Chapter 4
Global Perspective for the Use of Aquatic Macrophytes in Regulatory Risk Assessment for Contaminants

Verena Sesin, Judith L. Arnolds, and Gertie H. P. Arts

Abstract Macrophytes (aquatic plants) perform key structural and functional roles in aquatic and semi-aquatic ecosystems, and they also provide important ecosystem services for humans. It is therefore pertinent that macrophytes are considered in the ecological risk assessment (ERA) for chemicals and other contaminants that could impact their services. Macrophytes can display a range of morphologies and growth forms, and depending on those, require water, sediment (including pore water), and/or air to thrive in their environment; this diversity must be considered in ERAs. This chapter provides an overview of the use of macrophytes for ERAs as part of regulatory procedures. For several decades, free-floating *Lemna* spp. have been used as a "default" standard test species in phytotoxicity assays and ERA. During the last 15 years, additional species as well as toxicity endpoints beyond morphology and biomass have been included in regulatory approaches for potential contaminants of concern. Furthermore, increasingly complex, "higher-tier" ecological effects assessment approaches were developed, including species sensitivity distributions, microcosm and mesocosm studies, and modeling approaches. This chapter summarizes these developments and provides a global perspective on macrophyte use for risk assessments. It concludes with three recommendations for future ERAs with macrophytes: to educate young scientists in and raise awareness of ERA frameworks and testing methods for macrophytes, on a global scale; to fill knowledge gaps in the toxicity assessment with focus on submerged and emergent species and local species or varieties and climates; and to consider the complexity of stressor exposures and ecological contexts.

V. Sesin (✉)
The School of the Environment, Trent University, Peterborough, ON, Canada
e-mail: verena.sesin@cnsc-ccsn.gc.ca

Canadian Nuclear Safety Commission, Ottawa, ON, Canada

J. L. Arnolds
South African National Biodiversity Institute, Cape Town, South Africa

G. H. P. Arts
Wageningen Environmental Research, Wageningen University and Research, Wageningen, The Netherlands

M. L. Menone and C. D. Metcalfe (eds.), *The Ecotoxicology of Aquatic Macrophytes*, Environmental Contamination Remediation and Management,
https://doi.org/10.1007/978-3-031-27833-4_4

4.1 Introduction to Aquatic Macrophytes as Relevant to Risk Assessment

4.1.1 Macrophyte Growth Forms

Macrophytes (aquatic plants) are growing in or near water and might be growing with upright positions above the water surface (e.g., sediment-rooted, emergent), below the water surface (e.g., sediment-rooted, submerged, or non-rooted, submerged) or floating (e.g., rooted, floating-leaved, or free-floating) (see Fig. 4.1). Several examples of macrophytes include coontail (*Ceratophyllum demersum* L.), cattail (*Typha* L.), waterthyme (*Hydrilla verticillata* (L.f.) Royle), common water hyacinth (*Pontederia crassipes* Mart.), and duckweed (*Lemna* L.). Aquatic ecosystems provide essential services and macrophytes perform a key role in their functioning (Jackson et al. 2001; Maltby et al. 2010; Borst et al. 2018; Temmink et al. 2021). Abiotic and biotic factors influence the natural occurrence and abundance of macrophytes, and thereby affect ecosystem services (Temmink et al. 2021). Relevant abiotic conditions include water transparency (i.e., light availability), water temperature, carbon species, nutrient enrichment availability in surface water and sediment, water movement, and sediment and water phytotoxicity. Biotic factors that influence plant occurrence, distribution, and growth include herbivory and bioturbation by water birds, large fish, and crayfish (Lamers et al. 2013; Dar et al. 2014; Bakker et al. 2016; Temmink et al. 2021).

Macrophytes are adapted to growing in water-saturated sediments. The major difference between water-saturated and well-drained sediments is oxygen availability. The pore spaces are filled with air with a relatively high oxygen content in well-drained soils. Microorganisms that inhabit the soil and roots of plants growing in the soil are able to get oxygen directly from their surroundings. In water-saturated sediments, pore spaces are water-filled, and because of the slow rate of oxygen diffusion in water, the water-saturated sediments become anaerobic. The root systems of macrophytes growing in water-saturated substrates therefore must use oxygen from their aerial parts via internal transport. These macrophytes are morphologically adapted to grow in water-saturated sediment through large internal spaces for transportation of oxygen and rhizomes (Brix 1994). Some macrophytes are also able to radiate oxygen from their roots to the root environment to oxidize the sediment around the root tips.

4.1.2 Macrophyte Plasticity

The successful distribution of aquatic plants in new environments is often linked to multiple introductions and a diverse gene pool that facilitates adaptation to variable environmental conditions (Riis et al. 2010). However, there are two distinctive adaptive mechanisms that improve the survival, reproduction, and dispersal of plant

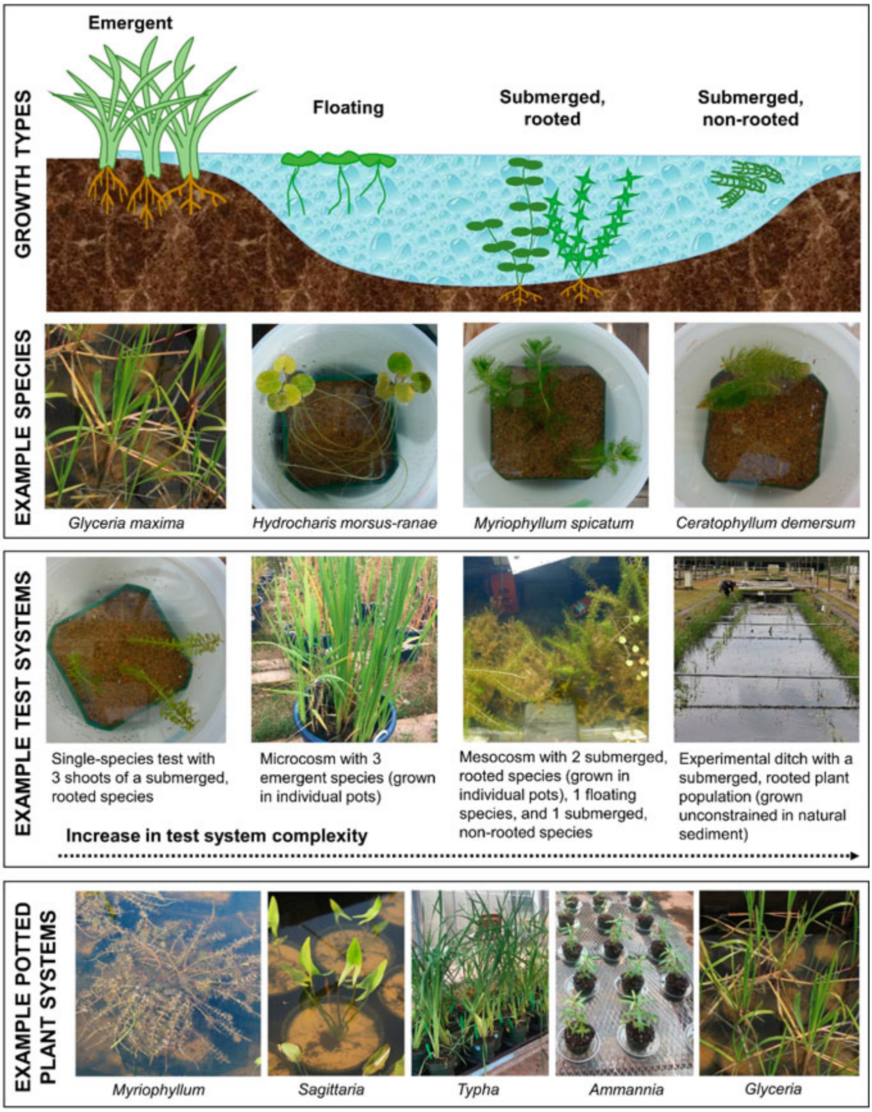

Fig. 4.1 Overview of macrophyte species used for phytotoxicity testing and ecological risk assessments. The figure displays examples of different growth forms and respective species, test systems of varying complexity, and potted plant systems

species: phenotypic plasticity and local adaptation (i.e., the capacity of a species to rapidly adapt genetically by virtue of a diverse gene pool) (Ward et al. 2008; Riis et al. 2010).

The capacity of a given genotype to express different phenotypes in different environments is called phenotypic plasticity (Sultan 2000). Plants are capable of rapidly changing their phenotypic characters if phenotypic plasticity is the primary adaptive mechanism for plants to spread into a range of habitats. The change is caused by environmental conditions in the habitat (Ward et al. 2008; Riis et al. 2010). Phenoplastic species can change their physiology or morphology in response to variations in environmental conditions (Schlichting 1986). The number of introductions of a species is essential in determining whether phenotypic plasticity or local adaptation is the most adaptive mechanism for invasive plant species (Kawecki and Ebert 2004). A morphologically plastic plant can display either competitor or stress-tolerant phenological traits, depending on the environmental conditions (Kautsky 1988; Garbey et al. 2004). One of the most important environmental conditions determining plasticity in aquatic systems is disturbance (Barrat-Segretain 2001).

4.1.3 Role of Macrophytes in Aquatic Ecosystems

Macrophytes are important components of aquatic and wetland ecosystems (Lesiv et al. 2020; Rejmankova 2011; Thomaz 2021) and play a diverse role in determining the structure and function of these systems through, for example, oxygenation of water, productivity, and nutrient recycling (Meena and Rout 2016; Ceschin et al. 2020). Macrophytes are involved in ecosystem processes such as biomineralization, transpiration, and sedimentation. Among biotic components of aquatic ecosystems, higher aquatic plants are one of the main factors of the formation and regulation of water quality and oxygen content in natural water (Rejmankova 2011). They are primary producers and provide food to invertebrates, fish, and birds, as well as organic carbon for bacteria. Macrophytes are at the bottom of herbivorous and detritivorous food chains, and their stems, roots, and leaves serve as substrate for periphyton, and as shelter for several invertebrates and different stages of fish, amphibians, and reptiles (Timms and Moss 1984; Rejmankova, 2011). Certain macrophytes are valuable for their direct contributions to human societies by providing food, biomass, and building materials (Egertson et al. 2004; Bornette and Puijalon 2011; Rejmankova, 2011). Moreover, macrophytes can accumulate heavy metals and other toxic substances from water bodies and play an important role in bioindication and phytoremediation (Kurilenko and Osmolovskaya 2005; Ceschin et al. 2020, 2021; Kumar et al. 2022).

The occurrence and growth forms of macrophytes influence the biogeochemical processes and movements in the water column and sediments. Submerged macrophytes play an important role in maintaining good water quality and high biodiversity in shallow ecosystems, and act as biofilters. Sediments represent an important source of nitrogen and phosphorus for rooted aquatic macrophytes (Barko & Smart 1981). The accumulation of nutrients in an aquatic system causes eutrophication which results in substantial growth of macrophytes and weeds. Submerged macrophytes could play a role in alleviating the adverse effects of phosphorus resuspension and release from bottom sediments. Particles resuspended from the bottom could increase turbidity and deteriorate the underwater light field. Resuspension processes influence nutrient flux at the sediment-water interface and in the water column, and then affect primary production by macrophytes (Zhu et al. 2015). Submerged macrophytes can also prevent the growth of algal blooms through the reduction of nutrients, allelopathy, and shading (Dhote 2007; Lv et al. 2019).

4.1.4 Ecosystem Services Provided by Macrophytes

The Millennium Ecosystem Assessment (MEA 2003, 2005) describes ecosystem services as "the benefits people obtain from ecosystems." These benefits can be classified into four broad categories: supporting, provisioning, regulating, and cultural services. Supporting services include soil and sediment formation, photosynthesis, primary production, nutrient cycling, water cycling, and provisioning of habitat. Regulating services include climate regulation (e.g., through carbon sequestration), water regulation, erosion regulation on shores, water purification, waste treatment, disease regulation through filtration of pollutants and pathogens, pest regulation, and biological control. Provisioning services include food, fiber, genetic resources, and environmental monitoring. Lastly, cultural services include educational value and cultural heritage value (MEA 2003, 2005; Dhote and Dixit 2009; Thomaz 2021; Kumar et al. 2022). The multiple benefits provided by macrophytes are often associated with ecosystems such as wetlands and shallow lakes (Taillardat et al. 2020).

4.2 Current Use of Macrophytes in Ecological Risk Assessments

4.2.1 Overview and Rationale for Ecological Risk Assessments

Effective environmental protection strategies face diverse ecological issues, including climate change, loss of biodiversity, and ubiquitous pollution by anthropogenic substances (Hope 2006). Ecological risk assessments (ERAs) use scientific knowledge and tools to generate informed conclusions that can support environmental decision-makers in designing effective protection strategies (Suter 2006). The ERA process is designed to evaluate how likely adverse ecological effects occur following exposure to one or more environmental stressors (Suter 2006; Quanz et al. 2020), with the goal to generate transparent, objective, and reliable information for decision-makers. Frameworks to guide this process are established by government authorities, including in Europe (e.g., European Chemicals Agency, European Food Safety Authority), North America (e.g., United States Environmental Protection Agency, Canadian Pest Management Regulatory Agency), Asia (e.g., Fan et al.

2019), Africa (e.g., Utembe and Gulumian 2015), Australia (e.g., Australian Government 2021), and across countries (e.g., Organisation for Economic Co-operation and Development, International Organization for Standardization). While there is no single internationally accepted framework (Quanz et al. 2020), most jurisdictions follow similar principles.

The three general principles of ERAs are problem formulation, analysis of exposure and effects, and risk characterization (Hope 2006; Suter 2006) (Fig. 4.2). Problem formulation specifies the issue to be solved, defines environmental components to be protected, and outlines a plan to obtain the necessary data to perform the assessment (Hope 2006; Suter 2006). The analysis of exposure and effects characterizes the spatio-temporal fate as well as interactions of a stressor in the environment, and then assesses the response of environmental components to exposures of realistic durations and magnitudes (Hope 2006; Suter 2006). Finally, risk characterization integrates all obtained information and estimates the ecological risks (Hope 2006; Suter 2006).

The ERA process consists of several levels, so-called tiers, that range from lower-tier screening methods employing hazard quotients calculated from laboratory-derived exposure and effect data, to higher-tier approaches such as ecological modeling, mesocosm and field studies, and weight-of-evidence analysis (Hope 2006; Suter 2006). The progression from lower to higher tiers increases complexity and costs but also results in higher accuracy, realism (risk-based), and predictive power for environmental decision-making (Solomon et al. 2008). Risk assessments are designed to be iterative, where decisions are refined through the acquisition of additional data (Solomon et al. 2008). The process starts with a lower-tier assessment, which if it suggests a potential risk, triggers a higher-tier assessment to further investigate the nature and extent of the risk. This progression promotes efficient use of resources while ensuring that risks are sufficiently characterized for an informed decision (Hope 2006). Scientific ERAs thereby represent an important part of regulatory environmental protection decisions that ultimately consider multiple factors, including economic benefits associated with an activity that results in environmental stress, possible human health risks, and the options for impact mitigation and management.

4.2.2 Macrophyte Use in Ecological Risk Assessments

Macrophytes play an important role in aquatic ecosystems, contributing to structural complexity, biogeochemical cycles, and overall productivity of waterbodies (Carpenter and Lodge 1986; Thomaz and Da Cunha 2010; Lewis and Thursby 2018), as outlined in previous sections. Risk assessments intend to ensure that these ecosystem services are not compromised by any activities that directly or indirectly affect macrophytes and their habitat. Macrophytes are therefore considered in risk assessments and monitoring of water quality including nutrient loading and eutrophication (Delmail 2014; Szoszkiewicz et al. 2020), wastewater discharges

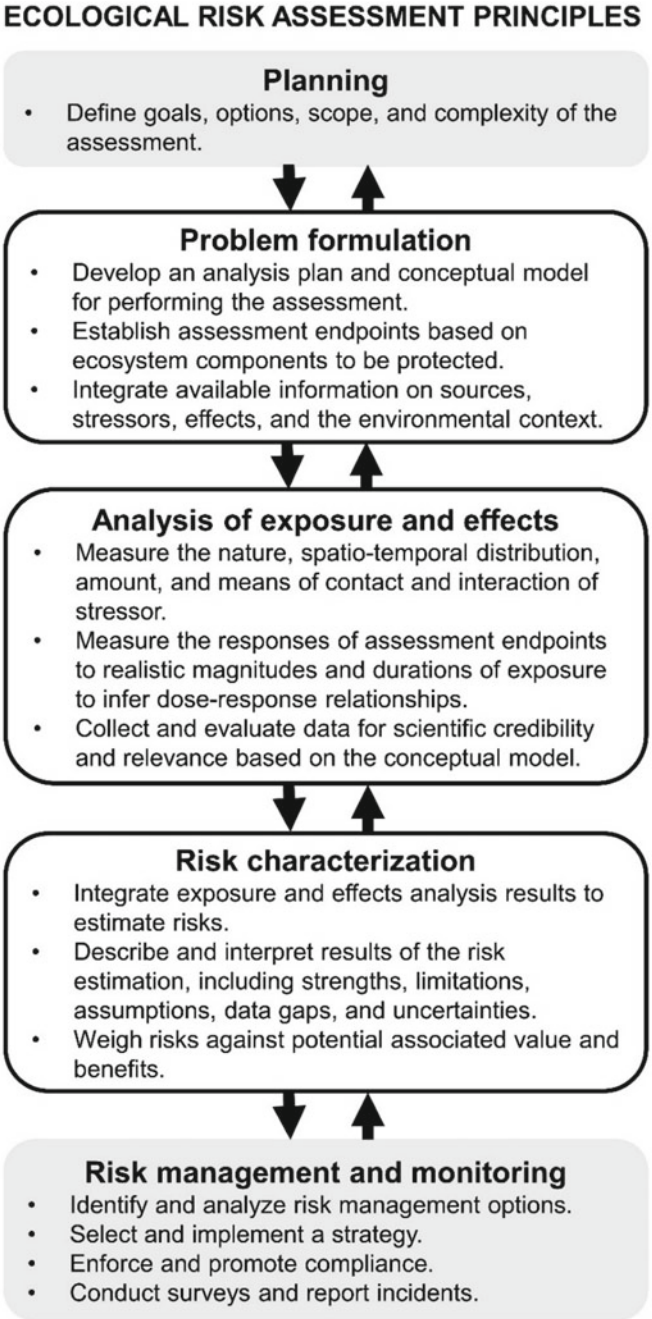

Fig. 4.2 General principles of ecological risk assessments including the core steps of problem formulation, analysis of exposure and effects, and risk characterization, as well as pre-assessment planning and post-assessment risk management and monitoring. The arrows indicate that this is an iterative process. Modified from Hope (2006), Suter (2006), and Health Canada (2021c)

(EPA Victoria 2009), contaminated sites (Government of Canada 2012), nuclear facilities and activities (CSA 2022), and chemicals that may be released into the environment (Australian Environment Agency 2009; ECHA 2017).

One important stressor to macrophytes that typically warrants ERA is pesticide use. Pesticides are anthropogenic chemicals designed for the control of pest organisms, including weeds, insects, rodents, and fungi. Pesticides are applied in agricultural, industrial, urban, and residential areas, in both terrestrial and aquatic settings. Their use is regulated through a complex registration process and tiered ERA performed by many government authorities worldwide, including Australia, Brazil, China, Europe, India, Japan, South Africa, and the United States, and efforts toward global harmonization of procedures and standards are ongoing (Handford et al. 2015). Macrophytes are an integral part of ERAs for pesticides in Canada (Health Canada 2021a), the United States (US EPA 2017), and Europe (EFSA PPR 2013). Pesticides with an herbicidal mode of action, such as herbicides, plant growth regulators, and certain fungicides, must undergo ERA on macrophytes prior to their registration (European Commission 2013; Health Canada 2021b; US EPA 2021).

4.2.3 Phytotoxicity Assessment Using Standardized Test Protocols

A key step in macrophyte ERA is phytotoxicity assessment, which determines the hazards posed by a stressor (e.g., pesticide, heavy metal, pharmaceutical, plastic) to selected non-target macrophyte species using a range of realistic exposure concentrations (e.g., Health Canada 2021a). Hereby, in lower-tier assessments, government agencies frequently rely on data from phytotoxicity studies following standardized test protocols which are designed to produce reliable and reproducible data for regulatory decisions (Taylor and Scroggins 2013; Rudén et al. 2017). The development of a standardized test protocol can be spearheaded by government agencies, industry, scientific societies, or any group of scientists, to address a lack of data in risk assessments (OECD 2009; Taylor and Scroggins 2013). Development procedures involve the selection of a suitable test species, establishing technical procedures of how to conduct the test, and validating that the method produces consistent results across laboratories (Taylor and Scroggins 2013). The final test protocol is typically published by an internationally recognized organization, such as the Organisation for Economic Co-operation and Development (OECD) or the American Society for Testing and Materials (ASTM) International. A selection of internationally recognized, standardized macrophyte tests are listed in Table 4.1. Development of standardized protocols has primarily focussed on testing on the laboratory to greenhouse scale, although a few testing guidelines have been proposed for higher-tier testing such as mesocosms (e.g., OECD 2003; Coors et al. 2006; EFSA PPR 2013); some of these have been standardized among continents, such as the *Myriophyllum* *spicatum* water-sediment test protocol (OECD 2014b) and the *Lemna* sp. growth inhibition test (OECD 2006). Standardization of test protocols aims to harmonize ERAs across countries (Taylor and Scroggins 2013) and continents, which can increase international cooperation as well as save costs and resources.

Table 4.1 Examples of internationally recognized, standardized macrophyte tests, listing key features of the tests: macrophyte species, duration of the test, exposure type, and assessment endpoints

Publisher	Year	Protocol	Macrophyte	Duration	Exposure type	Endpoint(s)	Reference
Floating species							
ASTM	2013	E1415-91	*Lemna gibba*	7 days	Aqueous	Growth inhibition	ASTM (2013)
EC	2007	EPS 1/RM/37	*Lemna minor*	7 days	Aqueous	Frond number and frond dry weight	EC (2007)
ISO	2005	ISO 20079	*Lemna minor*	7 days	Aqueous	Growth rate based on frond number, frond area, chlorophyll, and dry weight	ISO (2005)
OECD	2006	TG 221	*Lemna gibba*, *Lemna minor*	7 days	Aqueous	Frond number, total frond area, and fresh and dry weight	OECD (2006)
US EPA	2012	OCSPP 850.4400	*Lemna gibba*, *Lemna minor*	7 days	Aqueous	Growth rate and yield based on frond number, frond area, and frond dry weight	US EPA (2012)
Submerged species							

(continued)

Table 4.1 (continued)

Publisher	Year	Protocol	Macrophyte	Duration	Exposure type	Endpoint(s)	Reference
ASTM	2017	E1913-97	*Myriophyllum sibiricum*	14 days	Aqueous	Plant growth, shoot length, root number and length, fresh and dry weight, oxygen production, membrane permeability, and chlorophyll and carotenoid content	ASTM (2017)
ISO	2013	ISO 16191	*Myriophyllum aquaticum*	10 days	Sediment	Growth rate based on fresh weight, length, and number of new shoots and roots	ISO (2013)
OECD	2014	TG 238	*Myriophyllum spicatum*	14 days	Aqueous	Growth rate and yield based on shoot length, shoot fresh, and dry weight	OECD (2014a)
OECD	2014	TG 239	*Myriophyllum spicatum*	14 days	Aqueous, sediment	Growth rate and yield based on shoot length, shoot fresh. and dry weight	OECD (2014b)

ASTM: American Society for Testing and Materials International; EC: Environment Canada; ISO: International Organization for Standardization; OECD: Organisation for Economic Co-operation and Development; US EPA: United States Environmental Protection Agency

4.2.4 Standard Test Species for Phytotoxicity Assessment

It is not practical for an environmental assessment to analyze all species within the area under consideration; therefore, a set of representative “reference” animals and plants, in some sectors also referred to as “surrogates”, is typically used (Charrasse et al. 2022). Standard test species are species for which standardized test protocols have been developed. Typically, these species are model organisms, which are species that have been extensively studied to understand biological processes. To assess the suitability of a species for standardized testing, a set of criteria are routinely considered (Table 4.2). These criteria ensure that testing can be easily and reliably performed across facilities, producing ecologically meaningful results to inform environmental protection decisions. Ideally, phytotoxicity assessment is performed with a range of macrophytes representing the community, because no species is consistently the most sensitive to stressors (Fairchild et al. 1998; Arts et al. 2008; Lewis and Thursby 2018). Depending on the stressor mode of action and relevant exposure pathways, testing should include macrophytes of differing morphology and growth forms (e.g., free-floating and floating-leaved; sediment-rooted, submerged; non-rooted, submerged; sediment-rooted, emergent) (Fig. 4.1). Moreover, species should be chosen to reflect realistic environmental conditions, such as freshwater or saltwater, temperate or tropical environments. Marine macrophyte species are often neglected in toxicity testing (Vonk and Kraak 2020), although saltwater macrophytes can be more sensitive to several stressors, including cadmium, copper, diuron, and irgarol, compared to freshwater species (Lewis and Thursby 2018). Moreover, tropical species are commonly underrepresented in phytotoxicity studies, although they can be more sensitive than temperate species to some stressors (Binet et al. 2018; Mooney et al. 2019). When selecting a test species, consideration should also be given to the differing sensitivities of ecotypes (Kanoun-Boulé et al. 2009), as well as genotypic, intraspecific variation (Roubeau Dumont et al. 2019).

The internationally most commonly used standardized test species are free-floating, non-rooted *Lemna* spp. and submersed-rooted *Myriophyllum* spp. (see also Table 4.1). These standard tests typically quantify growth and biomass changes following 7–14 days of static exposure to a range of concentrations of a stressor. To address a lack of test protocols for emergent species, a guideline is currently under development for a 14-day test with *Glyceria maxima* (Hartm.) Holmb. (Davies et al. 2019). Another proposed emergent macrophyte is *Typha* that fulfilled many of the selection criteria (Sesin et al. 2021), although test methods are not yet developed. Several other macrophyte species and test procedures have been proposed for standardized testing. These proposals include a 48-h phytotoxicity test method using root-regrowth as a sensitive endpoint for *Lemna* spp. (Park et al. 2013) (updated to 72-h in Park et al. 2022 and ISO/DIS 4979), a 7-day test with the macrophyte *Salvinia natans* (L.) All. (Cui et al. 2020), and a bioassay with the tropical, marine seagrass *Halophila ovalis* (Wilkinson et al. 2015). Further unpublished testing protocols have been developed in research centers and by industry (Maltby et al. 2010), covering a range of floating species belonging to various genera including *Azolla*, and submerged species of the genera *Egeria*, *Elodea*, and *Ceratophyllum*, as well as emergent species of the genera *Sparganium*, *Sagittaria*, and *Phragmites* (Table 4.3); however, standardized test methods are not yet available for these species, but tests are based upon existing protocols such as the *Myriophyllum spicatum* water-sediment test (OECD 2014b) for submerged macrophytes or the *Glyceria maxima* water-sediment test (in development) for emergent macrophytes (Arts et al. 2022).

Table 4.2 Criteria to select macrophyte standard test species for phytotoxicity assessment (summarized from Powell et al. 1996; Maltby et al. 2010; Sesin et al. 2021)

Criterion	Explanation
Ecological relevance	The macrophyte should be relevant to the ecosystem and stressor exposure under investigation. Selection considers a species' role and importance in the ecosystem, geographical relevance (e.g., temperate or tropical areas), as well as its morphology and physiology
Suitability for different exposure pathways	Macrophytes can be exposed via different exposure routes depending on their growth form (e.g., emergent, submerged, floating). Selection considers if a species is likely to be exposed to the stressor via realistic routes (e.g., soil, water, air, spray drift, sediment, pore water)
Availability of material	Macrophyte material should ideally be available year-round to allow for continuous, timely testing. Selection considers the availability of material from natural populations as well as whether stock cultures can be established for continuous supply
Ease of cultivation	Standardized testing relies on protocols that minimize cost, workload, and space. Selection considers if a species can be cultivated under controlled conditions such as a growth chamber or greenhouse, and if cultivation is straightforward with high return of usable test material
Uniform growth	Macrophytes with low inherent variability in morphology and biomass are preferred as this can facilitate the statistical discernment between natural and stressor-related changes that are measured in the test. Selection considers the variation in these growth parameters to ensure it is acceptable for the test design (e.g., sample size). This criterion is transferrable to non-growth-related endpoints that may be assessed
Appropriate assessment endpoints	Endpoints are measured variables that reflect the performance of the macrophyte during the test. Selection considers if the endpoints are toxicologically sensitive to the stressor, exhibit low variability within treatments, and are biologically meaningful (i.e., useful for interference of effects on the individual to community level)

(continued)

Table 4.2 (continued)

Criterion	Explanation
Sensitivity toward stressors	The test species is ideally among the most sensitive species toward the stressor, so that the test results are protective of other co-occurring macrophytes. Selection considers the relative sensitivities of species, taking into account any "safety factors" to account for uncertainty that may be applied during ecological risk calculation or to extrapolate to other macrophyte species

Moreover, experimental conditions generally adopted in freshwater toxicity tests with macrophytes were recently summarized in a review by Ceschin et al. (2021).

4.2.5 Tier 1 (Lower-Tier) Phytotoxicity Tests with Macrophytes

Tier 1 tests are short-term, laboratory-based, and single-species phytotoxicity tests used to screen for major toxic effects. Typically, these lower-tier tests employ standardized test protocols as outlined in Table 4.1. Various potential stressors have been tested for their phytotoxicity using simple testing approaches that follow, or are modified from, standardized test protocols; these stressors include heavy metals, pharmaceuticals and personal care products, pesticides, hydrocarbons, surfactants, and plastics (summarized in Ceschin et al. 2021). Of all aquatic plants used in ecotoxicity testing, the majority (60%) are microalgae (Ceschin et al. 2021). *Lemna* spp., *Myriophyllum* spp., and *Hydrilla* spp. collectively account for one third (33%) of test species (Ceschin et al. 2021).

The small size, simple anatomy, and ease of culturing make *Lemna* spp. ideal test organisms for ecotoxicological investigations (Mkandawire et al. 2014). However, *Lemna* spp. are not appropriate test organisms in all cases and additional testing options with other macrophytes are needed. The AMRAP (Aquatic Macrophyte Risk Assessment for Pesticides) workshop (Maltby et al. 2010), held in 2008, triggered the development of test protocols for sediment-rooted aquatic macrophytes. Namely, the AMRAP workshop concluded upon regulatory concerns that risk assessments solely based on *Lemna* spp. and algal data at Tier 1 might underestimate the risk of plant protection products to aquatic macrophytes. One concern was that *Lemna* spp. are monocotyledonous species, while herbicides might also and sometimes specifically target dicotyledonous species (e.g., 2,4-D; Belgers et al. 2007). Moreover, concern was raised that *Lemna* spp. may not be sensitive to pesticides that form residues in sediment; because of considerable knowledge and experience with *Myriophyllum spicatum* L., this species was recommended as an additional Tier 1 test species (Maltby et al. 2010). After extensive test development and ring-testing among

Table 4.3 Macrophyte species previously used in laboratory studies that are potentially suitable for toxicity tests. Common names may not reflect all names used globally

Growth type	Species	English common name(s)
Floating	*Azolla**	Water fern
	*Lemna**	Duckweed
	Salvinia	Watermoss, kariba weed
	*Spirodela**	Duckmeat, duckweed
Submerged, non-rooted	*Ceratophyllum**	Coontail, hornwort
	Chara	Stonewort
Submerged, rooted	*Callitriche*	Water-starwort
	*Egeria**	Waterweed
	*Elodea**	Waterweed, pondweed
	Hydrilla	Hydrilla, water thyme
	*Heteranthera**	Mud plantain, ducksalad
	Hottonia	Water violet, featherfoil
	Hygrophila	Swampweed, starhorn
	*Lagarosiphon**	Oxygen weed, African elodea, curly waterweed
	*Myriophyllum**	Water milfoil
	Najas	Water nymph, naiad
	Potamogeton	Pondweed, ribbonleaf
	Ranunculus	Water crowfoot
	*Vallisneria**	Eelgrass, tape grass, vallis
Emergent	*Glyceria**	Sweet-grass, mannagrass
	Phragmites	Common reed
	Sagittaria	Arrowhead, duck potato, katniss, swamp potato, tule potato
	Sparganium	Bur-reed
	Typha	Bulrush, cattail, reedmace, cumbungi

The asterisk identifies species that are available from commercial suppliers, although import licences may be required. Table modified from Maltby et al. (2010)

several laboratories, a sediment-water guideline was published (OECD 2014b) and the test was adopted by the European Aquatic Guidance document (EFSA PPR 2013) and included in the data requirements of the pesticide regulation in Europe (EC 2013). These data requirements specifically include additional aquatic macrophyte species tests to be undertaken on a dicotyledonous species, such as *M. spicatum, M. aquaticum,* or a monocotyledonous species, such as the aquatic grass *G. maxima*, as appropriate. Research has shown that *G. maxima* is a suitable candidate for testing grass-specific herbicides (Mohr et al. 2015). The need to perform studies with rooted, submerged, and emergent macrophytes is to be discussed with the national competent authorities.

The *M. spicatum* water-sediment test (OECD 2014b) uses an artificial sediment (OECD 2004) with an overlying Smart and Barko test medium (Smart and Barko 1985). The sediment is enriched with nutrients (phosphorus and nitrogen) (OECD 2014b) to enable optimum growth of the macrophytes, while the overlying Smart and Barko medium only includes carbon. The protocol can be used as a blueprint for testing other sediment-rooted macrophytes in the laboratory, such as *Elodea nuttallii* (Planch.) H. St. John and *Elodea canadensis* Michx. In addition, exposure via the sediment may be simulated by spiking the artificial sediment with a test chemical and transplanting plants into this spiked sediment. As stated above, a *G. maxima* test is under development and has been ring-tested since 2016 (Arts et al. 2022).

Submerged macrophytes are easy to propagate, as each side shoot can develop new roots and can grow into a new shoot. Emergent macrophytes are different: mother plants need to be propagated to develop enough young shoots of similar length and leaf number to perform a test. Besides *G. maxima* as a potential emergent test species, *Typha* species turned out to be promising (Sesin et al. 2021). *Typha* spp. are increasingly used to assess the phytotoxicity of pollutants. *Typha* is easy to grow and suitable for water, soil, and air exposure tests. It enables a suite of morphological and physiological toxicity endpoints to be measured (Sesin et al. 2021). A drawback might be that *Typha* species have an ability to hybridize, which might be an issue in certain geographical regions. No species within the *Typha* genus is consistently the most sensitive to a range of stressors although comparable data is currently limited (Sesin et al. 2021). Selection of a *Typha* test species may therefore be based on local availability, and on the feasibility to propagate enough young shoots with an initial variability low enough to perform a toxicity test following regulatory requirements. This latter issue is relevant for all emergent macrophytes for use in laboratory toxicity tests.

4.2.6 Higher-Tier Phytotoxicity Tests with Macrophytes

In the risk assessment for pesticides, microcosm and mesocosm test systems can be used as a suitable reference tier. Maltby et al. (2010) stressed that the required endpoints for macrophytes need to be studied as naturally as possible, considering competition, predation, and natural stressors. Microcosms and mesocosms enable this type of studies as species are considered within their community. Microcosm and mesocosm studies with aquatic macrophytes can be performed as two different test designs: one option is the inclusion of the macrophytes as free-growing natural populations; the second option introduces the macrophytes as potted plants (Fig. 4.1). The first design limits the number of macrophyte species that can be studied, because free-living populations require a large surface area. It is only achievable in larger mesocosms such as experimental ditches. The second design with potted plants excludes below-ground competition between the rooted macrophyte species. Both approaches might be combined in a mesocosm when it is divided into two parts: one part is reserved for free-growing populations and the other part for potted plants (e.g., in the form of bioassays). Both design options allow the study of effects on free-living algae populations, phytoplankton and periphyton. Maltby et al. (2010; see Table 3.3 therein) gives an overview of advantages and limitations of assessing phytotoxicity in microcosms and mesocosms using potted plants *versus* plants rooted in natural sediment.

The earliest mesocosm studies were performed in the United States in the 1980's (Graney 1990; Solomon et al. 1996). There was only a short time window from 1988 to 1992 in which the United States Environmental Protection Agency (US EPA) required aquatic field studies (Boyle and Fairchild 1997). Afterward, the interest for mesocosm studies declined, partly due to difficulties in interpretation; however, there is a recent revival of the interest (see recent sessions and presentations in SETAC scientific congresses). Microcosm and mesocosm studies targeted for aquatic macrophytes have been comparably rare. An overview of mesocosm studies in ecotoxicology by Caquet et al. (2000) only mentions aquatic macrophytes as structural elements for other groups of organisms, but not as a sensitive or vulnerable taxonomic group to be studied. Studies performed in the 1990s addressed the effects of linuron, an herbicide, on primary producers including aquatic macrophytes in experimental ditches (Brink et al. 1997; Cuppen et al. 1997). Fairchild and Sappington (2002) studied the fate and effects of the triazinone herbicide metribuzin in experimental pond mesocosms including the effect on the inhabiting macrophytes. Mohr et al. (2007, 2009) performed studies in experimental pond and stream mesocosms. An alternative is to use bioassays with aquatic macrophytes in mesocosms (Coors et al. 2006). Bioassays are in situ tests and include the use of, for example, potted plants (Fig. 4.1).

The AMRAP workshop and its publication (Maltby et al. 2010) drew renewed attention to the inclusion of aquatic macrophytes in the risk assessment for pesticides and stressed the need for a proper higher-tier risk assessment for aquatic macrophytes. It is only recently, after the publication of the Aquatic Guidance document in Europe (EFSA PPR 2013) and a publication about the Minimum Detectable Difference approach for mesocosm studies (Brock et al. 2015), that potted-plant studies are presented by regulators as the only way forward for higher-tier aquatic macrophyte mesocosm studies to meet the European regulatory requirements of the inclusion of eight sensitive species.

Mesocosm studies were also performed in tropical climates (Daam et al. 2009a, b). The comparison of microcosm and mesocosm studies between temperate and tropical climates does not generate an unambiguous conclusion (Daam and van den Brink 2010). Pesticide dissipation rates and vulnerability of freshwaters do not appear consistently higher or lower in tropical regions compared to their temperate counterparts (Daam and van den Brink 2010). However, differences in fate and effects may occur for individual pesticides and taxa. Moreover, intensive agricultural practices in tropical countries lead to a higher input of pesticides and spread of contamination over watersheds (Daam and van den Brink 2010).

4.2.7 Ecotoxicological Endpoints for Macrophytes

Endpoints are explicit expressions of environmental values that environmental managers wish to protect (CSA 2022). An ecotoxicological endpoint can be defined as a variable reflecting macrophyte performance and development during and after exposure to a toxic compound. Several different categories of endpoints can be distinguished (Arts et al. 2008). Assessment endpoints are directly related to environmental management goals but they are typically stated in terms of population and community attributes (e.g., population success, community success, diversity) (CSA 2022). However, it is not always practical to quantify those attributes; therefore, more readily measurable or predictable surrogates, so-called measurement endpoints, can be selected (CSA 2022). For instance, for an assessment endpoint of "probability of greater than 10% reduction" in recruitment, the related measurement endpoints could be "% mortality" in exposed habitats (CSA 2022). Measurement endpoints should be defined in terms of survival, growth, or reproduction (CSA 2022). Examples for plants include biomass, frond number, number of leaves, area of leaves, shoot height, fresh weight (related to growth), mortality (related to survival), and number of new ramets, or number of seeds (related to reproduction). When defining measurement endpoints, priority should be given to those that are closely linked to assessment endpoints; for example, survival, growth, and reproduction are generally considered to be closely linked to population success (CSA 2022).

Assessment endpoints are used in the formal risk assessment. These may include the LOEC (Lowest Observed Effect Concentration), NOEC (No Observed Effect Concentration), or ECx (effect concentration for x% of the test population). For primary producers, both growth rate (ErC_{50}) and yield/biomass endpoints (EyC_{50} or EbC_{50}) are assessment endpoints. ErC_{50} is the preferred endpoint for primary producers (EFSA PPR 2013; OECD 2006, 2014b) and is a protective endpoint in most cases (Van Wijngaarden and Arts 2018). In order to use growth rate as an endpoint, exponential growth in the control plants should be demonstrated (EFSA 2015, 2019). Growth rate endpoints are independent of test duration, while yield or biomass endpoints decrease with test duration (Bergtold and Dohmen 2011). This is a consequence of mathematical calculation and not sensitivity (EFSA PRR 2013).

Effects of pesticides, other organic chemicals, as well as other pollutants on macrophytes generally do not cause mortality if environmental concentrations are applied (Maltby et al. 2010). Only at very high doses, macrophytes cannot survive. This means that endpoints for aquatic macrophytes are sub-lethal by nature (Arts et al. 2008). A range of endpoints is available to test the response and fitness of macrophytes. However, the endpoints included in toxicity tests should meet a number of criteria. They need to be sensitive to the stressor(s), exhibit low variability, and allow for easy measurement in standardized laboratory tests (Arts et al. 2008). In the *M. spicatum* test protocol (OECD 2014b), measurement endpoints are shoot fresh weight, total shoot dry weight, and total shoot length. In the ring-tests performed for the validation of this test protocol, these endpoints performed best in terms of achieving a low variability and appropriate sensitivity. These endpoints might slightly differ per plant species and growth form. For example, for the *G. maxima* protocol that is currently in development, shoot height was not an appropriate and sensitive endpoint and was replaced by total leaf length (Davies et al. 2017; Arts et al. 2022). Root endpoints were considered in some studies and are a sensitive endpoint (e.g., Belgers et al. 2007); however, limitations include potential high variability (Arts et al. 2008) and difficulty to continuously measure if plants are grown in soil (Sesin et al. 2020).

The following are examples of endpoints used for various contaminants: growth rate and biomass endpoints were used to assess toxicity of heavy metals, pharmaceuticals, pesticides, surfactants, and plastics (Ceschin et al. 2021). Measurements of enzymatic activity were performed to assess toxicity of heavy metals, pharmaceuticals, hydrocarbons, and pesticides (Ceschin et al. 2021). Antioxidant enzymes (e.g., superoxide dismutase, catalase, peroxidase) scavenge reactive oxidant species and thereby prevent oxidative damage, and can serve as biomarkers for exposure, particularly for stressors that target the photosynthetic chain by disrupting electron flow (Brain and Cedergreen 2009). Chlorophyll fluorescence was measured to assess toxicity of heavy metals, pharmaceuticals, and surfactants (Ceschin et al. 2021). Chlorophyll and carotenoid pigments absorb light energy for photosynthesis; stressors can affect their content and composition (Brain and Cedergreen 2009). Moreover, a review by Sesin et al. (2021) summarized morphological and physiological endpoints that can be used for ecotoxicological tests for various stressors (e.g., chemicals, heavy metals, carboxylic acids, xenobiotics, pharmaceuticals, persistent organic pollutants, wastewater, and algal toxins) with the emergent macrophyte *Typha* spp.

4.2.8 Sensitivity of Macrophyte Species and Endpoints

Macrophyte species might differ in their sensitivity to pollutants. We already discussed, as an example, the potential differences between monocotyledonous and dicotyledonous macrophytes in sensitivity to specific herbicides. Depending on the endpoint, sensitivity can vary greatly within a species, and pollutant- and species-specific endpoints should be considered in ERA (Berghahn et al. 2007; Dumont et al. 2019). Giddings et al. (2013) state that endpoints might differ in sensitivity by a factor of 10–1000. These authors compared the sensitivity of different aquatic primary producers (macrophytes and algae) to a series of herbicides by using the species sensitivity distribution (SSD) approach. They used the lowest reported reliable EC_{50} for each species after calculation of the geometric mean of identical measurement endpoints as recommended by Brock et al. (2011). This methodology gives insight into the sensitivity of standard test species used in the risk assessment for pesticides compared to other algae and macrophyte species. They found that no single species consistently represents the most sensitive aquatic plant species. For 12 of 14 chemicals, *Lemna gibba* L. and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) algae (i.e., the algae used in the risk assessment under the United States *Federal Insecticide, Fungicide, and Rodenticide Act*; *Pseudokirchneriella subcapitata*, *Anabaena flos-aquae*, *Navicula pelliculosa*, and *Skeletonema* *costatum*) included an EC_{50} near or below the lowest macrophyte EC_{50} and the macrophyte HC_5 (i.e., hazardous concentration for 5% of species). For the other compounds, *M. spicatum* was the most sensitive species of all aquatic plants considered. Overall, these results support the usefulness of testing *L. gibba*, *M. spicatum*, and the FIFRA algae for assessing pesticide risks to aquatic primary producers.

4.3 Global Examples of the Use of Macrophytes in Regulatory Risk Assessment

4.3.1 North America

Macrophytes are an important part of pesticide risk assessments in Canada that applies a tiered ERA approach (Health Canada 2021c). An initial screening level identifies non-target organisms for which there may be a potential risk. The screening uses conservative exposure scenarios and sensitive toxicity effects endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value, which is then compared to the level of concern (LOC). If the RQ is equal to or greater than the LOC, then a refined risk assessment is warranted to further characterize the risk. The refined assessment considers more realistic exposure scenarios and different toxicity endpoints. Refined methods include exposure modeling, monitoring data, mesocosm or field study data, and probabilistic approaches. The refinement process continues until either the risk is judged to be adequately characterized, or no further refinement is possible due to limited available data.

Currently, testing with aquatic vascular plants in Canadian pesticide risk assessments is only required if there is potential for freshwater exposure. From a regulatory perspective, testing with macrophytes of the genus *Lemna* is sufficient to meet the requirements (Sauvé 2012; Whiteside 2017), although data from other macrophytes are considered in assessments, if available. For example, the re-evaluation of the pesticide glyphosate (HC PMRA 2015) included data from the floating *Nymphaea odorata* Aiton, and emergent *Pontederia cordata* L. and *Carex comosa* Boott, all of which turned out to be more sensitive than *Lemna* spp. to the formulated product as compared by their respective EC_{50} values.

Macrophytes are also an important consideration in ERAs for nuclear facilities and activities regulated by the Canadian Nuclear Safety Commission (CNSC). The ERAs evaluate exposure and effects on representative biota and valued ecosystem components (CNSC 2020; CSA 2022), which in many cases include aquatic plants. Moreover, the assessments must specifically consider vulnerable, threatened, and endangered species, including plants, listed under the Government of Canada's *Species at Risk Act* as well as corresponding provincial and territorial statutes and regulations (CSA 2022).

The United States use a similar approach to Canada for most pesticide risk assessments. For aquatic macrophytes, the screening level RQ is routinely based on the lowest EC_{50}, although other toxicological endpoints may be used if they can be linked to assessment endpoints in a reasonable and plausible manner (US EPA 2022a). Typically, *Lemna gibba* is used in Tiers 1 and 2 (US EPA 2022b). In Tier 3, aquatic field tests are performed on a case-by-case basis if macrophytes show greater than 50% adverse effects on plant growth (US EPA 2022b). The US EPA has also developed the Plant Assessment Tool to better align macrophyte exposure models to pesticide fate and transport (Moore et al. 2021). The tool can be used to estimate pesticide exposures to plants inhabiting semi-aquatic areas that are adjacent to treated sites (Hook 2020). In a refined risk assessment, probabilistic tools and methods are used to estimate the variability and uncertainty in factors that influence risk (US EPA 2022a).

The US EPA specifically considers threatened and endangered species listed under the *Endangered Species Act*. Under this Act, all federal agencies must ensure that their regulatory actions are not likely to jeopardize the continued existence of listed species or destroy or adversely modify their critical habitat (US EPA 2022a). For threatened or endangered macrophytes, the NOEC is used in the RQ calculation. However, toxicity data are rarely available for listed species, and therefore surrogate species are often used, such as *Lemna* spp. (US EPA 2004). For data-deficient species, expert knowledge can also fill gaps and support decision-making (Fitzgerald et al. 2021).

4.3.2 South America

Risk assessment, especially for pesticides, is rapidly developing in South America (Carriquiriborde et al. 2014; Casallanovo et al. 2021a, 2021b). In Brazil, the current process is mainly hazard-based, but risk assessment guidelines for aquatic, terrestrial, and soil organisms are expected to be published by regulators within the next two years (Casallanovo et al. 2021b). A workshop held in 2014 (Carriquiriborde et al. 2014) recommended including macrophytes in the first tier of the risk assessment in the form of required tests for *Lemna* spp. Brazil uses procedures adapted from the European scheme (Topping et al. 2020).

4.3.3 Europe

In Europe, a tiered risk assessment procedure for pesticides has been established and is in force (EFSA PPR 2013; European Commission 2013). For compounds with an herbicidal mode of action, the first tier requires tests with *Lemna* spp. (*L. gibba* or *L. minor*). For substances with an herbicidal mode of action for which *Lemna* spp. are not sensitive or there is expected uptake from sediment by the roots of macrophytes, toxicity testing is required with another macrophyte, either *M. spicatum* or *G.*

maxima. The regulatory endpoint used in the risk assessment is the regulatory acceptable concentration (RAC), which—for macrophytes—is the EC_{50} with an assessment factor of 10. This RAC is compared with the predicted environmental concentrations (PECs) resulting from modeling of FOCUS scenarios for the use of the specific compound under evaluation. This results in tables with conclusions about the safe or unsafe use of the compound in the different scenarios.

In the aquatic risk assessment, the second tier provides several methods to refine the risk assessment (EFSA PPR 2013). If primary producers are the most sensitive group of organisms in Tier 1, a geomean approach can be followed if more macrophyte or algae endpoints are available, but less than eight. If at least eight endpoints from macrophytes and algae are available, an SSD curve can be generated. Rules are in place which organisms can be combined in one SSD. The best approach is to make an SSD with all primary producers first. If one of the groups of primary producers (e.g., algae, diatoms, macrophytes) are more sensitive than the others, separate curves for these groups need to be generated and the HC_5 of the most sensitive curve can be used in the risk assessment. A third option in the second tier is modified exposure tests. These tests include the standard test species but have a modified, more realistic exposure. These tests can be combined with TKTD (i.e., Toxicokinetic–Toxicodynamic) modeling. For example, for *Lemna* spp. a fit-for-purpose model is available (Schmitt et al. 2013), while this is in development for *M. spicatum* (Heine et al. 2015).

The third tier includes microcosm and mesocosm studies, which are described in Sect. 4.2.6. The highest tier might be on the landscape level, including a multi-species and multi-compound approach. This landscape approach is currently under debate and development in Europe.

4.3.4 Africa

In developing countries, risk assessment on pesticides has not been adequately studied due to the situation that concentrations and fate of pesticides in the environment are often undetermined. South Africa is facing challenges with significant pressures on its freshwater and agricultural resources, which enhances the prospects of pesticide effects. A few studies have been performed in South Africa in terms of pesticide risk assessment. The majority of the work concentrated on the estuaries and rivers of the Western Cape (Bollmohr et al. 2007; Malherbe et al. 2013). The PRIMET model is currently used in South Africa to predict risk to the aquatic environment. Models that are used to predict risks must be validated through field monitoring of pesticide exposure and effects. Most studies on macrophytes have focused on these plants as invasive alien species and very little work has been done on risk assessment.

In Ethiopia, the risk assessment that is currently being implemented is based on European principles on aquatic risk assessment and the registration procedure for pesticides. Risks for aquatic organisms are calculated by using water concentrations demonstrating the 90th percentile probability of occurrence in Ethiopia. This percentile is standard in the European Union's registration procedures for risks in the aquatic ecosystem and reflects a less strict requirement for the protection of aquatic organisms compared to humans. Pesticide toxicity to rooted macrophytes is not currently considered as a future addition to the risk assessment procedure (Teklu et al. 2015). Moreover, there is no pesticide monitoring system in place for the environment, primarily due to poor institutional capacity, and a lack of coordination on the safe use of pesticides among federal and regional governments (Negatu et al. 2021). A need to raise awareness of the public on issues of pesticide misuse was identified by scientists (Negatu et al. 2021).

4.3.5 Australia

Australia has a well-developed risk assessment process that evaluates the impacts associated with licensed activities that include various potential stressors such as radioactive substances, pesticides, and hazardous chemicals (NSW EPA 2022). For example, in the pesticide risk assessment, non-target macrophyte toxicity tests are integral to the hazard assessment (APVMA 2019). Notably, Australia uses a site-specific, "eco-regionalized" approach that recognizes the wide range of ecosystem types (e.g., tropical, temperate, arid environments) within their jurisdiction, and associated differences in water quality characteristics (Water Quality Australia 2019). As one example, an ERA of tebuthiuron in tropical Australian wetlands considered specifically tropical species including the macrophyte *Lemna aequinoctialis* Welw. (Dam et al. 2004).

4.3.6 Global Perspective on the Risk Assessment for Macrophytes

The United States, Canada, and the European Union were pioneers in developing sound risk assessment schemes (Casallanovo et al. 2021b). The procedures developed in these countries are taken as examples and adapted to other countries and their specific circumstances, such as in Brazil (Topping et al. 2020; Casallanovo et al. 2021b). However, macrophytes are often not included in risk assessment schemes, and if they are, then it is usually limited to requiring the standard test species *Lemna* spp. in the first tier of the risk assessment. Europe also considers rooted macrophytes in the risk assessment when triggered by the fate of the compound and/or the sensitivity of the standard test species *Lemna* spp., while in North America toxicity data from other macrophytes might be used in the risk assessment when available. The comparably minor role of macrophytes in ecotoxicological investigations does not reflect the major role macrophytes play in ecosystems. Many contaminants enter ecosystems via plants which are a key link in food webs (Ceschin et al. 2021). The usefulness of macrophytes goes beyond simple toxicity tests; they can also serve as bioindicators of water quality and phytoremediation agents (Ceschin et al. 2021). Moreover, there is a potential to establish large-scale monitoring programs to verify risk assessment predictions on a global level; for example, South African scientists called for intensifying and expanding water monitoring for pesticides using chemical, toxicological, and biological techniques (Ansara-Ross et al. 2012). Lastly, risk assessments on a global scale are heavily relying on standard test toxicity data produced in Europe or North America, and there is a lack of locally adapted and indigenous species being tested (e.g., Daam and van den Brink 2010; Ansara-Ross et al. 2012) which would be most relevant to the local risk assessment context.

4.4 Conclusion and Outlook: Future Ecological Risk Assessments with Macrophytes

Macrophytes are important components of aquatic and wetland ecosystems and sustain many ecosystem services, and therefore need to be an integral part of ERAs. Yet, ERAs tend to overlook the complexity of macrophytes, their growth forms and plasticity on an individual to community level, possibly resulting in insufficient protection measures. On an individual level, macrophyte growth forms (e.g., emergent, submerged, floating) and classes (e.g., monocots or dicots) influence exposure pathways and responses to stressors. On a community level, co-occurring species can influence community dynamics through competition for light or resources. As this chapter outlined, ERA approaches have been updated to try to address these factors, such as through the addition of new single-species tests with submerged and emergent species, as well as higher-tier, multi-species testing and modeling methods.

Scientific knowledge is continuously evolving, and the scientific community regularly proposes new ERA processes and tools to align approaches with environmental reality (Topping et al. 2020). However, regulatory frameworks are rarely modernized. This causes a time-lag of incorporating the most recent scientific knowledge into regulatory decisions. In addition, the widely used tiered risk assessment process is primarily based on single-stressor, single-use assessments (Topping et al. 2020), although multiple chemical products are typically used on the landscape scale. If the goal of ERA is to protect macrophyte populations and communities and ultimately biodiversity, then the current approach can be ineffective (Frische et al. 2018; Schäfer et al. 2019; Topping et al. 2020). Moreover, regulatory progress is not equivalent on a global level, and many countries have not yet established ERA frameworks for macrophytes (e.g., South Africa, Ethiopia, and countries in Latin America, possibly also in Asia).

We have three key recommendations for ERAs with macrophytes that can be considered in the adaptation of current regulatory processes as well as in the establishment of new frameworks, which should be relevant across countries.

First, we recommend educating young scientists all over the globe in ERA frameworks, in the effects of pollutants on individual, population, and ecosystem levels, on how these can be assessed (experimental and modeling tools), and on how a risk assessment process could look like in practice (see also Fig. 4.2). Awareness needs to be raised about the diversity of species and ecosystems in the environment and how these organisms can be protected from adverse effects. Knowledge exchange could be facilitated through bilateral or multilateral collaboration and training. One recent example is the collaboration between the International Institute for Sustainable Development and the African Center for Aquatic Research and Education to strengthen freshwater science in large lakes, addressing pollution at local, regional, and global scales (IISD 2020). Education can also extend to the public, and outreach and engagement efforts can include local residents, naturalist and stewardship groups, and indigenous communities. These stakeholders already have tremendous knowledge and experience with the local environment and plant communities. Acknowledging that communication is a two-way process, stakeholders' knowledge can in turn be linked to the ERA framework and could inform the selection of macrophyte test species as well as monitoring sites, frequency, and sample types. One example is the ERA conducted for certain nuclear facilities in Canada, which is periodically reviewed and revised using site-specific knowledge and indigenous knowledge, among other sources (CNSC 2020).

Secondly, we recommend developing scientific approaches to fill the gaps in our knowledge related to risk assessment for aquatic macrophytes. We have identified the following knowledge gaps: (1) we need more understanding of the sensitivity of different macrophyte growth forms, (indigenous) species, macrophyte ecotypes, and genotypes to herbicide exposure and exposure to other contaminants such as pharmaceuticals, nanoparticles, or radionuclides. (2) We need more knowledge on how to do a proper risk assessment on a local level, especially in different climatic zones all over the globe. Compared to temperate zones, tropical zones and tropical macrophyte species are less studied. For example, the applied field rates of pesticides and associated exposure routes differ locally, influenced by the climate, crop production, and government laws and regulations, among other factors. (3) We need to develop statistical and TKTD models for rooted (submerged and emergent) macrophytes to be used in risk assessment. (4) We need to revive aquatic microcosm and mesocosm studies with aquatic macrophytes as an important intermediate step between the lower-tier risk assessment for individual species and the risk assessment at the landscape level. (5) We need to develop approaches to perform a risk assessment for aquatic macrophytes at the landscape level.

Third, while further developing risk assessment for aquatic macrophytes, we recommend that future ERAs reflect the complexity of stressors that may expose macrophytes, as well as their ecological context. Macrophytes are typically exposed to a mixture of stressors, including anthropogenic pollutants, habitat disturbances and loss, climatic changes, and competition by invasive species. Exposure to stressors can be highly variable in temporal and spatial dimensions, and accounting for these in an assessment can increase environmental realism. Moreover, ERAs should ideally consider the ecological context, such as species interactions and community composition, as well as the landscape context, including habitat types and connectivity (Milner and Boyd 2017; Schäfer et al. 2019). As the case of South Africa shows, on a local level, invasive macrophyte species represent a significant pressure on freshwater ecosystems, and these issues should be considered in ERAs for co-occurring stressors, for example through a cumulative risk assessment. Renewed interest in microcosm and mesocosm studies is also promising in this regard. While accounting for all these factors is challenging, partly due to limited data availability, an approach that reflects the complexity and interdependence of ecosystem components, however, is needed to provide effective, long-term environmental protection.

Acknowledgements Selected sections of this chapter are inspired by Verena Sesin's Ph.D. thesis (Sesin 2021) which was funded by the Ontario Trillium Scholarship and co-supervised by Dr. Joanna Freeland and Dr. Christina Davy. The authors thank two anonymous reviewers for improving an earlier version of the manuscript.

References

Ansara-Ross TM, Wepener V, van den Brink PJ, Ross MJ (2012) Pesticides in South African fresh waters. Afr J Aquat Sci 37(1):1–16. https://doi.org/10.2989/16085914.2012.666336

Arts GHP, Belgers JDM, Hoekzema CH, Thissen JTNM (2008) Sensitivity of submersed freshwater macrophytes and endpoints in laboratory toxicity tests. Environ Pollut 153(1):199–206. https://doi.org/10.1016/j.envpol.2007.07.019

Arts G, Davies J, Gonsior GG, Isemer R, Kubitza J, Ratte M, Wedeking R (2022) Results of the 2021 Ring-test with Glyceria maxima Grown in a Water-Sediment System and Exposed to Imazapyr. Final report. 59. https://research.wur.nl/en/publications/an-oecd-test-guideline-for-glyceria-maxima-results-of-a-final-rin

ASTM International (2013) Standard guide for conducting static toxicity tests with *Lemna gibba* G3. West Conshohocken, PA.

ASTM International (2017) Guide for conducting static, axenic, 14-day phytotoxicity tests in test tubes with the submersed aquatic macrophyte, *Myriophyllum sibiricum* Komarov. West Conshohocken, PA. https://doi.org/10.1520/E1913-97

Australian Environment Agency (2009) Environmental risk assessment guidance manual for agricultural and veterinary chemicals. http://www.nepc.gov.au/system/files/resources/bffdc9e9-7004-4de9-b94f-b758140dbc8c/files/cmgt-nchem-eragm-agricultural-and-veterinary-chemicals-200902.pdf. Accessed 23 Dec 2021

Australian Government (2021) Environmental risk assessment of chemicals. https://www.environment.gov.au/protection/chemicals-management/environment-assessments. Accessed 18 Feb 2021

Australian Pesticides and Veterinary Medicines Authority [APVMA] (2019) APVMA risk assessment manual. https://apvma.gov.au/sites/default/files/publication/46416-risk_assessment_manual_environment_0.pdf. Accessed 15 May 2022

Bakker ES, Wood KA, Pagès JF, Veen GC, Christianen MJ, Santamaría L, Nolet BA, Hilt S (2016) Herbivory on freshwater and marine macrophytes: A reviewand perspective. Aquat Bot 135: 18–36. https://doi.org/10.1016/j.aquabot.2016.04.008

Barko JW, Smart RM (1981) Sediment-based nutrition of submersed macrophytes. Aquat Bot 10:339–352

Barrat-Segretain MH (2001) Biomass allocation in three macrophyte species in relation to the disturbance level of their habitat. Freshw Biol 46(7):935–945. https://doi.org/10.1046/j.1365-2427.2001.00728.x

Belgers JDM, Van Lieverloo RJ, Van der Pas LJT, Van den Brink PJ (2007) Effects of the herbicide 2,4-D on the growth of nine aquatic macrophytes. Aquat Bot 86(3):260–268. https://doi.org/10.1016/j.aquabot.2006.11.002

Berghahn R, Mohr S, Feibicke M, Meinecke S, Sperfeld E (2007) Endpoint 'floating leaves' of *Potamogeton natans*: a new method to evaluate the development of macrophytes in pond mesocosms. Environ Sci Pollut Res 14(3):190–193. https://doi.org/10.1065/espr2006.07.324

Bergtold M, Dohmen GP (2011) Biomass or growth rate endpoint for algae and aquatic plants: relevance for the aquatic risk assessment of herbicides. Integr Environ Assess Manag 7:237–247. https://doi.org/10.1002/ieam.136

Binet MT, Adams MS, Gissi F, Golding LA, Schlekat CE, Garman ER, Merrington G, Stauber JL (2018) Toxicity of nickel to tropical freshwater and sediment biota: a critical literature review and gap analysis. Environ Toxicol Chem 37(2):293–317. https://doi.org/10.1002/etc.3988

Bollmohr S, Day JA, Schulz R (2007) Temporal variability in particle-associated pesticide exposure in a temporarily open estuary, Western Cape, South Africa. Chemosphere 68(3): 479–488. https://doi.org/10.1016/j.chemosphere.2006.12.078

Bornette G, Puijalon S (2011) Response of aquatic plants to abiotic factors: a review. Aquat Sci 73(1): 1–14. https://doi.org/ https://doi.org/10.1007/s00027-010-0162-7

Borst AC, Verberk WC, Angelini C, Schotanus J, Wolters JW, Christianen MJ, van der Zee EM, Derksen-Hooijberg M, van der Heide T (2018) Foundation species enhance food web complexity through non-trophic facilitation. PLoS One 13(8):e0199152. https://doi.org/10.3389/fmars.2020.549478

Boyle TP, Fairchild JF (1997) The role of mesocosms studies in ecological risk analysis. Ecol Appl 7(4):1099–1102. https://doi.org/10.1890/1051-0761(1997)007[1099:TROMSI]2.0.CO;2

Brain RA, Cedergreen N (2009) Biomarkers in aquatic plants: selection and utility. Rev Environ Contam Toxicol 198:49–109. https://doi.org/10.1007/978-0-387-09647-6_2

van den Brink PJ, Hartgers EM, Crum SJH, Van Donk E, Brock TCM (1997) Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide linuron. Ecotoxicol Environ Saf 38(1):13–24. https://doi.org/10.1006/eesa.1997.1555

Brix H (1994) Functions of macrophytes in constructed wetlands. Water Sci Technol 29(4):71–78

Brock TCM, Arts GHP, ten Hulscher TEM, de Jong FMW, Luttik R, Roex EWM, Smit CE, van Vliet PJM (2011) Aquatic effect assessment for plant protection products. Dutch proposal that addresses the requirements of the Plant Protection Regulation and Water Framework Directive. Alterra-report 2235. Wageningen, The Netherlands, 140 p

Brock TCM, Hammers-Wirtz M, Hommen U, Preuss TG, Ratte HT, Roessink I, Strauss T, Van den Brink PJ (2015) The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. Environ Sci Pollut Res 22(2):1160–1174. https://doi.org/10.1007/s11356-014-3398-2

Canadian Nuclear Safety Commission [CNSC] (2020) Environmental protection: environmental principles, assessments and protection measures. REGDOC-2.9.1, version 1.2. Ottawa, Ontario, Canada, 57 p

Canadian Standards Association [CSA] (2022) CSA N288.6:22. Environmental risk assessments at nuclear facilities and uranium mines and mills. https://www.csagroup.org/store/product/2703416/

Caquet T, Lagadic L, Sheffield SR (2000) Mesocosms in ecotoxicology (1): outdoor aquatic systems. In GW Ware (eds) Reviews of environmental contamination and toxicology, vol 165. Springer, New York, NY. https://doi.org/10.1007/978-1-4612-1172-3_1

Carpenter SR, Lodge DM (1986) Effects of submersed macrophytes on ecosystem processes. Aquat Bot 26:341–370. https://doi.org/10.1016/0304-3770(86)90031-8

Carriquiriborde P, Mirabella P, Waichman A, Solomon K, Van den Brink PJ, Maund S (2014) Aquatic risk assessment of pesticides in Latin America. Integr Environ Assess Manag 10(4):539–542. https://doi.org/10.1002/ieam.1561

Casallanovo F, Simone DM, Santos GS, Kaminski TSD, Cione AP, Peranginangin N (2021a) Estimating pesticide environmental concentrations in Latin America: The importance of developing local scenarios. Integr Environ Assess Manag 17(5):901–904. https://doi.org/10.1002/ieam.4396

Casallanovo F, Simone DM, Santos, GS, Kaminski, TSD, Cione, AP (2021b) Pesticide environmental risk assessment in Latin American countries. The importance of developing local scenarios. GLOBE 22(4):2. SETAC.

Ceschin S, Crescenzi M, Iannelli MA (2020) Phytoremediation potential of the duckweeds *Lemna minuta* and *Lemna minor* to remove nutrients from treated waters. Environ Sci Pollut Res 27(13):15806–15814. https://doi.org/10.1007/s11356-020-08045-3

Ceschin S, Bellini A, Scalici M (2021) Aquatic plants and ecotoxicological assessment in freshwater ecosystems: a review. Environ Sci Pollut Res 28:4975–4988. https://doi.org/10.1007/s11356-020-11496-3

Charrasse B, Mora JC, Anderson T, Bonchuk Y, Telleria D (2022) Bounding uncertainties around the conceptual representation of species in radiological assessment in the context of routine atmospheric release. J Radiol Prot 42(2):020506. https://doi.org/10.1088/1361-6498/ac5dd1

Coors A, Kuckelkorn J, Hammers-Wirtz M, Strauss T (2006) Application of in-situ bioassays with macrophytes in aquatic mesocosm studies. Ecotoxicology 15(7):583–591. https://doi.org/10.1007/s10646-006-0095-z

Cui R, Nam, S-H, An Y-J (2020) *Salvinia natans*: a potential test species for ecotoxicity testing. Environ Pollut 267:115650. https://doi.org/10.1016/j.envpol.2020.115650

Cuppen JGM, van den Brink PJ, van der Woude H, Zwaardemaker N, Brock TCM (1997) Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide linuron. Ecotoxicol Environ Saf 38(1):25–35. https://doi.org/10.1006/eesa.1997.1556

Daam MA, Van den Brink PJ, Nogueira AJA (2009a) Comparison of fate and ecological effects of the herbicide linuron in freshwater model ecosystems between tropical and temperate regions. Ecotoxicol Environ Saf 72(2):424–433. https://doi.org/10.1016/j.ecoenv.2008.07.008

Daam MA, Rodrigues AMF, Van den Brink PJ, Nogueira AJA (2009b) Ecological effects of the herbicide linuron in tropical freshwater microcosms. Ecotoxicol Environ Saf 72(2):410–423. https://doi.org/10.1016/j.ecoenv.2008.07.009

Daam MA, van den Brink PJ (2010) Implications of differences between temperate and tropical freshwater ecosystems for the ecological risk assessment of pesticides. Ecotoxicology 19(1):24–37. https://doi.org/10.1007/s10646-009-0402-6

Dam RA, Camilleri C, Bayliss P, Markich SJ (2004) Ecological risk assessment of tebuthiuron following application on tropical Australian wetlands. Hum Ecol Risk Assess Int J 10(6):1069–1097. https://doi.org/10.1080/10807030490887140

Dar AD, Pandit AK, Ganai BA (2014) Factors affecting the distribution patterns of aquatic macrophytes. Limnol Rev 14(2):75–81. https://doi.org/10.2478/limre-2014-0008

Davies J, Arts G, Kubitza J, Kuhl K (2017) Results of a pre-ringtest using *Glyceria maxima* grown in a sediment-water-system. 94 p. Internal report.

Davies J, Arts G, Isemer RJ, Kubitza J, Ratte M (2019) WP275 testing the emergent macrophyte, *Glyceria maxima*, in a water-sediment system: progress towards development of an OECD test guideline. SETAC North America 40th Annual Meeting, November 6, Toronto, ON.

Delmail D (2014) Risk management of European inland waters using macrophyte biomonitoring. Front Environ Sci 2(31):1–4. https://doi.org/10.3389/fenvs.2014.00031

Dhote S (2007) Role of macrophytes in improving water quality of an aquatic ecosystem. J Appl Sci Environ Manag 11(4):133–135

Dhote S, Dixit S (2009) Water quality improvement through macrophytes—a review. Environ Monit Assess 152(1):149–153. https://doi.org/10.1007/s10661-008-0303-9

Dumont ER, Larue C, Lorber S, Gryta H, Billoir E, Gross EM, Elger A (2019) Does intraspecific variability matter in ecological risk assessment? Investigation of genotypic variations in three macrophyte species exposed to copper. Aquat Toxicol 211:29–37. https://doi.org/10.1016/j.aquatox.2019.03.012

Egertson CJ, Kopaska JA, Downing JA (2004) A century of change in macrophyte abundance and composition in response to agricultural eutrophication. Hydrobiologia 524(1):145–156. https://doi.org/10.1023/B:HYDR.0000036129.40386.ce

Environment Canada [EC] (2007) Biological test method: test for measuring the inhibition of growth using the freshwater macrophyte, *Lemna minor*: Report EPS 1/RM/37. 2nd edn. Environmental Protection Series, Ottawa, ON

European Chemicals Agency [ECHA] (2017) Guidance on information requirements and chemical safety assessment: Chapter R.7b: Endpoint specific guidance. Helsinki, Finland. https://echa.europa.eu/documents/10162/13632/information_requirements_r7b_en.pdf/1a551efc-bd6a-4d1f-b719-16e0d3a01919. Accessed 23 Dec 2021

European Commission (2013) Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, revised 2015. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32013R0284. Accessed 22 Apr 2021

European Food Safety Authority Panel on Plant Protection Products and their Residues [EFSA PPR] (2013) Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFS2. 11(7):3290, 268. https://doi.org/10.2903/j.efsa.2013.3290

European Food Safety Authority, Arena M, Auteri D, Barmaz S, Chaideftou E, Ctverackova L, Villamar-Bouza L (2019) Outcome of the Pesticides Peer Review Meeting on general recurring Issues in ecotoxicology. 16(7):1673E.

Environment Protection Authority Victoria [EPA Victoria] (2009) Guidelines for risk assessment of wastewater discharges to waterways: publication 1287. Victoria, Australia. https://www.epa.vic.gov.au/about-epa/publications/1287. Accessed 23 Dec 2021

Fairchild JF, Ruessler DS, Carlson AR (1998) Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor, and metolachlor. Environ Toxicol Chem 17(9):1830–1834. https://doi.org/10.1002/etc.5620170924

Fairchild J, Sappington L (2002) Fate and effects of the triazinone herbicide metribuzin in experimental pond mesocosms. Arch Environ Contam Toxicol 43:198–202. https://doi.org/10.1007/s00244-002-1208-1

Fan M, Liu Z, Dyer S, Federle T, Wang X (2019) Development of environmental risk assessment framework and methodology for consumer product chemicals in China. Environ Toxicol Chem 38(1):250–261. https://doi.org/10.1002/etc.4300

Fitzgerald DB, Smith DR, Culver DC, Feller D, Fong DW, Hajenga J, Niemiller ML, Nolfi DC, Orndorff WD, Douglas B, Maloney KO, Young JA (2021) Using expert knowledge to support Endangered Species Act decision-making for data-deficient species. Conserv Biol 35(5):1627–1638. https://doi.org/10.1111/cobi.13694

Frische T, Egerer S, Matezki S, Pickl C, Wogram J (2018) 5-point programme for sustainable plant protection. Environ Sci Eur 30(1):8. https://doi.org/10.1186/s12302-018-0136-2

Garbey C, Thiébaut G, Muller S (2004) Morphological plasticity of a spreading aquatic macrophyte, *Ranunculus peltatus*, in response to environmental variables. Plant Ecol 173(1):125–137. https://doi.org/10.1023/B:VEGE.0000026336.44576.ff

Giddings J, Arts G, Hommen U (2013) The relative sensitivity of macrophyte and algal species to herbicides and fungicides: an analysis using species sensitivity distributions. Integr Environ Assess Manag 9(2):308–318. https://doi.org/10.1002/ieam.1387

Government of Canada (2012) Federal Contaminated Sites Action Plan (FCSAP) ecological risk assessment guidance. Ottawa, ON. https://www.canada.ca/content/dam/eccc/migration/fcs-scf/B15E990A-C0A8-4780-9124-07650F3A68EA/ERA-20Guidance-2030-20March-202012_FINAL_En.pdf. Accessed 23 Dec 2021

Graney RL (1990) Aquatic mesocosm studies in ecological risk assessment. CRC Press

Handford CE, Elliott CT, Campbell K (2015) A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integr Environ Assess Manag 11(4):525–536. https://doi.org/10.1002/ieam.1635

Health Canada (2021a) Environmental toxicology. https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/protecting-your-health-environment/pesticide-registration-process/reviews/environmental-toxicology.html. Updated 28 June 2018; accessed 4 Jan 2022

Health Canada (2021b) Use site category (DACO tables)—agriculture and forestry. https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/registrants-applicants/product-application/use-site-category-daco-tables/agriculture-forestry-pesticides.html. Accessed 22 Apr 2021

Health Canada (2021c) PMRA guidance document. A framework for risk assessment and risk management of pest control products. https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/policies-guidelines/risk-management-pest-control-products.html. Accessed 4 Jan 2022

Health Canada Pest Management Regulatory Agency [HC PMRA] (2015) Glyphosate. Proposed re-evaluation decision. PRVD2015-01. Ottawa, ON.

Heine S, Schmitt W, Schaffer A, Gorlitz G, Buresova H, Arts G, Preuss TG (2015) Mechanistic modelling of toxicokinetic processes with *Myriophyllum*. Chemosphere 120:292–298. https://doi.org/10.1016/j.chemosphere.2014.07.065

Hook J (2020) EPA modeling approaches for wetland plants. https://ag.purdue.edu/arge/SiteAssets/EPA%20Modeling%20Approaches%20for%20Wetland%20Plants.pdf. Accessed 25 Feb 2022

Hope BK (2006) An examination of ecological risk assessment and management practices. Environ Int 32(8):983–995. https://doi.org/10.1016/j.envint.2006.06.005

International Institute for Sustainable Development [IISD] (2020) Scientists from two continents to work together to improve the health of the African Great Lakes. https://www.iisd.org/articles/scientists-two-continents-work-together-improve-health-african-great-lakes#:~:text=The%20IISD%2DACARE%20collaboration%20will,countries%20in%20which%20they%20reside. Accessed 25 Feb 2022

International Organization for Standardization [ISO] (2005) Water quality—determination of the toxic effect of water constituents and waste to duckweed (*Lemna minor*)—Duckweed growth inhibition test: ISO 20079:2005. Geneva, Switzerland

International Organization for Standardization [ISO] (2013) Water quality—determination of the toxic effect of sediment on the growth behaviour of *Myriophyllum aquaticum*: ISO 16191:2013. 1st edn. Geneva, Switzerland.

Jackson RB, Carpenter SR, Dahm CN, McKnight DM, Naiman RJ, Postel SL, Running SW (2001) Water in a changing world. Ecol Appl 11(4):1027–1045. https://doi.org/10.2307/3061010

Kanoun-Boulé M, Vicente JAF, Nabais C, Prasad MNV, Freitas H (2009) Ecophysiological tolerance of duckweeds exposed to copper. Aquat Toxicol 91(1):1–9. https://doi.org/10.1016/j.aquatox.2008.09.009

Kautsky L (1988) Life strategies of aquatic soft bottom macrophytes. Oikos 53:126–135. https://doi.org/10.2307/3565672

Kumar R, Ivy N, Bhattacharya S, Dey A, Sharma P (2022) Coupled effects of microplastics and heavy metals on plants: Uptake, bioaccumulation, and environmental health perspectives. Sci Total Environ 836: 155619.

Kurilenko VV, Osmolovskaya NG (2005) Ecological-biogeochemical role of macrophytes in aquatic ecosystems of urbanized territories (an example of small water bodies of St. Petersburg). Russ J Ecol 37(3):147–151. https://doi.org/10.1134/S1067413606030015

Lamers LP, Govers LL, Janssen IC, Geurts JJ, Van der Welle ME, Van Katwijk MM, Van der Heide T, Roelofs JG, Smolders AJ (2013) Sulfide as a soil phytotoxin—a review. Front Plant Sci 4: 268. https://doi.org/10.3389/fpls.2013.00268

Lesiv MS, Polishchuk AI, Antonyak HL (2020) Aquatic macrophytes: ecological features and functions. Biologichni Studii 14(2):79–94. https://doi.org/10.30970/sbi.1402.619

Lewis M, Thursby G (2018) Aquatic plants: test species sensitivity and minimum data requirement evaluations for chemical risk assessments and aquatic life criteria development for the USA. Environ Pollut 238:270–280. https://doi.org/10.1016/j.envpol.2018.03.003

Lv T, He Q, Hong Y, Liu C, Yu D (2019) Effects of water quality adjusted by submerged macrophytes on the richness of the epiphytic algal community. Front Plant Sci 9:1980. https://doi.org/10.3389/fpls.2018.01980

Malherbe W, Van Vuren JHJ, Wepener V (2013) Preliminary risk assessment of common-use pesticides using PRIMET and PERPEST pesticide risk models in a semi-arid subtropical region. Water Sa. 39(5):599–610. https://doi.org/10.4314/wsa.v39i5.3

Maltby L, Arnold D, Arts G, Davies J, Heimbach F, Pickl C, Poulsen V (2010) Aquatic macrophyte risk assessment for pesticides: SETAC Europe Workshop AMRAP Wageningen, Netherlands. CRC Press, Boca Raton, London, New York

Meena T, Rout J (2016) Macrophytes and their ecosystem services from natural ponds in Cachar district, Assam, India. Indian J Tradit Knowl 15(4):553–560

Millennium Ecosystem Assessment (2003) Ecosystems and human well-being: a framework for assessment/Millennium Ecosystem Assessment; authors, Joseph Alcamo et al. contributing authors, Elena M. Bennett et al. World Resources Institute, Washington, DC.

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: synthesis. Island Press, Washington, DC, 155 p

Milner AM, Boyd IL (2017) Toward pesticidovigilance. Science 357(6357):1232–1234. https://doi.org/10.1126/science.aan2683

Mkandawire M, Teixeira da Silva JA, Dudel EG (2014) The *Lemna* bioassay: Contemporary issues as the most standardized plant bioassay for aquatic ecotoxicology. Crit Rev Environ Sci Technol 44(2):154–197. https://doi.org/10.1080/10643389.2012.710451

Mohr S, Berghahn R, Feibicke M, Meinecke S, Ottenstroer T, Schmiedling I, Schmiediche R, Schmidt R (2007) Effects of the herbicide metazachlor on macrophytes and ecosystem function in freshwater pond and stream mesocosms. Aquat Toxicol 82:73–84. https://doi.org/10.1016/j.aquatox.2007.02.001

Mohr S, Berghahn R, Mailahn W, Schmiediche R, Feibicke M, Schmidt R (2009) Toxic and accumulative potential of the antifouling biocide and TBT successor irgarol on freshwater macrophytes: a pond mesocosm study. Environ Sci Technol 43(17):6838–6843. https://doi.org/10.1021/es900595u

Mohr S, Schott J, Hoenemann L, Feibicke M (2015) *Glyceria maxima* as new test species for the EU risk assessment for herbicides: a microcosm study. Ecotoxicology 24(2):309–320. https://doi.org/10.1007/s10646-014-1379-3

Mooney TJ, Pease CJ, Hogan AC, Trenfield M, Kleinhenz LS, Humphrey C, van Dam RA, Harford AJ (2019) Freshwater chronic ammonia toxicity: a tropical-to-temperate comparison. Environ Toxicol Chem 38(1):177–189. https://doi.org/10.1002/etc.4313

Moore DRJ, McCarroll-Butler CA, Avanasi R, Chen W, White M, Brain RA (2021) How protective to the environment is the pesticide risk assessment and registration process in the United States? J Regul Sci 9(2):1–20. https://doi.org/10.21423/jrs-v09i2moore

Negatu B, Dugassa S, Mekonnen Y (2021) Environmental and health risks of pesticide use in Ethiopia. J Health Pollut 11(30):210601. https://doi.org/10.5696/2156-9614-11.30.210601

NSW Environmental Protection Authority [NSW EPA] (2022) Risk assessment process. https://www.epa.nsw.gov.au/licensing-and-regulation/licensing/environment-protection-licences/risk-based-licensing/risk-assessment-process. Accessed 15 May 2022

Organisation for Economic Co-operation and Development [OECD] (2003) Draft guidance document on simulated freshwater lentic field tests (outdoor microcosms and mesocosms). https://www.oecd.org/chemicalsafety/testing/21476053.pdf. Accessed 23 Dec 2021

Organisation for Economic Co-operation and Development [OECD] (2004) Test No. 219: sediment-water chironomid toxicity using spiked water, OECD guidelines for the testing of chemicals, Section 2, OECD Publishing, Paris. https://doi.org/10.1787/9789264070288-en

Organisation for Economic Co-operation and Development [OECD] (2006) Test No. 221: *Lemna* sp. growth inhibition test. OECD (OECD guidelines for the testing of chemicals, Section 2). ISBN: 9789264016194

Organisation for Economic Co-operation and Development [OECD] (2009) Guidance document for the development of OECD guidelines for the testing of chemicals: ENV/JM/MONO(2006)20/REV1. OECD Environment Health and Safety Publications, Series on Testing and Assessment, Paris. https://www.oecd.org/chemicalsafety/testing/49803789.pdf

Organisation for Economic Co-operation and Development [OECD] (2014a) Test No. 238: sediment-free *Myriophyllum spicatum* toxicity test. OECD Publishing (OECD guidelines for the testing of chemicals, Section 2), Paris. ISBN: 9789264224131

Organisation for Economic Co-operation and Development [OECD] (2014b) Test No. 239: water-sediment *Myriophyllum spicatum* toxicity test. OECD Publishing (OECD guidelines for the testing of chemicals, Section 2), Paris. ISBN: 9789264224155

Park A, Kim Y-J, Choi E-M, Brown MT, Han T (2013) A novel bioassay using root re-growth in *Lemna*. Aquat Toxicol 140–141:415–424. https://doi.org/10.1016/j.aquatox.2013.06.004

Park J, Yoo E-J, Shin K, Depuydt S, Li W, Appenroth K-J, Lillicrap AD, Xie L, Lee H, Kim G, Saeger JD, Choi S, Kim G, Brown MT, Han T (2022) Interlaboratory validation of toxicity testing using the duckweed lemna minor root-regrowth test. Biology 11(1):37. https://doi.org/10.3390/biology11010037

Powell RL, Kimerle RA, Moser EM (1996) Development of a plant bioassay to assess toxicity of chemical stressors to emergent macrophytes. Environ Toxicol Chem 15(9):1570–1576. https://doi.org/10.1002/etc.5620150921

Quanz M, Willis R, Burr D, Recoskie R, Walker TR (2020) Aquatic ecological risk assessment frameworks in Canada: a case study using a single framework in South Baymouth, Ontario, Canada. Environ Monit Assess 192(8):530. https://doi.org/10.1007/s10661-020-08500-1

Rejmankova E (2011) The role of macrophytes in wetland ecosystems. J Ecol Field Biol 34(4):333–345. https://doi.org/10.5141/JEFB.2011.044

Riis T, Lambertini C, Olesen B, Clayton JS, Brix H, Sorrell BK (2010) Invasion strategies in clonal aquatic plants: are phenotypic differences caused by phenotypic plasticity or local adaptation? Ann Bot 106(5):813–822. https://doi.org/10.1093/aob/mcq176

Roubeau Dumont E, Larue C, Lorber S, Gryta H, Billoir E, Gross EM, Elger A (2019) Does intraspecific variability matter in ecological risk assessment? Investigation of genotypic variations in three macrophyte species exposed to copper. Aquat Toxicol 211:29–37. https://doi.org/10.1016/j.aquatox.2019.03.012

Rudén C, Adams J, Ågerstrand M, Brock TC, Poulsen V, Schlekat CE, Wheeler JR, Henry TR (2017) Assessing the relevance of ecotoxicological studies for regulatory decision making. Integr Environ Assess Manag 13(4):652–663. https://doi.org/10.1002/ieam.1846

Sauvé DG (2012) PMRA environmental risk assessment. Health Canada Pest Management Regulatory Agency, Ottawa, ON. http://adamoliverbrown.com/wp-content/uploads/2012/03/ottawa-U_march_2012_3.pdf. Accessed 4 Jan 2022

Schäfer RB, Liess M, Altenburger R, Filser J, Hollert H, Roß-Nickoll M, Schäffer A, Scheringer M (2019) Future pesticide risk assessment: narrowing the gap between intention and reality. Environ Sci Europe 31(1). https://doi.org/10.1186/s12302-019-0203-3

Schlichting CD (1986) The evolution of phenotypic plasticity in plants. Annu Rev Ecol Syst 17(1):667–693

Schmitt W, Bruns E, Dollinger M, Sowig P (2013) Mechanistic TK-TD-model simulating the effect of growth inhibitors on *Lemna* populations. Ecol Model 255:1–10. https://doi.org/10.1016/j.ecolmodel.2013.01.017

Sesin V, Davy CM, Stevens KJ, Hamp R, Freeland JR (2020) Glyphosate toxicity to native nontarget macrophytes following three different routes of incidental exposure. Integr Environ Assess Manag 17(3):597–613. https://doi.org/10.1002/ieam.4350

Sesin V (2021) Assessing effects and fate of environmental contaminants in invasive, native, and endangered macrophytes. PhD Dissertation, Trent University. http://digitalcollections.trentu.ca/objects/etd-992?solr_nav%5Bid%5D=78ce053f5eba0e171867&solr_nav%5Bpage%5D=0&solr_nav%5Boffset%5D=0

Sesin V, Davy CM, Freeland JR (2021) Review of *Typha* spp. (cattails) as toxicity test species for the risk assessment of environmental contaminants on emergent macrophytes. Environ Pollut 284:117105. https://doi.org/10.1016/j.envpol.2021.117105

Smart RM, Barko JW (1985) Laboratory culture of submersed freshwater macrophytes on natural sediments. Aquat Bot 21(3):251–263. https://doi.org/10.1016/0304-3770(85)90053-1

Solomon KR, Brock TCM, Zwart D de, Dyer SD, Posthuma L, Richards SM, Sanderson H, Sibley PK, van den Brink PJ (2008) Chapter 1. Extrapolation in the context of criteria setting and risk assessment. In KR Solomon, TCM Brock, D de Zwart, SD Dyer, L Posthuma, S Richards, H Sanderson, P Sibley, PJ van den Brink (eds). Extrapolation practice for ecotoxicological effect characterization of chemicals. CRC Press, pp 1–32

Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall LW Jr, Williams WM (1996) Ecological risk assessment of atrazine in North American surface waters. Environ Toxicol Chem 15(1):31–76. https://doi.org/10.1897/1551-5028

Sultan SE (2000) Phenotypic plasticity for plant development, function and life history. Trends Plant Sci 5(12):537–542. https://doi.org/10.1016/S1360-1385(00)01797-0

Suter GW (2006) Ecological risk assessment of contaminated sites, 2nd edn. CRC Press, Boca Raton, 643 p. ISBN: 1-56670-634-3.

Szoszkiewicz K, Jusik S, Pietruczuk K, Gebler D (2020) The macrophyte index for rivers (MIR) as an advantageous approach to running water assessment in local geographical conditions. Water 12(1):108–123. https://doi.org/10.3390/w12010108

Taillardat P, Thompson BS, Garneau M, Trottier K, Friess DA (2020) Climate change mitigation potential of wetlands and the cost-effectiveness of their restoration. Interface Focus 10:e20190129. https://doi.org/10.1098/rsfs.2019.0129

Taylor LN, Scroggins RP (2013) Standardization of ecotoxicological tests: the process. In: Blaise C (ed) Encyclopedia of aquatic ecotoxicology: 2 volume-set. Springer, New York, pp 1073–1080

Teklu BM, Adriaanse PI, Ter Horst MM, Deneer JW, Van den Brink PJ (2015) Surface water risk assessment of pesticides in Ethiopia. Sci Total Environ 508:566–574. https://doi.org/10.1016/j.scitotenv.2014.11.049

Temmink RJ, Dorenbosch M, Lamers LP, Smolders AJ, Rip W, Lengkeek W, Didderen K, Fivash GS, Bouma TJ, van der Heide T (2021) Growth forms and life-history strategies predict the occurrence of aquatic macrophytes in relation to environmental factors in a shallow peat lake complex. Hydrobiologia 848(17):3987–3999. https://doi.org/10.1007/s10750-021-04618-6

Thomaz SM, Da Cunha ER (2010) The role of macrophytes in habitat structuring in aquatic ecosystems. Methods of measurement, causes and consequences on animal assemblages' composition and biodiversity. Acta 22(2):218–236. https://doi.org/10.4322/actalb.02202011

Thomaz SM (2021) Ecosystem services provided by freshwater macrophytes. Hydrobiologia. https://doi.org/10.1007/s10750-021-04739-y

Timms RM, Moss B (1984) Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. Limnol Oceanogr 29:472–486. https://doi.org/10.4319/lo.1984.29.3.0472

Topping CJ, Aldrich A, Berny P (2020) Overhaul environmental risk assessment for pesticides. Science 367(6476):360–363. https://doi.org/10.1126/science.aay1144

United States Environmental Protection Agency [US EPA] (2004) Overview of the ecological risk assessment process in the Office of Pesticide Programs, U.S. Environmental Protection Agency.

Endangered and threatened species effects determinations. https://www.epa.gov/sites/default/files/2014-11/documents/ecorisk-overview.pdf. Accessed 25 Feb 2022

United States Environmental Protection Agency [US EPA] (2012) OCSPP 850.4400: aquatic plant toxicity test using *Lemna* spp. Ecological Effects Test Guidelines.

United States Environmental Protection Agency [US EPA] (2017) Technical overview of ecological risk assessment—analysis phase: ecological effects characterization. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-risk-assessment-0. Updated 27 Oct 2017. Accessed 18 June 2020

United States Environmental Protection Agency [US EPA] (2021) Code of federal regulations. Title 40 protection of environment. Chapter I. Subchapter E. Part 158. Subpart G—Ecological Effects. https://www.ecfr.gov/cgi-bin/text-idx?SID=0331fe3d75b55e3a661228b871b3bdb7&node=sp40.24.158.g&rgn=div6. Accessed 22 Apr 2021

United States Environmental Protection Agency [US EPA] (2022a) Technical overview of ecological risk assessment: risk characterization. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-risk-assessment-risk#foot_2. Accessed 25 Feb 2022

United States Environmental Protection Agency [US EPA] (2022b) Technical overview of ecological risk assessment—analysis phase: ecological effects characterization. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-risk-assessment-0. Accessed 25 Feb 2022

United States Environmental Protection Agency [US EPA] (2022c) National aquatic resource surveys. https://www.epa.gov/national-aquatic-resource-surveys/indicators-macrophytes. Accessed 1 Feb 2022

Utembe W, Gulumian M (2015) Challenges and research needs for risk assessment of pesticides for registration in Africa. Humand Ecol Risk Assess 21(6):1518–1541. https://doi.org/10.1080/10807039.2014.958940

Vonk JA, Kraak MHS (2020) Herbicide exposure and toxicity to aquatic primary producers. In de Voogt P (eds) Reviews of environmental contamination and toxicology, vol 250. Springer, Cham. https://doi.org/10.1007/398_2020_48

Ward SM, Gaskin JF, Wilson LM (2008) Ecological genetics of plant invasion: what do we know? Invasive Plant Sci Manag 1(1):98–109. https://doi.org/10.1614/IPSM-07-022.1

Water Quality Australia (2019) Your location—ecoregional water quality guidance and guideline values for Australia and New Zealand. https://www.waterquality.gov.au/anz-guidelines/your-location. Accessed 15 May 2022

Whiteside M (2017) Environmental risk assessments at the Pest Management Regulatory Agency, November 9, Ottawa, ON. http://adamoliverbrown.com/wp-content/uploads/2017/11/OttawaU.Nov2017-3-slides.pdf. Accessed 4 Jan 2022

Wilkinson AD, Collier CJ, Flores F, Mercurio P, O'Brien J, Ralph PJ, Negri AP (2015) A miniature bioassay for testing the acute phytotoxicity of photosystem II herbicides on seagrass. PLoS One 10(2):e0117541. https://doi.org/10.1371/journal.pone.0117541

van Wijngaarden RPA, Arts GHP (2018) Is the tier-1 effect assessment for herbicides protective for aquatic algae and vascular plant communities? Environ Toxicol Chem 37(1):175–183. https://doi.org/10.1002/etc.3936

Zhu M, Zhu G, Nurminen L, Wu T, Deng J, Zhang Y, Qin B, Ventelä AM (2015) The influence of macrophytes on sediment resuspension and the effect of associated nutrients in a shallow and large lake (Lake Taihu, China). PLoS One 10(6):e0127915. https://doi.org/10.1371/journal.pone.012791