Recovery of duckweed following chronic herbicide exposure: Implications for risk assessment

by

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Abstract

Herbicide exposure in surface water usually occur as pulses, which may allow aquatic primary producers to recover from the exposure. In this thesis, the quality of published studies on the recovery of aquatic primary producers (macrophyte, periphyton and phytoplankton) after herbicide exposure, as well as the recovery potential of two duckweed species after 2,4-D and atrazine exposure were examined. Forty peer-reviewed recovery studies on aquatic primary producers were reviewed and the reliability and ecological relevance of these studies were evaluated using transparent criteria. The literature review revealed a low percentage of recovery studies from both exposure (i.e., 30%) and recovery (i.e., 35%) periods with >50% of total strength for the method score and >50% for the total endpoint ecological relevance score for risk assessment. We recommend the development of standard protocols for recovery and guidelines to interpret recovery results in ecological risk assessment to improve the quality of future studies. In addition, the recovery potential of *Lemna gibba* and *Lemna minor* after 7, 14, 21 and 28 days of 2,4-D and atrazine exposure, followed by a transfer to clean media for 7 days were investigated. The EC50s and NOECs from the recovery period on the relative growth rate of *Lemna* sp. after the exposure were ≥ 558 µg/L and ≥ 117 µg/L for 2,4-D, and ≥ 271 µg/L and ≥ 89 µg/L for atrazine. The toxicity values suggest that the duckweed had no adverse effect after 2,4-D exposure, and were able to recover after atrazine exposure. We also evaluated the toxicity effect of 2,4-D and atrazine on duckweed after a 60-day continuous exposure at regulation concentrations with test concentrations up to 8 µg/L for 2,4-D and 20 µg/L for atrazine. We conclude that the current regulations are likely able to protect freshwater macrophytes from chronic exposure. Overall, we recommend that the recovery of macrophytes be considered in ecological risk assessments to ensure that future decisions are made with realistic and relevant data.

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Chapter 1 Introduction

This thesis is an investigation into the recovery of primary producers following herbicide exposure. It is presented in two parts. Chapter 2 presents a critical literature review of studies reporting on the recovery of aquatic primary producers, specifically periphyton, phytoplankton, and macrophytes, after herbicide exposure. The current literature was evaluated using an objective scoring rubric to determine study quality based on test species, design, statistics, and relevance of endpoint to community-level effects. The evidence for recovery, the state of the science, recommendations for future studies, and the implications of current testing on risk assessment were evaluated. Chapter 3 investigates the recovery potential of two duckweed species (*Lemna gibba* and *Lemna minor*) after 7, 14, 21, and 28 days of exposure to two different modes of action herbicide (2,4-D and atrazine) under laboratory conditions. In addition, the toxicity of 2,4-D and atrazine on duckweed after 60-days of continuous and pulsed exposure in laboratory was investigated. The results were compared to environmental concentrations and existing guidelines on herbicide concentrations in surface water provided by the Canadian Council of Ministers of the Environment (CCME) and the United States Environmental Protection Agency (USEPA) to assess their ability to protect macrophytes from herbicide exposure.

1.1 Herbicides

1.1.1 General background

Herbicides are typically a chemical used to suppress unwanted plants herein after referred to as weeds in agriculture and other contexts. Weeds can reduce crop productivity in a number of ways including competing for resources (e.g. sunlight, nutrients, and space), releasing toxic compounds into the soil, fouling of agricultural equipment, and providing a host for pests and diseases. (Song, 2014; Vats, 2015). The greatest estimated global crop production losses for 2001 - 2003 were generated by weeds (~34%), followed by animal pests (~18%) and pathogens (~16%) (Oerke, 2006).

In early agriculture, weeds were removed by hand but over time, hand tools and machines were invented. Biological and chemical weed controls were developed and synthesized (Vats, 2015) later on with the majority of chemical weed killers herein after referred to as herbicides being created during the post-World War II “chemical era”. commencing around 1947 (Vats, 2015) and continuing to this day. In 2012, herbicides represented the greatest proportion of pesticide sales in the world at 44%), followed by insecticides (25%), fungicides (16%), and fumigants (2%) (USEPA, 2017). In addition, herbicide active ingredients amounted to 57% and 49% of pesticide active ingredients applied in the United States and globally, respectively (USEPA, 2017). Globally the use of herbicides has increased from 1.0 billion kgs in 2008 to 1.3 billion kgs in 2012, and in the United States use has increased from 245 million kgs in 2008 to 308 million kgs in 2012 (USEPA, 2017). The majority of herbicides are currently being used for agricultural crop production (USEPA, 2017) but are also registered for industrial, commercial, government, and personal uses (USEPA, 2017).

1.1.2 Application period

A variety of herbicides have been developed to target plants with different modes of action. The application period of an herbicide depends on its targets, mode of action, geographic region, and regulatory restrictions. There are three general application periods for herbicides; pre-plant, pre-emergence, and post-emergence (Ware, 1989; Vats, 2015). Pre-plant herbicides are sprayed in early spring before crops are planted or seeded to prevent degradation by sunlight and/or evaporation (Ware, 1989; Vats, 2015). Pre-emergent herbicides are applied prior to seed germination targeting weeds but not affecting the seed (Ware, 1989; Vats, 2015) while post-emergent herbicides are sprayed after seeds have germinated (Ware, 1989, Vats, 2015).

With the application of herbicides in agriculture there is the risk of movement off-field. Environment and Climate Change Canada (ECCC) monitors pesticide concentrations in Canadian surface water (Government of Canada, 2022). Their 2011 report mentioned that, between 2003-2005,10 out of the 12 most detected pesticides in surface water, precipitation, groundwater, and runoff samples were herbicides, which means they could be affecting non-target aquatic organisms (ECCC, 2011). Challis et al. (2018a) used passive sampling to monitor the atrazine concentration in Red River, Manitoba in 2014 – 2015. The study reported the concentrations peaked in June, which was shown to be associated with the application period of atrazine in May upstream in the United States. The study also showed that the United States was the main contributor to the atrazine concentration in Red River, and contribution from Western Canada was low (Challis et al., 2018a). Therefore, greatest herbicide concentrations tend to be found in aquatic ecosystems during/after the application period, and are not year-round exposures.

1.1.3 Mode of action

Herbicides have different modes of action to target different weed physiologies. Herbicides are categorized into groups based on their modes of action (Table 1.1). The majority of herbicides interrupt plant-unique biological mechanisms by binding at specific sites of action. In general, there are two categories of herbicides, non-selective and selective. Non-selective herbicides, for example glyphosate, do not have specific targets and are able to control many types of plants (Ross and Childs, 1996). Early herbicides tended to be non-selective, with more selective herbicides being invented after World War II (Vats, 2015). In contrast, selective herbicides are more toxic to certain plant species (De Carvalho et al. 2009). The selectivity is typically due to the mode of action and a plant’s traits (De Carvalho et al, 2009). This allows the selective herbicide to be effective on certain plants but not others during the time of application or applied in a method that only affected the target weeds (Ware, 1989). For example, dicamba is a selective herbicide that mimics a plant's growth hormones and mainly targets broadleaf weeds (Ross and Childs, 1996).

1.1.4 Inputs to the aquatic environment

There are several ways for herbicides to enter the aquatic environment following application onto agricultural land. Primarily, herbicides can enter nearby surface waters via overspray, spray drift, and runoff after rainfall events (Baker, 1983; Leonard, 1990; Peterson et al., 1994). Also, herbicides can contaminate groundwater through leaching (Graymore et al., 2001) which can then migrate to surface waters. The United States Geological Survey (USGS) and USEPA monitored the herbicide concentration in stormwater runoff in 17 American states, from August 2016 - December 2017, to estimate the potential effects on aquatic ecosystems (Masoner et al., 2019). The study revealed that most of the herbicide concentrations were within a range of 0.1 – 1000 ng/L (Masoner et al., 2019).

Once an herbicide enters an aquatic ecosystem, and depending on its modes of action (e.g., photosystem II inhibitor, growth regulator), it may affect non-target aquatic plants (Ross and Childs, 1996). The effect on non-target primary producers may reduce the supply of oxygen, food, and habitat for other aquatic organisms, and lead to long-term effects on the aquatic ecosystem (Harper and Bolen, 1996; Newbold, 1975). Most of the time, herbicides are present in the aquatic environment at relatively low concentrations, often below toxicity thresholds for aquatic organisms (USEPA, 2018b); however, exceedances can occur. Andrus et al. (2013) measured the atrazine concentration in the midwestern United States streams near agricultural lands that were identified as likely to be exposed to atrazine due to application. The study indicated that the concentration in streams was pulsed, with a peak concentration of 70.53 μg/L in mid-June, which was related to the application timing and higher than the regulatory concentration in surface water from CCME (e.g., 1.8 μg/L), and USEPA (e.g., <1 μg/L and 10 μg/L) (Andrus et al., 2013; CCME, 1999; USEPA 2018a; USEPA, 2021a).

1.1.5 2,4-Dichlorophenoxyacetic acid (2,4-D)

2,4-Dichlorophenoxyacetic acid (2,4-D) is a selective herbicide targeting broadleaf eudicotyledonous weeds. Its chemical structure is shown in Figure 1.1 (Song, 2014). 2,4-D is a pre-emergent and post-emergent herbicide that kills target plants by mimicking growth regulating auxins which affects cell division and elongation (Grossmann, 2010; Islam et al., 2018; Song, 2014). Once 2,4-D comes into contact with the plant it will act as an auxin and stimulate production of ethylene that affects the growth of the plant (Michel et al., 2004). As the concentration of ethylene increases, the plant growth is inhibited, ultimately resulting in plant death (Michel et al., 2004).

2,4-D has a high water solubility (569 mg/L at 20°C), and a low soil adsorption coefficient (0.067 – 1.1 cm^3/g). This means that it has a higher tendency to dissolve in water than to adsorb to soil, meaning that it can enter a water body through runoff or groundwater via leaching (Islam et al., 2018; USEPA, 2005b; Walters, 1999). The half-life of 2,4-D in aquatic environments ranges from 13 – 312 days, depending on the conditions (Walters, 1999). The half-life of 2,4-D in oxygenated freshwater is 15 days (USEPA, 2005a).

2,4-D has been available on the market since 1948 and it is still widely used because it is a cost-effective means to control broadleaf weeds (Islam et al., 2018; USEPA, 2005b). 2,4-D is used on cropland including fruits and vegetables, and non-cropland, which includes turf and lawns (USEPA, 2005b). In 2012, in the United States, 2,4-D was the fifth most commonly used agricultural pesticide, increasing from 9.5-14.1 million kgs applied in 2005 to 13.6–18.1 million kgs applied (USEPA, 2017). With the extensive use of 2,4-D and its relatively high water solubility, greater concentrations of 2,4-D are likely to be present in the aquatic environment, with the potential to cause impacts on non-target biota (Challis, 2018b).

Measured concentrations of 2,4-D in the aquatic environment have been reported across Canada. Between 2003-2005 the ECCC conducted a pesticide-monitoring program to characterize the pesticide concentration in aquatic environments in provinces across Canada. In Alberta, Saskatchewan, and Manitoba 2,4-D was detected in 100% of water samples collected in 2004 from wetlands near agricultural lands, and in 92.2% of water samples collected from rivers (ECCC, 2011). The detected concentrations of 2,4-D in wetlands ranged from <0.47 to 4290 ng/L (ECCC, 2011) while 2,4-D concentrations in rivers were reported to have a range of <0.47 to 457 ng/L while reservoirs were reported to have a range of <0.47 to 1850 ng/L (ECCC, 2011). The 2,4-D concentrations in the Lower Fraser Valley and the Okanagan Basin, in British Columbia, ranged from <0.5 to 1230 ng/L (ECCC, 2011). The 2,4-D concentrations in Ontario and Quebec surface water were 0.71 – 8240 ng/L, and <10 – 340 ng/L, respectively (ECCC, 2011). Zero detection rates for 2,4-D have been found in surface water in Prince Edward Island, Nova Scotia, and New Brunswick (ECCC, 2011). From April to October 2016 Challis (2018b) sampled the water along the Red River to investigate herbicide concentrations. The time-weighted-average concentrations of 2,4-D was reported as ranging from <4.1 ng/L to 197.7 ng/L with a LOD of 4.1 ng/L (Challis, 2018b). Glozier et al. (2012) measured the concentration of 2,4-D in 16 watersheds in British Columbia, Alberta, Saskatchewan, Ontario, Quebec, New Brunswick, Prince Edward Island, and Nova Scotia, with a total of 19 sampling sites; 15 sites downstream of an urban area, 2 agriculture sites, and 2 reference sites. Three samples were collected per site during or after rainfall events between April and September 2007 (Glozier et al., 2012). The highest and lowest concentrations of 2,4-D were 1,960 ng/L and <0.47 ng/L, respectively with an average of 172.1 ng/L across the 19 sites (Glozier et al., 2012).

Based on the results of toxicity studies and risk assessments, regulations and guidelines have been established to protect aquatic ecosystems. The Canadian Council of Ministers of the Environment (CCME) has established water quality guidelines for the protection of aquatic life in freshwater and marine environments for some herbicides and pesticides. The surface water guideline concentration for 2,4-D is 4 μg/L (CCME, 1999; 2007b). The acute (<10 days) aquatic life benchmark of 2,4-D for vascular plants is 299.2 μg/L (USEPA, 2021a). The reported toxicity values of *Lemna* sp. after 2,4-D exposure are provided in Table 1.2.

1.1.6 Atrazine

Atrazine (Figure 1.2) is a triazine herbicide that targets broadleaf weeds by reversibly inhibiting photosynthesis (CCME, 1999). The presence of the herbicide prevents the transport of electrons between the D1 protein and plastoquinone within photosystem II (Brain et al., 2012b). Atrazine is a selective and systemic herbicide that is usually applied to corn, sorghum, sugarcane and blueberry crops. In Canada it was used on non-cropland prior to 2004; however, (CCME, 1999; LeBaron et al., 2008) after 2004, Health Canada's Pest Management Regulatory Agency (PMRA) limited the application of atrazine to corn, located mainly in Ontario and Quebec (PMRA, 2003). Atrazine was first registered in the United States in 1958 and has been available for weed control in North America since 1959 (USEPA, 2006). The herbicide has been widely used in North America as pre- or post-emergent herbicide (LeBaron et al., 2008). In 2012, 29.0 – 33.6 million kgs of atrazine was used by the United States agricultural industry, making it the second most commonly applied pesticide in the country (USEPA, 2017). Due to its popularity, the probability of atrazine reaching the aquatic ecosystem and non-target organisms is relatively high.

Atrazine has a moderate solubility (33 mg/L) and long half-life, ranging from 3 to >90 days in aquatic environments (CCME, 1999; Solomon et al., 1996; Yalkowsky et al., 2010). Studies have reported atrazine in groundwater, surface water, and soil across Canada. Between 2003-2005, the concentration of atrazine in the Lower Fraser Valley and the Okanagan Basin in British Columbia ranged from <0.016 to 64.5 ng/L (ECCC, 2011) while between 2003-2004 the atrazine concentration in Ontario and Quebec surface water ranged from 6.43 – 14900 ng/L and <20 – 2200 ng/L, respectively (ECCC, 2011). Atrazine was not detected in surface water in Prince Edward Island or Nova Scotia between 2003 – 2005 (ECCC, 2011) but it was detected in 25% of surface water samples from New Brunswick in the same period (ECCC, 2011). Between 2003-2004 atrazine was not detected in rivers or wetlands in Alberta, Saskatchewan or Manitoba. However, during the same period atrazine was detected in reservoir water in Alberta, Saskatchewan, and Manitoba with a range of 1.7 – 52.7 ng/L (ECCC, 2011). Challis et al. (2018a) measured the concentration of atrazine in the Red River which flows from the United States to Lake Winnipeg, Manitoba. In 2014 and 2015 five sampling sites in Manitoba were established along the Red River and water samples were collected from April to October. Atrazine was detected in all of the samples. The greatest time-weight concentration was reported to be 520 ng/L while the average was 63.0 ng/L (Challis et al., 2018a).

In the United States, Andrus et al. (2013) monitored the concentration of atrazine as part of the Atrazine Ecological Exposure Monitoring Program. Three watersheds located near high-intensity corn production areas were monitored for 16 weeks between May and August 2011. The USEPA selected the watersheds because of their high quality soil, small streams and high applications of atrazine (Andrus et al., 2013; USEPA, 2018a). Pulses of atrazine with peak concentrations up to 70.53 μg/L were recorded in the watershed, usually lasting less than 15 days, with an average concentration of 20 μg/L (Andrus et al., 2013).

The CCME water quality guideline for atrazine in surface water is 1.8 μg/L (CCME, 1999). Likewise, the USEPA has established a Concentration Equivalent Level of Concern (CE-LOC) to control the atrazine concentration in the watershed to protect the aquatic plant species. The CE-LOC states the average atrazine concentration over a 60-day period should not exceed 10 μg/L (USEPA, 2018a). Mitigation activities, including training sessions, and education and outreach for farmers and distributors on proper atrazine application, would be required to decrease the atrazine concentration in the watershed when the atrazine concentration exceeds 10 μg/L in a 2-year monitoring program (USEPA, 2018a). In addition, the acute (<10 day) aquatic life benchmark for atrazine on nonvascular plants is <1 μg/L (USEPA, 2021a). The reported toxicity values of *Lemna* sp. after atrazine exposure levels are listed in Table 1.3.

1.2 Primary producers in ecological risk assessment

1.2.1 Role in freshwater aquatic ecosystems

Primary producers (periphyton, phytoplankton, macrophytes) are important components of an aquatic ecosystem. Primary producers provide oxygen, supply food, and give shelter and protection to aquatic species (Harper and Bolen, 1996; Hanson, 2013). Abubakr (2010) examined the relationship between aquatic macrophytes and water quality. The study showed that the aquatic macrophytes improved the aquatic ecosystem by stabilizing the sediment at the bottom of lakes and rivers, remobilizing nutrients, cations and anions, and removing nutrients (e.g., nitrogen and phosphorus) in part by changing the pH and conductivity (Abubakr, 2010; Pérez et al., 2011).

The mode of action of herbicides usually removes weeds by inhibiting plant growth and synthesis of biochemistry in plants (Armstrong, 2019; Sherwani et al., 2015; Ross and Childs, 1996). Herbicides that enter surface water may cause adverse effects on non-target aquatic primary producers and organisms at higher tropic levels (Fairchild et al., 2011). If a population of macrophytes or periphyton are affected by an herbicide other aquatic organisms could be impacted through ecological mechanisms. Studies have found that the presence of atrazine in the water can reduce the population of primary producers, leading to a decrease in oxygen and an increase in turbidity (Graymore et al., 2001). This in turn may result in insufficient oxygen for other organisms, and a lower penetration of sunlight in the water column.

1.2.2 Role of primary producers in ecological risk assessment

Risk assessment is intended to protect aquatic plant species and primary producers from herbicide toxicity by evaluating the potential effect of herbicides in an environmental context prior to registration. Before an herbicide is brought to market an ecological risk assessment is required. Given the importance of primary producers in maintaining aquatic ecosystem structure and function, toxicity tests on primary producers, including duckweed (*Lemna* sp.) or algae is a requirement of lower tier risk assessment for herbicide registration in Europe (1107/2009/EC) (EC, 2009) and North America (PCPA, 1985; USEPA, 2007a). Governments request acute and/or chronic toxicity data from certain species of different trophic levels to characterize toxicity and set guidelines. Data on aquatic primary producers is usually one of the requirements (USEPA, 2020a; CCME, 2007a). In addition, the toxicity effect of herbicides on aquatic primary producer plants could be used to predict indirect effects on other species (e.g., mussel) (USEPA, 2007b).

The USEPA requires acute toxicity testing data (e.g., EC50) from five aquatic plant species for ecological risk assessment of herbicides in Tier I (USEPA, 2020a). The five aquatic plant species required by the USEPA are *Skeletonema costatum* (marine diatom), *Lemna gibba* (vascular plant), *Anabaena flos-aquae* (blue-green algae), *Pseudokirchneria subcapitata* (green algae), and one freshwater diatom (USEPA, 2020a). The Canadian Water Quality Guideline requires at least one aquatic vascular plant or algal study in the lower tier for establishing a pesticide guideline; however, this is not mandatory when there is not enough toxicity data (CCME, 2007a; Dobbins et al., 2010). Conversely, three toxicity studies from algal or freshwater plants are required if aquatic plants are the most sensitive species to the pesticide in question, for example an herbicide. (CCME, 2007a; Dobbins et al., 2010). The European Council Directive 91/414/EEC requires the risk assessment of herbicides to include toxicity testing with two species of algae including one green algae (e.g., *Pseudokirchneriella subcapitata*), one alga from another taxonomic group (e.g., diatom), and one species of macrophyte (e.g., *Lemna* spp.) (EC, 2002; 2013). Other macrophyte studies may be needed if the herbicide targets eudicots or other monocots, for example aquatic grass (EC, 2013). The toxicity data required for the risk assessment are EC10, EC20, EC50 and NOEC on growth rate and yield based on algal biomass. *Lemna* frond number and one more *Lemna* endpoint (EC, 2013) are also required.

To ensure that toxicity data considered in ecological risk assessment are of sufficient quality and reliability, organizations have different approaches to assess the data. The CCME categorizes toxicity data based on the test design (e.g., measurement of the exposure concentrations, endpoints that are relevant to the ecological effects) and statistics (e.g., provision of a concentration-response relationship) as primary data, secondary data, and unacceptable data (CCME, 2007a). Both primary and secondary data can be used to plot species sensitivity distribution, but more weight is placed on primary data (CCME, 2007a). Assessing toxicity testing data quality is important for improving the quality of future studies and establishing more reliable guidelines.

1.2.3 Duckweed toxicity testing

1.2.3.1 Test design and history

To help protect freshwater aquatic ecosystems and inform ecological risk assessment, toxicity testing is used to evaluate the potential effect of chemicals on biological receptors (ECCC, 2007). Duckweed toxicity testing is one of the most common toxicity tests used. It was developed in the 1930s and was first used to evaluate the phytotoxicity effect after exposure to phenoxy herbicide (Blackman and Robertson-Cummingham, 1955; ECCC, 2007; Moody and Miller, 2005). Duckweed *(Lemna* spp*.*) is a floating monocot that usually has 2-3 fronds per plant (ECCC, 2007). It is characterized by its wide global distribution, with approximately 40 different species, its high growth rate, ease of culturing, and sensitivity to chemicals. The characteristics make it a model species for toxicity testing (ECCC, 2007; Wang, 1990). Duckweed is an important protein source for aquatic birds, more specifically, for the growth of bone and tissue of female waterfowl during the early stages of the breeding period (Harper and Bolen, 1996). Duckweed chemically influences its environment by sequestering nutrients in the system (Arts et al., 2010). Guidelines and protocols have been developed for duckweed (mainly for *Lemna gibba* and *Lemna minor*) toxicity testing by ASTM International (ASTM, 2003), ECCC (ECCC, 2007), Organization for Economic Co-operation and Development (OECD, 2002), and the USEPA (USEPA, 2012a). Environmental conditions in duckweed toxicity tests can vary by protocol, for example static or continuous water renewal (ECCC, 2007). Details of toxicity testing conditions are listed in Table 1.4. The duration of the toxicity testing can be categorized as acute (<7 d) and chronic (≥7 d) to represent intense and short-term or long-term exposure at lower concentrations, respectively.

Researchers have expressed concerns regarding the use of duckweed and its sensitivity to the chemicals because of its physiology. Duckweed is a monocot and a floating plant without an extensive root structure (Hanson, 2013). There is concern that toxicity results may not be representative of eudicot plant species, which may contact chemicals through their roots in the sediment, or may be more sensitive to the toxicity of herbicides (e.g., dicamba; growth regulator) that target eudicots (Hanson, 2013; Ross and Childs, 1996). In addition, the floating characteristic of duckweed may reduce the exposure to chemicals that tend to partition into sediment (Hanson, 2013; Ross and Childs, 1996). Numerous studies have been performed to compare the sensitivity of duckweed to other algal and macrophytes species. The sensitivity of duckweed (*Lemna minor*) was compared to algae, diatoms and cyanobacteria by exposing all species to a suite of 23 pesticides (Peterson et al., 1994). The authors reported that duckweed was more adversely affected by the majority of the pesticides than other species (Peterson et al., 1994). They concluded that the sensitivity of duckweed was the same or greater than the other species for many pesticides. In contrast, Fairchild et al. (1998) studied the sensitivity of five macrophytes and six algae to atrazine, metribuzin, alachlor, and metolachlor and found that duckweed (*Lemma minor*) had a moderate sensitivity when compared to other tested macrophytes (Fairchild et al., 1998). In the study, the duration of exposure was 4 days for duckweed and 7 days for other macrophytes (Fairchild et al., 1998). The exposure duration of duckweed to the herbicides was shorter than for other species, which can explain the lower toxicity to duckweed under the tested conditions.

1.2.3.2 Endpoints

Endpoints are the measurements on the test species that could be used to evaluate the toxicity effect of the chemicals (ECCC, 2007). Endpoints in duckweed toxicity testing are mainly related to the growth or reproduction of duckweed, including plant number, frond number, total frond area, wet weight, dry weight, and their associated relative growth rates (RGR) (Brain et al., 2012b; Mohr et al., 2007; Teodorović et al., 2012). Endpoints can also be associated with physiological or metabolic processes, including chlorophyll production and carbon uptake (Jones et al., 1986). Endpoints could be used to represent the data relevance of the studies by connecting the reported endpoints to community or population level of effect (Hanson et al., 2019). An endpoint with high relevance could represent possible toxicity at a community or population level and be more useful for risk assessment (Hanson et al., 2019). In the case of duckweed, endpoints associated with growth would have a higher relevance than endpoints with chlorophyll production.

Typically, the median effective concentration (EC50), the lowest observable effect concentration (LOEC), and the no observed effect concentration (NOEC) can be calculated for the test substance (ECCC, 2007). The EC50 is the concentration where 50% of the test organisms are affected lethally or sub-lethally by the chemical and can be determined by plotting a dose-response curve (ECCC, 2007). The LOEC is the lowest tested concentration that is statistically significant from the control (ECCC, 2007). The NOEC is the highest tested concentration that is not statistically significant from the control (ECCC, 2007). These values can be used in the determination of hazard and risk for herbicides to aquatic primary producers.

Concern about the use of NOECs in ecological risk assessment was discussed in an OECD workshop in 1996 (OECD, 1998b). The reasons for concern included the fact the NOEC could be limited by the test design, including test concentration and replication. and it was recommended it be replaced by ECX determined by the concentration-response curve (DeForest et al., 2008;

OECD, 1998b). Previous studies were performed to determine the relationship between NOEC (i.e., the concentration that would not cause a significant toxicity effect) and ECX values (EC10 and EC20), in order to suggest ECX values that could be used as a surrogate for the NOEC in the risk assessment (DeForest et al., 2008; Beasley et al., 2015). Beasley et al. (2015) compared EC10 and EC20 to NOEC from 200 *Daphnia* toxicity tests The study concluded that EC10 was more closely related to NOEC than EC20 (Beasley et al., 2015). DeForest et al., (2008) also analyzed the relationship between NOEC to EC10 and EC20 on the toxicity of selenium on mallard ducks. The study concluded that mallards were not likely to be significantly affected at or below the concentration of EC10 and could be used in risk assessment to protect the mallard population. We would compare the exposure and recovery data after atrazine exposure EC10s to their NOECs in order to understand if the EC10 could be to be used as an effective surrogate for risk assessment.

1.3 Recovery testing

1.3.1 Assessing recovery in primary producers

Herbicides in the environment usually occur as pulsed events, in other words a rapid increase in concentration followed by a sharp drop in concentration, following a rainfall and spray drift event (e.g., Andrus et al., 2013). A pulsed exposure provides an opportunity for primary producers to recover from the exposure should effects have occurred (Brain et al., 2012b). Examining the recovery of primary producers could help to better predict the long-term effect of herbicides on aquatic ecosystems and inform more fully the duration of effects as it relates to risk assessment.

Recovery testing is used to evaluate the potential for recovery following exposure to a test chemical. Tests have been conducted to examine the recovery potential on different aquatic plant species, including the periphyton community, duckweed, seagrass (Baxter et al., 2014, Burns et al., 2015, Haynes et al., 2000). A general recovery testing procedure is to transfer the exposed test species from media containing an herbicide to clean medium. The test species is then allowed to continue to grow in the absence of the chemical stressor (Baxter et al., 2014, Brain et al., 2012b, Burns et al., 2015, Haynes et al., 2000). The test species is deemed fully recovered from the exposure if the endpoint of interest is determined to be not statistically significant from the control group at the end of the recovery period (Brain et al., 2012b). However, recovery is not mandatory in risk assessments, therefore, it is rarely considered in ecological risk assessment for herbicide registration (Brain et al., 2012b).

For herbicides acting by reversible modes of action, recovery becomes possible for aquatic organisms following an exposure. Van Oorschot (1965) performed an experiment using CO_2 uptake and transpiration of plants to evaluate the recovery potential after herbicide exposure. The CO_2 uptake of maize was observed during an exposure period with simazine and an increase in CO_2 measured after the simazine was removed (Van Oorschot, 1965). The results suggest recovery of photosynthesis in maize after exposure to simazine (Van Oorschot, 1965). Similarly, Brain et al. (2012b) performed a recovery study on duckweed after exposure to atrazine. The result showed that a majority of *Lemna gibba* growth rates recovered at the end of the recovery period, except for those exposed at the highest concentration (160 μg/L) (Brain et al., 2012b). Brain et al. (2012a) also explored the recovery potential of green (*Pseudokirchneriella subcapitata*), blue-green (*Anabaena flos-aquae*), and diatomaceous (*Navicula pelliculosa*) algae after a pulsed atrazine exposure. Photosynthesis (measured by photosystem II quantum yield) and growth rate of the tested algae recovered at all concentrations (5 – 250 μg/L for *P. subcapitata and N. pelliculosa*; 25 – 1000 μg/L for *A. flos-aquae*) during the 48-hour recovery period after being exposed to atrazine for 48 hours (Brain et al., 2012a). Brain et al. (2012a; 2012b) concluded that atrazine is unlikely to have adverse effects on primary producers under a pulsed exposure. Burns et al. (2015) examined the recovery potential of duckweed (*Lemna gibba* and *Lemna minor*) after 7-days of diuron (photosystem II inhibitor) exposure. An inhibition of biomass and growth were observed in both species after the exposure. After being transferred to clean medium, recovery was observed at all the test concentrations (0.4 – 208 µg/L) for *Lemna gibba*, and most of the test concentrations for *Lemna minor* (0.4 – 111 µg/L) with the exception of highest concentration (208 µg/L) (Burns et al., 2015). Boxall et al. (2013) examined the toxicity effect of herbicides (isproturon, metsulfuron-methyl, and pentachlorophenol) on *Lemna minor* with repeated pulsed exposure. *L. minor* was exposed to either a two or four consecutive day pulses every 7 days for 42 days under laboratory conditions (Boxall et al., 2013). The results indicated that samples that been exposed to high concentrations had not recovered after the 4-day pulses at time-weighted-average concentrations of ≥ 50 µg/L isoproturon, ≥ 0.5 µg/L metsulfuron-methyl, ≥1000 µg/L, and 2-day pulses at ≥100 µg isoproturon/L, ≥ 0.5 µg/L metsulfuron-methyl, ≥1000 µg/L (Boxall et al., 2013).

Except for duckweed, recovery of other primary producer species after herbicide exposure have also been studied. King et al. (2016) investigated the periphyton and phytoplankton communities in artificial streams after 60-days of three 4-days pulsed atrazine exposures. Each pulse was separated by 7-days. The water used in the experiment was collected from a nearby river and then put through a constructed wetland (King et al., 2016). The tested pulse concentrations were 0, 50, 100, and 150 µg/L and the corresponding time-weighted-average concentrations were 0, 10, 20, and 30 µg/L (King et al., 2016). The study concluded that communities with the treatment were not statistically significant from the control, at all pulse concentrations, at the end of the 60-day experiment (King et al., 2016).

1.4 Data quality and reporting

Scientific data on the toxicity of pesticides on non-target organisms and environmental concentrations of pesticides are required to predict risk. They are also critical in making decisions to establish regulations and guidelines on the use of chemicals, pesticides, and physical agents to protect non-target organisms in aquatic ecosystems (USEPA, 2016; National Research Council (U.S.), 2007). With toxicity data predicted no-effect concentrations are determined to make decisions on industrial registration, and to establish environmental quality standards for regulating chemical concentrations in the environment (Moermond et al., 2016; Ågerstrand et al., 2011). The USEPA has established Aquatic Life Benchmarks to identify sites that need to be monitored to protect aquatic species from pesticides in freshwater (USEPA, 2021a). To determine the Aquatic Life Benchmarks exposure toxicity data of pesticides on different freshwater species, including aquatic plants, fish and invertebrates, are used to evaluate the risk of the pesticides on freshwater organisms (USEPA, 2021a; USEPA, 2021b). As a result, the quality of the toxicological data in the ecological risk assessment is critical (National Research Council (U.S.), 2007; Kase et al., 2016).

To ensure quality data, the Organisation of Economic Co-operation and Development (OECD) developed Good Laboratory Practices (GLPs) to encourage quality studies that would produce valid, consistent, reliable, reproducible data (OECD, 1998a; Durada and Preziosi, 2000). These test guidelines and principles promote testing transparency by encouraging documentation of test procedures and reporting of raw values (Klimisch et al., 1997; Moermond et al., 2016; OECD, 1998a; Roncaglioni et al, 2011). With the standard guidelines in place, OECD countries could now use resources more effectively by preventing replication of studies as well as improving the quality of data being considered in first-tier risk assessments of pest control products (OECD, 1998a; Roncaglioni et al, 2011).

To better determine the quality and value of the data being used for risk assessments Klimisch et al. (1997) suggested three main factors be considered; reliability, relevance, and adequacy. Reliability is based on the amount of detail provided to determine whether a test is valid; relevance is if the endpoints collected are suitable for the purpose of the risk assessment; and adequacy is if the test result is vulnerable for the risk assessment (Klimisch et al., 1997). Previous studies have mentioned the issues regarding data quality and transparency. Generally, these studies found that by increasing the transparency of testing, by providing more detailed experimental methods, there is an increase in the reproducibility and credibility of the study (Klimisch et al., 1997; Moermond et al., 2016). Klimisch et al. (1997) proposed a system to analyze and rank the toxicity data and categorized the reliability of data into four groups based on established GLPs and/or standard protocols and the information provided (e.g., relevant test organisms). Later studies began to find that following GLP practices and/or standard protocols alone was not enough to identify if the toxicity data are reliable or relevant for risk assessment (Moermond et al., 2016; Ågerstrand et al., 2011). Also, the system proposed by Klimisch et al. (1997) did not explain well how to sort the toxicity data into categories (Schneider et al., 2009). Therefore, more criteria with higher transparency and consistency were developed to evaluate toxicity data quality as noted in Klimisch et al. (1997) (see Ågerstrand et al., 2011; Durda and Preziosi, 2000; Moermond et al., 2016; Hanson et al., 2019; Kase et al., 2016; Schneider et al., 2009).

A consideration for toxicity data is that the exposure concentration should be relevant to the environmental concentration to be useful and relevant for ecological risk assessment (Ågerstrand et al., 2011; Burden et al., 2020; Durda and Preziosi, 2000). Numerous studies have found that herbicide concentrations in freshwater pulse over time and are associated with the times of application and runoff (Andrus et al., 2013; Challis, 2018b; Brain et al., 2012b). Due to the mode of action of herbicides, aquatic plants are usually most likely to be affected because of their similar growth mechanisms (Ross and Childs, 1996; Brain et al., 2012a). Recovery of aquatic primary plants after herbicide exposure has been observed in previous studies and indicates that plants have the ability to recover between herbicide pulses (see Baxter et al., 2013; Brain et al., 2012a; Brain et al., 2012b; Burns et al., 2015; Cedergreen et al., 2005). With the pulses in nature, aquatic species could recover between the pulses and it should be included in the risk assessment (Barnthouse, 2004). However, recovery is rarely included or described in the standard protocol (e.g., biological test method on *Lemna minor* from ECCC; algal toxicity testing guideline from USEPA) because recovery data is not being required during the regulatory process (Barnthouse, 2004; Brain et al., 2012b; ECCC, 2007; Moermond et al., 2016; USEPA, 2012a). Recovery is mentioned in the guidelines for risk assessment on plant protection products of the European Commission but has been rarely considered in the decision-making process due to the lack of guidelines to integrate recovery data into risk assessment (Brain et al., 2012b; Moermond et al., 2016). Additionally, few standard protocols for toxicity testing on aquatic plants have detailed procedures to analyse recovery after exposure, except for a brief description in algae testing from the USEPA (ECCC, 2007; USEPA, 2012a; USEPA 2012b). The lack of standard protocols and guidelines to interpret the data in risk assessment could lead to fewer recovery data having high reliability and relevance to be considered in risk assessment. Therefore, there is a need to evaluate the reliability and relevance of the recovery data on aquatic plants using robust and transparent criteria and consistent observations of recovery in the studies. This will then identify where testing components are lacking and improve the reliability and relevance of the data for risk assessment.

1.5 Objectives and hypotheses

With the increased use of herbicides around the world, understanding their effects on primary producers is essential. The wide use of herbicides in agriculture and urban activities results in their presence in surface water due to overspray, spray drift, runoff, and discharge into groundwater (Baker, 1983; Leonard, 1990; Peterson et al, 1994). As a result, there are concerns on the toxicity of herbicides on non-target organisms. Governments around the world have performed ecological risk assessments on herbicides and have established guidelines on herbicide concentrations in surface water. High quality data are needed to reduce uncertainty and ensure environmental protection.

The objectives of the thesis are: (1) to review and evaluate the quality of data around the recovery potential of macrophytes, periphyton, and phytoplankton after herbicide exposure based on peer-reviewed studies; (2) to characterize the recovery potential of duckweed after different durations of herbicide exposure; and (3) to assess current government regulations and guidelines on herbicide concentration in surface water. Our hypotheses are: (1) recovery studies on macrophytes, algae, and periphyton indicate that aquatic plants have the potential to recover from herbicide exposure at environmentally relevant concentrations due to reversible inhibition as observed in previous studies; (2) duckweed can recover from atrazine and 2,4-D exposure at environmentally relevant concentrations regardless of duration based on the observations of previous studies (e.g., Brain et al., 2012b, Mohammad et al., 2010); and (3) current government regulations and guidelines can protect aquatic ecosystems from long-term effects caused by herbicide exposure because the guidelines were established with toxicity data from various aquatic species.

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1.7 Tables

Table 1.1. The modes of action for different groups of herbicides currently registered for use in North America (taken from Armstrong, 2019; Iowa State University, 2005; Lewis et al., 2016; Miller and Westra, 1998, Neal, 2014; Ross and Childs, 1996; Sherwani et al., 2015; Syngenta, 2022; USEPA, 2020b; USEPA, 2021c; Willowood, 2012; WSSA, 2022).

Chemical family[a]	**Mode of action**	**Application time (mainly)**	**Target weed example**	**Crops**	**Examples**
Group 1	Hinder the activity of acetyl-CoA carboxylase (ACCase) and prevent lipid synthesis	Post-emergence	Broadleaf weed	Non-grass crop	Diclodop Quizalofop
Group 2	Inhibit the synthesis of branched-chain amino acid by preventing the activity of acetolactate synthase (ALS)	Pre-plant, pre-, post-emergence	Wild garlic, Canada thistle, broadleaf weed, johnson grass, quackgrass, wirestem muhly	Soybeans, corn, alfalfa, wheat, barley, non-crop	Chlorsulfuron Imazaquin.
Group 3, 23	Root growth inhibitors which interrupt the functions of microtubules to prevent the cell division and inhibit root growth	Pre-plant, pre-emergence	Broadleaf weed, seedling grasses	Soybeans, peanuts, cotton, alfalfa, lettuce, woody species	Trifluralin Pendimethalon
Group 4	Synthetic growth regulator that shares the same structures as auxins. The synthetic auxins interrupt the activity of indole acetic acid, and affect cell growth	Post-emergence	Annual, simple perennial, and creeping perennial broadleaf weed	Grass crops (e.g., corn, sorghum, turf) and non-crop	2,4-D Dicamba

Table 1.1. Continued.

Chemical family	**Mode of action**	**Application time (mainly)**	**Target weed example**	**Crops**	**Examples**
Groups 5, 6, 7	Photosystem II inhibitors bind to the quinone protein at photosynthesis II to interrupt the electron transport chain, resulting in disruption of pathways (e.g., CO_2 fixation, and ATP generation) for plant growth and survival.	Pre-plant, Pre-, post-emergence,	Weeds, general vegetation	Corn, soybeans, potatoes, celery, parsnips, carrots, cotton, alfalfa, asparagus, mint, non-crop	Atrazine Simazine
Groups 8, 15	Inhibit their shoot growth at the seedling stage	Pre-plant, pre-emergence	Seedling grasses, broadleaf weed	Corn, soybeans, large and seeded legumes, beets, spinach, potatoes	Metolachlor Alachlor
Group 9	Inhibit the synthesis of amino acids	Post-emergence	Annual grasses and broadleaf, johnson grass, turf, cattail, Canada thistle, hemp dogbane, poison ivy, multiflora rose	Soybean	Glyphosate
Group 10	Interrupt the glutamine synthetase, an enzyme required in nitrogen metabolism	Post-emergence	Non-selective	Non-crop, apples, grapes	Glufosinate

Table 1.1. Continued.

Chemical family	Mode of action	Application time (mainly)	Target weed example	Crops	Examples
Group 11, 12, 13, 27	Inhibit the enzymes that catalyze synthesis of the pigment carotenoid	Pre-plant, pre-emergence	Poison ivy, aquatic weed	Soybeans, cotton	Norflurazon Isoxaflutole
Group 14	Inhibit protoporphyrinogen oxidase (PPO), an enzyme within the synthesis of chlorophyll and heme, resulting in impairment to the cell membrane	Pre-, post-emergence	Broadleaf weed	Soybeans, peanuts, rice	lactofen Sulfentrazone.
Group 16, 17, 25, 26	Unknown	Pre-plant, pre-, post-emergence	Annual broadleaf weed, annual grass weed	Sugar beets, garden beets, onion, garlic	Ethofumesate
Group 18	Inhibit dihydropteroate synthetase	Post-emergence	Alexander grass, Johnson grass, crabgrass	Sugarcane, turf, non-crop	Asulam
Group 19	Inhibit auxin transport	Post-emergence	Annual and biennial broadleaf weed	Corn	Diflufenzopyr
Group 20, 21, 28, 29	Inhibit cell wall synthesis	Post-, pre-emergence	Annual and perennial broadleaf weed	Woody ornamentals, nut, non-crop	Dichlobenil

Table 1.1. Continued.

Chemical family	Mode of action	Application time (mainly)	Target weed example	Crops	Examples
Group 22	Photosystem I inhibitor	Post-emergence	Non-selective	Soybeans, corn, cotton	Paraquat Diquat
Group 24	Uncouple oxidative phosphorylation	Pre-emergence	Annual broadleaf weed	Grapes, legumes, maize	Dinoterb

[a]The herbicides that have the same mode of action but belongs to different chemical family group (e.g., carbamate, triazine, urea). The groups are coded by the Global Herbicide Resistance Action Committee (HRAC) and Weed Science Society of America (WSSA).

Table 1.2 The reported toxicity values of 2,4-dichlorophenoxyacetic acid to *Lemna* spp. from the peer-reviewed literature.

Species	**Endpoint**	**Level of effect**	**Duration**	**Toxicity (µg/L)**	**Reference**
Lemna paucicostata	Total frond area	EC50	7 days	2210[a]	Michel et al., 2004
Lemna gibba	Biomass[b]	EC50	4 days	>100,000	Fairchild et al., 1997
Lemna trisulca	Biomass	EC50	28 days	>3000	Belgers et al., 2007
Lemna trisulca	Relative growth	EC50	28 days	>3000	Belgers et al., 2007

[a]Estimated value due to failure to be calculated by regression model,

[b]Biomass was estimated by frond number.

Table 1.3. The reported toxicity values of herbicide atrazine to *Lemna* spp. from the peer-reviewed literature.

Species	Endpoint	Level of effect	Duration	Toxicity (95%C.I.) (μg/L)[a]	Reference
Lemna gibba	Average growth rate on frond number	EC10	7 days	45 (32 -56)	Brain et al., 2012b
		EC50	7 days	124 (90 – 150)	
		EC10	14 days	53 (48 – 58)	
		EC50	14 days	>75 (NC[b])	
Lemna minor	Frond number	EC50	96 hrs	92 (80-104)	Fairchild et al., 1998

[a]Effective concentrations and 95% confidence interval,

[b]NC is not determined due to only two concentrations were tested.

Table 1.4. The settings of standard duckweed toxicity testing from different organizations' guidelines and protocols (taken from ECCC, 2007).

Test Component	**ASTM**[a]	**ECCC**[b]	**OECD**[c]	**USEPA**[d]
Test type	Static	Static, static-renewal	Static, static-renewal	Static-renewal
Test duration	7-day	7-day	7-day	7-day
Test species	*L. gibba*	*L. minor*	*L. gibba*, *L. minor*	*L. gibba*, *L. minor*
Test medium	Hoagland's E+, Modified Hoagland's, or 20X-AAP	Modified Hoagland's E+ medium	20X-AAP (for *L. gibba*); SIS medium (for *L. minor*)	Modified Hoagland's
Test temperature (°C)	25 ± 2	25 ± 2	24 ± 2	25 ± 2
Light intensity	Continuous 6200 – 6700 lux	Continuous 4000 – 5600 lux	Continuous 6500–10000 lux	Continuous 4200–6700 lux
Test concentration	≥5 plus control(s); geometric series; ≥0.6 dilution factor	≥7 plus control(s)	≥5 plus control(s); geometric series; ≥0.3 dilution factor	≥5 plus control(s); geometric series; ≥0.67 – 0.5 dilution factor

[a]American Society for Testing and Materials,

[b]Environment and Climate Change Canada,

[c]Organisation for Economic Co-operation and Development,

[d]United States Environmental Protection Agency.

1.8 Figures

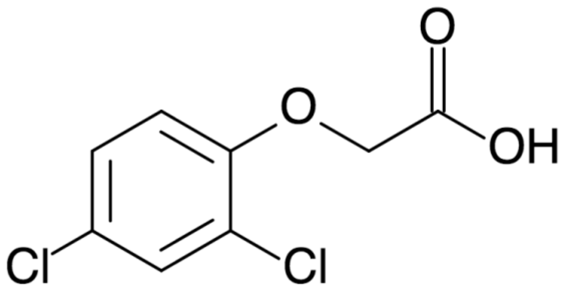

Figure 1.1. Chemical structure of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D).

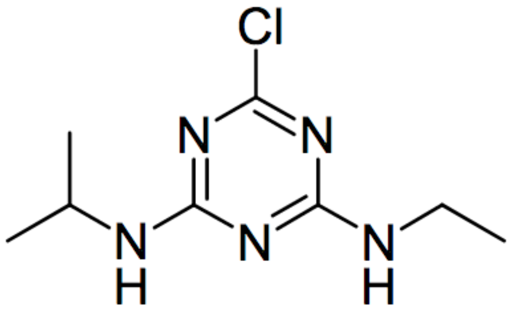

Figure 1.2. Chemical structure of the herbicide atrazine.

Chapter 2 Critical literature review of recovery studies for macrophytes, periphyton, and phytoplankton after exposure to herbicides in laboratory and field

2.1 Abstract

Herbicides are widely used in agriculture and may pose a threat to non-target plants and algae in aquatic ecosystems. Numerous studies have characterized the effects of herbicides and the ability of plants, algae, and periphyton to recover following the cessation of exposure. We sought to assess the strength and ecological relevance of these studies; specifically, as they relate to the evidence that recovery can occur in primary producers exposed to herbicides. A literature review of the peer reviewed literature on the evidence, and quality of evidence, for recovery of primary producers (macrophyte, periphyton, phytoplankton) exposed to herbicides was performed. A total of 40 recovery studies published between 1986 to 2019 were included in the review. The relevance of endpoints and strength of methods for the recovery studies were evaluated using a scoring rubric based on three main categories: (1) test substance; (2) test organism and experimental system; and (3) test design, statistics, and results, with a maximum score of 15. Relevance of endpoints were based on the association of reported endpoints to community/population levels of effect, with a maximum score of 5. A total of 41 test species and 42 herbicides were evaluated among the reviewed recovery studies. The most tested herbicide group were PSII inhibitors for 38%, 83%, and 67% of macrophyte, periphyton, and phytoplankton studies, respectively. In total, 80% of macrophyte and phytoplankton, and 60% of periphyton studies reported recovery after being transferred to clean media, indicating that many primary producers can recover following an acute exposure to tested herbicides. Approximately 30% of recovery testing scored >50% on both strength of method scores and relevance of endpoint scores, which was the threshold for recommending a study be used in ecological risk assessment. Overall, we recommend that standard protocols and guidelines for the assessment of recovery in primary producers be developed to

improve the strength of methods, relevance of endpoint for risk assessment, and reporting of toxicity data.

2.2 Introduction

Laboratory toxicity testing is performed to evaluate the effects of herbicides on organisms as a first tier in ecological risk assessment. The resulting data are used to approve for use and sale of these compounds and can also be used to establish guidelines and regulations around allowable herbicide concentrations in surface water. In the United States, the Tier I ecological risk assessment is determined by the USEPA based on terrestrial and aquatic plant toxicity data to evaluate the possible impacts of pesticides on non-target plants (USEPA, 2004; USEPA, 2021b). Toxicity data from five aquatic primary producers (*Skeletonema costatum*, *Lemna gibba*, *Anabaena flos-aquae*, *Pseudokirchneris subcapitata*, and one diatom species) are used in the USEPA Tier 1 risk assessment for herbicide registration (USEPA, 2021b). Toxicity data from aquatic plant and phytoplankton bioassays are first reviewed and then considered for inclusion in risk assessment, based in part on data quality (USEPA, 2021b). After evaluation, the data can be used to develop an aquatic life benchmark for freshwater species for registered pesticides. An example is the Concentration Equivalent Level of Concern (CE-LOC) near agricultural lands to ensure that the compound is not having adverse effects on the functions and structure of constituent communities (Farruggia et al., 2016, USEPA, 2021a). Aquatic primary producer toxicity testing is also required in the lower tier risk assessments for herbicides in the European Commission (EC, 2002; 2013). There, algae (i.e., *Pseudokirchneriella subcapitata* and a diatom) and one macrophyte (i.e., *Lemna* sp.), if it was the most sensitive, or else other monocots or eudicots are required in the Tier 1 ecological risk assessment (EC, 2002; 2013). In both regulatory systems the potential for the recovery of the plants following exposure is not a formal consideration.

Herbicides are used to control weeds for crop production globally. One of the common concerns is that herbicides could cause adverse effects on aquatic primary production (macrophytes, periphyton, and phytoplankton) once the chemicals enter the aquatic ecosystem by runoff, spray drift, overspray, or discharge with groundwater

(Baker, 1983; Leonard, 1990; Peterson et al, 1994). Studies have found that concentrations of herbicides usually exist as a pulsed event in the aquatic environment due to the temporal nature of the application periods and runoff events (Andrus et al., 2013; Challis, 2018b; Schottler et al., 1994). Challis et al. (2018a) observed greater time-weighted concentrations of atrazine in the Red River in Manitoba between June and July of 2014 and 2015 compared to other sampled months from the same years. The higher concentrations in June and July were associated with the application period and precipitation (Challis et al., 2018a). Similarly, Andrus et al. (2013) reported atrazine concentrations peaking in mid-June with a return to low concentrations (i.e., <20 μg/L) within 15 days in regions of high use. The time between each pulse could create an opportunity for aquatic plants to recover from those effects (Burns et al., 2015).

In lab studies a common definition of recovery is an exposure, that after being transferred to clean medium, is not statistically different from the control (Brain et al., 2012a; 2012b; Burns et al., 2015). This is also known as a NOEC from the recovery phase (Brain et al., 2012a; 2012b; Burns et al., 2015). Studies showed that recovery of aquatic plants was observed during the recovery period. The recovery of algae (*P. subcapitata*, *A. flos-aquae*, and *Navicula pelliculosa*) after atrazine exposure was studied, and results indicate that the PSII quantum yields and growth rates of the tested algae were not significantly different from the control at the end of the recovery period (Brain et al., 2012a). Recovery of duckweed was also found at most of the tested concentrations after atrazine and diuron exposure in the recovery phase (Brain et al., 2012b; Burns et al., 2015).

Recovery of aquatic plants following herbicide exposure is commonly observed (Brain et al., 2012a; 2012b; Burns et al., 2015). However, current herbicide registration and risk assessment mainly focuses on evaluating toxicity of herbicides on aquatic plants (e.g., algae, and duckweed) from exposure (Burns et al. 2015). Assessing recovery is mentioned in European guidance but recovery is rarely included due to lack of guidelines to incorporate recovery data into risk assessment (Brain et al., 2012b; Burns et al., 2015; EC, 2002). Recovery assessment is outlined in algal toxicity testing protocols from ASTM and USEPA, and is evaluated by determining the NOEC, and algicidal and algistatic concentrations in the recovery period (ASTM, 2012; USEPA, 2012a; Payne and Hall, 1979). The algistatic concentration is when there is no net increase of a test endpoint occurring during the exposure period and net growth is observed after the test species is transferred to clean medium (Payne and Hall, 1979). However, the procedure for testing the recovery potential after herbicide exposure is rarely described in standard protocols. For example, the duckweed and algae toxicity testing protocols from the Environment and Climate Change Canada and USEPA (ECCC, 2007; USEPA 2012a) do not explicitly outline how to assess for recovery.

In Canada, the Canadian Water Quality Guidelines for the Protection of Aquatic Life are established by reviewing the toxicity data, prioritizing the data by categorizing it based on the quality, and employing species sensitivity distribution (SSD) curves to define the Hazard Concentration (CCME, 2007). Toxicity data is categorized as either primary or secondary data based on the test design, such as confirmation of test concentrations on the first and last day of exposure, measurement of environmental conditions, sensitivity of endpoints, and proper controls (CCME. 2007). Data categorized as primary are more reliable and would be weighted higher in the risk assessment than secondary data (CCME, 2007). At least three toxicity data from aquatic plant or algae are required to determine guidelines for an herbicide (i.e., most sensitive to the chemicals) but when data is insufficient two studies are used (CCME, 2007). Therefore, the quality of plant toxicity testing is important for accuracy of decisions, and for robustness of guidelines and regulations (Hanson et al., 2019; Klimisch et al., 1997). The lack of standard protocols for assessing recovery in aquatic plants could result in lower quality studies. Therefore, reviewing published recovery studies using a transparent scoring method would help to identify common issues and standard procedures for future recovery studies.

As mentioned, the quality of toxicity data is important for establishing robust guidelines. A common approach to determine the quality of published toxicity testing is using a scoring method. Klimisch et al. (1997) created a scoring method to classify the reliability of toxicity and ecotoxicity studies into four categories (reliable without restriction, reliable with restrictions, not reliable, not assignable) based on the information provided in the studies. One of the criteria for reliability is adherence to a standard protocol (e.g., OECD, USEPA) in toxicity testing (Klimisch et al., 1997). Transparency is one of the main criteria to determine the reliability of testing (Klimisch et al., 1997; Schreider et al., 2010). Increasing the transparency by providing raw data would improve data credibility and assist policy makers to apply such data during the decision-making process (Schreider et al., 2010). Relevance is also evaluated by classifying the association of the endpoints to their level of biological organization levels, and therefore the ability of the data to represent more relevant population-level changes (Klimisch et al. 1997; Hanson et al. 2019). Identifying the reliability and credibility of a study increases the confidence of policy makers to consider the study's scientific data in ecological risk assessment (Hanson et al., 2019; Klimisch et al., 1994; Schreider et al., 2010).

Determining the quality of recovery studies is important to improve data reliability (Hanson et al., 2019). A critical literature review of published peer-reviewed studies, to determine the recovery potential of aquatic plants (macrophytes, periphyton, and phytoplankton) after herbicide exposure, was performed. The objectives of this review were to determine the strength and ecological relevance of herbicide recovery studies for primary producers, and to identify data gaps and common problems of these studies to improve the quality of future recovery studies. Addressing these data gaps would lead to more recovery data being considered in ecological risk assessment (Hanson et al., 2019). With sufficient high-quality recovery data, policy makers could use the data to establish more credible guidelines and regulations (CCREM, 1987; Hanson et al., 2019).

2.3 Materials and Methods

We assessed both the strength of methods and ecological relevance of endpoints from peer-reviewed recovery studies performed on primary producers exposed to herbicides. For the purposes of this exercise, we defined 'recovery' as measured endpoints not statistically different from control at the end of the recovery period following an exposure phase. Scoring rubrics for strength and relevance were developed and applied to each published study that met our inclusion criteria. The rubrics were modified from the scoring criteria of Hanson et al. (2019). In total, forty peer-reviewed papers from our literature search were reviewed, scored, and the resulting data was interpreted.

2.3.1 Literature search

The search for relevant literature was performed using a number of databases, specifically The University of Manitoba libraries, CRKN Wiley Online Library, Google Scholar, ScienceDirect, Scopus, and SpringerLink. Published studies that could be accessed through University of Manitoba libraries database were reviewed. The search terms employed were combinations of “herbicide”, “recovery”, “exposure”, “aquatic primary producer”, “algae”, “macrophytes”, “periphyton”, and “phytoplankton”. References in the scored papers were also reviewed for possible literature to be assessed. The inclusion criteria for scoring of studies required papers to have exposure and recovery periods (e.g., a pulse exposure period) for a single herbicide (no mixtures), reported effects on aquatic primary producers (i.e., macrophytes, periphyton, or phytoplankton), be written in English, and published in a peer-reviewed journal. The final search for published articles was performed on September 30th, 2019.

In total, forty studies, published between 1986 and 2019, were found to meet these criteria for inclusion in evaluation. Eight studies on recovery of primary producers after herbicide exposure, and three tested herbicides in Gustavson et al. (2003) study (listed in Appendix A, Table A1) were excluded from the review due to the lack of a recovery period (e.g., the test species were not transferred to herbicide-free medium). One of the potential papers (Drost et al., 2003) was not reviewed because it was not accessible through the University of Manitoba database and its document delivery service.

The included studies examined the recovery potential of macrophytes (20 species), phytoplankton (13 species), and periphyton (8 species) with a total of forty-one primary producer species. Some of the recovery studies included more than one species in their experiment (e.g., Brain et al., 2012a; Burns et al., 2015). A total of forty-two herbicides were tested in the reviewed studies, and some studies examined recoveries after multiple types of single herbicide exposure. The most commonly tested herbicides were atrazine (18 papers), isoproturon (7 papers), metsulfuron-methyl (4 papers), and diuron (4 papers). A summary of the type of tested herbicide is provided in Table 2.1.

A strength score was assigned for each paper based on the information provided in the published articles and their respective supplementary files. The rubric for scoring strength was developed based on previously published studies (Hanson et al., 2019) and can be found in Table 2.2, along with justifications for the scoring categories. The same rubric was used to evaluate all studies, regardless of species, herbicide, or reported endpoints.

The criteria were divided into three main groups (Group 1: Test Substances, Group 2: Test Organisms and Test System, and Group 3: Test Design, Statistics, and Results; Table 2.3). For Group 1, scores were given based on the provided information related to the source and purity of test substances, confirmation of test concentrations, number of test concentrations, and the ecological relevance of the exposures. For Group 2, the criteria were the provenance of test organisms, initial condition of test organisms, adherence to standard protocols, and test conditions. For Group 3, scores were based on number of replicates, statistical methods, concentration-response model, reported raw values, and control performance. A score of one was given to the study when a criterion was met, and a score of zero was given when the criterion was not met or not reported in the paper.

The score for ecological relevance of exposures is one criterion that will be highly context dependent (e.g., compound, time of year, geographic location). This criterion reflects the proximity of the recovery studies to a ‘real world’ situation and consequent relevance for the purposes of ecological risk assessment. In risk assessment, demonstrating toxicity and recovery, or the lack thereof, at ecologically relevant concentrations helps reduce uncertainty. This is highlighted in studies where unrealistic concentrations cause toxicity, and no subsequent recovery is observed. In this case, the potential for recovery is not truly captured, nor can the responses reported be easily extrapolated to the field to make predictions related to actual herbicide exposure. In the case where toxicity occurs, but recovery also occurs at unrealistic concentrations, the uncertainty about recovery at lesser concentrations has in fact been resolved (i.e., it can occur).

For this review, a score of one was given when at least one of the tested concentrations in the recovery testing was equal to or less than 20 μg/L (i.e., an 'environmentally relevant concentration'), regardless of the herbicide. The level of 20 μg/L was chosen based on available data for herbicides in surface water (in general and for the compounds tested; see Table 2.1 for herbicides tested), which indicates that most environmental exposures will be at or below this level for these compounds.

There are numerous monitoring programs reporting herbicide concentrations in surface water that support using 20 μg/L as a cut-off. For example, concentrations of atrazine in midwestern streams near agricultural lands with intensive atrazine application were measured May – August 2011. It was reported that atrazine tends to occur as a pulse in the streams, with concentrations typically below 20 μg/L (Andrus et al., 2013). After rainfall, runoff concentrations could increase up to 70 μg/L but would return to under 20 μg/L in a short period of time (Andrus et al., 2013). Schuler and Rand (2008) summarized the herbicide concentrations in South Florida's surface water from 1990 – 2006. The authors reported that most herbicides in ten counties of South Florida had a maximum concentration that was lower than 20 μg/L, with a range of 0.003 – 18 μg/L (Schuler and Rand, 2008). The exception was diuron in Hendry County, which had a maximum of 76 μg/L and a 90^{th} percentile of 0.15 μg/L (Schuler and Rand, 2008). The 90^{th} percentile for other herbicides (atrazine, atrazine desethyl, atrazine deisopropyl, ametryn, simazine, prometryn, hexazinone, metribuzin, diuron, linuron, bromacil, and norflurazon) ranged from 0.003 – 1.91 μg/L (Schuler and Rand, 2008). The monitoring program from Environment Canada in 2003 – 2005 showed that the majority of herbicide concentrations in surface waters across Canada were below 14.9 μg/L (ECCC, 2011). Based on the reported values from these studies, it can be reasonably concluded that concentrations of herbicide in North America's surface waters are usually below 20 μg/L (ECCC, 2011; Schuler and Rand, 2008; Solomon et al., 1996). Therefore, 20 μg/L was set as a generic environmentally relevant concentration in the strength of methods rubric.

There were also critical criteria that were considered integral to identifying a strong study and would provide confidence for the policy makers to use the data. The critical criteria were any confirmation of tested concentrations, ≥3 test concentrations, ≥3 replicates, and analysed with appropriate statistical methods. The critical criteria are highlighted in red in Table 2.2. To better evaluate the strength of the study, total scores were reduced if a critical criterion was not met. When criterion 2, 5, 11, and/or 12 was not met, the total score was multiplied by 0.5 for each missed criterion. If two critical criteria were not met, the total score would be multiplied by 0.25. The total score would be multiplied by an additional factor of 0.5 if expert judgment deemed there were additional study flaws that were not captured in the standard rubric criteria. The additional study flaws could include test substance detected in control, lack of proper control performance, or a statistical flaw (e.g., pseudoreplication), as examples. However, no further fundamental errors were found among reviewed papers, so no scores were reduced based on the judgment of the reviewer.

2.3.2 Scoring for endpoint relevance

The endpoints monitored in the studies were used to assign a score to the data for its relevance to ecological risk assessment. We worked from the assumption that the endpoints that are associated with higher levels of biological or ecological organization (i.e., population or community-level) are most useful for risk assessment (Hanson et al., 2019). For each study, each reported response was assigned a relevance score. The scores for each endpoint were between 0 and 5 (Table 2.3). The greater the relevance score for the endpoint, the more the response was conceptually and objectively linked to population and community-level responses that best inform ecological risk assessment.

2.3.3 Review QC/QA

Once the strength and relevance scores were assigned, the resulting spreadsheet (see Supplemental Information) underwent a quality control and quality assurance (QA/QC) exercise. Once the scores were assigned, the spreadsheets with scores and the scoring details (and papers) were reviewed by a separate individual with experience in ecotoxicology studies to help ensure accuracy of interpretation and reporting.

2.3.4 Data analysis

2.3.4.1 Data quality and relevance

The strength and relevance scores for each study were evaluated and recorded in MS Excel. With the collected scores, graphs (e.g., scatter plot, bar graphs, bubble plots, and box plots) were generated with the use of R (R Development Core Team, 2019) to examine the distribution of strength and relevance scores among aquatic primary producers (macrophytes, periphyton, and phytoplankton) over the years. In addition, plots were generated to examine the number of studies that received a score for each criterion. A Kruskal-Wallis test ($\alpha = 0.05$) followed by the post-hoc Dunn test as appropriate, were performed to determine if the strength of method score and test duration (exposure and recovery) were significantly different among the primary producer groups. Significant differences between exposure and recovery test durations in each group were determined by the Mann-Whitney Rank Sum Test ($\alpha = 0.05$). Statistical analysis was performed in R (R Development Core Team, 2019).

2.4 Results

2.4.1 Summary of reviewed studies

A total of 40 studies published between 1986 – 2019 met the inclusion criteria and were reviewed. The number of unique experiments, test substances, and endpoints are summarized in Table 2.4. The number of recovery studies has steadily increased during the above time frame (Figure 2.1).

2.4.1.1 Test organism class and species

Macrophytes were the most commonly tested (n = 76) in the studies and periphyton were least (n = 29) (Table 2.4; Figure 2.2). Macrophytes were tested with the most types of herbicides (n = 33) and were evaluated for the widest range of endpoints (n = 58), while periphyton was studied with the fewest herbicides (n = 4) and endpoints (n = 14) in Table 2.4. The most commonly tested species were *Lemna* sp. (n = 17), periphyton (n = 16) and *Lemna minor* (n = 15). In addition, the most test species in phytoplankton were *Pseudokirchneriella subcapitata* (n = 6) and *Oophila amblystomatis* (n = 6).

2.4.1.2 Test substances

Figure 2.3 shows the herbicide groups tested in the recovery studies over time. Over time there was increasing diversity of herbicide groups included in the recovery studies. It increased from a single herbicide group in the 1980s to 10 herbicide groups in the 2010s. Among all of the herbicide groups, photosystem II inhibitors were the most popular between the 1980s to 2010s, and acetolactate synthase (ALS) synthase inhibitor was the second most tested group in the 2000s and 2010s.

Photosystem II inhibitors were also the most studied herbicides among the three primary producer groups, with 29 for macrophytes, 24 for phytoplankton, and 22 for periphyton (Figure 2.2). The second most tested herbicide group for macrophytes was ALS synthesis inhibitors (with 21 experiments), and for periphyton and phytoplankton were shoot-growth inhibitors (with 5 experiments for both groups).

2.4.1.3 Test duration

The durations for exposure and recovery periods are seen in Figures 2.4A and 2.4B. The exposure and recovery test durations were significantly different ($p < 0.05$) between all the primary producer groups. The exposure durations for macrophytes (n = 296), periphyton (n = 103), and phytoplankton (n = 101) had mean of 18, 3, and 5 days, respectively, and medians of 7, 1, and 3 days, respectively (Figure 2.4A). Statistically significant differences ($p<0.05$) were observed between the exposure and recovery test durations for macrophytes and periphyton. The exposure and recovery test durations were not significantly different ($p>0.05$) for phytoplankton, with mean and median durations for the recovery period of 13 and 7 days for macrophytes (n = 359), 2 and 1 days for periphyton (n = 139), and 5 and 4 days for phytoplankton (n = 66), respectively (Figure 2.4B).

2.4.2 Endpoint relevance scores

The majority of relevance scores for macrophytes from exposure and recovery ranged between 3 and 4 (Figure 2.5, 2.6). The most common were reproduction (44%, n = 130 for exposure; 42%, n = 183 for recovery) and growth (29% for exposure; 31% for recovery) (Figure 2.6). For periphyton, the mean and median of relevance scores were 2.7 and 2 (Figure 2.5) with 57% and 63% of endpoints from exposure (n = 35) and recovery (n = 43) of periphyton being physiological (e.g., effective quantum yield of PSII, concentration of chlorophyll a) (Figure 2.6). The relevance scores for phytoplankton had median scores at 4 and mean scores at 3.3 (Figure 2.5), with most related to reproduction at 57% for both exposure (n = 44) and recovery (n = 49) (Figure 2.6). The relevance scores among herbicide groups for exposure and recovery are shown in Appendix A, Figures A1 and A2. Carotenoid biosynthesis inhibitor from exposure (mean = 2.6; n = 11) and recovery (mean = 2.8, n = 6) had the lowest mean of relevance scores in Appendix A, Figures A1 and A2. The herbicide groups that had the highest mean relevance scores of 4 from exposure were acetyl coenzyme A carboxylase (ACCase) inhibitor (n = 2), root-growth inhibitor (n = 4), and synthetic auxins (n = 1) in Appendix A, Figure A1. For recovery, ACCase inhibitor (n = 2), root-growth inhibitor (n = 4), synthetic auxins (n = 1), and protoporphyrinogen oxidase (PPO) inhibitor (n = 2) had the highest mean relevance scores of 4 in Appendix A, Figure A2.

2.4.3 Strength of method scores

The mean and median of strength of method scores for macrophytes, periphyton, and phytoplankton combined (n = 138) were 6.4 and 5, respectively (Figure 2.7). The mean for each primary producer group were 6.0 for macrophytes (n = 76), 7.3 for periphyton (n = 29), and 6.8 for phytoplankton (n = 33). The percentage of individual tests that received a score greater than 7.5 out of 15 (i.e., >50%) were 36.8% for macrophytes (n = 76), 31.0 % for periphyton (n = 29), and 24.2% for phytoplankton (n =

33). The strength of method score was not significantly different (p>0.05) among all primary groups (Figure 2.7).

The highest strength score in each primary producer group by each herbicide is listed in Table 2.5. A photosynthesis II inhibitor had the highest top strength of method score among all three primary producer groups with a score of 15 (i.e., full marks) as noted in Table 2.5. The herbicide group with the lowest top strength of method score for macrophytes was acetyl CoA carboxylase inhibitors, with a score of 1.75, and for periphyton was shoot-growth inhibitor with a score of 5. The herbicide groups with lowest top score for phytoplankton were ALS synthase inhibitors and synthetic auxins with scores of 5.5.

The scores as percentages for each criterion are found in Figures 2.8 – 2.10. Criterion 8 (the initial test organism characteristics were described) was met the most commonly for macrophytes (92%, n = 76). The criteria that met the most for periphyton (100%, n = 29) were Criterion 7 (test organisms strain/source were identified), 11 (≥ 3 replicate in each concentration) and 12 (Appropriate test statistics for NOEC, LOEC, ECX) for periphyton. Criterion 12 (appropriate test statistics for NOEC, LOEC, ECX) was met the most frequently for phytoplankton (100%, n = 33). The criterion least likely to be met was Criterion 15 (Control criteria and performance) for macrophytes (18%, n=76), periphyton (3%, n=29), and phytoplankton (36%, n=33). Phytoplankton also had the lowest met percentage (36%, n=33) on Criteria 4 (Reported the measured final concentration).

For critical criteria, of the studies that received a score in Criterion 2 (Any concentrations were measured and reported), periphyton had the highest percentage (66%; n = 29), followed by phytoplankton (52%, n = 33) and macrophytes (43%, n = 76). The percentage of macrophytes studies meeting Criterion 5 (≥ three test concentrations, excluded control) was 87%, followed by 76% for phytoplankton, and 66% for periphyton (Figure 2.8). All studies with periphyton met Criterion 11 (≥ 3 replicate in each concentration), while 83% and 94% of macrophyte and phytoplankton studies met Criterion 11, respectively (Figure 2.10). All reviewed periphyton and phytoplankton studies reviewed met Criterion 12 (Appropriate test statistics for NOEC, LOEC, ECX), while only 78% of macrophyte studies were successful.

2.4.4 Overall assessment

Relevance by strength of methods scores was plotted as bubble graphs (Figures 2.11A and 2.11B). The studies that scored 2.5 or greater for relevance and 7.5 for strength of method are located at the top-right of the graphs and are considered those studies most useful for risk assessment and identifying the potential for recovery. For the exposure phase, 30% (n = 209) of the studies were high relevance (≥ 2.5 marks) and strong of methods (≥ 7.5 marks). The percentage of recovery studies (n = 275) with high relevance and strength of method score was 35%.

By primary producer group, macrophytes had the largest number of studies achieving the scores for relevance and strength of method score above the 2.5 and 7.5 thresholds in exposure testing (36%, n = 130), followed by phytoplankton (25%, n = 44), and periphyton (11%, n = 35) (Figure 2.12A). Similar to exposure, macrophytes also had the highest percentage (44%, n = 183) of studies achieving scores for relevance and strength of method above the respective high-relevance 2.5 and 7.5 thresholds in the recovery phase, followed by phytoplankton (20%, n = 49) and periphyton (9%, n = 43) (Figure 2.12B).

2.4.5 Recovery

A summary of studies demonstrating evidence of recovery potential of primary producers to herbicides was generated for each primary producer group (Tables 2.6 – 2.8; Appendix A, Table A2). The results showed that a similar percentage of testing for macrophytes (83%; n = 76) and phytoplankton (81%; n = 32) had provided evidence of recovery either by mentioning that recovery was observed, or by providing NOEC/ECX values after exposure and recovery. Around 60% of the periphyton (n = 29) studies did as well.

2.5 Discussion

Previous studies have expressed concerns around the quality of ecotoxicology studies and recommended criteria to determine the reliability and relevance of data for risk assessment (Ågerstrand et al., 2014; Hanson et al., 2017; Hanson et al., 2019; Harris et al., 2014). We performed a critical review applying the criteria from Hanson et al. (2019) to identify the data gaps in the recovery of aquatic primary producers (macrophyte, periphyton, and phytoplankton) after exposure to herbicides. We found that most studies were performed on macrophytes and phytoplankton, compared to periphyton. Overall, recovery after herbicide exposure was observed consistently in studies among the three primary producer groups. We found that a relatively low percentage of studies met quality thresholds to be recommended for risk assessment. The critical review recognized the data gaps in the recovery studies and the need to increase the relevance and reliability of recovery data for risk assessment. Further details are presented below.

2.5.1 Recovery

2.5.1.1 Herbicide group

An increasing number of herbicide groups were found to be tested over time. PSII inhibitors (e.g., atrazine, diuron) were the most tested herbicides in all primary producer groups from 1986 to 2019. We surmise this is due to atrazine being one of the most widely used herbicides in agriculture and is therefore often detected in surface water (USEPA, 2017; ECCC, 2011; Challis et al., 2018a). Also, studies have consistently observed a reversible mode of action after exposure to PSII inhibitors, which allows for the occurrence of recovery once the test species are transferred to clean media (Brain et al., 2012a; Burn et al., 2015).

However, other herbicide groups are also commonly detected in surface waters. Ensminger et al. (2013) measured herbicide concentrations in surface water near an urban area in California, US in 2008 – 2011 and reported that three of the most frequently detected herbicides were synthetic auxins (i.e., 2.4-D, triclopyr, dicamba). Also, ECCC reported the most frequently detected herbicide in Alberta, Saskatchewan, and Manitoba surface waters in 2003 and 2004 were 2,4-D and MCPA (ECCC, 2011). Challis (2018b) detected 2,4-D in the surface water in the Red River, Winnipeg, Canada in 2016. These observations indicate that other herbicide groups in surface water could be affecting aquatic primary producer communities, and recovery studies would be required to investigate the possibility of recovery after exposure. Among the 40 reviewed studies, one study from Nema et al. (2019) tested the recovery potential of phytoplankton (e.g., *Oophila amblystomatis*) after 2,4-D exposure and recovery was observed in the experiment, but there were no studies for macrophyte or periphyton. No recovery experiment was performed on dicamba, MCPA, or triclopyr. More studies are required to focus on the herbicides that are detected frequently in freshwater. Therefore, our recommendation is to include additional herbicide groups (e.g., synthetic auxins) in the future to understand the recovery potential of primary producers after exposure to herbicides with other modes of action.

2.5.1.2 Test duration

Our results showed longer exposure and recovery test durations were performed for studies with macrophytes than those with phytoplankton and periphyton. The longer exposure and recovery test duration for macrophytes likely relates to their lifecycles and the suggested test duration in the standard protocols. For example, the biological toxicity testing duration for *Lemna minor*, according to the ECCC protocols, is 7 days, and that on green algae, according to USEPA protocols is 96 hours (i.e., 4 days) (ECCC, 2011; USEPA, 2012b). Andrus et al. (2013) monitored the atrazine concentration in the streams around agricultural lands in the midwestern United States. The study reported that atrazine peaked at a high concentration (i.e., 70 µg/L) and then returned to a low concentration (>20 µg/L) around 15 days (Andrus et al., 2013). In the environment, aquatic primary producers could be exposed to herbicides for days to weeks before the concentration returned to a low enough level for recovery. As a result, more recovery studies of exposure duration could be performed to determine the recovery potential of aquatic primary producers, especially for periphyton and phytoplankton.

2.5.1.3 Endpoints

Relative growth rate was commonly reported in the studies to determine the recovery potential by comparing the growth rate after exposure and recovery (Brain et al., 2012b; Burns et al., 2015). Our results showed that most of the reported endpoints from macrophyte and phytoplankton were related to reproduction (e.g., relative growth rate). For periphyton, the majority of endpoints were physiological, such as the effective quantum yield of photosystem II. For example, Laviale et al. (2011) and Prosser et al. (2013) examined the effective quantum yield of photosystem II, which resulted in lower relevance scores because this endpoint is less likely to be linked to a population-level effect (Van der Kraak et al., 2014; Hanson et al., 2019). As a result, more endpoints in growth and reproduction categories could be considered in the future for periphyton testing.

2.5.1.4 Toxicity values

The percentage of studies that reported recovery (i.e., mentioned recovery or/and provided NOEC/ECX from exposure and recovery) was 80% for macrophyte and phytoplankton and 60% for periphyton. This shows that recovery of aquatic primary producers is possible after herbicide exposure, especially during the gaps between pulses that had been observed in previous studies (see Andrus et al., 2013). When assessing the studies for evidence of recovery, we were not able to determine if recovery had occurred in some of the studies. Those are studies that did not provide enough toxicity information or/and values (i.e., /NOEC/ECX) for an exposure and recovery. For example, the reported NOECs from exposure had different test durations than NOECs from recovery, so determining the recovery potential is not possible (see Boxall et al., 2013). Another possibility is the lack of responses during the exposure phase and therefore no ability to access recovery. For example, Cedergreen et al. (2015) exposed *Lemna minor* to diquat and no significant effects at the highest concentration (~ 0.345 μg/L) after the 3 hr exposure period were reported. In addition, some of the studies (see Mohammad et al., 2006) presented the recovery results in figures only without providing toxicity values (e.g., NOEC and ECX values). With the figures only, estimating was required to evaluate the recovery, and this could result in inaccurate data for the risk assessment. To increase the reliability and relevance of recovery studies for the risk assessment, a guideline could be created on reporting toxicity information or values that could allow for the evaluation of recovery potential.

2.5.2 Data quality

Around 45% of individual tests provided sufficient information to score >7.5 in their strength of method score and were considered reliable studies. The criterium for which most of the studies lost marks was performance of controls, as less than 30% of studies reported the control performance for all three primary producer groups. A similar observation was found in Hanson et al. (2019) with many atrazine primary producer exposure studies missing information on the control performance. Reporting the control value is important to show that the test organisms were healthy by meeting standard protocol requirements (i.e., ≥8 times increase of *Lemna minor* frond number within a week and healthy-looking plants). This increases the reliability of the study (ECCC, 2007; Hanson et al., 2019). Two other criteria that had lower scoring percentages (less than 40%) were providing a concentration-response model for macrophytes and confirming final concentrations for macrophytes and phytoplankton. Both of these criteria are important to inform decision makers on the toxic effects over a range of different concentrations. Providing concentration-response models and confirming the final exposure concentration would give policy makers greater confidence in using the data in the risk assessment.

A total of two individual tests received full strength of method scores. These were the recovery of *Pseudokirchneriella subcapitata* and *Navicula pelliculosa* after atrazine exposure by Brain et al. (2012a) and reported endpoints (e.g., average growth rate and cell density) that were assigned as reproduction. The scores showed that there were reliable and relevant recovery studies on aquatic primary producers that could be considered in the risk assessment. Overall, 30% and 35% of recovery studies from exposure and recovery provided enough information to be recognized as good quality with both a high method of strength score (i.e., >7.5) and a high relevance score (i.e., >2.5). Similar results were also observed in other literature reviews on ecotoxicology studies. For example, 22 out of 147 studies provided sufficient information for risk assessment in exposure studies of aquatic primary producers to atrazine (Hanson et al., 2019). To further increase the amount of reliable and relevant studies, researchers should receive strict guidance for reporting and conducting basic toxicity studies.

2.5.3 Review criteria

The criteria used in this literature review were taken from Hanson et al. (2019) for exposure studies on aquatic primary producers after atrazine exposure. Some modifications could be considered in future literature reviews on recovery studies. As mentioned in Hanson et al. (2019), test organisms collected in the field could have been exposed to other chemicals that could have had an effect the toxicity result. As a result, one more criterion could represent information on the environment where specimens were collected from, or the culturing process after collection to ensure the test organisms were sterile (i.e., free of other organisms) and healthy. In addition, scores were given to studies when the ranges of temperature and light conditions were reported. However, it would be better if the test condition were provided for each growth chamber/tank if more than one growth chamber/tank was used instead of providing measurements as a whole (Hanson et al., 2019). Also, the test conditions when the growth chamber was malfunctioned or turned off due to a power outage should be included, so the test results would be more replicable.

Overall, all the criteria were applicable for recovery studies in this literature review. The criteria could also be used to determine the quality of exposure and recovery studies on other concerning chemicals in freshwater, for example, pharmaceutical, oil-spill related products, inorganic and organic contaminants.

2.5.4 Recovery in ecological risk assessment

To capture recovery in ecological risk assessment, the decision maker considers the recovery of function (e.g., rate of decomposition) and structure (e.g., keystone population) in the community via internal and external recovery mechanisms (Barnthouse, 2004; Brock et al., 2018). Internal and external mechanisms represent recovery of the impaired organisms after exposure, or immigration of new individuals from other uncontaminated areas, respectively (Barnthouse, 2004; Brock et al., 2018).

The recovery studies in this review mainly evaluated recovery of aquatic primary producers after herbicide exposure in the lab under controlled conditions. Recovery was observed in both monocot (e.g., *Lemna minor*) and eudicot (e.g., *Myriophyllum spicatum*) plants and showed that both physiologies have the potential to recover after exposure to herbicides. One of the main concerns was that the majority of the endpoints from the recovery studies were not at the community/population level (e.g., the greatest relevance for endpoints). Using lower ecological relevance endpoints may increase the uncertainty of recovery potential in the ecosystem for risk assessment.

We reviewed recovery studies that were performed in both the laboratory and field. Recovery between laboratory and field could be different, as the test species are typically in an environment under optimum light and temperature (Barnthouse, 2004). Also, the laboratory studies are usually focused on an individual species, such as the studies from Boxall et al. (2013) and El-Dib et al. (1999). It is rare for laboratory studies to evaluate the toxicity and recovery effect with the interaction of various species from the same (e.g., competition) or different (e.g., predator pressure) trophic levels (Barnthouse, 2004). As a result, we recommend conducting mesocosm studies to validate the recovery potential of an aquatic plant in a real environment. We also encourage including test species with different trophic levels (e.g., phytoplankton, zooplankton, fish) to have greater data with higher ecological relevance of endpoints to understand the recovery potential within a community.

2.6 Conclusions

This literature review evaluated the reliability and relevance of published recovery studies on aquatic primary producers (macrophytes, periphyton, phytoplankton) by assigning strength of method scores and relevance scores based on the provided information and reported endpoints. Our results are similar to those of previous studies, as we observed a low percentage of studies that provided adequate information to be recognized as reliable and relevant. Recovery was observed in more than half of the studies, but we found that studies were mainly focused on PSII inhibitors, and some of the studies failed to provide enough toxicity values or information to determine the recovery. As a result, we recommend providing a standard protocol/guideline on recovery testing procedures, endpoint selection, and toxicity value (i.e., control performance) reporting. This review found that recovery of aquatic primary producers was consistently observed in the evaluated studies and that a majority of the studies were performed in the laboratory. To further validate recovery in a real environment, we suggest performing mesocosm studies with various trophic levels to determine the recovery of the structure and function of aquatic ecosystems at the community level for ecological risk assessment. Furthermore, the scoring criteria for strength of methods and relevance in this critical review could be used to evaluate the recovery and exposure studies of aquatic plants on other chemicals (e.g., pharmaceutical, oil-spill related products, inorganic, and organic contaminants) in surface water.

2.7 References

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2.8 Tables

Table 2.1. Summary of tested herbicides from 40 reviewed studies, published between 1986 – 2019) on aquatic primary producers (macrophytes, periphyton, and phytoplankton).

Herbicide group (mode of action)	Number of papers in group[a]	Number of herbicides tested	Number of species tested	Number of macrophytespecies tested	Number of periphyton species tested	Number of phytoplankton species tested	Number of papers tested by herbicide
Photosynthesis II inhibitor	38	12	37	15	8	14	atrazine (18), isoproturon (7), diuron (4), bromacil (1), chlortoluron (1), linuron (1), chlorbromuron (1), metribuzin (1), metobromuron (1), simazine (1), simetryn (1), terbuthylazine (1),
Acetolactate synthase (ALS) inhibitor	21	13	6	5	0	1	metsulfuron-methyl (4), bensulfuron-methyl (3), cyclosulfamuron (3), thifensulfuron-methyl (2), ethoxysulfuron (1), flazasulfuron (1), flupysulfuron-methyl (1), imazamox (1), imazosulfuron (1), iofensulfuron-sodium (1), nicosulfuron (1), pyrazosulfuron-ethyl (1), rimsulfuron (1)
Photosynthesis I inhibitor	6	4	5	3	0	2	Irgarol (2), paraquat (2), diquat (1), quinoclamine (1)

Table 2.1. Continued.

Herbicide group (mode of action)	Number of papers in group[a]	Number of herbicides tested	Number of species tested	Number of macrophytes species tested	Number of periphyton species tested	Number of phytoplankton species tested	Number of papers tested by herbicide
Shoot-growth inhibitor	4	3	12	2	5	5	alachlor (2), s-metolachlor (2), pretilachlor (1)
Root growth inhibitor	3	3	1	1	0	0	pendimethalin (1), propyzamide (1), trifluralin (1)
Carotenoid biosynthesis inhibitor	2	1	8	8	0	0	fluridone (2)
Pigment synthesis inhibitor	2	1	2	2	0	0	norflurazon (2)
Acetyl Coenzyme A Carboxylase (ACCase) inhibitor	2	2	1	1	0	0	cyhalofop-butyl (1), thiobencarb (1)
Mitochondrial ATP-ase activity inhibitor	1	1	1	1	0	0	pentachlorophenol (1)
Protoporphyrinogen oxidase (PPO) inhibitor	1	1	1	0	0	1	pentoxazone (1)
Synthetic auxins	1	1	1	0	0	1	2,4-D (1)

[a]More than one herbicide was tested in some studies.

Table 2.2. Scoring criteria for strength of method scores of peer reviewed recovery studies of aquatic primary producers after herbicide exposure. The criteria highlighted in red are critical criteria considered to be more important for determining the reliability of the data for risk assessment. (Taken from Hanson et al., 2019, ECCC, 2007; USEPA, 2012a)

Assessment criteria[a]		Brief explanation	Rationale	Detailed Explanation
Group A - Test substance				
1	>95% pure	Source and purity of test substance reported	The purity of test chemical should be high to increase the confidence of the study's result by concluding that the test substance was the one that caused the effect on the test species, instead of other impurities (e.g., adjuvants, formulants) in the test substance. In addition, reagent grade chemicals (≥95% purity) are required in the standard protocol (e.g., *Lemna minor* toxicity testing from ECCC, algal toxicity testing guideline from USEPA)	1 - If the percentage and source of the test substance were reported with a percentage greater than 95%; 0 - if purity and/or source of test source were not reported, or the purity was less than or equal to 95%
2[b]	Measured concentrations reported – any	Any analytical confirmation and reporting of tested concentrations	Confirmation of the test concentration would increase the accuracy of the reported toxicity value and ensure the toxicity effect on the test species was caused by the test chemical.	1 - If the analysis of stock/test concentration solution were reported at any stage of the experiment; 0 - if the analysis of stock/test concentration was/were not reported
3	Measured concentrations reported - individual initial	Initial concentrations in individual test units reported (pooled or separate)	Measuring the initial concentration would decrease the errors (e.g., wrong dilution, incorrect test chemical) in the result. With the initial and final concentration, a time-average concentration could be calculated and applied to calculations.	1 - If initial tested/stock concentrations were reported; 0 - if initial tested/stock concentration were not reported
4	Measured concentrations reported - individual final	Final concentrations in individual test units reported (pooled or separate)	Measuring the final concentration would decrease the errors (e.g., wrong dilution, incorrect test chemical) in results. With the initial and final concentration, time-average concentration could be calculated and applied to calculations.	1 - If the test concentration(s) were measured and reported at the end of experiment; 0 - if the test concentration were not reported
5[b]	No. of concentrations tested (exclude control)	3 or more concentrations, plus control	Having at least 3 concentrations would assist the determination of concentration-response model.	1 - If number of tested concentration treatments (exclude control) was 3 or greater; 0 - If number of test concentrations treatments were fewer than 3

Table 2.2. Continued.

Assessment criteria [a]		Brief explanation	Rationale	Detailed Explanation
6[b]	Ecological relevance	At least 1 exposure concentration at 20 µg/L or less	Based on previous studies, the herbicide concentration in surface water is often at or lower than 20 µg/L (e.g., Andrus et al., 2013, Environment Canada, 2011). Therefore, 20 µg/L set as environmentally relevant concentration.	1 - If at least one of the tested concentrations was less than or equal to environmental concentration (e.g., 20 µg/L); 0 - if none of the tested concentration was less than environmental concentration (e.g., 20 µg/L)
Group B - Test organism and test system				
7	Strain/source identified	Provenance of test organism (e.g., wild-collected location; laboratory strain ID)	Information on location for collection of test species would help to understand if the test species were previously exposed to test chemical and which species were being tested.	1 - If the source of the test organisms was provided; 0 - if no mention of the source of test organisms
8	Initial test organism characteristics described	As relevant, size, density, mass, feeding protocols	Reporting of the initial size, density, mass of test species can be used to compare the exposed test species to access the toxicity effect.	1 - If initial frond number or cells counts were reported; 0 - if no information on the initial frond number, cell counts, or mass of the organisms
9	Standard protocol followed	e.g., EPA, OECD, ASTM; deviations acceptable if described	The testing was standardized by following a standard protocol and the result could be used to compare with other studies.	1 - If test procedures followed standard protocol; 0 - if the procedures were based on previous studies and/or not followed any standard protocol
10	Test conditions	Temp, light, pH; TOC if sediment	Reporting the test condition is essential because the condition of the test environment (e.g., temperature, light) could affect the growth rate and toxicity effect on primary producers.	1 - If temperature and/or light intensity, and media type were reported, and the error values of light intensity and/or temperature were reported; 0 - if temperature, light intensity, and media were not reported without error values of temperature and/or light intensity was not reported
Group C - Test design, statistics, and results				
11[b]	Replication	Minimum of 3 replicates per exposure	The testing should be repeated to confirm the toxicity effect, by the test chemical, is consistent.	1 - Replicates ≥3; 0 - Replicates <3
12[b]	Statistical methods described	Appropriate test reported and employed for NOEC, LOEC, and EC/LCX; appropriate controls employed	Proper statistical approach should be used to prevent incorrect results and misleading results for risk assessment.	1 - If the statistical method to calculate NOEC/LOEC/ECX/LCX or other endpoints were described and reported; 0 - if the statistical method to calculate NOEC/LOEC/ECX/LCX were not described and/or reported

Table 2.2. Continued.

Assessment criteria [a]		Brief explanation	Rationale	Detailed Explanation
13	Concentration-response	Concentration-response model and parameters provided	Providing concentration-response model to evaluate the toxicity effect and response.	1 - If formula of concentration-response model and parameters was reported or graphs with the concentration-response model equation information; 0 - If equation of concentration-response model and parameters were not showed or reported
14	Measured response (raw) values	Key measured raw values (e.g., not % of control) reported (all or avg. with error; graph or table)	Raw values (data without transformation) should be reported to assess the performance of the testing and toxicity effect of the test chemical.	1 - If raw values (average raw values is accepted but not % of control) were reported in tables/figures; 0 - If only the % of control was reported in graph/figure and none of the raw values (includes average) were reported or plotted in figures
15	Control performance	Control values reported and performance criteria met	Controls need to be reported to ensure the culture met the standard performance and was healthy in the testing, and if it is showing the toxicity effect appropriately.	1 - If control values (included solvent control, if solvent was used to prepare the test solution) were reported and the reported values showed to meet the control performance requirements; 0 - if control values were not reported and not mention on control performance requirement

[a]Full mark is fifteen and one mark would be given for meeting each criterion; if not met, a score of zero was assigned,

[b]The total score would be multiplied by 0.5 for each missed critical criterion.

Table 2.3. Scoring criteria for the ecological relevance of endpoints of peer reviewed recovery studies of aquatic primary producers after herbicide exposure.

Scoring criteria for relevance score				
Score	*Phytoplankton*	*Periphyton*	*Macrophytes*	*Detailed Explanation*
0	No known linkage to survival, development, growth, and/or reproduction.	No known linkage to survival, development, growth, and/or reproduction.	No known linkage to survival, development, growth, and/or reproduction.	If a response has no real or hypothetical linkage to higher-level effects, then it has little to no value in elucidating ecological risk.
1	Biomarker response that has limited linkage to higher-level effects (e.g., genomic, proteomic, metabolomic)	Biomarker response that has limited linkage to higher-level effects (e.g., genomic, proteomic, metabolomic)	Biomarker response that has limited linkage to higher-level effects (e.g., genomic, proteomic, metabolomic)	While informative from a mechanistic perspective, the relevance of these responses to population, community and ecosystem-level effects is considered very low. In many cases, the responses characterized are regular processes to detoxify or adapt to a transient stressor, which in and of themselves are not adverse.
2	Biomarker responses such as enzymatic changes (e.g., nitrogenase activity) or general physiology (e.g., PSII quantum yield, chlorophyll-a concentrations) or functional responses, (e.g., rate of oxygen production).	Biomarker responses such as enzymatic changes (e.g., nitrogenase activity) or general physiology (e.g., PSII quantum yield, chlorophyll-a concentrations) or functional responses, (e.g., rate of oxygen production).	Biomarker responses such as enzymatic changes (e.g., alkaline phosphatase) or general physiology (e.g., PSII quantum yield, chlorophyll-a concentrations) or functional responses, (e.g., rate of oxygen production).	While informative from a mechanistic perspective, the relevance of these responses to population, community and ecosystem-level effects is considered very low. In many cases, the responses characterized are regular processes to detoxify or adapt to a transient stressor, which in and of themselves are not adverse.
3	Changes in growth and development, such as biomass.	Changes in growth and development, such as biomass.	Changes in growth and development, such as biomass.	These responses are typically highly relevant to the success or sustaining populations and communities in an ecological context.
4	Changes in reproduction, such as growth rate or cell density.	Changes in reproduction, such as growth rate or cell density.	Changes in reproduction, such as seed production, seed viability, flower production, frond number, plant number and related metrics (e.g., growth rates).	These responses are typically highly relevant to the success or sustaining populations and communities in an ecological context.
5	Mortality and/or community level changes such as shifts in species diversity/composition.	Mortality and/or community level changes such as shifts in species diversity/composition.	Mortality and/or community level changes such as shifts in species diversity/composition.	Loss of individuals is highly relevant to the success or sustaining populations and communities in an ecological context.

Table 2.4. Summary of 40 peer reviewed studies published between 1986 - 2019 on aquatic primary producers (macrophytes, periphyton and phyplankton) exposed to herbicide and followed by a recovery period.

Organism type	**Number of peer reviewed papers**	**Number of experiments**	**Number of species tested**	**Number of herbicides tested**	**Number of endpoints reported**
Macrophytes	25	76	20	33	58
Periphyton	6	29	8	4	14
Phytoplankton	14	33	13	11	17
Total	*40*	*138*	*41*	*42*	*64*

Table 2.5. List of reviewed studies with the highest strength of method score in each primary producer group (macrophytes, periphyton and phytoplankton) and herbicide group. The studies with a strength of method score (full mark of 15) greater than 7.5 marks are in boldface.

Primary producer group	**Herbicide group**	**Study**	**Herbicide**	**Test species**	**Endpoints**	**Overall score**
Macrophytes	ACCase inhibitor	Mohammad et al., 2008	Cyhalofop-butyl, Thiobencarb	*Lemna sp.*	Daughter fronds production (relative growth rate)	1.75*
	ALS synthase inhibitor	**Wieczorek et al., 2017**	**Iofensulfuron-sodium**	***Myriophyllum spicatum, Elodea canadensis***	**Dry weight of total shoots (growth rate), main shoot length (growth rate), side shoot length (growth rate), total shoot length (growth rate),**	**12**
	Carotenoid biosynthesis inhibitor	**Netherland et al., 2015**	**Fluridone**	***Hydrilla verticillata***	**Biomass, chlorophyll florescence yield, shoot biomass**	**11**
	Mitochondrial ATP-ase activity inhibitor	**Boxall et al., 2013**	**Pentachlorophenol**	***Lemna minor***	**Growth rate, total frond area**	**10**
	Pigment synthesis inhibitor	**Wilson and Koch, 2013**	**Norflurazon**	***Lemna minor***	**Frond production**	**12**
	PSI inhibitor	Mohammad et al., 2010	Paraquat	*Lemna gibba*	Frond number, frond number (relative growth rate)	5
	PSII inhibitor	**Brain et al., 2012b**	**Atrazine**	***Lemna gibba***	**Average growth rate, biomass, frond number**	**13**
		Burns et al., 2015	**Diuron**	***Lemna gibba, Lemna minor***	**Plant number, frond number, fresh weight, dry weight, plant number (relative growth rate), frond number (relative growth rate), fresh weight (relative growth rate), dry weight (relative growth rate)**	**13**

Table 2.5. Continued.

Primary producer group	Herbicide group	Study	Herbicide	Test species	Endpoints	Overall score
Macrophytes	**PSII inhibitor**	**Burns et al., 2015**	**Diuron**	***Lemna gibba, Lemna minor***	**Plant number, frond number, fresh weight, dry weight, plant number (relative growth rate), frond number (relative growth rate), fresh weight (relative growth rate), dry weight (relative growth rate)**	**13**
		King et al., 2016	**Atrazine**	***Ceratophyllum demersum***	**Dry mass**	**13**
	Root-growth inhibitor	**Knežević et al., 2016**	**Trifluralin**	***Lemna minor***	**Frond number (relative growth rate), fresh weight (relative growth rate)**	**12**
	Shoot-growth inhibitor	Mohammad et al., 2010	Alachlor	*Lemna gibba*	Frond number, frond number (relative growth rate)	5
Periphyton	**PSII inhibitor**	**Brain et al., 2012a**	**Atrazine**	***Navicula pelliculosa***	**Average growth rate, cell density, PSII effective quantum yield**	**15**
	Shoot-growth inhibitor	Debenest et al., 2009	S-metolachlor	Benthic diatom, sensitive autotroph, tolerant autotroph, facultative heterotroph, obligate heterotroph	Chlorophyll a concentration, chlorophyll c concentration, viable cell density, abundance	5*
Phytoplankton	ALS synthase inhibitor	Nagai et al., 2011	Bensulfuron-methyl	*Pseudokirchneriella subcapitata*	Cell density (growth rate)	5.5*
	PPO inhibitor	Nagai et al., 2011	Pentoxazone	*Pseudokirchneriella subcapitata*	Cell density (growth rate), cell death rate	6*
	PSI inhibitor	Nagai et al., 2011	Quinoclaimine	*Pseudokirchneriella subcapitata*	Cell density (growth rate), cell death rate	6*
	PSII inhibitor	**Brain et al., 2012a**	**Atrazine**	***Pseudokirchneriella subcapitata***	**Average growth rate, cell density, PSII effective quantum yield**	**15**

Table 2.5. Continued.

Primary producer group	**Herbicide group**	**Study**	**Herbicide**	**Test species**	**Endpoints**	**Overall score**
Phytoplankton	**Shoot-growth inhibitor**	**Copin et al., 2016**	**S-metolachlor**	***Scenedesmus vacuolatus***	**Growth rate, size of algae cells**	**11**
	Synthetic auxin	Nema et al., 2019	2,4-D	*Oophila amblystomatis*	Cell density (growth rate)	5.5*

*The only study for the category.

Table 2.6. Summary of evidence for recovery from 76 aquatic macrophyte individual tests from reviewed recovery studies after herbicide exposure.

Reference	Herbicide	Test species	Did the study report evidence of recovery
Belgers et al., 2011	Metsulfuron-methyl	*Myriophyllum spicatum*	Yes (Mentioned recovery, provided exposure & recovery NOEC)
Boxall et al.,2013	Isoproturon	*Lemna minor*	NA (No information on exposure NOEC with the same exposure duration)
	Metsulfuron-methyl	*Lemna minor*	NA (No information on exposure NOEC with the same exposure duration)
	Pentachlorophenol	*Lemna minor*	NA (No information on exposure NOEC with the same exposure duration)
Brain et al., 2012b	Atrazine	*Lemna gibba*	Yes (Provided exposure & recovery NOEC)
Burns et al., 2015	Diuron	*Lemna minor*	Yes (Provided exposure & recovery NOEC)
		Lemna gibba	Yes (Provided exposure & recovery NOEC)
Cedergreen et al., 2005	Diquat	*Lemna minor*	NA (No significant effect from exposure)
	Terbuthylazine	*Lemna minor*	Yes (Mentioned recovery, but no exposure NOEC)
	Imazamox	*Lemna minor*	Yes (Mentioned recovery, but no exposure NOEC)
	Metsulfuron-methyl	*Lemna minor*	Yes (Mentioned recovery, but no exposure NOEC)
	Propyzamide	*Lemna minor*	Yes (Mentioned recovery, but no exposure NOEC)
	Pendimethalin	*Lemna minor*	Yes (Mentioned recovery, but no exposure NOEC)
Haynes et al., 2000	Diuron	*Cymodocea serrulata*	Yes* (Mentioned recovery; recovery NOEC same as exposure NOEC)
		Halophila ovalis	Yes (Provided exposure & recovery NOEC)
		Zoestera capricorni	Yes (Provided exposure & recovery NOEC)
Hughes et al., 1988	Atrazine	*Lemna gibba*	Yes (Provided exposure & recovery NOEC)
Jones et al., 1986	Atrazine	*Potamogeton perfoliatus* L.	Yes (Mentioned recovery, provided recovery NOEC)
King et al., 2016	Atrazine	*Ceratophyllum demersum*	NA (No significant effect from exposure)
Knežević et al., 2016	Atrazine	*Lemna minor*	Yes (Provided exposure & recovery NOEC)
	Isoproturon	*Lemna minor*	Yes (Provided exposure & recovery NOEC)
	Trifluralin	*Lemna minor*	Yes* (Mentioned recovery, but recovery NOEC same as exposure NOEC)
Macinnis-Ng and Ralph, 2003	Atrazine	*Zostera capricorni*	Yes (Mentioned on some endpoints)
	Diuron	*Zostera capricorni*	Yes (Mentioned recovery)
	Irgarol	*Zostera capricorni*	Yes (Mentioned recovery on some endpoints)

Table 2.6. Continued.

Reference	Herbicide	Test species	Did the study report evidence of recovery
Macinnis-Ng et al., 2004	Irgarol	*Zostera capricorni*	Yes (Mentioned recovery, provided on exposure & recovery NOEC)
Mohammad et al., 2006	Bensulfuron-methyl	*Lemna* sp.	Yes (Mentioned recovery but did not report exposure & recovery NOEC)
	Cyclosulfamuron	*Lemna* sp.	Yes (Mentioned recovery but did not report exposure & recovery NOEC)
	Ethoxysulfuron	*Lemna* sp.	Yes (Mentioned recovery but did not report exposure & recovery NOEC)
	Flazasulfuron	*Lemna* sp.	Yes (Mentioned recovery but did not report exposure & recovery NOEC)
	Imazosulfuron	*Lemna* sp.	Yes (Mentioned recovery but did not report exposure & recovery NOEC)
	Nicosulfuron	*Lemna* sp.	Yes (Mentioned recovery but did not report exposure & recovery NOEC)
	Pyrazosulfuron-ethyl	*Lemna* sp.	Yes (Mentioned recovery but did not report exposure & recovery NOEC)
	Thifensulfuron-methyl	*Lemna* sp.	Yes (Mentioned recovery but did not report exposure & recovery NOEC)
Mohammad et al., 2008	Alachlor	*Lemna* sp.	Yes* (Recovery NOEC same as exposure NOEC)
	Atrazine	*Lemna* sp.	Yes* (Recovery NOEC same as exposure NOEC)
	Bensulfuron-methyl	*Lemna* sp.	Yes* (Mentioned recovery; recovery NOEC same as exposure NOEC)
	Cyclosulfamuron	*Lemna* sp.	Yes (Provided exposure & recovery NOEC)
	Cyhalofop-butyl	*Lemna* sp.	Yes (Provided exposure & recovery NOEC)
	Diuron	*Lemna* sp.	Yes (Provided exposure & recovery NOEC)
	Paraquat	*Lemna* sp.	Yes* (Recovery NOEC same as exposure NOEC)
	Simetryn	*Lemna* sp.	Yes* (Mentioned recovery; recovery NOEC same as exposure NOEC)
	Thiobencarb	*Lemna* sp.	Yes (Provided exposure & recovery NOEC)
Mohammad, 2010	Alachlor	*Lemna gibba*	Yes (Provided exposure & recovery NOEC)
	Atrazine	*Lemna gibba*	Yes (Mentioned recovery, provided exposure & recovery NOEC)
	Cyclosulfamuron	*Lemna gibba*	Yes (Mentioned recovery, provided exposure & recovery NOEC)
	Paraquat	*Lemna gibba*	Yes (Mentioned recovery, provided exposure & recovery NOEC)
Netherland et al.,1997	Fluridone	*Myriophyllum spicatum* L	Yes*(Mentioned recovery; recovery NOEC same as exposure NOEC)
		Elodea canadensis Michaux	Yes (Provided exposure & recovery NOEC)
		Potamogeton nodosus Poiret	Yes (Provided exposure & recovery NOEC)

Table 2.6. Continued.

Reference	Herbicide	Test species	Did the study report evidence of recovery
Netherland et al.,1997	Fluridone	*Potamogeton pectinatus* L	Yes (Provided exposure & recovery NOEC)
		Vallisneria americana Michaux	Yes (Provided exposure & recovery NOEC)
		Chara spp. and *Najas guadalupensis* Magnus	NA (No significant effect from exposure)
		Total plant community	NA (Did not report recovery NOEC)
Netherland, 2015	Fluridone	*Hydrilla verticillata* (Lake Gaston, NC)	NA (Did not report recovery NOEC)
		Hydrilla verticillata (J. Strom Thurmond Lake in Georgia/South Carolina border)	No recovery (Provided exposure & recovery NOEC)
		Hydrilla verticillata (Smith Mountain Lake, VA)	NA (Did not report recovery NOEC)
		Hydrilla verticillata (Lake Gaston, NC)	NA (Did not report recovery NOEC)
Rosenkrantz et al., 2013	Flupysulfuron-methyl	*Lemna gibba*	Yes (Provided exposure & recovery NOEC)
	Metsulfuron-methyl	*Lemna gibba*	Yes (Provided exposure & recovery NOEC)
	Rimsulfuron	*Lemna gibba*	Yes* (Mentioned recovery; recovery NOEC same as exposure NOEC)
	Thifensulfuron-methyl	*Lemna gibba*	Yes* (Mentioned recovery; recovery NOEC same as exposure NOEC)
Snel et al. 1998	Linuron	*Elodea nuttallii* (Planchon) *St. John*	Yes (Provided exposure EC10 & EC50 and recovery NOEC)
		Myriophyllum spicatum L.	Yes (Provided exposure EC10 & EC50 and recovery NOEC)
		Potamogeton crispus L.	Yes (Provided exposure EC10 & EC50 and recovery NOEC)
		Ranunculus circinatus Sibth.	Yes (Provided exposure EC10 & EC50 and recovery NOEC)
		Chara globularis (Thuill.)	Yes (Provided exposure EC10 & EC50 and recovery NOEC)
Teodorovic et al., 2012	Atrazine	*Lemna gibba*	Yes (Provided exposure & recovery NOEC)
		Myriophyllum aquaticum	No recovery (Provided exposure & recovery NOEC)

Table 2.6. Continued.

Reference	Herbicide	Test species	Did the study report evidence of recovery
Varga et al., 2019	Isoproturon	*Lemna minor*	Yes (Provided exposure & recovery NOEC)
Wieczorek et al., 2017	Iofensulfuron-sodium	*Myriophyllum spicatum*	Yes (Mentioned recovery and provided NOEC))
		Elodea canadensis	Yes (Mentioned recovery and provided NOEC))
Wilson et al, 2006	Norflurazon	*Vallisneria americana*	Yes* (Mentioned recovery, but some of the endpoints were not significant different from exposure)
Wilson and Wilson, 2010	Bromacil	*Vallisneria americana michx*	Yes* (Mentioned recovery, but some of the endpoints were not significant different from exposure)
	Simazine	*Vallisneria americana michx*	Yes (Provided exposure & recovery of NOEC)
Wilson and Koch, 2013	Norflurazon	*Lemna minor*	Yes (Provided exposure & recovery of NOEC)

[NA]Not applicable due to insufficient toxicity values,

*Individual testing that mentioned recovery but no observed effect concentration (NOEC) from exposure and recovery was at the same test concentration.

Table 2.7. Summary of evidence for recovery from 29 aquatic periphyton individual tests from reviewed recovery studies after herbicide exposure.

Reference	Herbicide	Test species	Did the study report evidence of recovery
Brain et al., 2012a	Atrazine	*Navicula pelliculosa*	Yes (Provided exposure & recovery NOEC)
Debenest et al.,2009	Isoproturon	Benthic diatom	No recovery (Mentioned no recovery)
		Sensitive autotroph	NA (No information on exposure NOEC)
		Tolerant autotroph	NA (No information on exposure NOEC)
		Facultative heterotroph	NA (No information on exposure NOEC)
		Obligate heterotroph	NA (No information on exposure NOEC)
	S-metolachlor	Benthic diatom	Yes (Mentioned recovery but did not report some exposure NOEC)
		Sensitive autotroph	NA (No information on exposure NOEC)
		Tolerant autotroph	NA (No information on exposure NOEC)
		Facultative heterotroph	NA (No information on exposure NOEC)
		Obligate heterotroph	NA (No information on exposure NOEC)
Gustavson et al., 2003	Metribuzin	Periphyton community (September 1999)	Yes (Mentioned recovery and provided recovery NOEC)
		Periphyton community (May 2000)	Yes (Mentioned recovery and provided exposure & recovery NOEC
Hughes et al., 1988	Atrazine	*Navicula pelliculosa*	Yes (Provided exposure & recovery NOEC)
King et al., 2016	Atrazine	Periphyton	NA (No significant effect from exposure)
		Metaphyton	NA (No significant effect from exposure)
Laviale et al., 2011	Atrazine	Periphyton	Yes (Provided exposure & recovery NOEC)
	Isoproturon	Periphyton	Yes (Provided exposure & recovery NOEC)
Prosser et al., 2013	Atrazine	Periphyton (U of G microcosm)	Yes (Provided exposure & recovery NOEC)
		Periphyton (Bronte Creek)	Yes (Provided exposure & recovery NOEC)
		Periphyton (MO-05)	Yes (Provided exposure & recovery NOEC)
		Periphyton (IL-10)	Yes (Provided exposure & recovery NOEC)
		Periphyton (IA-03)	Yes (Provided exposure & recovery NOEC)

Table 2.7. Continued.

Reference	Herbicide	Test species	Did the study report evidence of recovery
Prosser et al., 2015	Atrazine	Periphyton (IA-03)	Yes (Provided exposure EC10, EC50 values and recovery NOEC)
		Periphyton (IL-10)	Yes (Provided exposure EC10, EC50 values and recovery NOEC)
		Periphyton (IL-17)	Yes (Provided exposure EC10, EC50 values and recovery NOEC)
		Periphyton (MO-07N)	Yes (Provided exposure EC10, EC50 values and recovery NOEC)
		Periphyton (OH-05)	Yes (Provided exposure EC10, EC50 values and recovery NOEC)
		Periphyton (MO-05)	No recovery (Provided exposure EC10, EC50 values and recovery NOEC)

[NA]Not applicable due to insufficient toxicity values,

*Individual testing that mentioned recovery but no observed effect concentration (NOEC) from exposure and recovery was at the same test concentration.

Table 2.8. Summary of evidence for recovery information from 32 aquatic phytoplankton individual testing from reviewed recovery studies after herbicide exposure.

Reference	Herbicide	Test species	Did the study report evidence of recovery
Baxter et al., 2013	Atrazine	*Pseudokirchneriella subcapitata*	Yes (Provided exposure & recovery NOEC)
Baxter et al., 2014	Atrazine	*Oophila* sp.	Yes (Provided exposure & recovery NOEC)
Brain et al., 2012a	Atrazine	*Pseudokirchneriella subcapitata*	Yes (Provided exposure & recovery NOEC)
		Anabaena flos-aquae	Yes (Provided exposure & recovery NOEC)
Copin et al., 2016	S-metolachlor	*Scenedesmus vacuolatus*	Yes (Mentioned recovery on cell density and algal cells size)
Debenest et al., 2009	Isoproturon	*Melosira varians*	No (Mentioned no recovery on algal biomass and diatoms, and no information on other endpoints exposure NOEC)
		Nitzschia dissipata	No (Mentioned no recovery on algal biomass and diatoms, and no information on other endpoints exposure NOEC)
		Eolimna minima	No (Mentioned no recovery on algal biomass and diatoms, and no information on other endpoints exposure NOEC)
	S-metolachlor	*Melosira varians*	Yes (Mentioned recovery on algal biomass and diatoms, and no information on other endpoints exposure NOEC)
		Nitzschia dissipata	Yes (Mentioned recovery on algal biomass and diatoms, and no information on other endpoints exposure NOEC)
		Eolimna minima	Yes (Mentioned recovery on algal biomass and diatoms, and no information on other endpoints exposure NOEC)
El-Dib et al., 1991	Chlortoluron	*Scenedesmus spp.*	Yes (Recovery on chlorophyll a based on exposure & recovery NOEC)
	Chlorbromuron	*Scenedesmus spp.*	Yes (Mentioned recovery on algal cells)
	Patoran	*Scenedesmus spp.*	Yes (Mentioned recovery on algal cells)
Hughes et al., 1988	Atrazine	*Anabaena flos-aquae*	Yes (Based on exposure & recovery NOEC)
		Dunaliella tertiolecta	Yes (Based on exposure & recovery NOEC)
King et al., 2016	Atrazine	Phytoplankton	NA (No significant effect from exposure
		Algal	NA (No significant effect from exposure

Table 2.8. Continued.

Reference	Herbicide	Test species	Did the study report evidence of recovery
Klaine et al., 1997	Atrazine	*Selenastrum capricornutum*	Yes (Based on exposure & recovery NOEC)
Nagai et al., 2011	Pretilachlor	*Pseudokirchneriella subcapitata*	Yes (Based on exposure & recovery NOEC)
	Bensulfuron-methyl	*Pseudokirchneriella subcapitata*	Yes (Based on exposure & recovery NOEC)
	Pentoxazone	*Pseudokirchneriella subcapitata*	NA (No significant effect from exposure)
	Quinoclaimine	*Pseudokirchneriella subcapitata*	Yes (Based on exposure & recovery NOEC)
Nema et al., 2019	2,4-D	*Oophila amblystomatis* (from Kinston)	Yes (Based on exposure & recovery NOEC)
		Oophila amblystomatis (from Sudden Tract)	Yes (Based on exposure & recovery NOEC)
		Oophila amblystomatis (from Bruce Peninsula)	Yes (Based on exposure & recovery NOEC)
	Atrazine	*Oophila amblystomatis* (from Kinston)	Yes (Based on exposure & recovery NOEC)
		Oophila amblystomatis (from Sudden Tract)	Yes (Based on exposure & recovery NOEC)
		Oophila amblystomatis (from Bruce Peninsula)	Yes (Based on exposure & recovery NOEC)
Vallotton et al., 2008	Atrazine	*Scenedesmus vacuolatus*	Yes (Based on exposure & recovery NOEC, and exposure EC50)
	Isoproturon	*Scenedesmus vacuolatus*	Yes (Based on exposure & recovery NOEC, and exposure EC50)
Vallotton et al., 2009	Isoproturon	*Scenedesmus vacuolatus*	Yes (Based on exposure EC50 and recovery NOEC)

[NA]Not applicable due to insufficient toxicity values,

*Individual testing that mentioned recovery but no observed effect concentration (NOEC) from exposure and recovery was at the same test concentration.

2.9 Figures

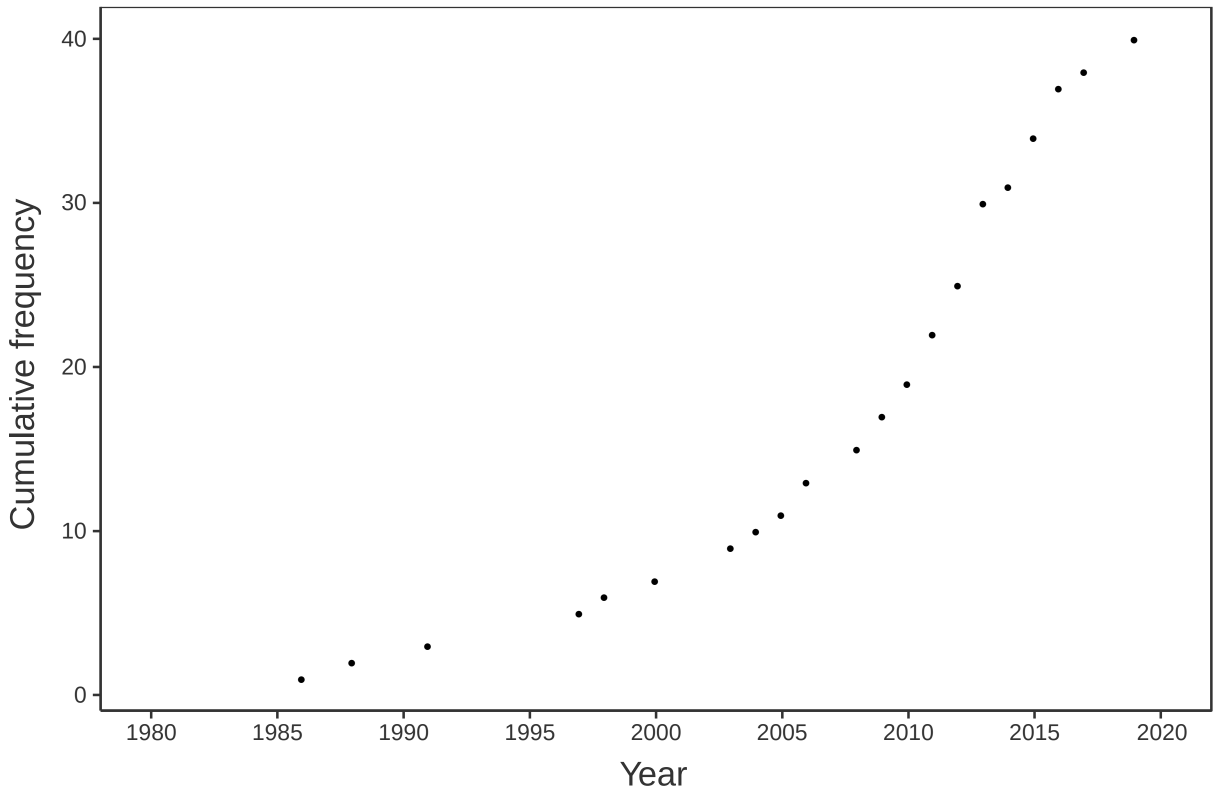

Figure 2.1. The cumulative number of published peer-reviewed recovery studies on aquatic primary producers exposed to herbicides (macrophytes, periphyton, phytoplankton) between 1986 to 2019. The total number of papers found was 40.

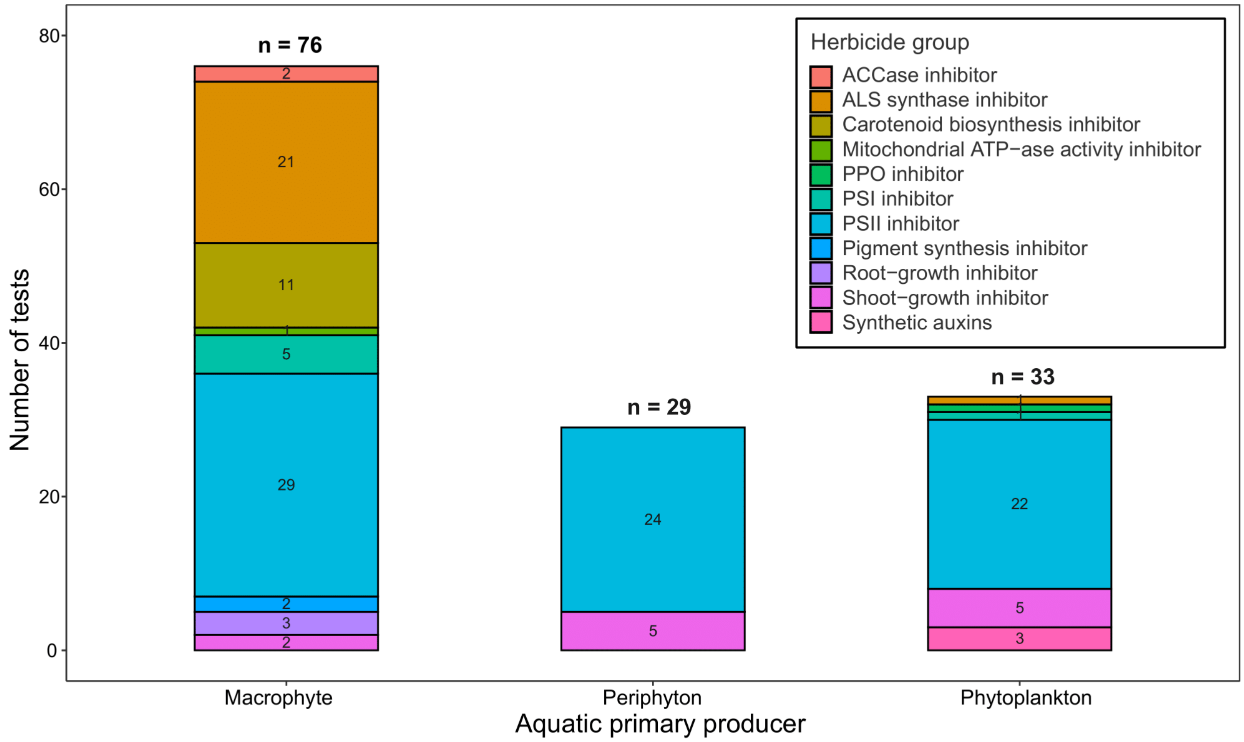

Figure 2.2. The number of unique recovery tests among herbicides groups for aquatic primary producers (macrophyte, periphyton and phytoplankton) exposed to herbicides from papers published in the peer review literature between 1986 and 2019. The n above each bar is the total number of unique tests in each primary producer group, and the number in each stacked bar is the number of tests in each herbicide group in the corresponding primary producer group.

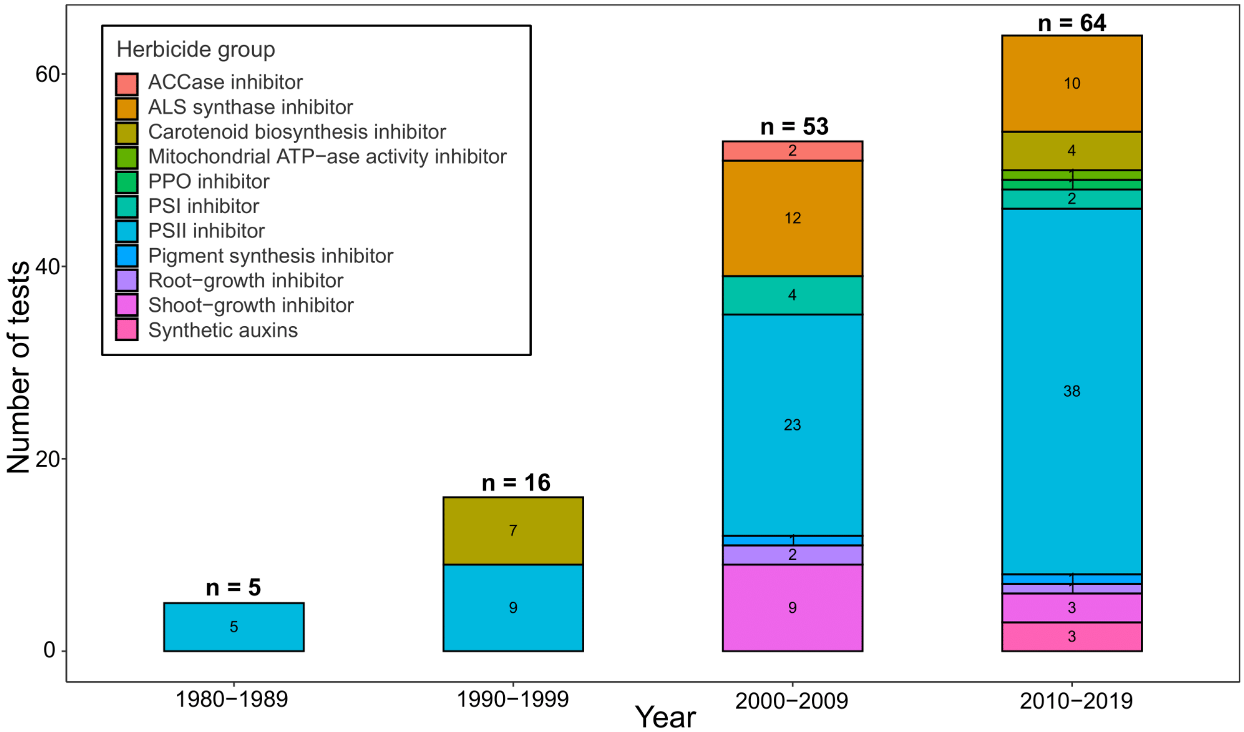

Figure 2.3. The number of aquatic primary producer herbicide recovery studies by herbicide groups between 1986 – 2019. The n above each bar is the total number of unique tests in each year group, and the number in each stacked bar is the number of tests in each herbicide group in the corresponding year group.

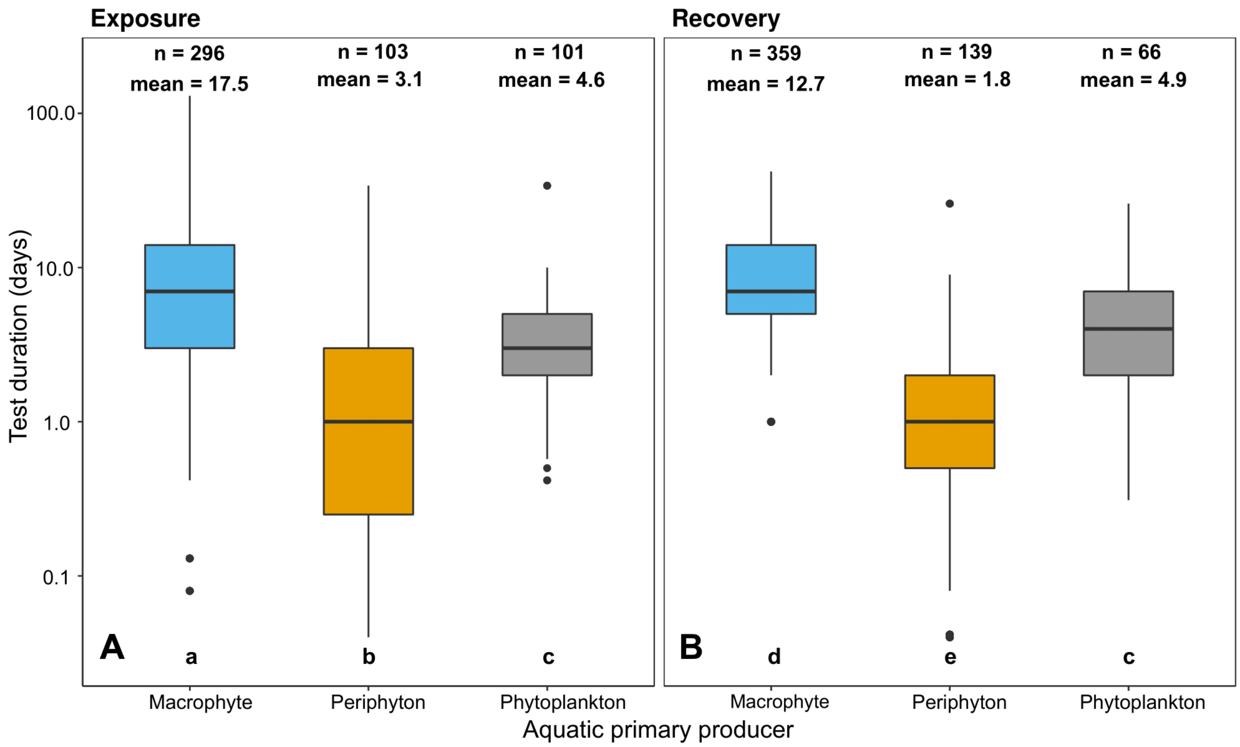

Figure 2.4. Boxplot with A) exposure and B) recovery test durations on aquatic primary producer (macrophytes, periphyton, and phytoplankton) herbicide exposure recovery studies. The n and mean listed at the top of the boxplot are the total number and mean unique responses by time-point reported. Significant differences among the groups from exposure and recovery were tested by Kruskal-Wallis test (α = 0.05) with by Dunn test with Bonferroni *p* adjustment as appropriate, and are indicated by lower case letters at the bottom of the box plot. The Mann-Whitney Rank Sum Test (α = 0.05) was used to determine significant difference between exposure and recovery in each group.

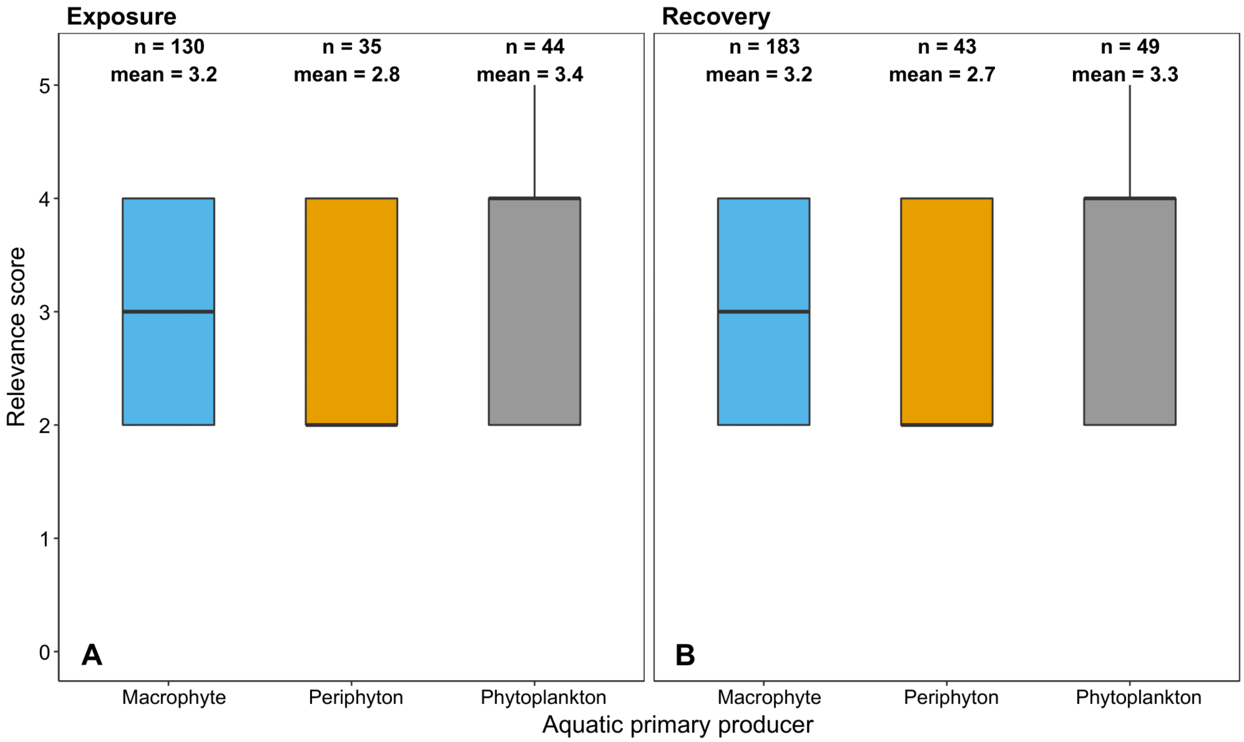

Figure 2.5. Boxplot with relevance score in each aquatic primary producer (macrophytes, periphyton, phytoplankton) from the A) exposure and B) recovery period. The n and mean listed at the top of the boxplot are the total number and mean of individual endpoints in each study.

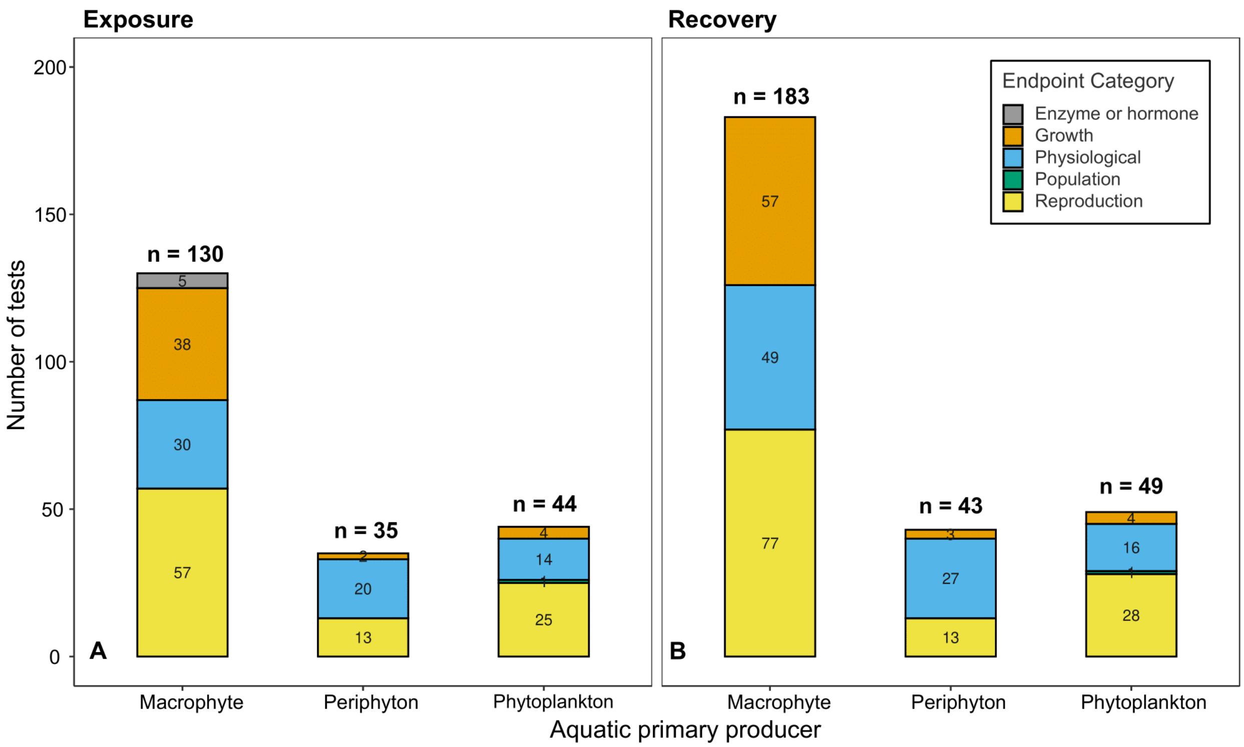

Figure 2.6. The number of recovery data points by endpoint category for each aquatic primary producer (macrophytes, periphyton, and phytoplankton) from the A) exposure and B) recovery period. The n listed at the top of the bar are the total number of individual endpoints in each study.

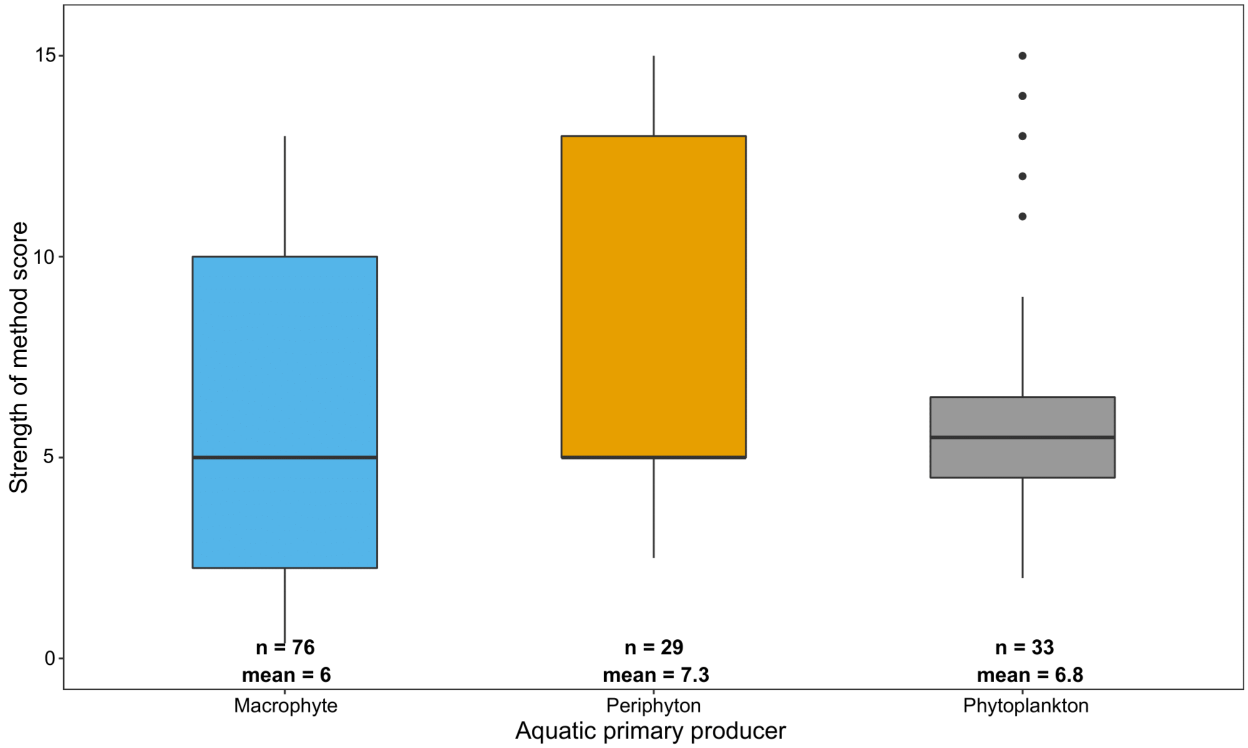

Figure 2.7. Boxplot with strength of method score for each aquatic primary producer group (macrophytes, periphyton, and phytoplankton). The n and mean listed below each boxplot are the total number and mean of individual test species in the studies. No significant difference ($p > 0.05$) between the groups were detected as tested by Kruskal-Wallis test.

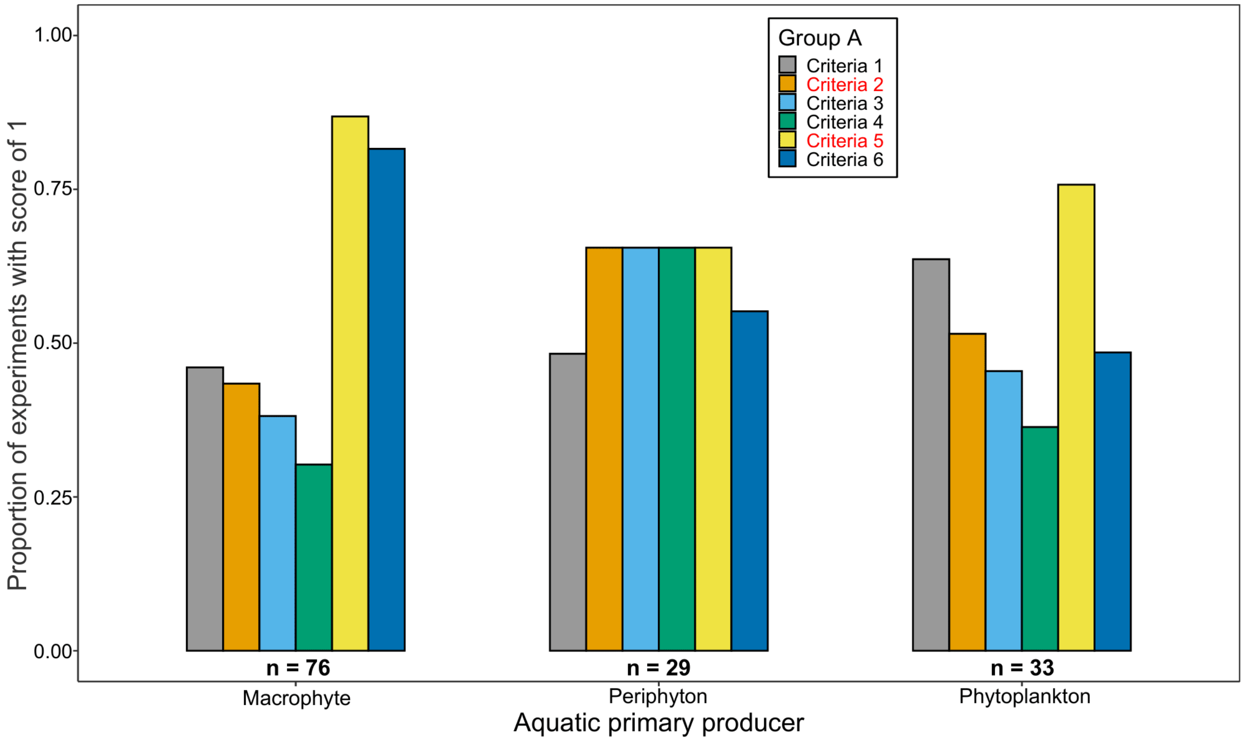

Figure 2.8. The proportion of experiments with score of 1 for Criteria 1 – 6 from Group A test substances by aquatic primary producer (macrophytes, periphyton, and phytoplankton). Criteria 2 and 5 are critical criteria and are highlighted in red. The n is the total number of unique tests in each primary producer group.

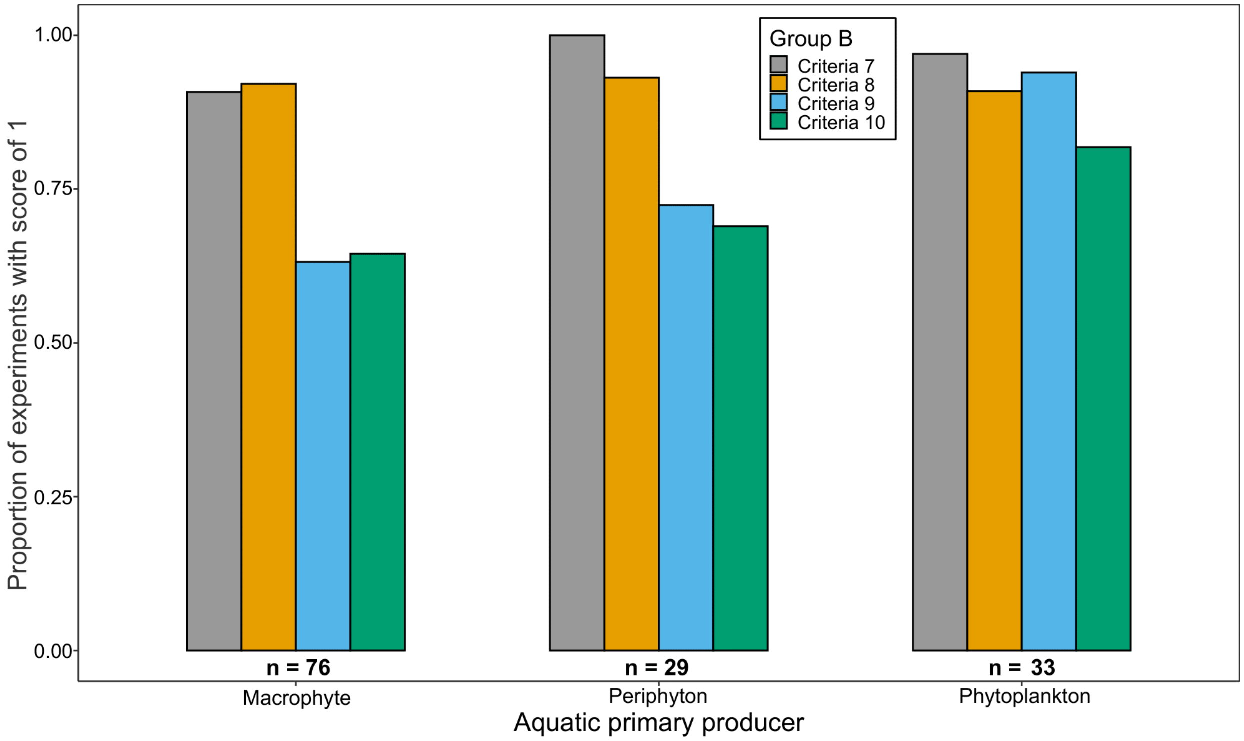

Figure 2.9. The proportion of experiments with score of 1 for Criteria 7 – 10 from Group B test organism and test system (n = 138) by aquatic primary producer (macrophytes, periphyton, and phytoplankton). The n is the total number of unique tests in each primary producer group.

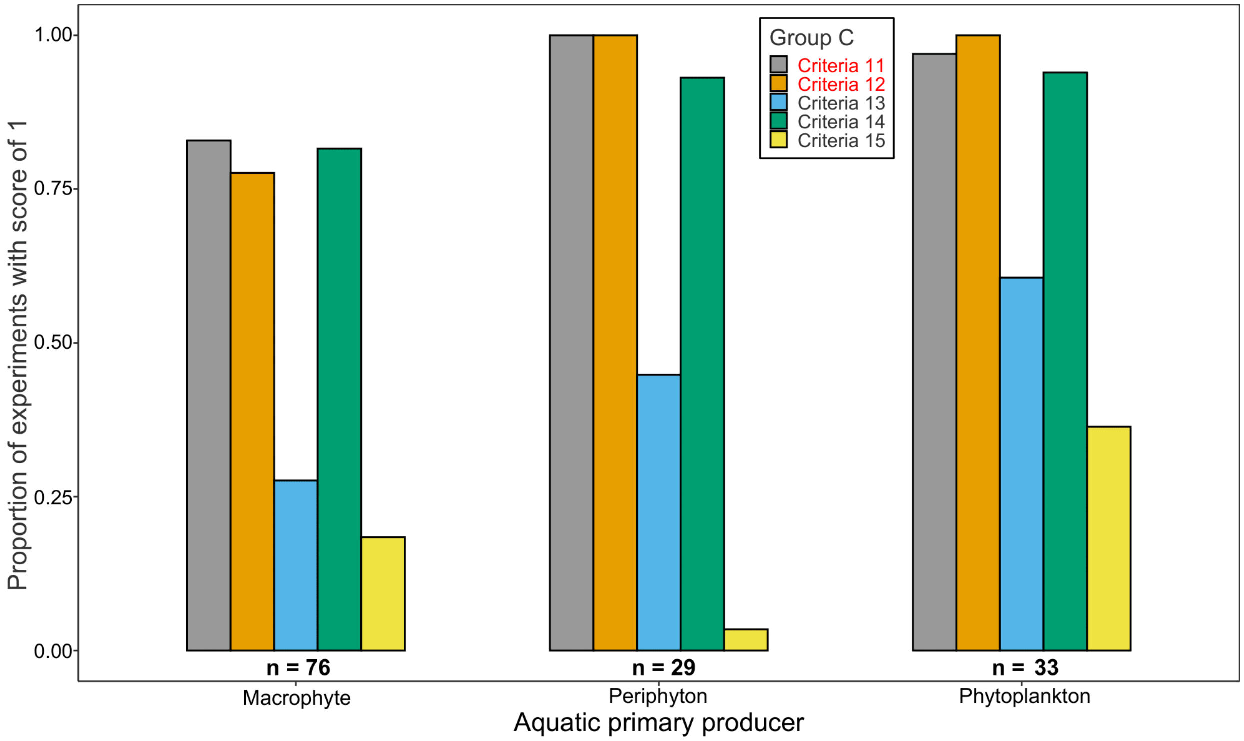

Figure 2.10. The proportion of experiments with score of 1 for Criteria 11 – 15 from Group C test design, statistics, and results (n = 138) by aquatic primary producer (macrophytes, periphyton, and phytoplankton). Criteria 11 and 12 are critical criteria and highlighted in red. The n is the total number of unique tests in each primary producer group.

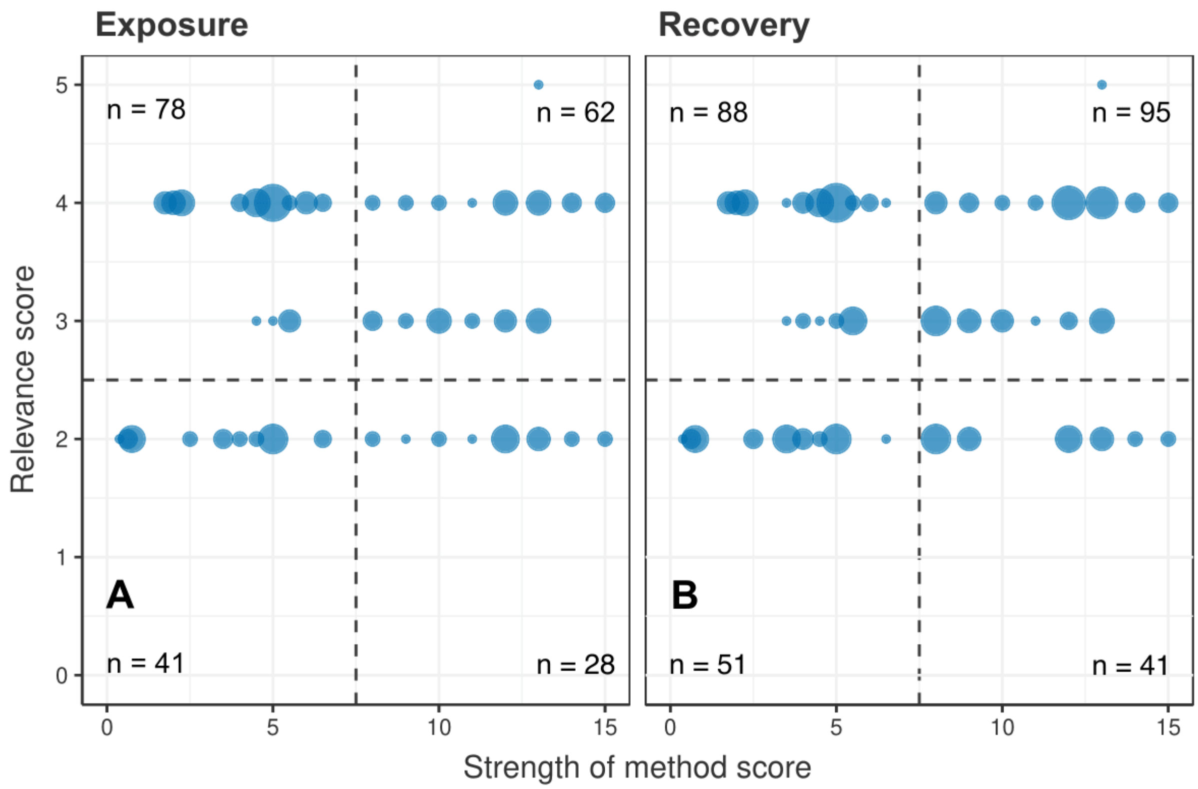

Figure 2.11. Bubble plot with relevance score versus strength of method score from A) exposure (n = 209 of total number of individual endpoint in exposure) and B) recovery (n = 275 of total number of individual endpoint in recovery) period. The n in each corner is the total number of individual endpoints in each quadrant.

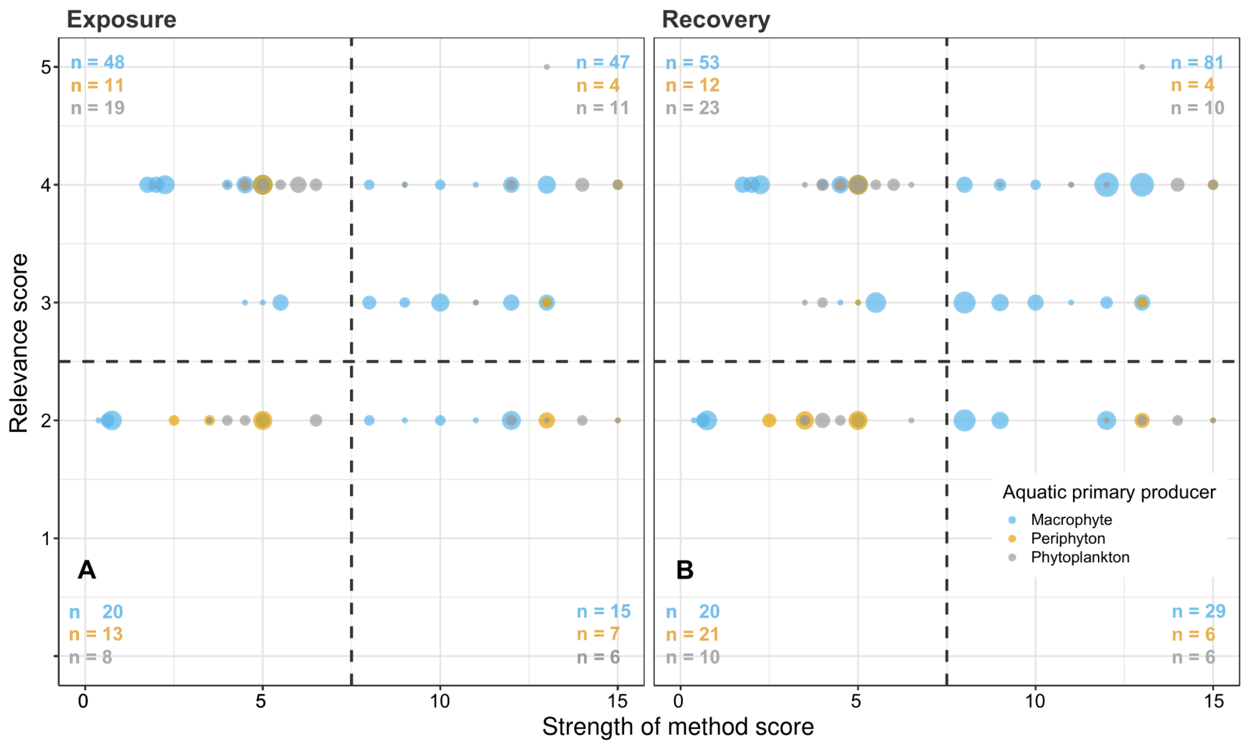

Figure 2.12. Bubble plot with relevance score against strength of method score from A) exposure (n = 209) and B) recovery (n = 275) period for aquatic primary producers (macrophytes, periphyton, and phytoplankton). The n in each corner is the total number of individual endpoints for each primary producer group in each quadrant.

Chapter 3 Recovery of duckweed following acute, pulsed, and chronic exposure to 2,4-D or atrazine: Implications for the risk assessment of herbicides

3.1 Abstract

Duckweed (*Lemna gibba* and *Lemna minor*) was exposed for 7, 14, 21, and 28 days to herbicides (2,4-D or atrazine) and then transferred to clean media for 7 days to investigate its potential to recover from effects in the laboratory. The 2,4-D and atrazine exposure concentrations ranged from 12 – 731 μg/L and 5 - 187 μg/L, respectively. The EC50s and NOECs for relative growth rate (RGR) following the recovery phase were ≥ 558 μg/L and ≥ 117 μg/L for 2,4-D, and ≥ 271 μg/L and ≥ 45 μg/L for atrazine, respectively, for up to 28 days of exposure. We also exposed *L. gibba* and *L. minor* to each herbicide (2,4-D: 2, 4, and 8 μg/L and atrazine: 3, 5, 11, and 22 μg/L) for up to 60 days continuously, including pulse regimens. The majority of endpoints (dry mass, frond number and plant number) were not significantly different from control after 60 days. Overall, the toxicity values from 2,4-D and atrazine for all tests were greater than the regulation guidelines from the Canadian Council of Ministers of the Environment Water Quality Guidelines for the Protection of Aquatic Life from 1999 (i.e., 4 μg/L, and 1.8 μg/L for 2,4-D and atrazine, respectively) and the aquatic life benchmark from USEPA (i.e., 299.2 μg/L for 2,4-D on acute (<10 days) vascular plants, and <1 μg/L for atrazine on acute nonvascular plants). We also found no effects for the USEPA 60-day guideline values for atrazine of 10 μg/L and at 2,4-D of 8 μg/L. We conclude that duckweed can recover after exposures above current environmental exposure levels, and that existing regulatory levels are likely protective of aquatic eudicots and monocots for atrazine. For 2,4-D, the current regulatory concentration should not cause an adverse effect on monocots. We also compared EC10 and NOEC after atrazine exposure and recovery period on *Lemna sp.* and concluded that EC10 could be used as a substitute for NOEC in risk assessment.

3.2 Introduction

Ecological risk assessments are performed in part to protect non-target aquatic primary producer species from the effects of herbicides prior to registration and sale in most jurisdictions (see USEPA, 2020). Herbicide manufacturers provide toxicity data from standard laboratory studies on non-target species such as duckweed, and information on the fate of herbicides in the aquatic environment, including predicted concentrations. While toxicity is characterized, the potential for recovery from observed effects by primary producers is not part of the formal assessment at this time (ECCC, 2007; Brain et al., 2012; Burns et al., 2015). Eventually, guideline values for herbicides may be developed (e.g., concentrations not to be exceeded) to protect aquatic life. These are updated as more data are gathered, and environmental exposures better understood. An example is the USEPA 60-day regulation for aquatic plant Concentration Equivalent Level of Concern (CE-LOC) for atrazine of 10 μg/L in watersheds, especially those located at areas with agricultural activities (USEPA, 2021b). To our knowledge, chronic regulations such as the 60-day CE-LOC have not been confirmed by laboratory testing (e.g., exposing duckweed to an herbicide for 60 days) nor have they been shown to inform the potential for recovery following exposure. In this chapter, we set out to examine the efficacy of current regulations to protect aquatic primary producers, specifically the monocot duckweed, and the potential for recovery using two herbicides as models: 2,4-dichlorophenoxyacetic acid (2,4-D) and atrazine.

The potential for recovery of plants after herbicide exposure is typically evaluated by comparing the response of exposed test species after being transferred to clean medium for a set recovery period. Full recovery is usually assumed to have occurred when the endpoint is not statistically significant from the control at the end of a defined recovery period. Recovery has been observed after herbicide exposure in previous studies but depends on the reversibility and severity of the specific toxic effect under investigation (Wilson and Koch, 2013). In addition, studies have found that exposure to herbicides usually takes place as pulsed inputs (Andrus et al., 2013), meaning there are windows for recovery to occur following an effect. Pesticide concentrations usually peak during periods of agricultural application and after rainfall events, with a later return to low concentrations, which would allow for potential recovery (Andrus et al., 2013; Nowell et al., 2018). Considering recovery in ecological risk assessment could permit regulatory agencies not just to capture more realistic exposure scenarios, but also to avoid missing possible latent effects, where the response continues or increases after the exposure period (Zhao and Newman, 2004).

Regulatory agencies use a variety of approaches to set guideline values to protect aquatic life from the impacts of herbicides. The Canadian Council of Ministers of the Environment (CCME) established the Canadian Water Quality Guidelines for the Protection of Aquatic Life (CWQG-PAL) based on water chemistry in Canadian lakes, rivers and streams, the usage of herbicides, the physical-chemical properties of herbicides, the fate of herbicides in water, and toxicological data of local aquatic organisms after exposure to herbicides (CCME, 2007a; CCREM, 1987). This process involves gathering existing toxicity data, categorizing the data (i.e., primary, secondary, and unacceptable), and then using the data to create a species sensitivity distribution (SSD) from which to derive the guideline value (CCME, 2007a). Primary data are the data that met more criteria (e.g., reported the test concentrations in the beginning and at the end of the exposure, and proper replications) and showed to be more reliable than secondary data (CCME, 2007a). Unacceptable data are the data that did not meet the criteria for primary and secondary data and not being considered by decision makers (CCME, 2007a). During the decision-making process, the primary data would be more valuable than secondary data. Another example is that of the USEPA where guidelines are developed based on a pesticide’s toxicity and degradation data (USEPA, 2021a).

In most cases, the resulting benchmarks, or recommendations, are not explicitly tested in the lab to further confirm the lack of impact on aquatic plants, especially under chronic exposure scenarios that can dramatically exceed typical lab exposures (e.g., Duckweed 7 days versus 60 days CE-LOC; a >8-fold increase in exposure) (ECCC, 2007). A further consideration is the nature of herbicide exposure, which tends to be pulsed over longer durations, as opposed to uniform over time (Boxall et al., 2013; Sanford et al., 2021). These types of exposures are not formally captured in current testing requirements used to inform water quality guideline derivation, including possible recovery.

Two herbicides were chosen for this study. The first being 2,4-D, a synthetic auxinthat mimics the plant growth hormone, auxin, affecting the plant cell growth (Grossman, 2010, Islam et al., 2018; Song, 2014). 2,4-D is a selective herbicide that targets broadleaf eudicotyledon weeds (Belgers et al., 2007). It is one of the most commonly used herbicides in the world and has high water solubility, meaning it is likely to enter aquatic ecosystems and potentially impair primary producers (USEPA, 2017). It is commonly detected in surface waters. For example, Challis et al. (2018b) measured 2,4-D concentrations along the Red River in Manitoba, Canada at 4.6-197.7 ng/L from April – September 2016. Between 2018 – 2019 Sanford and Prosser (2020) reported the greatest concentration of 2,4-D in small streams near agricultural activities in Ontario at 2.9 μg/L as a brief pulse. Fairchild et al. (1997) reported the toxicity of 2,4-D on *Lemna gibba* after a 96h exposure which resulted in an EC50 on frond numbers >10,000 μg/L. Michel et al. (2004) observed a 7-day EC50 for *Lemna paucicostata* total frond area after a 2,4-D exposure of 2210.3 μg/L. To our knowledge, the recovery of aquatic plants after 2,4-D exposure has not been studied. The current CCME water quality guideline for 2,4-D in freshwater is 4 μg/L (CCME, 2007b) and the USEPA aquatic ecotoxicity benchmark 2,4-D value for acute (<10 day) exposure on vascular plants is 299.2 μg/L (USEPA, 2021a).

Atrazine is a reversible photosystem II (PSII) inhibitor with moderate water solubility and is commonly used for weed control in North America (CCME, 1999; Yalkowsky et al., 2010). In 2012 it was the second most commonly used herbicide in the United States (USEPA, 2017). Atrazine is one of the most detected pesticides in North American surface waters (CCME, 1999; Challis et al., 2018a). For example, it was detected in the Red River in Manitoba, Canada, between 2014 - 2015 at concentrations ranging from 0.6 to 520 ng/L (Challis et al., 2018a). When exposed to atrazine *Lemna gibba* has a reported EC50 of 124 μg/L after 7 days exposure and >75 μg/L for 14 days exposure on average growth rate of frond number (Brain et al., 2012). Recovery of duckweed was studied previously and observed consistently, but not under chronic exposures (e.g., >14 days exposure) (Mohammad et al., 2010). The water quality guideline from CCME for atrazine for the protection of aquatic organisms in surface water is 1.8 μg/L (CCME, 1999). The Concentration Equivalent Level of Concern (CE-LOC), established by the USEPA to protect plant communities in watersheds from long-term atrazine exposure, regulates the atrazine concentration in watersheds near agricultural land to an average concentration of 10 μg/L or lower for 60 consecutive days (USEPA, 2021b).

This study also aimed to compare the results of different data reporting approaches. In risk assessment, the no observed effect concentration (NOEC) is the most commonly used effect measure to determine the concentration unlikely to cause adverse effects on non-target organisms (Beasley et al., 2015). A concern for using NOEC in risk assessment is that it could be affected by test design (i.e., distribution of test concentrations) and variation in the data (OECD, 1998; Hanson et al., 2003; DeForest et al., 2008). An ECX, or the concentration causing an x% change in the measured endpoint, is thought of as a more reliable approach because it is calculated from a concentration-response curve (OECD, 1998), and hence not as dependent on the original study design. Studies have found ECX values to be more suitable to use as a substitute for NOEC and so there is a trend for regulatory agencies to replace NOEC with ECX in their risk assessments (Beasley et al., 2015). Beasley et al. (2015) evaluated the association of NOECs and EC10s from 200 chronic *Daphnia* toxicity tests and concluded that the values of EC10s and NOECs were not significantly different to each other. DeForest et al. (2008) analyzed the relationship between NOECs and EC10s for the toxicity of selenium on aquatic birds and found that EC10s were suitable to consider as not causing significant effects from the control on the test species. As a result, there has been a desire to phase-out the use of NOEC values in the risk assessment process (OECD, 1998; Beasley et al., 2015), but this warrants further investigation.

The objectives of this study were: 1) to determine the ability of duckweed to recovery after exposure to herbicides with different modes of action (i.e. 2,4-D and atrazine) under laboratory conditions and different exposure regimens (acute, chronic continuous, and pulsed); 2) to examine the ability of current regulations and guidelines from the CCME and the USEPA for 2,4-D and atrazine in freshwater to protect aquatic plant communities; and 3) contrast NOEC and ECX estimates of toxicity to assess their relative sensitivity and suitability for risk assessment. These data will help to evaluate the recovery potential of duckweed after herbicide exposure to better inform risk assessments, as well as test the effectiveness of current risk assessment approaches and decision-making around select herbicide water quality guidelines.

3.3 Methods and materials

3.3.1 Chemicals and reagents

Exposures were conducted using analytical-grade 2,4-D (CAS: 94-75-7, 97% purity) and atrazine (CAS: 001912-24-9, >95% purity) purchased from Tokyo Chemical Industry (TCI) America and Ultra Scientific, respectively. The 2,4-D (> 98% purity) and 2,4-D-$_{13}C_6$ (>99% isotopic purity) standards for analytical confirmation were from Sigma-Aldrich (Oakville, ON) and Cambridge Isotopes (Andover, MA), respectively. The atrazine (>98% purity) and atrazine-d5 (>99% isotopic purity) standards for analytical confirmation were from MP Biomedicals (Montreal, QC) and C/D/N Isotopes Inc. (Pointe-Claire, QC), respectively. The Hutner's medium used in the testing was prepared with certified ACS grade chemicals purchased from Fisher Chemical.

3.3.2 Test stock solutions

Stock solutions were prepared for each herbicide by dissolving 10 mg of herbicide in 1L of sugar-free Hutner's medium (recipe in Brain and Solomon, 2007) without the use of a carrier solvent. The initial 10 mg/L parent stock solutions were autoclaved before being used to prepare the daughter stocks for subsequent tests. A magnetic stir bar was used to dissolve the herbicide into the medium in the dark for 48h. The stock solutions were kept at 4°C and covered with aluminum foil. These initial stock solutions were not renewed throughout the experimental period and remained clear of any visible precipitate.

All test solutions were prepared by serial dilution with the above parent stock solutions and sugar-free Hutner's medium. The test exposure media were renewed each month and after any power outage where the fridge temperature increased above 4°C. Test solutions of 2,4-D and atrazine were not remade during the 28-day exposure and recovery experiment, but test solutions for the 60-day exposure were renewed four and three times for 2,4-D and atrazine, respectively.

3.3.3 Recovery of duckweed following herbicide exposure for up to 28 days

A series of laboratory experiments were conducted to investigate the recovery potential of two duckweed species (*Lemna gibba* and *Lemna minor*) after chronic herbicide exposure. A static-renewal test was performed to compare outcomes after 7, 14, 21, and 28 days of exposure to 2,4-D or atrazine. Plants were transferred to clean media on Days 7, 14, 21, and 28 for a 7-day recovery period, or to fresh test media with herbicide to continue the exposure on Days 7, 14, and 21 (see Figure 3.1). The procedures of culturing and testing followed the protocols of Brain and Solomon (2007) coupled with guidance from Environment and Climate Change Canada (ECCC, 2007).

3.3.3.1 Culturing

Axenic *L. gibba* (CPCC 310) and *L. minor* were obtained from cultures maintained at the University of Waterloo and the University of Guelph, respectively. Following arrival at the University of Manitoba, the cultures were maintained in 100 mL of autoclaved half-strength sugared Hutner's media in 250-mL Erlenmeyer flasks. The sugared media is to ensure the cultures were sterile for testing. The flasks were placed in an environmental growth chamber (Conviron CMP 6050) at 25 ± 5 °C under continuous lighting with a measured intensity of 67 ± 5 $\mu mol/m^2/s$ (Brain and Solomon, 2007; ECCC, 2007). The temperature in the growth chamber was measured continuously using a water temperature data logger (Tidbit v2 UTBI-001) submerged in water during exposures (refer to Appendix B, Figure B1). The experiment was performed between August 3 - November 29, 2019. Brief power outages occurred during the culturing period on July 29, 2019, and during the experiment period on October 11 - November 15, 2019. One of the growth chambers was shut down briefly due to low pressure alarms on September 25 - November 26, 2019. In all cases, the interruptions were less than 12 hours with no discernable impact on controls or overall test performance.

The Erlenmeyer flask for culturing was capped with cotton wrapped in cheesecloth. Paraflim was used to seal the gap between the flask and the cotton by wrapping it around the flask mouth to avoid other materials from entering the flask while allowing air exchange. Prior to conducting any test, the health of the cultures was evaluated and found to meet the healthy culture criterion of greater than eight-fold increase of frond numbers after 7 days of culturing (ECCC, 2007). In all tests, plants were taken from 7 – 10 days old cultures for test initiation (ECCC, 2007).

3.3.3.2 Preliminary Range-Finding Tests

An initial 7-day range-finding test was performed for each herbicide with *L. gibba* and *L. minor* to determine the definitive test exposure concentrations. Test concentrations for the range-finding exercise were selected based on reported toxicity values from previous studies with 2,4-D and atrazine and the current Canadian and American guideline values for these active ingredients in surface water. Based on the 2,4-D and atrazine guidelines, CWQG-PAL from the CCME and CE-LOC from the USEPA, the nominal concentrations in the 7-day range-finding test were 0, 10, 50, 100, 200, 400, and 600 µg/L for 2,4-D and 0, 10, 20, 40, 80, 160, and 320 µg/L for atrazine, (CCME, 1999, 2007b; USEPA, 2021b). These test concentrations were not confirmed analytically. Two healthy plants with six fronds each were placed in each petri dish (n=3) with corresponding test solution for 7 days and placed in the growth chamber with continuous lighting under the same conditions as for culturing. Frond number, plant number, and dry mass were assessed at the end of the 7 days of exposure.

3.3.3.3 Definitive Toxicity and Recovery Testing

In the definitive toxicity and recovery tests, *L. gibba* and *L. minor* were exposed separately to 2,4-D or atrazine for 7, 14, 21 and 28 days followed by a 7-day recovery period using a standard protocol (ECCC, 2007). The measured test concentrations were control, 12, 69, 117, 259, 489, and 731 µg/L for 2,4-D, and control, 5, 11, 21, 45, 89, and 187 µg/L for atrazine (see Table 3.1 for more details). Each treatment concentration and control had three replicates (n=3). In each replicate, two visually healthy duckweed plants (no chlorosis) with three fronds each were placed in a 50-mL petri dish with 40 mL of test solution. The positions of Petri dishes in the growth chamber were assigned by a random number generator. On Days 7, 14, 21 and 28 of exposure, plants from each treatment group were transferred to either continuous exposure or recovery. Specifically, two plants were transferred to herbicide-free, sugar-free Hutner's media for 7 days for recovery assessment and two were put in a Petri dish with renewed fresh media test solution to continue the exposure. The remaining plants were used to assess dry mass and PSII effective quantum yield. Prior to transfer total plant number and frond number in each replicate was also recorded. A laminar flow hood was used to complete the duckweed transfer during the exposure and recovery to prevent contamination. The experiment was performed between August 17 - September 21, 2019 for atrazine, and October 1 - November 5, 2019 for 2,4-D. Brief power outages occurred during the culturing period on July 29, 2019, and during the experiment period on October 11, 2019. One of the growth chambers was shut down briefly due to low pressure alarms on September 25, 2019 and again on November 26, 2019. In all cases, the interruptions were less than 12 hours with no discernable impact on controls or overall test performance.

Frond number, dry mass, and PSII effective quantum yield were measured on Days 1, 7, 14, 21 and 28 of exposure and after 7 days of recovery in fresh media. Duckweed control performance was used to verify the testing culture health with more than eight-fold increase of frond number for both toxicity and recovery testing. To determine dry mass *Lemna* were dried in an oven at 60°C for at least 24 hr.

The photosystem II effective quantum yield (PSII yield) was measured with a Multi-colour PAM fluorometer (Heinz Walz, Effeltrich, Germany). Measurements were performed on light-adapted and dark-adapted fronds. Settings can be found in Appendix B, Table B1. Six fronds from each corresponding treatment were put onto a filter paper placed diagonally at the bottom of a cuvette to form a 45-degree angle between the emitter and detector. The light-adapted samples were measured directly after removal from the growth chamber. For the dark-adapted samples, the Petri dishes with fronds were placed in a box with the inner walls colored black for 30 minutes or more according to Murchie and Lawson (2013) before the taking PSII measurements under dark-adapted conditions.

3.3.4 Chronic and pulsed exposures of duckweed to herbicides for up to 60 days

A 60-day chronic exposure experiment to evaluate the effectiveness of the USEPA's CE-LOC and the CCME guidelines for 2,4-D and atrazine in surface water was performed (see Figure 3.2 and below for more details) (CCME, 1999, 2007b; USEPA, 2018b). In addition, as herbicides usually enter aquatic ecosystems via runoff, very often as a pulse, a pulsed exposure test was performed with a single concentration of each herbicide (see Figure 3.3 and below for more details).

3.3.4.1 Test concentrations

The selection of test concentrations was based on the current USEPA CE-LOC regulations and CWQG-PAL from CCME (CCME, 1999, 2007b; USEPA, 2018b). The pulsed testing setting was modeled on King et al. (2016) with three 4-day pulses of exposure. The nominal atrazine concentrations for the 60-day exposure were 0, 2.5, 5, 10, and 20 μg/L. The concentration for the 60-day pulsed atrazine test was three 4-day pulse concentrations at 50 μg/L with a time-weighted average of 10 μg/L. The 2,4-D nominal test concentrations for continuous exposure were 0, 2, 4, and 8 μg/L. The concentration for the 60-day pulsed 2,4-D test was three 4-day pulses at 20 μg/L with a time-weighted average of 4 μg/L. The nominal 2,4-D test concentration for continuous and pulsed exposure was 4 μg/L which is based on the CWQG-PAL of 2,4-D concentration from the CCME in freshwater (CCME, 2007b). The nominal atrazine test concentrations of 2.5 μg/L and 10 μg/L in continuous and pulsed exposure of atrazine came from the CCME guidelines on atrazine in freshwater (1.8 μg/L) and the CE-LOC from the USEPA on atrazine in watersheds (10 μg/L), respectively (CCME, 1999; USEPA, 2018b).

3.3.4.2 Testing

The 60-day continuous and pulsed experiment was performed between August 3 - November 29, 2019. Brief power outages occurred during the culturing period on July 29, 2019, and during the experiment period on October 11 - November 15, 2019. One of the growth chambers was shut down briefly due to low pressure alarms on September 25 and again on November 26, 2019. In all cases the interruptions were less than 12 hours with no discernable impact on controls or overall test performance.

3.3.4.2.1 Continuous testing

A total of two *Lemna* with three fronds each were placed in a 50-mL Petri dish with 40 mL of test media. Additionally, there were three replicate dishes at each test concentration. Test media was renewed every 7 days by transferring two random plants into new media to continue exposure until Day 60. Plant and frond number, dry mass, and PSII effective quantum yield were measured every 7 days during media renewal and at the end of the 60-day exposure.

3.3.4.2.2 Pulsed testing

Over the course of the 60-day study three pulses were performed, each with a duration of 4 days. Two *Lemna* plants with three fronds each were first placed in a 50-mL Petri dish with 40 mL of test media. There were three replicate dishes for each concentration. After 7 days in clean media, two duckweeds with three fronds were transferred back to the test media. Plants were placed in test media for 4-day pulses on Days 1, 11, and 22 and transferred back to clean media on Days 4, 15, and 26. After Day 26 two *Lemna* plants were kept in clean media for recovery. The media was renewed every 7 days by transferring two random plants into new media to continue exposure till Day 60. The number of plants and frond, dry mass, and PSII yield from the pulsed exposure were assessed at each media renewal until the 60-day exposure was completed.

3.3.5 Confirmation of Exposures

Chemical analysis of the herbicides in the test media was done at the beginning and end of each test (28-day exposure and recovery test, and 60-day continuous and pulsed tests) to confirm exposure concentrations. Samples of 2,4-D and atrazine were collected on Days 1 and 28 of the 28-day exposure period and recovery testing (October 1 and October 29, 2019 for 2,4-D and August 17 and September 14, 2019 for atrazine). For the 60-day testing, the herbicide samples were collected on Days 1 and 26 (the last day of pulse). The sample dates were October 1 and November 29, 2019 for 2,4-D, and August 3 and October 1, 2019 for atrazine. One sample was taken from each concentration and kept at 4°C.

The test concentrations of 2,4-D and atrazine were measured with liquid chromatography-tandem mass spectrometry (LC/MS) following published protocols (Carlson et al., 2013; Challis et al., 2016). Solid phase extraction (SPE) was performed to clean up the samples, whereby 2 mL of methanol and Milli-Q water were used to pre-condition an OASIS® HLB™ 3cc (60mg) extraction cartridge, then 25 μL of trace element and 5 mL of test solution were loaded and drawn through the pre-conditioned cartridges. Trace element was added to evaluate the efficiency of extraction. A 3-mL aliquot of methanol was added to the cartridges to elute the analytes into ashed centrifuge tubes. Nitrogen was used to evaporate the eluent at 40°C before reconstituting with 1 mL of 50:50 methanol: Milli-Q water and filtering with a 0.22 μm PTFE syringe filter (Carlson et al., 2013; Challis et al., 2016). The extracted samples were kept at 4 °C before the LC/MS analysis. A blank sample with MilliQ water was prepared with the same procedures for each set of test concentration samples to ensure no contamination from the extraction process.

The LC used in the analysis was an Agilent 1200 Series (Agilent Technologies, Mississauga, ON) binary pump, degasser, and column heater with a Phenomenex (Torrance, CA) Kinetex XB-C18 analytical column (50 mm x 2.1 mm x 1.7 μm particle size). The MS was an Agilent 6410B MS with electrospray ionization source in positive (for atrazine) and negative (for 2,4-D) ionization modes. The flow rate was 0.45 mL/min at a column temperature of 42°C, and the injection volume was 20 μL (Carlson et al., 2013; Challis et al., 2016). Details of the LC/MS settings, including limits of detection (LOD) and limits of quantitation (LOQ), are listed in Appendix B, Tables B2, B3, B4, and B5.

3.3.6 Statistics

Absolute values for the endpoints (e.g., plant number, frond number, and dry mass) for Days 7, 14, 21, and 28 of the exposure and recovery testing, and day 60 of the continuous and exposure testing, were calculated based on the data collected every 7 days (medium renewal) and at the end of the testing. Relative growth rates (RGR) of each endpoint (frond number and dry mass) were calculated to evaluate the recovery potential for Days 7, 14, 21, and 28 of exposure and recovery using Equation 1.

$$RGR = \frac{\left((\ln N2) - (\ln N1)\right)}{t} \quad \text{.........(1)}$$

where N_2 is the final frond number or dry mass,

N_1 is the initial frond number or dry mass, and

t is the number of days between the initial and final day.

The function drm() in package ‘drc’ (Ritz et al., 2015) in R-studio (R Development Core Team, 2019) were used to fit six possible concentration-response models (with and without hormetic effects) and to derive the ECX toxicity values for each endpoint and their respective RGR based on the mean measured concentrations. The final model chosen to determine the ECX value for each test was selected based on the lowest Akaike information criterion (AIC) values as well as visual inspection of the fit to the data. The six tested models were the Cedergreen-Ritz-Streibig modified log-logistic model with five different alpha values (α = 0.10, 0.25, 0.50, 0.75, 1.00) and a log-logistic model. The Cedergreen-Ritz-Streibig model was used to determine if hormesis was observed in the data. Hormesis is when the test organisms try to compensate with the interruption of mechanisms by prompting its growth and is often observe at low concentrations (Calabrease, 2001; Cedergreen et al., 2005). The Cedergreen-Ritz-Streibig modified log-logistic model ‘cedergreen’ (Cedergreen et al., 2005) was modified from the four-parameter logistic model to account the hormesis effect with inverse U- or j-shaped (Cedergreen et al., 2005; Ritz and Streibig, 2005). The model was used to compute the ECX values after herbicide exposure and recovery using Equation 2 (Cedergreen et al., 2005; Ritz and Streibig, 2005).

$$f(x) = c + \frac{d-c+f\ \exp\left(-1/x^{\alpha}\right)}{1+\exp\left(b(\log(x)-\log(e))\right)} \text{......... (2)}$$

where b is the slope after hormesis effect is at maximum

c is the lower limit of dose-response curve,

d is the upper limit of dose-response curve,

f is the size of the hormesis effect (i.e. no hormesis effect when f = 0),

e is the lower limit of EC50,

α is the steepness of the hormesis peak with a range of 0 to 1.

The log-logistic model ‘LL.4’ (Seber and Wild, 1989) was used to compute the ECX values using Equation 3 (Ritz et al., 2015).

$$\mathrm{f(x)} = \mathrm{c} + \frac{\mathrm{d-c}}{1+\exp\left(\mathrm{b}(\log(\mathrm{x})-\log(\mathrm{e}))\right)} \text{......... (3)}$$

where b is the slope of the linear curve,

c is the lower limit,

d is the upper limit,

e is the EC50 value.

3.3.6.1 Effect of *Lemna* species after herbicide exposure

With the calculated absolute numbers and RGR values, the endpoints (frond number, plant number, and dry mass) from each treatment level were compared with their corresponding control using one-way analysis of variance (ANOVA) with Dunnett’s test (α = 0.05) if the assumptions of normality (Shapiro-Wilk test) and equal variance (Levene’s test) were met. If the assumptions were not met, a non-parametric Kruskal-

Wallis test and Wilcoxon test were used (α = 0.05). The result from statistical significance was used to calculate the no observed effect concentrations (NOECs) and lowest observed effect concentration (LOECs) for each endpoint. The NOEC and LOEC values were determined based on the lowest test concentration eliciting a statistically significant effect on the respective endpoint from control.

The presence of statistically significant differences between the exposure and control in pulsed exposure were determined by Student's t-test (α = 0.05) at each timepoint for parametric data, and Wilcoxon test for non-parametric data.

In comparison between EC10s and NOECs, the NOEC value was not included when the corresponding EC10 was not calculated due to lack of response. To identify the significant difference between EC10 and NOEC, the data set was tested with Shapiro-Wilk test for normality followed by the paired t-test (α = 0.05) for significant differences.

3.3.6.2 Recovery of *Lemna* species after herbicide exposure

Recovery was considered to be achieved for a particular endpoint when there was no statistically significant difference from the control at the end of the recovery period (i.e., Days 7, 14, 21, and 28 of exposure testing). Therefore, the NOECs from the recovery were the concentration at which *Lemna* sp*.* was determined to have achieved full recovery after the exposure and recovery periods. For the NOEC and LOECs, the dataset was first accessed with Shapiro-Wilk test for normality and Levene's test for equality of variances. Then one-way ANOVA followed by Dunnett's test (α = 0.05) for parametric data and Kruskal-Wallis test followed by the Dunn test (α = 0.05) for non-parametric data.

3.4. Results

3.4.1 Recovery of **Lemna** *sp. following herbicide exposure up to 28 days*

3.4.1.1 Chemical analysis

The mean measured test concentrations for 2,4-D and atrazine are found in Table 3.1. There was no detection of 2,4-D or atrazine in control media. The percentage between the nominal and measured concentration for 2,4-D were ranged from 91.1 - 158% for initial and 103 – 131 % for final. For atrazine, the percentage between the nominal concentration to initial and final measured concentrations ranged from 98.9 – 120 % and 104 – 113 %, respectively. The changes between the initial and final measured 2,4-D test concentrations were larger (4.90 – 33.0%) due to 2,4-D having a shorter half-life in aerobic water (~ 15 days) (USEPA, 2005). The changes between the initial and final measured atrazine test concentrations were smaller (-5.30 – 12.2%) which could be due to the longer half-life in freshwater (i.e., ranging from 3 to >90 days) (Solomon et al., 1996).

3.4.1.2 2,4-D tests

The EC50s, NOECs, LOECs and the parameters for the log-logistic models for *L. gibba* and *L. minor* exposed to 2,4-D in the range-finding test, at 7, 14, 21, and 28 days, and following 7 days recovery in clean media can be found in Appendix B, Tables B6, B7, B8, B9, and B10, respectively. Figure 3.4 shows the response of absolute and relative growth rate endpoints (dry mass, frond number, and plant number) after exposure and recovery period. The EC50s for all endpoints in *L. gibba* and *L. minor* after exposure to 2,4-D were ≥506.0 μg/L, ≥336.3 μg/L, ≥302.7 μg/L, and ≥290.1 μg/L for days 7, 14, 21, and 28, respectively. The EC50 values decreased when the test duration increased in general, which indicated that no resistance was developed on *Lemna* sp. to the toxicity of 2,4-D overtime. The most sensitive endpoint from *L. gibba* and *L. minor* tended to be absolute frond number, and the least was PSII yield on light- and dark-adaptive samples. The recovery EC50s for all endpoints were ≥500.2 μg/L, ≥272.8 μg/L, ≥299.8 μg/L, and ≥278.7 μg/L for days 7, 14, 21, and 28, respectively. Full recoveries were observed based on the relative growth rate (RGR) and these were not significantly reduced from the control at concentrations up to 259.4 μg/L for Days 7, 14, and 21 and up to 116.8 μg/L for Day 28. The 7-day EC50 values were greater than USEPA benchmark on acute vascular plants (USEPA, 2021a). All toxicity and recovery EC50s and NOECs for *L. gibba* and *L. minor* were greater than the 2,4-D guideline value for the protection of aquatic life of 4 μg/L in freshwater (CCME, 1999). We also observed hormesis effect on 60% and 48% of *L. gibba* (n = 47) and *L. minor* (n = 48) endpoints after exposure and recovery period at concentrations up to 194 μg/L and 239 μg/L, respectively (refer Appendix B, Figure B3 for examples).

3.4.1.3 Atrazine tests

The EC50s, NOECs, and LOECs and the parameters for the log-logistic model for *L. gibba* and *L. minor* exposed to atrazine for the range-finding test, at 7, 14, 21, and 28 days, and following 7 days recovery in clean media are provided in Appendix B, Tables B6, B11, B12, B13, B14, respectively. The EC50s for absolute and relative growth rate endpoints (dry mass, frond number, and plant number) after atrazine exposure and recovery period are found in Figure 3.5. The toxicity EC50s for all endpoints on *L. gibba* and *L. minor* after exposure period were ≥74.5 μg/L, ≥35.5 μg/L, ≥46.2 μg/L, and ≥40.1 μg/L for Days 7, 14, 21, and 28, respectively. The most and least sensitive endpoints were absolute dry mass, and PSII yield on dark-adapted samples, respectively. The recovery EC50s for all absolute and relative growth rate endpoints were observed at a concentration of ≥80.0 μg/L, ≥62.9 μg/L, ≥50.0 μg/L, and ≥36.78 μg/L for Days 7, 14, 21, and 28, respectively. Full recovery was observed at concentrations of up to 186.8 μg/L for Day 7 and 88.63 μg/L for Days 14, 21, and 28. All toxicity and recovery EC50s for *L. gibba* and *L. minor* were greater than the atrazine guideline value for the protection of aquatic life in fresh water from CCME (i.e., 1.8 μg/L), and the acute benchmark and CE-LOC from the USEPA (i.e., <1 μg/L and 10 μg/L) (CCME, 1999; USEPA, 2021a, 2021b). The EC50s of light-adapted PSII yield after atrazine exposure and recovery is found in Appendix B, Figure B2.

The EC10s from Days 7, 14, 21, and 28 of the atrazine exposure and recovery periods on *Lemna gibba* and *Lemna minor* were not statistically significant from the NOECs, as noted in Figure 3.6A and 3.6C. The EC10s from *Lemna minor* after exposure were not significantly different from the NOECs (Figure 3.6B); however, EC10s after recovery were significantly lower than the NOECs (Figure 3.6D). As with 2,4-D, a hormetic effect was found at ≤51 μg/L and ≤45 μg/L for 23% (n = 48) of *L. gibba* and 60% (n = 48) of *L. minor* endpoints after atrazine exposure and recovery (refer to Appendix B, Figure B4).

3.4.2 Chronic 60-day testing

3.4.2.1 Chemical analysis

The mean measured concentration in the 60-day continuous testing can be found in Table 3.2. There was no detection of 2,4-D or atrazine in control media. The percentage between the nominal and measured 2,4-D concentrations ranged from 105 – 121% for initial and 71.5 – 90.8% for final. The percentage between the nominal and measured atrazine concentrations for initial and final ranged from 104 – 124%, and 91 – 102%, respectively. The concentration changes between Day 1 and Day 60 ranged from 27.1 – 32.1% for 2,4-D and 2.3 – 18.9% for atrazine. Greater change in concentration between Day 1 to 60 were observed in 2,4-D than atrazine. This may be due to 2,4-D has a shorter half-life in oxygenated aquatic environment (USEPA, 2005).

3.4.2.2 2,4-D tests

No statistically significant reduction from the control ($p>0.05$, one-way ANOVA with Dunnett's test) was observed for the absolute endpoints (dry mass, frond number and plant number) in *L. gibba* and *L. minor* at all tested concentrations for the 2,4-D pulse exposure, with the exception of absolute dry mass for *L. gibba* at 8.32 μg/L on day 21 (Figure 3.7 and Appendix B, Table B15). *L. gibba* and *L. minor* were not significantly impaired at concentrations greater than or equal to current regulation (i.e., 4 μg 2,4-D/L) after 60 days of exposure (CCME, 2007b). The NOECs and LOECs for light- and dark-adapted PSII yield during the 60 days of 2,4-D exposure are listed in Appendix B, Table

B16. The relative growth rate of endpoint (dry mass, frond number and plant number), light- and dark-adapted PSII yield after 60 days of 2,4-D continuous exposure are found in Appendix B, Figures B5 – B9.

3.4.2.3 Atrazine tests

The absolute endpoints for *L. gibba* and *L. minor* after 60 days of atrazine exposure had not significantly decreased from the control during the medium renewal or at the end of the 60-day exposure period, as seen in Figure 3.8. The majority of NOECs and LOECs for absolute and RGR endpoints were ≥21.75 μg/L and >21.75 μg/L, respectively which can be found in Appendix B, Table B17. There were some exceptions, including absolute dry mass, absolute frond number, absolute plant number, RGR dry mass and RGR frond number, that were greater than the control at the lower test concentrations (2.60μg/L and 5.08 μg/L). The NOECs and LOECs for all endpoints after atrazine exposure are found in Appendix B, Tables B17 and B18. *L. gibba* and *L. minor* were not significantly impaired after 60 days of atrazine exposure at a concentration that is greater than or equal to the current regulations for atrazine (i.e., 1.8 μg/L and 10 μg/L) (CCME, 1999; USEPA, 2021b). The NOECs and LOECs for light- and dark-adapted PSII yield during the 60 days of atrazine exposure are listed in Appendix B, Table B18. The relative growth rate endpoints (dry mass, frond number and plant number) and light- and dark-adapted PSII yield after 60 days of continuous atrazine exposure are found in Appendix B, Figures B10 – B14.

3.4.3 Pulsed testing

3.4.3.1 Chemical analysis

The measured concentrations of 2,4-D and atrazine can be found in Table 3.3. There was no detection of 2,4-D or atrazine in control media. The percentage differences between the nominal and measured 2,4-D concentration were 155% for initial and 95.5% for final. The percentage differences for atrazine between the nominal and measured concentration were 121% for initial and 106% for final. It should also be noted that 2,4-D (38.5%) had a greater decrease of concentration between Day 1 to Day 60 than atrazine (12.2%).

3.4.3.2 2,4-D tests

The relative growth rate endpoints (dry mass, frond number and plant number) for *L. gibba* and *L. minor* were not statistically different from controls ($p>0.05$) during and after the pulse periods, except for frond number for *L. minor* on Day 22 (the first day of third pulse; Figure 3.9) *L. gibba* and *L. minor* were not significantly impaired at pulse concentrations greater than or equal to current regulations (i.e. 4 μg 2,4-D/L) after 60 days of exposure (CCME, 2007b). The light- and dark-adapted PSII yield after 60 days of pulsed 2,4-D exposure are found in Appendix B, Figure B15.

3.4.3.3 Atrazine tests

The RGR of dry mass on *L. gibba* and *L. minor* were significantly reduced from the control at the end of each pulse (i.e., Days 4, 15 and 26), and significantly increased from the control after being transferred to clean media on Day 22 for *L. gibba* and on Days 11, 22, 33 and 40 for *L. minor* (Figure 3.10). Significant differences (decrease) from the control for RGR (frond number) were observed on the last day of each pulse (i.e., Day 4, 15, and 26) for *L. gibba* and Day 26 for *L. minor*. The RGR values returned to a similar level (not statistically different) as corresponding control after the pulses. The RGR of plant number were not statistically different during and after the pulse periods, except for a significant difference (increase) than the control on Day 33 for *L. minor*. *L. gibba* and *L. minor* were not significantly impaired after 60 days of atrazine pulse exposures at a time-weighted average concentration that is greater than or equal to the current regulations (i.e., 1.8 μg atrazine/L and 10 μg atrazine/L) (CCME, 1999; USEPA, 2021b). The light- and dark-adapted PSII yield after 60 days of pulsed atrazine exposure are found in Appendix B, Figure B15.

3.5. Discussion

We found that duckweed (*L. gibba* and *L. minor*) is not likely to be adversely affected following exposure to 2,4-D or atrazine ranging from 7 to 60 days at or above current regulatory values. Where effects were observed, full recovery was often observed in duckweed that has undergone an exposure duration up to 28 days at concentrations significantly greater than both environmental levels, as well as regulatory values. Overall, this study supports the position that current regulatory approaches in North America for the risk assessment of herbicides are protective of non-target aquatic macrophytes for both acute and chronic exposures.

3.5.1 2,4-D Tests

3.5.1.1 2,4-D toxicity and recovery following 7, 14, 21 and 28 days exposures

The toxicity of 2,4-D in *L. gibba* and *L. minor* in our study is consistent with that in previous reports. Tunić et al. (2015) reported that the 7-day EC50 absolute and RGR endpoints (frond number, fresh weight and frond area) for *L. minor* after 2,4-D exposure were 7080 to >10,000 μg/L and >10,000 μg/L, respectively. Fairchild et al. (1997) reported that the 96 hr EC50 values for *Lemna* sp. were >10,000 μg/L and Sanford et al. (2021) reported the 24 hr EC50 on *L. minor* was >2360 μg/L. We observed 7-day EC50s were >506 μg/L for *L. gibba* and >512 μg/L for *L. minor*. The majority of the 7-day EC50s on absolute and RGR endpoints were similar to or greater than our highest test concentration (731.1 μg/L) which implies there was a lack of response during the exposure period and the EC50s were extrapolated. Our highest test concentration was lower than previous studies by Tunić et al. 2005 and Fairchild et al. 1997 and could have resulted in the lower 7-day EC50 values observed in our study. To our knowledge there are no studies examining responses in duckweed greater than a 7-day exposure. As 2,4-D is a synthetic auxin and growth regulator that targets eudicot plants (e.g., *Myriophyllum* sp.), and not monocots such as *Lemna* sp., the general lack of toxicity is expected. A hormetic effect was observed after exposure and the recovery period in our study and a similar result was also found in Nema et al. (2019). This study reported that the growth rate of exposed *Oophila* sp. was higher than the control after 96 hr of 2,4-D exposure followed by a 96 hr recovery period at a concentration of 3000 μg/L (Nema et al., 2019).

Due to the lack of response to the 2,4-D toxicity the potential for duckweed to recover from effects induced by 2,4-D exposure remains uncertain. Our test concentrations were not sufficiently high enough for calculation of EC50s for most of the time points and therefore extrapolation of the concentration-response curve was required. Sanford et al. (2021) found a similar result with insignificant toxicity observed in *L. minor* after a 24 hr 2,4-D exposure at concentrations up to 2360 μg/L. They were also unable to test for recovery potential (Sanford et al., 2021).

L. gibba and *L. minor* frond number and PSII yield were the most and least sensitive endpoints for 2,4-D exposure, respectively. Sanford et al. (2021) concluded that dry mass was the most sensitive endpoint for *L. minor* after 2,4-D exposure and frond number was most sensitive for *L. minor* for 3 out of 6 test herbicides. The insensitivity of PSII yield in light- and dark-adapted *Lemna sp.* between control and treatment is reasonable because 2,4-D is a synthetic growth regulator that targets cell growth, not photosynthesis (Grossman, 2010).

3.5.1.2 60-day continuous and pulse testing with 2,4-D

Dry mass, frond number and plant number exhibited no significant reduction after 60 days of continuous exposure at all test concentrations (1.77 – 8.32 μg/L). The results agree with previous studies that 2,4-D is not toxic to *Lemna* sp. as noted above. There were also no significant effects observed after pulsed 2,4-D exposure. These results suggested that three 4-day pulses at 25.09 μg/L with an average concentration of 5.02 μg/L over 60 days does not cause adverse effects on *L. gibba or L. minor.*

3.5.1.3 Implications for current regulations and environmental exposures of 2,4-D on aquatic plants

The highest 2,4-D concentration reported in small Ontario streams near agricultural activities was 2.9 μg/L with duration of 1 day. This was observed in 2019 (Sanford and Prosser, 2020). In 2016, the time weighted average concentrations in Manitoba's Red and Assiniboine Rivers ranged from <0.0041 – 0.197 μg/L (Challis, 2018b). Between 2003 – 2005, surface water concentrations of 2,4-D across Canada were reported in the range of <0.00047 – 8.24 μg/L (ECCC, 2011). The reported 2,4-D concentrations in surface water were lower than the concentrations at which toxicity was observed in this study, regardless of duration of exposure. Therefore, 2,4-D concentrations in aquatic environments at this time are not likely to have an adverse effect on monocot aquatic plants under acute or chronic exposures.

The guideline for 2,4-D in surface water has been established in Canada and set at 4 μg/L to protect aquatic life from long-term exposure (CCME, 1987). Our data show that EC50 values after 7, 14, 21 and 28 days of exposure were all greater than the CCME value. Our results also show that 7-day EC50s (i.e., >394.24 μg/L for *L. gibba* and >512 μg/L for *L. minor*) are greater than the acute (<10 day) benchmark for vascular plants (USEPA, 2021a). In addition, the results from continuous and pulse exposure suggest that *Lemna* sp. is unlikely to be affected adversely at the regulation concentration after 60 days of exposure. Therefore, the CCME and USEPA regulations likely protect aquatic plants from long-term 2,4-D exposure. Since 2,4-D targets eudicots and the tested species were monocots this was not unexpected. Future work should focus on eudicot species to address issues of sensitivity.

3.5.2 Atrazine tests

3.5.2.1 Atrazine toxicity and recovery following 7, 14, 21 and 28 day exposures

The toxicity values for atrazine exposure and recovery from our study are similar to those from previous studies. Brain et al. (2012) reported *L. gibba* 7 and 14 day EC50s on endpoints (frond number, growth rate and biomass) of >57 μg/L and >64 μg/L, respectively. Mohammad et al. (2010) reported a *L. gibba* 7 day EC50 of 89 μg/L on frond number. We observed 7 and 14 day EC50s for all endpoints of >77 μg/L and >35 μg/L for *L. gibba* and >75 μg/L and >61 μg/L for *L. minor*. Longer exposure periods (14, 21 and 28 days) were studied by Mohammad et al. (2010) but the EC50s were not reported. In addition, the observation of a hormetic effect after atrazine exposure and recovery period in our study was also found in other studies. Brain et al. (2012) reported a hormesis effect in exposed *L. gibba* at concentrations up to 40 μg/L after a 5- and 7-

day of atrazine exposure. Hormesis effect was also reported on *Oophila* sp. photosystem II yield after 96h of atrazine exposure and recovery period up to 300 µg/L (Nema et al., 2019).

Recovery of *Lemna* sp. was observed after different exposure durations when comparing the NOECs from the post-exposure and recovery periods. Our results are consistent with those of previous studies. Photosynthesis inhibition by atrazine is reversible allowing plants to recover after exposure (Brain et al., 2020; Van Oorschot, 1965; Mohammad et al., 2010). Recovery of photosynthesis from the effect of atrazine exposure was shown in maize after being transferred to herbicide-free media (Van Oorschot, 1965). Mohammad et al. (2010) observed a 43% recovery for *L. gibba* relative to control in RGR frond number following a 28-day exposure at a concentration of 3,200 μg/L. Brain et al. (2012) reported that recovery of *L. gibba* was observed on growth rate at all test concentrations and durations after 7 and 14 day recovery periods. Recovery was also observed on frond number in all test concentrations except for concentrations of 80 and 160 μg/L after recovery from 5 and 7 days of exposure (Brain et al., 2012). As with frond number, recovery on biomass was observed at all concentrations, except for duckweed exposed to 80 and 160 μg/L for 7 days, followed by recovery (Brain et al., 2012). In the same study, Brain et al. (2012) concluded that the recovery of *L. gibba* was not associated with exposure duration as recovery was observed after all exposure durations (i.e., 1 to 14 days of exposure). Similar results were observed in our study. We observed that the RGR dry mass on *L. gibba* and all RGR endpoints in *L. minor* were recovered at all test concentrations after the 7 day recovery period following 7, 14, 21 and 28 day of exposures. Recovery of other RGR endpoints on *L. gibba* was observed at ≤ 44.88 µg/L for frond number and ≤ 88.63 µg/L for plant number.

The most and least sensitive endpoints for *L. gibba* and *L. minor* after atrazine exposure and recovery period were absolute dry mass and PSII yield on dark-adaptive samples, respectively. As mentioned, dry mass was observed to be the most sensitive endpoint in other studies with duckweed (i.e., Sanford et al., 2021, and Burns et al., 2015).

3.5.2.2 Continuous and pulsed 60-day atrazine exposure

As with 2,4-D continuous exposure, the absolute endpoints of dry mass, frond number and plant number were not significantly reduced at the end of the 60-day continuous atrazine exposure. Brain et al. (2012) found a similar result and reported that the LOEC and NOEC after 14 days of exposure were 37 and 18 μg/L, respectively.

For the 60-day pulse exposure the RGR dry mass was significantly reduced after each pulse for both *Lemna* sp. and recovered to a level that was higher or similar to the control after 7 days of recovery. Significant reductions were also found on RGR frond number on *L. gibba* at the end of all three pulses and on *L. minor* at the end of the third pulse. All endpoints returned to values similar to or higher than control values after a 7 day recovery period. A similar result was observed in field studies. King et al. (2016) reported no significant changes in periphyton and phytoplankton community structure in an outdoor mesocosm on the last day of three 4-day atrazine pulses where total test duration was 60 days and the NOEC was ≥ 30 μg/L. Results imply that *L. gibba* and *L. minor* are unlikely to have a long-term effect at pulse concentrations of 56.58 μg/L with an average concentration of 11.32 μg/L.

3.5.2.3 Implications for current regulations and environmental exposures of atrazine on aquatic plants

Between 2014 – 2015 the concentrations of atrazine in the Red River in Manitoba, Canada were reported to range between <0.0013 – 0.52 μg/L (Challis et al., 2018a). The atrazine concentrations in Canadian surface waters, between 2003 – 2005, were reported to range from <0.00002 – 14.9 μg/L (ECCC, 2011). The reported concentrations of atrazine in surface water were, in the majority of cases, lower than toxicity values and not likely to cause adverse effects. Greater concentrations have been observed in surface waters near agricultural areas during application time and are deemed worst-case scenarios for corn production in the United States (Andrus et al., 2013). Andrus et al. (2013) measured atrazine concentrations in surface waters near agricultural lands and found them to be below 20 μg/L for the majority of the time studied with the exception of pulses that had a peak concentration of up to 70.5 μg/L decreasing below 20 μg/L within a short period (<15 days) of time. The concentration peaked at 70.5 μg/L which was greater than our 7 and 14 days NOECs (i.e., 44.88 μg/L) and may cause temporary effects to duckweed. Our results show that *Lemna* sp. is likely to recover after the dissipation of similar pulses. As a result, the likelihood of *Lemna* sp. being significantly impacted by atrazine exposures currently found in the environment is low. The pulse exposure from Andrus et al. (2013) demonstrated the importance of considering recovery when performing ecological risk assessment in surface waters near agricultural areas.

The CCME guideline on atrazine concentrations in fresh water is 1.8 μg/L (CCME, 1999). In the United States the aquatic life benchmark on acute nonvascular plants and regulation for a 60-day average atrazine concentration in surface water near agricultural lands are <1 μg/L and 10 μg/L, respectively (USEPA, 2020). The results from continuous and pulse exposure showed that the likelihood of atrazine causing adverse effects on *Lemna* sp., at regulatory concentrations set by the CCME and USEPA, was low. As a result, the existing atrazine regulations are likely protective of non-target aquatic plants.

3.5.2.4 Comparing EC10s to NOECs

The toxicity values (EC10, NOEC) from the exposure and recovery periods were compared. Our results were similar to those of previous studies. Beasley et al. (2015) gathered toxicity values (EC10, EC20 and NOEC) from 200 chronic *Daphnia* toxicity data and suggested that EC10 was closer to NOEC than EC20. DeForest et al. (2008) investigated the association between EC10, EC20 to NOEC from toxicity testing on the hatching success of aquatic birds after exposure to selenium. The study concluded that the EC10s were not significantly different from the control and could be used to represent the concentration that would not cause adverse effects on organisms (DeForest et al., 2008). Our result also indicates that most of the NOEC values were not significantly different from EC10 with the exception of EC10s after recovery on *L. minor* which were significantly lower than NOECs. This indicates that EC10 could be considered as not significantly different from the control in the risk assessment.

3.6. Conclusions

In summary, toxicity values from previous studies and our results indicate that reported 2,4-D and atrazine concentrations in the environment are not likely to cause adverse effects on *L. gibba or L. minor*. As well, current regulations from the CCME and USEPA have the ability to protect these aquatic macrophytes from acute and chronic 2,4-D and atrazine exposure. Our work showed dry mass on *L. gibba* and all the endpoints on *L. minor* recovered after 7, 14, 21 and 28 days of exposure, followed by 7 days of recovery at all tested concentrations. It indicates that the recovery potential of *Lemna* sp. after atrazine exposure was not likely to be associated with the exposure durations. We also compared the EC10s with NOECs and showed that EC10s were lower than, or not statistically significant to, the NOECs. Therefore, EC10 could be considered as not significant different from the control in ecological risk assessment.

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3.8 Tables

Table 3.1. The measured test concentration of 2,4-D and atrazine on day 1 and 28 and the mean in stock solutions. The mean value was used in subsequent assessment of effects and recovery. No herbicide was detected in controls for either test. Limit of detection of 0.0041 μg/L and 0.0013 μg/L for 2,4-D and atrazine, respectively.

2,4-D (μg/L)				**Atrazine (μg/L)**			
Nominal	Day 1	Day 28	Mean	Nominal	Day 1	Day 28	Mean
10	12.66	12.04	12.35	5	4.94	5.21	5.08
50	72.14	65.78	68.96	10	11.80	9.78	10.79
100	130.46	103.04	116.75	20	22.30	20.51	21.40
200	310.58	208.23	259.40	40	47.46	42.30	44.88
400	550.51	426.90	488.71	80	91.81	85.44	88.63
600	791.01	671.09	731.05	160	193.40	180.29	186.84

Table 3.2. The measured initial, final, and mean test herbicide (2,4-D and atrazine) concentrations in stock solutions for 60-day continuous testing. The mean value was used in subsequent assessment of effects and recovery. No herbicide was detected in controls for either test. Limit of detection of 4.1 ng/L and 1.3 ng/L for 2,4-D and atrazine, respectively.

2,4-D (μg/L)				**Atrazine (μg/L)**			
Nominal	Day 1	Day 60	Mean	Nominal	Day 1	Day 60	Mean
2	2.10	1.43	1.77	2.5	2.61	2.55	2.58
4	4.98	3.62	4.30	5	5.61	4.55	5.08
8	9.65	6.98	8.32	10	12.40	10.12	11.26
-	-	-	-	20	23.06	20.44	21.75

Table 3.3. The measured initial, final and mean test herbicide (2,4-D and atrazine) concentrations in stock solutions for the 60-day pulsed (three 4-day pulses). The mean value was used in subsequent assessment of effects and recovery. No herbicide was detected in controls for either test. Limit of detection of 4.1 ng/L and 1.3 ng/L for 2,4-D and atrazine, respectively.

2,4-D (μg/L)				**Atrazine (μg/L)**			
Nominal	Day 1[a]	Day 26[b]	Mean	Nominal	Day 1[a]	Day 26[b]	Mean
20	31.1	19.1	25.1	50	60.3	52.1	56.6

[a]First day of first pulse,

[b]Last day of third pulse.

3.9 Figures

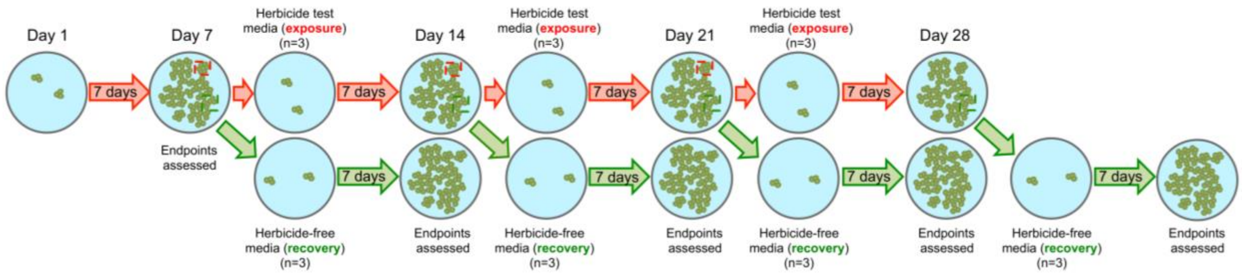

Figure 3.1. Conceptual model of the experimental design and timeline for the exposure and recovery study. Duckweed (*Lemna gibba* and *Lemna minor*) was exposed for 7, 14, 21 and 28 days to 2,4-D or atrazine followed by 7 days of recovery in clean media.

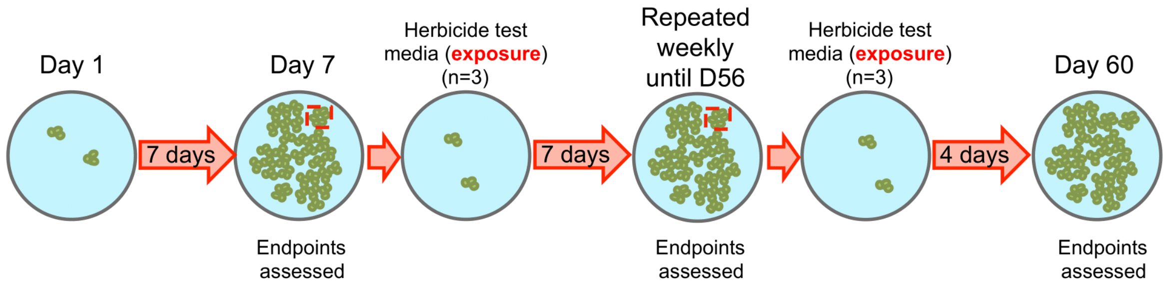

Figure 3.2. Conceptual model of the 60-day continuous herbicide (2,4-D or atrazine) exposure with duckweed (*Lemna gibba* and *Lemna minor*).

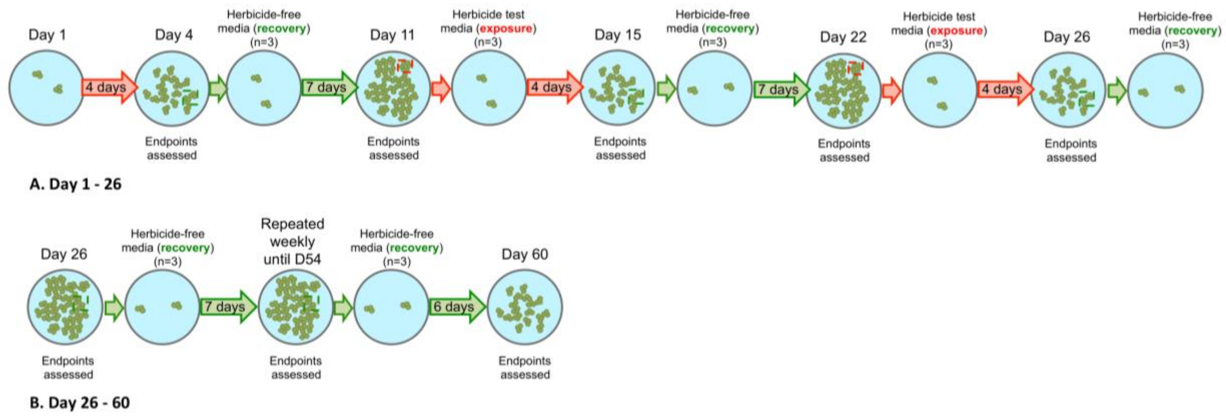

Figure 3.3. Conceptual model of the 60-day pulsed (three 4-day pulses) herbicide (2,4-D or atrazine) exposure on duckweed (*Lemna gibba* and *Lemna minor*) from (A) Day 1 – 26 and (B) Day 26 – 60.

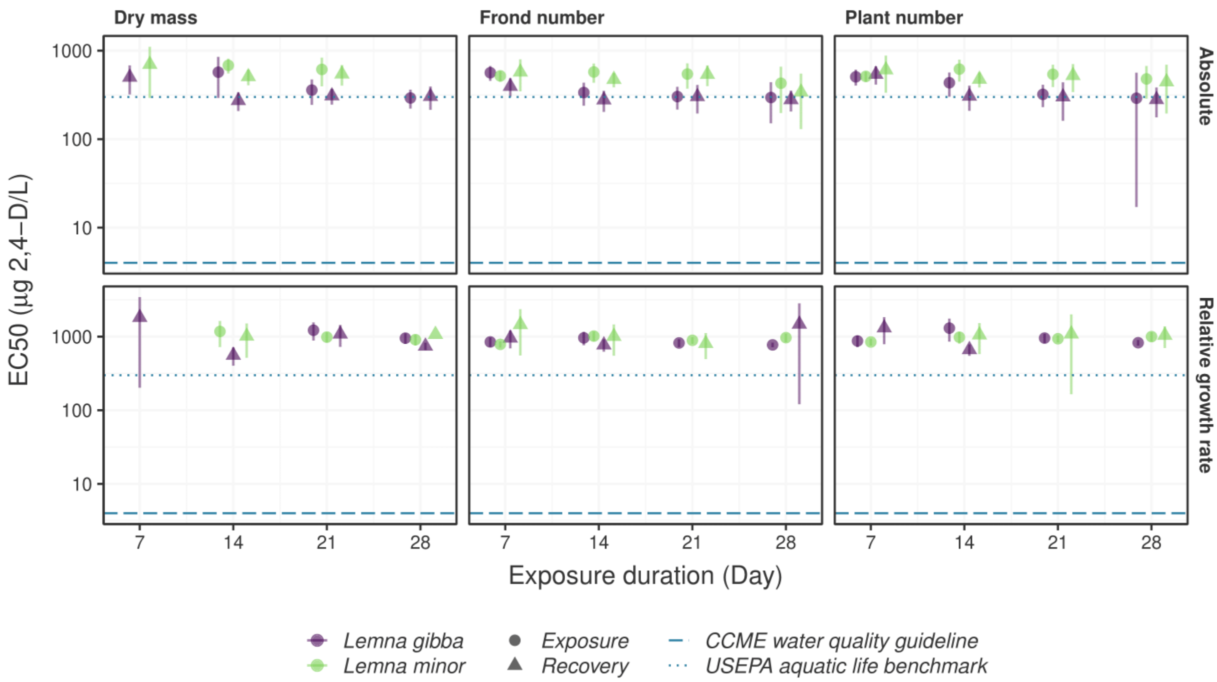

Figure 3.4. EC50 values for *Lemna gibba* and *Lemna minor* endpoints (dry mass, frond number and plant number) after 7, 14, 21 and 28 day 2,4-D exposure periods followed by transfer to clean media for a 7-day recovery period. The error bars represent 95% confidence intervals. The blue dashed lines indicate the acute (i.e., < 10 days) aquatic life benchmark on vascular plants for 2,4-D at 299.2 µg/L from the USEPA. The blue long-dashed line indicates the Canadian water quality guidelines for the protection of aquatic life (chronic) from the CCME on 2,4-D at 4 µg/L.

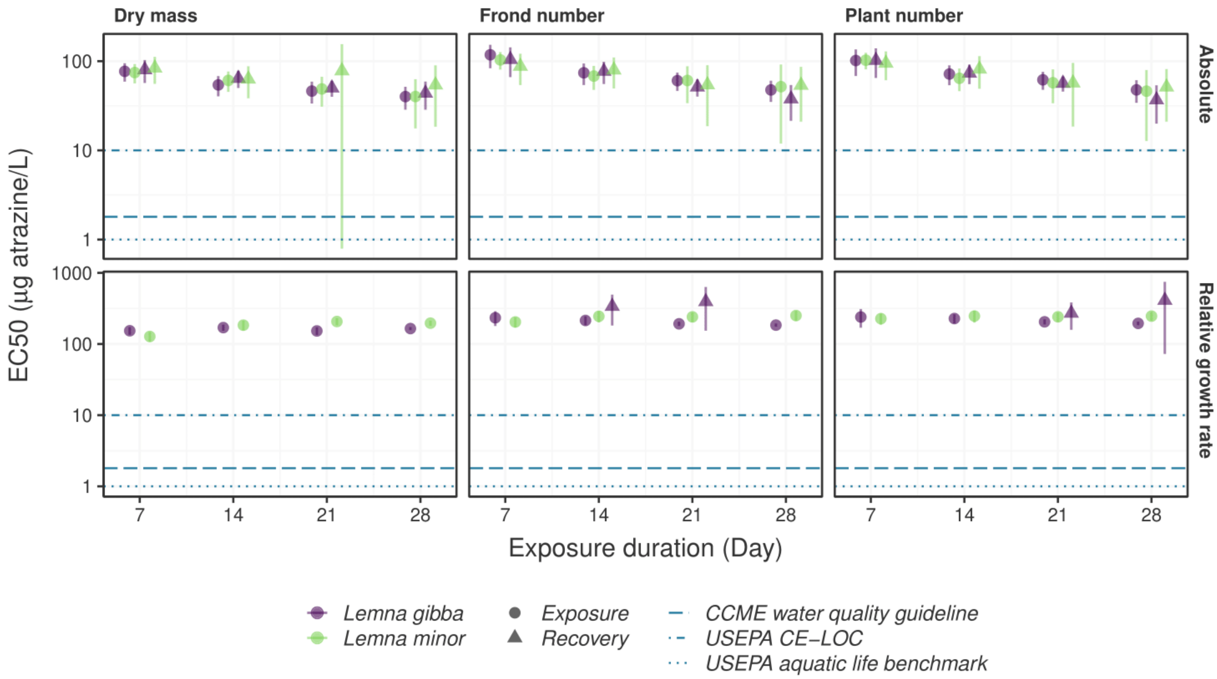

Figure 3.5. EC50 for *Lemna gibba* and *Lemna minor* endpoints (dry mass, frond number and plant number) after 7, 14, 21 and 28 day atrazine exposure periods followed by transfer to clean media for a 7-day recovery period. The error bars are 95% confidence intervals. The blue dashed and two dashed lines indicated the USEPA aquatic life benchmark for acute (i.e., < 10 day) nonvascular plants at 1 µg/L and the USEPA Concentration Equivalent Level of Concern (CE-LOC) at 10 µg/L, respectively. The blue long-dashed line indicated the Canadian water quality guidelines for the protection of aquatic life (chronic) from CCME on atrazine at 1.8 µg/L.

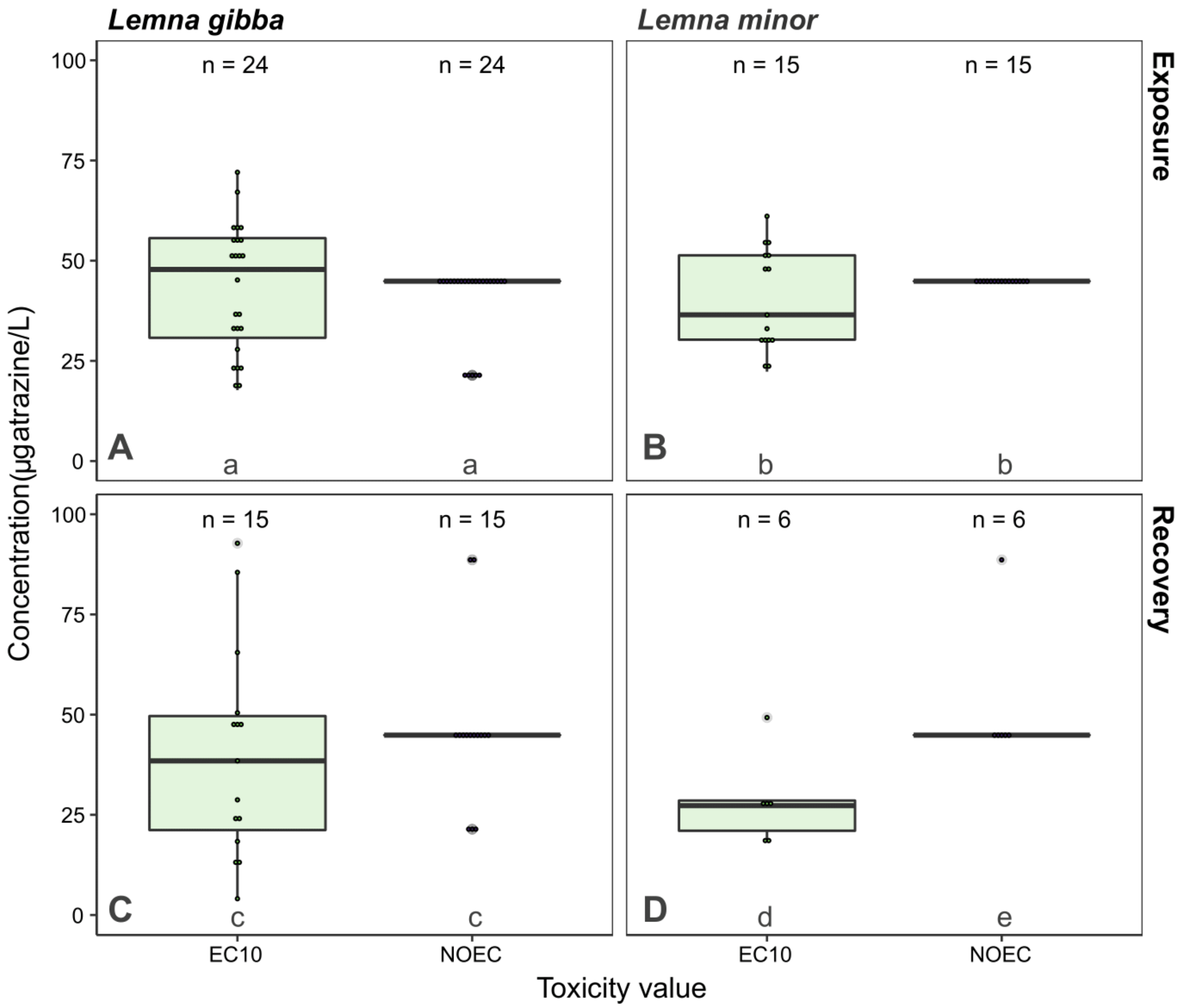

Figure 3.6. The boxplot with EC10 and NOEC for dry mass, frond number and plant number on *Lemna gibba* and *Lemna minor* after 7, 14, 21 and 28 days of atrazine exposure (A for *Lemna gibba*, B for *Lemna minor*) and recovery (C for *Lemna gibba*, D for *Lemna minor*) period. NOEC is the highest concentration that is not statistically significant from the control. Note that toxicity values (i.e. EC10 and NOEC) of endpoints with NOEC that were significantly higher than the control and where no EC10 values were calculated due to insufficient response are not included. The significant differences were determined by paired t-tests ($\alpha = 0.05$) for parametric data between the EC10 and the NOEC for *L. gibba* and *L. minor* after exposure and recovery, and are indicated by a lower-case letter (e.g., not significantly different were represented by the same letter) located under the boxplot.

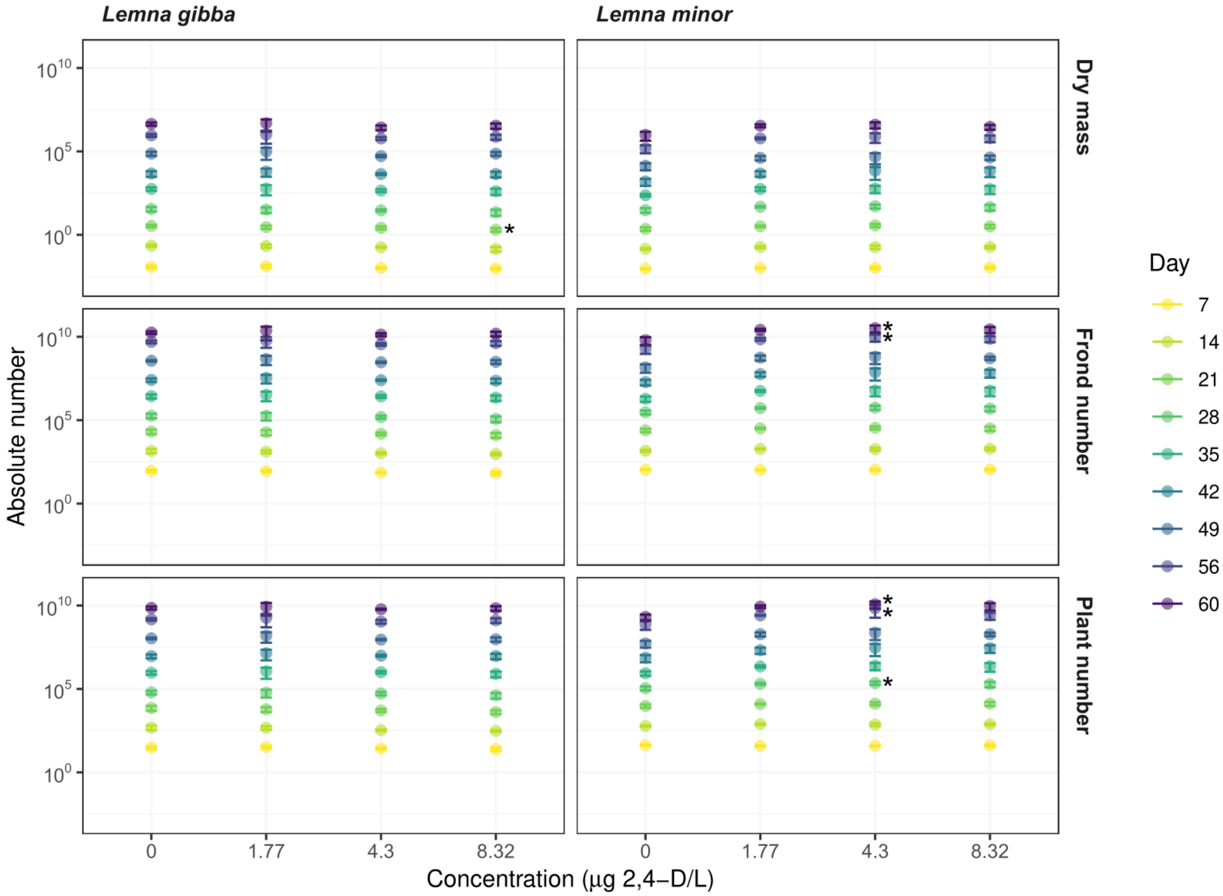

Figure 3.7. Mean *L. gibba* and *L. minor* responses (dry mass, frond number and plant number) after 60-day of continuous 2,4-D exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from the control are indicated with an asterisk and were determined with one-way ANOVA with Dunnett's test (α = 0.05) for parametric data, and a Kruskal-Wallis test by rank with Wilcoxon Test (α = 0.05) for non-parametric data. The absolute number for endpoints was calculated based on the values determined every 7 days.

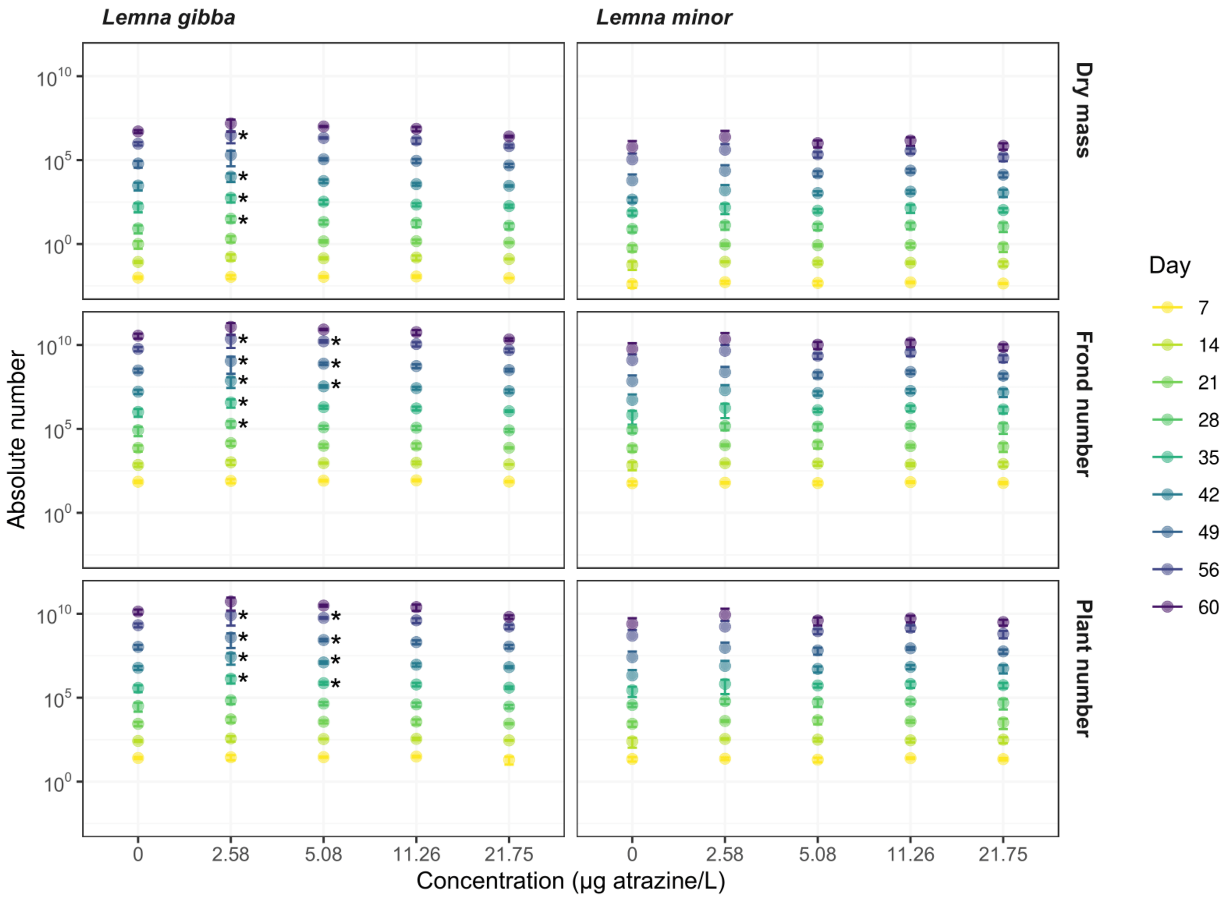

Figure 3.8. Mean *L. gibba* and *L. minor* responses (dry mass, frond number and plant number) after 60-day of continuous atrazine exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from the control are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05) for parametric data, and a Kruskal-Wallis test by rank with Wilcoxon Test (α = 0.05) for non-parametric data. The absolute number for endpoints was calculated based on the values determined every 7 days.

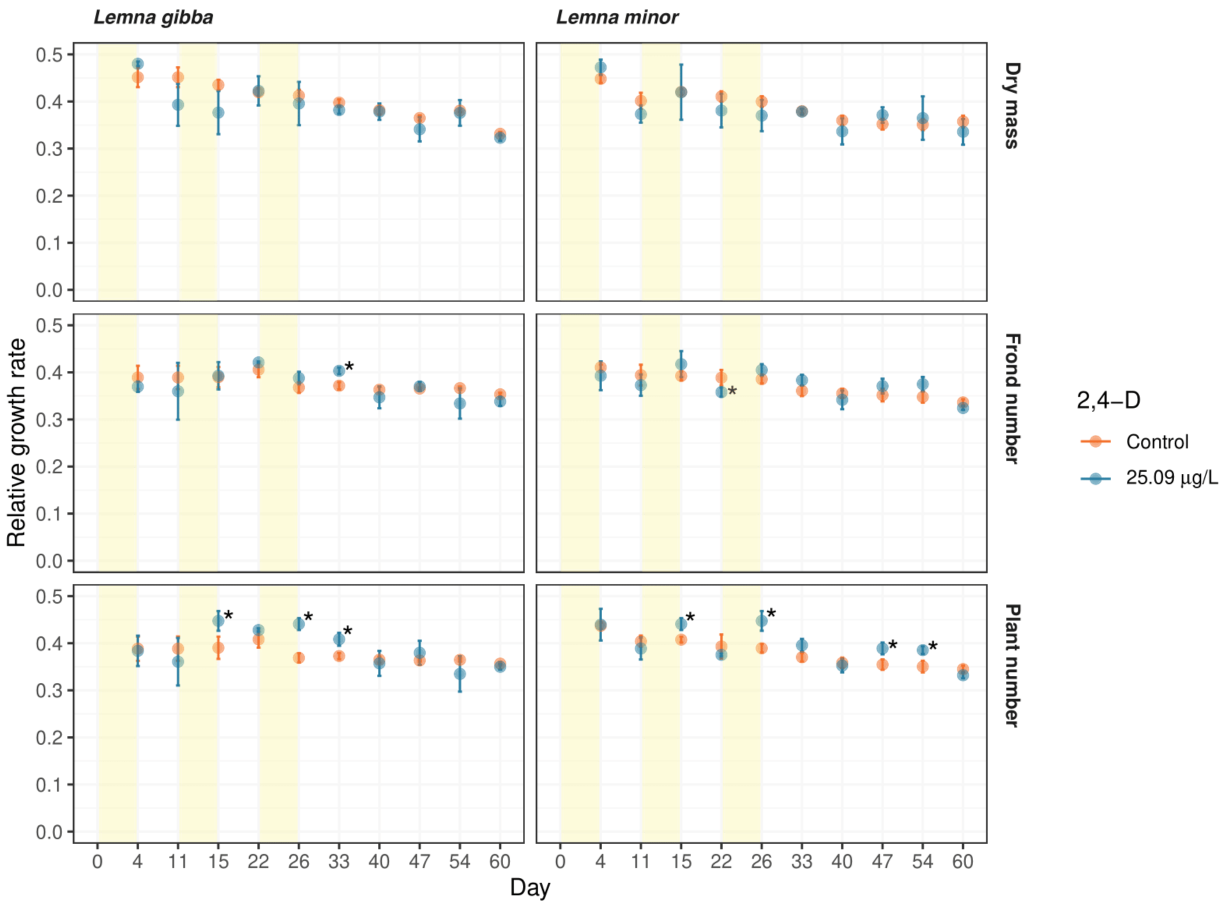

Figure 3.9. Mean relative growth rate (RGR) for *L. gibba* and *L. minor* endpoints (dry mass, frond number and plant number) after three 4-day pulses of 2,4-D with total test duration of 60 days. The pulse periods are highlighted in yellow. Error bars are the standard deviation (n = 3). Statistically significant differences from the control are indicated with an asterisk and were determined with a Student's t-test (α = 0.05) for parametric data and a Wilcoxon test (α = 0.05) for non-parametric data.

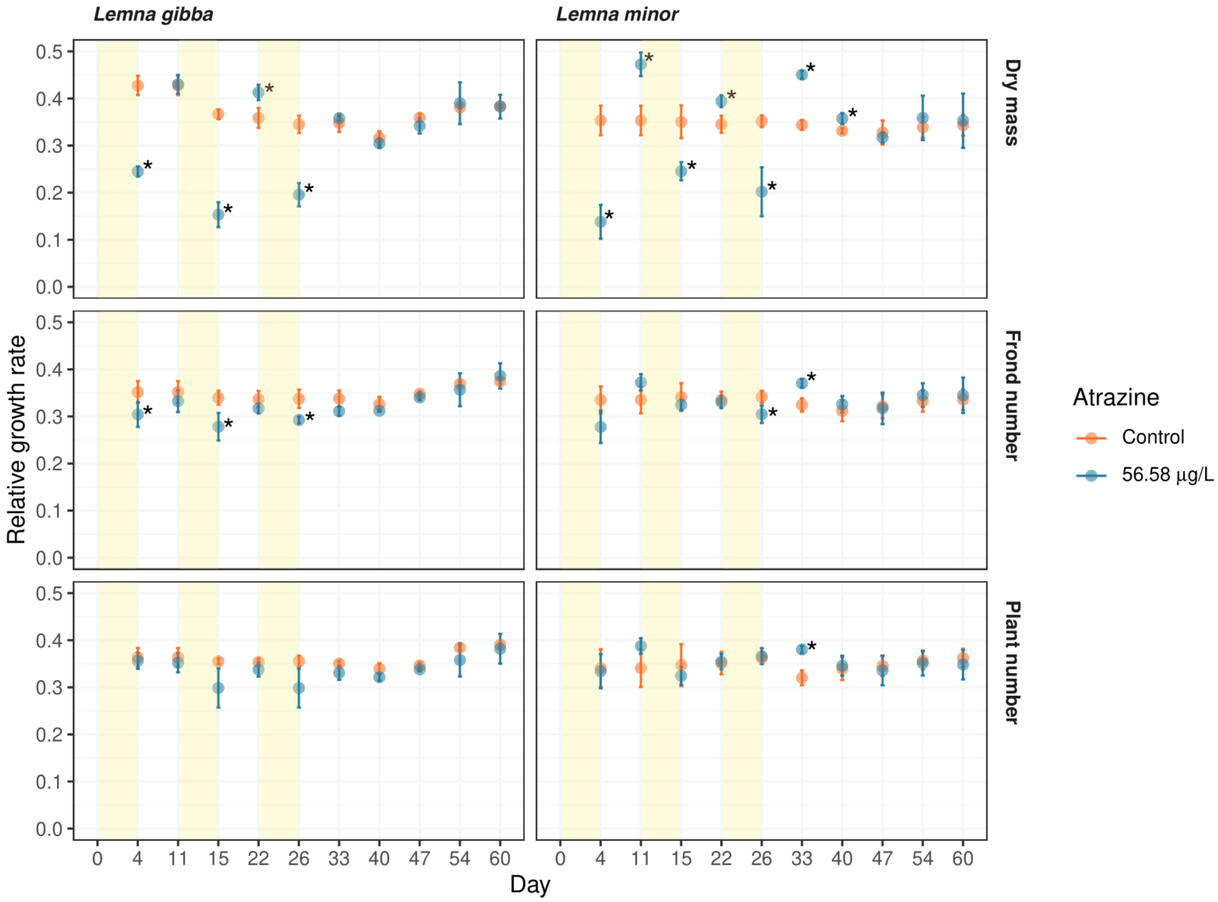

Figure 3.10. Mean relative growth rate (RGR) for *L. gibba* and *L. minor* endpoints (dry mass, frond number and plant number) after three 4-day pulses of atrazine with total test duration of 60 days. The pulse periods are highlighted in yellow. Error bars are the standard deviation (n = 3). Statistically significant differences from the control are indicated with an asterisk and were determined with a Student's t-test (α = 0.05) for parametric data and a Wilcoxon test (α = 0.05) for non-parametric data.

Chapter 4 Synthesis and Conclusions

This thesis examined the potential for aquatic macrophyte recovery following herbicide exposure under laboratory conditions. The predominantly pulsed nature of herbicide exposure in freshwater has been shown to allow for recovery of aquatic primary producers between exposure events (Brain et al., 2012). However, recovery is seldom considered in ecological risk assessment. This is due, in part, to insufficient standard protocols on recovery and guidelines that consider recovery into the decision-making process (Brain et al., 2012). As a result, we investigated the reliability and relevance of peer-reviewed recovery studies of aquatic primary producers (macrophytes, periphyton and phytoplankton) after herbicide exposure and we identified knowledge gaps in the published studies., The recovery potential of two duckweed species (*Lemna gibba* and *Lemna minor*) after exposure to 2,4-D and atrazine (herbicides with different modes of action) was determined. The toxicity after 60 days of both continuous and pulsed exposure at the regulation concentrations of 2,4-D and atrazine was also investigated. Together, these contributions helped us to recognize that aquatic primary producers have the potential to recover after an exposure. This observation has been made in many of the recovery studies that were reviewed as part of our literature review and is supported by our own recovery testing. The literature review revealed that more relevant and reliable studies are required for risk assessment and the criteria obtained through our review could provide a guideline for future studies. We recommend conducting mesocosm studies to validate the recovery potential in a field environment and the development of guidelines for such studies. The pulsed exposure of herbicide in surface water allows primary producers to recover between the pulses As a result, recovery should be included in risk assessment to have a more realistic assessment on the impact of the ecosystem.

4.1 Summary of main findings

4.1.1 Literature review on recovery studies

Our critical review found a total of 41 test species, 42 herbicides and 64 endpoints reported in 40 peer-reviewed recovery studies of aquatic primary producers (macrophyte, periphyton and phytoplankton) following an herbicide exposure. The number of individual experiments with unique test species and herbicides for macrophytes, periphyton and phytoplankton were 76, 29 and 33, respectively. We found that the percentages of studies reporting recovery were 80% for macrophyte and phytoplankton and 60% for periphyton. This shows that recovery of aquatic primary producers is consistently observed.

The majority of previous recovery studies on macrophytes, periphyton and phytoplankton were related to photosynthesis II inhibitors. The mean of exposure and recovery duration were 18 and 13 days for macrophyte, 3 and 2 days for periphyton, and 5 and 5 days for phytoplankton, respectively. This indicated to us that more herbicide groups need to be included in recovery studies to understand if recovery potential of aquatic plants after exposure is consistent across herbicides with different mechanisms of inhibition. Also, the exposure and recovery duration could be greater (e.g., > 7 days) for periphyton and phytoplankton as it could take up to 15 days for an herbicide's concentration in freshwater to return to normal levels (Andrus et al., 2013).

The quality of studies is also important for decision makers to consider. To assess quality our team assigned strength of method and endpoint relevance scores to each study using a set of criteria referenced from Hanson et al., (2019). The score was based on the test information provided including test substance, organisms, system, design, statistics, and results. The percentage of exposure and the recovery studies that were considered sufficient in quality for risk assessment (i.e., > 7.5 of strength of method score and > 2.5 of total endpoint ecological relevance score) were low; 30% from exposure and 35% from recovery. A total of two of the individual studies received full marks on strength of method score with high endpoint relevance scores. This indicates that high quality studies do exist and could be considered in ecological risk assessment. However, the low percentage of quality studies could reduce the quantity of recovery data being considered during ecological risk assessment.

4.1.2 Recovery testing of duckweed on 2,4-D and atrazine exposure

Recovery of *Lemna gibba* and *Lemna minor* after exposure to 2,4-D and atrazine for 7, 14, 21 and 28 days in the laboratory was investigated. The EC50 and NOEC values from the recovery period were ≥ 558 μg/L and ≥ 117 μg/L for 2,4-D, and ≥ 271 μg/L and ≥ 89 μg/L for atrazine. The relative growth rate of dry mass on *Lemna gibba* and all RGR endpoints on *Lemna minor* were recovered at the end of recovery period after 7, 14, 21 and 28 days of exposure. The results indicate that duckweed has the potential to recover after atrazine exposure at concentrations greater than the regulations from the CCME and the USEPA. Also, recovery after atrazine exposure was not associated with the exposure duration. In the case of 2,4-D the results showed that exposure has no adverse effect to either test species. The lack of adverse effects on duckweed (a monocot) after 2,4-D exposure is linked to the mode of action as 2,4-D is a synthetic auxin that targets eudicots (Song, 2014). Finally, we compared the EC10 and EC20 values to NOECs from the exposure and recovery testing. The results indicated that EC10s were not statistically different from or consistently more sensitive than NOECs. Therefore EC10 or NOECs could be used as an estimate of low toxicity in ecological risk assessment.

In addition, we addressed the toxicity of 2,4-D and atrazine on duckweed after 60-day continuous and pulsed (i.e., three 4-day pulses) exposures. The results showed that the current regulations on 2,4-D and atrazine concentrations in freshwater from the CCME and the USEPA could protect aquatic macrophytes from experiencing adverse effects. Testing showed that *Lemna* species had the ability to recover from exposure so including the recovery component in risk assessment would be beneficial to better understanding the duration of toxicity and could help create regulations that are more realistic.

We assessed the reliability and relevance of our 7-, 14-, 21- and 28-day recovery laboratory studies using the set of criteria outlined in Chapter 2. The strength of method scores were determined to be 14 for 2,4-D and atrazine. A score of one was given in all criteria, except for criterion 14 (reporting the raw values). The raw values of the absolute endpoints (dry mass, frond number and plant number) were not provided, but the majority of the other data were. To determine relevance of the study, absolute frond number, absolute plant number and all RGR endpoints received a score of 4 (i.e., reproduction). The absolute dry mass was given a score of 3 (i.e., growth), and light- and dark-adapted PSII yield were given a score of 2 (i.e., physiology). The strength of method and relevance scores showed that our recovery testing received >50% of strength of method scores for 2,4-D and atrazine and >50% of relevance of endpoint score for the majority of endpoints with the exception of the light- and dark-adapted PSII yield. We conclude that our recovery laboratory study met the thresholds to be considered for risk assessment.

4.2 Improvements and recommendations

4.2.1 Literature review on recovery studies

We were able to use a set of transparent and robust criteria to identify the quality of recovery studies of aquatic primary producers after herbicide exposure for ecological risk assessment. Efforts were made to ensure that studies were scored consistently by using detailed scoring guidelines for each criterion; however, some changes could be considered to improve the review. First, a score of 1 was given when a study reported the range of temperature and light intensity for Criterion 10. Reporting the temperature and light intensity range may be insufficient to represent the variation in test temperature or light intensity that can occur when a growth chamber has a light bulb burnout or experiences some type of malfunction or power outage. (Hanson et al., 2019). The variation of temperature and/or light intensity overtime should be reported so results are more reliable and reproducible. Secondly, a criterion could be added to reflect the health condition of test species that were collected in the field. Test organisms obtained from the field, as opposed to laboratory specimens, may have been pre-exposed to other chemicals that could affect the toxicity of the test chemical (Hanson et al., 2019). Additional criteria to be considered would be providing a description of the culturing of the test species after collection (e.g., steps to clean the test species to make it axenic) to ensure the specimen was axenic and healthy. Overall, the criteria used in this literature review were suitable to evaluate the strength and relevance of endpoints for the recovery studies that were reviewed. In addition to herbicide exposure, the criteria used in this study could also be used to determine the reliability and relevance of studies that investigate recovery after exposure to other chemicals including pharmaceuticals, oil-spill related products and other organic and inorganic contaminants. In this case some criteria may need to be adjusted. For example, Criterion 6 (ecological relevance) would need to be modified based on the environmental concentration for the target chemicals, as other contaminants may have a different environmental concentration than an herbicide in an aquatic environment.

Our review concluded that the evidence of primary producer recovery, after an herbicide exposure, is strong. However, due to the fact that some studies did not provide sufficient detail, doubts remain. Ideally, the same toxicity value (i.e., NOEC or ECX from both exposure and recovery periods and the same exposure duration should be reported when evaluating recovery potential. We found that studies reporting toxicity values from exposure and recovery had different exposure durations. Also, some studies presented results in graphical format without providing the toxicity values which required interpolating to determine the recovery potential. Finally, there were studies that reported ECX from the exposure period but NOEC from the recovery period. Inconsistencies in reported toxicity values may lead to uncertainties in integrating data for ecological risk assessment. As a result, we recommend developing a standardized test protocol (i.e., transferring the test species into clean media) and endpoints (i.e., relative growth rate) to increase the quality of future studies. Also, guidelines should be developed for decision makers to integrate qualified recovery results into risk assessments. Additionally, guidelines should be developed for researchers so that there is a clear understanding about what information should be reported.

4.2.2 Recovery studies

The recovery potential of duckweed after 7, 14, 21 and 28 days of atrazine exposure was determined, but the recovery potential after 2,4-D exposure remains uncertain due to lack of response during the exposure period. As a result, we recommend increasing the test concentrations or switching the test species from monocot (e.g., *Lemna minor*) to eudicot (e.g., *Myriophyllum spicatum*) given that eudicots are more sensitive to the toxicity of 2,4-D. This recent recovery study was performed in a controlled laboratory environment and the duckweed was tested in a nutrient-rich environment with optimum light and temperature. Optimum conditions may encourage recovery. It is rare for aquatic plants in the field to grow in a nutrient-rich environment and the recovery potential could be different under sub-optimal temperature and light conditions, recognizing that there is also additional pressure from predators (Barnthouse, 2004). Therefore, we suggest performing mesocosm recovery studies with duckweed to validate the recovery potential under more realistic field conditions. We encourage field testing with test organisms at different trophic levels (e.g., phytoplankton, zooplankton and fish) to evaluate the recovery potential at the community or population level. A field study could be performed during the spring and summer seasons because that is when herbicides are applied and higher concentrations have been observed in aquatic environments after application (Andrus et al., 2013; Challis, 2018).

Finally, only one test concentration at the regulation level (e.g., 25.1 μg/L for 2,4-D and 56.6 μg/L for atrazine) was tested in the 60-day pulse concentration for each herbicide. Previous studies have reported that the atrazine concentration may be as high as 70.53 μg/L which is higher than the tested concentration (Andrus et al., 2013). Higher pulse concentrations (e.g., 70 μg/L or higher) could be tested to further investigate the recovery potential of duckweed after repeated exposures at a higher concentration.

4.3 Conclusion

Our critical review as able to identify the reliability and relevance of recovery studies for possible inclusion in ecological risk assessment. We encourage researchers to refer the strength of methods and relevance criteria presented in this paper to ensure that the reported data allow for a full assessment of reliability and ecological relevance for risk assessment. To further increase the reliability and relevance of recovery studies, a standard protocol should be created for test procedures, endpoint (e.g., relative growth rate) and reported toxicity values to provide more data for decision makers to consider. Our laboratory recovery tests found that duckweed is not likely to be affected adversely at current regulations for atrazine or 2,4-D. Recovery after herbicide exposure was observed consistently in the recovery studies and in our recovery testing after atrazine exposure. With pulsed exposure in the environment and recovery potential, it is recommended that the inclusion of recovery data at higher tiers of risk assessment be considered.

4.4 References

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Appendix A: Literature Review

Tables

Table A1. List of excluded recovery studies from the critical review.

Paper	Reference	Study title	Reason for exclusion
1	Cunningham et al.,1984	Temporal responses of the macrophyte, *Potamogeton perfoliatus* L., and its associated autotrophic community to atrazine exposure in Estuarine microcosms	No recovery phase. The test species were not transferred to clean media.
2	Gustavson et al., 2003	Effects of exposure duration of herbicides on natural stream periphyton communities and recovery	The other three tested herbicides (pendimethalin, isoproturon, and hexazinone) were excluded due to lack of recovery phase.
3	Halstead et al., 2014	Community ecology theory predicts the effects of agrochemical mixtures on aquatic biodiversity and ecosystem properties	No recovery phase. The test species were not transferred to clean media.
3	Hamilton et al., 1988	The impact of two applications of atrazine on the plankton communities of in situ enclosures	No recovery phase. The test species were not transferred to clean media.
3	Kemp et al., 1985	Effects of atrazine and linuron on photosynthesis and growth of the macrophytes, *Potamogeton perfoliatus* L. and *Myriophyllum spicatum* L., in an estuarine environment	No recovery phase. The test species were not transferred to clean media.
4	Knauer and Hommen, 2012	Sensitivity, variability, and recovery of functional and structural endpoints of an aquatic community exposed to herbicides	No recovery phase. The test species were not transferred to clean media.
5	Mohr et al., 2007	Effects of the herbicide metazachlor on macrophytes and ecosystem function in freshwater pond and stream mesocosms	No recovery phase. The test species were not transferred to clean media.
6	Wellmann et al., 1998	Primary and secondary effects of methabenzthiazuron on plankton communities in aquatic outdoor microcosms	No recovery phase. The test species were not transferred to clean media.
7	Wood et al., 2017	Chronic effects of atrazine exposure and recovery in freshwater benthic diatoms from two communities with different pollution histories	No recovery phase. The test species were not transferred to clean media.
8	Sobrero et al., 2007	Effects of the glyphosate active ingredient and a formulation on *Lemna gibba* L. at different exposure levels and assessment end-points	No recovery phase. The test species were not transferred to clean media.
9	Takahashi et al., 2007	Impact of pretilachlor herbicide and pyridaphenthion insecticide on aquatic organisms in model streams	No recovery phase. The test species were not transferred to clean media.

Table A2. The evidence for recovery of aquatic primary producers (macrophyte, periphyton and phytoplankton) from the reviewed recovery studies.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
Baxter et al., 2013	Sensitivity of a green alga to atrazine is not enhanced by previous acute exposure	Atrazine	*Pseudokirchneriella subcapitata*	Phytoplankton	Mean growth rate	Reproduction	1	1	Y	300	Y
			Pseudokirchneriella subcapitata	Phytoplankton	Mean growth rate	Reproduction	1	2	Y	300	Y
			Pseudokirchneriella subcapitata	Phytoplankton	Mean growth rate	Reproduction	1	3	Y	300	Y
			Pseudokirchneriella subcapitata	Phytoplankton	Mean PSII quantum yield	Physiological (Chlorophyll)	1	1	Y	300	Y
			Pseudokirchneriella subcapitata	Phytoplankton	Mean PSII quantum yield	Physiological (Chlorophyll)	1	2	Y	300	Y
Baxter et al., 2014	Response of the green alga *Oophila sp.*, a salamander endosymbiont, to a PSII-inhibitor under laboratory conditions.	Atrazine	*Oophila* sp.	Phytoplankton	Growth rate (Test 1)	Reproduction	4	4	Y	300	Y
			Oophila sp.	Phytoplankton	Growth rate (Test 2)	Reproduction	4	4	Y	300	Y
			Oophila sp.	Phytoplankton	Optical density (Test 1)	Reproduction	4	4	Y	100	Y
			Oophila sp.	Phytoplankton	Optical density (Test 2)	Reproduction	4	4	Y	300	Y
			Oophila sp.	Phytoplankton	PSII yield (Test 1)	Reproduction	4	4	N	<3	N
			Oophila sp.	Phytoplankton	PSII yield (Test 2)	Reproduction	4	4	Y	300	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
Belgers et al., 2011	Can time-weighted average concentrations be used to assess the risks of metsulfuron-methyl to *Myriophyllum spicatum* under different time-variable exposure regimes?	Metsulfuron-methyl	*Myriophyllum spicatum*	Macrophyte	Total plant dry weight	Growth and Development	1	41	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight main shoot	Growth and Development	1	41	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight new shoots	Growth and Development	1	41	NA (Not information on exposure NOEC)	0.1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight new roots	Growth and Development	1	41	NA (Not information on exposure NOEC)	0.01	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Total length new shoots	Growth and Development	1	41	Y	<0.0001* (recovered at higher conc.0.001, 0.01, 0.1)	Y (recovered at higher conc.0.001, 0.01, 0.1)
			Myriophyllum spicatum	Macrophyte	Total length new roots	Growth and Development	1	41	Y	<0.0001* (recovered at higher conc.0.001, 0.01)	Y (recovered at higher conc.0.001, 0.01, 0.1)
			Myriophyllum spicatum	Macrophyte	Total plant dry weight	Growth and Development	3	39	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight main shoot	Growth and Development	3	39	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight new shoots	Growth and Development	3	39	NA (Not information on exposure NOEC)	0.1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight new roots	Growth and Development	3	39	NA (Not information on exposure NOEC)	0.1	NA (Not information on exposure NOEC)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Myriophyllum spicatum	Macrophyte	Total length new shoots	Growth and Development	3	39	Y	0.1	Y
			Myriophyllum spicatum	Macrophyte	Total length new roots	Growth and Development	3	39	NA (Not information on exposure NOEC)	0.01	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Total plant dry weight	Growth and Development	7	35	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight main shoot	Growth and Development	7	35	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight new shoots	Growth and Development	7	35	NA (Not information on exposure NOEC)	0.1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight new roots	Growth and Development	7	35	NA (Not information on exposure NOEC)	0.1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Total length new shoots	Growth and Development	7	35	Y	< 0.0001* (recovered at higher conc.0.001, 0.01, 0.1)	Y (recovered at higher conc.0.001, 0.01
			Myriophyllum spicatum	Macrophyte	Total length new roots	Growth and Development	7	35	NA (Not information on exposure NOEC)	< 0.0001*	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Total plant dry weight	Growth and Development	14	28	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight main shoot	Growth and Development	14	28	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Myriophyllum spicatum	Macrophyte	Dry weight new shoots	Growth and Development	14	28	NA (Not information on exposure NOEC)	0.01	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight new roots	Growth and Development	14	28	NA (Not information on exposure NOEC)	0.01	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Total length new shoots	Growth and Development	14	28	Y	0.01	Y
			Myriophyllum spicatum	Macrophyte	Total length new roots	Growth and Development	14	28	Y	0.0001 (recovered at 0.01)	Y
			Myriophyllum spicatum	Macrophyte	Total plant dry weight	Growth and Development	21	21	NA (Not information on exposure NOEC)	0.01	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight main shoot	Growth and Development	21	21	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight new shoots	Growth and Development	21	21	NA (Not information on exposure NOEC)	0.01	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Dry weight new roots	Growth and Development	21	21	NA (Not information on exposure NOEC)	1	NA (Not information on exposure NOEC)
			Myriophyllum spicatum	Macrophyte	Total length new shoots	Growth and Development	21	21	N	< 0.0001* (recovered at 0.001, 0.01)	N
			Myriophyllum spicatum	Macrophyte	Total length new roots	Growth and Development	21	21	NA (No information on exposure NOEC)	<0.0001	NA (No information on exposure NOEC)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
Boxall et al., 2013	Effects of repeated pulsed herbicide exposures on the growth of aquatic macrophytes	Isoproturon	*Lemna minor*	Macrophyte	Growth rate	Reproduction	24	18	Y	25	NA (No information on exposure NOEC)
			Lemna minor	Macrophyte	Growth rate	Reproduction	12	30	Y	50	NA (No information on exposure NOEC)
		Metsulfuron-methyl	*Lemna minor*	Macrophyte	Growth rate	Reproduction	24	18	Y	0.25	NA (No information on exposure NOEC)
			Lemna minor	Macrophyte	Growth rate	Reproduction	12	30	Y	0.25	NA (No information on exposure NOEC)
		Pentachlorophenol	*Lemna minor*	Macrophyte	Growth rate	Reproduction	24	18	Y	500	NA (No information on exposure NOEC)
			Lemna minor	Macrophyte	Growth rate	Reproduction	12	30	N	100	NA (No information on exposure NOEC)
Brain et al., 2012	Recovery of photosynthesis and growth rate in green, blue -green and diatom algae after exposure to atrazine	Atrazine	*Pseudokirchneriella subcapitata* (green algae)	Phytoplankton	PSII effective quantum yield	Physiological (Chlorophyll)	2	2	Y	232	Y
			Pseudokirchneriella subcapitata (green algae)	Phytoplankton	Average growth rate	Reproduction	2	2	Y	232	Y
			Pseudokirchneriella subcapitata (green algae)	Phytoplankton	Cell Density	Reproduction	2	2	N	<5.06	N
			Anabaena flos-aquae (blue-green algae)	Phytoplankton	PSII effective quantum yield	Physiological (Chlorophyll)	2	2	Y	887	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Anabaena flos-aquae (blue-green algae)	Phytoplankton	Average growth rate	Reproduction	2	2	Y	887	Y
			Anabaena flos-aquae (blue-green algae)	Phytoplankton	Cell Density	Reproduction	2	2	Y	230	Y
			Navicula pelliculosa (diatom)	Periphton	PSII effective quantum yield	Physiological (Chlorophyll)	2	2	Y	237	Y
			Navicula pelliculosa (diatom)	Periphton	Average growth rate	Reproduction	2	2	Y	237	Y
			Navicula pelliculosa (diatom)	Periphton	Cell Density	Reproduction	2	2	Y	237	Y
Brain et al., 2012	Recovery of duckweed from time-varying exposure to atrazine	Atrazine	*Lemna gibba*	Macrophyte	Frond number	Reproduction	1	7	NA	146 (Lack of responses from exposure)	NA
			Lemna gibba	Macrophyte	Frond number	Reproduction	3	7	Y	127	Y
			Lemna gibba	Macrophyte	Frond number	Reproduction	5	7	N	34	N
			Lemna gibba	Macrophyte	Frond number	Reproduction	5	14	N	18	N
			Lemna gibba	Macrophyte	Frond number	Reproduction	7	7	Y	<4.7* (recovered at 9.4, 19)	N/Y (recovered at 9.4, 19)
			Lemna gibba	Macrophyte	Frond number	Reproduction	7	14	N	38 (Same as exposure NOEC)	NA
			Lemna gibba	Macrophyte	Frond number	Reproduction	9	7	Y	77	Y
			Lemna gibba	Macrophyte	Frond number	Reproduction	14	7	Y	75	Y
			Lemna gibba	Macrophyte	Frond number	Reproduction	14	14	Y	75	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Lemna gibba	Macrophyte	Average growth rate	Reproduction	1	7	N	72	N
			Lemna gibba	Macrophyte	Average growth rate	Reproduction	3	7	Y	127	Y
			Lemna gibba	Macrophyte	Average growth rate	Reproduction	5	7	Y	137	Y
			Lemna gibba	Macrophyte	Average growth rate	Reproduction	5	14	Y	18* (Recovered at 68)	Y
			Lemna gibba	Macrophyte	Average growth rate	Reproduction	7	7	Y	157	Y
			Lemna gibba	Macrophyte	Average growth rate	Reproduction	7	14	Y	157	Y
			Lemna gibba	Macrophyte	Average growth rate	Reproduction	9	7	Y	77	Y
			Lemna gibba	Macrophyte	Average growth rate	Reproduction	14	7	Y	75	Y
			Lemna gibba	Macrophyte	Average growth rate	Reproduction	14	14	Y	4.4*(Recovered at 18, 37, 75)	Y
			Lemna gibba	Macrophyte	Biomass	Growth and Development	1	7	NA	38* (Recovered at 146)	NA
			Lemna gibba	Macrophyte	Biomass	Growth and Development	3	7	Y	127	Y
			Lemna gibba	Macrophyte	Biomass	Growth and Development	5	7	NA	NA	NA
			Lemna gibba	Macrophyte	Biomass	Growth and Development	5	14	Y	137	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Lemna gibba	Macrophyte	Biomass	Growth and Development	7	7	NA	NA	NA
			Lemna gibba	Macrophyte	Biomass	Growth and Development	7	14	N	38 (same as exposure NOEC)	NA
			Lemna gibba	Macrophyte	Biomass	Growth and Development	9	7	Y	77	Y
			Lemna gibba	Macrophyte	Biomass	Growth and Development	14	7	Y	75	Y
			Lemna gibba	Macrophyte	Biomass	Growth and Development	14	7	Y	75	Y
Burns et al., 2015	Growth recovery of *Lemna gibba* and *Lemna minor* following a 7-Day Exposure to the Herbicide Diuron	Diuron	*Lemna minor*	Macrophyte	Plant number	Reproduction	7	7	Y	111	Y
			Lemna minor	Macrophyte	Frond number	Reproduction	7	7	Y	60	Y
			Lemna minor	Macrophyte	Fresh weight	Growth and Development	7	7	Y	111	Y
			Lemna minor	Macrophyte	Dry weight	Growth and Development	7	7	Y	111	Y
			Lemna gibba	Macrophyte	Plant number	Reproduction	7	7	Y	111	Y
			Lemna gibba	Macrophyte	Frond number	Reproduction	7	7	Y	208	Y
			Lemna gibba	Macrophyte	Fresh weight	Growth and Development	7	7	Y	60	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Lemna gibba	Macrophyte	Dry weight	Growth and Development	7	7	Y	60	Y
			Lemna minor	Macrophyte	Plant number (RGR)	Reproduction	7	7	Y	111	Y
			Lemna minor	Macrophyte	Frond number (RGR)	Reproduction	7	7	Y	60	Y
			Lemna minor	Macrophyte	Fresh weight (RGR)	Growth and Development	7	7	Y	111	Y
			Lemna minor	Macrophyte	Dry weight (RGR)	Growth and Development	7	7	Y	60	Y
			Lemna gibba	Macrophyte	Plant number (RGR)	Reproduction	7	7	Y	111	Y
			Lemna gibba	Macrophyte	Frond number (RGR)	Reproduction	7	7	Y	≥208	Y
			Lemna gibba	Macrophyte	Fresh weight (RGR)	Growth and Development	7	7	Y	208	Y
			Lemna gibba	Macrophyte	Dry weight (RGR)	Growth and Development	7	7	Y	111	Y
Cedergreen et al., 2005	Does the effect of herbicide pulse exposure on aquatic plants depend on K-ow or mode of action?	Diquat	*Lemna minor*	Macrophyte	Chlorophyll fluorescence parameter	Physiological (Chlorophyll)	0.125	6.875	NA	NA (Lack of response during exposure)	NA

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
		Terbuthylazine	*Lemna minor*	Macrophyte	Chlorophyll fluorescence parameter	Physiological (Chlorophyll)	0.125	6.875	Y	4000	NA (No information on exposure NOEC)
			Lemna minor	Macrophyte	Frond area (RGR)	Reproduction	0.125	6.875	NA	4000 (Lack of response during exposure)	NA (Didn't compared with control)
		Imazamox	*Lemna minor*	Macrophyte	Frond area (RGR)	Reproduction	0.125	6.875	Y	1000	NA (Didn't compared with control)
		Metsulfuron-methyl	*Lemna minor*	Macrophyte	Frond area (RGR)	Reproduction	0.125	6.875	Y	25	NA (Didn't compared with control)
		Propyzamide	*Lemna minor*	Macrophyte	Frond area (RGR)	Reproduction	0.125	6.875	Y	10000	NA (Didn't compared with control)
		Pendimethalin	*Lemna minor*	Macrophyte	Frond area (RGR)	Reproduction	0.125	6.875	Y	5001	NA (Didn't compared with control)
Copin et al., 2016	Modeling the effect of exposing algae to pulses of S-metolachlor: How to include a delay to the onset of the effect and in the recovery	S-metolachlor	*Scenedesmus vacuolatus*	Phytoplankton	Size of algae cells (growth rate)	Reproduction	1.042	0.833	N	<3000	NA (No information on exposure NOEC)
			Scenedesmus vacuolatus	Phytoplankton	Size of algae cells (growth rate)	Reproduction	1.25	0.833	N	<3000	NA (No information on exposure NOEC)
			Scenedesmus vacuolatus	Phytoplankton	Size of algae cells (growth rate)	Reproduction	2	0.833	N	<525	NA (No information on exposure NOEC)
			Scenedesmus vacuolatus	Phytoplankton	Size of algae cells (growth rate)	Reproduction	1.042	2	Y	3000	(No information on exposure NOEC)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Scenedesmus vacuolatus	Phytoplankton	Size of algae cells (growth rate)	Reproduction	1.25	2	Y	3000	NA (No information on exposure NOEC)
			Scenedesmus vacuolatus	Phytoplankton	Size of algae cells (growth rate)	Reproduction	2	2	Y	24500	NA (No information on exposure NOEC)
Debenest et al., 2009	Sensitivity of freshwater periphytic diatoms to agricultural herbicides	Isoproturon	Benthic diatom	Periphyton	Biomass	Growth and Development	3	3	N	<30	N
			Benthic diatom	Periphyton	Chlorophyll a concentration	Physiological (Chlorophyll)	3	3	N	<30	N
			Benthic diatom	Periphyton	Chlorophyll c concentration	Physiological (Chlorophyll)	3	3	N	<30	N
			Benthic diatom	Periphyton	Viable cell density	Growth and Development	3	3	N	<30	N
			Melosira varians	Phytoplankton	Abundance	Growth and Development	3	3	N	30	NA (not enough toxicity values from exposure)
			Nitzschia dissipata	Phytoplankton	Abundance	Growth and Development	3	3	N	30	NA (not enough toxicity values from exposure)
			Eolimna minima	Phytoplankton	Abundance	Growth and Development	3	3	N	30	NA (not enough toxicity values from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Sensitive autotroph	Periphyton	Abundance	Growth and Development	3	3	NA (not enough toxicity values from exposure)	30	NA (not enough toxicity values from exposure)
			Tolerant autotroph	Periphyton	Abundance	Growth and Development	3	3	NA (not enough toxicity values from exposure)	30	NA (not enough toxicity values from exposure)
			Facultative heterotroph	Periphyton	Abundance	Growth and Development	3	3	NA (not enough toxicity values from exposure)	30	NA (not enough toxicity values from exposure)
			Obligate heterotroph	Periphyton	Abundance	Growth and Development	3	3	NA (not enough toxicity values from exposure)	30	NA (not enough toxicity values from exposure)
		S-metolachlor	Benthic diatom	Periphyton	Chlorophyll a concentration	Physiological (Chlorophyll)	3	3	Y (Mentioned recovery on algal biomass and diatoms)	30	NA (not enough toxicity values from exposure)
			Benthic diatom	Periphyton	Chlorophyll c concentration	Physiological (Chlorophyll)	3	3	Y (Mentioned recovery on algal biomass and diatoms)	30	NA (not enough toxicity values from exposure)
			Benthic diatom	Periphyton	Viable cell density	Growth and Development	3	3	Y (Mentioned recovery on algal biomass and diatoms)	30	NA (not enough toxicity values from exposure)
			Melosira varians	Phytoplankton	Abundance	Growth and Development	3	3	Y (Mentioned recovery on algal biomass and diatoms)	30	NA (not enough toxicity values from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Nitzschia dissipata	Phytoplankton	Abundance	Growth and Development	3	3	Y (Mentioned recovery on algal biomass and diatoms)	30	NA (not enough toxicity values from exposure)
			Eolimna minima	Phytoplankton	Abundance	Growth and Development	3	3	Y (Mentioned recovery on algal biomass and diatoms)	30	NA (not enough toxicity values from exposure)
			Sensitive autotroph	Periphyton	Abundance	Growth and Development	3	3	NA (not enough toxicity values from exposure)	30	NA (not enough toxicity values from exposure)
			Tolerant autotroph	Periphyton	Abundance	Growth and Development	3	3	NA (not enough toxicity values from exposure)	30	NA (not enough toxicity values from exposure)
			Facultative heterotroph	Periphyton	Abundance	Growth and Development	3	3	NA (not enough toxicity values from exposure)	30	NA (not enough toxicity values from exposure)
			Obligate heterotroph	Periphyton	Abundance	Growth and Development	3	3	NA (not enough toxicity values from exposure)	30	NA (not enough toxicity values from exposure)
El-Dib et al., 1991	Response of freshwater aglae (*Scenedesmus spp.*) to phenylurea herbicides	Chlortoluron	*Scenedesmus spp.*	Phytoplankton	Growth rate	Reproduction	10	10	N (Mentioned no recovery)	500	N
					Chl a @ max growth	Physiological (Chlorophyll)	10	10	Y	500	Y
					% chl a of control	Physiological (Chlorophyll)	10	10	NA (No information on exposure NOEC)	500	NA (No information on exposure NOEC)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
					Dry weight	Growth and Development	10	10	NA (No information on exposure NOEC)	500	NA (No information on exposure NOEC)
		Chlorbromuron	*Scenedesmus spp.*	Phytoplankton	Growth rate	Reproduction	10	10	Y	500	NA (No information on exposure NOEC)
					Chl a @ max growth	Physiological (Chlorophyll)	10	10	Y	500	Y
					% chl a of control	Physiological (Chlorophyll)	10	10	Y	500	NA (No information on exposure NOEC)
					Dry weight	Growth and Development	10	10	Y	500	NA (No information on exposure NOEC)
		Patoran	*Scenedesmus spp.*	Phytoplankton	Growth rate	Reproduction	10	10	Y	1000	NA (No information on exposure NOEC)
			Scenedesmus spp.	Phytoplankton	Chl a @ max growth	Physiological (Chlorophyll)	10	10	Y	1000	Y
			Scenedesmus spp.	Phytoplankton	% chl a of control	Physiological (Chlorophyll)	10	10	Y	1000	NA (No information on exposure NOEC)
			Scenedesmus spp.	Phytoplankton	Dry weight	Growth and Development	10	10	Y	1000	NA (No information on exposure NOEC)
Gustavson et al., 2003	Effects of exposure duration of herbicides on natural stream periphyton communities and recovery	Metribuzin	Periphyton community (September 1999)	Periphyton	Pigment concentration (all algae: chlorophyll a)	Physiological (Chlorophyll)	0.0833	2	Y (Mentioned recovery on photosynthetic activity)	50	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton community (September 1999)	Periphyton	Pigment concentration (all algae: chlorophyll a)	Physiological (Chlorophyll)	0.9583	2	Y (Mentioned recovery on photosynthetic activity)	50	Y
			Periphyton community (May 2000)	Periphyton	Pigment concentration (all algae: chlorophyll a)	Physiological (Chlorophyll)	2	2	Y (Mentioned recovery on photosynthetic activity)	10	Y
			Periphyton community (September 1999)	Periphyton	Pigment concentration (diatoms: fucoxanthin)	Physiological (Chlorophyll)	0.0833	2	Y (Mentioned recovery on photosynthetic activity)	50	Y
			Periphyton community (September 1999)	Periphyton	Pigment concentration (diatoms: fucoxanthin)	Physiological (Chlorophyll)	0.958	2	Y (Mentioned recovery on photosynthetic activity)	10	Y
			Periphyton community (May 2000)	Periphyton	Pigment concentration (diatoms: fucoxanthin)	Physiological (Chlorophyll)	2	2	Y (Mentioned recovery on photosynthetic activity)	10	Y
			Periphyton community (September 1999)	Periphyton	Pigment concentration (chlorophytes: chlorophyll b)	Physiological (Chlorophyll)	0.0833	2	N	<0.4	N

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton community (September 1999)	Periphyton	Pigment concentration (chlorophytes: chlorophyll b)	Physiological (Chlorophyll)	0.958	2	N	<0.4	N
			Periphyton community (May 2000)	Periphyton	Pigment concentration (chlorophytes: chlorophyll b)	Physiological (Chlorophyll)	2	2	N	<0.4	N
			Periphyton community (September 1999)	Periphyton	Pigment concentration cyanobacteria: zeaxanthin)	Physiological (Chlorophyll)	0.0833	2	Y (Mentioned recovery on photosynthetic activity)	50	Y
			Periphyton community (September 1999)	Periphyton	Pigment concentration cyanobacteria: zeaxanthin)	Physiological (Chlorophyll)	0.958	2	Y (Mentioned recovery on photosynthetic activity)	50	Y
			Periphyton community (May 2000)	Periphyton	Pigment concentration cyanobacteria: zeaxanthin)	Physiological (Chlorophyll)	2	2	Y (Mentioned recovery on photosynthetic activity)	50	Y
Haynes et al., 2000	The impact of the herbicide diuron on photosynthesis in three species of tropical seagrass	Diuron	*Cymodocea serrulata*	Macrophyte	Effective quantum yield	Physiological (Chlorophyll)	5	5	Y	1 (same as exposure NOEC)	NA
			Halophila ovalis	Macrophyte	Effective quantum yield	Physiological (Chlorophyll)	5	5	Y	10	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (μg/L)	Did the endpoint recovered to the control
			Zoestera capricorni	Macrophyte	Effective quantum yield	Physiological (Chlorophyll)	5	5	Y	0.1	Y
Hughes et al., 1988	An evaluation of appropriate expressions of toxicity in aquatic plant bioassays as demonstrated by the effects of atrazine on algae and duckweed	Atrazine	*Anabaena flos-aquae*	Phytoplankton	Cell counts	Reproduction	5	9	Y	3200	Y
			Navicula pelliculosa	Periphyton	Cell counts	Reproduction	5	9	Y	3200	Y
			Dunaliella tertiolecta	Phytoplankton	Cell counts	Reproduction	5	5	Y	1600	Y
			Lemna gibba	Macrophyte	Frond counts	Reproduction	5	9	Y	3200	Y
Jones et al., 1986	Atrazine uptake, photosynthetic inhibition, and short-term recovery for the submersed vascular plant, *Potamogeton perfoliatus L.*	Atrazine	*Potamogeton perfoliatus* L.	Macrophyte	Photosynthesis	Physiological (Chlorophyll)	0.0833	1	NA (Not enough toxicity values from exposure)	5	NA
			Potamogeton perfoliatus L.	Macrophyte	Photosynthesis	Physiological (Chlorophyll)	0.0833	3.208	Y	100	Y
King et al., 2016	Effects of pulsed atrazine exposures on autotrophic community structure, biomass, and production in field-based stream mesocosms	Atrazine	Phytoplankton	Phytoplankton	Species: composition	Population or Community Level Metrics	1.417	1.083	NA (Lack of responses from exposure)	30 (Same as LOEC from exposure)	NA (Lack of responses from exposure)
			Phytoplankton	Phytoplankton	Biomass (Ash-free dry mass)	Growth and Development	1.417	1.083	NA (Lack of responses from exposure	30	NA (Lack of responses from exposure
			Periphyton	Periphyton	Chlorophyll a	Physiological (Chlorophyll)	1.417	1.083	NA (Lack of responses from exposure	30	NA (Lack of responses from exposure
			Metaphyton	Periphyton	Dry mass	Growth and Development	1.417	1.083	NA (Lack of responses from exposure	30	NA (Lack of responses from exposure
			Ceratophyllum demersum	Macrophyte	Dry mass	Growth and Development	1.417	1.083	NA (Lack of responses from exposure	30 (Same as NOEC from exposure)	NA (Lack of responses from exposure

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Algae	Phytoplankton	Max daily Dissolved Oxygen	Physiological (Oxygen)	1.417	0.333	NA (Lack of responses from exposure	30	NA (Lack of responses from exposure
			Algae	Phytoplankton	Max daily Dissolved Oxygen	Physiological (Oxygen)	1.417	0.75	NA (Lack of responses from exposure	<10	NA (Lack of responses from exposure
Klaine et al., 1997	Agrochemical fate and effects in terrestrial, aquatic and estuarine ecosystems	Atrazine	*Selenastrum capricornutum*	Phytoplankton	Cell density (cells/mL)	Reproduction	4	4	Y	50	Y
Knežević et al., 2016	Getting more ecologically relevant information from laboratory tests: recovery of *Lemna minor* after exposure to herbicides and their mixtures	Atrazine	*Lemna minor*	Macrophyte	Frond number RGR	Reproduction	7	5	Y	320	Y
			Lemna minor	Macrophyte	Frond weight RGR	Growth and Development	7	5	Y	1000	Y
			Lemna minor	Macrophyte	Frond number RGR	Reproduction	7	5	N	80 (Same as NOEC from exposure)	N
			Lemna minor	Macrophyte	Frond weight RGR	Growth and Development	7	5	Y	80	Y
		Isoproturon	*Lemna minor*	Macrophyte	Frond number RGR	Reproduction	7	5	Y	320	Y
			Lemna minor	Macrophyte	Frond weight RGR	Growth and Development	7	5	Y	320	Y
			Lemna minor	Macrophyte	Frond number RGR	Reproduction	7	5	Y	100	Y
			Lemna minor	Macrophyte	Frond weight RGR	Growth and Development	7	5	Y	100	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
		Trifluralin	*Lemna minor*	Macrophyte	Frond number RGR	Reproduction	7	5	Y	100 (same as 7d exposure NOEC)	NA
			Lemna minor	Macrophyte	Frond weight RGR	Growth and Development	7	5	Y	100 (7d exposure NOEC)	NA
			Lemna minor	Macrophyte	Frond number RGR	Reproduction	7	5	Y	100 (same as 7d exposure NOEC)	NA
			Lemna minor	Macrophyte	Frond weight RGR	Growth and Development	7	5	Y	100 (same as 7d exposure NOEC)	NA
Laviale et al., 2011	Short term recovery of periphyton photosynthesis after pulse exposition to the photosystem II inhibitors atrazine and isoproturon	Atrazine	Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.0417	0.0417	Y	216	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.0417	0.5	Y	21600	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.125	0.0417	Y	216	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.125	0.5	Y	21600	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.292	0.0417	Y	216	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.292	0.5	Y	21600	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton	Periphyton	Optimal quantum yield of PSII	Physiological (Chlorophyll)	0.125	0.0417	N	21.6 (same as exposure NOEC)	NA
			Periphyton	Periphyton	Optimal quantum yield of PSII	Physiological (Chlorophyll)	0.125	0.5	Y	21600	Y
			Periphyton	Periphyton	Optimal quantum yield of PSII	Physiological (Chlorophyll)	0.292	0.0417	Y	2160	Y
		Isoproturon	Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.0417	0.0417	Y	21.6	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.0417	0.5	Y	2160	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.125	0.0417	Y	21.6	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.125	0.5	Y	216	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.292	0.0417	Y	21.6	Y
			Periphyton	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	0.292	0.5	Y	216	Y
			Periphyton	Periphyton	Optimal quantum yield of PSII	Physiological (Chlorophyll)	0.125	0.0417	Y	2160	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton	Periphyton	Optimal quantum yield of PSII	Physiological (Chlorophyll)	0.125	0.5	Y	21600	Y
			Periphyton	Periphyton	Optimal quantum yield of PSII	Physiological (Chlorophyll)	0.292	0.0417	N	<21.6	N
			Periphyton	Periphyton	Optimal quantum yield of PSII	Physiological (Chlorophyll)	0.0417	0.0417	Y	21600	Y
Macinnis-Ng and Ralph 2003	Short-term response and recovery of *Zostera capricorni* photosynthesis after herbicide exposure	Atrazine	*Zostera capricorni*	Macrophyte	Maximum quantum yield (Fv/Fm)	Physiological (Chlorophyll)	0.417	4	NA	100 (lack of response in exposure)	NA
			Lemna sp.	Macrophyte	Effective quantum yields	Physiological (Chlorophyll)	0.417	4	Y	10	Y
			Lemna sp.	Macrophyte	Effective quantum yields	Physiological (Chlorophyll)	0.417	4	NA	100 (lack of response in exposure)	NA
			Lemna sp.	Macrophyte	Total chlorophyll concentrations	Physiological (Chlorophyll)	0.417	4	Y	100	Y
			Lemna sp.	Macrophyte	Total chlorophyll concentrations	Physiological (Chlorophyll)	0.417	4	NA	100 (lack of response in exposure)	NA
		Diuron	*Zostera capricorni*	Macrophyte	Maximum quantum yield (Fv/Fm)	Physiological (Chlorophyll)	0.417	4	Y	100	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Zostera capricorni	Macrophyte	Effective quantum yields	Physiological (Chlorophyll)	0.417	4	Y	100	Y
			Zostera capricorni	Macrophyte	Effective quantum yields	Physiological (Chlorophyll)	0.417	4	NA	10 (same as exposure NOEC)	NA
			Zostera capricorni	Macrophyte	Total chlorophyll concentrations	Physiological (Chlorophyll)	0.417	4	Y	100	Y
			Zostera capricorni	Macrophyte	Total chlorophyll concentrations	Physiological (Chlorophyll)	0.417	4	Y	100	Y
		Irgarol	*Zostera capricorni*	Macrophyte	Maximum quantum yield (Fv/Fm)	Physiological (Chlorophyll)	*Zostera capricorni*	Macrophyte	Y	100	Y
			Zostera capricorni	Macrophyte	Effective quantum yields	Physiological (Chlorophyll)	0.417	4	Y	10	Y
			Zostera capricorni	Macrophyte	Effective quantum yields	Physiological (Chlorophyll)	0.417	4	Y	10	Y
			Zostera capricorni	Macrophyte	Total chlorophyll concentrations	Physiological (Chlorophyll)	0.417	4	NA	100 (lack of response in exposure)	NA
			Zostera capricorni	Macrophyte	Total chlorophyll concentrations	Physiological (Chlorophyll)	0.417	4	N	<10	N

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
Macinnis-Ng et al., 2004	In situ impact of multiple pulses of metal and herbicide on the seagrass, *Zostera capricorni*	Irgarol	*Zostera capricorni*	Macrophyte	Chlorophyll a fluorescence	Physiological (Chlorophyll)	0.417	4	N	<100	N
			Zostera capricorni	Macrophyte	Chlorophyll a fluorescence	Physiological (Chlorophyll)	0.417	8	Y	100	Y
			Zostera capricorni	Macrophyte	Chlorophyll a fluorescence	Physiological (Chlorophyll)	0.833	8	N	<100	N
Mohammad et al., 2006	Recovery of *Lemna sp.* after exposure to sulfonylurea herbicides	Bensulfuron-methyl	*Lemna* sp.	Macrophyte	Frond number (RGR)	Reproduction	7	10	Y	<10	NA (Didn't access the significant different to control
		Cyclosulfamuron	*Lemna* sp.	Macrophyte	Frond number	Reproduction	7	10	N	<1	NA (Didn't access the significant different to control
			Lemna sp.	Macrophyte	Frond number (RGR)	Reproduction	7	10	Y	1	NA (Didn't access the significant different to control
		Ethoxysulfuron	*Lemna* sp.	Macrophyte	Frond number (RGR)	Reproduction	7	10	Y	10	NA (Didn't access the significant different to control
		Flazasulfuron	*Lemna* sp.	Macrophyte	Frond number (RGR)	Reproduction	7	10	Y	100	NA (Didn't access the significant different to control
		Imazosulfuron	*Lemna* sp.	Macrophyte	Frond number (RGR)	Reproduction	7	10	Y	10	NA (Didn't access the significant different to control

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
		Nicosulfuron	*Lemna* sp.	Macrophyte	Frond number (RGR)	Reproduction	7	10	Y	100	NA (Didn't access the significant different to control
		Pyrazosulfuron-ethyl	*Lemna* sp.	Macrophyte	Frond number (RGR)	Reproduction	7	10	Y	1000	NA (Didn't access the significant different to control
		Thifensulfuron-methyl	*Lemna* sp.	Macrophyte	Frond number (RGR)	Reproduction	7	10	Y	1000	NA (Didn't access the significant different to control
Mohammad et al., 2008	Comparative effects of different families of herbicides on recovery potential in *Lemna sp.*	Alachlor	*Lemna* sp.	Macrophyte	Frond number	Reproduction	7	7	N	1 (Same as exposure NOEC)	NA
			Lemna sp.	Macrophyte	Daughter fronds production (RGR)	Reproduction	7	7	Y	1 (Same as exposure NOEC)	NA
		Atrazine	*Lemna* sp.	Macrophyte	Daughter fronds production (RGR)	Reproduction	7	7	Y	10 (Same as exposure NOEC)	NA
		Bensulfuron-methyl	*Lemna* sp.	Macrophyte	Daughter fronds production (RGR)	Reproduction	7	7	Y	1 (Same as exposure NOEC)	NA
		Cyclosulfamuron	*Lemna* sp.	Macrophyte	Frond number	Reproduction	7	7	Y	10	Y
			Lemna sp.	Macrophyte	Daughter fronds production (RGR)	Reproduction	7	7	Y	10	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
		Cyhalofop-butyl	*Lemna* sp.	Macrophyte	Daughter fronds production (RGR)	Reproduction	7	7	Y	1000	N/Y (at <100 µg/L)
		Diuron	*Lemna* sp.	Macrophyte	Daughter fronds production (RGR)	Reproduction	7	7	Y	10	N / Y (for 1 µg/L)
		Paraquat	*Lemna* sp.	Macrophyte	Frond number	Reproduction	7	7	Y	10 (Same as exposure NOEC)	NA
			Lemna sp.	Macrophyte	Daughter fronds production (RGR)	Reproduction	7	7	Y	10 (Same as exposure NOEC)	NA
		Simetryn	*Lemna* sp.	Macrophyte	Daughter fronds production (RGR)	Reproduction	7	7	Y	10 (Same as exposure NOEC)	NA
		Thiobencarb	*Lemna* sp.	Macrophyte	Daughter fronds production (RGR)	Reproduction	7	7	Y	1000	N / Y (for 1, 10 µg/L)
Mohammad et al., 2010	Effects of herbicides on *Lemna gibba* and recovery from damage after prolonged exposure	Alachlor	*Lemna gibba*	Macrophyte	Frond number	Reproduction	7	7	Y	50	N / Y (at 6.25 µg/L)
			Lemna gibba	Macrophyte	Frond number	Reproduction	14	7	Y	50	N / Y (at 6.25 µg/L)
			Lemna gibba	Macrophyte	Frond number	Reproduction	21	7	Y	25	N
			Lemna gibba	Macrophyte	Frond number	Reproduction	28	7	Y	12.5	N
			Lemna gibba	Macrophyte	Relative growth rate (Frond number)	Reproduction	28	7	N	<6.25	N

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
		Atrazine	*Lemna gibba*	Macrophyte	Frond number	Reproduction	7	7	Y	3200	N / Y (at < 200 µg/L)
			Lemna gibba	Macrophyte	Frond number	Reproduction	14	7	Y	3200	N
			Lemna gibba	Macrophyte	Frond number	Reproduction	21	7	Y	3200	N
			Lemna gibba	Macrophyte	Frond number	Reproduction	28	7	Y	3200	N
			Lemna gibba	Macrophyte	Relative growth rate (Frond number)	Reproduction	28	7	Y	3200	N
		Cyclosulfamuron	*Lemna gibba*	Macrophyte	Frond number	Reproduction	7	7	Y	50	N / Y (at 1 µg/L)
			Lemna gibba	Macrophyte	Frond number	Reproduction	14	7	Y	10	N
			Lemna gibba	Macrophyte	Frond number	Reproduction	21	7	Y	1	N
			Lemna gibba	Macrophyte	Frond number	Reproduction	28	7	Y	1	N
		Paraquat	*Lemna gibba*	Macrophyte	Frond number	Reproduction	7	7	Y	10	N / Y (at 2.5 µg/L)
			Lemna gibba	Macrophyte	Frond number	Reproduction	14	7	Y	10	N
			Lemna gibba	Macrophyte	Frond number	Reproduction	21	7	Y	5	N
			Lemna gibba	Macrophyte	Frond number	Reproduction	28	7	Y	5	N
			Lemna gibba	Macrophyte	Frond number (RGR)	Reproduction	28	7	N	<2.5	N
Nagai et al., 2011	Effects of four rice paddy herbicides on algal cell viability and the relationship with population recovery	Pretilachlor	*Pseudokirchneriella subcapitata*	Phytoplankton	Cell density (growth rate)	Reproduction	3	7	Y	16	Y
		Bensulfuron-methyl	*Pseudokirchneriella subcapitata*	Phytoplankton	Cell density (growth rate)	Reproduction	3	7	Y	960	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
		Pentoxazone	*Pseudokirchneriella subcapitata*	Phytoplankton	Cell density (growth rate)	Reproduction	3	7	NA	12.8* (Lack of responses at exposure)	NA
		Quinoclaimine	*Pseudokirchneriella subcapitata*	Phytoplankton	Cell density (growth rate)	Reproduction	3	7	Y	400	Y
Nema et al., 2019	Phylogeny of the egg-loving green alga *Oophila amblystomatis* (Chlamydomonadales) and its response to the herbicides atrazine and 2,4-D.	2,4-D	*Oophila amblystomatis* (from Kinston)	Phytoplankton	Cell density (growth rate)	Reproduction	4	4	Y	100	Y
			Oophila amblystomatis (from Sudden Tract)	Phytoplankton	Cell density (growth rate)	Reproduction	4	4	Y	100	Y
			Oophila amblystomatis (from Bruce Peninsula)	Phytoplankton	Cell density (growth rate)	Reproduction	4	4	Y	100	Y
		Atrazine	*Oophila amblystomatis* (from Kinston)	Phytoplankton	Cell density (growth rate)	Reproduction	4	4	Y	405	Y
			Oophila amblystomatis (from Sudden Tract)	Phytoplankton	Cell density (growth rate)	Reproduction	4	4	Y	360	Y
			Oophila amblystomatis (from Bruce Peninsula)	Phytoplankton	Cell density (growth rate)	Reproduction	4	4	Y	270	Y
			Oophila amblystomatis (from Kinston)	Phytoplankton	PSII yield	Physiological (Chlorophyll)	4	4	Y	405	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Oophila amblystomatis (from Sudden Tract)	Phytoplankton	PSII yield	Physiological (Chlorophyll)	4	4	Y	360	Y
			Oophila amblystomatis (from Bruce Peninsula)	Phytoplankton	PSII yield	Physiological (Chlorophyll)	4	4	Y	270	Y
Netherland et al. 1997	Mesocosm evaluation of the species-selective potential of fluridone	Fluridone	*Myriophyllum spicatum* L	Macrophyte	Dry weight biomass	Growth and Development	60	40	N	<5	N
			Elodea canadensis Michaux	Macrophyte	Dry weight biomass	Growth and Development	60	40	N	20 (same as exposure NOEC)	NA
			Potamogeton nodosus Poiret	Macrophyte	Dry weight biomass	Growth and Development	60	40	Y	10	Y
			Potamogeton pectinatus L	Macrophyte	Dry weight biomass	Growth and Development	60	40	N	5 (same as exposure NOEC)	NA
			Vallisneria americana Michaux	Macrophyte	Dry weight biomass	Growth and Development	60	40	Y	20	Y
			Myriophyllum spicatum L	Macrophyte	Dry weight biomass	Growth and Development	60	40	N	<5	N
			Elodea canadensis Michaux	Macrophyte	Dry weight biomass	Growth and Development	60	40	Y	5	Y
			Potamogeton nodosus Poiret	Macrophyte	Dry weight biomass	Growth and Development	60	40	Y	10	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Potamogeton pectinatus L	Macrophyte	Dry weight biomass	Growth and Development	60	40	Y	5	Y
			Vallisneria americana Michaux	Macrophyte	Dry weight biomass	Growth and Development	60	40	Y	20	Y
Netherland 2015	Laboratory and greenhouse response of monoecious *Hydrilla* to fluridone	Fluridone	*Hydrilla verticillata*	Macrophyte	Chlorophyll floresce nce yield	Physiological (Chlorophyll)	18	17	N	<6	N
			Hydrilla verticillata	Macrophyte	Chlorophyll floresce nce yield	Physiological (Chlorophyll)	12	23	N	<6	N
			Hydrilla verticillata	Macrophyte	Chlorophyll floresce nce yield	Physiological (Chlorophyll)	6	29	N	<6	N
Prosser et al. 2013	Assessing sensitivity and recovery of field-collected periphyton acutely exposed to atrazine using PSII inhibition under laboratory conditions	Atrazine	Periphyton (U of G microcosm)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.0833	Y	320	Y
			Periphyton (U of G microcosm)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.0833	Y	320	Y
			Periphyton (Bronte Creek)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.0833	Y	20	Y
			Periphyton (Bronte Creek)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.0833	Y	20	Y
			Periphyton (U of G microcosm)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.25	Y	320	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton (U of G microcosm)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.25	Y	320	Y
			Periphyton (Bronte Creek)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.25	N	<20	N
			Periphyton (Bronte Creek)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.25	Y	80	Y
			Periphyton (U of G microcosm)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	1	Y	320	Y
			Periphyton (U of G microcosm)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	1	Y	320	Y
			Periphyton (Bronte Creek)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	1	Y	320	Y
			Periphyton (Bronte Creek)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	1	Y	320	Y
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.0833	Y	40	Y
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.25	Y	160	Y
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	1	Y	40	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton (IL-10)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.0833	Y	80	Y
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.25	Y	80	Y
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	1	Y	80	Y
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.0833	Y	80	Y
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	0.25	Y	80	Y
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	1	1	N	20 (same as exposure NOEC)	NA
Prosser et al. 2015	Assessing temporal and spatial variation in sensitivity of communities of periphyton sampled from agroecosystem to, and ability to recover from, atrazine exposure	Atrazine	Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	Y (Based on ECX values)	320	Y
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	Y (Based on ECX values)	320	Y
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (IA-03)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y
			Periphyton (IL-10)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (IL-10)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y
			Periphyton (IL-10)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	Y (Based on ECX values)	320	Y
			Periphyton (IL-10)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y
			Periphyton (IL-10)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	NA (no information on exposure NOEC)	20	NA (no information on exposure NOEC)
			Periphyton (IL-10)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton (IL-10)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	N (Based on ECX values)	<20	N
			Periphyton (IL-10)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (IL-17)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	Y (Based on ECX values)	320	Y
			Periphyton (IL-17)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y
			Periphyton (IL-17)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	Y (Based on ECX values)	320	Y
			Periphyton (IL-17)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y
			Periphyton (IL-17)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	NA (no information on exposure NOEC)	320	NA (no information on exposure NOEC)
			Periphyton (IL-17)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	NA (no information on exposure NOEC)	320	NA (no information on exposure NOEC)
			Periphyton (MO-07N)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (MO-07N)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton (MO-07N)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	N (Based on ECX values)	<20	N
			Periphyton (MO-07N)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	N (Based on ECX values)	<20	N
			Periphyton (MO-07N)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	Y (Based on ECX values)	320	Y
			Periphyton (MO-07N)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y
			Periphyton (MO-07N)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	N (Based on ECX values)	320	N
			Periphyton (MO-07N)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	NA (no information on exposure NOEC)	320	NA (no information on exposure NOEC)
			Periphyton (OH-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (OH-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (OH-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	Y (Based on ECX values)	320	Y
			Periphyton (OH-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Periphyton (OH-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (OH-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (OH-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	NA (no information on exposure NOEC)	80	NA (no information on exposure NOEC)
			Periphyton (OH-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	Y (Based on ECX values)	320	Y
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	N (Based on ECX values)	320	N
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	N (Based on ECX values)	320	N
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	N (Based on ECX values)	320	N
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	N (Based on ECX values)	320	N
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	1	N (Based on ECX values)	320	N
			Periphyton (MO-05)	Periphyton	Effective quantum yield of PSII	Physiological (Chlorophyll)	2	2	N (Based on ECX values)	320	N

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
Rosenkrantz et al. 2013	Growth inhibition and recovery of *Lemna gibba* after pulse exposure to sulfonylurea herbicides	Flupysulfuron-methyl	*Lemna gibba*	Macrophyte	Biomass	Growth and Development	1	6	Y	1.1	Y
		Metsulfuron-methyl	*Lemna gibba*	Macrophyte	Biomass	Growth and Development	1	6	Y	1.8	Y
		Rimsulfuron	*Lemna gibba*	Macrophyte	Biomass	Growth and Development	1	6	N	0.16 (same as exposure NOEC)	NA
		Thifensulfuron-methyl	*Lemna gibba*	Macrophyte	Biomass	Growth and Development	1	6	N	0.44 (same as exposure NOEC)	NA
Snel et al. 1998	Inhibition of photosystem II (PSII) electron transport as a convenient endpoint to assess stress of the herbicide linuron on freshwater plants	Linuron	*Elodea nuttallii* (Planchon) *St. John*	Macrophyte	Efficiency of PSII electron flow	Physiological (Chlorophyll)	1	1	Y (Based on EC50 values from exposure)	50	Y
			Myriophyllum spicatum L.	Macrophyte	Efficiency of PSII electron flow	Physiological (Chlorophyll)	1	1	Y (Based on EC50 values from exposure)	50	Y
			Potamogeton crispus L.	Macrophyte	Efficiency of PSII electron flow	Physiological (Chlorophyll)	1	1	Y (Based on EC50 values from exposure)	50	Y
			Ranunculus circinatus Sibth.	Macrophyte	Efficiency of PSII electron flow	Physiological (Chlorophyll)	1	1	Y (Based on EC50 values from exposure)	50	Y
			Chara globularis (Thuill.)	Macrophyte	Efficiency of PSII electron flow	Physiological (Chlorophyll)	1	1	Y (Based on EC50 values from exposure)	50	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
Teodorovic et al. 2012	*Myriophyllum aquaticum* versus *Lemna minor*: sensitivity and recovery potential after exposure to atrazine	Atrazine	*Lemna gibba*	Macrophyte	Fresh weight (RGR)	Reproduction	3	4	Y	320	Y
			Lemna gibba	Macrophyte	Fresh weight (RGR)	Reproduction	3	6	Y	1280	Y
			Lemna gibba	Macrophyte	Fresh weight (RGR)	Reproduction	7	5	N	80 (same as exposure NOEC)	NA
			Lemna gibba	Macrophyte	Frond number (RGR)	Reproduction	3	4	Y	640	Y
			Lemna gibba	Macrophyte	Frond number (RGR)	Reproduction	3	6	Y	640	Y
			Lemna gibba	Macrophyte	Frond number (RGR)	Reproduction	7	5	Y	80	Y
			Lemna gibba	Macrophyte	Total frond area (RGR)	Reproduction	3	4	Y	640	Y
			Lemna gibba	Macrophyte	Total frond area (RGR)	Reproduction	3	6	Y	1280	Y
			Lemna gibba	Macrophyte	Total frond area (RGR)	Reproduction	7	5	N	80 (same as exposure NOEC)	NA
			Myriophyllum aquaticum	Macrophyte	Fresh weight (RGR)	Reproduction	3	9	N	40	N
			Myriophyllum aquaticum	Macrophyte	Fresh weight (RGR)	Reproduction	3	12	N	40	N
			Lemna gibba	Macrophyte	Fresh weight (Yield)	Growth and Development	3	4	Y	640	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Lemna gibba	Macrophyte	Fresh weight (Yield)	Growth and Development	3	6	Y	640	Y
			Lemna gibba	Macrophyte	Fresh weight (Yield)	Growth and Development	7	5	N	<80	N
			Lemna gibba	Macrophyte	Frond number (Yield)	Reproduction	3	4	Y	640	Y
			Lemna gibba	Macrophyte	Frond number (Yield)	Reproduction	3	6	Y	640	Y
			Lemna gibba	Macrophyte	Frond number (Yield)	Reproduction	7	5	N	<80	N
			Lemna gibba	Macrophyte	Total frond area (Yield)	Reproduction	3	4	Y	640	Y
			Lemna gibba	Macrophyte	Total frond area (Yield)	Reproduction	3	6	Y	640	Y
			Lemna gibba	Macrophyte	Total frond area (Yield)	Reproduction	7	5	N	<80	N
			Myriophyllum aquaticum	Macrophyte	Fresh weight (Yield)	Growth and Development	3	9	N	40 (same as exposure NOEC)	NA
			Myriophyllum aquaticum	Macrophyte	Fresh weight (Yield)	Growth and Development	3	12	N	40	N

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
Vallotton et al. 2008	Effect of pulse herbicidal exposure on *Scenedesmus vacuolatus*: A comparison of two photosystem II inhibitors	Atrazine	*Scenedesmus vacuolatus*	Phytoplankton	Optical density (growth rate)	Reproduction	1	1	Y	510	Y
			Scenedesmus vacuolatus	Phytoplankton	PSII effective quantum yield	Physiological (Chlorophyll)	1	1	Y	510* (Based on exposure EC50 and recovery NOEC)	Y
		Isoproturon	*Scenedesmus vacuolatus*	Phytoplankton	Optical density (growth rate)	Reproduction	1	0.917	Y	320	Y
			Scenedesmus vacuolatus	Phytoplankton	PSII effective quantum yield	Physiological (Chlorophyll)	1	0.917	Y	320* (Based on exposure EC50 and recovery NOEC)	Y
Vallotton et al., 2009	Effect of Squential Isoproturon Pulse Exposure on Scenedesmus vacuolatus	Isoproturon	*Scenedesmus vacuolatus*	Phytoplankton	Cell density (Growth rate)	Reproduction	1.25	4.75	Y	1000* (Based on exposure EC50 and recovery NOEC)	Y
Varga et al. 2019	Phytotoxicity assessment of isproturon on growth and physiology of non-targeted aquatic plant *Lemna minor L.* - A comparison of continuous and pulsed exposure with equivalent time-averaged concentrations	Isoproturon	*Lemna minor*	Macrophyte	Fresh weight	Growth and Development	2	5	NA (Not enough toxicity value from exposure)	200	NA (Not enough toxicity value from exposure)
			Lemna minor	Macrophyte	Frond number	Growth and Development	2	5	NA (Not enough toxicity value from exposure)	<50	NA (Not enough toxicity value from exposure)
			Lemna minor	Macrophyte	Chlorophyll a	Physiological (Chlorophyll)	2	5	NA (Not enough toxicity value from exposure)	150	NA (Not enough toxicity value from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Lemna minor	Macrophyte	Chlorophyll b	Physiological (Chlorophyll)	2	5	NA (Not enough toxicity value from exposure)	100	NA (Not enough toxicity value from exposure)
			Lemna minor	Macrophyte	Carotenoids	Physiological (Pigment other than chlorophyll)	2	5	NA (Not enough toxicity value from exposure) NA (Not enough toxicity value from exposure)	200	NA (Not enough toxicity value from exposure)
			Lemna minor	Macrophyte	Total soluble proteins	Enzyme function or hormone responses	2	5	NA (Not enough toxicity value from exposure)	100	NA (Not enough toxicity value from exposure)
			Lemna minor	Macrophyte	Activity of catalase	Enzyme function or hormone responses	2	5	NA (Not enough toxicity value from exposure)	150	NA (Not enough toxicity value from exposure)
			Lemna minor	Macrophyte	Activity of guaiacol peroxidase	Enzyme function or hormone responses	2	5	NA (Not enough toxicity value from exposure)	<50	NA (Not enough toxicity value from exposure)
			Lemna minor	Macrophyte	Activity of ascorbateperoxidase	Enzyme function or hormone responses	2	5	NA (Not enough toxicity value from exposure)	100	NA (Not enough toxicity value from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (μg/L)	Did the endpoint recovered to the control
			Lemna minor	Macrophyte	H_2O_2 concentration	Enzyme function or hormone responses	2	5	NA (Not enough toxicity value from exposure)	100	NA (Not enough toxicity value from exposure)
			Lemna minor	Macrophyte	TBARS concentration	Enzyme function or hormone responses	2	5	NA (Not enough toxicity value from exposure) NA (Not enough toxicity value from exposure)	150	NA (Not enough toxicity value from exposure)
			Lemna minor	Macrophyte	Found number (Growth rate)	Reproduction	2	1	Y	50	Y
			Lemna minor	Macrophyte	Found number (Growth rate)	Reproduction	2	2	Y	50	Y
			Lemna minor	Macrophyte	Found number (Growth rate)	Reproduction	2	3	Y	50	Y
			Lemna minor	Macrophyte	Found number (Growth rate)	Reproduction	2	4	Y	200	Y
			Lemna minor	Macrophyte	Found number (Growth rate)	Reproduction	2	5	Y	200	Y
			Lemna minor	Macrophyte	Fresh weight	Growth and Development	3	4	NA (not enough toxicity values from exposure)	<50	NA (not enough toxicity values from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Lemna minor	Macrophyte	Frond number	Growth and Development	3	4	NA (not enough toxicity values from exposure)	<50	NA (not enough toxicity values from exposure)
			Lemna minor	Macrophyte	Chlorophyll a	Physiological (Chlorophyll)	3	4	NA (not enough toxicity values from exposure)	50	NA (not enough toxicity values from exposure)
			Lemna minor	Macrophyte	Chlorophyll b	Physiological (Chlorophyll)	3	4	NA (not enough toxicity values from exposure)	50	NA (not enough toxicity values from exposure)
			Lemna minor	Macrophyte	Carotenoids	Physiological (Pigment other than chlorophyll)	3	4	NA (not enough toxicity values from exposure)	150	NA (not enough toxicity values from exposure)
			Lemna minor	Macrophyte	Total soluble proteins	Enzyme function or hormone responses	3	4	NA (not enough toxicity values from exposure)	100	NA (not enough toxicity values from exposure)
			Lemna minor	Macrophyte	Activity of catalase	Enzyme function or hormone responses	3	4	NA (not enough toxicity values from exposure)	100	NA (not enough toxicity values from exposure)
			Lemna minor	Macrophyte	Activity of guaiacol peroxidase	Enzyme function or hormone responses	3	4	NA (not enough toxicity values from exposure)	<50	NA (not enough toxicity values from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Lemna minor	Macrophyte	Activity of ascorbateperoxidase	Enzyme function or hormone responses	3	4	NA (not enough toxicity values from exposure)	<50	NA (not enough toxicity values from exposure)
			Lemna minor	Macrophyte	H_2O_2 concentration	Enzyme function or hormone responses	3	4	NA (not enough toxicity values from exposure)	50	NA (not enough toxicity values from exposure)
			Lemna minor	Macrophyte	TBARS concentration	Enzyme function or hormone responses	3	4	NA (not enough toxicity values from exposure)	50	NA (not enough toxicity values from exposure)
			Lemna minor	Macrophyte	Found number (Growth rate)	Reproduction	3	4	N	<50 (Same as exposure NOEC)	NA
			Lemna minor	Macrophyte	Found number (Growth rate)	Reproduction	3	4	Y	100	Y
			Lemna minor	Macrophyte	Found number (Growth rate)	Reproduction	3	4	Y	150	Y
			Lemna minor	Macrophyte	Found number (Growth rate)	Reproduction	3	4	Y	150	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
Wieczorek et al., 2017	Response and recovery of the macrophytes *Elodea canadensis* and *Myriophyllum spicatum* following a pulse exposure to the herbicide iofensulfuron-sodium in outdoor stream mesocosms.	Iofensulfuron-sodium	*Myriophyllum spicatum*	Macrophyte	Main shoot length (growth rate)	Reproduction	1	1	Y	30	NA (not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Main shoot length (growth rate)	Reproduction	1	3	Y	30	NA (not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Main shoot length (growth rate)	Reproduction	1	7	Y	10	NA (not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Main shoot length (growth rate)	Reproduction	1	14	NA (Not enough information to determine)	1	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Main shoot length (growth rate)	Reproduction	1	42	Y	10	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Side shoot length (growth rate)	Reproduction	1	1	Y	10	Y
			Myriophyllum spicatum	Macrophyte	Side shoot length (growth rate)	Reproduction	1	3	NA (Not enough information to determine)	3	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Side shoot length (growth rate)	Reproduction	1	7	NA (Not enough information to determine)	3	NA (Not enough information to determine)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Myriophyllum spicatum	Macrophyte	Side shoot length (growth rate)	Reproduction	1	14	NA (Not enough information to determine)	3	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Side shoot length (growth rate)	Reproduction	1	42	Y	30	Y
			Myriophyllum spicatum	Macrophyte	Total shoot length (growth rate)	Reproduction	1	1	Y	10	Y
			Myriophyllum spicatum	Macrophyte	Total shoot length (growth rate)	Reproduction	1	3	NA (Not enough information to determine)	3	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Total shoot length (growth rate)	Reproduction	1	7	NA (Not enough information to determine)	1	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Total shoot length (growth rate)	Reproduction	1	14	NA (Not enough information to determine)	1	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Total shoot length (growth rate)	Reproduction	1	42	Y	30	Y
			Myriophyllum spicatum	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	1	Y	30	NA (Not enough information to determine)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Myriophyllum spicatum	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	3	Y	30	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	7	Y	30	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	14	Y	30	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	42	NA (Not enough information to determine)	10	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Root length (growth rate)	Reproduction	1	1	Y	30	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Root length (growth rate)	Reproduction	1	3	Y	30	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Root length (growth rate)	Reproduction	1	7	Y	30	NA (Not enough information to determine)
			Myriophyllum spicatum	Macrophyte	Root length (growth rate)	Reproduction	1	14	NA (Not enough information to determine)	10	NA (Not enough information to determine)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Myriophyllum spicatum	Macrophyte	Root length (growth rate)	Reproduction	1	42	Y	30	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Main shoot length (growth rate)	Reproduction	1	1	Y	30	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Main shoot length (growth rate)	Reproduction	1	3	Y	30	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Main shoot length (growth rate)	Reproduction	1	7	Y	10	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Main shoot length (growth rate)	Reproduction	1	14	Y	10	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Main shoot length (growth rate)	Reproduction	1	42	NA (Not enough information to determine)	1	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Side shoot length (growth rate)	Reproduction	1	1	Y	30	Y
			Elodea canadensis	Macrophyte	Side shoot length (growth rate)	Reproduction	1	3	Y	10	Y
			Elodea canadensis	Macrophyte	Side shoot length (growth rate)	Reproduction	1	7	Y	10	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Elodea canadensis	Macrophyte	Side shoot length (growth rate)	Reproduction	1	14	Y	10	Y
			Elodea canadensis	Macrophyte	Side shoot length (growth rate)	Reproduction	1	42	Y	30	Y
			Elodea canadensis	Macrophyte	Total shoot length (growth rate)	Reproduction	1	1	Y	30	Y
			Elodea canadensis	Macrophyte	Total shoot length (growth rate)	Reproduction	1	3	Y	10	Y
			Elodea canadensis	Macrophyte	Total shoot length (growth rate)	Reproduction	1	7	NA (Not enough information to determine)	3	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Total shoot length (growth rate)	Reproduction	1	14	Y	10	Y
			Elodea canadensis	Macrophyte	Total shoot length (growth rate)	Reproduction	1	42	Y	30	Y
			Elodea canadensis	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	1	Y	30	NA (Not enough information to determine)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Elodea canadensis	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	3	Y	30	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	7	Y	30	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	14	Y	30	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Dry weight of total shoots (growth rate)	Reproduction	1	42	Y	30	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Side shoot number (growth rate)	Reproduction	1	1	Y	30	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Side shoot number (growth rate)	Reproduction	1	3	N	3	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Side shoot number (growth rate)	Reproduction	1	7	N	3	NA (Not enough information to determine)
			Elodea canadensis	Macrophyte	Side shoot number (growth rate)	Reproduction	1	14	Y	10	NA (Not enough information to determine)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Elodea canadensis	Macrophyte	Side shoot number (growth rate)	Reproduction	1	42	Y	30	NA (Not enough information to determine)
Wilson et al., 2006	Toxicity of the Norflurazon to the Aquatic Macrophyte Vallisneria americana (Michx.)	Norflurazon	*Vallisneria americana*	Macrophyte	Fresh weight gains (14d postexposure)	Growth and Development	14	14	Y	80	N
			Vallisneria americana	Macrophyte	New leaf production (14d postexposure)	Reproduction	14	14	NA	80 (Lack of responses in exposure)	NA
			Vallisneria americana	Macrophyte	Total leaf growth (14d postexposure)	Growth and Development	14	14	NA	100 (Lack of responses in exposure)	NA
			Vallisneria americana	Macrophyte	Leaf greenness (14d postexposure)	Physiological (Chlorophyll)	14	14	Y	100	Y
			Vallisneria americana	Macrophyte	Offspring quantity (14d exposure + 14d postexposure)	Reproduction	14	14	NA (not enough toxicity values from exposure)	80	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Offspring fresh weight (14d exposure + 14d postexposure)	Reproduction	14	14	NA (not enough toxicity values from exposure)	100	NA (not enough toxicity values from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (μg/L)	Did the endpoint recovered to the control
			Vallisneria americana	Macrophyte	Stolon fresh weight (14d exposure + 14d postexposure)	Growth and Development	14	14	NA (not enough toxicity values from exposure)	80	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Shoot dry weight (14d exposure + 14d postexposure)	Growth and Development	14	14	NA (not enough toxicity values from exposure)	100	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Roots dry weight (14d exposure + 14d postexposure)	Growth and Development	14	14	NA (not enough toxicity values from exposure)	80	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Stolons dry weight (14d exposure + 14d postexposure)	Growth and Development	14	14	NA (not enough toxicity values from exposure)	60	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Chlorophyll a (after 14d of exposure + 14d postexposure)	Physiological (Chlorophyll)	14	14	NA (not enough toxicity values from exposure)	100	NA (not enough toxicity values from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Vallisneria americana	Macrophyte	Total soluble sµgars (Carbohydrates in shoots) (14d of exposure + 14d postexposure)	Enzyme function or hormone responses	14	14	NA (not enough toxicity values from exposure)	20	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Hexose (Carbohydrates in shoots) (14d of exposure + 14d postexposure)	Enzyme function or hormone responses	14	14	NA (not enough toxicity values from exposure)	20	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Sucrose (Carbohydrates in shoots) (14d of exposure + 14d postexposure))	Enzyme function or hormone responses	14	14	NA (not enough toxicity values from exposure)	100	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Starch (Carbohydrates in shoots) (14d of exposure + 14d postexposure)	Enzyme function or hormone responses	14	14	NA (not enough toxicity values from exposure)	100	NA (not enough toxicity values from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (μg/L)	Did the endpoint recovered to the control
			Vallisneria americana	Macrophyte	Total soluble sμgars (Carbohydrates in roots) (14d of exposure + 14d postexposure)	Enzyme function or hormone responses	14	14	NA (not enough toxicity values from exposure)	80	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Hexose (Carbohydrates in roots) (14d of exposure + 14d postexposure)	Enzyme function or hormone responses	14	14	NA (not enough toxicity values from exposure)	100	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Sucrose (Carbohydrates in roots) (14d of exposure + 14d postexposure))	Enzyme function or hormone responses	14	14	NA (not enough toxicity values from exposure)	80	NA (not enough toxicity values from exposure)
			Vallisneria americana	Macrophyte	Starch (Carbohydrates in roots) (14d of exposure + 14d postexposure)	Enzyme function or hormone responses	14	14	NA (not enough toxicity values from exposure)	100	NA (not enough toxicity values from exposure)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
Wilson and Wilson, 2010	Toxicity of the herbicides bromacil and simazine to the aquatic macrophyt, Vallisneria americana michx	Bromacil	*Vallisneria americana michx*	Macrophyte	Fresh weight gain	Growth and Development	13	15	N	<20	N
			Vallisneria americana michx	Macrophyte	New leaf production	Reproduction	13	15	N	20	N
			Vallisneria americana michx	Macrophyte	Length increase	Growth and Development	13	15	N	<20	N
			Vallisneria americana michx	Macrophyte	SPAD units (Greenness) - Leaf greenness/chlorophyll a	Physiological (Chlorophyll)	13	15	N	92 (same as exposure NOEC)	NA
			Vallisneria americana michx	Macrophyte	Offspring quantity	Reproduction	13	15	Y	77	Y (at concentration of 20)
			Vallisneria americana michx	Macrophyte	Offspring fresh weight	Reproduction	13	15	Y	77	N
			Vallisneria americana michx	Macrophyte	Stolon fresh weight	Growth and Development	13	15	Y	77	Y (at concentration of 20)
			Vallisneria americana michx	Macrophyte	Total plant (dry weight)	Growth and Development	13	15	NA (not enough information to determine)	<20	NA (not enough information to determine)
			Vallisneria americana michx	Macrophyte	Shoot (dry weight)	Growth and Development	13	15	NA (not enough information to determine)	92	NA (not enough information to determine)

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Vallisneria americana michx	Macrophyte	Root (dry weight)	Growth and Development	13	15	NA (not enough information to determine)	20	NA (not enough information to determine)
			Vallisneria americana michx	Macrophyte	Stolon (dry weight)	Growth and Development	13	15	NA (not enough information to determine)	20	NA (not enough information to determine)
			Vallisneria americana michx	Macrophyte	Total chlorophyll - Leaf greenness/chlorophyll a	Physiological (Chlorophyll)	13	15	NA	92 (lack of responses in exposure)	NA
			Vallisneria americana michx	Macrophyte	Roots and shoots (soluble sugar and starch allocation)	Physiological	13	15	NA	92 (lack of responses in exposure)	NA
			Vallisneria americana michx	Macrophyte	Total soluble sugars (soluble sugar and starch allocation)	Physiological	13	15	NA	92 (lack of responses in exposure)	NA
			Vallisneria americana michx	Macrophyte	Hexose (Soluble sugar and starch allocation)	Physiological	13	15	NA	92 (lack of responses in exposure)	NA

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Vallisneria americana michx	Macrophyte	Sucrose (soluble sugar and starch allocation)	Physiological	13	15	NA	92 (lack of responses in exposure)	NA
			Vallisneria americana michx	Macrophyte	Starch (soluble sugars and starch allocation)	Physiological	13	15	NA	92 (lack of responses in exposure)	NA
		Simazine	*Vallisneria americana michx*	Macrophyte	Fresh weight gain	Growth and Development	13	14	N	<58	N
			Lemna minor	Macrophyte	New leaf production	Reproduction	13	14	Y	592	Y
			Lemna minor	Macrophyte	Length increase	Growth and Development	13	14	Y	58 (same as exposure NOEC)	NA
			Lemna minor	Macrophyte	SPAD units (Greenness) - Leaf greenness/chlorophyll a	Physiological (Chlorophyll)	13	14	N	<58	NA
			Lemna minor	Macrophyte	Offspring quantity	Reproduction	13	14	Y	229	Y
			Lemna minor	Macrophyte	Offspring fresh weight	Reproduction	13	14	Y	592	Y
			Lemna minor	Macrophyte	Stolon fresh weight	Growth and Development	13	14	Y	58	Y

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (µg/L)	Did the endpoint recovered to the control
			Lemna minor	Macrophyte	Total plant (dry weight)	Growth and Development	13	14	NA (not enough toxicity values for exposure)	58	NA (not enough toxicity values for exposure)
			Lemna minor	Macrophyte	Shoot (dry weight)	Growth and Development	13	14	NA (not enough toxicity values for exposure)	58	NA (not enough toxicity values for exposure)
			Lemna minor	Macrophyte	Root (dry weight)	Growth and Development	13	14	NA (not enough toxicity values for exposure)	229	NA (not enough toxicity values for exposure)
			Lemna minor	Macrophyte	stolon (dry weight)	Growth and Development	13	14	NA (not enough toxicity values for exposure)	58	NA (not enough toxicity values for exposure)
			Lemna minor	Macrophyte	Total chlorophyll - Leaf greenness/chlorophyll a	Physiological (Chlorophyll)	13	14	NA	592 (lack of responses from exposure)	NA
			Lemna minor	Macrophyte	Roots and shoots (soluble sµgar and starch allocation)	Physiological	13	14	NA	592 (lack of responses from exposure)	NA
			Lemna minor	Macrophyte	Total soluble sµgars (soluble sµgar and starch allocation)	Physiological	13	14	NA	592 (lack of responses from exposure)	NA

Table A2. Continued.

Reference	Study title	Herbicide	Test species	Species group	Endpoint	Endpoint categories	Exposure duration (day)	Recovery duration (day)	Recovered (Y/N)	At which concentration (μg/L)	Did the endpoint recovered to the control
			Lemna minor	Macrophyte	Hexose (Soluble sμgar and starch allocation)	Physiological	13	14	NA	592 (lack of responses from exposure)	NA
			Lemna minor	Macrophyte	Sucrose (soluble sμgar and starch allocation)	Physiological	13	14	NA	592 (lack of responses from exposure)	NA
Wilson and Koch, 2013	Influence of exposure concentration and duration on effects and recovery of *Lemna minor* exposed to the herbicide norflurazon	Norflurazon	*Lemna minor*	Macrophyte	Frond production	Reproduction	10	4	N	10	N
			Lemna minor	Macrophyte	Frond production	Reproduction	10	8	N	10	N
			Lemna minor	Macrophyte	Frond production	Reproduction	10	17	Y	250	Y
			Lemna minor	Macrophyte	Frond production	Reproduction	10	28	Y	500	Y

Figures

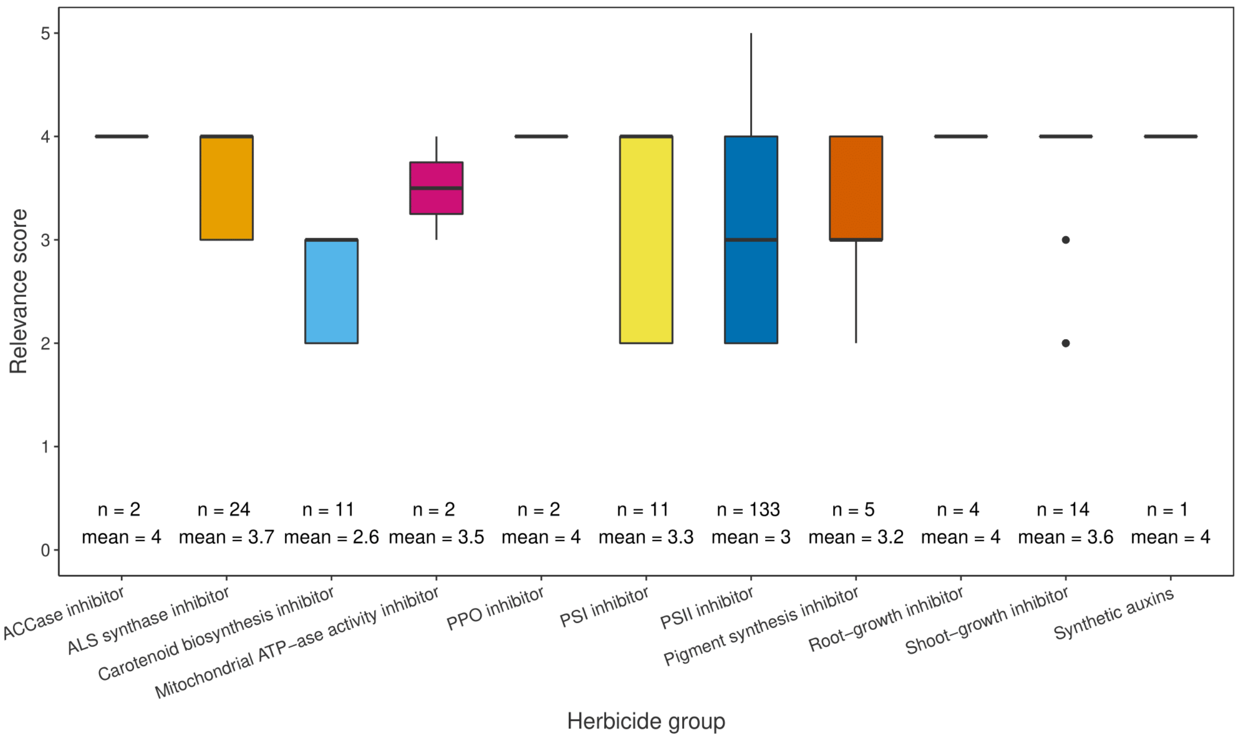

Figure A1. Boxplot of relevance scores for each herbicide group from exposure phase of testing. The n and mean were the total number and mean score of individual test species in the studies, respectively.

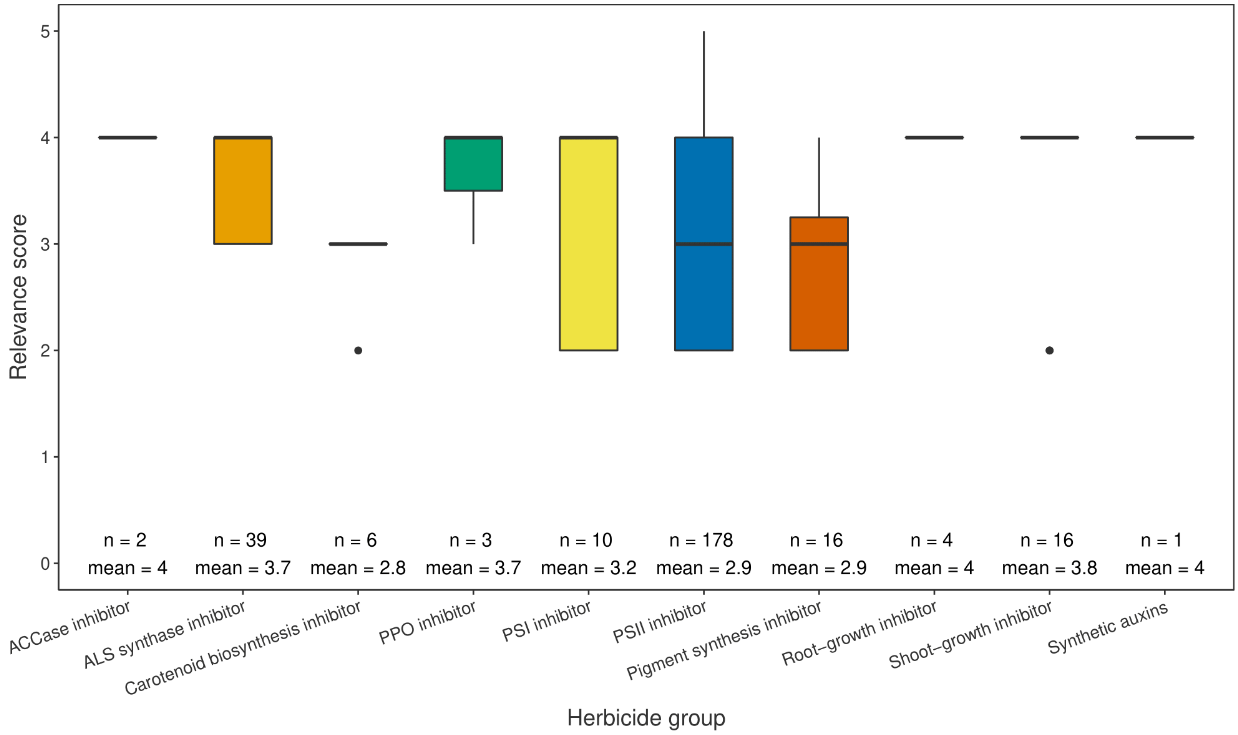

Figure A2. Boxplot of relevance scores for each herbicide group from recovery phase of testing. The n and mean were the total number and mean score of individual test species in the studies, respectively.

Appendix B: Laboratory recovery study

Tables

Table B1. Settings for photosystem II effective quantum yield (PSII yield) on multi-colour pulse amplitude modulation (PAM) fluorometer.

Measuring light intensity (levels)	**System Parameters**		**Actinic light intensity**	**Saturation pulse intensity**	**Int (Fo, Fm)**	**SP-Width (10ms)**	**AL color (nm)**	**ML color (nm)**	**Analysis mode**
	Damping	Gain							
2	2	4	6	10	5	30	440	440	SAT-Pulse

Table B2. The liquid chromatography-tandem mass spectrometry (LC/MS) setting for positive mode. LC was Aglient 1200 Series (Aglient Technologies, Mississauga, ON) binary pump, degasser, and column heater with an analytical column of Phenomenex (Torrance, CA) Kinetex XB-C18 column (50 mm x 2.1 mm x1.7 μm particle size). MS was Agilent 6410B MS with electrospray ionization source positive (for atrazine) + negative (for 2,4-D). The flow rate setting was 0.45 mL/min at a column temperature of 42°C, and the injection volume was 20 μL (taken from Carlson et al., 2013; Challis et al., 2016).

Time	**B%**
0.00	6.0
1.00	6.0
2.80	50.0
3.50	50.0
6.50	95.0
7.50	95.0
7.51	6.0
11.50	6.0
Run Time: 11.5 min Solvent A: 95% H20:5% MeOH w/ 0.05% Formic acid Solvent B: MeOH w/ 0.05% Formic acid	

Table B3. The liquid chromatography-tandem mass spectrometry (LC/MS) setting for negative mode. LC was Aglient 1200 Series (Aglient Technologies, Mississauga, ON) binary pump, degasser, and column heater with an analytical column of Phenomenex (Torrance, CA) Kinetex XB-C18 column (50 mm x 2.1 mm x1.7 μm particle size). MS was Agilent 6410B MS with electrospray ionization source positive (for atrazine) + negative (for 2,4-D). The flow rate setting was 0.45 mL/min at a column temperature of 42°C, and the injection volume was 20 μL (taken from Carlson et al., 2013; Challis et al., 2016).

Time	**B%**
0.00	6.0
0.50	6.0
6.00	95.0
7.00	95.0
7.01	6.0
11.00	6.0
Run Time: 11 min Solvent A: 95% H_2O: 5% MeOH Solvent B: 100% MeOH	

Table B4. The *m/z* transition, limits of detection (LOD), limit of quantitation (LOQ), fragmentor voltage (Frag), collision energy (CE), and retention time (RT) details for the tandem mass spectrometry (MS/MS) positive mode dynamic MRM method (taken from Challis et al., 2016). MS was Agilent 6410B MS.

Compound Name	**Precursor Ion**	**Product Ion**	**LOD (ng/L)[a]**	**LOQ (ng/L)[b]**	**Frag (V)**	**CE (V)**	**RT (min)**
Atrazine (Q)	216.1	174.1	1.3	4.4	130	16	4.3
Atrazine (q)	216.1	146.2	1.3	4.4	130	20	4.3
Atrazine-d_5 (IS)	221.1	179.1	1.3	4.4	130	16	4.3

Q = quantifier ion; q = qualifier ion; IS = internal standard,

[a] Limit of detection (LOD) = $(X_{BLK} + 3\sigma_{BLK})$/slope,

[b] Limit of quantitation (LOQ) = $(X_{BLK} + 10\sigma_{BLK})$/slope.

Table B5. The m/z transition, limit of detection (LOD), limit of quantitation (LOQ), fragmentor voltage (Frag), collision energy (CE), and retention time (RT) details for the tandem mass spectrometry (MS/MS) negative mode dynamic MRM method (taken from Challis et al., 2016). MS was Agilent 6410B MS.

Compound Name	**Precursor Ion**	**Product Ion**	**LOD (ng/L)[a]**	**LOQ (ng/L)[b]**	**Frag (V)**	**CE (V)**	**RT (min)**
2,4-D (Q)	219.0	161.0	4.1	14	75	5	3.1
2,4-D (q)	219.0	124.9	4.1	14	75	24	3.1
2,4-D-$^{13}C_6$ (IS)	225.0	167.0	4.1	14	75	5	3.1

Q = quantifier ion; q = qualifier ion; IS = internal standard,

[a] Limit of detection (LOD) = (X_{BLK} + 3σ_{BLK})/slope,

[b] Limit of quantitation (LOQ) = (X_{BLK} + 10σ_{BLK})/slope.

Table B6. The EC50 (95% confidence interval), no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC) values for *L. gibba* and *L. minor* absolute and relative growth rate (RGR) endpoints after 7 days range-finding tests with 2,4-D (2,4-Dichlorophenoxyacetic acid) and atrazine.

Herbicide	Species	Toxicity values (μg/L)	Endpoints						
			Absolute frond number	***Absolute plant number***	***Absolute dry mass***	***RGR frond number***	***RGR plant number***	***RGR dry mass***	***PSII yield (dark)***
2,4-D	*Lemna gibba*	EC50 (95% C.I.)	591.7 (338.8 - 844.6)[a]	549.5 (301.9 - 797.07)[b]	892.48* (323.71 - 1461.26)[a]	1532.46* (321.76 - 2743.15)[a]	2267.10* (-513.73 - 5047.92)[a]	2668.71* (-1191.88 - 6529.29)[a]	-[c]
		NOEC	200	200	200	200	200	200	≥600[e]
		LOEC	400	400	400	400	400	400	600[e]
	Lemna minor	EC50 (95% C.I.)	711.4* (365.6 - 1057.3)[a]	714.4* (338.7 - 1090.2)[b]	799.1* (279.7 - 1318.4)[a]	1401.0* (102.6 - 2699.4)[a]	1515.8* (-460.1 - 3491.7)[a]	-[c]	-[c]
		NOEC	≥600	400	≥600	400	400	≥600	≥600[d]
		LOEC	>600	600	>600	600	600	>600	>600[d]
Atrazine	*Lemna gibba*	EC50 (95% C.I.)	62.79 (46.09 - 79.48)[a]	71.35 (53.25 - 89.46)[a]	45.91 (34.68 - 57.14)[a]	106.42 (86.75 - 126.09)[a]	131.25 (107.36 - 155.14)[a]	82.32 (66.00 - 98.65)[a]	2819.24* (649.77 - 4988.7)[a]
		NOEC	20	40	20	40	40	20	40
		LOEC	40	80	40	80	80	40	80
	Lemna minor	EC50 (95% C.I.) (μg/L)	44.83 (26.07 - 63.59)[a]	52.30 (29.94 - 74.66)[a]	39.61 (24.27 - 54.96)[a]	104.06 (73.57 - 134.55)[a]	125.92 (91.91 - 159.93)[a]	90.08 (68.84 - 111.31)[a]	965.29* (664.3 - 1266.28)[a]
		NOEC	20	20	20	40	40	20	40
		LOEC	40	40	40	80	80	40	80

PSII yield – Photosystem II effective quantum yield,
* The ECX values greater than the tested concentration (i.e. 2,4-D: 600 μg/L and atrazine: 320 μg/L),
[a] Log-logistic model,
[b] The Cedergreen-Ritz-Streibig model (α=1.00),
[c] Not calculated because of lack of responses to determine the EC50,
[d] Significantly greater than control at 50 μg/L,
[e] Significantly greater than control at 200, 400, and 600 μg/L.

Table B7. The toxicity values for absolute and relative growth rate (RGR) on *L. gibba* after 7, 14, 21 and 28 days of 2,4-D (2,4-Dichlorophenoxyacetic acid) exposure and followed by a 7-day period of recovery.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
Lemna gibba	7	E	CV (%)	20.4	19.6	12.1	7.8	7.3	4.0	1.7	1.2
			EC01 (µg/L)	265.1 (116.2 - 414.1)[b]	104.1 (14.7 - 193.5)[f]	NC	277.3 (132.9 - 421.6)[b]	103.5 (28.5 - 178.6)[f]	NC	NC	NC
			EC10 (µg/L)	322.6 (198.1 - 447.1)[b]	237.6 (130.3 - 344.8)[f]	NC	405.6 (307.4 - 503.7)[b]	315.2 (217.5 - 412.9)[f]	NC	NC	NC
			EC25 (µg/L)	408.2 (303.1 - 513.2)[b]	346.7 (246.5 - 446.9)[f]	NC	569.4 (498.2 - 640.6)[b]	525.0 (442.8 - 607.2)[f]	NC	NC	NC
			EC50 (µg/L)	562.7 (455.6 - 669.8)[b]	506.0 (402.9 - 609.1)[f]	NC	847.9*** (723.2 - 972.6)[b]	874.4*** (718.5 - 1030.3)[f]	NC	NC	NC
			EC90 (µg/L)	1196.4*** (650.0 - 1742.8)[b]	1077.7*** (605.4 - 1550.0)[f]	NC	1996.1*** (1094.7 - 2897.4)[b]	2425.3*** (1135.8 - 3714.74)[f]	NC	NC	NC
			NOEC (µg/L)	259.40	259.40	116.75	259.40	259.40	≥731.05	≥731.05	≥731.05
			LOEC (µg/L)	488.71	488.71	259.40	488.71	488.71	>731.05	>731.05	>731.05
		R	CV (%)	16.5	22.1	19.7	1.2	1.7	3.1	1.0	1.0
			EC01 (µg/L)	50.0 (4.4 - 95.57)[f]	101.8 (-14.4 - 217.9)[f]	29.8 (-22.6 - 82.1)[f]	12.7 (-3.7 - 29.0)[f]	42.1 (-14.8 - 99.0)[f]	1.8 (-4.9 - 8.5)[f]	NC	NC

Table B7. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC10 (µg/L)	146.8 (73.9 - 219.8)[f]	242.9 (97.8 - 388.0)[f]	129.8 (12.4 - 247.2)[f]	120.8 (51.4 - 190.3)[f]	253.4 (122.7 - 384.2)[f]	65.9 (-37.0 - 168.7)[f]	NC	NC
			EC25 (µg/L)	240.6 (160.7 - 320.5)[f]	361.8 (227.4 - 496.3)[f]	254.8 (113.5 - 396.1)[f]	339.6 (239.6 - 439.5)[f]	576.9 (441.8 - 712.0)[f]	346.1 (102.0 - 590.3)[f]	NC	NC
			EC50 (µg/L)	394.2 (301.0 - 487.5)[f]	539.0 (413.4 - 664.6)[f]	500.2 (319.7 - 680.6)[f]	954.3*** (692.1 - 1216.5)[f]	1313.4*** (788.5 - 1838.2)[f]	1819.0*** (202.2 - 3435.8)[f]	NC	NC
			EC90 (µg/L)	1058.4*** (591.2 - 1525.7)[f]	1196.3*** (550.1 - 1842.5)[f]	1927.1*** (230.8 - 3623.4)[f]	7537.7*** (1003.0 - 14072.3)[f]	6806.8*** (-1008.4 - 14622.0)[f]	50234.9*** (-92502.3 - 192972.0)[f]	NC	NC
			NOEC (µg/L)	116.8	259.4	≥731.1	116.8	116.8	≥731.1	≥731.1	≥731.1
			LOEC (µg/L)	259.4	488.7	>731.1	259.4	259.4	>731.1	>731.1	>731.1
	14	E	CV (%)	35.8	32.5	31.4	6.4	5.9	4.9	1.5	1.6
			EC01 (µg/L)	168.4 (69.3 - 267.4)[b]	214.4 (94.0 - 334.7)[b]	36.3 (-37.1 - 109.7)[f]	169.74 (53.6 - 285.9)[b]	217.8 (75.3 - 360.4)[b]	NC	NC	NC
			EC10 (µg/L)	197.4 (108.6 - 286.2)[b]	249.6 (137.4 - 361.7)[b]	152.8 (-1.7 - 307.3)[f]	305.6 (211.5 - 399.8)[b]	394.0 (276.4 - 511.5)[b]	NC	NC	NC
			EC25 (µg/L)	244.6 (161.7 - 327.3)[b]	309.80 (201.64 - 417.96)[b]	295.3 (111.4 - 479.3)[f]	517.5 (434.2 - 600.8)[b]	680.5 (555.8 - 805.1)[b]	NC	NC	NC

Table B7. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC50 (µg/L)	336.32 (238.6 - 434.1)[b]	433.55 (301.3 - 565.8)[b]	570.7 (291.8 - 849.6)[f]	961.9*** (763.0 - 1160.8)[b]	1305.2*** (853.7 - 1756.7)[b]	NC	NC	NC
			EC90 (µg/L)	745.2*** (329.9 - 1160.5)[b]	1034.6*** (427.2 - 1642.0)[b]	2131.2*** (-385.7 - 4648.1)[f]	3663.4*** (1334.0 - 5992.8)[b]	5368.7*** (333.2 - 10404.6)[b]	NC	NC	NC
			NOEC (µg/L)	259.4	259.4	≥731.1	259.4	259.4	≥731.1	488.7	≥731.1
			LOEC (µg/L)	488.7	488.7	>731.1	488.7	488.7	>731.1	731.1	>731.1
		R	CV (%)	46.0	46.1	37.5	3.4	5.4	2.4	2.0	2.5
			EC01 (µg/L)	185.7 (91.6 - 279.9)[b]	193.7 (97.6 - 289.8)[b]	62.8 (2.4 - 123.2)[f]	117.0 (0.9 - 233.2)[f]	118.4 (-10.1 - 246.8)[f]	116.4 (-98.8 - 331.6)[f]	NC	NC
			EC10 (µg/L)	203.8 (128.0 - 279.6)[b]	214.6 (132.8 - 296.4)[b]	135.2 (67.1 - 203.2)[f]	312.9 (173.5 - 452.2)[f]	291.5 (139.6 - 443.4)[f]	263.8 (12.3 - 515.3)[f]	NC	NC
			EC25 (µg/L)	230.8 (173.1 - 288.4)[b]	247.0 (174.0 - 320.1)[b]	192.0 (129.8 - 254.3)[f]	490.9 (380.5 - 601.3)[f]	440.5 (315.2 - 565.8)[f]	383.8 (167.4 - 600.2)[f]	NC	NC
			EC50 (µg/L)	277.2 (203.0 - 351.4)[b]	305.3 (208.7 - 401.9)[b]	272.8 (207.5 - 338.0)[f]	770.3*** (622.8 - 917.8)[f]	665.7 (547.7 - 783.6)[f]	558.3 (402.2 - 714.4)[f]	NC	NC
			EC90 (µg/L)	436.7 (93.3 - 780.1)[b]	520.3 (153.3 - 887.3)[b]	550.5 (268.2 - 832.8)[f]	1896.7*** (682.0 - 3111.4)[f]	1520.4*** (613.5 - 2427.2)[f]	1181.7*** (227.1 - 2136.4)[f]	NC	NC

Table B7. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			NOEC (µg/L)	259.4	259.4	116.8	488.7	259.4	259.4	≥731.1	≥731.1
			LOEC (µg/L)	488.7	488.7	259.4	731.1	488.7	488.7	>731.1	>731.1
	21	E	CV (%)	44.1	49.9	44.7	5.2	5.9	4.8	1.0	0.5
			EC01 (µg/L)	231.6 (169.2 - 294.0)[b]	234.4 (167.2 - 301.5)[b]	235.7 (138.5 - 332.9)[b]	218.4 (131.4 - 305.3)[b]	230.6 (149.6 - 311.6)[b]	235.2 (102.4 - 368.1)[b]	NC	NC
			EC10 (µg/L)	243.5 (187.8 - 299.1)[b]	248.2 (184.5 - 311.8)[b]	255.9 (164.2 - 347.5)[b]	330.3 (261.4 - 399.3)[b]	354.4 (287.0 - 421.9)[b]	409.5 (306.6 - 512.4)[b]	NC	NC
			EC25 (µg/L)	263.7 (209.5 - 317.9)[b]	272.2 (207.9 - 336.5)[b]	290.3 (200.0 - 380.5)[b]	497.1 (439.5 - 554.8)[b]	550.8 (490.7 - 611.0)[b]	675.8 (574.6 - 777.0)[b]	NC	NC
			EC50 (µg/L)	302.7 (215.7 - 389.8)[b]	320.7 (229.8 - 411.5)[b]	358.3 (244.0 - 472.6)[b]	821.3*** (721.2 - 921.4)[b]	956.2*** (817.1 - 1095.3)[b]	1220.9*** (882.0 - 1559.8)[b]	NC	NC
			EC90 (µg/L)	448.5 (111.0 - 786.1)[b]	516.4 (181.8 - 850.9)[b]	639.3 (245.4 - 1033.2)[b]	2485.0*** (1567.5 - 3402.4)[b]	3270.1*** (1870.0 - 4670.2)[b]	4380.5*** (1091.1 - 7670.0)[b]	NC	NC
			NOEC (µg/L)	488.7	259.4	259.4	259.4	259.4	259.4	≥731.1	≥731.1
			LOEC (µg/L)	731.1	488.7	488.7	488.7	488.7	488.7	>731.1	>731.1
		R	CV (%)	21.6	35.5	19.3	10.8	12.3	11.1	1.4	0.9
			EC01 (µg/L)	259.3 (225.1 - 293.5)[b]	260.0 (219.4 - 300.6)[b]	226.2 (176.7 - 275.8)[b]	NC	NC	51.9 (-13.1 - 116.9)[f]	NC	NC

Table B7. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC10 (µg/L)	265.8 (227.2 - 304.5)[b]	266.0 (218.5 - 313.6)[b]	239.3 (192.7 - 285.8)[b]	NC	NC	253.2 (125.1 - 381.3)[f]	NC	NC
			EC25 (µg/L)	277.7 (221.5 - 333.8)[b]	277.0 (205.4 - 348.6)[b]	262.0 (216.5 - 307.6)[b]	NC	NC	523.5 (399.6 - 647.3)[f]	NC	NC
			EC50 (µg/L)	302.2 (194.6 - 409.9)[b]	299.8 (161.1 - 438.5)[b]	308.0 (245.7 - 370.3)[b]	NC	NC	1082.3*** (725.6 - 1439.0)[f]	NC	NC
			EC90 (µg/L)	397.8 (35.2 - 760.3)[b]	388.7 (-75.6 - 853.1)[b]	494.3 (255.7 - 732.9)[b]	NC	NC	4626.6*** (64.9 - 9188.4)[f]	NC	NC
			NOEC (µg/L)	≥731.1	≥731.1	12.4*	≥731.1	≥731.1	259.4	≥731.1	≥731.1
			LOEC (µg/L)	>731.1	>731.1	70.0*	>731.1	>731.1	488.7	>731.1	>731.1
	28	E	CV (%)	42.6	36.6	45.6	3.9	3.3	3.7	1.6	1.9
			EC01 (µg/L)	268.0 (223.1 - 312.9)[b]	265.2 (206.3 - 324.0)[b]	203.4 (141.3 - 265.4)[b]	305.6 (244.2 - 367.0)[b]	292.8 (224.7 - 361.0)[b]	222.4 (117.3 - 327.6)[b]	NC	NC
			EC10 (µg/L)	272.1 (214.7 - 329.5)[b]	269.0 (180.8 - 357.1)[b]	216.9 (157.1 - 276.7)[b]	399.3 (351.2 - 447.2)[b]	397.1 (343.8 - 450.4)[b]	362.4 (282.2 - 442.6)[b]	NC	NC
			EC25 (µg/L)	279.6 (196.0 - 363.2)[b]	275.8 (130.0 - 421.7)[b]	241.2 (182.6 - 299.7)[b]	533.1 (495.6 - 570.6)[b]	548.3 (505.9 - 590.8)[b]	564.6 (499.5 - 629.7)[b]	NC	NC

Table B7. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC50 (µg/L)	295.3 (150.8 - 439.9)[b]	290.1 (17.1 - 563.1)[b]	291.8 (221.1 - 362.4)[b]	772.7*** (718.1 - 827.2)[b]	826.2*** (754.0 - 898.4)[b]	953.9*** (798.7 - 1109.2)[b]	NC	NC
			EC90 (µg/L)	355.7 (-56.5 - 767.8)[b]	343.6 (-459.6 - 1146.7)[b]	291.8 (221.1 - 362.4)[b]	1788.4*** (1403.5 - 2173.2)[b]	2076.2*** (1530.6 - 2621.9)[b]	2963.6*** (1495.6 - 4431.6)[b]	NC	NC
			NOEC (µg/L)	69.0*	12.4*	488.7	69.0*	69.0*	488.7	488.7	≥731.1
			LOEC (µg/L)	116.8*	69.0*	731.1	116.8*	116.8*	731.1	731.1	>731.1
		R	CV (%)	44.9	49.4	55.6	2.1	6.1	4.4	2.6	0.8
			EC01 (µg/L)	232.9 (174.7 - 291.0)[b]	248.0 (189.2 - 306.9)[b]	250.3 (193.6 - 307.0)[b]	11.2 (-24.4 - 46.7)[f]	NC	530.6 (402.3 - 658.8)[e]	NC	NC
			EC10 (µg/L)	239.4 (180.6 - 298.1)[b]	252.7 (205.4 - 300.0)[b]	257.9 (200.1 - 315.7)[b]	142.8 (-45.5 - 331.1)[f]	NC	574.1 (469.6 - 678.5)[e]	NC	NC
			EC25 (µg/L)	251.6 (190.2 - 313.1)[b]	261.3 (217.7 - 304.8)[b]	272.2 (208.7 - 335.6)[b]	459.2 (142.5 - 775.9)[f]	NC	637.1 (556.9 - 717.3)[e]	NC	NC
			EC50 (µg/L)	278.7 (204.7 - 352.8)[b]	279.62 (176.17 - 383.07)[b]	303.39 (214.2 - 392.6)[b]	1476.9*** (120.4 - 2833.4)[f]	NC	741.6*** (657.5 - 825.7)[e]	NC	NC
			EC90 (µg/L)	397.6 (207.2 - 588.1)[b]	353.3 (-118.5 - 825.06)[b]	439.4 (151.9 - 726.9)[b]	15277.3*** (-23596.6 - 54151.3)[f]	NC	1076.6*** (738.6 - 1414.7)[e]	NC	NC

Table B7. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			NOEC (µg/L)	<12.4*	<12.4*	<12.4*	≥731.1	259.4	488.7	≥731.1	≥731.1
			LOEC (µg/L)	≤12.4*	≤12.4*	≤12.4*	>731.1	488.7	731.1	>731.1	>731.1

PSII yield – PSII effective quantum yield,
NC Not calculated because the endpoint at the highest tested concentration did not reach sufficient inhibition to determine the ECX values,
* The LOEC was statistically different from the control, but the next higher concentration was not significantly different from the control,
*** ECX values greater the tested concentration (731.1 µg/L),
[a] The Cedergreen-Ritz-Streibig model (α=0.10),
[b] The Cedergreen-Ritz-Streibig model (α=0.25),
[c] The Cedergreen-Ritz-Streibig model (α=0.50),
[d] The Cedergreen-Ritz-Streibig model (α=0.75),
[e] The Cedergreen-Ritz-Streibig model (α=1.00),
[f] log-logistic model.

Table B8. The toxicity values for absolute and relative growth rate (RGR) on *L. minor* after 7, 14, 21 28 days of 2,4-D (2,4-Dichlorophenoxyacetic acid) exposure and followed by a 7-day period of recovery.

Species	**Duration (day)**	**Exposure (E) or Recovery (R)**	**Endpoint**	**Absolute frond number**	**Absolute plant number**	**Absolute dry mass**	**RGR frond number**	**RGR plant number**	**RGR dry mass**	**PSII yield (light)**	**PSII yield (dark)**
Lemna minor	7	E	CV (%)	11.5	15.0	5.0	5.0	6.3	1.6	1.1	0.8
			EC01 (µg/L)	109.8 (27.2 - 192.3)[f]	87.8 (26.2 - 149.4)[f]	NC	126.9 (61.5 - 192.3)[f]	104.2 (50.5 - 157.9)	NC	NC	NC
			EC10 (µg/L)	246.7 (150.2 - 343.2)[f]	220.3 (140.2 - 300.4)[f]	NC	328.6 (253.9 - 403.3)[f]	310.8 (242.3 - 379.3)[f]	NC	NC	NC
			EC25 (µg/L)	357.6 (272.3 - 442.8)[f]	335.8 (261.4 - 410.1)[f]	NC	508.1 (451.7 - 564.4)[f]	512.9 (459.7 - 566.1)[f]	NC	NC	NC
			EC50 (µg/L)	518.3 (446.6 - 589.9)[f]	511.8 (444.9 - 578.6)[f]	NC	785.7*** (708.2 - 863.1)[f]	846.4*** (754.8 - 938.0)[f]	NC	NC	NC
			EC90 (µg/L)	1088.8*** (713.5 - 1464.0)[f]	1188.8*** (799.6 - 1578.0)[f]	NC	1878.6*** (1241.9 - 2515.3)[f]	2304.8*** (1474.0 - 3135.5)[f]	NC	NC	NC
			NOEC (µg/L)	259.4	259.4	>731.1	259.4	259.4	>731.1	>731.1	>731.1
			LOEC (µg/L)	488.7	488.7	≥731.1	488.7	488.7	≥731.1	≥731.1	≥731.1
		R	CV (%)	6.9	2.8	13.0	7.7	4.8	6.4	1.6	2.5
			EC01 (µg/L)	314.5 (129.6 - 499.4)[a]	279.6 (85.9 - 473.2)[e]	28.3 (-45.1 - 101.6)[f]	82.6 (-38.3 - 203.5)[f]	NC	NC	NC	NC

Table B8. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC10 (µg/L)	354.2 (179.7 - 528.6)[a]	327.0 (140.28 - 513.71)[e]	150.8 (-37.0 - 338.5)[f]	369.4 (181.2 - 557.5)[f]	NC	NC	NC	NC
			EC25 (µg/L)	424.3 (254.9 - 593.7)[a]	413.5 (227.1 - 600.0)[e]	324.5 (90.0 - 559.0)[f]	733.7*** (501.3 - 966.1)[f]	NC	NC	NC	NC
			EC50 (µg/L)	572.1 (346.2 - 798.0)[a]	605.9 (333.8 - 878.0)[e]	698.5 (291.8 - 1105.3)[f]	1457.3*** (555.3 - 2359.3)[f]	NC	NC	NC	NC
			EC90 (µg/L)	1293.4*** (131.7 - 2455.0)[a]	1687.1*** (-165.7 - 3539.8)[e]	3236.8*** (-1888.0 - 8361.5)[f]	5749.6*** (-2921.5 - 14420.6)[f]	NC	NC	NC	NC
			NOEC (µg/L)	>731.1	>731.1	>731.1	259.40*	>731.1	259.40	>731.1	>731.1
			LOEC (µg/L)	≥731.1	≥731.1	≥731.1	488.71*	≥731.1	488.71	≥731.1	≥731.1
	14	E	CV (%)	16.4	17.6	13.3	3.3	3.4	2.3	4.4	2.3
			EC01 (µg/L)	404.5 (279.8 - 529.3)[b]	411.4 (251.1 - 571.7)[a]	320.8 (10.2 - 631.3)[f]	420.1 (329.4 - 510.7)[b]	263.3 (126.6 - 400.0)[f]	572.3 (439.3 - 705.3)[b]	NC	NC
			EC10 (µg/L)	430.9 (311.4 - 550.4)[b]	443.9 (293.1 - 594.8)[a]	475.3 (235.8 - 714.8)[f]	544.4 (479.8 - 609.0)[b]	523.1 (430.7 - 615.5)[f]	716.3 (630.7 - 801.9)[b]	NC	NC
			EC25 (µg/L)	477.6 (360.6 - 594.6)[b]	501.1 (357.7 - 644.5)[a]	569.1 (392.5 - 745.7)[f]	717.1 (658.5 - 775.6)[b]	716.4 (660.5 - 772.4)[f]	894.9*** (715.5 - 1074.3)[b]	NC	NC

Table B8. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC50 (µg/L)	574.0 (433.4 - 714.5)[b]	618.2 (446.1 - 790.2)[a]	681.4 (551.5 - 811.4)[f]	1017.8*** (864.7 - 1170.8)[b]	981.3*** (800.9 - 1161.7)[f]	1175.9*** (720.6 - 1631.2)[b]	NC	NC
			EC90 (µg/L)	984.9*** (503.1 - 1466.7)[b]	1125.0*** (436.0 - 1814.1)[a]	977.0*** (529.8 - 1424.1)[f]	2235.9*** (1352.1 - 3119.8)[b]	1840.8*** (924.6 - 2757.0)[f]	2145.0*** (355.6 - 3934.4)[b]	NC	NC
			NOEC (µg/L)	69.0*	69.0*	>731.1	69.0*	69.0*	488.7	>731.1	>731.1
			LOEC (µg/L)	116.8*	116.8*	≥731.1	116.8*	116.8*	731.1	≥731.1	≥731.1
		R	CV (%)	16.4	17.6	17.1	6.9	8.0	3.9	2.1	1.6
			EC01 (µg/L)	231.4 (-28.9 - 491.6)[f]	223.6 (-15.9 - 463.0)[f]	192.2 (20.8 - 363.6)[f]	268.3 (-47.6 - 584.1)[f]	196.9 (-38.7 - 432.5)[f]	96.6 (-84.2 - 277.4)[f]	NC	NC
			EC10 (µg/L)	335.6 (141.0 - 530.3)[f]	331.0 (148.6 - 513.4)[f]	318.9 (169.3 - 468.6)[f]	534.6 (326.5 - 742.8)[f]	472.6 (273.4 - 671.7)[f]	329.2 (89.9 - 568.5)[f]	NC	NC
			EC25 (µg/L)	398.0 (260.5 - 535.6)[f]	396.2 (264.9 - 527.4)[f]	402.2 (282.5 - 522.0)[f]	733.3*** (592.7 - 873.9)[f]	705.8 (548.5 - 863.0)[f]	577.2 (400.9 - 753.6)[f]	NC	NC
			EC50 (µg/L)	472.0 (385.0 - 559.0)[f]	474.2 (383.1 - 565.2)[f]	507.3 (405.8 - 608.8)[f]	1005.8*** (550.1 - 1461.4)[f]	1054.0*** (579.9 - 1528.2)[f]	1012.2*** (515.7 - 1508.7)[f]	NC	NC
			EC90 (µg/L)	663.7 (327.2 - 1000.3)[f]	679.3 (336.9 - 1021.7)[f]	806.9*** (453.5 - 1160.3)[f]	1892.1*** (-340.6 - 4124.8)[f]	2351.0*** (-380.8 - 5082.8)[f]	3112.3*** (-1589.8 - 7814.3)[f]	NC	NC

Table B8. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			NOEC (µg/L)	69.0*	69.0*	69.0*	488.7	259.4	259.4	>731.1	≥731.1
			LOEC (µg/L)	116.8*	116.8*	116.8*	731.1	488.7	488.7	≥731.1	>731.1
	21	E	CV (%)	28.6	21.4	14.9	1.8	3.5	1.8	1.1	3.8
			EC01 (µg/L)	462.3 (326.4 - 598.2)[b]	460.1 (342.0 - 578.2)[b]	521.6 (383.4 - 659.7)[b]	462.6 (371.2 - 554.1)	456.8 (362.8 - 550.7)[a]	536.6 (446.1 - 627.2)[b]	NC	NC
			EC10 (µg/L)	475.0 (348.6 - 601.5)[b]	472.8 (363.8 - 581.8)[b]	535.5 (393.0 - 678.0)[b]	564.4 (503.4 - 625.3)	573.3 (513.6 - 633.0)[a]	643.3 (586.8 - 699.9)[b]	NC	NC
			EC25 (µg/L)	497.8 (374.5 - 621.0)[b]	495.3 (389.0 - 601.6)[b]	560.8 (401.6 - 719.9)[b]	691.8 (647.5 - 736.1)[b]	715.4 (669.8 - 760.9)[a]	777.3*** (718.7 - 835.9)[b]	NC	NC
			EC50 (µg/L)	544.1 (371.2 - 716.9)[b]	540.4 (387.7 - 693.0)[b]	613.4 (394.3 - 832.4)[b]	891.6*** (800.7 - 982.5)[b]	936.4*** (831.8 - 1041.0)[a]	986.1*** (832.3 - 1139.9)[b]	NC	NC
			EC90 (µg/L)	720.6 (116.1 - 1325.0)[b]	708.4 (180.1 - 1236.7)[b]	820.3*** (234.7 - 1405.8)[b]	1566.2*** (1123.4 - 2009.0)[b]	1689.5*** (1188.4 - 2190.6)[a]	1677.1*** (1029.9 - 2324.3)[b]	NC	NC
			NOEC (µg/L)	69.0*	69.0*	69.0*	69.0*	69.0*	69.0*	>731.1	≥731.1
			LOEC (µg/L)	116.8*	116.8*	116.8*	116.8*	116.8*	116.8*	≥731.1	>731.1
		R	CV (%)	43.1	41.0	41.0	7.3	6.9	8.5	3.4	2.7
			EC01 (µg/L)	453.0 (313.2 - 592.8)[b]	437.9 (233.3 - 642.5)[b]	420.1 (189.9 - 650.3)[b]	613.1 (190.4 - 1035.8)[f]	361.3 (-163.9 - 886.4)[f]	NC	NC	NC

Table B8. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC10 (µg/L)	468.8 (354.8 - 582.7)[b]	451.5 (268.8 - 634.3)[b]	442.9 (246.9 - 638.9)[b]	707.1 (609.0 - 805.3)[f]	641.1 (436.6 - 845.5)[f]	NC	NC	NC
			EC25 (µg/L)	494.0 (401.4 - 586.6)[b]	475.4 (318.4 - 632.3)[b]	479.1 (328.7 - 629.4)[b]	754.9*** (651.7 - 858.2)[f]	833.7*** (549.6 - 1117.9)[f]	NC	NC	NC
			EC50 (µg/L)	539.0 (396.8 - 681.2)[b]	522.5 (340.4 - 704.6)[b]	543.9 (401.7 - 686.0)[b]	806.0*** (494.7 - 1117.2)[f]	1084.3*** (164.9 - 2003.7)[f] [f]	NC	NC	NC
			EC90 (µg/L)	684.5 (153.1 - 1215.8)[b]	696.0 (-8.3 - 1400.3)[b]	763.2*** (116.5 - 1409.9)[b]	918.6*** (95.5 - 1741.7)[f]	1834.0*** (-1688.6 - 5356.5)[f]	NC	NC	NC
			NOEC (µg/L)	488.7	69.0*	>731.1	488.7	>731.1	488.7	>731.1	>731.1
			LOEC (µg/L)	731.1	116.8*	≥731.1	731.1	≥731.1	731.1	≥731.1	≥731.1
	28	E	CV (%)	59.2	61.0	53.4	5.4	5.3	4.7	1.75	1.8
			EC01 (µg/L)	343.9 (185.4 - 502.3)[b]	373.5 (221.2 - 525.7)[b]	NC	383.8 (290.3 - 477.2)[b]	410.8 (313.7 - 507.9)[b]	351.4 (175.8 - 527.1)[f]	NC	NC
			EC10 (µg/L)	356.19 (190.6 - 521.8)[b]	389.2 (235.5 - 543.0)[b]	NC	507.4 (440.8 - 574.0)[b]	542.7 (477.8 - 607.7)[b]	577.5 (479.3 - 675.6)[f]	NC	NC
			EC25 (µg/L)	379.0 (196.5 - 561.5)[b]	418.0 (256.9 - 579.0)[b]	NC	676.2 (622.0 - 730.4)[b]	714.5 (660.1 - 769.0)[b]	725.0 (675.8 - 774.2)[f]	NC	NC

Table B8. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC50 (µg/L)	428.1 (197.2 - 659.0)[b]	478.94 (286.50 - 671.4)[b]	NC	968.3*** (838.2 - 1098.3)[b]	1000.9*** (856.6 - 1145.2)[b]	910.3*** (756.2 - 1064.3)[f]	NC	NC
			EC90 (µg/L)	638.9 (109.4 - 1168.4)[b]	738.3*** (282.6 - 1194.0)[b]	NC	2153.3*** (1361.2 - 2945.4)[b]	2101.3*** (1296.7 - 2905.9)[b]	1434.8*** (759.2 - 2110.4)[f]	NC	NC
			NOEC (µg/L)	69.0*	69.0*	69.0*	69.0*	69.0*	69.0*	≥731.1	≥731.1
			LOEC (µg/L)	116.8*	116.8*	116.8*	116.8*	116.8*	116.8*	>731.1	>731.1
		R	CV (%)	60.2	61.0	65.8	4.5	7.7	5.1	2.6	1.2
			EC01 (µg/L)	281.5 (178.9 - 384.2)[b]	367.5 (192.6 - 542.4)[b]	NC	NC	176.6 (9.9 - 343.4)[f]	13.9 (-58.2 - 86.1)[f]	NC	NC
			EC10 (µg/L)	290.4 (175.9 - 404.9)[b]	378.4 (195.3 - 561.5)[b]	NC	NC	445.4 (292.7 - 598.0)[f]	134.3 (-163.2 - 431.7)[f]	NC	NC
			EC25 (µg/L)	306.3 (165.1 - 447.5)[b]	398.8 (197.8 - 599.8)[b]	NC	NC	680.3 (564.8 - 795.9)[f]	379.23 (-22.0 - 780.4)[f]	NC	NC
			EC50 (µg/L)	339.4 (129.8 - 549.0)[b]	443.5 (194.5 - 692.6)[b]	NC	NC	1039.3*** (698.7 - 1379.8)[f]	1071.2*** (-135.2 - 2277.6)[f]	NC	NC
			EC90 (µg/L)	470.6 (-86.9 - 1028.0)[b]	638.4 (106.4 - 1170.5)[b]	NC	NC	2425.2*** (302.8 - 4547.5)[f]	8546.6*** (-22714.8 - 39808.1)[f]	NC	NC

Table B8. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			NOEC (μg/L)	69.0*	69.0*	69.0*	116.8	488.7	259.4*	≥731.1	≥731.1
			LOEC (μg/L)	116.8*	116.8*	116.8*	259.4	731.1	488.7*	>731.1	>731.1

PSII yield – Photosystem II effective quantum yield,
NC Not calculated because the endpoint at the highest tested concentration did not reach sufficient inhibition to determine the ECX values,
* The LOEC was statistically different from the control, but the next higher concentration was not significantly different from the control,
*** ECX values greater than the tested concentration (731.1 μg/L),
[a] The Cedergreen-Ritz-Streibig model (α=0.10),
[b] The Cedergreen-Ritz-Streibig model (α=0.25),
[c] The Cedergreen-Ritz-Streibig model (α=0.50),
[d] The Cedergreen-Ritz-Streibig model (α=0.75),
[e] The Cedergreen-Ritz-Streibig model (α=1.00),
[f] log-logistic model.

Table B9. The parameters for concentration-response models for *L. gibba* after 7, 14, 21 and 28 days of 2,4-D (2,4-Dichlorophenoxyacetic acid) exposure and followed by a 7-day period of recovery.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
Lemna gibba	7	E	b	2.8[b]	3.0	NC	2.5[b]	2.2[f]	NC	NC	NC
			Lower limit	6[b]	2[f]	NC	0[b]	0[f]	NC	NC	NC
			Upper limit	72.5[b]	28.6[f]	NC	0.4[b]	0.4[f]		NC	NC
			e	509[b]	506[f]	NC	810[b]	874[f]	NC	NC	NC
			f	13.0[b]	NA[f]	NC	0.026[b]	NA[f]	NC	NC	NC
		R	b	2.2[f]	2.8[f]	1.6[f]	1.1[f]	1.3[f]	0.7[f]	NC	NC
			Lower limit	6[f]	2[f]	0[f]	0[f]	0[f]	0[f]	NC	NC
			Upper limit	1160[f]	417[f]	0.2[f]	0.4[f]	0.4[f]	0.4[f]	NC	NC
			e	394[f]	539[f]	500[f]	954[f]	1310[f]	1820[f]	NC	NC
			f	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NC	NC
	14	E	b	2.57[b]	2.32[b]	1.67[f]	1.61[b]	1.52[b]	NC	NC	NC
			Lower limit	6[b]	2[b]	0[f]	0[b]	0[b]	NC	NC	NC
			Upper limit	1100[b]	390[b]	0.2[f]	0.4[b]	0.4[b]	NC	NC	NC
			e	287[b]	350[b]	571[f]	891[b]	1200[b]	NC	NC	NC
			f	348[b]	155[b]	NA[f]	0.029[b]	0.03[b]	NC	NC	NC
		R	b	4.5[b]	3.8[b]	3.1[f]	2.4[f]	2.7[f]	2.9[f]	NC	NC
			Lower limit	6[b]	2[b]	0[f]	0[f]	0[f]	0[f]	NC	NC
			Upper limit	16300[b]	5700[b]	3.5[f]	0.4[f]	0.4[f]	0.4[f]	NC	NC
			e	254[b]	273[b]	273[f]	770[f]	666[f]	558[f]	NC	NC
			f	4900[b]	1900[b]	NA[f]	NA[f]	NA[f]	NA[f]	NC	NC
	21	E	b	4.9[b]	4.0[b]	3.4[b]	1.9[b]	1.7[b]	1.7[b]	NC	NC
			Lower limit	6[b]	2[b]	0[b]	0[b]	0[b]	2[b]	NC	NC
			Upper limit	15600[b]	5600[b]	3.0[b]	0.4[b]	0.4[b]	0.4[b]	NC	NC
			e	260[b]	261[b]	296[b]	756[b]	862[b]	1130[b]	NC	NC
			f	11100[b]	4540[b]	1.7[b]	0.04[b]	0.044[b]	0.03[b]	NC	NC
		R	b	6.6[b]	7.0[b]	4.1[b]	NC	NC	1.5[f]	NC	NC
			Lower limit	6[b]	2[b]	0[b]	NC	NC	0[f]	NC	NC
			Upper limit	172000[b]	61100[b]	38.91[b]	NC	NC	0.4[f]	NC	NC
			e	250[b]	248[b]	250[b]	NC	NC	1080[f]	NC	NC
			f	277000[b]	108000[b]	32.7[b]	NC	NC	NA[f]	NC	NC
	28	E	b	9.56[b]	10.6[b]	3.4[b]	2.5[b]	2.3[b]	1.9[b]	NC	NC
			Lower limit	6[b]	2[b]	0[b]	0[b]	0[b]	2[b]	NC	NC
			Upper limit	185000[b]	71700[b]	44.5[b]	0.4[b]	0.4[b]	0.4[b]	NC	NC

Table B9. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			e	252[b]	253[b]	224[b]	713[b]	760[b]	893[b]	NC	NC
			f	422000[b]	149000[b]	40.6[b]	0.05[b]	0.05[b]	0.03[b]	NC	NC
		R	b	5.0[b]	7.6[b]	4.8[b]	0.9[f]	1.0[b]	5.5[e]	NC	NC
			Lower limit	6[b]	2[b]	0[b]	0[f]	0[b]	0[e]	NC	NC
			Upper limit	2070000[b]	742000[b]	402[b]	0.4[f]	0.3[b]	0.3[e]	NC	NC
			e	198[b]	225[b]	219[b]	1480[f]	914[b]	695[e]	NC	NC
			f	5930000[b]	1930000[b]	965[b]	NA[f]	0.08[b]	NA[e]	NC	NC

PSII yield – Photosystem II effective quantum yield,
NC Not calculated because the endpoint at the highest tested concentration did not reach sufficient inhibition to determine the ECX values,
[a] The Cedergreen-Ritz-Streibig model (α=0.10),
[b] The Cedergreen-Ritz-Streibig model (α=0.25),
[c] The Cedergreen-Ritz-Streibig model (α=0.50),
[d] The Cedergreen-Ritz-Streibig model (α=0.75),
[e] The Cedergreen-Ritz-Streibig model (α=1.00),
[f] log-logistic model.

Table B10. The parameters for concentration-response models for *L. minor* after 7, 14, 21 and 28 days of 2,4-D (2,4-Dichlorophenoxyacetic acid) exposure and followed by a 7-day period of recovery

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
Lemna minor	7	E	b	3.0[f]	2.6[f]	NC	2.5[f]	2.2[f]	NC	NC	NC
			Lower limit	6[f]	2[f]	NC	0[f]	0[f]	NC	NC	NC
			Upper limit	63.0[f]	24.0[f]	NC	0.3[f]	0.4[f]	NC	NC	NC
			e	518[f]	512[f]	NC	786[f]	846[f]	NC	NC	NC
			f	NA[f]	NA[f]	NC	NA[f]	NA[f]	NC	NC	NC
		R	b	2.4[a]	2.0[e]	1.4[f]	1.6[f]	1.1[b]	0.8[b]	NC	NC
			Lower limit	6[a]	2[e]	0[f]	0[f]	0[b]	0[b]	NC	NC
			Upper limit	1120[a]	457[e]	0.135[f]	0.4[f]	0.4[b]	0.4[b]	NC	NC
			e	441[a]	453[e]	699[f]	1460[f]	2020[b]	1720[b]	NC	NC
			f	834[a]	173[e]	NA[f]	NA[f]	0.072[b]	0.081[b]	NC	NC
	14	E	b	3.5[b]	3.2[a]	6.1[f]	2.7[b]	3.5[f]	3.6[b]	NC	NC
			Lower limit	6[b]	2[a]	0[f]	0[b]	0[f]	0[b]	NC	NC
			Upper limit	1090[b]	456[a]	0.154[f]	0.4[b]	0.4[f]	0.4[b]	NC	NC
			e	451[b]	489[a]	681[f]	948[b]	981[f]	1130[b]	NC	NC
			f	900[b]	436[a]	NA[f]	0.05[b]	NA[f]	0.04[b]	NC	NC
		R	b	6.5[f]	6.1[f]	4.7[f]	3.5[f]	2.7[f]	2.0[f]	NC	NC
			Lower limit	6[f]	2[f]	0[f]	0[f]	0[f]	0[f]	NC	NC
			Upper limit	20700[f]	7930[f]	1.9[f]	0.4[f]	0.4[f]	0.4[f]	NC	NC
			e	472[f]	474[f]	507[f]	1010[f]	1050[f]	1010[f]	NC	NC
			f	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NC	NC
	21	E	b	6.6[b]	6.9[b]	6.8[b]	3.9[b]	3.6[a]	4.0[b]	NC	NC
			Lower limit	6[b]	2[b]	0[b]	0[b]	0[a]	0[b]	NC	NC
			Upper limit	14100[b]	5700[b]	1.28[b]	0.4[b]	0.4[a]	0.4[b]	NC	NC
			e	458[b]	463[b]	501[b]	853[b]	900[a]	944[b]	NC	NC
			f	18300[b]	6640[b]	2.00[b]	0.04[b]	0.05[a]	0.04[b]	NC	NC
		R	b	8.2[b]	6.6[b]	5.9[b]	16.8[f]	4.18[f]	NC	NC	NC
			Lower limit	6[b]	2[b]	0[b]	0[f]	0[f]	NC	NC	NC
			Upper limit	276000[b]	86200[b]	26.1[b]	0.393[f]	0.375[f]	NC	NC	NC
			e	500[b]	451[b]	494[b]	806[f]	1080[f]	NC	NC	NC
			f	148000[b]	86400[b]	12.0[b]	NA[f]	NA[f]	NC	NC	NC
	28	E	b	4.5[b]	4.3[b]	NC	2.7[b]	2.9[b]	4.8[f]	NC	NC
			Lower limit	6[b]	2[b]	NC	0[b]	0[b]	0[f]	NC	NC

Table B10. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			Upper limit	208000[b]	84100[b]	NC	0.4[b]	0.4[b]	0.4[f]	NC	NC
			e	318[b]	364[b]	NC	907[b]	949[b]	910[f]	NC	NC
			f	366000[b]	116000[b]	NC	0.042[b]	0.04[b]	NA[f]	NC	NC
		R	b	5.6[b]	4.9[b]	NC	NC	2.6[f]	1.1[f]	NC	NC
			Lower limit	6[b]	2[b]	NC	NC	0[f]	0[f]	NC	NC
			Upper limit	3260000[b]	1060000[b]	NC	NC	0.4[f]	0.4[f]	NC	NC
			e	275[b]	320[b]	NC	NC	1040[f]	1070[f]	NC	NC
			f	4650000[b]	2580000[b]	NC	NC	NA[f]	NA[f]	NC	NC

PSII yield – Photosystem II effective quantum yield,
NC Not calculated because the endpoint at the highest tested concentration did not reach sufficient inhibition to determine the ECX values,
[a] The Cedergreen-Ritz-Streibig model (α=0.10),
[b] The Cedergreen-Ritz-Streibig model (α=0.25),
[c] The Cedergreen-Ritz-Streibig model (α=0.50),
[d] The Cedergreen-Ritz-Streibig model (α=0.75),
[e] The Cedergreen-Ritz-Streibig model (α=1.00),
[f] log-logistic model.

Table B11. The toxicity values for absolute and relative growth rate (RGR) on *L. gibba* after 7, 14, 21 and 28 days of atrazine exposure and followed by a 7-day period of recovery.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
Lemna gibba	7	E	CV (%)	20.4	19.6	12.1	7.8	7.3	3.9	1.2	1.1
			EC01 (µg/L)	41.6 (11.8 - 71.4)[c]	11.2 (-1.5 - 23.8)[f]	9.2 (0.4 - 18.1)[f]	41.5 (11.8 - 71.3)[c]	9.3 (-1.4 - 20.0)[f]	11.9 (4.6 - 19.1)[f]	0.8 (0.3 - 1.2)[f]	NC
			EC10 (µg/L)	54.0 (27.2 - 80.9)[c]	35.4 (14.9 - 55.8)[f]	27.9 (13.7 - 42.1)[f]	72.0 (46.2 - 97.9)[c]	50.7 (24.8 - 76.5)[f]	45.2 (31.6 - 58.8)[f]	30.6 (23.3 - 37.8)[f]	NC
			EC25 (µg/L)	74.8 (49.1 - 100.5)[c]	60.0 (37.1 - 82.9)[f]	46.3 (31.0 - 61.6)[f]	122.5 (97.4 - 147.6)[c]	110.2 (79.6 - 140.8)[f]	83.3 (68.6 - 98.0)[f]	167.4 (149.2 - 185.7) [f]	NC
			EC50 (µg/L)	117.9 (83.2 - 152.5)[c]	101.7 (68.1 - 135.3)[f]	76.9 (59.0 - 94.8)[f]	233.8*** (179.0 - 288.6)[c]	239.6*** (169.0 - 310.2)[f]	153.6 (134.7 - 172.5)[f]	917.4*** (717.2 - 1117.7)[f]	NC
			EC90 (µg/L)	351.2*** (121.4 - 580.9)[c]	292.5*** (87.4 - 497.6)[f]	212.0*** (109.8 - 314.1)[f]	967.1*** (300.3 - 1634.0)[c]	1133.1*** (195.2 - 2071.1)[f]	522.4*** (346.6 - 698.2)[f]	27542.1 *** (10989.7 - 44094.5)[f]	NC
			NOEC (µg/L)	44.9	44.9	44.9	44.9	44.9	44.9	5.1	5.1
			LOEC (µg/L)	88.6	88.6	88.6	88.6	88.6	88.6	10.8	10.8
		R	CV (%)	15.4	13.6	10.5	2.4	3.1	2.2	0.7	3.3
			EC01 (µg/L)	38.5 (8.5 - 68.5)[c]	39.2 (11.5 - 66.9)[c]	6.5 (-1.7 - 14.6)[f]	NC	NC	NC	NC	NC

Table B11. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC10 (µg/L)	48.8 (21.3 - 76.4)[c]	48.8 (22.8 - 74.7)[c]	24.0 (8.0 - 40.0)[f]	NC	NC	NC	NC	NC
			EC25 (µg/L)	66.6 (39.5 - 93.7)[c]	65.5 (39.5 - 91.5)[c]	43.8 (25.3 - 62.4)[f]	NC	NC	NC	NC	NC
			EC50 (µg/L)	104.5 (66.2 - 142.8)[c]	101.9 (64.8 - 139.1)[c]	80.0 (57.0 - 103.1)[f]	NC	NC	NC	NC	NC
			EC90 (µg/L)	316.8*** (70.3 - 563.3)[c]	309.5*** (70.6 - 548.4)[c]	266.7*** (99.8 - 433.5)[f]	NC	NC	NC	NC	NC
			NOEC (µg/L)	44.9	44.9	44.9	≥ 186.8	≥ 186.8	≥ 186.8	≥ 186.8	≥ 186.8
			LOEC (µg/L)	88.6	88.6	88.6	> 186.8	> 186.8	> 186.8	> 186.8	> 186.8
	14	E	CV (%)	17.5	13.6	22.3	3.5	2.6	3.7	1.9	1.2
			EC01 (µg/L)	18.3 (-2.4 - 38.9)[f]	15.4 (-0.6 - 31.5)[f]	9.2 (-1.1 - 19.6)[f]	36.2 (14.0 - 58.4)[c]	11.8 (4.2 - 19.5)[f]	24.9 (9.3 - 40.5)[c]	0.6 (-0.6 - 1.8)[f]	NC
			EC10 (µg/L)	37.9 (15.2 - 60.7)[f]	34.4 (15.6 - 53.2)[f]	23.3 (9.1 - 37.4)[f]	67.1 (49.4 - 84.8)[c]	55.4 (39.6 - 71.3)[f]	50.5 (38.7 - 62.2)[c]	47.0 (20.4 - 73.6)[f]	NC
			EC25 (µg/L)	53.0 (32.5 - 73.5)[f]	49.7 (32.2 - 67.1)[f]	35.5 (21.4 - 49.6)[f]	114.7 (98.4 - 131.0)[c]	112.4 (95.8 - 129.1)[f]	89.1 (78.1 - 100.2)[c]	343.6*** (221.2 - 466.0) [f]	NC

Table B11. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC50 (µg/L)	74.0 (54.0 - 94.1)[f]	71.7 (53.8 - 89.7)[f]	54.2 (40.3 - 68.0)[f]	214.2*** (183.3 - 245.1)[c]	228.1*** (194.7 - 261.4)[f]	170.1 (153.3 - 186.9)[c]	2513.5*** (283.0 - 4744.0)[f]	NC
			EC90 (µg/L)	144.5 (63.0 - 226.1)[f]	149.6 (69.4 - 229.8)[f]	126.2 (64.6 - 187.8)[f]	817.9*** (462.2 - 1173.7)[c]	938.4*** (521.1 - 1355.74)[f]	668.6*** (467.1 - 870.0)[c]	134481.1*** (-157243.2 - 425205.4)[f]	NC
			NOEC (µg/L)	44.9	44.9	44.9	44.9	44.9	44.9	5.1	≥ 186.8
			LOEC (µg/L)	88.6	88.6	88.6	88.6	88.6	88.6	10.8	> 186.8
		R	CV (%)	20.0	17.5	22.1	2.0	3.5	7.9	2.4	0.6
			EC01 (µg/L)	45.8 (29.8 - 61.8)[c]	41.3 (26.6 - 55.9)[c]	33.8 (22.1 - 45.6)[a]	36.4 (-5.9 - 78.7)[f]	83.4 (NC)[b]	NC	NC	NC
			EC10 (µg/L)	50.5 (34.9 - 66.1)[c]	46.2 (32.2 - 60.2)[c]	38.5 (27.1 - 49.8)[a]	116.5 (73.9 - 159.0)[f]	135.4 (96.9 - 173.9)[b]	NC	NC	NC
			EC25 (µg/L)	58.9 (42.9 - 74.9)[c]	55.0 (41.0 - 68.9)[c]	46.7 (35.5 - 57.8)[a]	198.4 (163.6 - 233.1)[f]	242.6 (NC)[b]	NC	NC	NC
			EC50 (µg/L)	76.9 (55.4 - 98.3)[c]	73.3 (55.2 - 91.5)[c]	64.0 (50.0 - 78.0)[a]	337.9*** (182.1 - 493.8)[f]	542.0*** (NC)[b]	NC	NC	NC

Table B11. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC90 (µg/L)	164.1*** (71.1 - 257.2)[c]	162.1*** (78.4 - 245.8)[c]	151.0 (81.1 - 220.8)[a]	980.6*** (-192.5 - 2153.8)[f]	3669.4*** (NC)[b]	NC	NC	NC
			NOEC (µg/L)	44.9	44.9	44.9	<5.1*	<5.1*	≥ 186.8	≥ 186.8	44.9
			LOEC (µg/L)	88.6	88.6	88.6	≤5.1*	≤5.1*	> 186.8	> 186.8	88.6
	21	E	CV (%)	22.6	20.5	19.9	2.8	2.6	2.2	3.5	1.1
			EC01 (µg/L)	15.7 (1.7 - 29.6)[f]	17.1 (2.2 - 31.9)[f]	8.1 (-1.4 - 17.5)[f]	16.3 (10.7 - 21.8)[f]	15.3 (9.2 - 21.3)[f]	16.0 (6.0 - 26.0)[f]	NC	NC
			EC10 (µg/L)	31.7 (16.4 - 47.0)[f]	33.4 (17.6 - 49.2)[f]	20.0 (6.9 - 33.2)[f]	59.0 (49.6 - 68.4)[f]	59.2 (48.5 - 69.9)[f]	51.9 (35.8 - 68.1)[f]	NC	NC
			EC25 (µg/L)	43.8 (29.7 - 57.9)[f]	45.4 (31.1 - 59.8)[f]	30.4 (17.1 - 43.7)[f]	106.5 (97.3 - 115.7)[f]	110.2 (99.6 - 120.8)[f]	89.1 (72.7 - 105.4)[f]	NC	NC
			EC50 (µg/L)	60.5 (46.4 - 74.6)[f]	61.8 (47.7 - 75.9)[f]	46.2 (33.5 - 58.9)[f]	192.3*** (179.3 - 205.3)[f]	205.0 (188.1 - 221.9)[f]	152.7 (134.0 - 171.5)[f]	NC	NC
			EC90 (µg/L)	115.32 (64.2 - 166.5)[f]	114.38 (65.4 - 163.4)[f]	106.43 (57.0 - 155.9)[f]	626.81*** (495.4 - 758.2)[f]	710.03*** (528.5 - 891.6)[f]	449.1*** (299.4 - 598.7)[f]	NC	NC
			NOEC (µg/L)	44.9	44.9	21.4	44.9	44.9	44.9	21.4	5.1*
			LOEC (µg/L)	88.6	88.6	44.9	88.6	88.6	88.6	44.9	10.8*
		R	CV (%)	23.5	21.4	21.8	0.3	0.3	1.0	0.6	2.5

Table B11. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC01 (µg/L)	0 (-0 - 0)[b]	13.7 (1.0 - 26.6)[f]	10.9 (0.3 - 21.4)[f]	9.3 (-9.4 - 27.9)[f]	28.8 (-17.9 - 75.5)[f]	NC	NC	NC
			EC10 (µg/L)	4.1 (-28.6 - 36.7)[b]	28.7 (14.4 - 43.0)[f]	24.1 (11.4 - 36.8)[f]	65.5 (18.3 - 112.7)[f]	92.8 (35.4 - 150.2)[f]	NC	NC	NC
			EC25 (µg/L)	33.1 (17.7 - 48.5)[b]	40.3 (27.6 – 53.0)[f]	34.7 (23.0 - 46.4)[f]	160.5 (116.8 - 204.3)[f]	158.5 (120.6 - 196.3)[f]	NC	NC	NC
			EC50 (µg/L)	51.3 (40.0 - 62.5)[b]	56.5 (45.5 - 67.4)[f]	50.0 (39.8 - 60.2)[f]	393.4*** (153.8 - 633.0)[f]	270.8*** (158.1 - 383.4)[f]	NC	NC	NC
			EC90 (µg/L)	100.2 (59.0 - 141.4)[b]	111.0 (65.0 - 156.9)[f]	103.8 (61.4 - 146.3)[f]	2362.9*** (-1855.9 - 6581.7)[f]	790.3*** (-249.4 - 1830.0)[f]	NC	NC	NC
			NOEC (µg/L)	21.4	21.4	44.9	44.9	88.6	88.6	<5.1*	88.6
			LOEC (µg/L)	44.9	44.9	88.6	88.6	186.8	186.8	≤5.1*	186.8
	28	E	CV (%)	28.6	33.0	25.6	2.7	3.2	2.3	2.2	1.4
			EC01 (µg/L)	10.4 (-2.2 - 23.0)[f]	10.7 (-3.1 - 24.6)[f]	7.2 (-1.2 - 15.7)[f]	15.4 (11.4 - 19.4)[f]	15.1 (10.4 - 19.7)[f]	14.5 (10.8 - 18.1)[f]	NC	NC
			EC10 (µg/L)	23.0 (7.8 - 38.2)[f]	23.4 (7.2 - 39.6)[f]	17.7 (6.1 - 29.3)[f]	56.2 (49.3 - 63.2)[f]	57.3 (49.2 - 65.5)[f]	51.5 (45.2 - 57.8)[f]	NC	NC

Table B11. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC25 (μg/L)	33.1 (18.9 - 47.3)[f]	33.4 (18.5 - 48.3)[f]	26.6 (14.8 - 38.4)[f]	101.8 (94.9 - 108.7)[f]	105.7 (97.7 - 113.8)[f]	92.2 (85.7 - 98.6)[f]	NC	NC
			EC50 (μg/L)	47.6 (34.9 - 60.3)[f]	47.7 (34.2 - 61.2)[f]	40.1 (28.6 - 51.6)[f]	184.3 (175.0 - 193.6)[f]	195.1 (183.4 - 206.8)[f]	164.9 (157.0 - 172.8)[f]	NC	NC
			EC90 (μg/L)	98.5 (47.7 - 149.2)[f]	97.2 (40.7 - 153.8)[f]	90.8 (48.3 - 133.3)[f]	604.2*** (509.9 - 698.4)[f]	663.9*** (537.6 - 790.3)[f]	527.7*** (453.6 - 601.8)[f]	NC	NC
			NOEC (μg/L)	44.9	44.9	21.4	21.4	21.4	21.4	5.1	5.1
			LOEC (μg/L)	88.6	88.6	44.9	44.9	44.9	44.9	10.8	10.8
		R	CV (%)	28.6	28.5	27.1	3.0	3.0	0.6	1.6	1.0
			EC01 (μg/L)	4.5 (-2.4 - 11.5)[f]	3.9 (-2.5 - 10.3)[f]	7.1 (-2.9 - 17.2)[f]	NC	15.5 (-20.9 - 51.8)[f]	NC	NC	NC
			EC10 (μg/L)	13.7 (1.7 - 25.7)[f]	12.6 (0.7 - 24.4)[f]	18.4 (3.7 - 33.0)[f]	NC	85.5 (20.8 - 150.2)[f]	NC	NC	NC
			EC25 (μg/L)	22.7 (8.8 - 36.6)[f]	21.5 (7.4 - 35.6)[f]	28.4 (13.0 - 43.7)[f]	NC	187.2*** (138.1 - 236.3)[f]	NC	NC	NC
			EC50 (μg/L)	37.7 (21.4 - 54.0)[f]	36.8 (19.9 - 53.6)[f]	43.8 (28.5 - 59.0)[f]	NC	410.0*** (72.5 - 747.6)[f]	NC	NC	NC

Table B11. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC90 (μg/L)	103.76 (35.4 - 172.2)[f]	107.58 (33.8 - 181.4)[f]	104.21 (47.9 - 160.6)[f]	NC	1966.4*** (-2532.3 - 6465.2)[f]	NC	NC	NC
			NOEC (μg/L)	44.9	21.4	44.9	88.6	88.6	44.9*	≥ 186.8	88.6
			LOEC (μg/L)	88.6	44.9	88.6	186.8	186.8	88.6*	> 186.8	186.8

PSII yield – Photosystem II effective quantum yield,
NC Not calculated because the endpoint at the highest tested concentration did not reach sufficient inhibition to determine the ECX values,
* LOEC was statistically different from the control, but the next higher concentration was not significantly different from the control,
*** ECX values greater than the tested concentration (186.8 μg/L),
[a] The Cedergreen-Ritz-Streibig model (α=0.10),
[b] The Cedergreen-Ritz-Streibig model (α=0.25),
[c] The Cedergreen-Ritz-Streibig model (α=0.50),
[d] The Cedergreen-Ritz-Streibig model (α=0.75),
[e] The Cedergreen-Ritz-Streibig model (α=1.00),
[f] log-logistic model.

Table B12. The toxicity values for absolute and relative growth rate (RGR) on *L. minor* after 7, 14, 21 and 28 days of atrazine exposure and followed by a 7-day period of recovery.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
Lemna minor	7	E	CV (%)	11.5	25.0	15.7	5.0	6.3	6.1	1.2	1.8
			EC01 (µg/L)	9.5 (-0.4 - 19.4)[f]	7.8 (0.3 - 15.3)[f]	5.97 (-1.7 - 13.7)[f]	10.3 (2.0 - 18.6)[f]	8.3 (1.5 - 15.2)[f]	9.3 (2.0 - 16.7)[f]	0.3 (-0.1 - 0.4)[f]	NC
			EC10 (µg/L)	33.0 (15.4 - 50.7)[f]	29.9 (15.1 - 44.7)[f]	22.3 (7.1 - 37.4)[f]	49.0 (31.1 - 67.0)[f]	46.8 (29.6 - 63.9)[f]	36.5 (21.9 - 51.1)[f]	18.1 (9.0 - 27.1)[f]	NC
			EC25 (µg/L)	58.4 (39.2 - 77.7)[f]	55.4 (38.4 - 72.3)[f]	40.8 (23.5 - 58.0)[f]	100.1 (80.7 - 119.4)[f]	103.1 (83.6 - 122.6)[f]	68.3 (51.8 - 84.7)[f]	154.7 (117.7 - 191.8)[f]	NC
			EC50 (µg/L)	103.4 (80.4 - 126.3)[f]	102.5 (81.0 - 124.1)[f]	74.5 (56.3 - 92.8)[f]	204.2*** (170.4 - 238.0)[f]	227.2*** (186.0 - 268.4)[f]	127.7 (107.5 - 147.8)[f]	1323.9*** (727.0 - 1920.8)[f]	NC
			EC90 (µg/L)	323.4*** (151.5 - 495.4)[f]	351.4*** (175.6 - 527.2)[f]	249.2*** (108.4 - 390.0)[f]	850.4*** (411.5 - 1289.3)[f]	1103.8*** (485.5 - 1722.0)[f]	446.8*** (264.1 - 629.4)[f]	96938.4*** (-22245.0 - 216121.8)[f]	NC
			NOEC (µg/L)	44.9	44.9	44.9	44.9	44.9	44.9	<5.1	88.6
			LOEC (µg/L)	88.6	88.6	88.6	88.6	88.6	88.6	≤5.1	186.8
		R	CV (%)	22.5	18.5	17.9	4.8	4.9	2.3	1.2	0.8

Table B12. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC01 (µg/L)	3.6 (-2.9 - 10.1)[f]	18.5 (1.1 - 35.9)[c]	3.40 (-2.04 - 8.8)[f]	NC	NC	NC	NC	NC
			EC10 (µg/L)	19.1 (1.1 - 37.1)[f]	27.8 (9.6 - 46.0)[c]	18.1 (2.8 - 33.4)[f]	NC	NC	NC	NC	NC
			EC25 (µg/L)	40.8 (16.5 - 65.2)[f]	46.1 (25.1 - 67.1)[c]	38.9 (18.3 - 59.6)[f]	NC	NC	NC	NC	NC
			EC50 (µg/L)	87.4 (53.9 - 121.0)[f]	94.9 (61.2 - 128.5)[c]	83.7 (55.7 - 111.8)[f]	NC	NC	NC	NC	NC
			EC90 (µg/L)	400.7*** (60.2 - 741.1)[f]	551.9*** (-0.6 - 1104.3)[c]	387.5*** (99.0 - 675.9)[f]	NC	NC	NC	NC	NC
			NOEC (µg/L)	44.9	44.9	44.9	≥ 186.8	≥ 186.8	≥ 186.8	≥ 186.8	<5.1*
			LOEC (µg/L)	88.6	88.6	88.6	> 186.8	> 186.8	> 186.8	> 186.8	≤5.1*
	14	E	CV (%)	21.6	21.1	10.3	4.7	4.4	1.9	1.8	2.0
			EC01 (µg/L)	24.6 (9.8 - 39.41)[a]	23.1 (7.7 - 38.4)[a]	24.7 (11.6 - 37.8)[a]	27.1 (12.0 - 42.3)[a]	24.18 (9.0 - 39.4)[b]	26.3 (8.4 - 44.2)[a]	NC	NC
			EC10 (µg/L)	30.7 (15.6 - 45.7)[a]	29.3 (14.3 - 44.4)[a]	30.0 (16.8 - 43.1)[a]	55.7 (40.3 - 71.0)[a]	52.3 (37.3 - 67.5)[b]	50.3 (34.1 - 66.5)[a]	NC	NC

Table B12. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC25 (µg/L)	41.9 (26.1 - 57.7)[a]	40.3 (25.2 - 55.5)[a]	39.5 (26.1 - 52.9)[a]	109.1 (92.5 - 125.8)[a]	106.1 (89.3 - 122.9)[b]	91.0 (74.7 - 107.4)[a]	NC	NC
			EC50 (µg/L)	68.1 (47.7 - 88.5)[a]	64.3 (46.1 - 82.5)[a]	60.8 (45.2 - 76.4)[a]	245.1*** (200.7 - 289.6)[a]	247.0*** (199.7 - 294.4)[b]	184.1 (155.0 - 213.2)[a]	NC	NC
			EC90 (µg/L)	241.0*** (64.6 - 417.4)[a]	203.7*** (65.8 - 341.7)[a]	187.1*** (68.3 - 305.8)[a]	1436.4*** (606.1 - 2266.8)[a]	1564.9*** (583.9 - 2545.9)[b]	847.5*** (436.3 - 1258.8)[a]	NC	NC
			NOEC (µg/L)	44.9	44.9	<5.1*	44.9	44.9	44.9	5.1	≥ 186.8
			LOEC (µg/L)	88.6	88.6	≤5.1*	88.6	88.6	88.6	10.8	> 186.8
		R	CV (%)	31.6	32.8	37.8	7.1	7.3	11.2	2.6	1.1
			EC01 (µg/L)	40.1 (19.1 - 61.0)[a]	44.0 (21.8 - 66.2)[a]	12.0 (-4.5 - 28.5)[f]	NC	NC	NC	NC	NC
			EC10 (µg/L)	45.6 (24.7 - 66.5)[a]	49.3 (27.2 - 71.4)[a]	28.5 (7.5 - 49.5)[f]	NC	NC	NC	NC	NC
			EC25 (µg/L)	55.9 (34.1 - 77.6)[a]	59.1 (36.0 - 82.2)[a]	42.4 (21.2 - 63.5)[f]	NC	NC	NC	NC	NC
			EC50 (µg/L)	79.6 (49.4 - 109.8)[a]	81.4 (49.0 - 113.7)[a]	62.9 (38.3 - 87.5)[f]	NC	NC	NC	NC	NC

Table B12. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC90 (µg/L)	219.2*** (33.1 - 405.2)[a]	206.6*** (29.6 - 383.7)[a]	138.9 (35.4 - 242.5)[f]	NC	NC	NC	NC	NC
			NOEC (µg/L)	<5.1*	88.6	<5.1*	≥ 186.8	≥ 186.8	44.9	≥ 186.8	≥ 186.8
			LOEC (µg/L)	≤5.1*	186.8	≤5.1*	> 186.8	> 186.8	88.6	> 186.8	> 186.8
	21	E	CV (%)	44.7	45.7	29.4	5.5	5.4	3.5	2.3	2.2
			EC01 (µg/L)	29.4 (11.7 - 47.1)[a]	27.0 (9.4 - 44.5)[a]	21.3 (8.6 - 34.0)[a]	35.3 (21.0 - 49.5)[a]	32.2 (17.9 - 46.4)[a]	26.4 (13.8 - 39.1)[a]	0.8 (-0.1 - 1.6)[f]	NC
			EC10 (µg/L)	33.4 (15.2 - 51.6)[a]	31.1 (13.2 - 48.9)[a]	25.0 (11.8 - 38.1)[a]	63.1 (49.0 - 77.3)[a]	61.1 (47.2 - 75.0)[a]	53.4 (41.8 - 65.0)[a]	40.3 (24.8 - 55.8)[f]	NC
			EC25 (µg/L)	41.3 (21.9 - 60.8)[a]	38.9 (20.4 - 57.4)[a]	32.0 (17.9 - 46.0)[a]	114.4 (99.4 - 129.4)[a]	113.5 (98.9 - 128.1)[a]	99.6 (87.9 - 111.4)[a]	250.1*** (198.7 - 301.5)[f]	NC
			EC50 (µg/L)	60.7 (33.8 - 87.7)[a]	57.3 (33.7 - 80.9)[a]	48.8 (30.9 - 66.8)[a]	240.7*** (203.2 - 278.3)[a]	241.3*** (204.8 - 277.7)[a]	207.1*** (183.2 - 230.9)[a]	1553.1*** (801.6 - 2304.6)[f]	NC
			EC90 (µg/L)	192.5*** (-5.1 - 390.0)[a]	172.7*** (5.6 - 339.8)[a]	160.9 (26.0 - 295.7)[a]	1268.2*** (661.4 - 1875.0)[a]	1267.5*** (676.2 - 1858.8)[a]	1002.0*** (639.8 - 1364.3)[a]	59885.4*** (-13728.9 - 133499.8)[f]	NC
			NOEC (µg/L)	<5.1*	44.9	<5.1*	<5.1*	44.9	44.9	10.8	≥ 186.8

Table B12. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			LOEC (µg/L)	≤5.1*	88.6	≤5.1*	≤5.1*	88.6	88.6	21.4	> 186.8
		R	CV (%)	32.1	36.2	49.2	5.7	4.7	7.9	2.7	0.8
			EC01 (µg/L)	20.6 (4.5 - 36.7)[a]	22.7 (0.5 - 44.9)[a]	31.6 (1.4 - 61.9)[a]	NC	NC	NC	NC	NC
			EC10 (µg/L)	24.1 (6.24 - 42.0)[a]	26.5 (2.6 - 50.5)[a]	36.28 (2.6 - 69.9)[a]	NC	NC	NC	NC	NC
			EC25 (µg/L)	31.7 (10.1 - 53.3)[a]	34.6 (7.6 - 61.7)[a]	46.6 (4.6 - 88.5)[a]	NC	NC	NC	NC	NC
			EC50 (µg/L)	54.3 (18.7 - 89.9)[a]	57.0 (18.5 - 95.5)[a]	77.9 (0.8 - 155.0)[a]	NC	NC	NC	NC	NC
			EC90 (µg/L)	322.9*** (-224.7 - 870.4)[a]	268.3*** (-248.5 - 785.1)[a]	477.1*** (-642.1 - 1596.2)[a]	NC	NC	NC	NC	NC
			NOEC (µg/L)	<5.1*	<5.1*	<5.1*	≥ 186.8	≥ 186.8	≥ 186.8	≥ 186.8	≥ 186.8
			LOEC (µg/L)	≤5.1*	≤5.1*	≤5.1*	> 186.8	> 186.8	> 186.8	> 186.8	> 186.8
	28	E	CV (%)	53.8	33.0	36.0	5.9	3.3	3.2	1.2	1.0
			EC01 (µg/L)	21.9 (3.7 - 40.1)[a]	19.1 (4.8 - 33.4)[a]	17.1 (6.5 - 27.7)[a]	34.6 (19.9 - 49.4)[a]	29.4 (17.2 - 41.6)[a]	27.7 (17.2 - 38.1)[a]	0.2 (0 - 0.5)[f]	NC

Table B12. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC10 (µg/L)	25.0 (5.1 - 45.0)[a]	21.9 (6.0 - 37.7)[a]	19.6 (7.9 - 31.2)[a]	64.4 (49.8 - 78.9)[a]	58.8 (46.6 - 70.9)[a]	54.6 (45.4 - 63.7)[a]	35.8 (24.2 - 47.42)[f]	NC
			EC25 (µg/L)	31.9 (7.9 - 55.9)[a]	27.9 (8.5 - 47.4)[a]	24.9 (10.92 - 38.8)[a]	118.5 (103.3 - 133.8)[a]	112.6 (99.7 - 125.5)[a]	98.6 (89.6 - 107.6)[a]	355.6*** (279.6 - 431.5)[f]	NC
			EC50 (µg/L)	51.8 (11.9 - 91.6)[a]	46.1 (12.7 - 79.4)[a]	40.1 (17.6 - 62.7)[a]	251.3*** (210.9 - 291.7)[a]	246.6*** (212.4 - 280.9)[a]	196.3*** (179.9 - 212.7)[a]	3552.3*** (1809.4 - 5255.3)[f]	NC
			EC90 (µg/L)	267.9 (-210.0 - 745.8)[a]	264.9 (-191.9 - 721.6)[a]	200.4 (-59.6 - 460.3)[a]	1323.4*** (666.4 - 1980.5)[a]	1369.1*** (767.6 - 1970.6)[a]	861.8*** (635.6 - 1087.9)[a]	348606.3*** (-60780.9 - 757993.3)[f]	NC
			NOEC (µg/L)	44.9	<5.1*	<5.1*	<5.1*	<5.1*	<5.1*	<5.1*	5.1
			LOEC (µg/L)	88.6	≤5.1*	≤5.1*	≤5.1*	≤5.1*	≤5.1*	≤5.1*	10.8
		R	CV (%)	77.8	78.6	69.5	9.8	17.4	15.2	2.2	0.4
			EC01 (µg/L)	25.4 (5.5 - 45.2)[a]	23.4 (4.2 - 42.5)[a]	24.1 (6.1 - 42.2)[a]	NC	NC	NC	NC	NC
			EC10 (µg/L)	28.8 (8.0 - 49.6)[a]	26.8 (6.8 - 46.9)[a]	27.5 (8.0 - 46.9)[a]	NC	NC	NC	NC	NC

Table B12. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			EC25 (µg/L)	35.8 (12.8 - 58.8)[a]	33.7 (11.7 - 55.7)[a]	34.5 (11.8 - 57.2)[a]	NC	NC	NC	NC	NC
			EC50 (µg/L)	53.6 (21.0 - 86.2)[a]	51.2 (21.0 - 81.4)[a]	54.0 (18.4 - 89.7)[a]	NC	NC	NC	NC	NC
			EC90 (µg/L)	187.5 (-67.2 - 442.2)[a]	181.7 (-63.7 - 427.1)[a]	237.2 (-115.0 - 589.3)[a]	NC	NC	NC	NC	NC
			NOEC (µg/L)	44.9	44.9	<5.1*	≥ 186.8	≥ 186.8	88.6	88.6*	≥ 186.8
			LOEC (µg/L)	88.6	88.6	≤5.1*	> 186.8	> 186.8	186.8	186.8*	> 186.8

PSII yield – Photosystem II effective quantum yield,
NC Not calculated because the endpoint at the highest tested concentration did not reach sufficient inhibition to determine the ECX values,
* LOEC was statistically different from the control, but the next higher concentration was not significantly different from the control,
*** ECX values greater than the tested concentration (186.8 µg/L),
[a] The Cedergreen-Ritz-Streibig model (α=0.10),
[b] The Cedergreen-Ritz-Streibig model (α=0.25),
[c] The Cedergreen-Ritz-Streibig model (α=0.50),
[d] The Cedergreen-Ritz-Streibig model (α=0.75),
[e] The Cedergreen-Ritz-Streibig model (α=1.00),
[f]log-logistic model.

Table B13. The parameters for concentration-response models for *L. gibba* after 7, 14, 21 and 28 days of atrazine exposure and followed by a 7-day period of recovery

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
Lemna gibba	7	E	b	1.9[c]	2.1[f]	2.2[f]	1.5[c]	1.4[f]	1.8[f]	0.6[f]	NC
			Lower limit	6[c]	2[f]	0[f]	0[c]	0[f]	0[f]	0[f]	NC
			Upper limit	72.6[c]	28.8[f]	0[f]	0.4[c]	0.4[f]	0.5[f]	0.8[f]	NC
			e	99.4[c]	102[f]	76.9[f]	211[c]	240[f]	154[f]	917[f]	NC
			f	13.9[c]	NA[f]	NA[f]	0.031[c]	NA[f]	NA[f]	NA[f]	NC
		R	b	1.9[c]	1.8[c]	1.8[f]	NC	NC	NC	NC	NC
			Lower limit	6[c]	2[c]	0[f]	NC	NC	NC	NC	NC
			Upper limit	1100[c]	406[c]	0.238[f]	NC	NC	NC	NC	NC
			e	85.0[c]	80.5[c]	80.0[f]	NC	NC	NC	NC	NC
			f	280[c]	120[c]	NA[f]	NC	NC	NC	NC	NC
	14	E	b	3.3[f]	3.0[f]	2.6[f]	1.6[c]	1.6[f]	1.6[c]	0.6[f]	NC
			Lower limit	6[f]	2[f]	0[f]	0[c]	0[f]	0[c]	0[f]	NC
			Upper limit	1030[f]	376[f]	0.2[f]	0.4[c]	0.4[f]	0.4[c]	0.7[f]	NC
			e	74.0[f]	71.7[f]	54.2[f]	200[c]	228[f]	160[c]	2510[f]	NC
			f	NA[f]	NA[f]	NA[f]	0.023[c]	NA[f]	0.022[c]	NA[f]	NC
		R	b	2.6[c]	2.5[c]	2.3[a]	2.1[f]	1.1[b]	NC	NC	NC
			Lower limit	6[c]	2[c]	0[a]	0[f]	0[b]	NC	NC	NC
			Upper limit	11000[c]	4300[c]	2.7[a]	0.4[f]	0.4[b]	NC	NC	NC
			e	56.9[c]	56.2[c]	49.0[a]	338[f]	403[b]	NC	NC	NC
			f	7130[c]	2250[c]	2.26[a]	NA[f]	0.083[b]	NC	NC	NC
	21	E	b	3.4[f]	3.6[f]	2.6[f]	1.9[f]	1.8[f]	2.0[f]	NC	NC
			Lower limit	6[f]	2[f]	0[f]	0[f]	0[f]	0[f]	NC	NC
			Upper limit	13700[f]	4970[f]	3.1[f]	0.4[f]	0.4[f]	0.4[f]	NC	NC
			e	60.5[f]	61.8[f]	46.2[f]	192[f]	205[f]	153[f]	NC	NC
			f	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NC	NC
		R	b	3.5[b]	3.3[f]	3.0[f]	1.2[f]	2.1[f]	NC	NC	NC
			Lower limit	6[b]	2[f]	0[f]	0[f]	0[f]	NC	NC	NC
			Upper limit	244000[b]	82400[f]	44.0[f]	0.4[f]	0.4[f]	NC	NC	NC
			e	56.3[b]	56.5[f]	50.0[f]	393[f]	271[f]	NC	NC	NC
			f	-49300[b]	NA[f]	NA[f]	NA[f]	NA[f]	NC	NC	NC

Table B13. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
	28	E	b	3.0[f]	3.1[f]	2.7[f]	1.9[f]	1.8[f]	1.9[f]	NC	NC
			Lower limit	6[f]	2[f]	0[f]	0[f]	0[f]	0[f]	NC	NC
			Upper limit	234000[f]	84900[f]	45.1[f]	0.38[f]	0.4[f]	0.4[f]	NC	NC
			e	47.6[f]	47.7[f]	40.1[f]	184[f]	195[f]	165[f]	NC	NC
			f	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NC	NC
		R	b	2.2[f]	2.1[f]	2.5[f]	NC	1.4[f]	NC	NC	NC
			Lower limit	6[f]	2[f]	0[f]	NC	0[f]	NC	NC	NC
			Upper limit	4250000[f]	1590000[f]	750[f]	NC	0.41[f]	NC	NC	NC
			e	37.7[f]	36.8[f]	43.7[f]	NC	410[f]	NC	NC	NC
			f	NA[f]	NA[f]	NA[f]	NC	NA[f]	NC	NC	NC

PSII yield – PSII effective quantum yield,
NC Not calculated because the endpoint at the highest tested concentration did not reach to sufficient inhibition to determine the ECX values,
[a] The Cedergreen-Ritz-Streibig model (α=0.10),
[b] The Cedergreen-Ritz-Streibig model (α=0.25),
[c] The Cedergreen-Ritz-Streibig model (α=0.50),
[d] The Cedergreen-Ritz-Streibig model (α=0.75),
[e] The Cedergreen-Ritz-Streibig model (α=1.00),
[f] log-logistic model.

Table B14. The parameters for concentration-response models for *Lemna minor* after 7, 14, 21, and 28 days of atrazine exposure and followed by a 7-day period of recovery

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
Lemna minor	7	E	b	1.9[f]	1.8[f]	1.8[f]	1.5[f]	1.4[f]	1.8[f]	0.5[f]	NC
			Lower limit	6[f]	2[f]	0[f]	0[f]	0[f]	0[f]	0[f]	NC
			Upper limit	63.9[f]	24.3[f]	0.0[f]	0.3[f]	0.4[f]	0.4[f]	0.8[f]	NC
			e	103[f]	103[f]	74.5[f]	204[f]	227[f]	128[f]	1320[f]	NC
			f	NC[f]	NC[f]	NC[f]	NC[f]	NC[f]	NC[f]	NA[f]	NC
		R	b	1.4[f]	1.2[c]	1.4[f]	NC	NC	NC	NC	NC
			Lower limit	6[f]	2[c]	0[f]	NC	NC	NC	NC	NC
			Upper limit	1100[f]	398[c]	0.1[f]	NC	NC	NC	NC	NC
			e	87.4[f]	68.3[c]	83.7[f]	NC	NC	NC	NC	NC
			f	NC[f]	103[c]	NC[f]	NC	NC	NC	NC	NC
	14	E	b	1.6[a]	1.8[a]	1.8[a]	1.2[a]	1.2[b]	1.4[a]	NC	NC
			Lower limit	6[a]	2[a]	0[a]	0[a]	0[b]	0[a]	NC	NC
			Upper limit	859[a]	351[a]	0.1[a]	0.4[a]	0.4[b]	0.4[a]	NC	NC
			e	49.2[a]	51.5[a]	45.6[a]	217[a]	216[b]	167[a]	NC	NC
			f	560[a]	164[a]	0.1[a]	0.051[a]	0[b]	0.05[a]	NC	NC
		R	b	1.9[a]	2.1[a]	2.8[f]	NC	NC	NC	NC	NC
			Lower limit	6[a]	2[a]	0[f]	NC	NC	NC	NC	NC
			Upper limit	11300[a]	4220[a]	1.50[f]	NC	NC	NC	NC	NC
			e	52.8[a]	54.3[a]	62.9[f]	NC	NC	NC	NC	NC
			f	12900[a]	5290[a]	NC[f]	NC	NC	NC	NC	NC
	21	E	b	1.7[a]	1.8[a]	1.6[a]	1.3[a]	1.3[a]	1.4[a]	0.6[f]	NC
			Lower limit	6[a]	2[a]	0[a]	0[a]	0[a]	0[a]	0[f]	NC
			Upper limit	12300[a]	5170[a]	1.4[a]	0.4[a]	0.4[a]	0.4[a]	0.7[f]	NC
			e	34.0[a]	37.0[a]	31.0[a]	208[a]	213[a]	189[a]	1550[f]	NC
			f	19200[a]	5880[a]	1.58[a]	0.067[a]	0.058[a]	0.046[a]	NA[f]	NC
		R	b	1.0[a]	1.2[a]	1.0[a]	NC	NC	NC	NC	NC
			Lower limit	6[a]	2[a]	0[a]	NC	NC	NC	NC	NC
			Upper limit	178000[a]	70500[a]	11.8[a]	NC	NC		NC	NC
			e	13.1[a]	22.4[a]	11.1[a]	NC	NC	NC	NC	NC
			f	591000[a]	146000[a]	67.0[a]	NC	NC	NC	NC	NC
	28	E	b	1.1[a]	1.0[a]	1.1[a]	1.3[a]	1.3[a]	1.5[a]	0.5[f]	NC
			Lower limit	6[a]	2[a]	0[a]	0[a]	0[a]	0[a]	0[f]	NC

Table B14. Continued.

Species	Duration (day)	Exposure (E) or Recovery (R)	Endpoint	Absolute frond number	Absolute plant number	Absolute dry mass	RGR frond number	RGR plant number	RGR dry mass	PSII yield (light)	PSII yield (dark)
			Upper limit	159000[a]	64700[a]	16.0[a]	0.4[a]	0.4[a]	0.4[a]	0.8[f]	NC
			e	12.0[a]	7.67[a]	10.2[a]	220[a]	219[a]	181[a]	3530[f]	NC
			f	640000[a]	345000[a]	60.2[a]	0.06[a]	0.053[a]	0.042[a]	NA[f]	NC
		R	b	1.5[a]	1.5[a]	1.3[a]	NC	NC	NC	NC	NC
			Lower limit	6[a]	2[a]	0[a]	NC	NC	NC	NC	NC
			Upper limit	2470000[a]	1050000[a]	240[a]	NC	NC	NC	NC	NC
			e	25.7[a]	26.2[a]	17.1[a]	NC	NC		NC	NC
			f	4910000[a]	1790000[a]	753[a]	NC	NC	NC	NC	NC

PSII yield – Photosystem II effective quantum yield,

NC Not calculated because the endpoint at the highest tested concentration did not reach sufficient inhibition to determine the ECX values,

[a] The Cedergreen-Ritz-Streibig model (α=0.10),

[b] The Cedergreen-Ritz-Streibig model (α=0.25),

[c] The Cedergreen-Ritz-Streibig model (α=0.50),

[d] The Cedergreen-Ritz-Streibig model (α=0.75),

[e] The Cedergreen-Ritz-Streibig model (α=1.00),

[f] log-logistic model.

Table B15. The NOEC and LOEC of endpoint (dry mass, frond number and plant number) and their relative growth rate (RGR) of *Lemna gibba* and *Lemna minor* following a 60 day 2,4-D (2,4-Dichlorophenoxyacetic acid) continuous exposure. Significantly different values from control are highlighted in red and the percent difference is indicated in brackets.

Species	**Day**	**Toxicity values (µg/L)**	**Endpoints**					
			Absolute dry mass	***Absolute frond number***	***Absolute plant number***	***RGR dry mass***	***RGR frond number***	***RGR plant number***
Lemna gibba	7	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3
	14	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3
	21	NOEC	4.3 (- 22.4%)	≥8.3	≥8.3	4.3 (- 3%)	≥8.3	≥8.3
		LOEC	8.3 (- 40.8%)	>8.3	>8.3	8.3 (- 6%)	>8.3	>8.3
	28	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3
	35	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3
	42	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3
	49	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3
	56	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3
	60	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3

Table B15. Continued.

Species	Day	Toxicity values (µg/L)	Endpoints					
			Absolute dry mass	***Absolute frond number***	***Absolute plant number***	***RGR dry mass***	***RGR frond number***	***RGR plant number***
Lemna minor	7	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3
	14	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3	>8.3
	21	NOEC	≥8.3	≥8.3	≥8.3	≥8.3[a]	≥8.3	≥8.3
		LOEC	>8.3	>8.3	>8.3	>8.3[a]	>8.3	>8.3
	28	NOEC	≥8.3	≥8.3	≥8.3[a]	≥8.3	≥8.3[b]	≥8.3[b]
		LOEC	>8.3	>8.3	>8.3[a]	>8.3	>8.3[b]	>8.3[b]
	35	NOEC	≥8.3	≥8.3	≥8.3	≥8.3	≥8.3[c]	≥8.3[c]
		LOEC	>8.3	>8.3	>8.3	>8.3	>8.3[c]	>8.3[c]
	42	NOEC	≥8.3	≥8.3	≥8.3	≥8.3[d]	≥8.3[d]	≥8.3[b]
		LOEC	>8.3	>8.3	>8.3	>8.3[d]	>8.3[d]	>8.3[b]
	49	NOEC	≥8.3	≥8.3	≥8.3	≥8.3[c]	≥8.3[c]	≥8.3[c]
		LOEC	>8.3	>8.3	>8.3	>8.3[c]	>8.3[c]	>8.3[c]
	56	NOEC	≥8.3	≥8.3[a]	≥8.3[a]	≥8.3[c]	≥8.3[c]	≥8.3[c]
		LOEC	>8.3	>8.3[a]	>8.3[a]	>8.3[c]	>8.3[c]	>8.3[c]
	60	NOEC	≥8.3	≥8.3[a]	≥8.3[a]	≥8.3[c]	≥8.3[c]	≥8.3[c]
		LOEC	>8.3	>8.3[a]	>8.3[a]	>8.3[c]	>8.3[c]	>8.3[c]

[a] Significantly higher than the control at 4.3 µg/L, but not significantly different at the higher concentrations (8.3 µg/L),
[b] Significantly higher than the control at 1.8 and 4.3 µg/L, but not significantly different at the higher concentrations (8.3 µg/L),
[c] Significantly higher than the control at 1.8, 4.3 and 8.3 µg/L,
[d] Significantly higher than the control at 4.3 and 8.3 µg/L.

Table B16. The NOEC and LOEC of photosystem II effective quantum yield (PSII yield) on light- and dark-adapted *Lemna gibba* and *Lemna minor* following a 60-day continuous 2,4-D (2,4-Dichlorophenoxyacetic acid) exposure.

Species	**Day**	**Toxicity values (μg/L)**	**Light PSII**	**Dark PSII**
Lemna gibba	7	NOEC	1.8	≥8.3
		LOEC	4.3	>8.3
	14	NOEC	4.3	≥8.3
		LOEC	8.3	>8.3
	21	NOEC	<1.8[a]	≥8.3
		LOEC	≤ 1.8[a]	>8.3
	28	NOEC	1.8[b]	≥8.3
		LOEC	4.3[b]	>8.3
	35	NOEC	≥8.3	≥8.3
		LOEC	>8.3	>8.3
	42	NOEC	4.3	≥8.3
		LOEC	8.3	>8.3
	49	NOEC	≥8.3	<1.8[a]
		LOEC	>8.3	≤ 1.8[a]
	56	NOEC	≥8.3	≥8.3
		LOEC	>8.3	>8.3
	60	NOEC	<1.8[a]	≥8.3
		LOEC	≤ 1.8[a]	>8.3
Lemna minor	7	NOEC	<1.8[a]	≥8.3
		LOEC	≤ 1.8[a]	>8.3
	14	NOEC	≥8.3	<1.8
		LOEC	>8.3	≤ 1.8
	21	NOEC	1.8[b]	≥8.3
		LOEC	4.3[b]	>8.3
	28	NOEC	≥8.3	≥8.3
		LOEC	>8.3	>8.3

Table B16. Continued.

Species	Day	Toxicity values (µg/L)	Light PSII	Dark PSII
	35	NOEC	≥8.3	≥8.3
		LOEC	>8.3	>8.3
	42	NOEC	≥8.3	≥8.3
		LOEC	>8.3	>8.3
	49	NOEC	≥8.3	≥8.3
		LOEC	>8.3	>8.3
	56	NOEC	≥8.3	≥8.3
		LOEC	>8.3	>8.3
	60	NOEC	≥8.3	≥8.3
		LOEC	>8.3	>8.3

[a] Significantly different from the control at 1.8 µg/L but not significantly different at the higher concentration at 4.3 and 8.3,
[b] Significantly different from the control at 4.3 µg/L, but not significantly different at the higher concentrations 8.3 µg/L.

Table B17. The NOEC and LOEC of endpoint (dry mass, frond number and plant number) and their relative growth rate (RGR) of *Lemna gibba* and *Lemna minor* following a 60 day continuous exposure to atrazine. Significantly different values from control are highlighted in red (if any) and the percent difference is indicated in brackets.

Species	**Day**	**Toxicity values (µg/L)**	**Endpoints**					
			Absolute dry mass	***Absolute frond number***	***Absolute plant number***	***RGR dry mass***	***RGR frond number***	***RGR plant number***
Lemna gibba	7	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8
	14	NOEC	≥21.8	≥21.8	≥21.8	≥21.8[a]	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8[a]	>21.8	>21.8
	21	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8
	28	NOEC	≥21.8[a]	≥21.8[a]	≥21.8	≥21.8[a]	≥21.8[a]	≥21.8
		LOEC	>21.8[a]	>21.8[a]	>21.8	>21.8[a]	>21.8[a]	>21.8
	35	NOEC	≥21.8[a]	≥21.8[a]	≥21.8[b]	≥21.8[a]	≥21.8[a]	≥21.8
		LOEC	>21.8[a]	>21.8[a]	>21.8[b]	>21.8[a]	>21.8[a]	>21.8
	42	NOEC	≥21.8[a]	≥21.8[b]	≥21.8[b]	≥21.8[a]	≥21.8[a]	≥21.8
		LOEC	>21.8[a]	>21.8[b]	>21.8[b]	>21.8[a]	>21.8[a]	>21.8
	49	NOEC	≥21.8	≥21.8[b]	≥21.8[b]	≥21.8[a]	≥21.8[a]	≥21.8
		LOEC	>21.8	>21.8[b]	>21.8[b]	>21.8[a]	>21.8[a]	>21.8
	56	NOEC	≥21.8[a]	≥21.8[b]	≥21.8[b]	≥21.8[a]	≥21.8[b]	≥21.8
		LOEC	>21.8[a]	>21.8[b]	>21.8[b]	>21.8[a]	>21.8[b]	>21.8
	60	NOEC	≥21.8	≥21.8	≥21.8	≥21.8[a]	≥21.8[a]	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8[a]	>21.8[a]	>21.8
Lemna minor	7	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8
	14	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8

Table B17. Continued.

Species	Day	Toxicity values (µg/L)	Endpoints					
			Absolute dry mass	***Absolute frond number***	***Absolute plant number***	***RGR dry mass***	***RGR frond number***	***RGR plant number***
	21	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8
	28	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8
	35	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8
	42	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8
	49	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8
	56	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8
	60	NOEC	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8	≥21.8
		LOEC	>21.8	>21.8	>21.8	>21.8	>21.8	>21.8

[a] Significantly higher than the control at 2.6 µg/L, but not significantly different at the higher concentrations,
[b] Significantly higher than the control at 2.6 and 5.08 µg/L, but not significantly different at the higher concentrations.

Table B18. The NOEC and LOEC of photosystem II effective quantum yield (PSII yield) on light- and dark-adapted *Lemna gibba* and *Lemna minor* following a 60-day continuous atrazine exposure.

Species	Day	Toxicity values (µg/L)	Light PSII	Dark PSII
Lemna gibba	7	NOEC	5.08	≥21.8
		LOEC	11.3	>21.8
	14	NOEC	2.6	5.08[c]
		LOEC	5.08	11.3[c]
	21	NOEC	5.08	≥21.8
		LOEC	11.3	>21.8
	28	NOEC	5.08	≥21.8
		LOEC	11.3	>21.8
	35	NOEC	2.6	5.08
		LOEC	5.08	11.3
	42	NOEC	<2.6	2.6
		LOEC	≤ 2.6	5.08
	49	NOEC	<2.6[a]	<2.6[d]
		LOEC	≤ 2.6[a]	≤ 2.6[d]
	56	NOEC	<2.6	≥21.8
		LOEC	≤ 2.6	>21.8
	60	NOEC	2.6	5.08
		LOEC	5.08	11.3
Lemna minor	7	NOEC	2.6	2.6
		LOEC	5.08	5.08
	14	NOEC	5.08	≥21.8
		LOEC	11.3	>21.8
	21	NOEC	5.08	≥21.8
		LOEC	11.3	>21.8
	28	NOEC	<2.6[a]	<2.6[b]
		LOEC	≤ 2.6[a]	≤ 2.6[b]

Table B18. Continued.

Species	Day	Toxicity values (µg/L)	Light PSII	Dark PSII
	35	NOEC	5.08	≥21.8
		LOEC	11.3	>21.8
	42	NOEC	5.08	<2.6
		LOEC	11.3	≤ 2.6
	49	NOEC	2.6	5.08
		LOEC	5.08	11.3
	56	NOEC	5.08	≥21.8
		LOEC	11.3	>21.8
	60	NOEC	2.6	11.3
		LOEC	5.08	21.8

[a] Significantly different from the control at 2.6, 11.3, 21.8 µg/L, but not significantly different at 5.08 µg/L,
[b] Significantly different from the control at 2.6 µg/L, but not significantly different at higher concentrations 5.08, 11.3, 21.8 µg/L,
[c] Significantly different from the control at 11.3 µg/L, but not significantly different at 21.8 µg/L,
[d] Significantly different from the control at 2.6 and 11.3 µg/L.

Figures

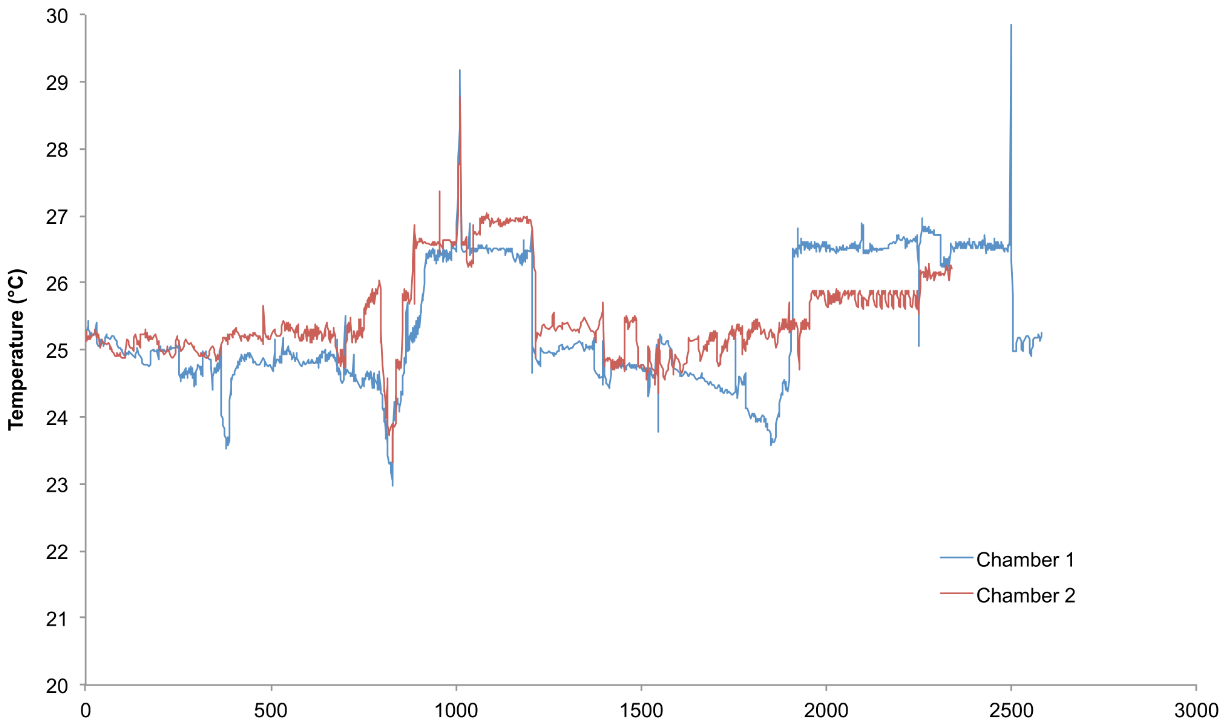

Figure B1. The temperature (°C) in growth chambers during the 28-d exposure and recovery and the 60-d exposure test. Temperature was measured by Tidbit v2 water temperature data logger (UTBI-001). Growth chamber 1 was used for the entire experiment while chamber 2 was used from August 3 to November 29, 2019.

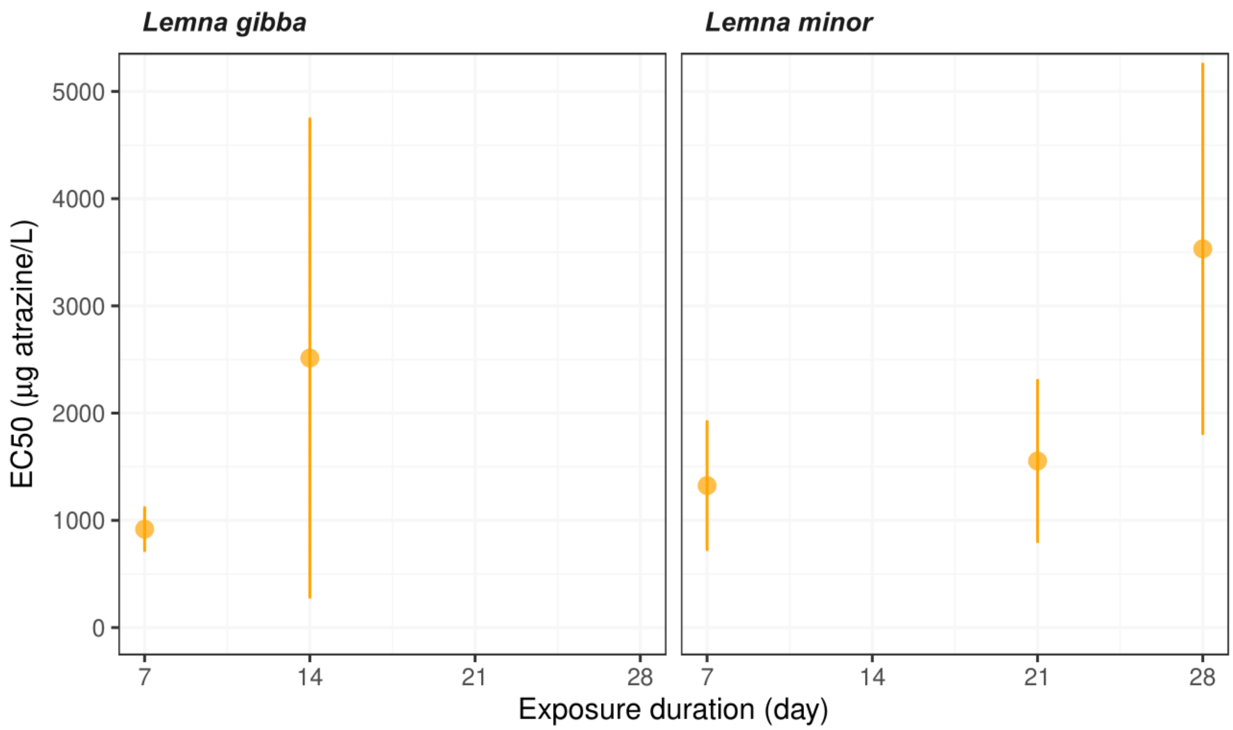

Figure B2. Scatter plot of EC50 values for PSII yield in light-adapted *L. gibba* and *L. minor* after 7-, 14-, 21- and 28-day atrazine exposure periods and followed by a transfer to clean media for a 7-day recovery period. The error bars indicate the 95% confidence interval. Some EC50s were not calculated due to a lack of concentration-response and model convergence.

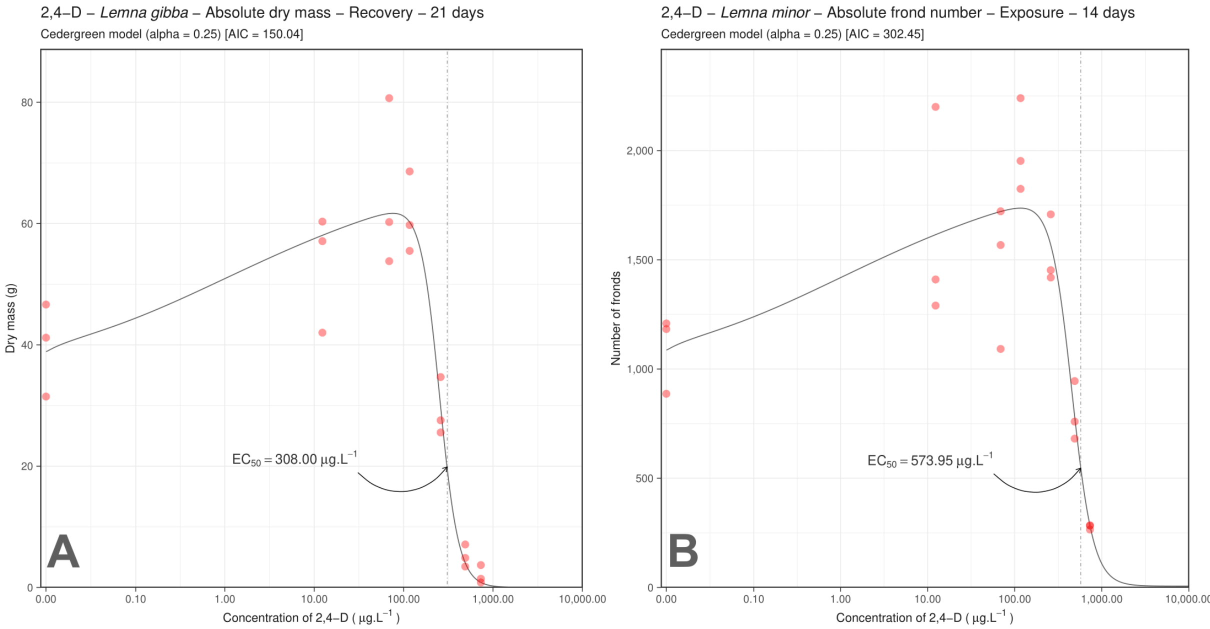

Figure B3. The concentration response curve on (A) *Lemna gibba* absolute dry mass after 21 days of 2,4-D exposure followed by a 7-day recovery period and (B) *Lemna minor* absolute frond number after 14 days of 2,4-D exposure. The curves were plotted by Cedergreen model with α = 0.25 and showed that hermetic effects were found up to concentrations of 75.7 µg/L for *Lemna gibba* and 116.3 µg/L for *Lemna minor*.

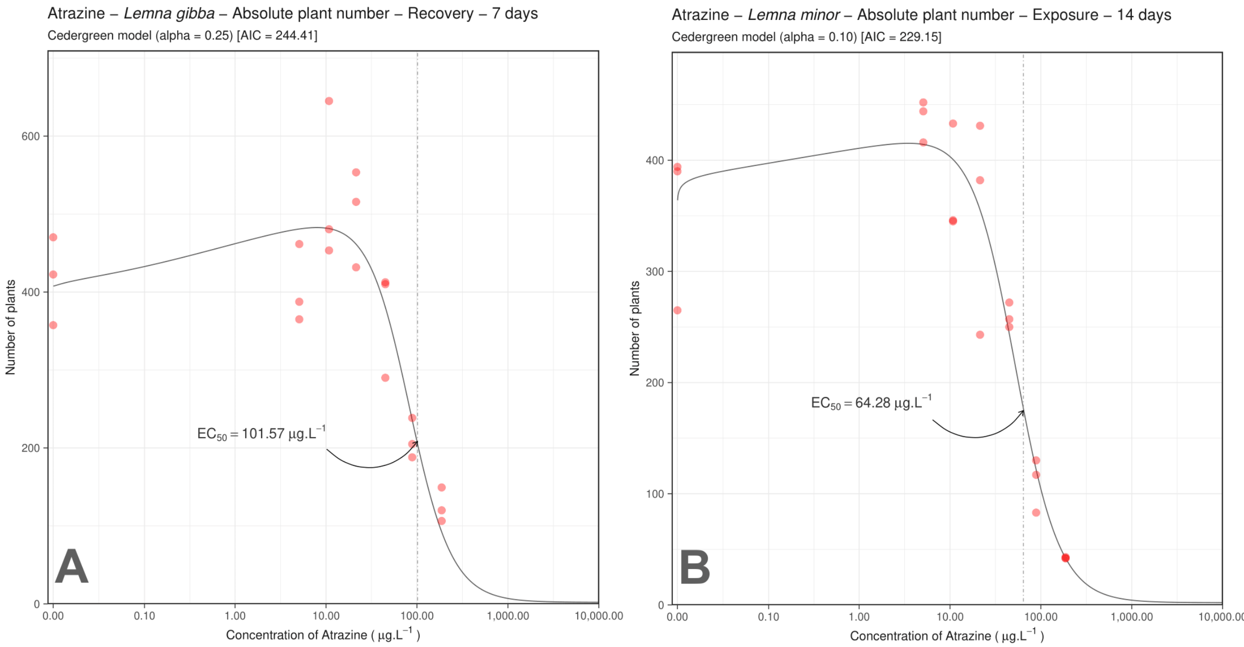

Figure B4. The concentration response curve on (A) *Lemna gibba* absolute plant number after 7 days of atrazine exposure and followed by a 7-day recovery period and (B) *Lemna minor* absolute frond number after 14 days of atrazine exposure. The *Lemna gibba* curve was plotted by Cedergreen model with α = 0.25 and showed that hermetic effects were found up to concentrations of 7.9 µg/L. For *Lemna minor*, the curve was plotted by Cedergreen model with α = 0.1 and showed that hermetic effects were found up to concentrations of 3.5 µg/L.

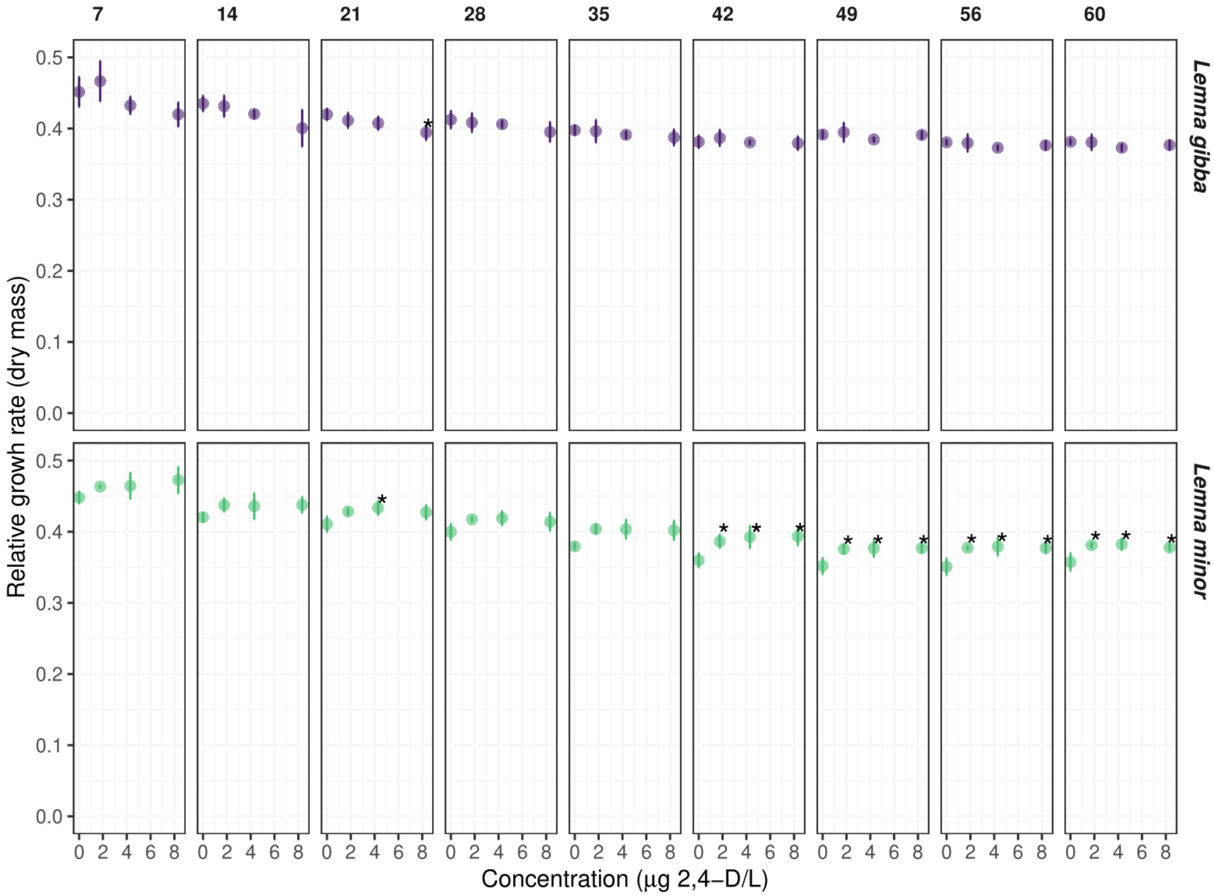

Figure B5. Mean relative growth rate (RGR) for *Lemna gibba* and *Lemna minor* dry mass over 60 days of continuous 2,4-D exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

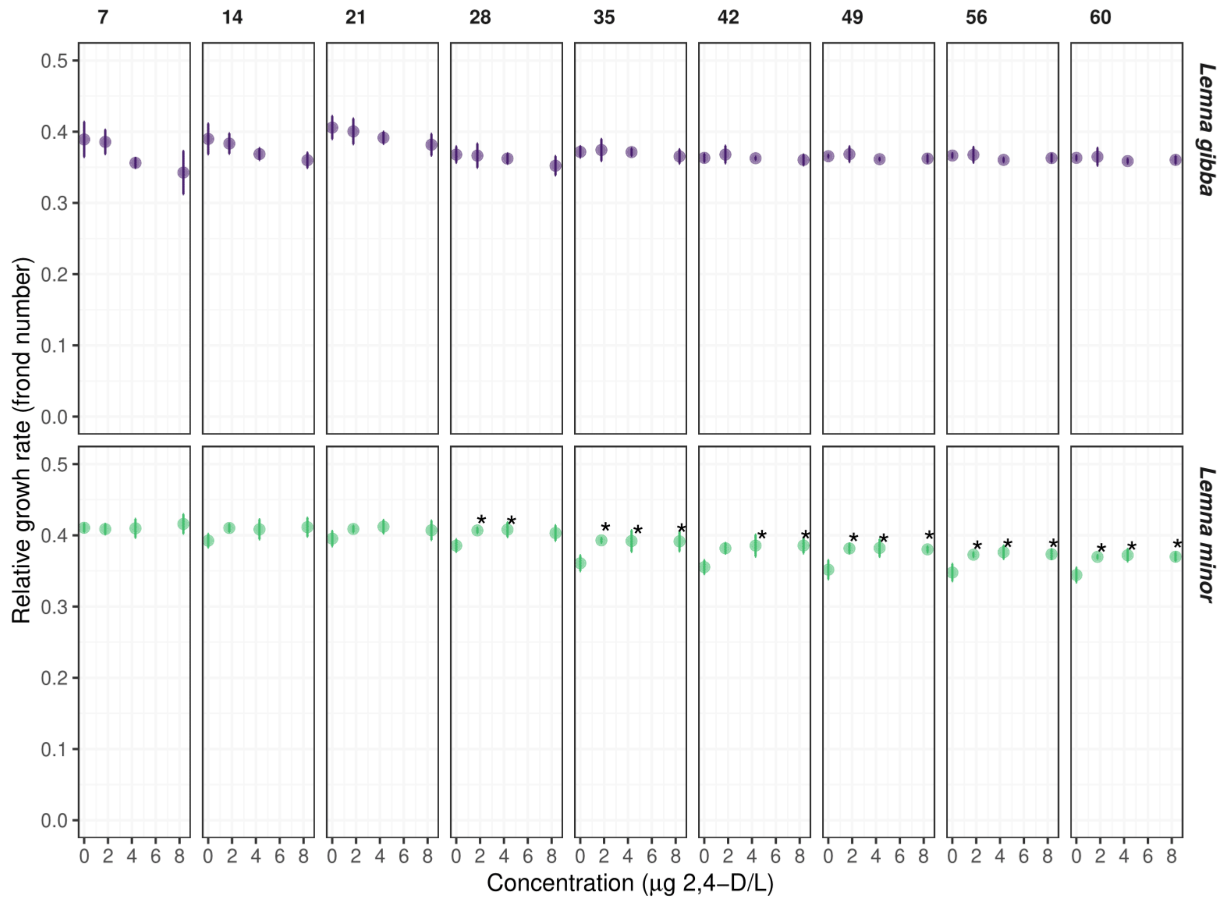

Figure B6. Mean relative growth rate (RGR) for *Lemna gibba* and *Lemna minor* frond number over 60 days of continuous 2,4-D exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

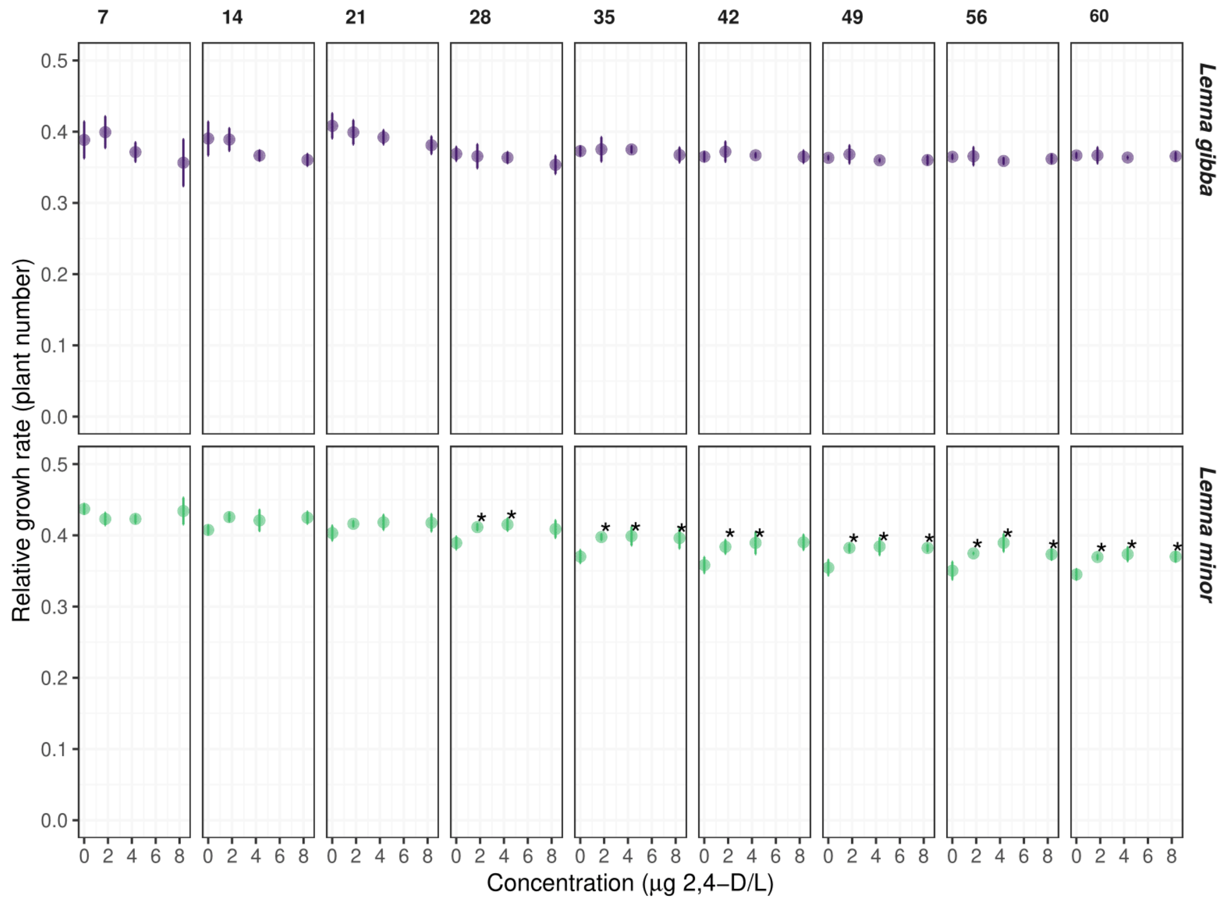

Figure B7. Mean relative growth rate (RGR) for *Lemna gibba* and *Lemna minor* plant number over 60 days of continuous 2,4-D exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

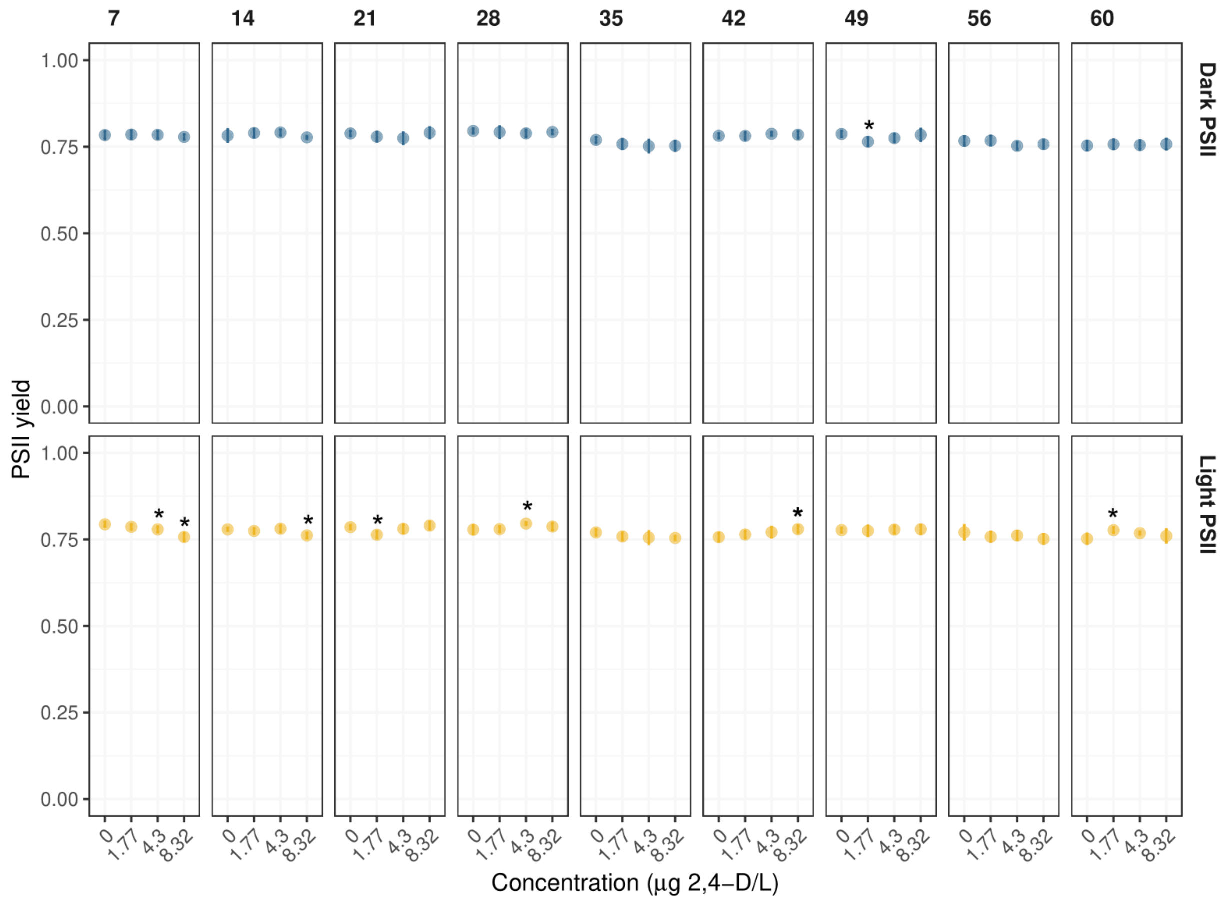

Figure B8. Mean *Lemna gibba* dark- and light-adapted photosystem II effective quantum yield (PSII yield) over 60 days of continuous 2,4-D exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

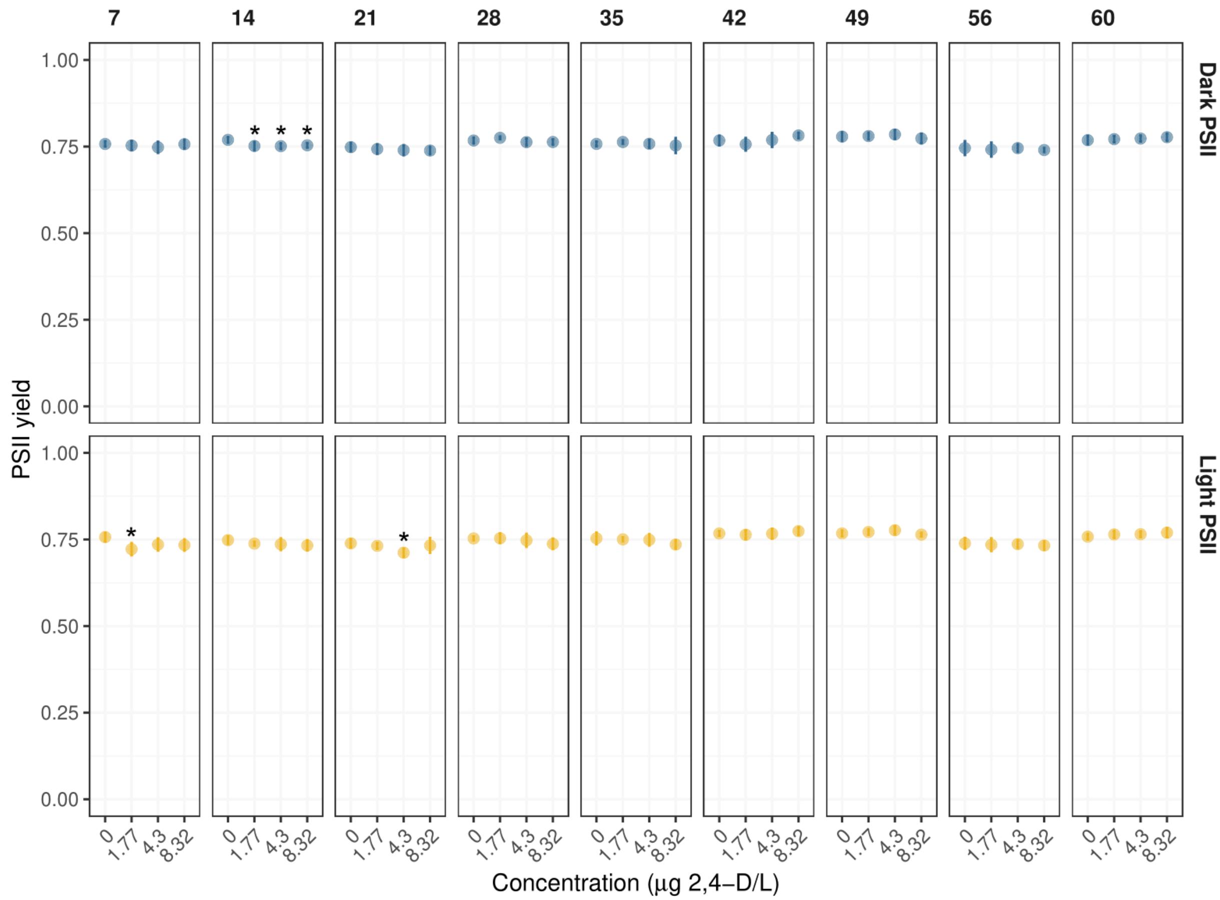

Figure B9. Mean *Lemna minor* dark- and light-adapted photosystem II effective quantum yield (PSII yield) over 60 days of continuous 2,4-D exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

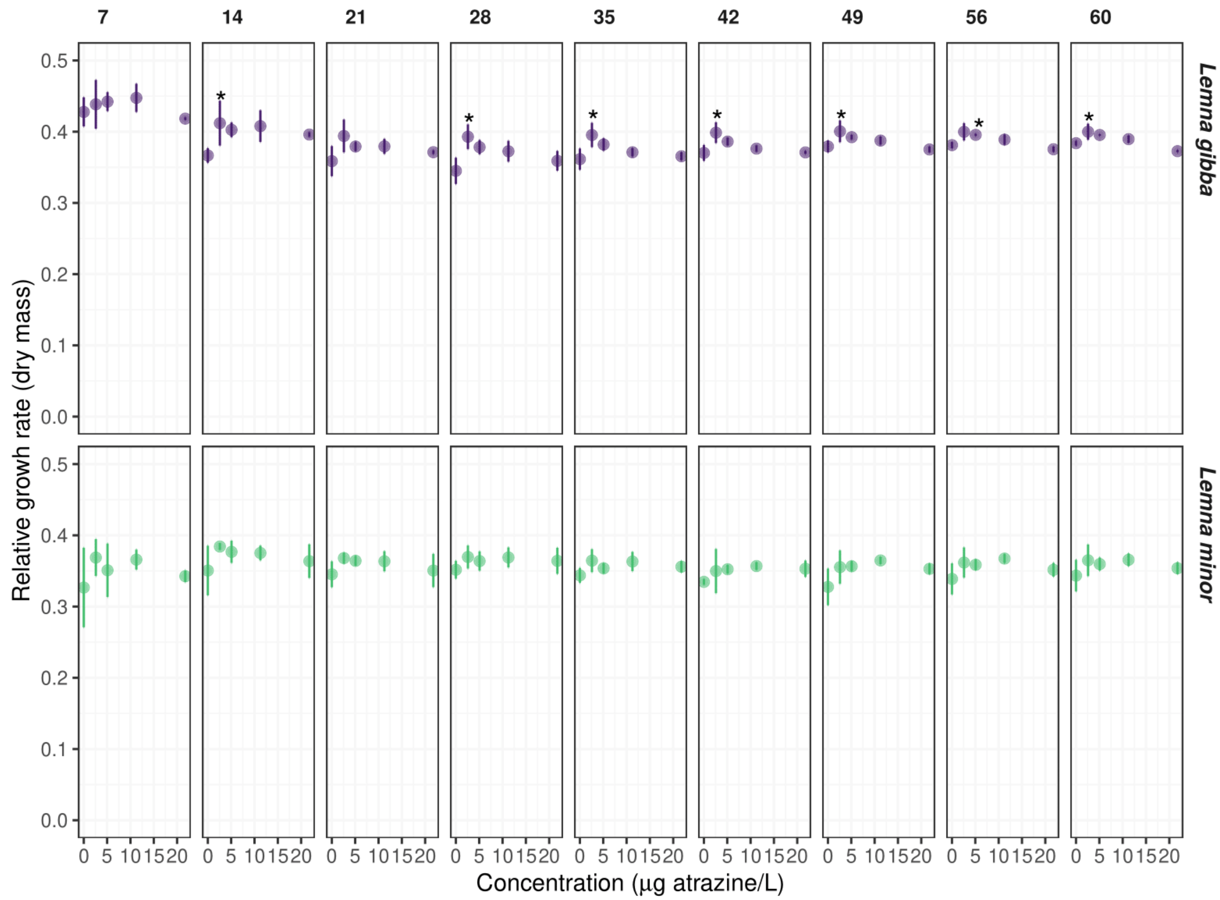

Figure B10. Mean relative growth rate (RGR) *Lemna gibba* and *Lemna minor* dry mass over 60 days of continuous atrazine exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

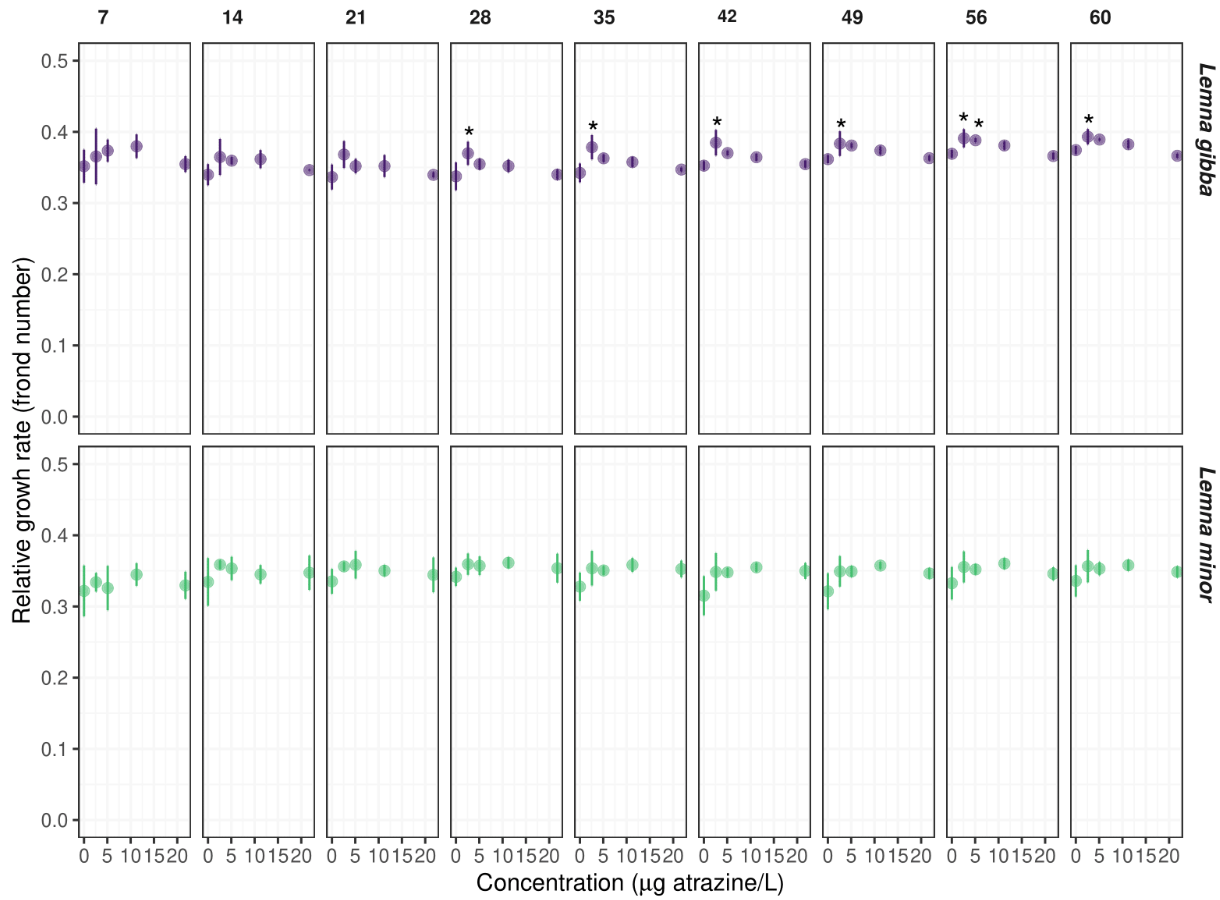

Figure B11. Mean relative growth rate (RGR) *Lemna gibba* and *Lemna minor* frond number over 60 days of continuous atrazine exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

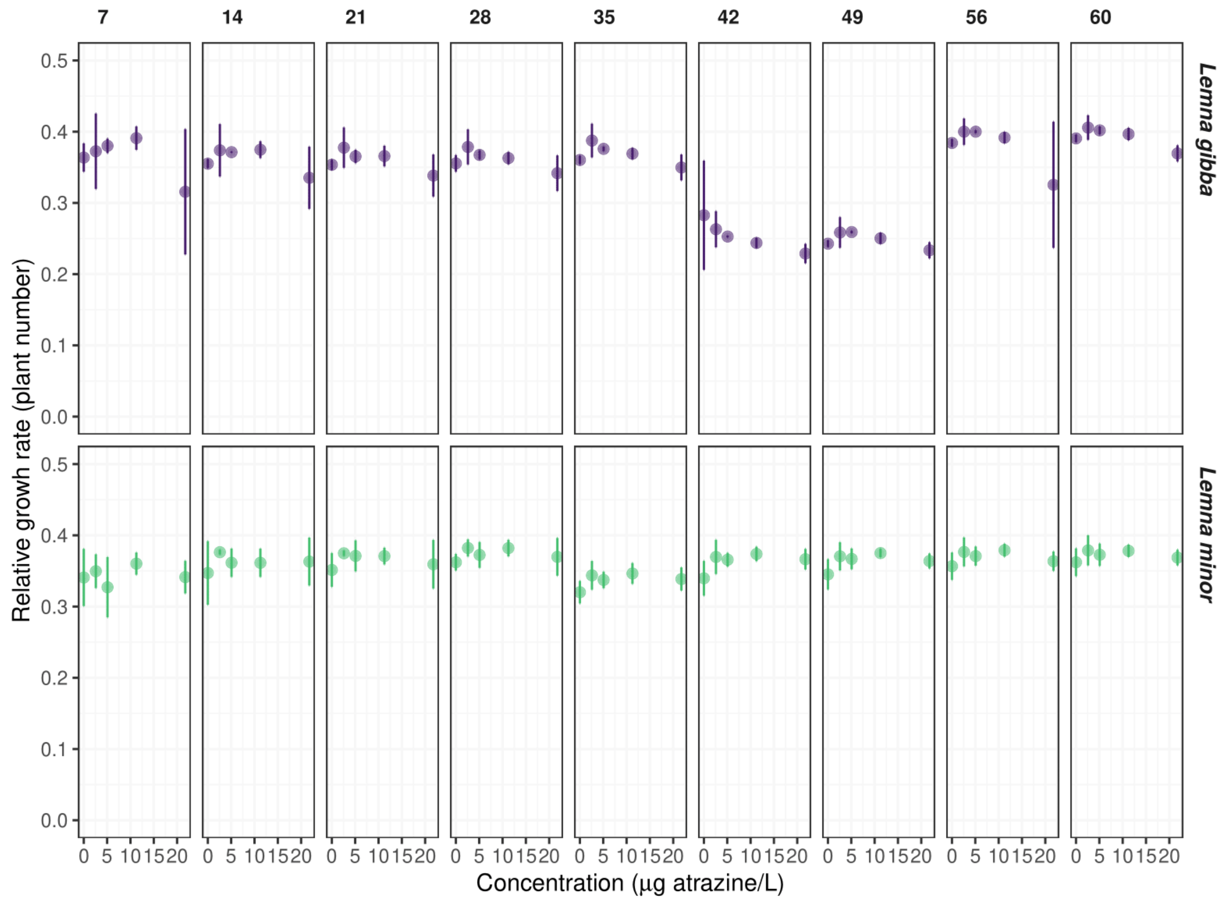

Figure B12. Mean relative growth rate (RGR) *Lemna gibba* and *Lemna minor* plant number over 60 days of continuous atrazine exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

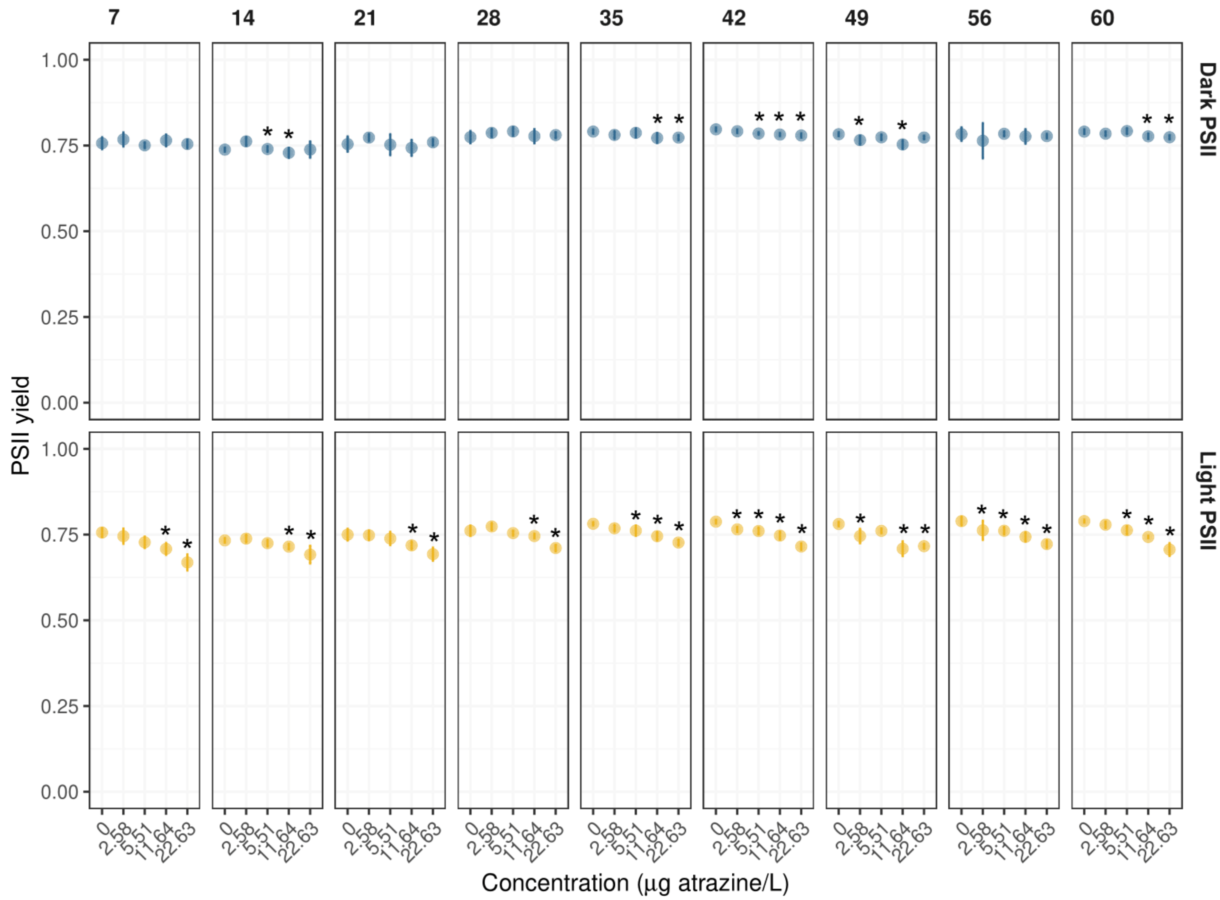

Figure B13. Mean *Lemna gibba* dark- and light-adapted photosystem II effective quantum yield (PSII yield) after 60 days of continuous atrazine exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

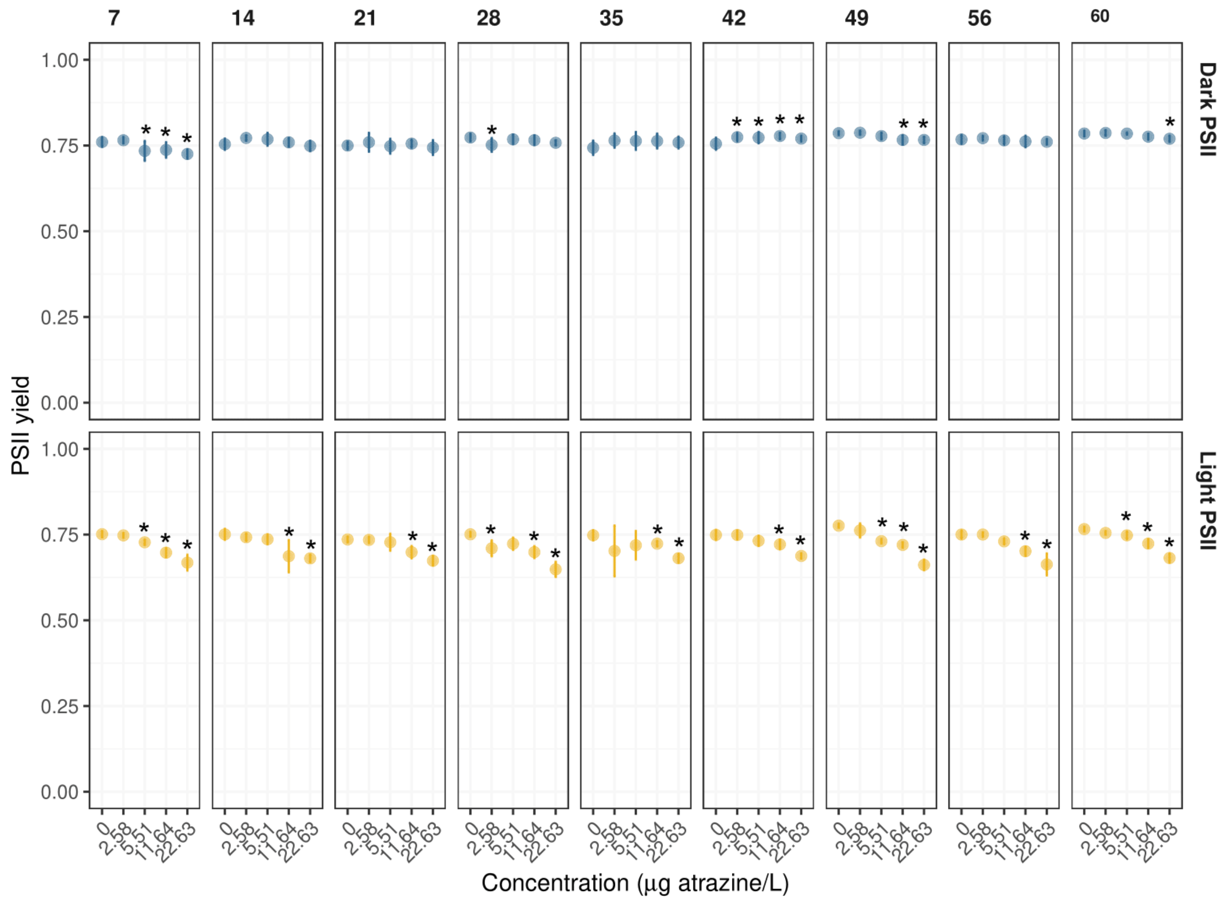

Figure B14. Mean *Lemna minor* dark- and light-adapted photosystem II effective quantum yield (PSII yield) over 60 days of continuous atrazine exposure. Error bars are the standard deviation (n = 3). Statistically significant differences from control for each time-period are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test ($\alpha = 0.05$).

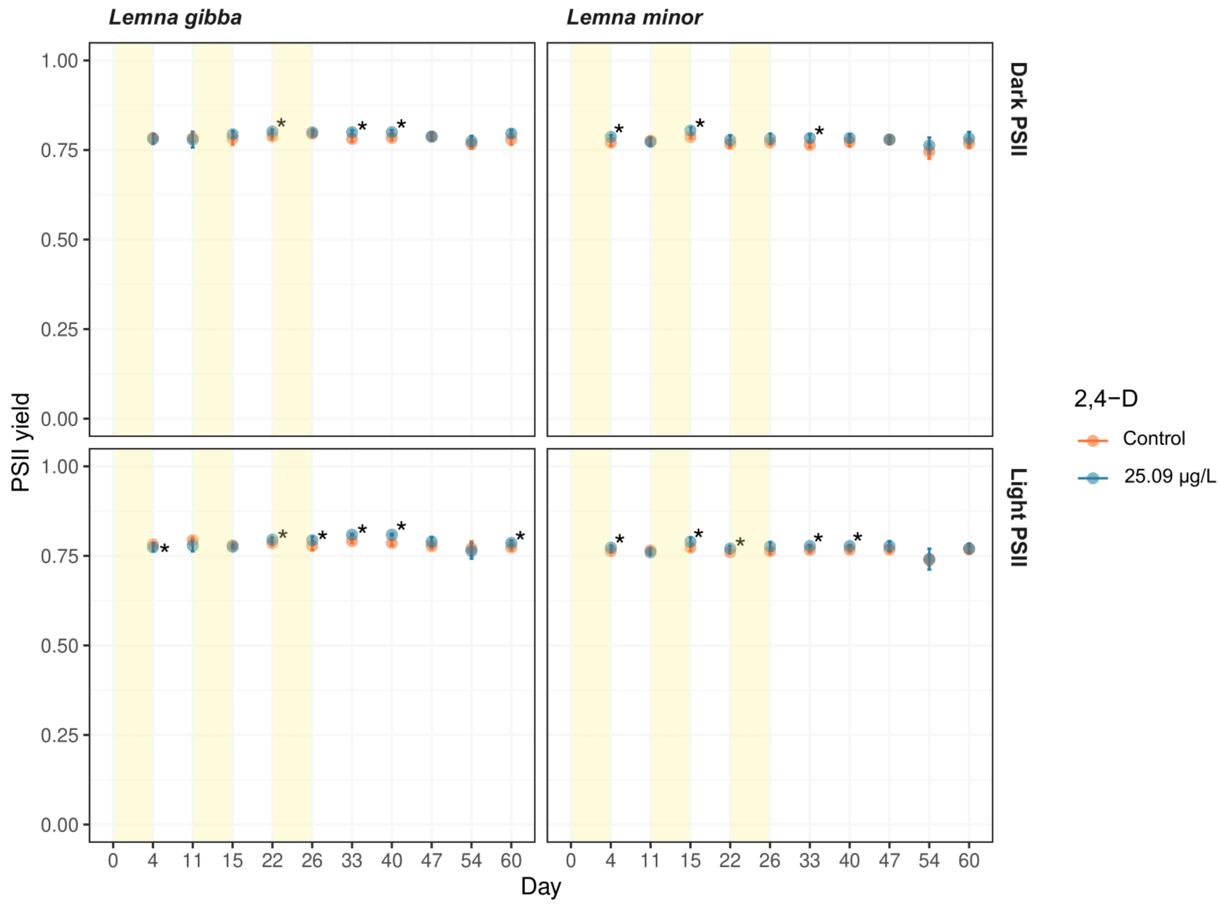

Figure B15. Mean *Lemna gibba* and *Lemna minor* light- and dark-adapted photosystem II effective quantum yield (PSII yield) after three 4-day pulsed 2,4-D exposure over 60 days. The 2,4-D concentration for each pulse was 25.09 μg/L. The pulse periods were on day 1 – 4, 11 – 15 and 22-26. These periods are highlighted in yellow. Error bars are the standard deviation (n = 3). Statistically significant differences from control are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).

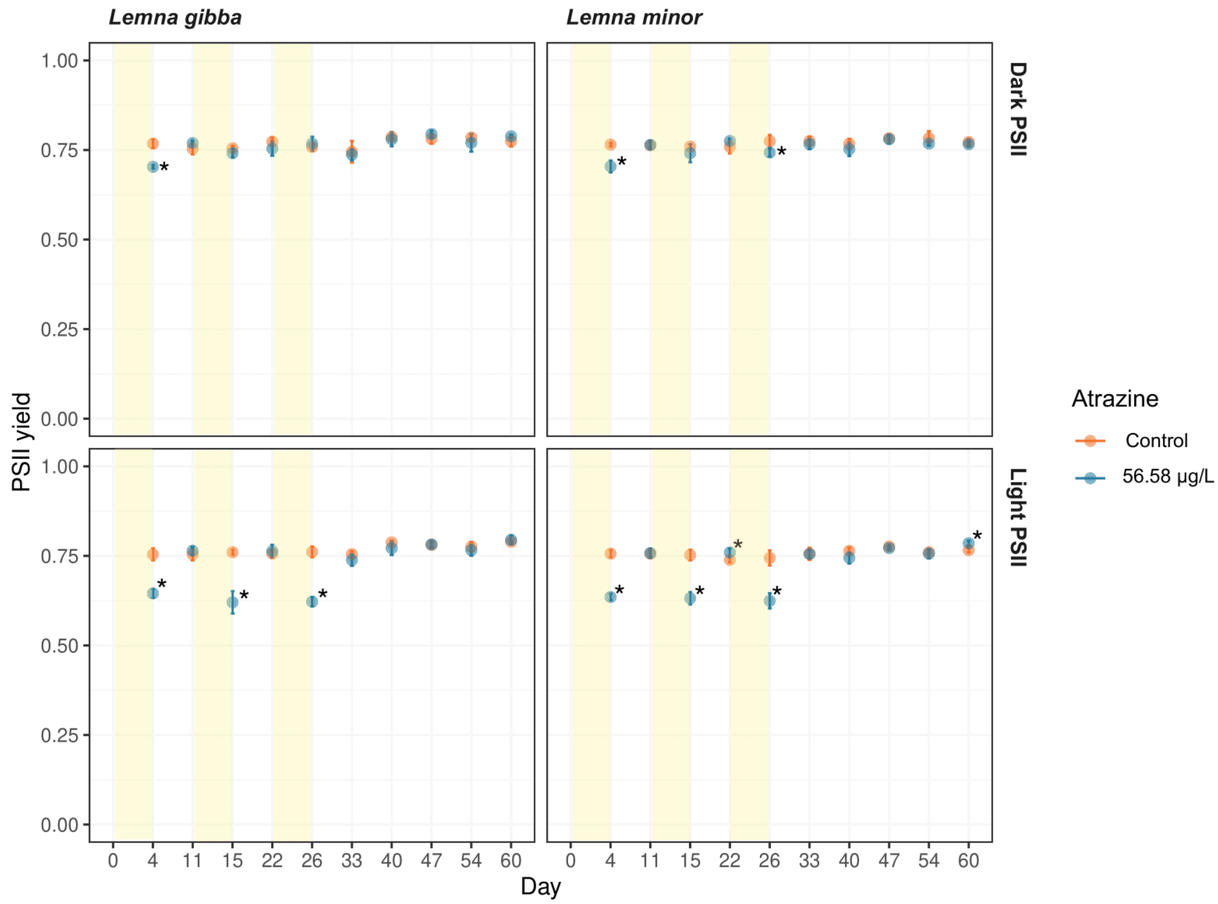

Figure B16. Mean *Lemna gibba* and *Lemna minor* light- and dark-adapted photosystem II effective quantum yield (PSII yield) after three 4-day pulsed 2,4-D exposure over 60 days. The atrazine concentration for each pulse was at 56.58 μg/L. The pulse periods were on day 1 – 4, 11 – 15 and 22-26. These periods are highlighted in yellow. Error bars are the standard deviation (n = 3). Statistically significant differences from control are indicated with an asterisk and were determined by one-way ANOVA with Dunnett's test (α = 0.05).