Proposed Hazard Classification of Metals in the Terrestrial Environment

Second Discussion Paper

by
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and
Lawrence A. Kapustka, Ph.D.

PLANTS AND INVERTEBRATES
SOIL SYSTEMS
NATURAL OCCURRENCE
BIOAVAILABILITY

International Council on Metals and the Environment
Promoting policies and practices towards sustainable development
ICME

The International Council on Metals and the Environment (ICME) has published this document as part of its ongoing efforts to provide information on environmental, health and related matters affecting the metals mining and producing industry. The contents of ICME publications range from general and technical information to discussions of policy and regulatory issues. The topics examined may be of interest not only to industry but also to others, including policy makers, regulators, educators and the public at large. ICME hopes that these publications provide insight into what are sometimes difficult and complex issues.

Although the views expressed are those of the authors, ICME welcomes questions and comments on the perspectives and information contained in its publications. ICME also appreciates suggestions regarding other issues of public importance for possible future publications.

Founded in 1991, ICME is a non-governmental organization that promotes sustainable development policies and practices related to the mining and production of primary metals, which will ensure the safe production, use, recycling and disposal of metals. In addition to publications, ICME's extensive information program includes a Web site and a quarterly newsletter with a worldwide distribution.

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Foreword

In May 1997, ICME published a discussion paper entitled *Hazard Classification of Metals in Terrestrial Systems*. The document initiated a process of consultation and discussion which in turn sparked new thinking and furthered understanding in this area.

In 1999, in order to further advance discussions on classification while improving the scientific understanding of hazard determination, ICME and other metal industry associations sponsored a Society of Environmental Toxicology and Chemistry (SETAC) workshop entitled "Test Methods for Hazard Determination of Metals and Sparingly Soluble Metal Compounds in Soils."

The present document is based on the results of that workshop, as well as on feedback received from the 1997 discussion paper and on scientific advancements in hazard assessment that have been made in other environmental media (e.g. aquatic). Its most significant recommendations are that the phenomena of bioavailability as well as of complexation, soil ageing and other transformation processes should be taken into account in the development of a hazard classification system for metals and metal compounds in the terrestrial environment.

The authors of the publication are Drs. Anne Fairbrother and Lawrence Kapustka, respectively experts in wildlife and plant ecotoxicology. Both are experts in environmental risk assessment.

ICME commissioned the work as a contribution to the ongoing dialogue concerning the development of a classification approach for metals and inorganic metal compounds with respect to their potential hazard to the terrestrial system. It invites feedback and comments and welcomes any opportunities to participate in discussions with interested organizations or agencies.

Gary Nash
Secretary General
ICME

Executive Summary

The Organisation for Economic Co-operation and Development (OECD) has requested the European Union to develop terrestrial hazard classification criteria for substances in commerce. This discussion paper contributes to the dialogue on this topic, with specific recommendations for incorporating metal-specific issues into a proposed classification and labelling scheme for substances that could affect soil organisms (plants and invertebrates).

Hazard classification should consider both short-term and long-term effects. Toxicity information for terrestrial organisms should be obtained from standardized tests conducted on organisms exposed to metals or metallic substances in soil. The toxicity tests recommended are designed to maximize metal bioavailability under standard conditions within naturally occurring ranges of soil parameters. Hazard categories are formed by combining toxicity values with the slope of the concentration–response curve, to incorporate information about the response of organisms to increasing metal concentrations.

Elements occur naturally in various oxidation states. Physico-chemical changes alter the form or species of the elements, which in turn influence toxicity. Physico-chemical conditions also govern rates of transformation of metallo-compounds. However, unlike the decomposition of synthetic organic substances, elements undergo biogeochemical transformations in complex cycles. Short-term effects can be evaluated through toxicity tests performed on soils immediately following incorporation of the test substance into the test soil. Evaluating long-term hazards of metal compounds is complicated by transformation processes.

Physico-chemical conditions also affect bioavailability. As metal substances transform and bind to soil constituents, bioavailability changes over time. Bioavailability is the most important determinant of potential hazard from metal exposures. Because the state-of-the-science is not sufficiently advanced to predict how different metals or metallic substances will behave in various soils, empirical testing is required to make reasonable hazard classification predictions.

One method of simulating some aspects of transformation has been proposed. Following incorporation of the substance to be tested into the test soil, saturation and leaching steps are performed to simulate ageing. Subsequent toxicity tests on the “aged” material can be performed to evaluate the potential long-term hazard.

The ageing process eliminates the need to modify hazard classification based on persistence and bioaccumulation as is done for organic substances. Many elements are essential for life. Those essential elements required in relatively low amounts are referred to as micronutrients. Generally, micronutrients are required at tissue concentrations that are greater than the concentration found in the environment and are actively concentrated and regulated by plants and other organisms. Typically, however, bioconcentration of metals up the food chain is far less than the several orders of magnitude observed for organic compounds such as the organochlorines.

Preface

This document was produced at the invitation of the International Council on Metals and the Environment. It is intended to add to the ongoing dialogue concerning the development of a scheme for classifying substances placed on the market regarding their potential hazard to the terrestrial environment. The particular emphasis of this document is on metals, inorganic metal compounds and other naturally occurring elements. Nevertheless, it is our hope that most of the basic principles are compatible with classification of organic substances as well. We highlight areas of particular concern for metals, but recognize that concepts of toxicity, test methods and hazard prediction are similar regardless of the substance of concern. Substances should be classified only when data from appropriate standardized terrestrial toxicity tests are available; the terrestrial environment is too heterogeneous and complex to extrapolate from data that were generated for other purposes. One of the reasons is the differences in natural occurrence of metals and the potential of organisms to acclimate to these conditions by the mechanism of homeostasis. We encourage the continued support of ecotoxicity data generation as well as research into geochemical principles regulating metal bioavailability and ecological responses to metal enrichment of terrestrial systems.

Anne Fairbrother, D.V.M., Ph.D.
Lawrence A. Kapustka, Ph.D.

SECTION 1
Introduction

The Organisation for Economic Co-operation and Development (OECD) has requested the European Union to develop terrestrial hazard classification criteria for substances in commerce. This stems from the public's right to know whether a substance may be hazardous to human health or the environment. Hazard classification and labelling are forms of Hazard and Risk communication, intended to inform users how to handle substances during manufacture, transport, use and disposal. Hazards may be defined for short-term effects, long-term effects, or both. While the concepts of hazard classification appear to be relatively straightforward, implementation is much more complex, particularly for metals. Metals are ubiquitous naturally occurring substances, many of which are required nutrients for organisms, as well as components of manufactured products. Additional complexity arises as metals partition into different parts of the environment, depending upon a number of physical-chemical factors that vary significantly from place to place and over time. Moreover, different plant and animal species respond differently to metal exposure depending upon their physiology, adaptations to variable natural background concentrations, and whether or not the metal is an essential element.

The OECD focused first on a methodology for the classification of hazard of materials to aquatic organisms, because aquatic systems had been studied more thoroughly than the terrestrial systems and standard test guidelines were available. Standard protocols were in place for organic substances that needed only slight modification to accommodate metal-specific issues, such as dissolution rates, bioavailability, biotransformation and adaptation to background levels.

Discussion for development of a similar system for terrestrial organisms began about three years ago with the publication of a draft scheme by the Terrestrial Effects Working Group of the OECD Hazard Assessment Advisory Body prepared by Sweden (see Lundgren, 1999). However, the authors recognized that this document was developed mainly for synthetic organic compounds and did not adequately address naturally occurring metals, minerals and their derivative compounds.

The International Council on Metals and the Environment (ICME) published a discussion paper a year later (Fairbrother and Kapustka, 1997) that proposed a modified scheme to be used for metals in terrestrial systems. This scheme was predicated on the assumption that no standardized test data were available for many substances and so suggested a method for initial prioritization of substances that subsequently would be classified once toxicity data became available. In practice, however, substances for which data are missing or inadequate will not be classified.

In the past two years, two scientific workshops on terrestrial hazard identification were held to discuss and describe the basic properties of soil organisms–contaminant interactions that are important in hazard identification and risk assessment, as well as to establish test methods specifically for hazard determination of metals in soils (Fairbrother et al., 1999; Saterbak et al., in press). Additionally, a workshop was convened in Madrid, 4–6 November 1998 by the European regulatory authorities to explore various options for a hazard identification-classification system for the terrestrial environment (Vega et al., 1999).

It is now appropriate to revisit the recommended terrestrial hazard classification scheme proposed by ICME in 1997 to incorporate advances in the state-of-the-science and regulatory policy concerning hazard identification for metals in soil, most particularly the availability of standardized testing protocols for soil organisms.

This discussion paper presents the objectives for a terrestrial classification scheme, describes a practical approach, and presents the rationale for selection of various options. Consensus views interpreted from recent workshops on the subjects of test methods and classification processes have been integrated into this paper.

SECTION 2
Objectives

The objectives of the terrestrial classification scheme proposed herein were:

- to classify metals and their inorganic metal compounds according to their potential hazard to soil organisms (specifically, plants and soil invertebrates);
- to identify which metals and inorganic metal compounds potentially are hazardous in the short-term and long-term periods;
- to be compatible with similar approaches and starting points for organic substance hazard classification; and
- to be consistent with the hazard classification approach for metals in aquatic systems.

The proposed scheme does not assess hazard to soil microbial communities or to above-ground organisms (e.g. birds, mammals, invertebrates). As proposed by Germany (Feibicke, 1999; Feibicke et al., 1999) and Sweden (Lundgren, 1999), the procedures required for hazard determination for these organisms for any substance are not sufficiently advanced to propose a scientifically valid approach. These compartments should be incorporated at a later date based on experiences gained with the soil compartment and necessary advances in test protocol design and interpretation.

SECTION 3
Classification System

The proposed classification system contains steps for determining short-term hazards of metals to soil organisms, a method to simulate ageing to evaluate long-term hazard, and criteria for assigning substances to different hazard categories. Justifications and technical support information for development of the proposed scheme are presented in Section 4.

Approaches to hazard classification for terrestrial systems are under active discussion (European Community, 1997; Tarazona et al., 1999). Although no final determinations have been made regarding the number of categories or specific cut-off values between categories, a consensus may be forming on key issues. Specifically, agreement may be emerging to use relevant data from chronic studies to set cut-off values and to “override” assumptions regarding persistence or bioaccumulation.

Our proposed system is not predicated on bioavailability and persistence criteria. Rather, we recommend using empirical data developed directly from toxicity tests on substances treated to simulate ageing processes. The concept of persistence as a key modifier of long-term hazard is important for evaluation of organic substances such as chlorinated hydrocarbons, but it should not be the foundation of hazard classification of metals. Transformation kinetics favour certain species of metals and determine the rate of change. But unlike degradation of organic compounds, metallo-species typically reoccur through rather complex biogeochemical cycles. Bioaccumulation of essential elements is a normal biological phenomenon, which occurs in actively growing organisms where exposure concentrations are low. As such, bioaccumulation potential is not particularly meaningful for hazard evaluation of metals and inorganic metal compounds. The complexity of biological interactions with metals requires that long-term hazard should be established only after conducting suitable bioassays as outlined in subsequent sections.

3.1 Short-term Hazard Identification

Our previous proposal (Fairbrother and Kapustka, 1997) emphasized using intrinsic toxicity as a means to classify substances based on the key element of interest (i.e. cadmium for all cadmium salts). Intrinsic toxicity refers to the inherent properties of a substance that cause adverse effects. This is distinguished from apparent toxicity (i.e. that which is observed in a test situation), which may be different from intrinsic toxicity due to modifying factors such as bioavailability. For example, intrinsic toxicity of divalent cadmium is the same for different forms such as cadmium chloride, cadmium nitrate or cadmium sulfate. But the apparent toxicity of these salts may differ with solubility and other factors influencing uptake and transport within an organism. With the objective to classify products on the market, intrinsic toxicity may be largely an academic interest. Also, as substances with inadequate data will not be classified, the benefits of the generalized classification scheme we presented previously would not be realized.

The approach presented here relies on direct measurement of toxicity for each product of interest (e.g. cadmium sulfate, lead acetate). Determination of the potential short-term hazard of a substance to soil organisms incorporates point estimates (e.g. EC_{20}) as well as the slope of the toxicity response.[1] Thus, the system proposed here expands on our previous proposal and includes recommendations adapted from the plant and invertebrate working groups at the recent workshop on test methods for hazard determination of metals in soils (Fairbrother et al., 1999). In many jurisdictions around the world, toxicity data used for evaluating effects of chemicals on ecological resources have been limited to point estimates (e.g. NOEAC, LOEAC or EC_x). This has been useful in identifying relative differences in threshold levels for different chemicals and among species. Such information identifies hexavalent chromium in aquatic systems to be more toxic than trivalent chromium; cadmium to be more toxic than zinc, etc. However, use of the point estimates alone ignores much of the data already generated in toxicity tests. Using the slope of the concentration–response relationship generated in the test would improve the technical foundation for regulation of chemicals. For example, the phytotoxic response to copper reveals a steep response relationship, whereas the response to zinc follows a gradual slope. The steepness of the response is an indication of ability of organisms to tolerate or cope with levels above the threshold concentrations. If the toxic response progresses from minor effects to death after only a small increase in exposure concentration, then organisms are considered to be sensitive and there is a greater potential hazard from adding that substance to the environment.

The design of toxicity tests should be given careful consideration. Many standardized toxicity tests use a design most suited for analysis of variance (ANOVA) approach to identify predicted no adverse effect (NOAEC) and lowest adverse effect values (LOAEC). Such designs have been criticized by many (e.g. Chapman et al., 1996). Alternative test designs that are tailored for regression analysis are preferred as they yield information on both effects threshold values and the slope of the response (i.e. intrinsic toxicity and tolerance).

1 Though differences in point estimates and slope are not unique to metals, there are special cases to consider, especially for essential nutrients. Physiological processes enable organisms to regulate the cellular concentrations of metals, which can produce element-specific response curves.

While many studies in the scientific literature report effects of metal-contaminated soils on terrestrial species, most have not followed standardized protocols. Thus, comparison or compilation of results are confounded by the different physical-chemical properties of soil, different environmental parameters, species tested and endpoints measured. Nevertheless, some of these data may be useful for hazard classification, provided the studies can be qualified as having appropriate quality control and reporting methods, as well as acceptable test designs such as are described in the following sections. For those substances requiring additional data, the use of standardized tests is recommended.

3.1.1 Test soil

Toxicity testing with metals presents complications not found in tests with organic compounds. For organic compounds, it is relatively easy to determine that the test soil has none of the test substance prior to treatment. However, each soil will have some level of metals in some form. For example, a test for toxic effects of magnesium sulfate would start with some background level of magnesium. Consequently, there is a critical need to standardize the test matrix to be used for hazard classification tests. We suggest that the OECD artificial soil should be used for all tests, modified to have the following properties:

- low organic matter content (2%);
- low cation exchange capacity (<50 mol/kg); and
- low amounts of iron, manganese and aluminum oxides.

For hazard classification of cationic metals (e.g. copper, cadmium, lead, nickel and zinc), soils should be acidic, with pH between 5.0 and 5.5. For anionic metalloids (e.g. arsenic and selenium), soils should be similar, but pH should be high (7.5 to 8.0). This will provide a soil within naturally occurring ranges, but at the highest practical bioavailability of metal ions. The properties specified here maximize bioavailability of metals and therefore provide a truer picture of the inherent hazard of a substance and is consistent with regulatory requirements to be protective. Higher levels of organic matter, clay content (and thus cation exchange capacity) and other ranges of pH would lower bioavailability and correspondingly lower risk in the environment.

For acute effects determination, the substance to be classified should be used in the tests. Soils should be allowed to equilibrate for 5 days after mixing, before the test substance is added. An additional 7-day equilibration period should be allowed after mixing the test substance with the soil, before the introduction of the test organisms. Top dressing applications should not be allowed even though this might simulate a spill situation. Without proper mixing, test organisms would be exposed to a wide range of concentrations (maximum concentration at the surface and potentially no elevation above background levels within the test container). In addition, transformation reactions would be limited with surface applications.

3.1.2 Invertebrates

Two species of invertebrates should be tested: *Eisenia foetida* (Lumbricidae; earthworm) and *Folsomia candida* (Collembola; springtail). Standard, acute LC_{50} protocols should be followed (OECD, 1984; Wiles and Krogh, 1998) to determine the acute lethality of the test substance. The concentration at which 50% mortality of the replicate treatments occurs should be calculated and the slope of the dose–response curve determined.

3.1.3 Plants

The short-term effects on plants may be measured as growth of seedlings. Many species have been used and selected in standardized test procedures. For maximum comparability with the long-term tests, we recommend using *Brassica rapa* as the test species (OECD, 1984; ASTM, 1999). Measurement endpoints to be taken include seedling emergence, survival, root length, shoot height, root mass and shoot mass in a 14-day post-emergence test. The study should be designed for regression analyses. The EC_{20} for each endpoint should be determined and the lowest value used for hazard classification. The slope of the dose–response relationship for the selected endpoint should also be calculated.

3.2 Long-term Hazard Identification

Determination of long-term hazard to the terrestrial environment consists of two parts. First, metals and metal substances change in regard to the amount bound to soil particles over time, thus altering their potential bioavailability. Therefore, testing must incorporate some estimate of whether the hazard might increase or decrease over time due to physical-chemical interactions of the substance with the soil. Second, organisms respond differently to long-term exposures to substances than they do to a short, acute insult. Toxic effects may become apparent only after a lengthy exposure period or, conversely, protective mechanisms (acclimation) may be activated conferring tolerance. Therefore, long-term hazard identification involves a longer equilibration time of the test substance with the soil and measures of chronic toxicity in the test organisms.

3.2.1 Ageing of test substance in soil

Transformation processes begin with the addition of the test substance to the test matrix. Integration of metals into soil components as well as association with cations and anions resident in the soil affect transport (e.g. leaching) and bioavailability. As these processes change over time, it is important to provide time for a significant amount of ageing to occur before toxicity tests are initiated. To achieve this, the test substance should be allowed to interact with the soil for a period of at least 60 days[2] following the 7-day

2 An equilibration period of 60 days was suggested based on expert judgement by attendees at the workshop "Test Methods for Hazard Determination of Metals and Sparingly Soluble Metal Compounds in Soils," Madrid, Spain, June 1999. The length of time may need adjustment following empirical testing.

incubation period used for the acute hazard studies. At the start of this 60-day period, the soil is leached with three porewater volumes of de-ionized water. This simulates environmental ageing and will remove any soluble portions of the test substances that would not be expected to persist in the natural environment. Chronic toxicity tests would then be conducted on soils that have equilibrated for the 60 days.

3.2.2 Invertebrates

The same two species used in the short-term tests should be used for the longer-term testing. Measurement endpoints will be sublethal effects related to reproductive output. Standardized testing protocols for reproduction effects are available and should be followed (ISO 1994, 1995). However, the equilibrated and leached test soil described above should be substituted for the immediately prepared artificial soil recommended in the published protocols. Because of the long-term nature of the test (e.g. eight weeks for earthworms), food will need to be added. Food (e.g. finely ground cattle manure or fermented alfalfa pellets) should be tested for metals and only those food sources low in metals should be used. The test endpoint is the number of juveniles produced at the end of the test period. The concentration at which 10% of the test replicates are affected (i.e. the EC_{10}) is used as the critical toxicity value. The slope of the dose–response curve for the selected endpoint should also be calculated, for the reasons discussed previously.

3.2.3 Plants

Chronic toxicity to plants should be determined using the same Brassica life-cycle test recommended for the acute toxicity studies (ASTM, 1999). However, the exposure period should be extended to 45 days. Measurement endpoints include those recorded for acute toxicity (seedling emergence, root elongation and vegetative growth), but also include reproductive endpoints such as number of flowers, amount of fruit set and seed production. The EC_{20} for each endpoint should be determined and the lowest value used for hazard classification. The slope of the dose–response curve for the selected endpoint should also be calculated.

3.3 Principles to Derive Cut-off Values

Consensus does not exist on either technical grounds or regulatory policy regarding the manner of selecting cut-off values. Because knowledge is always limited, the most rational procedure is to use a *weight-of-evidence* approach to consider the full range of data available from all properly conducted studies. Although this requires considerable technical evaluation on a case-by-case basis, it provides the strongest technical foundation for any decision that will be made regarding classification. Different terrestrial hazard classifications will be derived for acute and chronic effects to flora and for acute and chronic hazard to soil organisms. This will provide necessary information for the potential classification categories.

Toxicity threshold values will first be determined from the hazard identification tests as follows:

- acute toxicity to soil invertebrates – LC_{50} (acute lethality);
- acute toxicity to plants – EC_{50} (seedling emergence and early seedling growth);
- chronic toxicity to soil invertebrates – EC_{20} (reproduction effects); and
- chronic toxicity to plants – EC_{20} (full life-cycle study).

These threshold values can then be multiplied by 0.1, 1 or 10 to incorporate the slope factor information. The choice to use multipliers as discreet "class" values instead of an infinite set of slopes derived from individual tests was made to simplify the process and to acknowledge the level of precision typical in such toxicity tests. The term "slope factor" for the acute (short-term) classification is defined as the amount of increase in soil concentration necessary to move from the $L(E)C_{50}$ to the $L(E)C_{75}$ in the standard test from the species used to set the toxicity value (Fairbrother and Kapustka, 1997). Slope factors for long-term hazard are similar, but are based on the amount of increase in soil concentration between the EC_{20} and EC_{50}:

- multiple toxicity threshold value by 0.1 if: slope factor ≤ 100
- multiple toxicity threshold value by 1 if: 100 < slope factor ≤ 1000; or
- multiple toxicity threshold value by 10 if: slope factor > 1000.

Substances will then be classified into terrestrial hazard categories for plants as follows with the value for X to be agreed based on a thorough analysis of phytotoxicity data for metals and metallic substances:

- *very toxic*: slope-adjusted EC values ≤ X mg/kg soil;
- *toxic*: slope-adjusted EC values $X < EC_x \leq 10X$ mg/kg soil;
- *harmful*: slope-adjusted EC values $10X < EC_x \leq 100X$ mg/kg soil; or
- *not classified*: slope-adjusted EC values >100X mg/kg soil.

Substances will then be classified into terrestrial hazard categories for plants as follows with the value for Y to be agreed based on a thorough analysis of soil invertebrate toxicity data:

- *very toxic*: slope-adjusted L(E)C values ≤ Y mg/kg soil;
- *toxic*: slope-adjusted EC values $Y < EC_x \leq 10Y$ mg/kg;
- *harmful*: slope-adjusted EC values $10Y < EC_x \leq 100Y$ mg/kg soil; or
- *not classified*: slope-adjusted EC values >100 Y mg/kg soil.

This would result in final categorizations of *very toxic, toxic, harmful* or *not classified*.

SECTION 4
Rationale and Technical Support

This section explains the reasons for the suggested classification scheme, keeping in mind the stated objectives. Every attempt has been made to use sound scientific principles and to identify areas where policy decisions were made based on best professional judgement. We hope this will result in a transparent process that can be easily understood and supported by the regulatory community, industry and academic scientists.

4.1 Reliance on Standardized Tests

The capacity for metals to transfer from the soil into plants or invertebrates and cause toxicological responses is a function of the physical-chemical properties of the metal and the soil as well as biological properties of the organism (Anderson et al., 1999). Because both of these physical and biological systems are extremely complex, we are not yet able to predict with a high degree of certainty which metals are likely to be most hazardous to plants or soil organisms in any particular soil ecosystem. Empirical studies reported in the literature provide some insight into which types of soils have greater bioavailability of metals to soil organisms. However, they rarely follow the same study protocol, making it difficult to compare intrinsic toxicities of the various metals. This is a significant departure from the state-of-the-science in aquatic systems where standardized aquatic toxicity test methods have been available for many years, producing comparable test results.

There has been some success in applying a free ion-ligand binding model to predict toxicity in aquatic systems. It would be useful to extend the free ion model to the terrestrial system, but the dynamics and complex heterogeneity of soil systems will make this difficult to accomplish in the near term. Briefly, the free ion model postulates that toxicity to plants and soil invertebrates is directly proportional to the amount of free metal ions in the soil porewater. Porewater concentrations are, in turn, a function of the amount of substance present in the soil and physical properties such as soil texture, organic carbon content, cation exchange capacity, calcium carbonate equivalent and pH (Förstner, 1995). Soil mineralogy, including clay minerals, hydrous oxides, carbonates and type of humic substances, are also important determinants of metal mobility in soils (Sparks, 1995). If the assumption is made that all soil processes are in equilibrium, then soil sorption coefficients (K_Ds) can be derived to predict the amount of free ion in porewater as a function of all the influencing parameters listed above. In reality, soil processes are rarely (if ever) in equilibrium, so more complex kinetic reactions are needed to model the sorption processes (Sparks, 1995). This has been done in some contexts, primarily to model movement of metallic substances through soils into groundwater. However, the root zone adds an additional level of complexity to predictions of porewater concentrations as plants and micro-organisms associated with roots significantly alter this environment. Plants acidify the root zone, remove oxygen from the system, and set up counter-exchange gradients that move macronutrients such as phosphorus and nitrogen toward the plant. This changes the physical properties that cause metals to sorb or desorb from soil particles (Förstner, 1995). Furthermore, plants and invertebrates actively take up certain required elements (e.g. copper, nickel and zinc) from soils that are deficient in these metals or actively exclude them if the soil concentrations are too high. This introduces further complications to predicting free ion concentrations in root zone porewater.

Predictions of toxicity based on porewater concentrations require that bioassays be conducted in a manner such that plant or invertebrate responses can be correlated to the porewater concentrations, not just to bulk soil chemistry. Suggestions for doing this include: 1) conducting bioassays in a soil matrix, but measuring porewater concentrations rather than bulk soil chemistry; 2) conducting all bioassays in hydroponic solutions; and 3) comparing soil porewater concentrations to toxicity levels for aquatic organisms such as daphnids (to represent soil invertebrates) or algae (to represent terrestrial plants). All three of these approaches have significant drawbacks that preclude their use as predictive models for terrestrial hazards.

Measuring metal concentrations in soil porewater rather than bulk soil during bioassays either in glasshouses (i.e. pot studies) or in field plots is difficult to do in a manner that is reproducible and predictive of real-world situations. There are several methods for extracting porewater from soil, ranging from centrifugation to gentle suction through appropriately placed straws. There does not appear to be a consensus in the scientific community about which one of these methods most closely models the porewater that is extracted by soil organisms. The process of extracting the porewater changes the physical-chemical equilibria in the soil such that the measured K_D may differ by an order of magnitude or more depending upon the method used. Repeatable extractions of porewater from pot studies are particularly problematic as the structure of the soil matrix is disrupted by placing the soil into the pots, and is dependent upon the degree of compaction and hydration of the soil. Therefore, measuring soil porewater concentrations rather than bulk soil measurements may not increase the predictability of toxic response.

Use of hydroponic solutions for plant bioassays eliminates the need for extraction of soil porewater. However, these conditions are highly artificial, primarily because they cannot accommodate associations of mycorrhizal fungi with plant roots and short- as well as long-term equilibrium partitioning with soil particles, mineral lattices and others. Nearly all plants are associated with mycorrhizal fungi in soil systems, which significantly alters the interactions of roots with porewater constituents. Nutrients, metals and other substances must first pass through the fungi before reaching the absorptive region of the root, thereby allowing additional regulation of what is passed on to the plant. Thus, the uncertainties in predicting toxicity in soil systems are increased when using hydroponic bioassays, as extrapolations to mycorrhizal systems are added to the difficulties in estimating metal porewater concentrations. Furthermore, soil invertebrate bioassays cannot be done in hydroponic systems.

Therefore, the suggestion has been made that soil porewater concentrations (measured or estimated) could be compared to toxicity data from aquatic organisms to establish potential terrestrial system hazards (Vega et al., 1999). However, as clearly shown by Clausen (1999), there is no correlation between biological responses of aquatic and terrestrial organisms to metal exposure. Nor is there any reason to believe that a daphnid should respond to metal exposures in a similar way as an earthworm or springtail, nor that a unicellular algae should be an appropriate model for a multicellular terrestrial plant and its mycorrhizal associations. Binding of free metal ions to gills or cell surfaces of aquatic organisms is dominated by a passive competitive binding process, wherein the metal ion competes for binding sites with other ionic ligands. In plants and soil invertebrates, passive binding occurs but at a much lower rate. Of greater importance is active uptake or exclusion of metal ions by gastrointestinal cells, roots or fungal cells. Internal distribution and use of the various metals likely also differs among organisms. Therefore, there is no basis for pursuing this line of reasoning further.

In summary, there are very few reports in the literature of toxic responses of soil organisms to metal-contaminated soils that can be compared among metals to establish a consistent ranking and categorization of hazard. While there currently is interest and ongoing research in extending the free ion/biotic ligand model to terrestrial systems, sorption coefficients for metals in soils over a wide range of physical-chemical conditions have yet to be developed, particularly if systems are modelled in their dynamic state rather than assuming that equilibrium exists between free metal ions in the soil porewater and those sorbed to soil particles. Furthermore, use of the free ion model for hazard classification (i.e. basing predictions on relative K_Ds) would require much more extensive knowledge than is currently available about the complex relationship between soil porewater concentrations and plant or invertebrate responses. It is more likely that the current research on methods for measuring porewater or estimating K_Ds will be useful for site-specific risk assessment (e.g. extrapolating dose–response relationships developed in one soil type to the specific site soil) than for use in a generic hazard classification scheme.

Our recommendation for using a standard test procedure for the purposes of hazard classification will ensure that the intrinsic toxicity and organism tolerances for all metals and their derivative substances will be comparable. The proposed methods are based on protocols derived to test synthetic organic substances with modifications to accommodate some of the particular properties of metals as proposed by a group of scientific experts (Fairbrother et al., 1999). They are designed to maximize bioavailability without resorting to soil systems that would cause organism stress due to other, non-metallic factors and that are still representative of a reasonable amount of the world's soils.

4.2 Persistence and Bioaccumulation

As argued by Fairbrother and Kapustka (1997), and in accordance with concepts developed for the aquatic ecosystem, measures of persistence and bioaccumulation of metals and their inorganic metal compounds as indicators of potential chronic hazard are inappropriate for terrestrial systems. Metals are naturally persistent substances, some of which are required elements for plants and animals. Because of this, terrestrial organisms have evolved methods for actively taking up or excluding most of the elements in order to maintain biological homeostasis. At times, this means bioconcentrating materials to levels in tissues that exceed those in soils; at other times, tissue concentrations may be significantly less than those found in soils. Therefore, predicting possible long-term hazard based on relative persistence or bioaccumulation of metals from soils will not differentiate among the various substances, nor will it be an accurate estimate of chronic toxicity potential.

4.2.1 Persistence

The transformation/leaching step for soil preparation before conducting chronic toxicity tests provides a more reasonable assessment of the probability of continued hazard from long-term exposure of metal substances in the soil. For metals, persistence needs to be defined as the continued presence of a bioavailable toxic form. If the metal persists in a form that readily dissociates, but is not quickly leached from the soils, it will remain in a bioavailable state for a relatively longer time, thus increasing the potential hazard. On the other hand, a substance that is readily leached from the soil or remains in a sorbed or otherwise non-available state will not pose a hazard to soil organisms for very long. Therefore, the concept of persistence is really one of *transformation* and *bioavailability*. Given the difficulties described in the above section of currently being able to predict rates of transformation and leaching and subsequent changes in bioavailability from sorption kinetics, empirical test data should be used for hazard classification rather than reliance on K_Ds. In actuality, relatively few metals and almost no metallic substances (other than soluble salts) have K_Ds developed for comparative standard soils.

4.2.2 Bioaccumulation

Edwards and Bohlen (1996) summarized information on accumulation of heavy metals (cadmium, copper, lead and zinc) by earthworms. Accumulation factors (i.e. the ratio of amount of metal in the earthworm as compared to amount in soil) never exceeded 1 for copper or lead and were always less than 10 for zinc. Cadmium had the greatest accumulation factors, ranging up to nearly 200. Similarly, Förstner (1995) showed that accumulation factors in plants are greater than 1 only for zinc, cadmium and thallium (all of which are less than 10), but even these patterns differ among species with some plants designated as accumulators and others as excluders (Baker, 1981). Thus, with the possible exception of cadmium in some species and specific hyperaccumulator plants, metal bioaccumulation generally is not of great concern in terrestrial systems. It is more likely that direct toxic effects to plants and soil organisms will occur before observations of effects to higher trophic levels. This has been substantiated in site-specific studies around old zinc and lead smelters (e.g. Hunter et al., 1987; Beyer and Storm, 1995).

4.3 Essentiality and Tolerance

It is well known that many metals are required by plants and animals as micronutrients, playing an important role in enzyme function, electron transport and energy production, and other cellular and membrane processes (see Fairbrother and Kapustka, 1997 for complete listing of required elements). Different biological species have different requirements and may be unable to grow or survive in areas naturally deficient in certain elements. Naturally occurring soil constituents, including essential elements, differ on a global scale and interact with climatic factors to produce a variety of eco-regions around the world (Bailey, 1998). Each of these eco-regions supports different communities of plants, animals, fungi and bacteria in dynamic relationships that continually change and evolve in response to changing environmental pressures.

4.3.1 Acclimation

Because of the nutrient requirements of plants and soil invertebrates, test soils used in bioassays for hazard determination always will contain these metals. It is, however, important that the amounts of these nutrients be within the required and tolerated range for the test species so the organisms are not stressed by the test conditions, thus masking their response to the metal of interest. However, naturally occurring soil organisms tend to become adapted to different environments, often within a single generation (Posthuma and van Straalen, 1993). Therefore, during the conduct of toxicity tests for naturally occurring substances, attention must be paid to culture conditions of invertebrates or seed sources of plants to ensure that short-term adaptive changes to either high or low metal concentrations do not occur (Fairbrother et al., 1999). Additionally, the recommended test species are representative of organisms within the usual range of mineral requirements, as those that are exceptionally tolerant or require particularly large amounts of one or more essential elements would not be reasonable models for all terrestrial systems.

4.3.2 Tolerance and slope factors

A corollary to the concepts of essentiality and adaptation is that of tolerance. While the toxicity threshold (i.e. the soil concentration at which toxic responses begin to occur) may be similar among various species, the ability of organisms to tolerate increasing amounts of metal exposure may differ. This may be due to differences in which processes are disrupted by presence of excess metal, in capacity to regulate uptake of metals from soils, in detoxification mechanisms, or by other physiological processes. Not only will tolerance differ among species, but it will also differ within the same species, depending upon the metal. The degree of tolerance is an important component of hazard classification as it provides a means of assessing whether a small increase in the amount of metal or metallic substance in the environment will result in a large increase in toxic response. Because metals occur naturally in all soils, the release of such substances from anthropogenic sources (e.g. due to a spill) must always be viewed in the context of how much more material will be present. This is most important in site-specific risk assessments where similar-sized spills may increase the metal content at a particular site by less than one to several orders of magnitude, depending upon how much is spilled and the background concentrations already present. Because hazard classification is a generic assessment of potential risk and is not site-specific, a method is required that allows a prediction of whether a small addition of the metallic substance is likely to cause as great a hazard as a large amount. Hazard should be considered greater for a substance for which a small incremental increase would result in a large increase in toxic response than for a substance that is tolerated well by most species.

The classification scheme proposed here uses all the information of the dose–response relationship to generate a hazard classification, relying on the slope factor to provide a measure of tolerance while the $L(E)C_x$ identifies the toxicity threshold. Proposed slope factors are in accordance with those in Fairbrother and Kapustka (1997). Slope-factor categories were developed from a comparative study of earthworm toxicity data in Callahan et al. (1994), soil respiration effects in Shirazi et al. (1984) and phytotoxicity data. This incorporates the arguments that hazards caused by releasing substances to an environment that already contains that material (e.g. metals) is a function of both the sensitivity and the tolerance of the exposed organisms.

4.4 Soil Micro-organisms

The hazard classification scheme proposed here makes reference only to soil invertebrates and terrestrial plants; soil micro-organisms are not included.[3] While there is widespread agreement that soil micro-organisms (particularly bacteria and fungi) play an extremely important ecological role (e.g. carbon cycling and decomposition, nitrogen fixation), there is disagreement on whether or not methods are available for quantifying these processes and on how to interpret the measurement of pollutant effects. This is due to compensation processes where a specific ecological function is continued by different groups of organisms, non-specific assays that cannot differentiate pollutant effects from natural environmental fluctuations or responses, and an incomplete understanding of what constitutes an adverse ecological effect within the microbial community (see "The role of soil microbial tests in ecological risk assessment" in Vol. 5 of *Human and Ecological Risk Assessment* for further details). Hazard of metals and other pollutants to soil microbial communities and processes should be incorporated into substance classification when the state-of-the-science has progressed to the point where meaningful ecological interpretations of measured responses can be made (Fairbrother et al., 1999).

4.5 Cut-off Values

Suggesting appropriate cut-off values for hazard categories is difficult for terrestrial systems, given the lack of standardized data. Plant toxicity studies in the scientific literature range across a wide variety of soil types (i.e. highly variable bioavailability and background concentrations), use different test species grown under variable environmental conditions for different lengths of time, use different methods for mixing and equilibrating metals into soils, and are highly variable in regard to quality of study design. Moreover, nearly all of the reported studies were designed to determine no effect or lowest observable effect levels, rather than EC_x values and dose–response relationships.

Nevertheless, we made a general assessment of plant and invertebrate (primarily earthworm) toxicity for cadmium, copper, nickel, lead and zinc. Information for plants (Table 1) was derived from Kabata-Pendias and Pendias (1992), Aller et al. (1990), McIlveen and Negusanti (1994), Temple and Bisessar (1981), and a comprehensive review of the zinc and copper toxicity literature; information for additional elements was extracted from Kabata-Pendias and Pendias (1992). Invertebrate data (Table 2) were complied for earthworms by Edwards and Bohlen (1996) and include additional information on springtails, isopods and other species for copper and zinc, derived from a comprehensive review of the literature.

The strategy reported in the above mentioned tables represents a wide variety of study designs, soil types and measurement endpoints. However, they provide a relative assessment of comparative hazard among the various elements and give some direction for initial setting of cut-off values. The proposed standard tests would report LC_{50}s, EC_{50}s or

3 For a discussion of the topic, see "Invited Debate/Commentary: The role of soil microbial tests in ecological risk assessment" in *Human and Ecological Risk Assessment* 1999; 5: 657–727.

EC_{20}s from organisms exposed in soils with relatively high bioavailability. The selection of the 50th and 20th percentiles for acute and chronic effects tests, respectively, is consistent with suggestions made by the Nordic countries' proposal, as well as the German, Spanish and ECETOC (Vega et al., 1999). However, use of these values rather than the PNEC or lowest effect levels reported in the literature will likely produce toxicity threshold values within the mid- to high end of the ranges reported above.

Table 1. Trace Element Effects Concentrations (mg/kg) for Plants

Element	Toxic Range	Element	Toxic Range
As	5 – 20	Ni	10 – 300
B	50 – 200	Pb	30 – 1,000
Ba	500	Sb	150
Be	10 – 50	Se	5 – 30
Cd	5 – 30	Sn	60
Co	10 – 20	Ti	50 – 200
Cu	20 – 150	Tl	20
F	1 – 3	V	5 – 10
Hg	0.5 – 1	Zn	100 – 500
Mn	400 – 1,000		

Table 2. Trace Element Effects Concentrations (mg/kg) for Soil Invertebrates

Element	Toxic Range	Element	Toxic Range
Cd	11 – 280	Mn	> 50,000
Co	20	Ni	200 – 400
Cu	110 – 2,000	Pb	36 – 12,000
Cr	> 50,000	Zn	170 – 5,000
Hg	10 – 25		

SECTION 5

Conclusion

A scheme for classifying metals and inorganic metal compounds in regard to their hazard to the terrestrial environment has been proposed, building upon previous discussions, writings and workshops. This approach is compatible with proposed approaches for the classification for the aquatic environment, as well as initial proposals for a terrestrial classification system for organic substances. It also recognizes the need to move forward with classification of effects to the soil environment while methods are developed for hazard determination in above-ground invertebrates, birds and mammals. Metal-specific modifications were included through recommended methods for incorporation of metallic substances into test soils and in suggestions about treatment of fate parameters. The recommendations set forth here are in accordance with current scientific concepts of hazard of metals to the soil ecosystem. Specifically, we recommend:

1. for substances that lack adequate data, short-term and long-term effects should be characterized through the use of standardized toxicity tests using plants and soil invertebrates; studies of long-term effects should simulate ageing of metals in soils, which emulate transformation processes affecting bioavailability;

2. analysis of toxicity should incorporate the critical aspects of point estimates and slope of response; and

3. these data (point estimates and slope relationships) should be considered on a case-by-case basis to establish hazard classification categories such as *very toxic, toxic, harmful* or *not classified*.

SECTION 6

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