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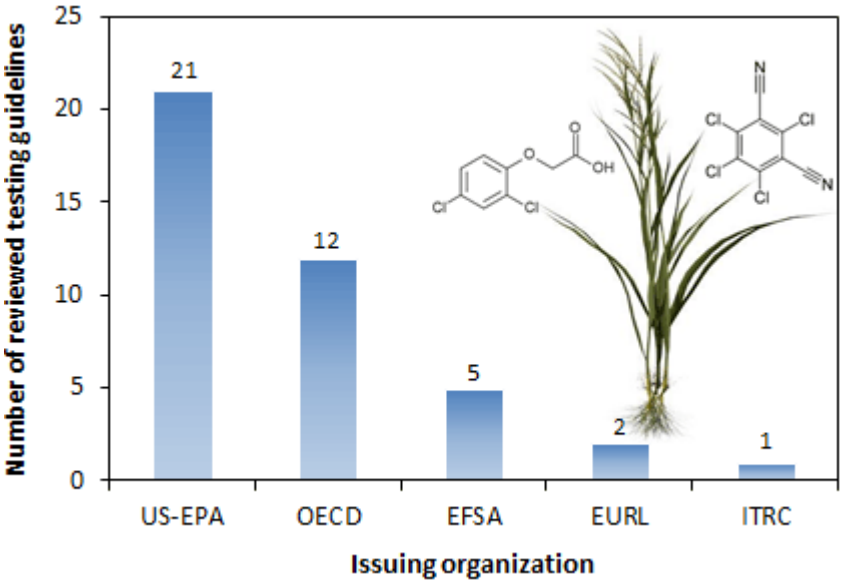

Abstract

Experimental data and models for plant bioaccumulation of organic contaminants play a crucial role for assessing the potential human and ecological risks associated with chemical use. Plants are receptor organisms and direct or indirect vectors for chemical exposures to all other organisms. As new experimental data are generated they are used to improve our understanding of plant-chemical interactions that in turn allows for the development of better scientific knowledge and conceptual and predictive models. The interrelationship between experimental data and model development is an ongoing, never-ending process needed to advance our ability to provide reliable quality information that can be used in various contexts including regulatory risk assessment. However, relatively few standard experimental protocols for generating plant bioaccumulation data are currently available and because of inconsistent data collection and reporting requirements, the information generated is often less useful than it could be for direct applications in chemical assessments and for model development and refinement. We review existing testing guidelines, common data reporting practices, and provide recommendations for revising testing guidelines and reporting requirements to improve bioaccumulation knowledge and models. This analysis provides a list of experimental parameters that will help to develop high quality datasets and support modeling tools for assessing bioaccumulation of organic chemicals in plants and ultimately addressing uncertainty in ecological and human health risk assessments.

Keywords: bioaccumulation modeling; biotransformation; plant uptake; organic contaminants; reporting requirements; testing guidelines

1. Introduction

Terrestrial plants constitute the largest global mass fraction of living organisms and are the primary food source for humans and most terrestrial animals (Houghton et al., 2009). Plants take up, translocate, transform, and accumulate organic chemicals that are not essential for plant growth and development (ITRC, 2011; U.S. EPA, 2012f), thereby contributing to the cycling of organic contaminants from local to global scales (Collins et al., 2011). Plants are subject to toxic effects from exposure to chemical stressors. Plants are also direct and indirect vectors for chemical exposures to higher trophic level organisms. Environmental concentrations and plant bioaccumulation (toxicokinetics) determine the likelihood for adverse effects to plants directly and to subsequent exposures and potential adverse effects to higher trophic level organisms. The extent of bioaccumulation is a function of substance-specific physicochemical properties, plant species-specific characteristics, and environmental conditions (Collins et al., 2011; Fantke et al., 2014; Trapp, 2015). Understanding plant uptake and bioaccumulation is crucial for a variety of regulatory applications including the authorization of formulations containing pesticides (EC, 2009) or biocides (EC, 1998), and for commercial chemicals falling under the REACH regulation (EC, 2006). Plant uptake has also been exploited to phytoremediate chemically contaminated sites and to delineate the extent of groundwater plumes using plants as biomonitors. The potential influence of plants in the overall fate and persistence of chemicals in the environment has been modelled at various scales but is largely unknown, particularly for chemicals that may be subject to degradation on or in plants (Cousins and Mackay, 2001; Undeman and McLachlan, 2011).

Experimentally, plant bioaccumulation data are collected from *in vivo* and *in vitro* studies. *In vivo* studies (field and greenhouse grown plants) usually focus on accumulation and dissipation from harvested plant components or whole plants and attempt to simulate realistic environmental conditions (Burrows et al., 2002). In contrast, *in vitro* studies (cell cultures) provide information on transport and degradation processes in plant cells under

66 controlled laboratory conditions (Schwitzguébel et al., 2011). Data from *in vivo* and *in vitro*
67 studies demonstrate the capacity of plants to biotransform and bioaccumulate a wide range of
68 organic contaminants (Bacci et al., 1990; Eggen et al., 2011; Fantke and Juraske, 2013; Jones
69 and Duarte-Davidson, 1997; Liu et al., 2009; Macherius et al., 2012; Mikes et al., 2009;
70 Samsøe-Petersen et al., 2002; Scheunert et al., 1994; Sharma et al., 2007; St-Amand et al.,
71 2007; Stahl et al., 2009; Willis and McDowell, 1987). For most chemical-plant species
72 combinations no experimental bioaccumulation and biotransformation data exist (Arnot et al.,
73 2013; Fantke et al., 2014) and in the few cases where data are available, the critical
74 information necessary to assess data reproducibility and interpretability are often lacking
75 (Fantke and Juraske, 2013).
76 Mathematical models are used to complement expensive and time-consuming
77 experimental studies for generalizing and extrapolating findings from specific experimental
78 scenarios and as input for decisions in exposure- and risk-related science-policy fields.
79 Models thereby show considerable potential for improving the basic understanding of
80 contaminant transport processes in plants (Gobas et al., 2016). In this study, we seek to help
81 identifying key test parameters that are required to improve the interpretation and evaluation
82 of plant bioaccumulation data, and to support the development, parameterization, application
83 and evaluation of plant bioaccumulation models.
84 We first review existing plant bioaccumulation testing guidelines and their reporting
85 requirements to identify whether information crucial for interpreting experimental data and
86 for supporting modeling is reported. Next, we give a brief overview of data that are essential
87 for developing and testing plant bioaccumulation models. Finally, we evaluate how data
88 reporting requirements in current test protocols can be improved to better support the
89 interpretation of experimental data and their use in plant bioaccumulation modeling. We will
90 thereby emphasize that reporting the most relevant additional data is usually feasible and does
91 not provide additional financial challenges. Overall, our study aims to improve the

understanding of plant bioaccumulation in support of various regulatory and non-regulatory applications.

2. Available bioaccumulation testing and data reporting guidelines

2.1. Existing guidelines and their scope

Current plant bioaccumulation testing guidelines were reviewed ($n = 41$) with focus on the following key question: Do the reporting recommendations in current testing guidelines include the key parameters needed to adequately interpret and quantify the experimental results and facilitate the use of measured data in models for risk and impact assessment? Guidelines were categorized according to their relevance for quantifying bioaccumulation and/or biotransformation in terrestrial plants via modeling approaches. High relevance is given when either plant uptake, accumulation or transformation/other removal was addressed in a quantitative way. Medium relevance is given when uptake, accumulation or transformation was addressed, but could not be quantitatively associated with chemical application (e.g. pesticides) or emission or when specifically residue sampling and analysis procedures were addressed. Low relevance is given when neither plant uptake, accumulation or transformation was the focus, but when other metrics associated with plant bioaccumulation were addressed, such as crop damage, human contact levels (as input for occupational exposure), or transformation products in soil that could enter the plant.

Few guidelines from national and international organizations address one or more aspects involved in the testing of uptake, translocation and bioaccumulation of chemicals in plants as listed in Table 1. The U.S. Environmental Protection Agency (US-EPA) established a series of ecological effect, residue chemistry, fate, transport and transformation, as well as occupational and residential exposure test guidelines developed by the Office of Chemical Safety and Pollution Prevention and the Office of Prevention, Pesticides and Toxic Substances for use in the experimental testing of chemicals (U.S. EPA, 1996a, b, c, d, e, f, g,

h, i, j, k, l, m, 2008a, b, 2012a, b, c, d, e, f). The EU Reference Laboratories for Residues of Pesticides (EURL) and the European Food Safety Authority (EFSA) developed guidelines focusing on the sampling of plants in the frame of pesticide residue testing (EFSA, 2010, 2012, 2013, 2014a, b; EU RLRP, 2011, 2013). The Interstate Technology and Regulatory Council (ITRC) provides guidelines for the evaluation of contaminated sediment sites, thereby also addressing plant uptake (ITRC, 2011). Finally the Organisation for Economic Co-operation and Development (OECD) established several guidelines for the testing of chemicals for use in studies measuring the distribution of chemicals in the plant environment (OECD, 1992, 1997, 2002, 2006a, b, 2007a, b, c, d, e, 2008, 2009).

Table 1 Existing guidelines and standards for different contexts of testing bioaccumulation of chemicals in plants, plant environments and plant-based commodities, and their relevance for quantification of bioaccumulation and/or biotransformation in terrestrial plants.

Issuing organization	Guideline	Purpose	Relevance
U.S. Environmental Protection Agency	Fate, Transport and Transformation Test Guidelines: Terrestrial Field Dissipation, OPPTS 835.6100 (U.S. EPA, 2008a)	Plant uptake is assessed as one of several field dissipation pathways; restricted to pesticides; bioaccumulation or biotransformation in plants not considered	Medium
	Fate, Transport and Transformation Test Guidelines: Forestry Dissipation, OPPTS 835.6300 (U.S. EPA, 2008b)	Uptake into tree litter assessed as one of several field dissipation pathways; restricted to bioaccumulation in tree litter, soil and water	High
	Ecological Effects Test Guidelines: Background and Special Considerations - Tests with Terrestrial and Aquatic Plants, Cyanobacteria, and Terrestrial Soil-Core Microcosms, OCSPP 850.4000 (U.S. EPA, 2012a)	Exposure damage to plants and other organisms including non-target plants is assessed; quantitative bioaccumulation or biotransformation not considered	Low
	Ecological Effects Test Guidelines: Seedling	Effects of substances on plants during early critical development	Low

Issuing organization	Guideline	Purpose	Relevance
	Emergence and Seedling Growth, OCSPP 850.4100 (U.S. EPA, 2012b)	stages are measured; quantitative bioaccumulation or biotransformation not considered	
	Ecological Effects Test Guidelines: Vegetative Vigor, OCSPP 850.4150 (U.S. EPA, 2012c)	Effects of foliar applied substances on plants during vegetative growth are measured; restricted to spray application (i.e. not applicable for root uptake); quantitative bioaccumulation or biotransformation not considered	Low
	Ecological Effects Test Guidelines: Early Seedling Growth Toxicity Test, OCSPP 850.4230 (U.S. EPA, 2012d)	Data on the phytotoxicity of substances are provided; quantitative bioaccumulation or biotransformation not considered	Low
	Ecological Effects Test Guidelines: Terrestrial Plants Field Study, OCSPP 850.4300 (U.S. EPA, 2012e)	Field experiments with focus on plant damage are conducted; bioaccumulation or biotransformation not considered	Low
	Ecological Effects Test Guidelines: Plant Uptake and Translocation Test, OCSPP 850.4800 (U.S. EPA, 2012f)	Data on the quantity of substances incorporated in plant tissues and the potential for entry into food chains are provided; consideration of quantitative plant uptake and bioaccumulation	High
	Residue Chemistry Test Guidelines: Nature of the Residue – Plants, Livestock, OPPTS 860.1300 (U.S. EPA, 1996d)	Qualitative metabolic fate of an active ingredient applied to a plant is assessed; quantitative bioaccumulation or biotransformation not considered; restricted to pesticides	High
	Residue Chemistry Test Guidelines: Residue Analytical Method, OPPTS 860.1340 (U.S. EPA, 1996e)	Analytical methods are tested to determine all components of the total toxic residue; quantitative bioaccumulation or biotransformation not considered	Medium
	Residue Chemistry Test Guidelines: Multiresidue Method, OPPTS 860.1360 (U.S. EPA, 1996f)	Analytical methods are applied to confirm the presence or absence of many pesticides and their metabolites in commodities; quantitative bioaccumulation or biotransformation not considered	Low
	Residue Chemistry Test Guidelines: Storage Stability Data, OPPTS 860.1380 (U.S. EPA, 1996g)	Stability or decomposition rate of total toxic residue in or on raw/processed agricultural commodity between harvest or	Medium

Issuing organization	Guideline	Purpose	Relevance
		sample collection and analysis are validated; quantitative bioaccumulation or biotransformation not considered	
	Residue Chemistry Test Guidelines: Water, Fish, and Irrigated Crops, OPPTS 860.1400 (U.S. EPA, 1996h)	Levels of pesticide residues are assessed in water, fish, and irrigated crops; restricted to application to water to control aquatic pests; restricted to pesticides	High
	Residue Chemistry Test Guidelines: Crop Field Trials, OPPTS 860.1500 (U.S. EPA, 1996i)	Magnitude of pesticide residues are assessed in or on raw agricultural commodities; designed for field experiments, but restricted to pesticides	High
	Residue Chemistry Test Guidelines: Processed Food/Feed, OPPTS 860.1520 (U.S. EPA, 1996j)	It is assessed whether residues in raw commodities may be expected to degrade or concentrate during food processing (i.e. not applicable for plant uptake); restricted to time after harvest, restricted to pesticides	High
	Residue Chemistry Test Guidelines: Proposed Tolerances, OPPTS 860.1550 (U.S. EPA, 1996k)	Tolerance levels are obtained based on maximum residues during field trials; quantitative bioaccumulation or biotransformation not considered	Low
	Residue Chemistry Test Guidelines: Confined Accumulation in Rotational Crops, OPPTS 860.1850 (U.S. EPA, 1996l)	Nature and amount of pesticide residue uptake in rotational crops are assessed; restricted to pesticides	High
	Residue Chemistry Test Guidelines: Field Accumulation in Rotational Crops, OPPTS 860.1900 (U.S. EPA, 1996m)	Amount of pesticide residue uptake in rotational crops is assessed under actual field-use conditions; restricted to pesticides	High
	Occupational and Residential Exposure Test Guidelines: Background for Post-application Exposure Monitoring Test Guidelines, OPPTS 875.2000 (U.S. EPA, 1996a)	Time necessary is assessed for pesticide residues at the treated site to decline to allowable human reentry levels (i.e. not applicable for plant uptake); restricted to pesticides	Low

Issuing organization	Guideline	Purpose	Relevance
	Occupational and Residential Exposure Test Guidelines: Foliar Dislodgeable Residue Dissipation, OPPTS 875.2100 (U.S. EPA, 1996b)	Pesticide residues are assessed which are deposited on and remain on surfaces after pesticide application (i.e. not applicable for plant uptake); restricted to pesticides	Medium
	Occupational and Residential Exposure Test Guidelines: Data Reporting and Calculations, OPPTS 875.2900 (U.S. EPA, 1996c)	Detectable dislodgeable residues are assessed of the pesticide on surfaces to which the pesticide was applied (i.e. not applicable for plant uptake); restricted to pesticides	Low
EU Reference Laboratories for Residues of Pesticides	Method Validation & Quality Control Procedures for Pesticide Residues Analysis in Food & Feed, SANCO/12495/2011 (EU RLRP, 2011)	Sampling procedure is evaluated as part of laboratory tests (i.e. not applicable for plant uptake or transformation tests); restricted to pesticides	Medium
	Guidance Document on Analytical Quality Control and Validation Procedures for Pesticide Residues Analysis in Food and Feed, SANCO/12571/2013 (EU RLRP, 2013)	Sampling procedure and sampling quality control are evaluated as part of laboratory tests (i.e. not applicable for plant uptake or transformation tests); restricted to pesticides	Medium
European Food Safety Authority	Standard Sample Description for Food and Feed (EFSA, 2010)	Sampling procedure is evaluated as part of laboratory tests (i.e. not applicable for plant uptake or transformation tests); restricted to pesticides	Medium
	Use of the EFSA Standard Sample Description for the Reporting of Data on the Control of Pesticide Residues in Food and Feed According to Regulation (EC) No 396/2005; including revision 1 and version 2013 data collection (EFSA, 2012, 2013, 2014b)	Sampling procedure is evaluated part of laboratory tests for the reporting of the national results of the pesticide monitoring (i.e. not applicable for plant uptake or transformation tests); restricted to pesticides	Medium
	EFSA Guidance Document for Evaluating Laboratory and Field Dissipation Studies to Obtain DegT50 Values of Active Substances of Plant Protection Products and Transformation Products of	Degradation rates of active substances and transformation products in soil are assessed and crop interception values are selected (i.e. not applicable for plant uptake or transformation tests); restricted to pesticides	Low

Issuing organization	Guideline	Purpose	Relevance
	these Active Substances in Soil (EFSA, 2014a)		
Interstate Technology and Regulatory Council	Incorporating Bioavailability Considerations into the Evaluation of Contaminated Sediment Sites (ITRC, 2011)	Plant uptake is assessed as one out of several considered pathways of sediment dissipation; bioaccumulation or biotransformation in plants not considered	Medium
Organisation for Economic Co-operation and Development	OECD Guidelines for the Testing of Chemicals, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, Test no. 208 (OECD, 2006a)	Negative effects on seedling emergence and growth are assessed; restricted to soil application (i.e. not applicable for foliar uptake); bioaccumulation or biotransformation not considered	Low
	OECD Guidelines for the Testing of Chemicals, Terrestrial Plant Test: Vegetative Vigour Test, Test no. 227 (OECD, 2006b)	Negative effects on vegetative vigor of plants are assessed; restricted to spray application (i.e. not applicable for root uptake); bioaccumulation or biotransformation not considered	Low
	OECD Guidelines for the Testing of Chemicals: Ready Biodegradability, Test no. 301 (OECD, 1992)	Chemicals are screened for ready biodegradability in an aerobic aqueous medium; not applicable for plant uptake or plant tissue sample testing	Medium
	OECD Guidelines for the Testing of Chemicals: Aerobic and Anaerobic Transformation in Soil, Test no. 307 (OECD, 2002)	Aerobic and anaerobic transformation in soil is evaluated; includes formation and decline of transformation products (i.e. not applicable for plant uptake or transformation tests)	Low
	OECD Guidelines for the Testing of Chemicals: Metabolism in Crops, Test no. 501 (OECD, 2007a)	Total radioactive residues, transformation products and pathways are estimated in crops after treatment; rates of uptake and degradation not considered; intended for pesticides	High
	OECD Guidelines for the Testing of Chemicals: Metabolism in Rotational Crops, Test no. 502 (OECD, 2007b)	Potential of chemicals and their soil transformation products to accumulate in rotational crops is assessed; restricted to pesticides	High
	OECD Guidelines for the Testing of Chemicals:	Residues from accumulation in rotational crops via soil uptake	High

Issuing organization	Guideline	Purpose	Relevance
	Residues in Rotational Crops, Limited Field Studies, Test no. 504 (OECD, 2007c)	under field conditions are assessed; restricted to pesticides	
	OECD Guidelines for the Testing of Chemicals: Stability of Pesticide Residues in Stored Commodities, Test no. 506 (OECD, 2007d)	Stability time period in crop commodities is analyzed between sampling and analysis (i.e. not applicable for plant uptake or transformation tests)	Low
	OECD Guidelines for the Testing of Chemicals: Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis, Test no. 507 (OECD, 2007e)	Magnitude of residues in processed food commodities is assessed (compared to raw agricultural commodities); restricted to post-harvest processes	Medium
	OECD Guidelines for the Testing of Chemicals: Magnitude of the Pesticide Residues in Processed Commodities, Test no. 508 (OECD, 2008)	Distribution of residues of active ingredients and degradation products is quantified in processed commodities resulting from processing; not applicable for plant uptake; restricted to post-harvest processes	High
	OECD Guidelines for the Testing of Chemicals: Crop Field Trial, Test no. 509 (OECD, 2009)	Magnitude of residues is assessed in or on raw agricultural commodities and dissipation rate after field application; restricted to pesticides	High
	OECD Series on Testing and Assessment, Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides During Agricultural Application, OCDE/GD(97)148 (OECD, 1997)	Worker exposure during and after field application of pesticides is assessed (i.e. not applicable for plant uptake or transformation tests); restricted to pesticides	Low

131

132 2.2. The role of data reporting requirements

133 Most test guidelines provide general reporting recommendations for test species,
134 pathway analysis and sample extraction. Of these, some US-EPA and OECD guidelines
135 provide a good starting point to improve the quantitative understanding of bioaccumulation
136 and biotransformation in plants. These guidelines focus on general bioaccumulation in plant

tissue (U.S. EPA, 2012f), biotransformation in crops (OECD, 2007a) and rotational crops (OECD, 2007b), accumulation of pesticides in rotational crops under confined (U.S. EPA, 1996l) and actual field conditions (OECD, 2007c; U.S. EPA, 1996m), and residual pesticide concentrations and biotransformation after harvest in raw (OECD, 2009; U.S. EPA, 1996i) and processed agricultural crop-based commodities (OECD, 2008; U.S. EPA, 1996j). EURL provides extensive reporting guidelines for sampling of pesticide residues in plants (EU RLRP, 2011, 2013). A key limitation is that none of the existing guidelines discusses or provides guidance on how to further use the experimental data (study conditions, measurement results, etc.) to support plant bioaccumulation modeling that is used to complement experimental data in several science-policy fields. Further, except EURL (EU RLRP, 2011, 2013), existing guidelines do not provide information on how to determine uncertainty associated with measurement, sampling and analytical tools with respect to a standardized interpretability of different testing designs and with respect to reporting measurement uncertainty.

All in all, there is no existing testing guideline that provides sufficient information of how to consistently report and interpret testing data or how to use experimental results as such and as input in plant bioaccumulation models applied in regulation and decision support. Most importantly, guidelines do not include requirements for relevant plant and exposure medium characteristics, relevant environmental condition parameters, and applied formulation and substance properties, although most of these aspects can be readily obtained and do not require additional experimental equipment. As a consequence, current data reporting gaps in experimental testing studies and underlying guidelines are recognized important limitations in plant bioaccumulation models (Arnot et al., 2013; Environment Agency, 2006; Fantke and Juraske, 2013; Fryer and Collins, 2003; Gobas et al., 2016; McKone and Maddalena, 2007). However, several existing guidelines already provide a good starting point in terms of data reporting requirements and these guidelines could be slightly modified to provide critical

information that could be used to improve plant bioaccumulation modeling. For that, it is important to understand the data that are required in bioaccumulation modeling, which is outlined in the following.

3. Plant bioaccumulation models and their application

3.1. Framework for plant bioaccumulation modeling

Mathematical models are often used to better understand experimental data obtained under defined test conditions. Models also help the extrapolation of experimental data from defined test conditions to specific environmental scenarios in an attempt to address various regulatory questions. Key processes described in plant bioaccumulation models are direct application onto the plant (e.g. agricultural pesticide applications), gaseous and dry/wet particle deposition from air onto cuticles, evaporation from cuticles and transpiration through leaf stomata, root uptake with soil pore water, diffusion between soil gas and root phases, chemical and microbial transformation in plant tissue, chemical partitioning between tissues and phases, as well as translocation with xylem transpiration and phloem assimilation streams. Furthermore, re-volatilization from soil, leaching toward groundwater, soil surface run-off, wash-off from plants, wind-drift in air and plant growth are often modeled processes influencing the distribution and accumulation of chemicals in plants. Detailed process descriptions are found elsewhere (Collins et al., 2006; Collins et al., 2011; Fantke et al., 2011; Riederer, 1990; Trapp and Legind, 2011; Trapp and Mc Farlane, 1995). Different types of plant bioaccumulation models are described elsewhere (e.g. Gobas et al., 2016).

Models are generally not accepted until they can be evaluated using results from tests collected under a variety of conditions. Most models rely on measured data from field and laboratory tests with respect to various input variables (e.g. air temperature, plant water content) and process-related parameters (e.g. degradation rates in plant components), depending on each model's scope and level of detail. Fig. 1 shows conceptually how key

uptake, partitioning, translocation and degradation processes measured in experimental plant bioaccumulation tests (Fig. 1a) can be translated into modeled systems based on interconnected environmental and plant compartments (Fig. 1b).

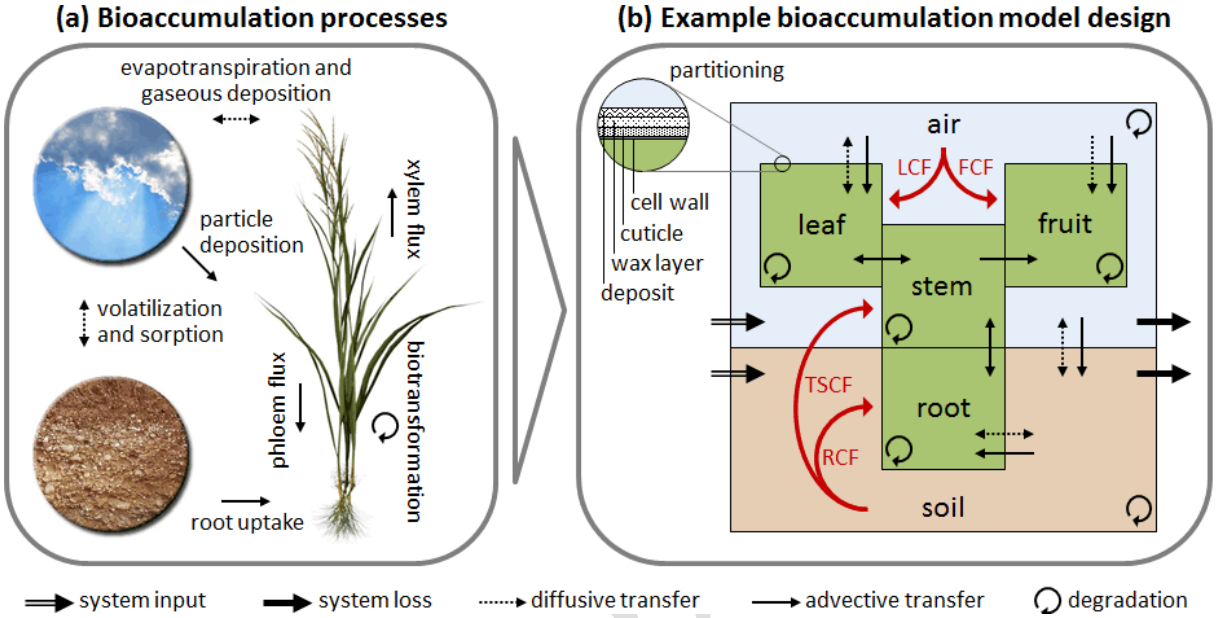

Fig. 1 – Schematic representation of main processes relevant in plant bioaccumulation studies (a) and their representation in a mechanistic plant bioaccumulation models (b): Red arrows indicate steady-state concentration ratios between leaf (LCF), fruit (FCF), stem (TSCF), root (RCF) and external solution, respectively; black arrows indicate process rates.

3.2. Input data requirements

Typically, when doing experiments more data are collected then reported in experimental plant bioaccumulation studies, often because it is not clear which of the measured data are in fact useful as relevant aspects for decisions and/or as input for models. To address the latter, the present section provides insight into typical input data requirements for plant bioaccumulation models.

In a typical mass balance model (Fig. 1b), bioaccumulation of a chemical is the net result of competing uptake and elimination processes. Plants take up chemicals from air (via

aerial surfaces, predominantly leaves) and soil (via roots). Elimination of chemicals from plants includes losses to the environment (e.g. volatilization), losses due to plant growth (biodilution), and degradation within plants. To quantify these processes, input data are required for each level of model detail and scope. For example, to estimate chemical uptake through the air-leaf interface, a simple model might require the leaf concentration factor (LCF, Fig. 1b) defined as the concentration ratio in leaf and in air at equilibrium (Calamari, 1993). In contrast, a more complex model might quantify each competitive process contributing to leaf uptake, such as dry and wet deposition, as a function of particle concentration, aerosol washout and rain occurrence in air (Fantke et al., 2011), diffusion through the leaf-air boundary layer derived from stomatal and cuticular resistances (Schreiber and Schönherr, 2009), and concentration dilution as function of plant growth rates. In any case, specific input variables must be given to model plant uptake. If these input variables cannot be estimated based on e.g. available regressions, models rely on experimental studies to obtain required input data. Input variables that are reported in 25 plant uptake modeling studies to strongly affect bioaccumulation processes and that typically have to be obtained from experimental testing studies are listed in Table 2.

Partition coefficients K_{OW} and K_{AW} along with half-lives in plants are by far the substance properties most frequently reported to be relevant for plant bioaccumulation modeling followed by molecular mass and pKa. Most frequently reported plant characteristics are plant lipid and water contents, growth rates, and xylem flow (transpiration stream). Air temperature and soil organic carbon (OC) content are the most frequently reported environmental conditions relevant for plant bioaccumulation modeling along with scenario-specific time between substance application (e.g. in case of intentionally applied pesticides) and plant harvest. Many additional parameters are less frequently reported to be relevant (see Table 2). This demonstrates that generally multiple parameters are required as input for bioaccumulation models including substance properties, plant characteristics, and

environmental and scenario conditions – most of these parameters need to be provided by experimental testing studies.

Table 2 Relevant input parameters identified in 25 plant bioaccumulation models.

Key parameters		Plant bioaccumulation modeling studies																								
		a	b	c	d	e	f	g	h[(1)]	i	j	k	l	m[(1)]	n[(1)]	o	p[(1)]	q	r	s	t	u	v	w	y	z
substance properties	Molecular mass		x			x		x	x													x				
	Vapor pressure	x							x																	
	pKa	x	x																x				x			
	K_{OW}		x	x		x	x	x	x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x
	K_{AW}		x	x								x			x		x			x	x		x		x	x
	K_{OC}						x														x					
	Half-life in plant					x	x	x		x	x	x	x			x							x			
	Half-life in soil						x																			
plant characteristics	Plant mass															x										
	Plant temperature																								x	
	Leaf area index											x											x	x		
	Leaf thickness	x																								
	Plant conductance																						x	x		
	Plant growth rate		x									x						x		x			x	x	x	
	Plant lipid content		x		x						x		x	x	x					x		x	x			
	Plant pH	x																	x				x			
	Plant water content				x								x	x	x							x	x	x		
	Plant xylem flow															x				x			x	x	x	x
environmental conditions	Soil mass															x										
	Air temperature								x				x	x	x	x	x						x		x	
	Air humidity												x													
	Soil OC content		x				x													x		x		x		
	Soil pH												x										x			
	Soil water content																			x				x		
	Sunlight				x																					
	Wind speed																								x	
	Time to harvest[(2)]						x	x		x		x						x					x		x	

[a]Buchholz and Trapp (2015); [b]Collins et al. (2011); [c]Czub and McLachlan (2004); [d]Doucette et al. (2005); [e]Fantke et al. (2011); [f]Fantke et al. (2012); [g]Fantke et al. (2013); [h]Fantke et al. (2014); [i]Fantke and Jolliet (2016); [j]Fryer and Collins (2003); [k]Jacobsen et al. (2015); [l]Juraske et al. (2008); [m]Kömp and McLachlan (1997b); [n]Kömp and McLachlan (1997a); [o]Legind et al. (2011); [p]McLachlan (1995); [q]Rein et al. (2011); [r]Rendal et al. (2011); [s]Takaki et al. (2014); [t]Trapp et al. (1990); [u]Trapp et al. (2007); [v]Trapp and Legind (2011); [w]Trapp (2015); [y]Undeman et al. (2009); [z]Undeman and McLachlan (2011).

[1]Studies refer to "plant characteristics" in general as key aspect influencing bioaccumulation.

[2]Specific for chemicals applied in pulses to plants, such as pesticides.

Not all data that are summarized in Table 2 as being relevant for bioaccumulation models are commonly reported in experimental studies. We seek to identify and close gaps between data provided by studies following current testing guidelines and data required for improving plant bioaccumulation science by adapting current experimental methods (and reporting requirements). In most cases these gaps can be addressed with minimal additional resources.

4. Current practice in plant bioaccumulation testing

4.1. Reviews of experimental plant bioaccumulation studies

Experimental plant bioaccumulation tests are usually conducted under well-defined environmental conditions (field and greenhouse studies) or under controlled conditions (laboratory studies). Laboratory studies are usually carried out at 25°C and 14 hours light cycle. Plants are exposed to known substance concentrations applied as a pulse or continuously over a certain time period; one example of significant differences in exposure design. Plants and the exposure media (soils or hydroponic solutions) are sampled at different times during and after exposure, but at least once at the end of the experimental period. Concentrations of contaminants are normally reported for plants and soil/hydroponic solution.

To highlight the state of science in experimental plant bioaccumulation testing, we summarize key findings from two recent compilations of experimental data. The first compilation focuses on plant bioaccumulation studies published in the peer-reviewed literature for a broad range of chemical classes including PAHs, legacy pesticides, current use pesticides (CUPs), PCBs, polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs), pharmaceutical and veterinary chemicals and others (Arnot et al., 2013). This review focused on key words pertaining to quantitative metrics of plant bioaccumulation, such as “bioconcentration factor” (BCF), “root concentration factor” (RCF), “transpiration stream concentration factor” (TSCF), and other plant/exposure medium-based metrics as well

as plant uptake and biotransformation rate constants. The resulting dataset includes 3,644 unique entries for 358 chemicals from 166 scientific references. Only 11 of the 166 studies included any mention of plant biotransformation and only 3 of the 11 included biotransformation rate information (for lindane and a series of phenols). Proximate composite analysis of the plants (i.e., lipid contents, water contents) was reported in only about 10% of all studies.

The second compilation focuses on experimentally derived pesticide dissipation half-lives in plants obtained from key word searches with regard to "dissipation", "persistence" and "degradation" of pesticides in plants or certain plant components. This compilation identified 4,513 unique data points for 346 substances applied to almost 200 different plant species collected from 811 scientific references (Fantke and Juraske, 2013). Key points are to analyze the variability across substances, plant species and harvested plant components as well as to discuss different substance, vegetation and environmental aspects influencing pesticide dissipation kinetics. Only 18% of all reviewed references assessed one or more of these aspects, such as the influence of temperature on pesticide dissipation from plants. Furthermore, most reported data regarding substance (e.g. purity), plant characteristics (e.g. growth stage), application and sampling settings (e.g. treated plant components), and environmental conditions (e.g. air humidity) were incomplete (see Table 3 for an example).

4.2. Limitations of reported data for use in bioaccumulation modeling

Screening various experimental studies reveals there are few parameters that are consistently reported, such as the sampled plant component and the substance application rate (typically for pesticide treatment) or assumed exposure concentrations (typically for non-pesticide contaminants). In contrast, many parameters considered essential for interpreting experimental data and serving as important input for plant bioaccumulation models are infrequently reported, such as mean air temperature, substance fraction that is intercepted by

plants or water content of the sampled plant components or soil characteristics. To demonstrate differences in reporting data, we compared six studies that assessed the same substance-plant species combination, namely cypermethrin applied to eggplant, and analyzed residues in the same sampled plant components, i.e. eggplant fruits (Arora, 2009; Kaur et al., 2011; Lu, 2011; Mukherjee et al., 2012; Sinha and Gopal, 2002; Walia et al., 2010). Data reported in each of the compared studies are summarized in Table 3.

Table 3 Comparison of data reported in experimental plant bioaccumulation test studies analyzing the same combination of chemical, plant species, and plant component, i.e. cypermethrin residues measured in sampled eggplant fruits. For full parameter descriptions see Table 4.

Parameter	Reported in experimental testing study					
	a	b	c	d	e	f
Study location(s)	✓[1]	✓[1]	✓[1]	✓[1]	✓[1]	✓[1]
Study year(s)		✓		✓		
Study characteristics	✓		✓	✓		
Application rate	✓	✓	✓	✓	✓	✓
Application date (or days after planting)		✓				
Application duration					✓	
Application type	✓[2]	✓[2]	✓[2]	✓[2]		✓[2]
Treated component			✓			
Formulation	✓	✓	✓	✓		✓
Substance purity	✓		✓			✓
Relative air humidity	✓	✓				
Rain rate	✓[3]	✓				
Wind speed		✓				
Air temperature	✓	✓				
Binomial plant name (including variety)	✓	✓	✓	✓	✓[4]	✓
Plant growth period		✓		✓		
Plant stage	✓			✓		✓
Planting density	✓	✓				
Sampled component	✓	✓	✓	✓	✓	✓
Sampled mass	✓	✓	✓	✓	✓	✓
Sampling date(s)/time(s)	✓	✓	✓	✓		✓
Sampling specifics	✓		✓	✓	✓	✓
Residue analysis setup	✓[5]	✓	✓	✓[5]	✓	✓[5]
Analysis temperature(s)	✓	✓	✓	✓		✓

Parameter	Reported in experimental testing study					
	a	b	c	d	e	f
Solvents used	✓	✓	✓	✓	✓	✓
Fate processes studied			✓	✓	✓	
Kinetic models used	✓					✓

[a]Sinha and Gopal (2002); [b]Arora (2009); [c]Walia et al. (2010); [d]Kaur et al. (2011); [e]Lu (2011); [f]Mukherjee et al. (2012).

[1]Reported in a way that does not allow deriving exact geographical coordinates; [2]Application height not given; [3]Total rainfall (mm) during experiment given, but explicit duration of experiment not stated; [4]Variety not given; [5]Analytical limits of detection not given.

Many aspects of the sampling and analysis methods are reported by all studies compared in Table 3. In contrast, several key parameters considered as important input to plant bioaccumulation models and required by existing testing guidelines, are not consistently reported (e.g. pesticide application dates, treated plant components, air temperature and relative humidity, plant growth stage during treatment and at sampling times), or not reported by any study (e.g. substance CAS number, pH of soil or hydroponic solution, plant root to shoot ratio, plant leaf area index). Inconsistent collection or presentation of data makes it difficult to use or compare results from different studies. For non-pesticide chemicals, there are generally even less data reported, because testing requirements are less stringent (Arnot et al., 2013). The inconsistency of key bioaccumulation information reported in the literature is primarily because studies either do not follow any official guideline or they do not comply with reporting recommendations when following existing guideline.

5. Toward consistent bioaccumulation testing data sets

5.1. Sampled plant components

With respect to harvested plant samples, most modeling approaches either require information on individual plant components, such as leaves, fruits, roots, etc. (Fantke et al., 2011; Trapp and Legind, 2011), or specific component parts or tissues like fruit peel, fruit pulp, epicuticular wax, nectar, etc. (Satchivi et al., 2006). In contrast, composite plant parts

(straw, shoot, etc.) are often mixed and homogenized before analysis, thus assigning chemical quantities in individual interconnected components is usually impossible. The best case scenario is when sampled plant components are well distinguished and terms like “rind” or “fruit-surface” are avoided as these are difficult to allocate to specific plant components. As an example of good practice, using “bark” or “peel” are unambiguous terms referring to specific plant components.

To get the maximum benefit from an experimental study, we recommend to separately sample and report plant components and to provide a description of each sampled component. However, when facing sample mass limitations, i.e. not enough mass of specific components or tissue is sampled to allow a proper analysis, the reporting focus should be on the tissue or component that is most relevant for subsequent exposure studies, such as fruits harvested for human or animal consumption. This would require consistently describing each sampled component in terms of sampled mass and composition (e.g. water content). Moreover, we recommend reporting not only the day of sampling, but also the day of planting or at least the different plant component growth stages at sampling time, such as flowering. This does not require additional equipment, but provides important information about for example growth dilution.

5.2. Considered (fate) processes

Most experimental studies measure overall dissipation from plant samples (Braun et al., 1980; Galietta et al., 2011; Lee and Cheng, 1983; Willis and McDowell, 1987) or focus on particular dissipation processes, such as volatilization (Bedos et al., 2010; Guth et al., 2004; Kubiak et al., 1995; Stork et al., 1998), photodecomposition (Burrows et al., 2002; Katagi, 2004; McCrady and Maggard, 1993) or microbial degradation (Azaizeh et al., 2011; Quistad et al., 1974; Roy et al., 2001). However, whereas this might be sufficient to ensure compliance with regulatory thresholds for plant uptake and bioaccumulation, it does not help

to understand bioaccumulation mechanisms as relevant in other science-policy fields. Moreover, mechanistic models typically rely on information of all contributing dissipation processes to arrive at a complete set of rate coefficients as input (Fantke et al., 2014). Such processes include dry and wet deposition, advective root and foliar uptake, volatilization (gas-exchange), wash-off from plant surfaces, chemical concentration dilution due to plant growth, direct and indirect photolysis, microbiological, chemical and photodecomposition, metabolism due to hydroxylation and oxidation, and plant-internal translocation in xylem and phloem (Collins et al., 2011; Fantke and Juraske, 2013). It is often impractical to simultaneously report rate constants for various individual dissipation processes. However, if this information is reported, it allows for a much more detailed analysis and understanding of the dynamics of chemicals in the plant-environment systems relevant for different science-policy fields.

We recommend reporting rate constants for specific processes whenever possible, e.g. for biodegradation when metabolites are known based on metabolite concentrations or for volatilization based on measuring air concentrations. When only overall dissipation can be reported, we recommend testing different kinetic models instead of simply assuming first-order kinetics for best interpretability of actual dissipation. While reporting data for specific processes may require additional equipment (e.g. when sampling air), testing different kinetic models can easily be implemented without additional costs, and an overview of different kinetic models is for example given in Fantke and Juraske (2013). Further, we recommend reporting environmental conditions to the extent feasible. This includes most importantly air temperature, air humidity, and soil properties like pH and organic carbon content. If air temperature cannot be measured directly, average temperature over the study duration at the study site can serve as proxy, and if air humidity is not available, recording the number of rain events can serve as alternative.

Partitioning of neutral organic chemicals is predominantly controlled by the quantity and quality of organic carbon; hence, organic carbon content of the soil can contribute to variance in the plant bioaccumulation of neutral organics exposed from soils (Seth et al., 1999). While analyzing soil samples for carbon content might come at the expense of additional resources, classifying the soil (e.g. as podzol) and providing a basic description of the soil horizons will already give some information about potential soil characteristics. Quantifying the environmental fate and sorption of ionizable organic chemicals is generally more uncertain. Evidence suggests that the cation exchange capacity (CEC) of the soil is a key determinant for the sorption of cations (Droge and Goss, 2013). For anionic chemicals, the sorption to soils may be adequately characterized by soil organic carbon and soil pH (Kah and Brown, 2007). At present, we recommend reporting CEC for soil exposures to cations. Revisions to guidelines and reporting requirements for plant bioaccumulation for ionizable organic chemicals should consider the emerging science on chemical distribution of these chemicals in multimedia environments.

Finally, bioaccumulation processes are usually chemical-specific and, hence, physicochemical properties need to be considered in modeling approaches. However, most if not all relevant chemical data are already reported elsewhere, e.g. in the database on registered substances of the European Chemicals Agency (http://echa.europa.eu/information-on-chemicals), except the CAS registry number that is essential to identify a chemical unambiguously. We hence recommend to at least reporting CAS registry numbers.

5.3. Recommendations for reported bioaccumulation testing data

Based on the findings of our review of experimental plant bioaccumulation testing studies and our knowledge regarding bioaccumulation models, we present a set of recommended parameters to be included in future testing studies (Table 4). Parameters that have been identified being of high relevance for interpreting test results and for developing

412 plant bioaccumulation models are specified in the "priority data list" of Table 4. Parameters
413 providing additional information for interpreting experimental results and for use in
414 bioaccumulation modeling are given in the "complementary data list" of Table 4.

415

416 Table 4 Priority and complementary data recommended to be reported in testing studies
417 referring to parameters relevant to improve the interpretation of measured data and to support
418 quantification of bioaccumulation in plants with modeling approaches.

Parameter (unit)	Description
PRIORITY DATA LIST (recommended to be reported by all testing studies)	
CAS-RN	Chemical Abstracts Service Registry Number; unique identifier of a tested chemical
Study location(s)	Location (geographic coordinates) or city/specific area within country) of experimental study site(s)
Study characteristics	Specific conditions, such as field or greenhouse study
Application or release rate ($kg\,day^{-1}$ or $L\,ha^{-1}\,day^{-1}$)	Application or release rate of chemical; number of applications during study
Application or release date(s)	For purposely applied chemicals (e.g. pesticides), application or release date(s) of chemical (exposure time of the plant) or application or release in days after planting; for single exposure events (e.g. spill), exposure concentration and duration
Treated plant component(s) or exposure medium	Treated (exposed) plant component (leaf, pulp, etc.) or environmental compartment/matrix (soil, hydroponic solution, etc.)
Formulation (%)	Fraction of applied or released substance/active ingredient, if applied or released as formulation (e.g. refers to active ingredient of interest plant protection product formulation)
Air temperature (°C)	Mean daily temperature in air (at soil surface level) and min/max range
Soil pH	pH of treated/exposed/sampled soil
Soil OC content ($kg\,kg^{-1}$)	Organic carbon content in treated/exposed/sampled soil for neutral organic chemicals; alternatively, the soil type and horizons can be described
Soil CEC ($meq\,g^{-1}$)	Cation exchange capacity of treated/exposed/sampled soil for ionizable organic chemicals
Binomial plant name	Unambiguous identification of plant species and, if required, variety or cultivar

Parameter (unit)	Description
Plant stage	Growth stage of the treated/exposed/sampled plant (mature, seedling, etc.)
Capture coefficient (–)	Substance capture coefficient as average substance fraction that is intercepted by plant during sampling period and min/max range
Plant transpiration ($L\ kg^{-1}$ or $L\ day^{-1}$)	Plant transpiration as inverse of weight unit of plant dry mass produced per weight unit of consumed water or as volumetric transpiration stream per time unit
Sampled component(s)	Sampled plant component(s) (leaf, pulp, etc.) or tissue(s) (wax layer, etc.) and proximate composition (lipids, organic carbon, carbohydrates, water)
Sampled mass (kg)	Dry and/or wet mass of plant sample(s)
Sampling date(s)/time(s)	Sampling date(s) or sampling days or times after application or release or exposure (day)
Sampling specifics	Specific sampling conditions, such as cold storage, washing or food processing after harvest/sampling
Fate processes studied	Considered fate processes (including post-harvest) either contributing to bioaccumulation (penetration, deposition, etc.) or biodilution (volatilization, metabolism, etc.)
Kinetic models used	Applied assessment models in case of calculating rate coefficients (pseudo-first order, second order, biexponential, etc.); this is only required if the underlying raw data (e.g. concentration at any sampled time) is not provided
COMPLEMENTARY DATA LIST (recommended to be reported when feasible)	
Study year(s)	Year(s) of experimental study
M ($g\ mol^{-1}$)	Molecular mass
log K_{AW} (–)	Air/water partition coefficient; alternatively, the Henry's law constant ($Pa\ m^3\ mol^{-1}$), or the combination of saturation vapor pressure (Pa) and water solubility ($g\ m^{-3}$)
log K_{OW} (–)	*N*-octanol/water partition coefficient
log K_{OA} (–)	*N*-octanol/air partition coefficient; alternatively, Koa can be calculated from Kaw and Kow as $\log Koa = \log Kow - \log Kaw$
K_{OC} ($L\ kg^{-1}$)	Organic carbon normalized soil sorption coefficient
pKa (–)	Acid dissociation constant
Chiral configuration	Specification of (*S*)-(+)-enantiomer and (*R*)-(−)-enantiomer status
Application or release duration (day)	Application or release duration of chemical (exposure duration of the plant)
Application or release type	Application or release type or method (for pesticides aerial spray, drip irrigation, soil injection, etc.) including release or application height (m)

Parameter (unit)	Description
Substance purity (%)	Purity of chemical analytical standard or substance/active ingredient as part of mixture; radio purity, if applicable
Rain rate ($\mathrm{mm\,day^{-1}}$)	Daily average precipitation rate ($1\,\mathrm{mm} = 1\,\mathrm{L\,m^{-2}}$) and min/max range; alternatively, average relative air humidity or number of rain events over the study duration can be reported
Wind speed ($\mathrm{m\,day^{-1}}$)	Mean wind speed at 2 m above soil surface level and min/max range
Soil temperature (°C)	Mean temperature of treated/exposed/sampled soil
Soil water content ($\mathrm{L\,L^{-1}}$)	Fraction of volumetric water in bulk soil
Soil porosity ($\mathrm{L\,L^{-1}}$)	Volumetric porosity in soil or fraction of volumetric pores in bulk soil
Plant growth rates ($\mathrm{day^{-1}}$)	Plant growth rates for different plant components the differences in plant component masses (kg) per time period(s) during the study (day)
Planting density ($n_{\mathrm{plants}}\,\mathrm{ha^{-1}}$)	Number of plants grown per defined area (only in field and greenhouse studies)
Root to shoot ratio (–)	Average ratio between below-ground and aerial plant components
Leaf fraction (–)	Average fraction of aerial plant components that is leaf
Fruit fraction (–)	Average fraction of aerial plant components that is fruit
Stem fraction (–)	Average fraction of aerial plant components that is stem/trunk
LAI (–)	Leaf area index at different times between substance application or release and plant harvest/sampling; for plants with only 1 leaf layer the leaf cover ($\mathrm{m^{-2}}$) can be reported instead
Leaf/fruit/stem/root water ($\mathrm{L\,kg^{-1}}$)	Average water content of plant leaf/fruit/stem/root
Leaf/fruit/stem/root lipid ($\mathrm{L\,kg^{-1}}$)	Average lipid content of plant leaf/fruit/stem/root
Stem height (m)	Average height of plant stem/trunk during study period
Residue analysis setup	Description of all post-sampling procedures and analysis steps including durations of individual processing and analysis steps and analytical detection limits
Analysis temperature(s) (°C)	Temperatures at all post-sampling processing and analysis stages
Solvents used	Solvents and solvent concentrations/purity used at all post-sampling processing and analysis stages

6. Conclusions and implications for future research and policy making

We have highlighted current data gaps that need to be addressed to improve the quantitative understanding of organic chemical bioaccumulation and biotransformation in plants. For non-organic contaminants, the reader is referred to the respective literature (Pulford and Watson, 2003; Raskin and Ensley, 2000; Salt et al., 1995; Weis and Weis, 2004). We emphasize the key experimental parameters that would need to be measured and reported in priority and without much additional effort or equipment in order to improve models for use in various regulatory and decision support contexts. The focus is on terrestrial plants, but similar concepts should also be considered for aquatic plants.

Our reporting recommendations (Table 4) are intended to optimize existing testing guidelines for improved mechanistic bioaccumulation knowledge in a cost-effective manner. This includes reducing unnecessary or redundant testing of the same chemical-plant combinations and to keep study areas and sampling mass reasonably small. Ultimately, the focus of future experimental testing should be to improve data quality and to better facilitate the interpretation and use of testing study results in decision support models.

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