The bioavailability of chemicals in soil for earthworms

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Abstract

The bioavailability of chemicals to earthworms can be modified dramatically by soil physical/chemical characteristics, yet expressing exposure as total chemical concentrations does not address this problem. In order to understand the effects of modifying factors on bioavailability, one must measure and express chemical bioavailability to earthworms in a consistent, logical manner. This can be accomplished by direct biological measures of bioavailability (e.g., bioaccumulation, critical body residues), indirect biological measures of bioavailability (e.g., biomarkers, reproduction), or indirect chemical measures of bioavailability (e.g., chemical or solid-phase extracts of soil). If indirect chemical measures of bioavailability are to be used, they must be correlated with some biological response. Bioavailability can be incorporated into ecological risk assessment during risk analysis, primarily in the estimation of exposure. However, in order to be used in the site-specific ecological risk assessment of chemicals, effects concentrations must be developed from laboratory toxicity tests based on exposure estimates utilizing techniques that measure the bioavailable fraction of chemicals in soil, not total chemical concentrations.

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1. Introduction

Soils are a tremendously heterogeneous environmental matrix with varying spatial and temporal gradients of organic carbon, pH, and particle size distribution. These soil physical and chemical characteristics, in concert with physiology and behavior, determine the bioavailability of chemicals in soils to earthworms. Although much effort has been expended standardizing and codifying protocols for conducting bioassays with earthworms (ASTM, 1999), little parallel effort has been invested in developing standard, meaningful measures of exposure that incorporate chemical bioavailability. One of the biggest problems in earthworm toxicity testing is determining the actual chemical exposure of an earthworm. As an example, effects in earthworm toxicity tests conducted according to standard protocols using the same total concentration of lead (Pb) ranged from complete acute mortality to no effects on cocoon production (Fig. 1)! The observed range of responses was due to differences in metal bioavailability resulting from Pb interactions with the physical/chemical matrix of the soil (e.g., pH, 4.1–7.7; organic carbon (%), 5–2.6; clay content (%), 4–59), assuming similar behavior of earthworms in each soil type. The broad definition of bioavailability is “a measure of the potential of a chemical for entry into biological receptors. It is specific to the receptor, the route of entry, time of exposure, and the matrix containing the contaminant” (Anderson et al., 1999). Within this broad definition of bioavailability, it becomes possible to define and measure bioavailability more specifically.

Bioavailability, as discussed by Landrum et al. (1992), can be presented as a concept diagram (Fig. 2; adapted from Lanno, 2002). Chemicals present in soil interact with specific soil constituents in a dynamic manner over time, resulting in the sequestration of a portion of the chemical, making it unavailable for interaction with...
biological receptors. Sequestration can be defined as “the state in which a contaminant is segregated from and rendered unavailable to a receptor, and arises from rate-limiting processes involving contaminant interactions with the surrounding matrix, such as phase transfer, complexation, and reversible chemical transformation. Sequestration is specific in relation to the combination of receptor, matrix, spatial and temporal scales, and route of exposure” (Lanno, 2003). The definition of sequestration may also be extended to include the biological context of the partitioning of specific chemicals inside an organism into inert forms or pools that are biologically unavailable to the earthworm. An example would be the sequestration of metals into inert granules consisting of an inorganic matrix in chloragogenous tissue. The portion of total chemical in the soil that is not sequestered may be referred to as the environmentally available fraction and remains available for all fate and transport processes, including interaction with earthworms. During movements through the soil, earthworms encounter and interact with only a specific portion of the environmentally available chemical. Interaction may be through either direct dermal contact with chemicals in soil solution or soil atmosphere or ingestion of soil or specific fractions of the soil, and the fraction of the environmentally available chemical that the earthworm interacts with is termed the environmentally bioavailable fraction. The proportion of total chemical in soil that is environmentally bioavailable is therefore dependent on the physiology and behavior of the earthworm and the route of exposure. As an example, only the chemical dissolved in the soil solution is thought to be environmentally bioavailable to the earthworm for dermal uptake (Belfroid et al., 1996). However, ingested soil will be subjected to the chemical conditions present in the gastrointestinal tract (e.g., presence of digestive enzymes and surfactants, difference in pH from ambient soil), theoretically resulting in a different proportion of the total chemical present in solution and available for uptake. Some portion of the environmentally bioavailable fraction of the chemical may be absorbed across the external membranes of the earthworm (e.g., epidermis, gastrointestinal tract). Once absorbed, the chemical may be metabolized and excreted, accumulated in other tissues, sequestered internally, or transported in the organism to the site(s) of toxic action (STA). Toxicological bioavailability refers to that portion of absorbed chemical that reaches and interacts with the STA. The STA (Fig. 2) represents a threshold level, with toxic effects only occurring when the amount of chemical present at the STA completely fills the box and exceeds the threshold. Thus, it is possible to have accumulation of a compound without toxicity. However, once the

Fig. 1. Range of earthworm responses to soils varying in physical/chemical characteristics and spiked with 2000 mg Pb/kg (unpublished data).

Fig. 2. Schematic model of bioavailability (with permission from Lanno, 2003).
chemical accumulates to a level above a theoretical toxic threshold, the measurable residue associated with an organismal response is termed a critical body residue (CBR) (McCarty and Mackay, 1993). Although the concept diagram may be useful in helping to define bioavailability, it is also important to develop tools that can actually be used to measure these various parameters. This review paper discusses chemical and biological approaches to assessing the bioavailability of chemicals for earthworms and some of the regulatory implications of measures of bioavailability.

Earthworms are the standard soil toxicity test organism and are ideally suited for assessing the bioavailability of many chemicals in soil for a number of reasons:

1. Earthworms reside in soil and are more or less in constant contact with some portion of the soil.
2. Earthworms reside in contaminated sites, allowing field validation of chemical bioavailability.
3. Earthworms are found in a wide variety of soil types and horizons.
4. The exterior epidermal surface of the earthworm is vascularized with no cuticle, allowing the uptake of contaminants directly from the soil.
5. Earthworms ingest soil or specific fractions of soil, providing a means for the dietary uptake of contaminants.
6. Earthworms have a large mass, so contaminant concentrations can be determined in individual organisms.
7. There is a low level of mixed-function oxidase (MFO) activity, allowing greater potential for the accumulation of organic compounds that would normally be metabolized in other organisms.
8. We have an understanding of their physiology and metabolism of metals.
9. Standardized toxicity testing protocols are available.
10. Many techniques are available for assessing effects at population, organismal, and suborganismal levels exist for earthworms.
11. Some species, such as Eisenia fetida, can be cultured in the laboratory under controlled conditions and are tolerant of many soil types, allowing the testing of different soils.

In addition, there exists an extensive literature providing background on the ecology, physiology, and ecotoxicology of earthworms, making them an excellent model for assessing the bioavailability of chemicals in soil systems.

2. Measures of bioavailability

Measures of bioavailability may be either direct or indirect and chemical or biological (Fig. 3). The advantages and disadvantages of each of these measures will be presented and discussed in the context of the conceptual model of bioavailability presented in Fig. 2.

2.1. Direct biological measures of bioavailability

Direct biological measures of bioavailability are determinations of the actual amount of chemical taken up by the earthworm and may provide the most accurate measure of bioavailability since they integrate all of the biotic (e.g., metabolism) and abiotic (e.g., pH, organic matter content of soil) modifying factors of chemical bioavailability. Included in this approach are measures such as bioaccumulation and CBRs. Bioaccumulation is a direct measure of chemical concentrations in an earthworm resulting from the net inward flux of chemicals from the soil due to the balance between uptake and depuration processes. CBRs are internal chemical concentrations that are associated with sublethal or lethal endpoints (McCarty and Mackay, 1993; Lanno et al., 1998; Wells and Lanno, 2001). In the model (Fig. 2), bioaccumulation is associated with chemical residues accumulated at the site of toxic action but below a toxic threshold, as well as residues in other tissues containing no site of toxic action (e.g., storage lipid). In contrast, CBRs are chemical residues at the STA at a level above a threshold required to elicit a toxic response and may also include residues present in storage tissues. These measures simply may be thought of as a continuum of chemical concentrations in earthworms ranging from no effect through to levels that may result in organismal-level responses such as impaired reproduction and mortality. CBRs represent toxicological bioavailability, while bioaccumulation represents an intermediate between environmental bioavailability and toxicological bioavailability. Results of bioaccumulation tests or tissue residues measured in organisms exposed to contaminated soil in toxicity tests
or in the field can be compared with the CBR to obtain an indication of bioavailability and potential risk. Although the CBR concept has been well established for organic chemicals in aquatic ecotoxicology (McCarty et al., 1992), the application of CBRs for soil-dwelling organisms has developed more recently (Van Wensem et al., 1994; Van Straalen, 1996). CBRs have been derived for earthworms exposed to pentachlorophenol (Fitzgerald et al., 1996) and pyrene (Wells and Lanno, 2001). Some CBRs also exist for soil invertebrates exposed to metals. Crommentuijn et al. (1994) determined lethal body concentrations for cadmium in six different soil arthropod species. CBRs for cadmium and/or zinc on the growth and/or reproduction of plants, earthworms, and Collembola have also been reported (Posthuma et al., 1998; Van Gestel and Hensbergen, 1997; Smit and Van Gestel, 1997, 1998). Although both bioaccumulation and CBRs explicitly integrate bioavailability over the duration of the exposure period, they are also limited to chemicals that actually bioaccumulate to a measurable level (e.g., hydrophobic compounds with a log $K_{ow}$ > 4. many nonessential metals). Bioaccumulation and CBR models also often make assumptions regarding chemical toxicokinetics with tissue or whole body residues assumed to be in a steady state, although bioaccumulation can be estimated from uptake and depuration rate constants. The determination of chemical levels in earthworm tissues may also be expensive, and if earthworms are exposed to chemicals of unknown identity it may be very difficult to conduct a chemical analysis.

2.2. Indirect biological measures of bioavailability

Measures of bioavailability can also be strictly biological in nature, determined by the response of the earthworm to presumed chemical exposure. As an example, exposure to soils collected from industrial sites containing varying levels of chemical mixtures may elicit a variety of biological responses by earthworms. Responses that have been observed in earthworms range from lethality to sublethal changes in biomarkers (e.g., induction and or inhibition of enzymes, metallothionein induction, retention time of neutral red by lysosomes) (Reinecke and Reinecke, 1998; Scott-Fordsmand and Weeks, 1998; Scott-Fordsmand et al., 1998; O’Halloran et al., 1999). Assuming the use of appropriate controls or reference exposures, we can say that earthworms in these tests are responding to some level of bioavailable chemical, even though the actual bioavailable chemical level remains unknown. Without knowing the chemical exposure level, these responses can be called indirect biological measures of bioavailability and span a continuum from subcellular, biochemical biomarkers through to whole-organism responses such as mortality and reproductive effects. In this case, a chemical present in the soil in a concentration gradient of bioavailable chemical may be well correlated with an observed biological response in a dose–response relationship, but causality is not proven, only assumed. Although there are many techniques for measuring organism responses (e.g., reproductive effects, biomarkers), none of these indirect biological measures of bioavailability allow the quantitative identification of the level of bioavailable chemical to which an earthworm has been exposed. Most indirect biological measures of bioavailability are nonspecific (e.g., mortality, reproductive effects), with others being specific for groups of chemicals (e.g., metallothioneins, MFO enzymes).

One promising area of research is the use of genes or gene products for the determination of chemical exposure to specific chemicals or groups of chemicals (Stürzenbaum et al., 1998; Kille et al., 1999). The premise of these methods is that exposure to a specific chemical (e.g., Cd) or concentration of a chemical will result in the expression of a specific pattern of genes or a specific quantitative amount of a specific pattern of proteins (e.g., metallothioneins, heat-shock proteins, etc.), providing a unique fingerprint for the chemical and/or concentration of exposure. However, promising as these methods may be, they are currently in early stages of development and require a substantial investment of time and money in molecular techniques that may be specific for each organism. From a regulatory context, there is also a need to correlate these molecular measures with sublethal whole-organism (e.g., reproduction) and population responses and with indirect chemical measures of bioavailability.

2.3. Indirect chemical measures of bioavailability

Indirect chemical measures of bioavailability are chemical concentrations determined in the exposure medium and may also be expressed as a nominal chemical concentration or as a percent dilution of a contaminated soil with a reference soil. Indirect chemical measures of bioavailability include a number of chemical methods for determining chemical concentrations in soil ranging from total chemical to some measure of the chemical concentration in soil solution. In the example given in the preceding section, if chemical levels were determined in the test soils and the relationship between the chemical level and earthworm response examined, then the chemical measurements could be considered as indirect chemical measures of bioavailability. This is an important concept, since chemical measures alone without correlation with a biological response are not measures of chemical bioavailability. Only organisms can determine whether a chemical is bioavailable. Indirect chemical measures of bioavailability that estimate the environmentally available fraction of a chemical include chemical determina-
tions conducted on the exposure medium, ranging from vigorous extractions thought to estimate total chemical levels, to various liquid and solid-phase extraction techniques that sample some fraction of the chemical present in the test medium. Even though total chemical measurements may greatly overestimate the bioavailable fraction of a chemical in soil (Alexander, 2000) and may not be useful in predicting toxicity, they are still used in the development of soil quality guidelines and for the expression of chemical exposure in most earthworm bioassays.

The actual bioavailable fraction of chemical for earthworms in soil has been suggested to reside in the pore water (Belfroid et al., 1996), with partitioning of chemical from soil sorbing phases into pore water prior to actual uptake by the earthworm. The concentration of a chemical in pore water has been found to be better correlated with bioaccumulation and effects in earthworms than total chemical measurements (Van Gestel and Ma, 1988, 1990; Belfroid et al., 1994). Rather than obtaining pore water from soil, which may be a difficult process, aqueous extracts also may be used for estimating chemical concentrations in the soil solution. Expressing toxicity as a function of the aqueous phase concentration may result in an interpretation of toxicity completely different from that expressing toxicity as a function of total chemical levels. Consider the acute toxicity of three metals (Cd, Pb, Zn) to *E. fetida* in artificial soil (Table 1). When the incipient lethal level (ILL) is expressed as a function of total metal present in the artificial soil, Zn is most toxic, followed by Cd, with Pb the least toxic. However, if toxicity is expressed as a function of a weak salt extract (0.1 M Ca(NO₃)₂), Pb is now the most toxic, followed by Zn and then Cd. The interpretation of responses in the same earthworm bioassay is essentially reversed depending upon how exposure is expressed. This is due to the fact that Pb is more tightly sorbed by the components of artificial soil, reducing the actual bioavailable fraction of Pb that results in toxicity. Regardless of the chemical measure of bioavailability, it is imperative that it is correlated with either the accumulation or metabolism of the chemical by earthworms or an observed effect in the earthworm.

In addition to aqueous extractions, nonaqueous extraction methods also have been used for nonpolar chemicals, assuming that the quantity of chemical extracted from soil by a mild or nonexhaustive extraction reflects the amount of chemical bioavailable to earthworms. Concentrations of nonpolar organic chemical in soil extractions with mild organic solvents have been correlated with the bioaccumulation of nonpolar organic chemicals in earthworms (Chung and Alexander, 1999; Tang and Alexander, 1999; Krauss et al., 2000). Supercritical-fluid extraction (SFE) and accelerated solvent extraction typically have been used to measure total organic chemical levels in soil, but alterations in extraction conditions allow various fractions of total chemical to be extracted from soil (Hawthorne et al., 1995), and mild SFE may serve as a rapid and useful tool to predict bioavailability of PAHs in contaminated soil (Hawthorne and Grabanski, 2000). Thermal desorption techniques also have been used to assess the environmental availability of organic chemicals in soil, with sequestered compounds requiring more time and/or energy to be removed from soil samples than that needed for easily desorbable compounds (Werth and Reinhard, 1997; Uzigiris et al., 1995).

Solid-phase extraction (SPE) techniques also may be used for measuring the environmentally available fraction of a chemical in soil. Chemical partitioning to SPE devices exposed in wet soil or a soil suspension for a period of time is assumed to be proportional to the chemical concentration in the pore water, providing an estimate of chemical environmental availability. Many

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Metal & Total metal (mg/kg) & Ca(NO₃)₂-extractable (mg/kg) & Ca(NO₃)₂-extractable (% of total) \\
\hline
Cd & 20 & 9.8 & 27–59 \\
Pb & 28 & 1.2 & 0.4–9.7 \\
Zn & 9.7 & 6.3 & 12–85 \\
Toxicity rank & Zn > Cd > Pb & Pb > Zn > Cd &  \\
\hline
\end{tabular}
\caption{Toxicity (incipient lethal level) of lead (Pb), cadmium (Cd), and zinc (Zn) to *E. fetida* in artificial soil (adapted from Conder and Lanno, 2002)}
\end{table}

Metal exposure is expressed as both total metal concentration and metal concentration in a 0.1 M Ca(NO₃)₂ extract of the soil.
different phases are available as SPE devices, with the amount of chemical that partitions to the solid phase determined by the mass and polarity of the solid phase and the ability of the soil to maintain pore water chemical concentrations. If the partitioning of chemicals to the SPE device is correlated with chemical uptake by the earthworm (Fig. 4), the SPE device may be termed biomimetic. Various SPE devices that have been used to assess the availability of organic compounds in soil include Tenax beads, C18 membranes, and solid-phase microextraction (SPME) fibers. Good correlations have been observed between the uptake of DDT and its metabolites, DDE, DDD, and PAHs, by *E. fetida* from a range of soils and the quantity of chemical sorbed by C18 membranes (Tang and Alexander, 1999; Morrison et al., 2000). Phenanthrene bioaccumulation by earthworms exposed in artificial soil varying in organic matter content also was well correlated with sorption of phenanthrene to SPME fibers (Wells and Lanno, 2001; Fig. 4). All of these techniques appear promising for detecting environmentally available fractions of organic chemicals in soil, but until they are correlated with bioaccumulation or toxicity and are validated in field situations they will remain only promising techniques.

2.4. Direct chemical measures of bioavailability

A direct chemical measure of bioavailability is, by definition, not possible since only an earthworm can actually determine how much chemical is bioavailable to an earthworm. This measure is represented by a question mark in Fig. 3 since it represents a theoretical value to be estimated through repetitive correlation of some indirect chemical measure with a direct biological measure of bioavailability. Once this correlation has been repeated sufficiently to allow the prediction of biological effects based upon chemical measures, only then can the chemical measure possibly be classified as a direct measure of bioavailability. The best chemical predictor of bioavailability is that which theoretically best represents the environmentally bioavailable fraction of chemical in soil and is correlated with the best estimate of toxicological bioavailability, or the number of moles of a chemical at the site of toxic action. Until good correlations have been developed, new chemical methods for measuring chemicals that are theoretically bioavailable to earthworms must be validated using indirect and direct biological measures.

3. The temporal nature of bioavailability

One of the biggest problems in assessing chemical bioavailability is that bioavailability is not simply a chemical concentration, but the dynamic integration of chemical uptake and metabolism over the duration of the exposure period. Given the heterogeneity of chemical distribution in soil systems, on both micro- and macroscales, it is incorrect to assume that a chemical concentration in a small sample of soil represents the chemical bioavailability to an earthworm. Any chemical measures in soil are simply surrogate measures for the theoretical environmentally bioavailable fraction of the chemical. Different extraction procedures may provide a snapshot of the different fractions of chemicals to which an earthworm may be exposed, but this does not account for the biological aspects of bioavailability, such as metabolism, behavior, and the duration of exposure. A true measure of bioavailability requires the incorporation of the temporal nature of chemical exposure. All of the preceding analytical chemistry techniques measure only chemical availability from a soil subsample, although they may measure partitioning from that sample over a period of time. However, earthworms are exposed to a continuously changing chemical gradient as they move through the soil, integrating exposure over the heterogeneity of chemical distribution in soil.

SPE devices also show some promise in the integration of a temporal component into bioavailability assessment. Although rate-of-release studies using XAD2 resin beads (Updyke and Loehr, 1999) examine the cumulative release of various chemicals bound to soil, these studies have not been correlated with earthworm uptake over a similar period of time. Semi-permeable membrane devices have been used to assess the environmental availability of nonpolar compounds in water and sediment (Huckins et al., 1990, 1993) and have recently been applied to mimic the uptake of phenanthrene by earthworms in artificial soil (Wells and Lanno, 2001). Similar devices comprised of low density polyethylene packets containing C18 have been used to examine partitioning of nonpolar contaminants in field soils (Johnson et al., 1995; Awata et al., 1999). In addition to measuring environmentally available fractions of nonpolar compounds in soil subsamples, SPME fibers have also been exposed in test soils for longer periods of time (weeks) in a biomimetic approach to compare uptake of chlorobenzenes by enchytraeids (Van der Wal et al., 2001). Sijm et al. (2000) have also suggested that partitioning of hydrophobic compounds to SPME fibers or C18 discs may serve as a surrogate for partitioning to an organism. However promising SPE methods may seem, continued development of these techniques must be coupled with chemical uptake and biological responses in earthworms. Since bioaccumulation and CBRs are determined in organisms that may be at or near a steady state with respect to internal chemical concentrations, these measures of bioavailability account for some of the temporal aspects of bioavailability.
4. Applications of bioavailability

The development of methods for the measurement and understanding of bioavailability is important for assessing the toxicity of contaminated soils, but an understanding of how this information will be applied in ecological risk assessment is also needed. The application of bioavailability in ecological risk assessment requires that

1. a dose–response relationship exist between toxicity and a chemical measure of bioavailability,
2. soil quality guidelines based on bioavailable levels of chemicals and a maximum allowable effect for soil organisms have been established, and
3. measures of bioavailability are available for the soils in question.

In the scheme of the USEPA (1998) risk paradigm, bioavailability considerations would be involved in the assessment of exposure and effects during the risk analysis phase and in the estimation of a risk (hazard) quotient during risk characterization. In its most simplistic form, risk is often expressed as a risk (hazard) quotient or a ratio of exposure concentrations and chemical levels associated with effects

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\text{Risk Quotient} = \frac{\text{Exposure concentration}}{\text{Effect concentration}} = \frac{\text{Total chemical}}{\text{Bioavailable chemical}} = \frac{\text{Body residue}}{\text{CBR}}.
\]

Using our example of earthworms exposed to metals in Table 1, the ratio of toxicity estimates for Pb based on total and Ca(NO\textsubscript{3})\textsubscript{2}-extractable (i.e., \(\text{ILL}_{\text{total}}/\text{ILL}_{\text{Ca(NO\textsubscript{3})\textsubscript{2}-extractable}}\)) Pb concentrations is approximately 23. In this estimation, ILL is the incipient lethal level, or time-independent LC\textsubscript{50}, estimated from acute toxicity tests with earthworms (Lanno et al., 1997). In other words, by utilizing total Pb levels in the specific soil used in the toxicity test, actual exposure was overestimated by more than 20-fold, assuming that the Ca(NO\textsubscript{3})\textsubscript{2}-extractable fraction of Pb was a more accurate estimate of bioavailability. Such an overestimation of exposure may have a significant effect on the interpretation of exposure and effects during risk characterization. However, for bioavailability to be used in exposure estimates for calculating risk factors, effects concentrations also must be measured in terms of bioavailable chemical levels. This would require that laboratory data used in generating effects concentrations also be based upon environmentally available chemical levels. Direct biological measures of bioavailability also can be used in assessing risk based upon organism residue levels in relation to CBRs. In the same manner, if biomimetic devices such as SPME fibers were used in the estimation of effects concentrations, exposure concentrations based on environmentally bioavailable chemical levels measured using SPE techniques should also be used in risk screening.

5. Conclusions and recommendations for future research

As total chemical measures are inadequate for expressing the chemical exposure of earthworms in soil due to various abiotic and biotic modifying factors, it becomes necessary to include chemical bioavailability in the expression of exposure. A theoretical framework for bioavailability (Landrum et al., 1992) is revisited and presented in schematic form (Fig. 2). Various biological and chemical measures that theoretically represent the three major compartments of Fig. 2 (environmental availability, environmental bioavailability, toxicological bioavailability) are presented in Fig. 3 and classified as direct and indirect biological measures and indirect chemical measures of bioavailability. The most important message that evolves from the discussion in this paper is that bioavailability needs to be considered for understanding exposure in soil systems and that biological and chemical measures of bioavailability must be correlated and not developed independently. Estimates of the environmentally available fraction of chemicals should be included in standard laboratory toxicity tests with earthworms or when determining chemical levels in field soils.

References


