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Review of Laboratory-Based Terrestrial Bioaccumulation Assessment Approaches for Organic Chemicals: Current Status and Future Possibilities

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EDITOR'S NOTE:

This paper is 1 of 3 articles resulting from a workshop sponsored by The Health and Environmental Sciences Institute (HESI) held in January 2013 in Miami, Florida, USA. The aim of the workshop was to review current practices, identify data gaps, and provide recommendations to improve current methods and develop new methods supporting both prospective and retrospective environmental assessments of organic chemical bioaccumulation in terrestrial ecosystems.

ABSTRACT

In the last decade, interest has been renewed in approaches for the assessment of the bioaccumulation potential of chemicals, principally driven by the need to evaluate large numbers of chemicals as part of new chemical legislation, while reducing vertebrate test organism use called for in animal welfare legislation. This renewed interest has inspired research activities and advances in bioaccumulation science for neutral organic chemicals in aquatic environments. In January 2013, ILSI Health and Environmental Sciences Institute convened experts to identify the state of the science and existing shortcomings in terrestrial bioaccumulation assessment of neutral organic chemicals. Potential modifications to existing laboratory methods were identified, including areas in which new laboratory approaches or test methods could be developed to address terrestrial bioaccumulation. The utility of "non-ecotoxicity" data (e.g., mammalian laboratory data) was also discussed. The highlights of the workshop discussions are presented along with potential modifications in laboratory approaches and new test guidelines that could be used for assessing the bioaccumulation of chemicals in terrestrial organisms. *Integr Environ Assess Manag* 2016;12:109–122. © 2015 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals, Inc. on behalf of SETAC.

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INTRODUCTION

Recently, interest has been renewed in approaches for the assessment of the bioaccumulation potential of chemicals, principally driven by the need to evaluate large numbers of chemicals as part of new chemical legislation, (e.g., Environment Canada DSL [Environment Canada 2003], REACH [ECHA 2007]). This renewed interest has led to a variety of research activities and advances in bioaccumulation science as exemplified by recent International Life Sciences Institute–Health and Environmental Sciences Institute (ILSI-HESI) (Nichols et al. 2007; Weisbrod et al. 2008; HESI 2011; Burkhard, Cowan-Ellsberry, et al. 2012) and Society of Environmental Toxicology and Chemistry (SETAC) workshops (Klecka and Muir 2008). Although much of this increased activity has focused on bioaccumulation in aquatic systems, increased interest also has been expressed in bioaccumulation assessment in terrestrial systems (Gottardo et al. 2012). This interest includes both the relevance of aquatic bioaccumulation data for predicting bioaccumulation in terrestrial systems and the identification of potential methods for directly evaluating bioaccumulation in terrestrial organisms and humans

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(Swackhamer et al. 2009; Tonnelier et al. 2012). Early research suggested that terrestrial and aquatic bioconcentration were correlated with the same physical chemical properties (e.g., K_{OC}, K_{OW}, water solubility) and that bioconcentration in terrestrial organisms (e.g., cattle, swine) was correlated with bioconcentration in fish (Kenaga 1980). More recent publications by Kelly and Gobas (2000, 2003) raised the potential importance of terrestrial food chain bioaccumulation in sensitive Arctic systems, and Kelly et al. (2007) suggested that aquatic bioaccumulation assessments may not be protective of terrestrial systems because of differences in digestive tract physiology, body temperature, and elimination mechanisms between aquatic and terrestrial vertebrates.

Laboratory studies provide opportunities to limit the number of variables potentially affecting the outcome of bioaccumulation assessment. In addition, laboratory testing facilitates direct manipulation of experimental variables (e.g., soil or sediment organic C concentrations) as well as the development of tiered testing approaches (e.g., screening vs definitive, in vitro vs in vivo, invertebrate vs vertebrate, and so forth). An example of a potential, tiered laboratory assessment scheme for aquatic bioaccumulation assessment is presented in Figure 1. Tiered testing approaches recognize the importance of increasing efficiency in resource utilization, including limiting animal use to comply with animal welfare considerations, such as the 3Rs—Reduce, Refine, Replace (Russell and Burch 1959), while maximizing data gathered and number of chemicals assessed. An example of such an approach applied to the assessment of bioconcentration in fish was presented by de Wolf et al. (2007). Development of similar approaches is no doubt possible for the assessment of bioaccumulation in terrestrial species. Given the number and variety of laboratory tests available for assessing exposure and toxicity to terrestrial organisms, adapting existing toxicity tests with terrestrial plants, invertebrates, and vertebrates (e.g., birds, mice, rats) to provide data relevant for terrestrial bioaccumulation assessments may be possible. Development of integrated testing and intelligent assessment approaches that maximize the potential use of data or expand the data collected during mandated regulatory testing is particularly important given recent animal welfare legislation and concerns, as well as guidance provided in chemical regulations such as REACH (Ahlers et al. 2008; Madden et al. 2012). Recognition of the animal welfare concerns associated with vertebrate toxicity testing makes support for "new" terrestrial bioaccumulation test methods or test guidelines with avian or mammalian species unlikely.

These ideas were discussed during the January 2013 ILSI-HESI Terrestrial Bioaccumulation Workshop. The principal areas of emphasis during Laboratory Assessment workgroup discussions revolved around identification of existing knowledge and data gaps, potential improvements or additional parameters for existing test methods, and identification of new nonvertebrate test methods to fill potential data gaps.

USEFUL PHYSICAL–CHEMICAL AND ENVIRONMENTAL FATE DATA

The potential for exposure of terrestrial (and aquatic) biota to a chemical depends on the chemical's environmental fate, which is determined by its release pattern into the environment, its chemical structure, and its physical and chemical characteristics. A variety of physical–chemical properties of chemicals provide useful insight into their potential for bioaccumulation (Opresko 1996; USEPA 2012a, Supplemental Data Table 1). Concern over the release of a chemical to the environment must take into account the toxicity of the chemical, the amount and mode (continuous, intermittent) of release, the environmental compartment to which the chemical is released (soil, atmosphere, water), and fate processes that may ameliorate the potential for exposure or transform the compound to a more or less toxic form. The important fate processes in terrestrial ecosystems include both abiotic and biotic processes, such as hydrolysis, photolysis, biodegradation, soil adsorption and mobility, volatilization from water or soil, and biodegradation. Various regulatory guidance documents

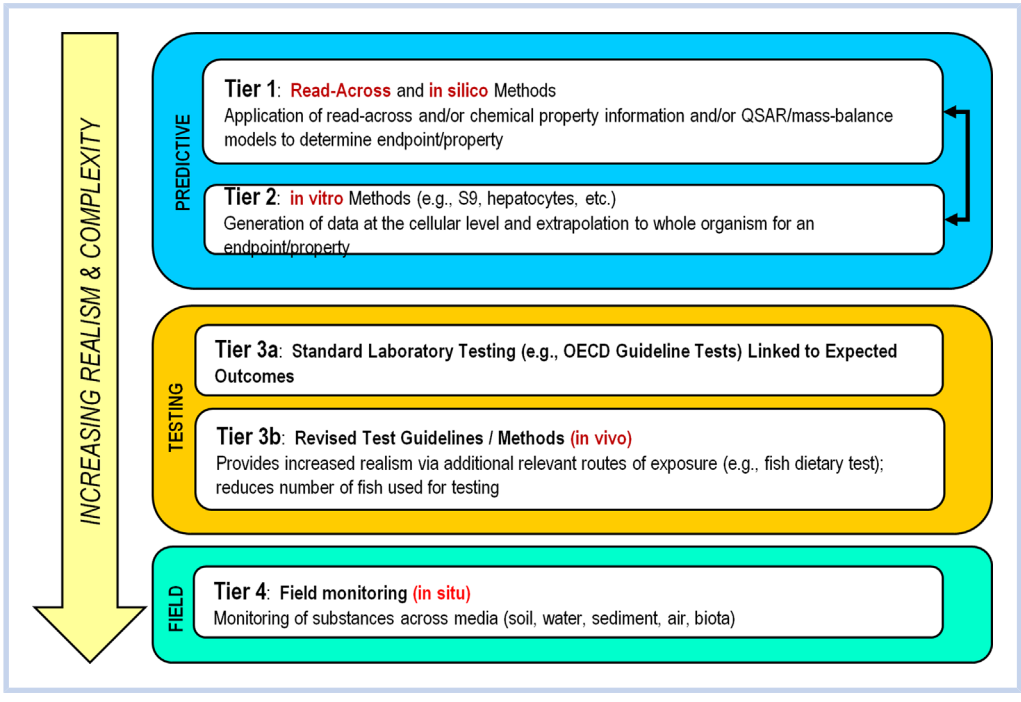

Figure 1. An example tiered framework for aquatic laboratory bioaccumulation assessment.

(Agriculture Canada 1987, http://www.oecd.org/env/ehs/risk-assessment/manualfortheassessmentofchemicals.htm, http://www.epa.gov/pesticides/science/efed/policy_guidance/team_authors/endangered_species_reregistration_workgroup/esa_reporting_fate.pdf) exist to help characterize the fate of substances in the terrestrial environment; these documents typically include information to assist in interpretation of data such as mobility estimates and half-lives of a substance and its transformation products.

BIOAVAILABILITY AND SOIL CHARACTERISTICS

When evaluating the environmental fate of chemicals in terrestrial ecosystems, the bioavailability of the chemical and the effects of soil characteristics are both important considerations in determining the potential for intercompartment transfer as well as the bioaccumulation potential of a chemical. Bioavailability of organic compounds in terrestrial environments is complicated by many factors that will also influence the outcome of bioaccumulation tests. In the work of Semple et al. (2003) and as adapted in recent European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) reports on extraction technique and bioavailability (ECETOC 2013a) and the inclusion of nonextractable residues in risk assessment (ECETOC 2013b), a bioavailable chemical "is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time that is available now (no constraints)." A chemical is thought to be bioaccessible if it "is available to cross an organism's cellular membrane from the environment it inhabits, if the organism has access to the chemical." Thus, the bioavailable fraction is a subset of the bioaccessible fraction (ECETOC 2013b) and is likely the fraction of greatest importance when conducting terrestrial exposure studies.

Inherent properties of natural soils such as pH, fertility, organic matter content, and texture can significantly influence the bioavailability of chemicals. However, the effects of these potential modifying influences can be factored into evaluations of bioavailability through proper soil characterization and experimental design (e.g., testing of multiple soil types). At a minimum, pH and organic matter content of test soils must be measured before use in terrestrial bioaccumulation testing. For plant bioaccumulation testing, soil fertility also must be known, given the important influence of nutrient availability to plant roots. The importance of soil type was demonstrated by Princz et al. (2014) in an earthworm bioaccumulation study in which uptake of the test chemical in tissue of earthworms exposed in a sandy soil was significantly greater than that in earthworms exposed to the chemical in clay loam soil where organic matter and clay content were significantly higher. The choice of test soils must be considered during the experimental design phase to ensure that critical parameters such as soil pH and organic matter content are not outside the tolerance limit of the test organism. In addition, changes in chemical bioavailability over time (i.e., "aging effects"), which are significant in, for example, metals and hydrocarbons, should be factored into the test design (Kraaij et al. 2002; Vickova and Hofman 2012; Sutton et al. 2013).

These concerns raise the issue of what fraction of the chemical, under what conditions and to what extent, is bound (i.e., across a spectrum from reversibly to irreversibly bound). Complexity arises from both the properties of the soil matrix (e.g., organic C content, pH) and the physical chemical properties of the chemical itself. Because a soil particle is composed of both organic matter and mineral surfaces, a wide range of intermolecular interactions, based on chemical structure and surface characteristics (e.g., van der Waals interactions, hydrogen bonding, electrostatic forces [e.g., dipole–dipole], the potential for ligand exchange and ionic and covalent bonding) may either make the chemical available for uptake into an organism or sequester it. Approaches exist that could be used to quantify the freely available concentration of the chemical in terrestrial test systems to facilitate more accurate characterization of exposure. These techniques have been validated mainly in aquatic and sediment systems (Muijs and Jonker 2012; Cui et al. 2013), but several studies also exist showing their applicability to the terrestrial environment (e.g., Maenpaa et al. 2011; Gomez-Eyles et al. 2012). Ultimately, field studies may be necessary to benchmark the accuracy of these estimates of bioavailability.

INDIRECT ASSESSMENT OF TERRESTRIAL BIOACCUMULATION

Aquatic Invertebrate Bioaccumulation Studies—Lumbriculus studies

Lumbriculus variegatus is the most widely used oligochaete for aquatic bioaccumulation tests (Chapman and Wang 2001). Because many oligochaetes ingest sediment with associated contaminants, this makes them particularly appropriate for bioaccumulation studies. Terrestrial oligochaetes (i.e., earthworms) also are routinely used in bioaccumulation testing, such as Organisation for Economic Co-operation and Development (OECD) TG 317 (OECD 2010a) and, in a few cases, studies have been done using both aquatic and terrestrial oligochaetes. Stanley et al. (2010) and Coleman et al. (2010) present laboratory data from both aquatic and terrestrial bioaccumulation of nano-aluminum in oligochaetes. Because ingestion of contaminants via sediment or soil is probably the most relevant bioaccumulation route for both species (Leppannen and Kukkonnen 1998), results from sediment oligochaete bioaccumulation studies normalized to organic C could potentially be used as an indication of the potential for bioaccumulation by terrestrial oligochaetes in terrestrial bioaccumulation assessments. However, a thorough comparison of data for both terrestrial and benthic species has not been performed, and insufficient data may exist for the relevant species with the same chemicals to conduct a meaningful evaluation.

Fish bioconcentration studies

Historically, bioaccumulation assessments have typically focused on the aquatic environment and used aqueous phase exposures with various fish species (e.g., bluegill sunfish, rainbow trout, common carp) as the target organisms for evaluation and the bioconcentration factor (BCF) from water as the evaluation endpoint, such as US Environmental Protection Agency (USEPA) 850.1730 (USEPA 1996a) and OECD TG 305 (OECD 2012). In this paradigm, exposure via the aqueous phase with partitioning into the lipid phase is the dominant mechanism for neutral organic chemical bioconcentration in fish. However, other potential routes of exposure for aquatic organisms (e.g., dietary; see later discussion) and types of chemicals (e.g., ionogenic and high log K_{OW} organic chemicals) may not be adequately addressed in this paradigm. Aquatic bioconcentration studies may not be appropriate for predicting potential bioaccumulation in terrestrial organisms

and ecosystems where dietary exposure, in conjunction with some limited potential for dermal exposure, predominates and different elimination mechanisms are operative (Kelly et al. 2007). However, Mackay et al. (2013) present a compelling argument for the importance and relevance of the BCF value, particularly the kinetic BCF value, BCF_k, as a principal determinant of chemical concentrations in aquatic food webs. Mackay et al. (2013) also highlight what is arguably the most important aspect of any bioaccumulation assessment: "Ultimately, however, it is the absolute concentrations, not their ratios, that are of concern from an exposure and risk assessment perspective." The concerns highlighted by Kelly et al. (2007) do not entirely negate the potential use of aquatic bioconcentration metrics for predicting bioaccumulation in terrestrial food webs but do suggest that chemicals with both a log K_{OA} of 10^6 or higher and a log K_{OW} greater than 10^2 may represent a class of chemicals for which traditional extrapolation of aquatic bioaccumulation metrics to terrestrial organisms is not appropriate, although relatively limited empirical data currently exist to support this hypothesis.

Potential Additional Parameters in Existing Guideline Studies with Fish

Fish early life stage studies, such as USEPA 850.1400 (USEPA 1996b) and OECD TG 210 (OECD 2013) and the USEPA OPPTS 890.1350 fish short-term reproduction assay (USEPA 2009) are commonly conducted studies for chemical registration purposes. These studies are conducted for 21 to 28 d, which is similar to the typical uptake phase duration in the OECD TG 305 study. Therefore, these studies provide an opportunity to collect tissue residue measurements consistent with the screening approach proposed in the revised OECD TG 305. With the same caveats already mentioned relative to the use of the BCF metric, these data may help inform the potential for chemical uptake by aquatic and possibly terrestrial organisms. In addition, an approach combining residue and toxicity data provides a unique opportunity to link biological responses (e.g., growth, reproduction) with body burden data in a tissue-residue-toxicity evaluation.

DIRECT ASSESSMENT OF TERRESTRIAL BIOACCUMULATION

Plants

Currently, no standardized test protocols are specifically designed to investigate the bioaccumulation of a chemical in plants, although some residue studies are designed to address livestock and human safety. A number of standardized test guidelines used to determine chemical residues in plants and toxicity to plants could potentially be used to screen or assess bioaccumulation in plants, but they would require significant modifications to meet this goal.

Potential additional parameters in existing plant test guidelines

A large number of studies present in the literature have examined bioaccumulation of organic chemicals in terrestrial plants. The general approach has been to measure either root or foliar uptake, depending on the properties of the chemical or the most relevant route of exposure. Therefore, a number of different metrics have been proposed to assess bioaccumulation in plants (Table 1). The data requirements to determine these different metrics vary; consequently, experiment design can vary widely as well.

The value of a single metric (e.g., bioaccumulation factor [BAF], BCF, biota–soil accumulation factor) has become an important parameter for regulatory agencies in the determination of whether a chemical should be deemed bioaccumulative (CEPA 1999; UNEP 2006; ECHA 2007). Currently, no standardized test guidelines are specifically designed to develop bioaccumulation metrics (e.g., BCF, BAF) in plants. For simplicity in the discussion that follows, the term BAF will be used as a surrogate to represent all potential measures of bioaccumulation that have been used with plants (Table 1).

Plant uptake, translocation, and metabolism testing. A number of existing test guidelines that address plant uptake, translocation, and metabolism of chemicals (OECD 2007; USEPA 2012b) could provide data useful in determining whether a chemical accumulates in plants. The USEPA test guideline (2012b) outlines procedures for conducting a mass balance study of the distribution of a chemical in environmental matrices and different components of the plant under root or foliar exposure for use in determining human and livestock food safety. Although these guidelines were not specifically designed to assess bioaccumulation in plants, they do evaluate the ability of pesticides to be taken up by and translocate throughout plants, using a maximum exposure scenario, or characterize metabolic or degradation pathways to identify residues of concern. The data collected could allow for the calculation of a bioaccumulation metric(s) based on the ratio of the concentration of the chemical in the plant relative to the concentration in the relevant environmental matrices. During the conducting of the test, the method of exposure (i.e., spraying, dusting, biosolids-amended soil, soil spiking), route of exposure (i.e., leaf and/or root), quantification of exposure, and characteristics of plant growth matrices would need to be considered carefully for the determination of a realistic bioaccumulation metric.

The OECD and the USEPA have developed a number of test guidelines to assess chemical residues in crop species (Supplemental Data Table 2). The purpose of these guidelines is to identify the concentration of parent test chemical and metabolites present in the portion of the crop that is to be consumed by humans or livestock. These guidelines provide quantitative exposure estimates (residue levels) in food commodities to inform human health dietary risk assessments and support enforcement activities. Regulatory agencies typically require that the type and number of field trials are representative of the crop and the region where the agricultural chemical is to be applied. The field trials attempt to account for the variability in results among field trials by selecting more than 1 test site. This facilitates the evaluation of the combined effects of such factors as soil type, weather, and regional cultural practices. The current guidelines, however, do not provide a ratio of concentrations between soil and whole plants so that a bioaccumulation metric can be reported. If the suggested considerations for bioaccumulation were addressed (Supplemental Data Table 2), a study following these guidelines could provide data useful in determining a BAF value based on the ratio of test chemical in the plant tissue relative to the growth substrate at the time of sampling.

The OECD, USEPA, Environment Canada, and other regulatory agencies have designed a number of tests to assess the effect of chemicals on terrestrial plants (Supplemental

Table 1. Examples of metrics used to quantify bioaccumulation in plants

Bioaccumulation metric	Formula
Root uptake pathway	
Transpiration stream concentration factor (TSCF)	Concentration in xylem sap (mg/L) Concentration in exposure solution (mg/L)[a]
Below ground tissue concentration factor: Root concentration factor (RCF) Tuber concentration factor (TCF)	 Concentration in specific tissue (mg/kg) Concentration in soil (mg/kg)[b] or exposure solution (mg/L)
Aboveground tissue concentration factor (leaf, fruit, stem, grain, seed, hull)	Concentration in specific tissue (mg/kg) Concentration in soil (mg/kg) or exposure solution (mg/L)
Aboveground (foliar or shoot) concentration factor (AGCF) or	Concentration aboveground tissues (mg/kg)
Plant uptake factor (PUF)	Concentration in soil (mg/kg) or exposure solution (mg/L)
Whole plant concentration factor (WPCF)	Concentration in whole plant materials (mg/kg) Concentration in soil (mg/kg) or exposure solution (mg/L)
Atmospheric Uptake Pathway	
Specific tissue concentration factor (leaf, cuticle, fruit, stem, grain, seed, hull)	Concentration in specific tissue (mg/kg) Concentration in air (mg/L) or particulate matter (mg/kg)
Aboveground (foliar or shoot) concentrations factor (AGCF) or	Concentration in aboveground tissues (mg/kg)
Plant uptake factor (PUF)	Concentration in air (mg/L) or particulate matter (mg/kg)

[a]Exposure solution = hydroponic solution (measured) or soil solution (calculated or estimated).
[b]Concentration in soils or tissues typically expressed on wet or dry basis.

Data Table 3). Modifications to these methods could provide information on chemical bioaccumulation in the plants used in the tests. The considerations indicated relative to the plant uptake and residue identification test guidelines mentioned previously also apply for the following plant toxicity test guidelines. The selection of appropriate treatment group(s) to assess bioaccumulation is an additional consideration for plant toxicity tests. Higher exposures of a test chemical may have adverse effects on plant physiology, causing a reduction in uptake and growth, both of which are important factors in the evaluation of potential bioaccumulation.

The residue and toxicity test guidelines mentioned previously were not designed to assess bioaccumulation in plants. None of the guidelines, even with the modifications suggested, provide the data necessary to determine a definitive steady state or kinetic BAF value. Therefore, even with the suggested considerations (Supplemental Data Tables 2, 3), these protocols will not provide a robust, standard method for the assessment of bioaccumulation in plants. Based on this observation, a clear need exists for the development of a regulatory test method that addresses potential bioaccumulation in plants.

Development of a plant bioaccumulation test method

A number of variables would need to be considered during the development of a method to assess bioaccumulation in plants, such as growth dilution, the fact that plants continue to increase in size over the course of their life cycle. Therefore, significant dilution of the test chemical may be observed because of growth of the plant during the test (Li et al. 2005; Collins et al. 2006). The amount of dilution will be influenced by the growth stage at which the plant is exposed and when tissue is sampled for analysis (Schwab et al. 1998; Collins et al. 2006). If the route of exposure is through the roots, then the choice of growth media becomes important. Growing plants hydroponically may allow for simplified uptake and elimination phase logistics. However, growing plants hydroponically does not represent an environmentally relevant mode of exposure. The bioavailability of the test chemical in nutrient solution, and therefore the ability of the chemical to bioaccumulate, can vary significantly compared with a natural growth substrate (Karnjanapiboonwong et al. 2011). If the plant is grown in soil, then the composition of soil and the number of soils to be used in the test need to be considered. Bioaccumulation in plants has been shown to vary with the texture, pH, and organic C content of soil; therefore, these factors need to be considered when choosing the type and number of test soils (Semple et al. 2003; Sjostrom et al. 2008; Karnjanapiboonwong et al. 2011; Hollings et al. 2012; Jakob et al. 2012; Smidova et al. 2012; Vickova and Hofman 2012). Exposure of the plant through the roots allows for the collection of data necessary to calculate a steady-state or kinetic BAF value. However, methods to calculate a BAF value for other routes of exposure (e.g., foliar exposure through liquid or gas) are not as well established and require further investigation (Bacci et al. 1990; McLachlan 1999; Collins et al. 2006). Bioaccumulation has been shown to vary across plant species (Huelster et al. 1994; Otani et al. 2007; Inui et al. 2008). For example, species from the Cucurbitaceae family have been shown to accumulate certain organic chemicals at higher levels than plants from other families (Huelster et al. 1994; Otani et al. 2007). Variation in accumulation has also been observed between cultivars of a plant species (Inui et al. 2008). Therefore, the choice of plant species and cultivar for the test becomes another important factor for consideration. The bioaccumulation of test chemical has been shown to vary among different tissues in the plant (Schroll et al. 1994; Aryal and Reinhold 2011; Tanoue et al. 2012). Consequently, a

standardized test needs to clearly define which tissues need to be sampled for analysis. Sampling and analysis of only the commodity portion of the plant to be consumed by humans or livestock limits the ability of the test to characterize possible exposure to other types of organisms (e.g., phytophagous invertebrates, birds, bees).

INVERTEBRATES

Terrestrial oligochaetes such as earthworms and potworms (enchytraeids) play an important role in soil structure development and microbial community function. As prey in the natural world, earthworms and potworms are eaten by other soil organisms (e.g., predatory mites and beetles) and vertebrate predators (e.g., voles, foxes, birds), which could lead to secondary poisoning in the terrestrial food web. Earthworm bioaccumulation tests add information on bioavailability and mobility of specific chemicals from soil to soil-dwelling organisms and provide data useful for assessing the potential for chemical transfer to highertrophic-level organisms.

The only existing regulatory test guideline for evaluation of bioaccumulation in terrestrial oligochaetes (i.e., earthworms) is OECD TG 317 (OECD 2010a). The test guideline consists of 2 parts: an uptake exposure phase in chemical-amended soil and the postexposure elimination phase in clean soil. The principal test endpoint is the steady-state bioaccumulation factor (BAF_{ss}). If steady state is not achieved, the kinetic BAF (BAF_k) is calculated based on the ratio of the uptake rate constant (K_u) and the elimination rate constant (K_e). The biota–soil accumulation factor also maybe calculated when the objective is to compare the results with other bioaccumulation tests with the same chemical because this endpoint is normalized for worm lipid content and soil organic C. Additional test guidance or requirements such as chemical amendment procedure, solvent usage, test organism health, feeding, density, minimum replication, soil and tissue sample storage, and example schedules for key test steps are provided in the test guideline (OECD 2010a).

The test is applicable to stable neutral organic chemicals, metallo-organics, metals, and other trace elements. Recent testing with an ionogenic organic chemical proved challenging for test endpoint determination because the chemical was highly water-soluble, had low lipophilicity, and did not depurate from the earthworm tissue during the elimination phase of the test (Princz et al. 2014). These results suggest the need for additional research on the use of the earthworm bioaccumulation test guideline or other bioaccumulation tests when evaluating the potential bioaccumulation of ionogenic compounds. Additional details on the development of the oligochaete bioaccumulation test guideline are provided in the Supplemental Data.

Potential additional parameters in existing invertebrate studies

Collection of additional parameters relevant to bioaccumulation is possible as part of the OECD TG 222 earthworm reproduction test (OECD 2004a) as demonstrated in Kinney et al. (2012). In this test guideline, earthworms are exposed for 28 d to the test chemical spiked into soil. After 28 d the adult worms are removed and assessed for mortality and growth (wet weight). At this point, assuming relevant analytical methods are available, the concentration of the test chemical in the adult worms could be determined to give an indication of uptake into the organism. The test concentration chosen for bioaccumulation assessment would be one in which no adverse effects on growth or reproduction are observed because test concentrations that are toxic may affect chemical uptake by the earthworms. Ideally, in any test replicate used for assessing bioaccumulation, mortality should be less than 10%, and body weight loss should be less than 20% over the 28-d exposure period (i.e., the test acceptance criteria for control performance). The additional tissue residue endpoint only measures uptake at test termination and does not consider any elimination of the chemical. Although the data from such a study should be interpreted with caution, the test could provide valuable screening information on chemical accumulation that could be used either to rule out bioaccumulation or as a rangefinder for more specific testing for bioaccumulation testing following OECD TG 317. This approach also could be used for other invertebrate species, such as dung beetles (OECD GD 122, 21-d larval survival test [OECD 2010b]), dung flies (OECD 228, developmental test with dipteran flies [OECD 2008]), or the collembollan OECD 232, reproduction test (OECD 2009; Schmidt et al. 2013), depending on the expected route of exposure. However, particularly for the collembolla, the small size of the test species (i.e., limited biomass) may present a technical challenge in terms of measuring tissue residues.

Although designed mainly for risk assessment of plant protection products, comprehensive guidance exists for considering adverse effects on honeybees in both laboratory and field-based experiments (EPPO 2010a). Guidance exists for when residue analysis of flowers, pollen, or nectar should be conducted as part of a field study depending on the specific properties of a chemical and existing data, for example, on plants (EPPO 2010b). Measurement of actual honeybee body burdens could therefore be a realistic additional parameter in such studies. Alternatively, more specific data on bioaccumulation in the honeybee via the dietary route might be obtained by using extended acute studies assuming relevant nontoxic test concentrations were included in the test design (OECD 1998). In principle, this approach could be applied to dietary exposure of other terrestrial invertebrates.

A potential route of exposure also exists from aquatic sediments into the terrestrial environment via emerging insects (Menzie 1980). Mayflies (*Hexagenia* spp.) have been shown to bioamplify polychlorinated biphenyls within their bodies during the process of emergence (Daley et al. 2011). Chironomids also have been shown to be an important route of transfer of contaminants, including polychlorinated biphenyls, with subsequent accumulation in terrestrial species such as tree swallows (Maul et al. 2006; Alberts et al. 2013) and birds that feed on terrestrial spiders (Walters et al. 2010). The importance of the transfer of contaminants from aquatic systems to terrestrial systems has been discussed in Sullivan and Rodewald (2012). Bioaccumulation via emerging chironomids could be assessed in the laboratory by additional body burden measurements of adult chironomids as they emerge during the current OECD TG 218 and TG 219 chironomid emergence tests (OECD 2004b, 2004c). This approach also may be used to rule out this pathway of bioaccumulation for some chemicals, as was the case for fluoranthene, for which the emergence process in chironomids was shown to be a route of chemical elimination (Bell et al. 2004). Similar measurements also could be considered as additional parameters for inclusion in the OECD TG 233

chironomid life cycle test (OECD 2010c) with appropriate test design modifications.

VERTEBRATES

Physiologically based toxicokinetic modeling

Physiologically based toxicokinetic (PBTK) models are increasingly being used as an effective tool in ecological risk assessments (Krishnan and Peyret 2009). Physiologically based toxicokinetic models are computational tools used to simulate the adsorption, distribution, metabolism, and excretion of chemicals in the body based on critical relationships between the chemical and the physiology of the species of concern. Specifically, the models predict the concentrations of chemicals over time in various body compartments such as blood, urine, liver, kidney, and fat, assuming specified intake amounts from diet, transdermal, or inhalation exposures. If the tissue-specific target dose for adverse effects (toxicants) or efficacy (pharmaceuticals) is known, the models also can be used to calculate species-specific effective environmental exposure concentrations (the concentrations at which the adverse or desired effects are likely to occur). Because PBTK models integrate internal concentrations over time for the various body compartments, they also are useful in predicting which chemicals may bioaccumulate either in the whole body or in specific tissue compartments.

Data hungry and computationally intensive, applications of PBTK models have, until recently, been limited to standard laboratory species (e.g., rats). Model input parameters include rate constants for the transfer of chemicals across body compartments and the volumes of the body compartments (e.g., blood volume). Because chemicals move around the body via blood flow, blood–tissue partition coefficients and the amount and rate of blood flow are critical input variables. Similarly, metabolism rates regulate the amount of the chemical available for transfer as well as the formation of metabolites that also may have toxicological properties. Elimination rates play a very important role in regulating the bioaccumulation of all chemicals, including metals and other nonlipophilic chemicals (Luoma and Rainbow 2005).

Physiologically based toxicokinetic modeling begins with a conceptual model of the species of interest and routes of exposure. Available data on the uptake, excretion, metabolism, and physicochemical properties of the chemical are evaluated and described by equations for each adsorption, distribution, metabolism, and excretion component. This includes the portal(s) of entry (e.g., inhalation→lungs; oral→gastrointestinal tract; dermal →skin) and target organs for parent compound and metabolites (e.g., liver) and excretory organs (e.g., kidney). For lipophilic chemicals, fat is shown as a separate compartment. A final compartment, called "rest of body," is included to facilitate the mass balance calculations. The physiological parameters required to parameterize the models include breathing rate, skin surface area, cardiac output, tissue blood flow rates, and tissue volumes. These can be found in the literature for some terrestrial species (see, for example, Mitruka and Rawnley 1977; Arms and Travis 1988; Brown et al. 1997; Krishnan and Peyret 2009). Partition coefficients (e.g., tissue–blood) may be obtained at steady state in repeat-dosing studies (Gallo et al. 1987); in vitro measurements using ultrafiltration, equilibrium dialysis, or headspace equilibrium technique (Sato and Nakajima 1979; Lin et al. 1982; Gargas et al. 1989); or from Quantitative Structure Activity Relationship modeling based on molecular and biological determinants (e.g., DeJongh et al. 1997; Payne and Kenny 2002; B'eliveau et al. 2003). However, estimating these metabolic constants continues to be a limiting step in developing PBTK models for many species of concern. Software can be used to solve the ordinary differential equations constituting the models (e.g., Krishnan and Andersen 2007; Krishnan and Peyret 2009) and the models should be validated by comparing the model simulations with experimental results.

Toxicokinetics studies

Contrary to the aquatic environment, for which a test guideline to determine dietary bioaccumulation potential in fish has recently been published as part of the revised OECD TG 305 (OECD 2012), no standard vertebrate test guidelines are available to specifically determine the potential for dietary bioaccumulation or biomagnification in terrestrial organisms. Data from rodent toxicokinetics studies, however, are available in the literature for pesticides, cosmetics, and pharmaceuticals, as well as some industrial chemicals and well-studied environmental pollutants (e.g., Yokel and McNamara 2001; Kudo and Kawashima 2003). These studies include a wide range of parameters that could provide useful empirical information on the assimilation, metabolism, and excretion of chemicals in nonaquatic environments and provide data to inform the PBTK modeling approaches discussed previously and in the modeling chapter from the HESI terrestrial bioaccumulation workshop (Gobas et al. this issue). Currently, toxicokinetics studies are used mainly in human health assessments, but they could provide useful information to help determine the potential for bioaccumulation in terrestrial food chains in a manner similar to the approach used for data from dietary fish studies (Weisbrod et al. 2009). Caution is warranted, however, because toxicokinetics studies are complex, and the parameters derived from them must be properly evaluated before conducting any comparison of aquatic and terrestrial bioaccumulation potential for a substance.

Basic toxicokinetic parameters, including elimination half-lives from various matrices and whole body total elimination half-lives, can be derived from mammalian toxicokinetics studies conducted according to OECD TG 417 (OECD 2010d). In addition, a supplemental in vitro component to OECD TG 417 can be conducted to obtain an enzymatic kinetic half-life value (K_m: Michaelis-Menten). Although this addresses enzymatic biotransformation only, these tests are cheaper and use fewer animals and are typically used in screening-level assessments. However, enzymatic transformation is only a component of overall elimination and thus does not represent a comprehensive assessment of potential elimination mechanisms.

The whole body elimination half-lives obtained as discussed previously can be used to determine whether the half-life is sufficiently short to permit elimination of the chemical or active metabolites from the body between exposures (Goss et al. 2013). The whole body and primary biotransformation half-lives are key parameters determining the extent of bioaccumulation, biological concentration, and risk from chemical exposure (Arnot et al. 2014). The relationship between whole body and biotransformation half-lives in mammals is examined in Arnot et al. (2014).

Several differences exist between the OECD TG 305 fish dietary bioaccumulation protocol (OECD 2012) and the

OECD TG 417 toxicokinetics protocol (OECD 2010d) that must be considered when using toxicokinetics data in characterizations of terrestrial bioaccumulation potential. Steady-state considerations and first-order kinetics, which are assumed in fish dietary studies, are not necessarily assured in toxicokinetics studies. Whole body measurements are typically used in dietary fish studies. In toxicokinetics studies, the exposed organism is compartmentalized to assess distribution of the chemical in the different organs, and concentrations in blood or plasma are measured over time to determine toxicant assimilation. The dynamic equilibrium between the blood and the (fast or slow equilibrating) organs defines the distribution of the chemical, and this distribution will change depending on the elimination rate. The elimination kinetics of a chemical also will change over time depending on the relationship between chemical distribution in the different organs and elimination. All of these parameters are measured in toxicokinetics studies, allowing for careful considerations of substance distribution and changes in elimination to evaluate bioaccumulation potential in the relevant tissues. However, the amount of information available from these studies makes it difficult to establish a single relevant bioaccumulation metric to be extracted from these studies.

However, some of the parameters obtained from such a study (Supplemental Data Table 4), such as the assimilation efficiency and elimination half-life, can be used to estimate the potential for a chemical to accumulate in the relevant terrestrial organism. The elimination rate for a chemical has recently been proposed as a relevant parameter to help characterize bioaccumulation potential in both aquatic and terrestrial organisms (Goss et al. 2013). If already available from toxicokinetics studies, the use of elimination half-life combined with the absorption of a chemical by the test organism may be used to determine the potential for bioaccumulation in an organism. Here, we use the examples of pyrene, fluoranthene, and benz[*a*]anthracene, for which data are available both from toxicokinetics studies (Lipniak and Brandys 1993; Viau et al. 1999) and fish dietary studies (EMBSI 2005, 2007) as a test of this hypothesis (Table 2). Polycyclic aromatic hydrocarbons (PAHs) are known not to accumulate in higher trophic levels of the aquatic food web because they are easily metabolized by fish (Wan et al. 2007). The comparison between dietary and toxicokinetics elimination half-lives for these substances shows that the half-lives for the 3 PAHs in the organisms were very low, even with the differences in metabolism rates between fish and rat. The elimination half-life for the 3 compounds in the rat ranged between 0.72 h (pyrene) and 1.7 h (fluoranthene), whereas in fish dietary studies the elimination half-life ranged from 9.6 h (fluoranthene) to 14.4 h (pyrene and benz[*a*]anthracene). A second toxicokinetics study on pyrene (Viau et al. 1999) demonstrated an elimination half-life of 4 h in fatty tissues that are considered to be the relevant tissue for bioaccumulation of nonpolar organic chemicals such as pyrene. These elimination half-lives are much lower than the 70 d proposed by Goss et al. (2013) as the threshold for bioaccumulation assessment in humans, as is expected for easily metabolized PAHs.

Based on the example, parameters obtained in toxicokinetics studies can provide valuable information to help assess the potential for bioaccumulation and dietary transfer in terrestrial organisms. This information could be used in both screening and weight of evidence approaches, which are relevant for persistence, bioaccumulation and toxicity (PBT) assessments, under various regulatory frameworks. However, a thorough analysis of available toxicokinetics data needs to be undertaken before threshold values for bioaccumulation assessment can be established for some of these parameters.

Mammalian studies and application to bioaccumulation screening

During the research and development process for chemicals, mammalian toxicity (e.g., rat, mouse, dog) studies may be conducted that could be useful in the bioaccumulation screening process. In vitro studies may be conducted to evaluate bioavailability and metabolism. In particular, liver microsomal and S9 fractions, as well as hepatocytes, are often used to assess metabolic stability. Derivation of metabolic rate constants and relevant metabolites are typical end-products from these types of studies. However, in vitro approaches have limitations because they do not adequately reflect the integration of metabolic pathways and physiological processes that is incorporated into in vivo studies. In vivo studies ranging in duration from days (i.e., acute lethality studies) to 2 y (i.e., rodent carcinogenicity studies) often measure internal dose levels, as well as the amount of the chemical excreted. Internal dosimetry analysis can focus on specific tissues as well as circulating plasma concentrations. Relevant metabolite levels also can be measured, which enables scientists to better understand metabolism of the parent chemical. While these data are collected in laboratory mammalian test species, efforts have been made to "read-across" these types of data to other species such as fish (Huggett et al. 2003).

When considering vertebrate testing and the "3Rs," data from laboratory mammals may be particularly useful in the bioaccumulation screening process and reduction in terrestrial vertebrate testing. For instance, if a chemical is found to not be absorbed appreciably in a rodent model, then the chemical may not be absorbed in a bird model. Detailed comparisons need to be conducted before the scientific community relies too heavily on the laboratory mammalian data sets, but these data can provide a first glimpse at potential bioaccumulation issues (or the lack thereof) for terrestrial vertebrates.

Agricultural livestock studies

The OECD TG 503 Metabolism in Livestock methodology is designed to assess both qualitative and quantitative

Table 2. PAH data from mammalian toxicokinetics and fish dietary studies

Substance	Dose (rat) mg/kg	Dose (fish) mg/kg	Half-life (rat) h	Half-life (fish) h	References
Fluoranthene	20	100	1.7	14.4	Lipniak and Brandys 1993; EMBSI 2007
Pyrene	20/10	75	0.72/4	9.6	Lipniak and Brandys 1993; Viau et al. 1999; EMBSI 2005
Benz[*a*]anthracene	20	100	0.76	14.4	Lipniak and Brandys 1993; EMBSI 2005

metabolism or degradation of a chemical (OECD 2007). This test guideline is generally used for pesticide assessment when the chemical is directly applied to livestock, used in feedstuffs, or for livestock facilities treatments. Tests are generally performed using radiolabeled compound on either poultry (laying hens) or ruminants (lactating goats), and to provide estimates of total residues in excreta and edible tissues such as meat, milk, and eggs. In addition, the test provides information on the relevant metabolic pathways as well as the terminal residues in the edible tissues. For these studies, the lowest dose level is defined as the level of exposure expected from feeding test animals treated crops with the highest observed residue levels. These studies provide data that could be used in either screening or weight of evidence assessments of bioaccumulation in terrestrial vertebrates.

Avian studies

No existing regulatory test guidelines directly address bioaccumulation in avian species. However, a number of existing regulatory test guidelines evaluate toxicity to avian species, including a 14-d, single-oral-dose acute toxicity study, a 5-d dietary acute toxicity study, and an approximately 4-month-long USEPA, OCSPP 850.2300 avian reproduction study (USEPA 2012c) based on dietary exposure. Numerous experimental and test species considerations make the acute studies impractical for use for bioaccumulation assessment. However, the chronic reproduction study design with mallard ducks, northern bobwhite quail, or Japanese quail is potentially amenable to adaptation for use in assessing bioaccumulation in avian species. Regulatory agencies have the flexibility to require that additional endpoints and measurements be added to protocols to address the specific concerns of a particular substance, for example, as part of Consent Decrees in the United States. In the case of a substance that is potentially bioaccumulative, additional endpoints or measurements to address the potential for bioaccumulation could be required as an add-on to an already existing chronic study (such as the avian reproduction study).

Two recent avian reproduction studies that included additional tissue residue measurements useful for the assessment of bioaccumulation were published by Newsted et al. (2007, 2008). In the 2007 study, adult mallard ducks and northern bobwhite quail were exposed to perfluorooctane sulfonate (PFOS) in the diet for up to 21 weeks. Endpoints not typically included in an avian reproduction study included measurement of PFOS in: 1) red blood cell and serum samples from whole blood samples collected over the course of the study, 2) blood and liver samples from the surviving adults of both species at test termination, and 3) 14-d-old chicks hatched from eggs laid during the study. Pooled egg yolk samples also were collected for measurement of PFOS concentrations in multiple fractions of the egg yolk. This study demonstrated that bobwhite quail were more sensitive to PFOS than mallard duck and that both species bioaccumulate PFOS from the diet. It was also possible to calculate preliminary kinetic parameters (serum steady-state concentration, uptake rate, elimination rate, and half-life)–based serum measurements. Newsted et al. (2008) reported on the results of a bobwhite quail reproduction study with perfluorobutane sulfonate (PFBS) dosed in feed for up to 21 weeks. Concentrations of PFBS in blood serum, liver, and eggs were determined to be dose dependent but were less than the concentration of PFBS in the diet, demonstrating biodilution.

Avian dietary reproductive studies represent an opportunity for evaluating the bioaccumulation potential of chemicals of concern in terrestrial food webs. The full utility of the test for this purpose may require alterations to existing test guidance as well as an expansion of the endpoints evaluated during the test. As an example, given that fish bioconcentration study guidance suggests using test concentrations that are between 0.1% and 1% of the 96-h acute 50% lethal concentration value, similar guidance on relevant concentrations may be required for the avian study. As is the case for studies that may evaluate chemical concentrations in individual tissues or organs of other organisms, a need exists to identify the most appropriate avian tissues for analysis and then develop appropriate bioaccumulation metrics for the evaluation of those tissue or organ concentrations. Chemical concentrations in individual tissues or organs may best be used for the development of bioaccumulation metrics if they can be directly linked to adverse toxicological outcomes in those same tissues or organs. A need also exists to evaluate approaches for integrating chemical concentrations in multiple tissues into an integrated whole-body bioaccumulation metric, because this metric may arguably be more important, based on organism feeding strategies, for trophic magnification factor development.

HIGHER-TIER STUDIES

Terrestrial mesocosm studies

The Terrestrial Model Ecosystem (TME) testing system uses intact soil cores with natural invertebrate and microbial communities under controlled environmental conditions or under field conditions. This intact soil core test system is known as a semi-field method that attempts to simulate the processes and interactions of components in a portion of the terrestrial environment exposed to chemicals such as pesticides. They are designed in such a way that the advantages of laboratory studies are combined with the advantages of field studies. Terrestrial mesocosm studies can provide a large array of assessment and measurement endpoints, depending on the study objectives and design. The test measures both fate and effect endpoints with a combination of structural and functional measurements of the terrestrial compartment as well as residue chemistry of the test chemical (Knacker et al. 2004; Weyers et al. 2004; Jänsch et al. 2006).

Although generation of bioaccumulation data is not a primary endpoint of this higher-tier test system, measurement of terrestrial bioaccumulation in exposed invertebrates and plants may be possible but would require some adaptation of the test design and endpoint estimates. Although the TME test system has been validated through an international ring test and TME results have been compared with data from full field scale trials with the same chemicals (principally pesticides), the standardized methodology was prepared as technical guidance published as part of a SETAC Europe workshop report (Schäffer et al. 2007) and not as a regulatory test guideline. As of January 2013, the OECD test guideline working group is considering a proposal from Germany to start the development of a TME test guideline for assessment of the fate and effects of chemicals.

Terrestrial field dissipation studies

In principle, higher-tier studies, such as field studies, have greater environmental relevance (Boethling et al. 2009). Laboratory studies are often used to inform study methods

for field studies, and the results obtained from field studies should be adequately explained and supported by the results of laboratory studies. Field studies are needed to substantiate the physical–chemical properties, mobility, and biotransformation data from laboratory studies (Agriculture Canada 1987).

Data on terrestrial field dissipation and/or accumulation are required by regulatory agencies worldwide for the registration of pest control products. The objective is to determine the fate and behavior and environmental exposures for a chemical when it is used according to label directions in representative use areas and when all environmental fate and transport processes are acting together. In the European Union, the objective is to determine the overall environmental degradation half-life (DegT50) and persistence under conditions in which surface losses of the chemical are minimized. The proposed methodology for these studies is based on a conceptual model and modular approach. Pesticide properties and laboratory data are used to develop a conceptual model of environmental behavior and fate, and field study design is based on the identified concerns. Guidance is provided on a basic study design to determine persistence, residue carryover, transformation, leaching, formation and decline of transformation products, and routes of dissipation. European-specific requirements for persistence and DegT50 under minimal surface losses (by incorporating test material into soil) are also covered in this basic study guidance. In addition, guidance is provided on modules to determine volatilization, surface runoff, leaching to groundwater, plant uptake, and so forth, as triggered by the conceptual model along with a detailed description of experimental layout, including region, site, and soil characterization, field design, sampling, analysis, and reporting of results.

These field studies are useful for characterizing behavior under field conditions (i.e., determining mobility, persistence, transformation, and dissipation, which can then be incorporated into exposure assessments). The plant-uptake module can provide information on the accumulation of a test substances and its transformation products from soil. However, the scope, complexity, and cost of these studies likely limits their routine application for the assessment of terrestrial bioaccumulation for substances other than pesticides.

CONCLUSIONS AND RESEARCH RECOMMENDATIONS

The goals of this manuscript were to 1) discuss existing knowledge and data gaps pertinent to the laboratory assessment of terrestrial bioaccumulation; 2) identify potential improvements or additional parameters that could be incorporated into existing test guidelines to facilitate assessments of potential bioaccumulation in terrestrial organisms; and 3) identify new nonvertebrate bioaccumulation test methods to fill identified data gaps. Renewed interest in bioaccumulation in terrestrial organisms and food chains is being driven by a perceived inadequacy of aquatic assessments and the need to evaluate large numbers of chemicals as part of recent chemical legislation. The overarching issue is whether assessment of bioaccumulation potential in aquatic organisms can be extrapolated to protect terrestrial organisms and ecosystems. The principal support for this hypothesis is derived from studies by Kelly and Gobas (2000, 2003) and Kelly et al. (2007) that evaluated historical persistent organic pollutant compounds in primarily an Arctic lichen–caribou–wolf food chain. However, relatively few additional empirical data support this hypothesis beyond those found in the publications cited previously.

Obtaining additional experimental bioaccumulation data from a variety of terrestrial systems for comparison with aquatic food chains is a high priority. This effort should include evaluations of when and why aquatic assessments may or may not be adequate, including whether specific chemical classes or physical-chemical properties could identify when aquatic-to-terrestrial extrapolations are feasible and when separate analyses would be required.

Regulatory programs such as REACH have galvanized the evaluation and compilation of physical-chemical property data for registered chemicals. One result is greater recognition of the need for, and importance of, improved data for partition coefficients such as K_{OW}, K_{OC}, and K_{OA}. Better guidance is also needed for evaluation of the importance of environmental fate processes in terrestrial bioaccumulation assessments, including the importance of chemical bioavailability in terrestrial ecosystems based on factors such as soil characteristics and climate.

No regulatory guidelines, other than the OECD earthworm bioaccumulation test, directly address bioaccumulation potential in terrestrial organisms (e.g., plants, invertebrates, mammals, birds). Therefore, using relevant existing data and data collected in an ongoing manner for other regulatory purposes to inform terrestrial bioaccumulation assessments will be important. Tissue residues of chemicals are occasionally measured in avian and mammalian laboratory toxicity studies; however, these measurements lack standardization, and an appropriate bioaccumulation metric for such measurements has not been defined. Standardized and validated approaches and endpoints for terrestrial bioaccumulation that would serve to "verify" or "refine" screening criteria or models are needed and must be in place if regulatory terrestrial bioaccumulation assessments are to be implemented in a meaningful manner.

In a number of areas, immediate progress in terrestrial bioaccumulation assessments could be made, including increased use of in silico and in vitro approaches. The ability of an organism to metabolize and eliminate xenobiotics plays an important role in bioaccumulation assessments. Hence metabolic transformation potential must be considered when extrapolating from modeling to laboratory outcomes and then to field measurements. Recently, considerable effort has been devoted to standardize procedures used to quantify the ability of fish to biotransform xenobiotics (Han et al. 2007; Johanning et al. 2012). Using subcellular (e.g., S9) and whole cell (e.g., hepatocytes) assays, one can obtain a biotransformation rate constant. The biotransformation rate can then be applied within a modeling context to refine bioaccumulation assessments. Similar standardization of in vitro metabolism assays for terrestrial vertebrates has not occurred. Some existing data are available in the literature on biotransformation in terrestrial species (e.g., Huan et al. 1998; Cortright and Craigmill 2006). If these types of studies could be compiled and used as a starting point, it would be possible to standardize, for example, in vitro biotransformation assays for quail and mallard ducks, 2 species that are frequently used in avian toxicity tests for chemical registration purposes. Development of these standardized methods and approaches could better inform the potential for bioaccumulation to occur in these species, as well as potentially refine or reduce animal use.

Many studies have evaluated bioaccumulation based on chemical concentrations in individual tissues of organisms

because of sampling constraints. A need exists to identify the most appropriate tissues for analysis and then develop appropriate bioaccumulation metrics for the evaluation of those tissue concentrations. Chemical concentrations in individual tissues may best be used for the development of bioaccumulation metrics if they can be directly linked to adverse toxicological outcomes in those same tissues. A need also exists to evaluate approaches for integrating chemical concentrations in multiple tissues into an integrated whole-body bioaccumulation metric because the whole-body metric is arguably more important (based on organism feeding strategies) and reflective of potential issues associated with assessments of food chain biomagnification.

Terrestrial bioaccumulation testing needs should be based on the relevant exposure pathways and physical-chemical and environmental fate properties of substances. This includes better guidance and approaches for assessment of chemical bioavailability in terrestrial ecosystems, including identification of the most important factors affecting bioavailability. Additional effort could be placed on the development of a standard regulatory test guideline to address bioaccumulation in plants or adaptation or extension of existing residue methods used for pesticides. Plants are undoubtedly one of the most important exposure pathways from soil contamination into the food web. This effort will require additional research to be conducted and decisions to be made on the relevant plant species and tissues (e.g., roots, stems, leaves, fruit) as well as the study designs and testing conditions (e.g., soil type and characteristics, lighting, humidity) for conducting meaningful bioaccumulation studies. Much of this work has already been done for agricultural crop plant species (e.g., relevant plant tissues eaten as livestock and human food), and leveraging this body of knowledge to bioaccumulation assessments involving noncrop plant species important in terrestrial food chains may be possible.

Finally, relevant and meaningful metrics for the expression and evaluation of the potential for bioaccumulation in terrestrial organisms and food chains need to be developed and agreed on by the scientific and regulatory communities. As part of this effort, evaluating the potential for extending the common thermodynamics basis (i.e., fugacity analysis) approach for the holistic, weight of evidence evaluation of bioaccumulation as discussed in Burkhard, Arnot, et al. (2012) for aquatic systems also would be useful. This approach has numerous benefits including avoiding biases inherent in K_{OW}-based assessments; ready identification of substance-specific data gaps, inconsistencies, and outliers; and the ability to integrate data from different species, food chains, and types of studies including laboratory, field, and monitoring studies.

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SUPPLEMENTAL DATA

Brief description of the history of the development of the OECD earthworm bioaccumulation test guideline.

Table S1. Details and references for test methods.

Table S2. Test guidelines for evaluating plant residues.

Table S3. Test guidelines for evaluating toxicity to plants.

Table S4. Comparison of data collected in OECD TG 305 versus TG 417.

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