.

Most organisms do not rely on a single transporter for an essential metal ion but have redundant functions. For example, zinc uptake in *E. coli* is accomplished by at least two transport systems, ZupT and ZnuABC (Grass et al., 2002). Moreover, many transporters have overlapping specificities and most do not transport a single metal species but rather are able to transport several metals with differing affinities. Examples include the natural resistance-associated macrophage protein (Nramp) and ZIP family of proteins discussed below. Metals also interact with each other in unexpected ways. One example is the copper requirement for high-affinity iron uptake first identified in yeast (Askwith et al., 1994; Dancis et al., 1994), but probably also present in other organisms. Copper must be taken up across the cytoplasmic membrane and inserted into a multicopper oxidase that is required for high-affinity iron uptake. Many other interactions have been noted. However, the multitude of transporters and interactions does not allow an extensive overview of handling and interactions of metals in different organisms, but the reader is referred to some recent reviews (Rensing et al., 1999; Williams et al., 2000). It is important to realize that transporters greatly influence how toxic a given metal is to an organism. In contrast, passive diffusion is not a major route of entry for most metals. For example, a metal needs to be taken up to exert a toxic effect on an organism. Furthermore, the presence of efflux pumps or metal-binding proteins also protects cells from metal-induced toxicity.

3.3.1. Uptake

Several ubiquitous families of proteins have been implicated in the uptake of trace metals. These include the Nramp, the ZIP family of proteins, and homologues of the yeast copper influx protein CTR1 (Williams et al., 2000). Metals can also enter cells accidentally through a nonspecific gate. Examples include the uptake of arsenic and antimonite by the glycerol facilitator in *E. coli* and yeast (Sanders et al., 1997; Tamas and Wyczocki, 2001).

Nramp describes a novel family of divalent metal-translocating proteins. The highly conserved Nramp gene family has been found in a wide range of organisms, including bacteria, yeast, insects, mammals, and higher plants (Govoni and Gros, 1998; Williams

et al., 2000). In yeast, three members of the Nramp family have been identified (SMF1, SMF2, and SMF3) whereas *E. coli* possesses one member (MntH) (Kehres et al., 2000; Supek et al., 1996; West et al., 1992). The physiological function of MntH in *E. coli* and SMF1 and SMF2 in *S. cerevisiae* appears to be manganese uptake. However, they have also been shown to mediate the uptake of other metal ions such as copper (SMF1, SMF2), cobalt (SMF2), and cadmium (MntH) (Kehres et al., 2000; Liu et al., 1997). A mammalian homologue, DCT1, was capable of transporting iron, manganese, zinc, and several other divalent cations in a pH-dependent, electrogenic manner, suggesting a proton-coupled carrier (Gunshin et al., 1997).

The ZIP family of proteins are metal transporters that are found in all three kingdoms. The IRTs also belong to the ZIP family and are involved in iron uptake. IRT1 is also able to transport iron, zinc, and manganese in yeast (Korshunova et al., 1999). The ZIPs have been shown to transport zinc but may also transport other metals such as cadmium and manganese (Fox and Guerinot, 1998).

3.3.2. Metabolism

Essential metal ions such as copper and iron have to be taken up and delivered to metal-requiring enzymes. Free metal ions are harmful to cells, since they can damage DNA or metabolic processes. Therefore, cells have intracellular metal-binding proteins. Ferritin is a protein used for the storage of iron and can contain several thousand iron atoms. Metallothioneins (MTs) are small gene-encoded, Cys-rich polypeptides and have the selective capacity to bind metal ions such as zinc, copper, and cadmium. In addition to detoxification by sequestering metal ions, MTs might also play a role in maintaining intracellular zinc in conditions of zinc deprivation (Suhy et al., 1999). Copper in eukaryotes is transported to specific destinations by cytosolic copper chaperones (Harris, 2000).

3.3.3. Compartmentalization and excretion

The most important protective mechanism to reduce metal toxicity is efflux of the metal ions from the cytoplasm. Cells can transport metals either into intracellular compartments such as the vacuole or across the cytoplasmic membrane. For example, *S. cerevisiae* has two independent transport systems for the removal of arsenite from the cytosol (Ghosh et al., 1999). The ABC transporter Ycf1p catalyzes the ATP-driven uptake of arsenite into the vacuole, and Acr3p is a plasma membrane transporter that confers resistance to arsenite by arsenite extrusion from cells (Ghosh et al., 1999). Other ubiquitous transporter families involved in metal efflux include the cation diffusion facilitator and the soft metal (or CPx-type) P-type ATPases (Paulsen and Saier, 1997; Solioz and Vulpe, 1996).

3.4. Methods for measuring biological parameters

As already outlined, there are several important exposure pathways and processes for organisms and humans. Chemical as well as biological parameters give information on exposure and the consequences of the exposure. Biological parameters include toxicity measurements, in vitro simulation test designs and specific investigations for single transport mechanisms. Furthermore, there are monitoring approaches, which are described in detail in the article "Methodologies to examine the importance of host factors to bioavailability of metals".

Toxicity can be observed at different levels. The level/parameter considered in the experiment determines the sensitivity with which the damage by contaminants is observed. Different levels are, for example, measurements of

- the molecular level
- the metabolic level (enzymatic activities or biomarkers)
- behavior (avoidance, reproduction, mortality, changes of the biocoenosis in the field).

In Fig. 7, one can see which chemical fractions of the contaminant can be determined and how these measured fractions are connected with the biological responses that are investigated. The scheme is restricted for water, soil, and sediment organisms as well as for plants.

With toxicity measurements mainly performed in routine assessment, only the available fraction in an organism is determined. Due to organism-specific uptake systems and the ability to change the environmental conditions in the near environment, chemical analyses with respect to the contaminants in water, soil, and sediment do not provide information on the portion that is taken up by the organisms. To obtain such an insight, body burden concentrations are determined. These fit, however, to toxicity only in cases in which no detoxified amounts (e.g., by storage) exist. Body burden, however, gives information on the amount of the contaminants available for bioaccumulation/biomagnification in the food web.

Uptake studies in microorganisms give information on the mechanisms (e.g., by specific or transport proteins) and on the fraction of the contaminant penetrating the organism. As further metabolic and elimination processes in a microbial cell can be inhibited, the determination of the chemical concentration in the organism gives information on specific uptake rates. The results, however, are specific for the organism, the metal, and the experimental conditions.

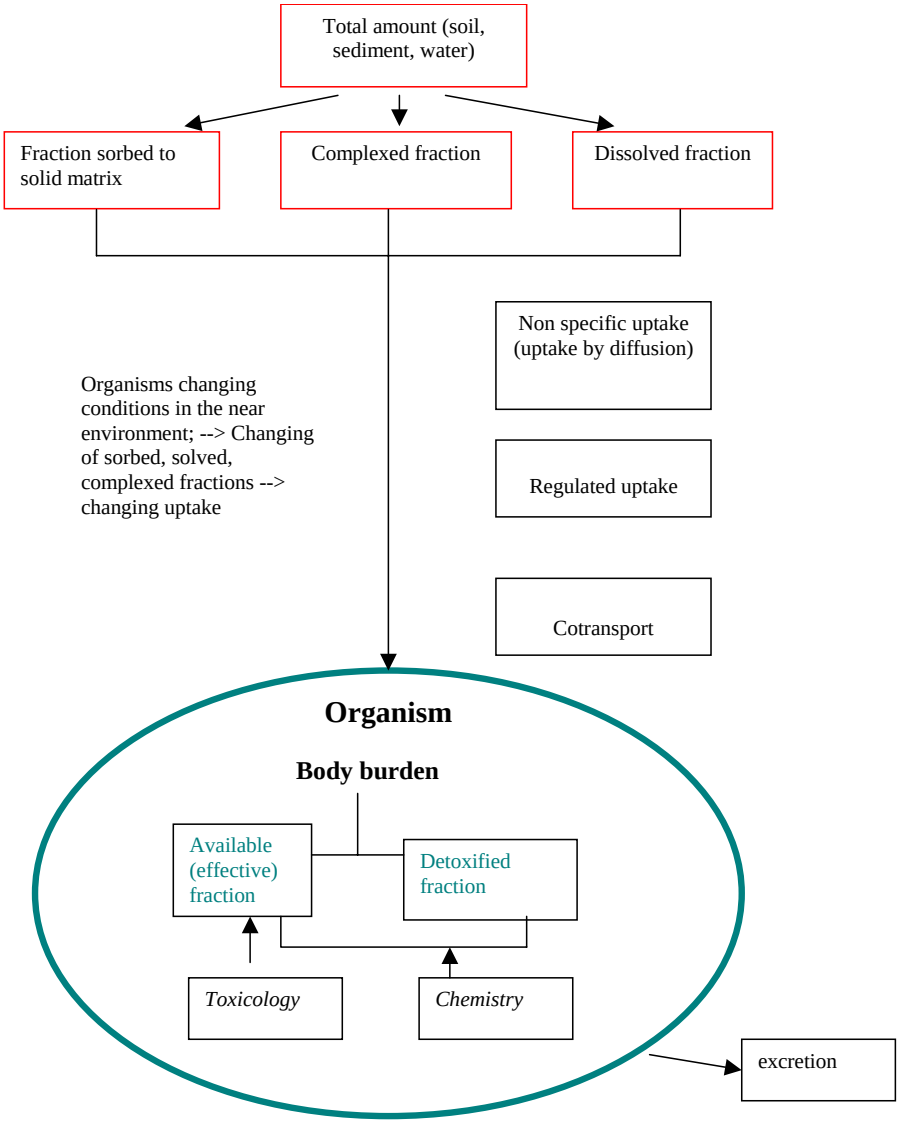

Fig. 7. Overview of chemical fractions connected with biological responses.

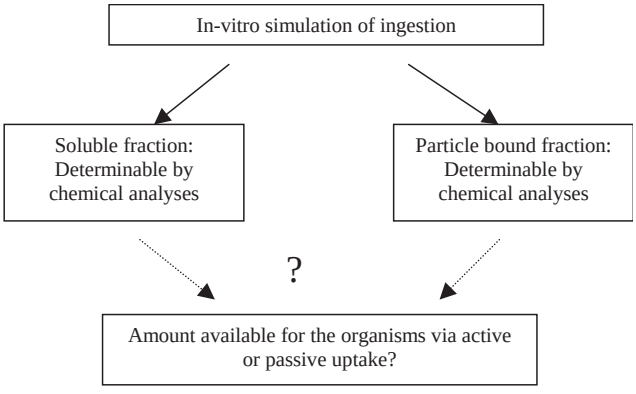

Fig. 8. Overview of the difficulties encountered in interpreting in-vitro tests.

For humans and mammals, the main uptake route is via the intestine or lung. As this uptake is difficult to study in vivo, in vitro test designs have been developed (see Fig. 8 for a schematic representation of the difficulties encountered in interpreting in vitro tests). In these experiments, using synthetic gastric juices and modifying the pH can simulate physical processes. Biological processes can be simulated on the level of enzymatic activities. The specific uptake from the intestine, however, can only be roughly simulated. Therefore, these model test designs are under critical discussion.

For many experiments, chemical and biological analyses refer to different fractions of the contamination and are difficult to compare. Only for microorganisms does a comparison seem to be possible, as via genetic modifications individual transport mechanisms can be studied. Recommendations to develop analytical methods differentiating between detoxified and available fractions in organisms in analogy to these for the investigation of soil have been recommended.

3.5. Monitoring approaches

Metal bioavailability and metal bioaccessibility are complex phenomena that are dependent upon a cascade of matrix-related, species-related, and metal-related issues. In turn, the properties affecting metal bioavailability and metal bioaccessibility often are interrelated and, as a consequence, the effect of these properties often is not straightforward. In practice, most (operationally defined) measures of bioavailability or bioaccessibility have limited applicability among a wide range of matrices, organisms, plants, or metals. For monitoring purposes it is therefore necessary to adequately consider the relevant end points of determination. Subsequently, it is possible to select the most appropriate techniques among the plethora of methods developed so far. It is essential that the methods of choice incorporate expressions of both the potentially and the actually available metal fractions. It should be noted that monitoring is not necessarily restricted to chemical measurements; bioassays and biosensors may for instance, also be methods of choice for specific metals when one wishes a direct insight into truly bioavailable metal fractions while integrating all aspects of bioavailability.

As there is no universal expression of bioavailable and bioaccessible metal fractions, it is not possible at present to develop a detailed monitoring strategy that is broadly applicable. Clearly, the determinants of bioavailability and bioaccessibility must be better understood before one is to monitor or, ultimately, predict the effects of metals.

4. Summary and recommendations

4.1. Use of exposure information in risk assessment

(i) Using descriptions of exposure for decisions in risk assessment, regulation, and policy requires that exposure be integrated into the risk assessment process. Accordingly, exposure must be related to the bioavailable dose in the dose-response relationship and to the effects of a specific metal in excess and, for essential trace elements, in homeostasis and deficiency.

(ii) For ETEs, exposure characterization is most critical for the regions of marginal deficiency and marginal excess (toxicity), since these regions define the lower and upper limits of the safe range. For nETEs, it is the region of marginal excess and toxicity only that is of concern.

(iii) Interspecies and intraspecies variabilities create uncertainties in both the exposure and dose-response assessments and in the risk assessments derived from them. Thus, the challenge of exposure assessment and the methods employed to measure exposure is to develop ways of accounting for variabilities and reducing uncertainty. In the end, developing better measurement methods that reduce uncertainty in exposure assessment will result in wiser risk, regulatory, and policy decisions and ultimately better protection of all biota.

4.2. Parameters determining (bio)availability

(i) There are a large number of parameters determining the effective bioavailable dose. Physicochemical factors affect the externally bioavailable dose to a varying degree. Also, opposite effects are notable and a number of factors covary in practice.

(ii) Although it is possible to assess the impact of each parameter semiquantitatively, it is only to a limited extent possible to truly quantify bioavailable fractions in specific ecosystems because of a lack of understanding of the fundamentals of the processes that determine the environmental fate of metals. In such cases, however, it usually is possible to identify the major parameters determining the effective dose, and quantitative bioavailability assessment should at least focus on these parameters.

(iii) A wide variety of methods for measuring the physicochemical parameters affecting bioavailable metal fractions and for measuring a large number of (often pragmatically defined) metal pools are available. Depending upon the purpose of the study (for instance, measuring total metal content versus actually available metal species for a specific organism), the most appropriate measurement technique may be selected. Prior to this selection, it is of utmost importance, however, that a proper monitoring approach is adapted.

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