

## Use of Terrestrial Field Studies In the Derivation of Bioaccumulation Potential of Chemicals

Nico W van den Brink,<sup>\*†</sup> Jennifer A Arblaster,<sup>‡</sup> Sarah R Bowman,<sup>§</sup> Jason M Conder,<sup>||</sup> John E Elliott,<sup>#</sup> Mark S Johnson,<sup>††</sup> Derek CG Muir,<sup>‡‡</sup> Tiago Natal-da-Luz,<sup>§§</sup> Barnett A Rattner,<sup>||||</sup> Bradley E Sample,<sup>##</sup> and Richard F Shore<sup>†††</sup>

<sup>†</sup>Subdepartment of Toxicology, Wageningen University, Wageningen, the Netherlands

<sup>‡</sup>Ramboll Environ Corp., Irvine, California, USA

<sup>§</sup>Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, Ohio, USA

<sup>||</sup>Geosyntec Consultants, Huntington Beach, California, USA

<sup>#</sup>Environment Canada, Delta, British Columbia, Canada

<sup>††</sup>US Army Public Health Center, Aberdeen Proving Ground, Aberdeen, Maryland

<sup>‡‡</sup>Environment Canada, Burlington, Ontario, Canada

<sup>§§</sup>Department of Life Sciences, Centre for Functional Ecology, University of Coimbra, Coimbra, Portugal

<sup>||||</sup>Patuxent Wildlife Research Center, US Geological Survey, Beltsville, Maryland

<sup>##</sup>Ecological Risk, Rancho Murieta, California, USA

<sup>†††</sup>NERC Centre for Ecology and Hydrology, Lancaster, United Kingdom

(Submitted 9 June 2015; Returned for Revision 13 July 2015; Accepted 25 September 2015)

### 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

Field-based studies are an essential component of research addressing the behavior of organic chemicals, and a unique line of evidence that can be used to assess bioaccumulation potential in chemical registration programs and aid in development of associated laboratory and modeling efforts. To aid scientific and regulatory discourse on the application of terrestrial field data in this manner, this article provides practical recommendations regarding the generation and interpretation of terrestrial field data. Currently, biota-to-soil-accumulation factors (BSAFs), biomagnification factors (BMFs), and bioaccumulation factors (BAFs) are the most suitable bioaccumulation metrics that are applicable to bioaccumulation assessment evaluations and able to be generated from terrestrial field studies with relatively low uncertainty. Biomagnification factors calculated from field-collected samples of terrestrial carnivores and their prey appear to be particularly robust indicators of bioaccumulation potential. The use of stable isotope ratios for quantification of trophic relationships in terrestrial ecosystems needs to be further developed to resolve uncertainties associated with the calculation of terrestrial trophic magnification factors (TMFs). Sampling efforts for terrestrial field studies should strive for efficiency, and advice on optimization of study sample sizes, practical considerations for obtaining samples, selection of tissues for analysis, and data interpretation is provided. Although there is still much to be learned regarding terrestrial bioaccumulation, these recommendations provide some initial guidance to the present application of terrestrial field data as a line of evidence in the assessment of chemical bioaccumulation potential and a resource to inform laboratory and modeling efforts. *Integr Environ Assess Manag* 2016;12:135–145. © 2015 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals, Inc. on behalf of SETAC.

**Keywords:** Biomagnification factors Biota-to-soil-accumulation factors BMF BSAF Chemical bioaccumulation Terrestrial food web TMF Trophic magnification factors

This article includes online-only Supplemental Data.

\* Address correspondence to nico.vandenbrink@wur.nl

Published online 5 October 2015 in Wiley Online Library

(wileyonlinelibrary.com).

DOI: 10.1002/ieam.1717

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

### INTRODUCTION

The potential of compounds to bioaccumulate in organisms and to transfer and biomagnify in food webs is a key consideration in chemical regulation (Weisbrod et al. 2009). Currently, the assessment of the bioaccumulation potential is primarily based on data derived from marine or freshwater organisms and food webs, and many assessments include field data collected from wild aquatic organisms. However, physiological and ecological factors affecting bioaccumulation in terrestrial ecosystems are considered to be very different from those in aquatic ecosystems. Hence, bioaccumulation

assessments derived from aquatic systems may not be predictive of bioaccumulation potential in terrestrial systems (Kelly and Gobas 2001, 2003; Kelly et al. 2007).

It is generally understood that soil properties such as organic C content and quality affect the bioavailability of chemicals, and thus uptake of organic compounds by soil organisms (Chung and Alexander 2002; Amorim et al. 2005; Cornelissen et al. 2005). Furthermore, the availability of organic compounds in soil may decrease over time due to aging and consecutive increased binding of the chemical to soil particles and weathering or degradation of the compound (Belfroid et al. 1995; Styriashve et al. 2008; Johnson, Salice et al. 2009). Current methods used to predict bioaccumulation potential of organics in aquatic systems rely on measures of hydrophobicity and coefficients such as  $\log K_{OW}$ . However, in terrestrial systems,  $\log K_{OW}$  alone does not explain or predict bioaccumulation (Belfroid et al. 1995). In terrestrial systems, biotransformation seems to have more profound effects on bioaccumulation and biomagnification in food webs (Kelly et al. 2007; McLachlan et al. 2011) (see Supplemental Data for more details).

Despite the possible discrepancy between bioaccumulation in aquatic and terrestrial ecosystems, explicit assessment of terrestrial bioaccumulation data are not specified in national legislations or specifically required in bioaccumulation assessments. In the European Union (EU), the amendment of Annex XIII in the current Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) requires consideration of all available bioaccumulation metrics as part of a weight of evidence analysis. As a result, terrestrial field studies or comparable laboratory simulations, if available, are recommended for EU chemical assessments (Moermond et al. 2012; Vierke et al. 2012; Gottardo et al. 2014), although formal guidance or recommendations on the use of terrestrial bioaccumulation data and thresholds are not available.

In January 2013, the ILSI Health and Environmental Sciences Institute (HESI) sponsored a workshop on terrestrial bioaccumulation in Miami, Florida, USA. The goal of the workshop was to compile information and inform upon a framework for the assessment of bioaccumulation in terrestrial food webs that would be useful in chemical registration programs. This article provides an overview on the different approaches to evaluate organic chemicals data generated from wild terrestrial organisms and abiotic media collected from field investigations. This article also addresses the advantages and opportunities for using terrestrial field data in bioaccumulation assessments and provides practical recommendations for generating and applying such data. Companion articles provide similar focus on the use of data generated from terrestrial laboratory studies (Hoke et al. this issue) and environmental modeling (Gobas et al. this issue).

#### **Importance terrestrial field data in bioaccumulation assessments**

Measurement of organic chemicals in aquatic organisms and abiotic media provide important data that have been used to evaluate bioaccumulation potential (Selck et al. 2012). Depending on the types of information collected, chemical concentration data from field-collected samples can be used to calculate bioaccumulation factors (BAFs), biomagnification factors (BMFs), and trophic magnification factors (TMFs) (Gobas et al. 2009; Conder et al. 2012). Bioaccumulation data from terrestrial settings can also be easily expressed in this manner, including biota-soil accumulation factors (BSAFs),

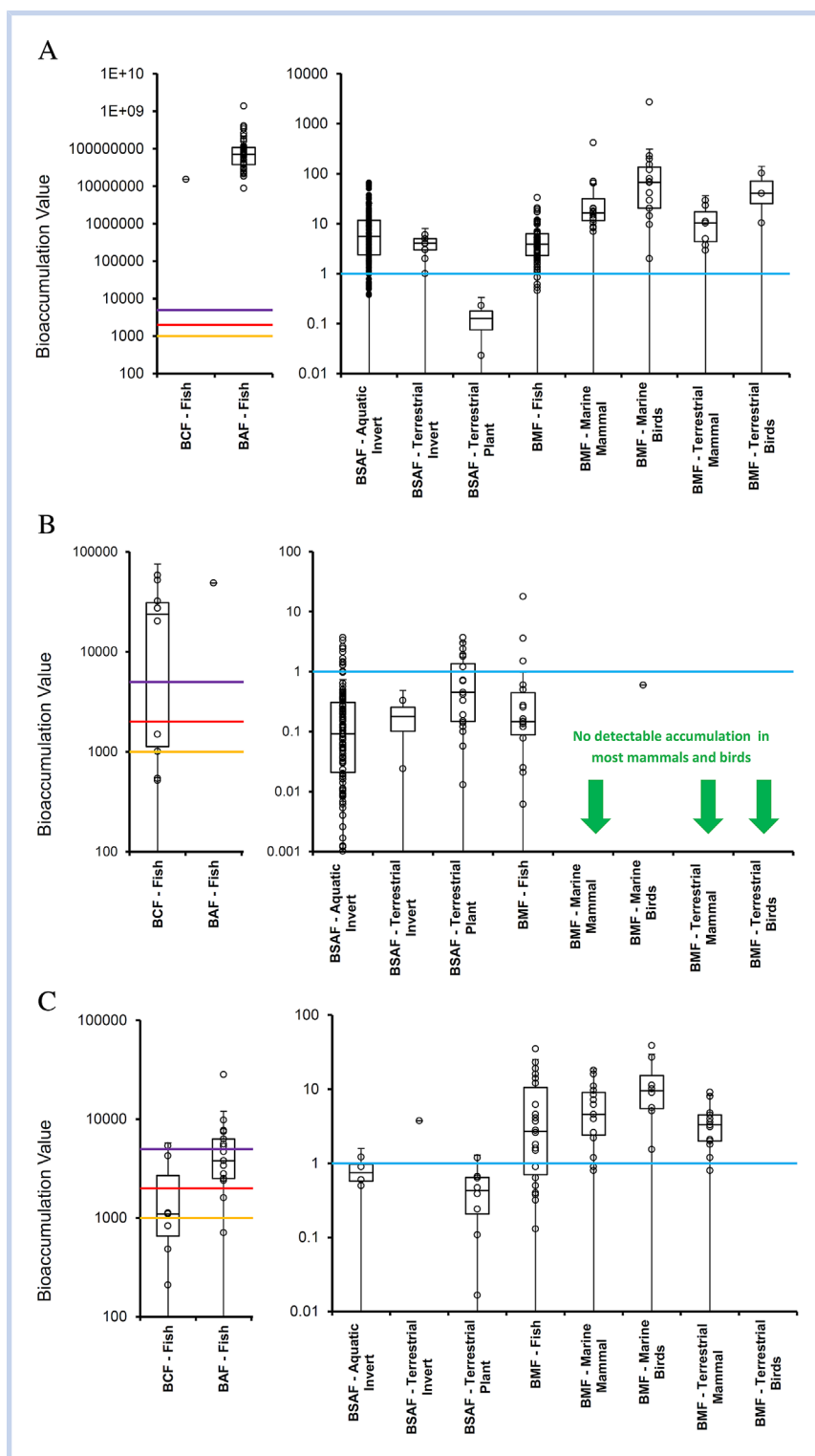
BAFs, BMFs, and TMFs (Gobas et al. this issue). When derived in terrestrial field studies, these metrics can be used in a weight of evidence approach to assess the bioaccumulation potential of chemicals that have been released in the environment in similar approaches as those used to evaluate data from aquatic bioaccumulation potential assessments. In addition, such data can be used to inform the development, validation, and refinement of laboratory tests and models for prospective assessments of chemicals that have yet to be released into the environment.

There is a wealth of terrestrial field data that can be used to assess bioaccumulation potential (see Supplemental Data for >20 different studies). The available evidence strongly suggests that terrestrial field data provide information that is not always consistent with data generated from aquatic studies. For example, field data compiled for 4 example chemicals, PCB-153, pyrene, and perfluorooctane sulfonic acid (PFOS), demonstrate both the utility of and need for consideration of terrestrial data in the regulatory assessment of bioaccumulation potential (Figure 1; for details on the studies and the derivation of the metrics, see the Supplemental Data). In the case of PCB-153, the BCFs derived from aquatic studies imply high potential for accumulation, but the BSAFs for invertebrates and plants (approximate terrestrial analogues to aquatic BCFs) indicate a much lower potential to bioaccumulate in terrestrial systems (Figure 1A). Furthermore, the examples indicate that aquatic derived BCFs and BAFs do not always match estimates of bioaccumulation potential derived from terrestrial (soil) organisms or avian and mammalian species. For pyrene, a metabolizable polycyclic aromatic hydrocarbon, bioaccumulation potential is supported by aquatic BCF and BAF estimates, but not by 1) BSAFs for terrestrial invertebrates and plants (generally <1) or 2) BMF-data from homeothermic animals in terrestrial and aquatic food webs (Figure 1B). The converse (bioaccumulation potential not indicated by aquatic BCFs and BAFs but instead by BMF data for homeotherms) is found with PFOS (Figure 1C).

#### **Recommendations for generating field study data useful to terrestrial bioaccumulation assessments**

Given that terrestrial field data have value in providing useful evidence in bioaccumulation potential assessment for particular chemicals, as well as information useful to modeling and laboratory methods in support of bioaccumulation assessments, workshop participants were able to provide some initial practical recommendations regarding the interpretation and generation of terrestrial field data. Workshop participants focused on several primary issues, as detailed in the remainder of this section: the selection of species and tissue types to include in investigations or to focus upon in existing data sets; considerations for spatial and temporal aspects of sampling and data analysis; available methods to determine food web relationships; considerations for sample sizes needed for robust bioaccumulation data analysis; and general practical advice on obtaining samples from terrestrial organisms. The guidance provided may need to be refined as advances are made in regulatory and technical aspects related to the assessment of bioaccumulation potential.

*Selection of species.* Central to species selection is the need to identify trophic guilds and predator-prey interactions that can generate data reflecting key food web relationships.



**Figure 1.** Bioaccumulation metric values for PCB-153 (A), pyrene (B), and PFOS (C). Line markers include one, the scientific definition of bioaccumulation (blue line); and values of 1000 (orange line), 2000 (red line), and 5000 (purple line) associated with various bioaccumulation levels of concern for US, Canadian, and/or European regulatory agencies.

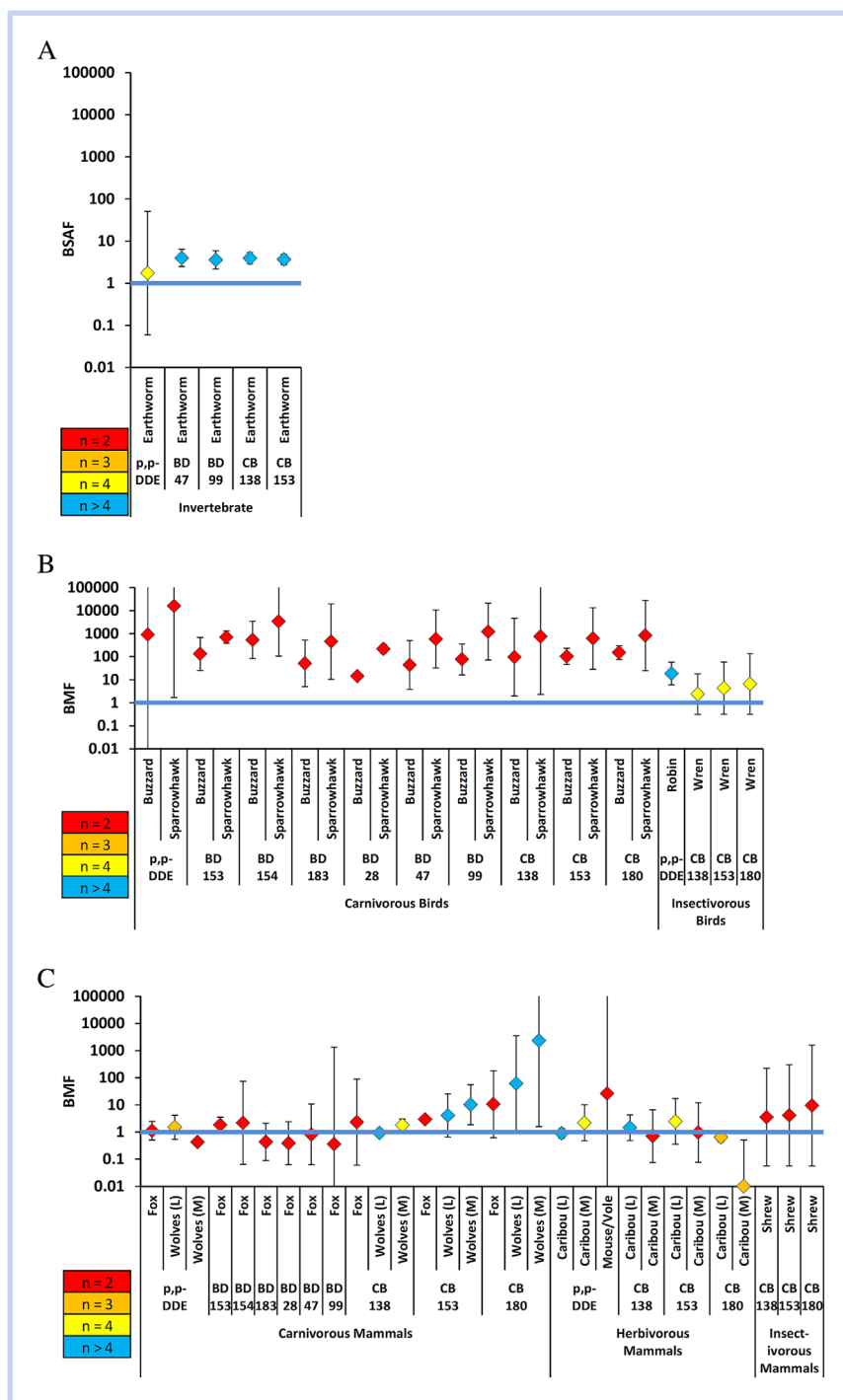
Measurements of plant tissues and soft-bodied soil invertebrates should be used to generate BSAFs, because these organisms are in direct contact with the soil. The use of BSAFs is not recommended for estimating bioaccumulation potential in higher trophic level vertebrate species (i.e., reptiles, birds, and mammals) because of the considerable uncertainties associated with exposure and uptake. The primary route of organic chemical exposure for these organisms is generally

dietary (USEPA 1997, 2005), with only a minor contribution from soil ingestion (Beyer et al. 1994). Consequently, chemical bioaccumulation for these species in terrestrial systems is better represented by BMFs or TMs.

Calculation of BMF values requires concentrations of chemical in a predator (or consumer) and its diet, usually represented by one or more food items. Selection of a predator-prey pair to measure is an option that should be considered in

experimental design and interpretation of BMFs from trophic level data sets. It is clear that BMF values in aquatic systems vary among trophic levels (Conder et al. 2012), suggesting that a particular species or component of a terrestrial food web may also be a more robust indicator of bioaccumulation. To evaluate this hypothesis, BMFs and BSAFs for hydrophobic chemicals typically considered bioaccumulative (e.g., PCBs, PBDEs, and DDTs) were compiled from 8 studies that evaluated chemical residues in terrestrial invertebrates (earthworms) exposed in

the laboratory to field-contaminated soils, and matched predator–diet pairs for mammalian insectivore, omnivore, and carnivore species as well as avian carnivore species (Hebert et al. 1994; Belfroid et al. 1995; Harris et al. 2000; Kelly and Gobas 2001; Matscheko et al. 2002; Blankenship et al. 2005; Voorspoels et al. 2007; White et al. 2007) (see Supplemental Data). These data suggest that soil invertebrates and avian carnivores provide the most compelling measures of bioaccumulation potential (lowest variance coefficient, Figure 2).



**Figure 2.** Mean (95% CI) BSAFs (g, soil OC/g, lipid) for soil invertebrates (A), BMFs (g, diet lipid/g, predator lipid) for birds (B) and BMFs for mammals (C) associated with p,p-DDE, PCBs congeners (noted “CB” plus congener number), and PDBE congeners (noted “BD” plus congener number). Blue line indicates a value of 1 (threshold for bioaccumulation potential). “L” and “M” labels note BMFs developed using liver and muscle sample data, respectively. Other values were based on averages of BMFs based on liver and muscle tissues (e.g., buzzard, sparrowhawk, and fox), tissue samples not identified by the source study (e.g., shrew, wren, robin), or soil-voided whole body and soil measurements (e.g., earthworms).

Approximately 80% of invertebrate BSAFs (Figure 2A) and avian BMFs (Figure 2B) were greater than 1 for bioaccumulative chemicals, compared to less than 20% of the BMFs for mammalian species (Figure 2C). The data indicate that carnivores exhibited greater bioaccumulation potential than other guilds and, thus, carnivores may be the most appropriate sentinel species for measuring bioaccumulation potential in terrestrial field studies. For example, data for avian invertivores tended to indicate bioaccumulation less often (only 1 of 4 avian invertivore BMFs indicated bioaccumulation potential) than data for avian carnivores (Figure 2B), and only 5 of the 30 BMFs for carnivorous mammals were indicative of bioaccumulation (Figure 2C).

Substantially greater BMF values for carnivores are not unexpected, as noted in modeling and empirical studies (Kelly and Gobas 2003; Debruyne and Gobas 2006; Kelly et al. 2007). Carnivores tend to exhibit high BMFs due to their top (and sometimes apex) position in terrestrial food webs (Kelly and Gobas 2003; Kelly et al. 2007). BMFs may increase for predators situated at the top of food webs due to a great number of trophic transfers from lower tier predators and prey species (Debruyne and Gobas 2006). Greater bioaccumulation in avian species compared to mammalian predators has been observed in other field studies of aquatic food webs (Hop et al. 2002; Hallanger et al. 2011), suggesting that avian carnivores may be an important guild to include in terrestrial studies evaluating bioaccumulation potential of chemicals. Larger avian BMFs could be related to a number of physiological or ecological differences between birds and mammals. For example, fish-eating birds were shown to have less cytochrome P450-associated monooxygenase activity compared to mammals (Walker 1980), leading to less metabolism and greater accumulation of chemicals. This may be related to a relatively low exposure to complex plant secondary metabolites and a lack of evolutionary selection for the capacity to detoxify these compounds. Avian species also require less water intake compared to mammals (Sample et al. 1997), and metabolites in urine can be re-absorbed in the cloaca (Walker 1983), possibly resulting in reduced urinary excretion pathways for chemicals. For terrestrial species, the respiratory elimination route is also of importance with respect to chemical accumulation (Kelly et al. 2007). However, the avian respiratory pathway is very efficient in  $O_2$ - $CO_2$  exchange, resulting in lower breathing rates in comparison to mammals (normalized to body weight) (Sample et al. 1997). Such lower breathing rates may result in less respiratory elimination of organic contaminants. However, it is clear that more modeling and empirical work is needed to evaluate the hypothesis that avian carnivores may be top bioaccumulators in most terrestrial food webs.

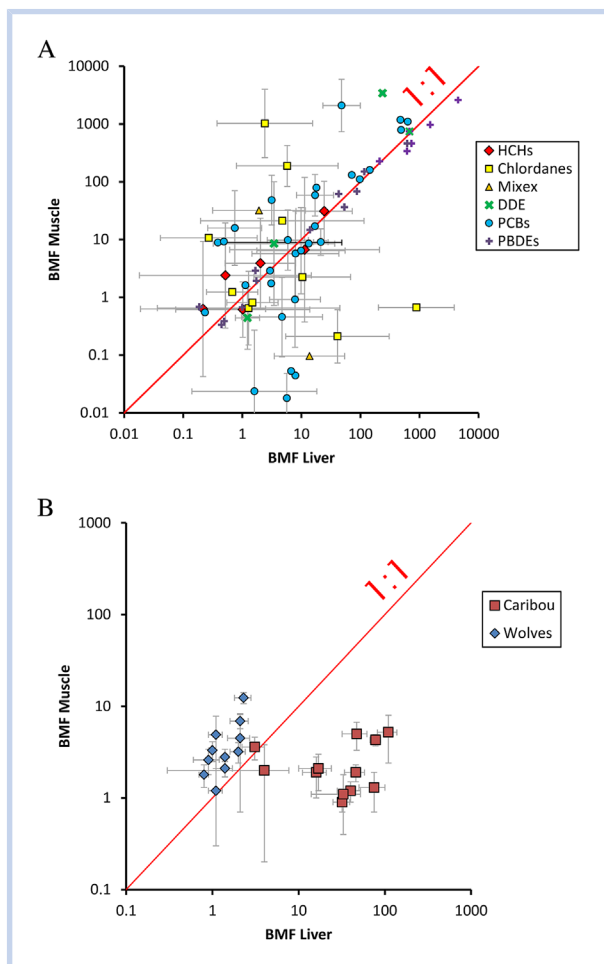
**Selection of tissue type.** Selection of tissue types and treatment of samples should be carefully evaluated for terrestrial bioaccumulation determinations. Field data used to derive BSAFs for earthworms should ideally be based on depurated (soil-free) whole body analyses. Chemicals associated with soil within the gut of earthworms have not been transferred to the biological compartment and, thus, do not truly represent the process of bioaccumulation. However, if earthworms are used to estimate a BMF, as prey item, this depuration process may not be appropriate because predators consume nondepurated prey. Plant-based BSAFs are usually established on the analysis of aboveground tissues such as leaves or fruits, because those

tissues are generally consumed by higher trophic level animals. However, BSAFs generated from aboveground tissues are generally lower than those based on roots due to preferential partitioning of nonionic organic chemicals to roots than other plant tissues (Simonich and Hites 1995; Collins et al. 2005). Plant and fruit BSAFs can be affected by aerial deposition of organics onto aboveground surfaces, and therefore, inaccurately represent the bioaccumulation process from soil.

In general, matched predator-diet samples used to calculate BMF values are more difficult to obtain compared to samples needed for BSAFs, and BMF data interpretation is more difficult. Several factors influence the collection and interpretation of BMF data, including spatial variation, behavior, habitat, time of year, reproductive status, and other characteristics of predator and diet (Borga et al. 2012). The tissue type targeted for sampling and testing also is an important consideration because it is rarely practical to analyze whole organisms, especially for larger predators. Theory suggests that concentrations of hydrophobic compounds in tissues can be lipid-normalized to account for differences in fugacity due to different lipid contents. If so, BMFs based on lipid normalized concentrations should be similar between tissue types and provide similar information for organic compounds. To demonstrate this important consideration, studies that included the concentrations of organic chemicals in both the liver and muscle tissues of organisms from terrestrial food webs were compiled to calculate paired  $BMF_{liver}$  and  $BMF_{muscle}$  values (see Supplemental Data). Figure 3A indicates that  $BMF_{muscle}$  and  $BMF_{liver}$  are related to each other and not significantly different for legacy, nonpolar organic contaminants. Nevertheless, even for nonpolar organic chemicals, rapid changes in the body condition of the organism may result in internal remobilization of chemicals, which will disturb the internal equilibrium (Crosse et al. 2013). In such cases, even lipid-normalized chemical concentrations may not be comparable between tissue types. Organic contaminants that do not preferentially partition into lipids (e.g., some perfluoroalkyl and polyfluoroalkyl substances [PFASs]), cannot be lipid normalized to account for differences in tissue concentration, and present challenges for evaluating bioaccumulation via individual tissues. For example, individual tissue BMFs vary widely for some PFASs due to differences in bioaccumulation patterns that are not yet understood (see Figure 3B,  $r = -0.06$  and  $p = 0.77$ ). To accommodate this uncertainty, BMFs are often evaluated on a whole body basis by estimating the concentration in the entire body on the basis of an organ mass balance and measurement of PFASs in several different tissues (Houde, Martin et al. 2006; Müller et al. 2011). Overall,  $BMF_{muscle}$  showed better agreement with  $BMF_{wholebody}$  than did  $BMF_{liver}$  for both wolves and caribou (Figure S1). Although whole body BMF values are preferred metrics for assessing bioaccumulation of PFASs,  $BMF_{muscle}$  may appear to be an acceptable surrogate for this specific class of chemicals.

**Selection of sampling location and timing.** Both spatial and temporal variation of chemical contaminants should be taken into account in terrestrial ecosystems when addressing bioaccumulation of compounds under field conditions. The first source of spatial variation is the spatial distribution of substances in soil, which may be related to the primary source of the compounds, their dispersal, and both soil and chemical properties (Heywood et al. 2006). Within-site variation in soil concentrations of organic compounds can be substantial (up to





**Figure 3.** Correlation between  $BMF_{Liver}$  and  $BMF_{Muscle}$ . (A) Data on nonpolar organic contaminants from different species. (B) Data on PFASs from caribou and wolves. Error bars represent 1 SD of the mean. Lines indicate a 1:1 relationship. Data from Kelly and Gobas (2001), Müller et al. (2011), and Voorspoels et al. (2007).

3 orders of magnitude) (Johnson, Korcz et al. 2009; Niemeyer et al. 2010) and will greatly influence bioaccumulation. A second source of spatio-temporal variation is the availability of prey items. For example, prey availability for the little owl (*Athene noctua*) varied significantly during season and among habitat types such that modeled chemical exposures varied by 3 orders of magnitude (Schipper et al. 2012). In a field study with small mammals, species-specific spatial habitat usage and diet composition also greatly influenced chemical uptake, in addition to metal bioavailability (van den Brink et al. 2011). Such environmental and ecological factors may influence bioaccumulation under field conditions for some chemicals to such extend that BMFs for particular predator-prey relationships may differ greatly from BMFs determined in other seasons or dissimilar habitats.

Evidence of spatio-temporal variability from these and other studies suggest that when calculating a BMF, it may be preferable to evaluate the locations of prey capture relative to the predator's foraging range. If predators obtain most prey from a relatively small but highly contaminated area comprising a tiny fraction of its foraging range, this may bias the calculation of the BMF. It is more appropriate to target the collection of prey from an area defined by its predator's foraging range, rather than focusing on the footprint of the total contaminated area. Additionally, it may be advantageous

to collect several different prey items, particularly if the predator diet varies seasonally, to derive a time-weighted average concentration in the diet.

**Assessment of food web relationships.** Determining BMF and TMF metrics that can be used to evaluate bioaccumulation potential requires information on the food web and trophic position to evaluate field data and design relevant and meaningful field studies. The 2 most widely used techniques to quantify these ecological factors are analysis of stable isotopes in tissues and food items and dietary characterization via gut or fecal analysis. A third technique showing promise is the use of molecular level analysis, although this approach has yet to be widely used in ecotoxicology studies.

At present, stable isotopes of N ( $^{15}N/^{14}N$ ;  $\delta^{15}N$ ) are widely used to characterize trophic position of terrestrial organisms, whereas C ( $^{13}C/^{12}C$ ;  $\delta^{13}C$ ) and S ( $^{34}S/^{32}S$ ;  $\delta^{34}S$ ) have been applied to characterize diets (Peterson and Fry 1987; Kelly 2000; Koch 2008). Sulphur has been less widely used because few laboratories routinely conduct this isotope ratio analysis. Increases in  $\delta^{15}N$  occur because of the preferential retention of the heavier isotope from the diet of the consumer. This fractionation is related to excretion of urea and other nitrogenous substances that are enriched in  $^{14}N$  relative to body N pools (Parker et al. 2005). In aquatic environments, this fractionation is relatively constant with an enrichment factor ( $\delta^{15}N$ ) of 3.0‰ to 5.0‰ between trophic levels. The  $\delta^{15}N$ , used to calculate trophic level (TL), is often assumed to be 3.4‰ to 3.8‰ based on a number of feeding experiments or syntheses of the literature (Hobson and Welch 1992; Post 2002; Jardine et al. 2006). However, feeding experiments on birds and mammals have shown that the magnitude of fractionation increases with increasing protein content in the diet, possibly because animals on low protein diets use most of their dietary N for protein synthesis, and consequently have a lower urea N flux (Koch 2008). Metabolic differences between taxa may also be important. For example, the  $\delta^{15}N$  between an avian diet and its muscle tissue was only 2.4‰ (Mizutani et al. 1991). This has implications for using a single  $\delta^{15}N$  to estimate the trophic position of organisms within terrestrial avian and mammalian food webs and contributes uncertainty in the calculation of the trophic enrichment factor within food webs.

In addition, environmental factors such as precipitation, temperature, soil characteristics, and nutrient availability determine plant community composition and influence  $\delta^{13}C$  and  $\delta^{15}N$  at the base of the terrestrial food web (Ben-David and Flaherty 2012), and the isotope composition in organisms can change seasonally with food availability (e.g., fasting in winter can increase  $\delta^{15}N$ ) (Hobson et al. 1993). These factors can differ both spatially and temporally resulting in variability of stable isotope signatures in terrestrial food webs. For example, the range of  $\delta^{13}C$  and  $\delta^{15}N$  in the vegetation-caribou-wolf food web, which has been studied extensively for biomagnification of organic contaminants (Kelly and Gobas 2001; Müller et al. 2011), is illustrative of the variation encountered in terrestrial food webs. In cottongrass (*Eriophorum vaginatum*), aquatic sedge (*Carex aquatilis*), and willow (*Salix pulchra*) from the same sampling sites,  $\delta^{13}C$  varied widely and was only moderately enriched along the food web (1‰ to 2‰) (Müller et al. 2011). Lichen, caribou, and wolf had similar  $\delta^{13}C$  values implying that the caribou were mainly feeding on lichen and the wolves mainly

on caribou or other lichen eating herbivores. In contrast, the  $\delta^{15}\text{N}$  difference between lichen and caribou were rather large (7‰ to 8‰) compared to usually assumed  $\delta^{15}\text{N}$  differences of 3.4‰ to 3.8‰, complicating the calculation of TL values used to calculate TMFs. Although additional source modeling (e.g., IsoSource) (Phillips et al. 2005) may be useful in understanding dietary contributions to isotopic mass balances, it is clear there are uncertainties that challenge the interpretation of stable isotope signals in terrestrial food webs, and additional study is needed before routine application for calculation of TMF values.

Visual gut content and fecal analysis have long been used in ecological and toxicological studies to determine the diet of organisms. These approaches rely on the identification and quantification of partially digested prey fragments and provide a snapshot of the diet at any given sampling time. They can be invasive (gut dissection) or noninvasive (collection of fecal samples or regurgitations). Although these procedures are inexpensive and do not require expensive instrumentation, they do have drawbacks. For example, the level of identification associated with gut content is sometimes limited by mastication and digestive processes that damage specimens resulting in fragments of tissue (Sample et al. 1993; Sample and Whitmore 1993). Furthermore, soft-bodied prey items may get digested more quickly than other items resulting in underestimation of these types of items in the diet. Considerable expertise in taxonomy is necessary to identify diet items based on tissue fragments, which makes the identification of specimens to species level difficult (Soininen et al. 2009). This can result in a somewhat subjective and even biased identification of specimens based on experience and professional judgment. Although fecal analysis is a noninvasive technique relative to analysis of gut content, such samples only contain fragments of tissue that were not digested and, thus, pose similar limitations and bias during identification. To avoid issues with digestion or partial digestion of items collected from gut content or fecal samples, predigestive samples can be obtained via throat ligation techniques, which have been successfully used for nestling birds (Mellott and Woods 1993; Powell 1984). This technique allows for an accurate determination of food items delivered to nestlings before digestion and can be used to ascertain site-specific concentrations of chemicals in food items.

Molecular methods can also be useful for determining diet and food web structure. With advances in DNA sequencing and polymerase chain reaction (PCR)-based technology, molecular methods have become more widely used by ecologists as tools for diet analysis. With the availability of free molecular databases, it is possible to use DNA barcoding (analyzing DNA-fragments) for organism identification even with short or degraded DNA sequences (Zaidi et al. 1999; Hajibabaei et al. 2006; Meusnier et al. 2008). Barcoding can be especially useful for species where the diet cannot be identified by gut-content analysis, observation, or other methods. Molecular methods are typically invasive in the case of gut dissections (Chen et al. 2000; Soininen et al. 2009; Carreon-Martinez et al. 2011) and noninvasive when using fecal samples (Corse et al. 2010; Zeale et al. 2011). PCR-based diet analysis is successfully accomplished in both aquatic and terrestrial systems and usually renders results with better taxonomic resolution than visual methods (Soininen et al. 2009; Carreon-Martinez et al. 2011). However, because each species may differ in the amount of DNA present per unit

biomass and/or in tissue digestibility, molecular techniques provide merely a qualitative description of the diet (Zaidi et al. 1999). Laboratory testing may be used to calibrate PCR techniques for each food type to achieve semiquantitative results (Soininen et al. 2009; Deagle et al. 2010). One disadvantage of this approach however, is the inability to detect and distinguish primary and secondary predation (Sheppard et al. 2005). Because contamination of the sample with the predator's DNA is likely (e.g., during gut dissection), detection of cannibalism can also be difficult (Deagle et al. 2010; Carreon-Martinez et al. 2011). There are other practical considerations that may affect results such as gene and primer selection, sample preservation, temperature, and time since ingestion (for review, see Sheppard and Harwood 2005; King et al. 2008; Valentini et al. 2009).

**Sample size.** Sample size is a critical factor in statistical interpretation of bioaccumulation data, which is of great importance given the high variability and low sample sizes that are more the rule than the exception in field data sets. For example, results in Figure 2 indicate that a single BMF value should not be taken at face value without accounting for measurement variation. Forty-eight of the 59 (>80%) BMF and BSAF values in Figure 2 appear to be greater than 1 (indicating bioaccumulation potential). However, nearly 40% of these values were not statistically greater ( $\alpha = 0.05$ ) than 1. Statistical comparison of field bioaccumulation information should be a requirement of all field studies reporting such information, and raw data, measures of variability and sample size should always be included to enable other researchers to use the data for assessing bioaccumulation potential (Borga et al. 2012; Conder et al. 2012).

The results shown in Figure 2 suggest that at least 3 or 4 replicates are the minimum sample size required for generating robust BMF data. Although avian BMFs in Figure 2B were variable (in part due to small sample sizes), higher values tended to offset variability and provide a statistically powerful estimate, capable of detecting significant bioaccumulation potential. Earthworm BSAFs for comparable chemicals tended to be 2 to 3 orders of magnitude lower than avian carnivore BMFs, but the large number of replicates ( $n > 10$ ) improved statistical power (Figure 2A). Mammalian BMFs (Figure 2C) exhibited considerable variability, suggesting the need for greater replication than required to estimate avian BMFs.

#### **Practical advice on obtaining samples**

Obtaining adequate numbers of samples may be a practical challenge in terrestrial bioaccumulation studies. For example, in marine bioaccumulation studies, colonial breeding birds are often studied because collection of samples from animals is generally most efficient when they occur in aggregations or colonies. However, with some exception (e.g., European bee-eater, *Merops apiaster*, at a mining site) (Lopes et al. 2010) most terrestrial avian species generally do not aggregate. Some terrestrial studies take advantage of the willingness of cavity-nesting avian species to use nest boxes (e.g., tawny owl [*Strix aluco*] [Bustnes et al. 2007]; American kestrel [*Falco sparverius*] [Hebert et al. 1994]; tree swallow [*Tachycineta bicolor*] [Custer 2011]; small passerines *Parus* and *Ficedula* spp. [van den Steen et al. 2009; Berglund et al. 2012] house wrens [*Troglodytes aedon*] and eastern bluebirds [*Sialia sialis*] [Fredricks et al. 2010]; and the widespread European starling [*Sturnus vulgaris*] [Eens et al. 2013]). However, in

contrast to avian species, most mammalian species will generally not use provided shelter or nesting structures, which leaves the collection of mammalian samples more difficult.

A particularly efficient sampling approach involves leveraging sampling efforts from other activities or sampling programs. For example, tissue samples may be obtained from hunters (Conder and Lanno 1999; Müller et al. 2011) or from biologists and citizen scientists that collect animals found dead (van den Brink and Ma 1998). There are a growing number of environmental specimen banks located around the world (Becker et al. 2006) and long-term environmental monitoring projects which may offer the opportunity to provide tissue samples from controlled effort. Such specimen banks and programs have generally been designed to collect samples that are of value in assessing spatial and temporal variation in contaminant concentrations either in sentinel species and/or in species of particular conservation concern or interest (Elliott et al. 2005; Hebert and Weseloh 2006; Norstrom and Hebert 2006; Braune et al. 2007; Anderson et al. 2009; Helgason et al. 2009; Crosse et al. 2012). Data from these samples can be used to derive information on bioaccumulation potential or efforts to support modeling or data interpretation approaches. For example, patterns of PCBs, PBDEs, and stable isotopes of H, C, and N in eggs from peregrine falcons (*Falco peregrinus*) varied markedly among eggs collected from “big city” versus “coastal” nests, and revealed the need for data on dietary variation to decipher pathways and processes of biomagnification in a terrestrial top predators (Park et al. 2011). Archived liver samples of terrestrial raptors, such as Cooper’s hawk (*Accipiter cooperi*), peregrine falcon, and Eurasian sparrowhawk (*A. nisus*), have been examined for spatial trends (urban to rural gradients) and accumulation patterns of POPs in relation to patterns of stable isotopes (Crosse et al. 2012, Crosse et al. 2013). When using archived samples from specimen banks, it should be made certain that concentrations of chemicals in the samples have not been affected by storage conditions and length of storage period. Especially in case of new emerging chemicals, without an analytical track-record issues with quality control and/or quality assurance should be considered when using archived samples.

Sublethal and minimally invasive sampling procedures should be considered for sampling programs, as lethal sampling may not be practical for many species, such as top predators, charismatic megafauna, and species of special conservation status (threatened and endangered). As long as species that are easily captured and handled in a manner that does not incur lethality, several types of tissues may be collected nondestructively (D’Have et al. 2006). For PFASs, blood is often used to determine trophic transfer based on the specific tissues (Tomy et al. 2004; Houde, Bujas et al. 2006). Relationships between levels of PFASs in avian feathers and liver have been established (Meyer et al. 2009). Feathers have also been used to monitor POPs in the chicks of predatory birds (Eulaers et al. 2011). Depending on the study purpose, the calibration of feathers, blood, and body tissues in target species is recommended particularly for less persistent contaminants that may occur at proportionally greater concentrations of these compounds than in other tissue types (Dauwe et al. 2005; Jaspers et al. 2007; Espín et al. 2010; Garcia-Fernandez et al. 2013). For mammals, hair can provide a noninvasive sample. For example, significant relationships have been established between concentrations of total PBDEs and PBDE congener-47 in the hair

of hedgehogs (*Erinaceus europaeus*) relative to internal tissues, although less persistent congeners were more predominant in hair than other tissues (D’Have et al. 2007). Preen oil has been used to analyze POPs in marine birds, and this may be applicable to terrestrial species as well (van den Brink et al. 1998).

Fecal matter is another noninvasive approach to monitor uptake and bioaccumulation in some mammalian species. For example, taking advantage of the use of regular latrine sites by river otters, a several researchers used scat samples to infer body burden of PCBs and other persistent contaminants (Mason et al. 1992; Smit et al. 1994; Elliott et al. 2008). Scats that have decreased relative levels of metabolizable PCB congeners reflect internal concentrations of otters and may therefore be used to assess accumulation in otters (van den Brink and Jansman 2006). The approach has been further developed using fecal DNA to identify individual animals and track their movement and contaminant exposure in time and space (Guertin et al. 2010).

## CONCLUSIONS

Regulatory assessment of chemical bioaccumulation potential can benefit from data provided by terrestrial field studies, and initial work indicates that aquatic data may not completely represent bioaccumulation potential in terrestrial ecosystems. Data from samples obtained from terrestrial field studies can be used to derive bioaccumulation metrics that can be interpreted in existing chemical registration programs, and can also be used in the development, validation, and refinement of laboratory tests and models for prospective assessments of chemicals that have not been released to the environment.

In this article, we present practical recommendations and key issues that should be considered by scientists involved in research that elucidates chemical bioaccumulation potential in terrestrial systems and by regulatory authorities involved in the assessment of bioaccumulation potential within programs designed to register chemicals. BSAFs, BAFs, and BMFs appear to be the most suitable metrics that can be generated from terrestrial field studies. BSAF values are robust when based on measurements of soft-bodied soil organisms or plants. For higher trophic-level organisms, the BMF currently appears to be more robust than the TMF, which may reflect uncertainty in quantifying the trophic level of terrestrial animals using stable isotope signals. BMF values for lipophilic, nonpolar chemicals can be calculated using a variety of sample types (e.g., muscle, liver) if concentrations are expressed on a lipid-normalized basis. BMF values for avian carnivores appear to be particularly useful values for understanding chemical bioaccumulation, as this trophic guild appears to accumulate chemicals to a greater degree than other trophic levels, although this hypothesis deserves further investigation.

Sampling programs and efforts to evaluate data should strive for maximum efficiency in experimental design. It is essential, nonetheless, to achieve the appropriate statistical and interpretive power in field studies to optimize the achievement of scientific goals and, ultimately, the information needed to inform regulatory decision making. For some terrestrial food webs, as few as 4 to 5 predator–prey sample pairs may be sufficient for estimation of a relatively precise BMF. For other food webs, researchers may be able to take advantage of specimen tissue banks and long-term monitoring programs to reduce species collection efforts and obtain the tissues to support their research. Noninvasive and nonlethal sampling



efforts are also possible using samples of blood, hair, feather, feces, and other tissues.

More work is needed to improve the design of terrestrial field studies that address chemical bioaccumulation, as well as the subsequent application of field data to improve decision making in chemical registration programs. Several sources of uncertainty remain challenging such as seasonal variability in the diets of terrestrial organisms and the high spatial heterogeneity of the distribution of chemicals in terrestrial environments. The determination of food web relationships and diet preferences for predators is critical for developing BMFs and TMFs. Studies on the use of stable isotope ratios for quantifying trophic relationships in terrestrial ecosystems are needed to resolve uncertainties in the calculations of TMFs. In conclusion, addressing recommendations provided in this overview, as well as future scientific and regulatory discourse, will facilitate the application of terrestrial field data as a line of evidence in the assessment of chemical bioaccumulation potential.

**Acknowledgment**—The workshop was organized by The Health and Environmental Sciences Institute (HESI). The authors would like to thank Michelle Embry and Megan Harries for their excellent support. Other participants of the workshop are greatly thanked for their contribution during the workshop.

## SUPPLEMENTAL DATA

**Data S1.** Specific aspects of environmental fate and behavior of compounds in terrestrial ecosystems

**Data S2.** Accumulation metrics (BSAF, BMF)

**Data S3.** Terrestrial field studies on bioaccumulation potential of chemicals

## REFERENCES

- Amorim MJB, Rombke J, Scheffczyk A, Soares A. 2005. Effect of different soil types on the enchytraeids *Enchytraeus albidus* and *Enchytraeus luxuriosus* using the herbicide Phenmedipham. *Chemosphere* 61:1102–1114.
- Anderson ORJ, Phillips RA, McDonald RA, Shore RF, McGill RAR, Bearhop S. 2009. Influence of trophic position and foraging range on mercury levels within a seabird community. *Mar Ecol Prog Ser* 375:277–288.
- Becker PR, Gunter EW, Schlüter C, Shibata Y, Wise SA. 2006. Environmental specimen banking. *J Environ Monitor* 8:776–778.
- Belfroid A, van Den Berg M, Seinen W, Hermens J, van Gestel K. 1995. Uptake bioavailability and elimination of hydrophobic compounds in earthworms (*Eisenia andrei*) in field-contaminated soil. *Environ Toxicol Chem* 14:605–612.
- Ben-David M, Flaherty EA. 2012. Stable isotopes in mammalian research: A beginner's guide. *J Mammal* 93:312–328.
- Berglund ÅMM, Rainio MJ, Eeva T. 2012. Decreased metal accumulation in passerines as a result of reduced emissions. *Environ Toxicol Chem* 31:1317–1323.
- Beyer WN, Connor EE, Gerould S. 1994. Estimates of soil ingestion by wildlife. *J Wildl Manage* 58:375–382.
- Blankenship AL, Zwiernik MJ, Coady KK, Kay DP, Newsted JL, Strause KD, Park C, Bradley PW, Neigh AM, Millsap SD, et al. 2005. Differential accumulation of polychlorinated biphenyl congeners in the terrestrial food web of the Kalamazoo River superfund site, Michigan. *Environ Sci Technol* 39:5954–5963.
- Borga K, Kidd KA, Muir DCG, Berglund O, Conder JM, Gobas FAPC, Kucklick J, Malm O, Powell DE. 2012. Trophic magnification factors: Considerations of ecology, ecosystems, and study design. *Integr Environ Assess Manag* 8:64–84.
- Braune BM, Mallory ML, Grant Gilchrist H, Letcher RJ, Drouillard KG. 2007. Levels and trends of organochlorines and brominated flame retardants in Ivory Gull eggs from the Canadian Arctic, 1976 to 2004. *Sci Total Environ* 378:403–417.
- Bustnes JO, Yoccoz NG, Bangjord G, Polder A, Skaare JU. 2007. Temporal trends (1986–2004) of organochlorines and brominated flame retardants in tawny owl eggs from Northern Europe. *Environ Sci Technol* 41:8491–8497.
- Carreon-Martinez L, Johnson TB, Ludsin SA, Heath DD. 2011. Utilization of stomach content DNA to determine diet diversity in piscivorous fishes. *J Fish Biol* 78:1170–1182.
- Chen Y, Giles KL, Payton ME, Greenstone MH. 2000. Identifying key cereal aphid predators by molecular gut analysis. *Mol Ecol* 9:1887–1898.
- Chung N, Alexander M. 2002. Effect of soil properties on bioavailability and extractability of phenanthrene and atrazine sequestered in soil. *Chemosphere* 48:109–115.
- Collins C, Fryer M, Grosso A. 2005. Plant uptake of non-ionic organic chemicals. *Environ Sci Technol* 40:45–52.
- Conder JM, Gobas FAPC, Borga K, Muir DCG, Powell DE. 2012. Use of trophic magnification factors and related measures to characterize bioaccumulation potential of chemicals. *Integr Environ Assess Manag* 8:85–97.
- Conder JM, Lanno RP. 1999. Heavy metal concentrations in mandibles of white-tailed deer living in the Picher Mining District. *B Environ Contam Toxicol* 63:80–86.
- Cornelissen G, Gustafsson O, Bucheli TD, Jonker MTO, Koelmans AA, van Noort PCM. 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ Sci Technol* 39:6881–6895.
- Corse E, Costedoat C, Chappaz R, Pech N, Martin J-F, Gilles A. 2010. A PCR-based method for diet analysis in freshwater organisms using 18S rDNA barcoding on faeces. *Mol Ecol Res* 10:96–108.
- Crosse JD, Shore RF, Jones KC, Pereira MG. 2013. Key factors affecting liver PBDE concentrations in sparrowhawks (*Accipiter nisus*). *Environ Pollut* 177:171–176.
- Crosse JD, Shore RF, Wadsworth RA, Jones KC, Pereira MG. 2012. Long-term trends in PBDEs in Sparrowhawk (*Accipiter nisus*) eggs indicate sustained contamination of UK terrestrial ecosystems. *Environ Sci Technol* 46:13504–13511.
- Custer CM. 2011. Swallows as sentinel species for contaminant exposure and effect studies. In: Elliott JE, Bishop CA, Morrissey CA, editors. *Wildlife ecotoxicology: Forensic approaches*. New York (NY): Springer. p 45–91.
- Dauwe T, Jaspers V, Covaci A, Schepens P, Eens M. 2005. Feathers as a nondestructive biomonitor for persistent organic pollutants. *Environ Toxicol Chem* 24:442–449.
- Deagle B, Chiaradia A, McInnes J, Jarman S. 2010. Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out? *Conserv Genet* 11:2039–2048.
- Debruyne AMH, Gobas F. 2006. A bioenergetic biomagnification model for the animal kingdom. *Environ Sci Technol* 40:1581–1587.
- D'Havé H, Scheirs J, Covaci A, Schepens P, Verhagen R, De Coen W. 2006. Nondestructive pollution exposure assessment in the European hedgehog (*Erinaceus europaeus*): III. Hair as an indicator of endogenous organochlorine compound concentrations. *Environ Toxicol Chem* 25:158–167.
- D'Havé H, Scheirs J, Covaci A, van den Brink NW, Verhagen R, De Coen W. 2007. Nondestructive pollution exposure assessment in the European hedgehog (*Erinaceus europaeus*): IV. Hair versus soil analysis in exposure and risk assessment of organochlorine compounds. *Environ Pollut* 145:861–868.
- Eens M, Jaspers VLB, van den Steen E, Bateson M, Carere C, Clergeau P, Costantini D, Dolenc Z, Elliott JE, Flux J, et al. 2013. Can starling eggs be useful as a biomonitoring tool to study organohalogenated contaminants on a worldwide scale? *Environ Int* 51:141–149.
- Elliott JE, Guertin DA, Balke JME. 2008. Chlorinated hydrocarbon contaminants in feces of river otters from the southern Pacific coast of Canada, 1998–2004. *Sci Tot Environ* 397:58–71.
- Elliott JE, Wilson LK, Wakeford B. 2005. Polybrominated diphenyl ether trends in eggs of marine and freshwater birds from British Columbia, Canada, 1979–2002. *Environ Sci Technol* 39:5584–5591.
- Espín S, Martínez-López E, Gómez-Ramírez P, María-Mojica P, García-Fernández AJ. 2010. Assessment of organochlorine pesticide exposure in a wintering population of razorbills (*Alca torda*) from the southwestern Mediterranean. *Chemosphere* 80:1190–1198.
- Eulaers I, Covaci A, Herzke D, Eens M, Sonne C, Moum T, Schnug L, Hanssen SA, Johnsen TV, Bustnes JO, et al. 2011. A first evaluation of the usefulness of feathers of nestling predatory birds for non-destructive biomonitoring of persistent organic pollutants. *Environ Int* 37:622–630.
- Fredricks T, Zwiernik M, Seston R, Coefield S, Plautz S, Tazelaar D, Shotwell M, Bradley P, Kay D, Giesy J. 2010. Passerine exposure to primarily PCDFs and

- PCDDs in the river floodplains near Midland, Michigan, USA. *Arch Environ Contam Toxicol* 58:1048–1064.
- García-Fernández AJ, Espin S, Martínez-López E. 2013. Feathers as a biomonitoring tool of polyhalogenated compounds: A review. *Environ Sci Technol* 47:3028–3043.
- Gobas FAPC, Burkhard LP, Doucette WJ, Sappington KG, Verbruggen EM, Hope BK, Bonnell MA, Arnot JA, Tarazona JV. 2015. Review of existing terrestrial bioaccumulation models and terrestrial bioaccumulation modeling needs for organic chemicals. *Integr Environ Assess Manag* 12:123–134.
- Gobas FAPC, de Wolf W, Burkhard LP, Verbruggen E, Plotzke K. 2009. Revisiting bioaccumulation criteria for POPs and PBT assessments. *Integr Environ Assess Manag* 5:624–637.
- Gottardo S, Hartmann N, Sokull-Kluttgen B. 2014. Review of available criteria for non-aquatic organisms in PBT/vPvB frameworks. Part 1: Bioaccumulation assessment, EUR-Scientific and Technical Research series. Luxembourg: European Commission Joint Research Centre.
- Guertin DA, Harestad AS, Ben-David M, Drouillard KG, Elliott JE. 2010. Fecal genotyping and contaminant analyses reveal variation in individual river otter exposure to localized persistent contaminants. *Environ Toxicol Chem* 29:275–284.
- Hajibabaei M, Smith MA, Janzen DH, Rodríguez JJ, Whitfield JB, Hebert PDN. 2006. A minimalist barcode can identify a specimen whose DNA is degraded. *Mol Ecol Note* 6:959–964.
- Hallanger IG, Warner NA, Ruus A, Evenset A, Christensen G, Herzke D, Gabrielsen GW, Borga K. 2011. Seasonality in contaminant accumulation in Arctic marine pelagic food webs using trophic magnification factors as measures of bioaccumulation. *Environ Toxicol Chem* 30:1026–1035.
- Harris ML, Wilson LK, Elliott JE, Bishop CA, Tomlin AD, Henning KV. 2000. Transfer of DDT and metabolites from fruit orchard soils to American robins (*Turdus migratorius*) twenty years after agricultural use of DDT in Canada. *Arch Environ Contam Toxicol* 39:205–220.
- Hebert CE, Weseloh DV, Kot L, Glooschenko V. 1994. Organochlorine contaminants in a terrestrial foodweb on the Niagara Peninsula, Ontario, Canada 1987–89. *Arch Environ Contam Toxicol* 26:356–366.
- Hebert CE, Weseloh DVC. 2006. Adjusting for temporal change in trophic position results in reduced rates of contaminant decline. *Environ Sci Technol* 40:5624–5628.
- Helgason LB, Polder A, Føreid S, Bæk K, Lie E, Gabrielsen GW, Barrett RT, Skaare JU. 2009. Levels and temporal trends (1983–2003) of polybrominated diphenyl ethers and hexabromocyclododecanes in seabird eggs from North Norway. *Environ Toxicol Chem* 28:1096–1103.
- Heywood E, Wright J, Wienburg CL, Black HJ, Long SM, Osborn D, Spurgeon DJ. 2006. Factors influencing the national distribution of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in British soils. *Environ Sci Technol* 40:7629–7635.
- Hobson KA, Alisauskas RT, Clark RG. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: Implications for isotopic analyses of diet. *Condor* 95:388–394.
- Hobson KA, Welch HE. 1992. Determination of trophic relationships within a high Arctic marine food web using delta-C13 and delta-N15 analysis. *Mar Ecol Prog Ser* 84:9–18.
- Hoke R, Huggett D, Brasfield S, Brown B, Embry M, Fairbrother A, Kivi M, Paumen ML, Prosser R, Salvito D, Scroggins R. 2015. Review of laboratory-based terrestrial bioaccumulation assessment approaches for organic chemicals: Current status and future possibilities. *Integr Environ Assess Manag* 12:95–108.
- Hop H, Borga K, Gabrielsen GW, Kleivane L, Skaare JU. 2002. Food web magnification of persistent organic pollutants in poikilotherms and homeotherms from the Barents Sea. *Environ Sci Technol* 36:2589–2597.
- Houde M, Bujas TAD, Small J, Wells RS, Fair PA, Bossart GD, Solomon KR, Muir DCG. 2006. Biomagnification of perfluoroalkyl compounds in the bottlenose dolphin (*Tursiops truncatus*) food web. *Environ Sci Technol* 40:4138–4144.
- Houde M, Martin JW, Letcher RJ, Solomon KR, Muir DCG. 2006. Biological monitoring of polyfluoroalkyl substances: A review. *Environ Sci Technol* 40:3463–3473.
- Jardine TD, Kidd KA, Fisk AT. 2006. Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. *Environ Sci Technol* 40:7501–7511.
- Jaspers VLB, Voorspoels S, Covaci A, Lepoint G, Eens M. 2007. Evaluation of the usefulness of bird feathers as a non-destructive biomonitoring tool for organic pollutants: A comparative and meta-analytical approach. *Environ Int* 33:328–337.
- Johnson MS, Korcz M, von Stackelberg K, Hope BK. 2009. Spatial analytical techniques for risk based decision support systems. In: Marcomini A, Suter GW II, Critto A, editors. Decision support systems for risk-based management of contaminated sites. New York (NY): Springer. p 75–93.
- Johnson MS, Salice CJ, Sample BE, Robidoux PY. 2009. Bioconcentration, bioaccumulation, and biomagnifications of nitroaromatic and nitramine explosives in terrestrial systems. In: Sunahara GI, Kupermand RG, Hawari J, editors. Ecotoxicology of explosives. Boca Raton (FL): CRC Press. p 227–252.
- Kelly BC, Gobas FAPC. 2001. Bioaccumulation of persistent organic pollutants in lichen-caribou-wolf food chains of Canada's Central and Western Arctic. *Environ Sci Technol* 35:325–334.
- Kelly BC, Gobas FAPC. 2003. An arctic terrestrial food-chain bioaccumulation model for persistent organic pollutants. *Environ Sci Technol* 37:2966–2974.
- Kelly BC, Ikononou MG, Blair JD, Morin AE, Gobas FAPC. 2007. Food web-specific biomagnification of persistent organic pollutants. *Science* 317:236–239.
- Kelly JF. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can J Zool* 78:1–27.
- King RA, Read DS, Traugott M, Symondson WOC. 2008. Molecular analysis of predation: A review of best practice for DNA-based approaches. *Mol Ecol* 17:947–963.
- Koch P. 2008. Isotopic study of the biology of modern and fossil vertebrates. In: Michener R, Laitha K, editors. Stable isotopes in ecology and environmental science. Boston (MA): Blackwell. p 99–155.
- Lopes I, Sedlmayr A, Moreira-Santos M, Moreno-Garrido I, Blasco J, Ribeiro R. 2010. European bee-eater (*Merops apiaster*) populations under arsenic and metal stress: Evaluation of exposure at a mining site. *Environ Mon Assess* 161:237–245.
- Mason CF, MacDonald SM, Bland HC, Ratford J. 1992. Organochlorine pesticide and PCB contents in otter (*Lutra lutra*) scats from western Scotland. *Water Air Soil Poll* 64:617–626.
- Matscheko N, Lundstedt S, Svensson L, Harju M, Tysklind M. 2002. Accumulation and elimination of 16 polycyclic aromatic compounds in the earthworm (*Eisenia fetida*). *Environ Toxicol Chem* 21:1724–1729.
- McLachlan MS, Czub G, MacLeod M, Arnot JA. 2011. Bioaccumulation of organic contaminants in humans: A multimedia perspective and the importance of biotransformation. *Environ Sci Technol* 45:197–202.
- Mellott RS, Woods PE. 1993. An improved ligature technique for dietary sampling in nestling birds. *J Field Ornithol* 64:205–210.
- Meusnier I, Singer GAC, Landry JF, Hickey DA, Hebert PDN, Hajibabaei M. 2008. A universal DNA mini-barcode for biodiversity analysis. *BMC Genom* 9:214.
- Meyer J, Jaspers VLB, Eens M, de Coen W. 2009. The relationship between perfluorinated chemical levels in the feathers and livers of birds from different trophic levels. *Sci Total Environ* 407:5894–5900.
- Mizutani H, Kabaya Y, Wada E. 1991. Nitrogen and carbon isotope composition relate linearly in cormorant tissues and its diet. *Isotopenpraxis* 27:166–168.
- Moermond CTA, Janssen MPM, de Knecht JA, Montforts MHMM, Peijnenburg WJGM, Zweers PGPC, Sijm DTHM. 2012. PBT assessment using the revised annex XIII of REACH: A comparison with other regulatory frameworks. *Integr Environ Assess Manag* 8:359–371.
- Müller CE, De Silva AO, Small J, Williamson M, Wang XW, Morris A, Katz S, Gamberg M, Muir DCG. 2011. Biomagnification of perfluorinated compounds in a remote terrestrial food chain: Lichen-caribou-wolf. *Environ Sci Technol* 45:8665–8673.
- Niemeyer J, Moreira-Santos M, Nogueira M, Carvalho G, Ribeiro R, Da Silva E, Sousa J. 2010. Environmental risk assessment of a metal-contaminated area in the Tropics. Tier I: Screening phase. *J Soil Sed* 10:1557–1571.
- Norstrom RJ, Hebert CE. 2006. Comprehensive re-analysis of archived herring gull eggs reconstructs historical trends in chlorinated hydrocarbon contamination in Lake Ontario and Green Bay, Lake Michigan, 1971–1982. *J Environ Monit* 8:835–847.
- Park J-S, Fong A, Chu V, Holden A, Linthicum J, Hooper K. 2011. Prey species as possible sources of PBDE exposures for peregrine falcons (*Falco peregrinus*) nesting in major California cities. *Arch Environ Contam Toxicol* 60:518–523.

- Parker KL, Barboza PS, Stephenson TR. 2005. Protein conservation in female caribou (*Rangifer tarandus*): Effects of decreasing diet quality during winter. *J Mammal* 86:610–622.
- Peterson BJ, Fry B. 1987. Stable Isotopes in Ecosystem Studies. *Annu Rev Ecol Syst* 18:293–320.
- Phillips DL, Newsome SD, Gregg JW. 2005. Combining sources in stable isotope mixing models: Alternative methods. *Oecologia* 144:520–527.
- Post DM. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* 83:703–718.
- Powell GVN. 1984. Reproduction by an altricial songbird, the red-winged blackbird, in fields treated with the organophosphate insecticide fenitrothion. *J Appl Ecol* 21:83–95.
- Sample BE, Aplin MS, Efroymson RA, Suter GW, Welsh CJ. 1997. Methods and tools for estimation of the exposure of terrestrial wildlife to contaminants, ORNL/TM-13391. Oak Ridge (TN): Oak Ridge National Laboratory. p 1–148.
- Sample BE, Cooper RJ, Whitmore RC. 1993. Dietary shifts among songbirds from a diflubenzuron-treated forest. *Condor* 95:616–624.
- Sample BE, Whitmore RC. 1993. Food habits of the endangered Virginia big-eared bat in West Virginia. *J Mammal* 74:428–435.
- Schipper AM, Wijnhoven S, Baveco JM, van den Brink NW. 2012. Contaminant exposure in relation to spatio-temporal variation in diet composition: A case study of the little owl (*Athene noctua*). *Environ Pollut* 163:109–116.
- Selck H, Drouillard KG, Eisenreich K, Koelmans AA, Palmqvist A, Ruus A, Salvito D, Schultz I, Stewart R, Weisbrod A, et al. 2012. Explaining variability of bioaccumulation measurements between laboratory and field using a modelling approach. *Integr Environ Assess Manag* 8:42–63.
- Sheppard SK, Bell J, Sunderland KD, Fenlon J, Skervin D, Symondson WOC. 2005. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. *Mol Ecol* 14:4461–4468.
- Sheppard SK, Harwood JD. 2005. Advances in molecular ecology: Tracking trophic links through predator-prey food-webs. *Funct Ecol* 19:751–762.
- Simonich SL, Hites RA. 1995. Global distribution of persistent organochlorine compounds. *Science* 269:1851–1854.
- Smit MD, Leonards PEG, van Hattum AGM, De Jongh AWJJ. 1994. PCBs in European otter (*Lutra lutra*) populations. Amsterdam, the Netherlands: Institute for Environmental Studies, Free Univ.
- Soininen EM, Valentini A, Coissac E, Miquel C, Gielly L, Brochmann C, Brysting AK, Sonstebo JH, Ims RA, Yoccoz NG, et al. 2009. Analysing diet of small herbivores: The efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. *Front Zool* 6:16.
- Styrishave B, Mortensen M, Krogh PH, Andersen O, Jensen J. 2008. Solid-phase microextraction (SPME) as a tool to predict the bioavailability and toxicity of pyrene to the springtail, *Folsomia candida*, under various soil conditions. *Environ Sci Technol* 42:1332–1336.
- Tomy G-T, Budakowski W, Halldorson T, Helm P-A, Stern G-A, Friesen K, Pepper K, Tittlemier S-A, Fisk A-T. 2004. Fluorinated organic compounds in an eastern Arctic marine food web. *Environ Sci Technol* 38:6475–6481.
- US Environmental Protection Agency. 1997. Ecological risk assessment guidance for Superfund sites. Washington (DC): USEPA. EPA-540-R-97-006.
- US Environmental Protection Agency. 2005. Guidance for developing ecological soil screening levels. Attachment 4.1—Exposure factors and bioaccumulation models for derivation of wildlife Eco-SSLs. Washington (DC): USEPA.
- Valentini A, Pompanon F, Taberlet P. 2009. DNA barcoding for ecologists. *Trends Ecol Evol* 24:110–117.
- van den Brink NW, Jansman HAH. 2006. Applicability of spraints for monitoring organic contaminants in free-ranging otters (*Lutra lutra*). *Environ Toxicol Chem* 25:2821–2826.
- van den Brink NW, Lammertsma DR, Dimmers WJ, Boerwinkel MC. 2011. Cadmium accumulation in small mammals: Species traits, soil properties and spatial habitat use. *Environ Sci Technol* 45:7497–7502.
- van den Brink NW, Ma WC. 1998. Spatial and temporal trends in levels of trace metals and PCBs in the European badger *Meles meles* (L. 1758) in the Netherlands: Implications for reproduction. *Sci Total Environ* 222:107–118.
- van den Brink NW, van Franeker JA, De Ruiter-Dijkman EM. 1998. Fluctuating concentrations of organochlorine pollutants during a breeding season in 2 Antarctic seabirds: Adelie penguin and southern fulmar. *Environ Toxicol Chem* 17:702–709.
- van den Steen E, Pinxten R, Jaspers VLB, Covaci A, Barba E, Carere C, Cichoń M, Dubiec A, Eeva T, Heeb P, et al. 2009. Brominated flame retardants and organochlorines in the European environment using great tit eggs as a biomonitoring tool. *Environ Int* 35:310–317.
- Vierke L, Stauda C, Biegel-Engler A, Drost W, Schulte C. 2012. Perfluorooctanoic acid (PFOA)—Main concerns and regulatory developments in Europe from an environmental point of view. *Environ Sci Eur* 24:16–26.
- Voorspoels S, Covaci A, Jaspers VLB, Neels H, Schepens P. 2007. Biomagnification of PBDEs in three small terrestrial food chains. *Environ Sci Technol* 41:411–416.
- Walker CH. 1980. Species variation in some hepatic microsomal enzymes that metabolize xenobiotics. In: Bridges JW, Chasseaud LF, editors. Progress in drug metabolism. New York (NY): John Wiley & Sons. p 113–164.
- Walker CH. 1983. Pesticides and birds—Mechanisms of selective toxicity. *Agric Ecosyst Environ* 9:211–226.
- Weisbrod AV, Woodburn KB, Koelmans AA, Parkerton TF, McElroy AE, Borga K. 2009. Evaluation of bioaccumulation using in vivo laboratory and field studies. *Integr Environ Assess Manag* 5:598–623.
- White JC, Peters R, Kelsey JW. 2007. Surfactants differentially impact p,p'-DDE accumulation by plant and earthworm species. *Environ Sci Technol* 41:2922–2929.
- Zaidi RH, Jaal Z, Hawkes NJ, Hemingway J, Symondson WOC. 1999. Can multiple-copy sequences of prey DNA be detected among the gut contents of invertebrate predators? *Mol Ecol* 8:2081–2087.
- Zeale MRK, Butlin RK, Barker GLA, Lees DC, Jones G. 2011. Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Mol Ecol Res* 11:236–244.