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Minimum Detectable Difference (MDD) in model ecosystems with different experimental designs

Dissertação para obtenção do Grau de Mestre em Engenharia do Ambiente, perfil de Engenharia Sanitária

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UNIVERSIDADE NOVA DE LISBOA

Janeiro de 2021

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Acknowledgments

To my supervisor, Professor Michiel Daam, for being present and givining me the necessary tools and materials to develop this dissertation, for guiding and teaching from beginning to end, and for the motivation, knowledge and professional advice.

To my co-supervisor, Professor António Rodrigues, for his help and guidance as well as the knowledge during my studies.

To Inês for putting up with me for all these years and for the endless friendship, for the happy and unforgettable moments and the laughs. You will always be in my heart no matter what.

To Rafaela, for the endless friendship, for the happy and unforgettable moments and laughs. There is always a place in my heart for you.

To Ana Rita, Beatriz, Patrícia, and Rita, for the friendship and the good moments and good and fun memories.

To Diogo, João Tiago, Manel, Manuel, Ricardo, and Tiago, for the friendship and good moments, and always correcting my slips.

To my family, for all the love and support and values that made me who I am today.

To Ricardo, for his love, support, dedication, and patience, and for always making me a better person.

To my sister, for her support and love.

To my parents, for their love and support and for always being an example for me. This dissertation is for you.

Resumo

O estudo de ecotoxicologia de substâncias químicas tem-se focado na abordagem de bioensaios, onde a toxicidade de um composto é avaliada numa única espécie em laboratório. No entanto, os ecossistemas naturais, são sistemas extremamente complexos, onde várias espécies interagem entre si e entre os componentes abióticos. Por outro lado, a avaliação dos efeitos de toxicidade no meio natural, ou seja, estudos de campo, tem como desvantagens a complexidade do mesmo, o que dificulta a interpretação das correlações de casuais entre exposição e efeitos de compostos individuais, e é bastante difícil encontrar um local em condições pristinas.

Modelos de ecossistemas, também referidos como microcosmos e mesocosmos, são ecossistemas experimentais que são construídos com componentes de ecossistemas naturais. Este desenho experimental, permite a avaliação das correlações casuais entre exposição e efeitos (quando comparados a estudos de campo) com algum realismo ecológico (quando comparados com os bioensaios em laboratório). Assim, os modelos de ecossistemas são fundamentais para perceber como funcionam os ecossistemas naturais e como a exposição a elementos tóxicos ou potencialmente tóxicos influencia o funcionamento e estruturas dos ecossistemas.

O Minimum Detectable Difference é a diferença mínima entre o controlo e os meios de tratamento para o composto estudado que deve de existir para ser estatisticamente significante. Este indicador foi desenvolvido de modo a proporcionar uma unidade de medida para o poder estatístico das experiências com microcosmos e mesocosmos. No entanto, são poucos os cálculos de MDD em modelos de ecossistemas experimentais. Assim, o objetivo desta dissertação é avaliar a influencia dos desenhos experimentais de estudos previamente realizados, com modelos de microcosmos e mesocosmos, podem ter nos seus valores de MDD.

O método apresentado pela EFSA em 2013, para pesticidas foi usado para o cálculo e comparação dos valores de MDD para os estudos com modelos de ecossistemas previamente realizados, com o pesticida clorpirifos, na Holanda e na Tailândia. Foram, também, procurados e posteriormente comparados os valores de MDD já calculados em estudos publicados. Desta análise de valores de MDD, foi possível identificar vários fatores que podem influenciar positivamente ou negativamente os valores de MDD. Na parte final desta dissertação, são fornecidas perspectivas futuras baseadas nas conclusões deste estudo.

Abstract

Ecotoxicological testing of chemicals have traditionally focused on the bioassay approach, where the ecotoxicity of a compound is evaluated on only a single species at the time in the laboratory. However, natural ecosystems are must more complex and include species interactions and the influence of ambient environmental factors. On the other hand, the evaluation of the toxic effects in the field has the disadvantage that this complexity hampers the interpretation of the correlations of treatment-related effects of individual stressors, and it is very hard to find a non-polluted field site.

Model ecosystems, also referred to as microcosms and mesocosms, are experimental ecosystems that are constructed with components of natural ecosystems. This experimental design allows the evaluation of the correlations of treatment-related effects (when compared to field trials) with a higher ecological realism (when compared with laboratory bioassay tests). Thus, the model ecosystems have often been used to evaluate how the natural ecosystems function and how the exposure to toxic elements or potentially toxic elements influences ecosystem structure and functioning.

The Minimum Detectable Difference (MDD) is the minimum difference between the control and treatments with a compound under study that must exist to be statistically significant. This indicator was developed in order to provide a measure for the statistical power of microcosms and mesocosms experiments. However, few MDD calculations on model ecosystem studies have been made so far. Therefore, the aim of this thesis work was to evaluate the influence of the experimental design of the previously conducted microcosms and mesocosms experiments on their MDD values.

The method presented in the Aquatic Guidance document for pesticides was used to calculate and compare MDD values from model ecosystem studies previously conducted with the same pesticide (the insecticide chlorpyrifos) in the Netherlands and Thailand. MDD values reported in previously published papers dealing with model ecosystems were also procured and compared. From these calculated and compiled MDD values, several factors that potentially increase or decrease the MDD values were identified. Several directions for future research based on the study findings are provided in the last section of this thesis.

List of Abbreviations and Acronyms

AIT	Asian Institute of Technology
CPF	Chlorpyrifos
EC_{50}	Half maximal effect concentration
ERA	Ecological risk assessment
EQS	environmental quality standards
FOCUS	Forum for the Co-ordination of pesticides fate models and their use
LC_{50}	Median lethal concentration
LOEC	Lowest observed effect concentration
MDD	Minimum detectable difference
MEC	Measured environmental concentration
NOEC	No observed effect concentration
PEC	Predicted environmental concentration
RAC	Regulatory acceptable concentration

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1. Introduction

1.1. Research scope

Resulting from the concepts of “ecology” and “toxicology” the term “ecotoxicology” was introduced in 1969 by Truhaut. Its appearance resulted from the growing concern and necessity to understanding how chemicals affect the environment and other species beyond man. This new science arose from a series of environmental accidents on the 1950s and 1960s, which created the need to understand them in order to reduce or mitigate the risk that future accidents could have. Today ecotoxicology is defined as the study of the destination of toxic substances negative effects on an ecosystem (Klaassen, 2003).

This field of science requires an understanding of the structure and functioning of ecosystems, ways and speeds of energy and matter transfer between ecosystem constituents and its changes, as well as the changes that happen on the different organization levels. It also requires the understanding of the effects of pollutants on individuals and the elements that compose them, as well as the mechanisms of harmful effects and conditions under which they occur. Thus, the ecotoxicological assessment of a compound is achieved with the use of species up to ecosystems that are sensitive to changes in their environment.

Of all the ecosystems that may be affected by pollutants, the aquatic system is the most sensitive to harmful effects, which causes a major concern worldwide, since the pollution of an aquatic ecosystem can represent a possible contaminated drinking water source (Klaassen, 2003). Subsequently, residual urban and industrial wastewater must be treated before it is discharged in the natural ecosystems or reused. However, the discharge of liquid effluents, even treated, can cause chronic effects in the aquatic environment, as the organisms present are exposed to low concentrations of pollutants for a prolonged period of time.

Thus, in order to understand the adverse effects of xenobiotic agents on aquatic species, several ecotoxicological tests have been developed and conducted according to the specific objectives under study.

Chlorpyrifos is an organophosphate insecticide used mainly to control foliage and soil-borne insects pests on a variety of food and feed crops. It has been used as a pesticide since 1965, in both agricultural and non-agricultural areas. According to EPA, is used mainly in corn fields, as well as soybeans, fruits and nuts trees, cranberries and other row crops, the non-agricultural use includes golf courses, green houses as well as non-structural wood treatments. In December 2019, the Member States of the European Union, voted against the renewal of the approval of the use of chlorpyrifos. That measure started to be in effect on the 10 of January 2020, where Member States had a month to stop the authorization of the pesticide use.

For herbicides, one of the most used ones is Linuron, which is used to control the growth of grass and weeds on crop fields. It can be used as a pre-emergent or pos-emergent herbicide and like

chlorpyrifos is used in many crops and plantations. It was classified by EPA as a Restricted Use Pesticide.

Several doubts have been raised in the past decades regarding which tests to conduct, which species to test and the statistical analysis of ecotoxicological tests. In literature review section, these issues will be discussed in more detail.

In this dissertation, it was calculated the MDD values for three microcosms studies, that used the pesticide chlorpyrifos in its experimental design, for the zooplankton community. It was also used an additional study that used both zooplankton and phytoplankton, in order to compare both communities.

1.2. Document structure

This dissertation is structed in 8 chapters.

Chapter 1 consists of the introduction to ecotoxicological tests used in wastewater treatment effluents and pesticides.

Chapter 2 consists of the literature review. This chapter presents scientific publication considered relevant in the elaboration of this dissertation.

Chapter 3 consists of the aims of this research work.

Chapter 4 consists of the methods used and conducted in this dissertation work. In this section all steps taken during this dissertation, the calculations are detailed.

Chapter 5 consists of the presentation of the results obtained in the development of this dissertation and their discussion in view of other scientific studies.

Chapter 6 consists of the conclusions drawn from the results.

Chapter 7 consists of the discussion and presentation of future perspectives and what can be improved in future studies for further investigation that could be deduced from this dissertation.

Chapter 8 consists of the bibliographic references that support this dissertation.

2. Research aim

The main aim of this dissertation was to evaluate to what extent the experimental design of model ecosystem studies influences MDD values and hence their statistical power to and ability to show chemical-related effects on aquatic populations. To this end, MDD values from model ecosystem studies previously conducted with the same pesticide (the insecticide chlorpyrifos) in the Netherlands and Thailand were calculated. In addition, MDD values previously published in papers using model ecosystems were also mined. Based on an evaluation of these MDD values, this dissertation also has the objective of listing the factors that would increase or decrease the MDD values and what can be done to maximize the statistical power of model ecosystem experiments. A last objective was to indicate directions for future research based on the study findings.

3. Literature review

3.1. Ecological risk assessment

The use of risk assessment studies have been conducted for the last five decades in order to protect human health, but ecological risk assessments (ERA) have mostly been carried out only in the last two decades (Solomon & Takacs, 2001). ERA includes the assessment of potential impacts and effects of potential stressors, including those related to the exposure to chemical substances. For the ecotoxicology of chemical substances, the aim of ERA is to estimate the probability of adverse effects, such as increased lethality or reduced growth of non-target, or beneficial, communities in habitats exposed to the substances and the probability of adverse.

ERAs are usually conducted for many reasons as shown in figure 1. These reasons range from the need for simple raking systems to the need for more complex probabilistic methods. It assessed the toxicity, when it is necessary to understand how a organism reacts to being exposed to a certain stressor. The assessment of hazards is based on a ration that relates the exposure to the toxicity of a stressor. The risk assessment are based on the probability of exposure and on the probability that the stressor has a toxic effect (Solomon & Takacs, 2001).

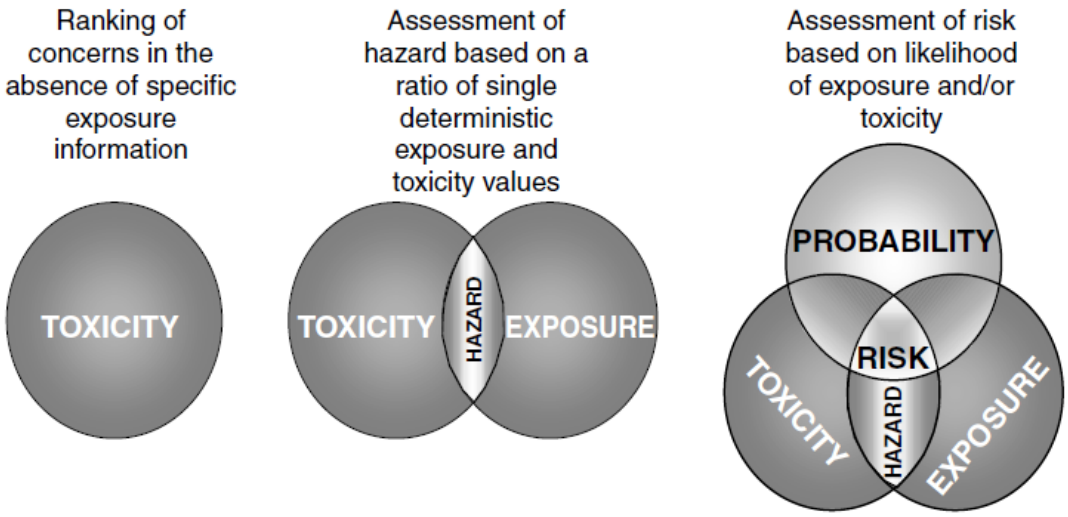

Figure 1 – Illustration of the types of approaches to ecological risk assessments (Source: Solomon and Takacs, 2001).

Subsequently, in an ecological risk assessment of chemicals, it is necessary to evaluate the exposures (section 2.2) and toxicity (section 2.3) of chemicals considering the probability in which these will occur in real-world settings.

3.2. Exposure assessment

In prospective chemical ERA, i.e., before allowing the use of a chemical on the market, the environmental exposure needs to be predicted using environmental fate models. For pesticides, a predicted environmental concentration (PEC) is usually calculated using computer models like those used by FOCUS (Forum for the Co-ordination of Pesticide Fate Models and Their Use; FOCUS, 2001) using pesticide characteristics, the recommended pesticide dose, and a simulated landscape scenario as input parameters (figure 2). The representativeness of these models and the scenarios used for all European agroecosystems has been often disputed (e.g. Daam et al., 2011; Pereira et al., 2017; Zubrod et al., 2019). Especially the South-European scenarios appear to be insufficiently developed since generic FOCUS scenarios were mostly based on North/Central European conditions (Daam et al., 2011; Pereira et al., 2017). For example, although spray drift is assumed to be the main route of edge-of-field surface water contamination in the Mediterranean countries, particularly after heavy rainfall following a period of drought (Ramos et al., 2000). Recently, EFSA published a scientific report on the “repair action” of the FOCUS surface water scenarios (EFSA, 2020). This report, however, indicates rather specific and detailed minor changes in the modelling exercise so that pesticide PECs calculated for South European countries using FOCUS models continue to need to be interpreted with caution and need valuation.

In retrospective ERA, i.e., after allowing the use of a chemical on the market, exposure assessments can be made by taking environmental samples and measure chemical concentrations, i.e., MECs: measured environmental concentrations. Measuring the chemical exposure is one of the most critical components of ERA. However, it can be subjected to errors thought improper sampling techniques and incorrect analysis. An unbiased and representative sample from an environmental matrix may be hard and costly, yet it is probably the most important part of any exposure characterization (Posthuma et al., 2001).

The sampling must consider both the temporal and the spatial distribution of the stressor, for example, the concentration of a compound by may vary by depth and/or distance from shore immediately after a spray-drift contamination of water. As such, the concentration of a stressor can decrease with increased distance from the source of contamination, because of factors like degradation in water, adsorption to sediments or the dilution from uncontaminated water entering the stream (Posthuma et al., 2001).

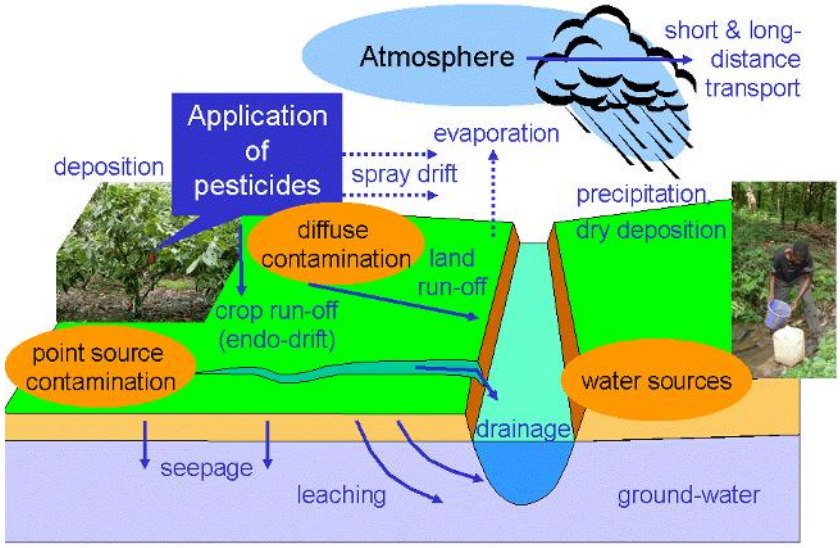

Figure 2 – Landscape scenario (Source: FOCUS 2001).

3.3. Effect assessment: Introduction

The most important factors in exposure are the type, duration, frequency, and concentration of the compound. Toxicity is dependent on the type of exposure the organism has to the compound. As for the solubility of the compound, these can be divided into two categories, water-soluble and lipophobic. The water-soluble compound is bioavailable for most organisms, as they may be ingested or absorbed, the lipophilic compounds are mostly found in aggregated particulate matter and organic matter. Thus, water-soluble compounds can be absorbed over the entire body surface of aquatic organisms and even ingested through the mouth or gills, depending on the species, while lipophilic compounds must be ingested and absorbed in the gastric system, if it exists (Rand, 1995).

The toxicity of a compound is also affected by the organism's exposure duration. In cases of acute (short-term) exposure, organisms come in contact with the compound only once, or several times in a reduced space of time. In these exposures, if the compound is rapidly absorbed, the effects are immediate, although it is possible to have delayed effects (also sometimes referred to as latent effects) similar to those resulting from prolonged exposures (Rand, 1995). In cases of chronic (long-term) exposures, the organisms are exposed to a compound for a long period of time, but at a low concentration; this happens when compounds are continuously discharged or released. In this type of exposure, there are usually effects that develop slowly and progressively, although there can also be immediate effects like after acute exposure (Rand, 1995).

The toxicity of a compound is also affected by the frequency of exposure. An acute exposure to a certain concentration can result in an immediate adverse effect, while two consecutive and cumulative exposures with an equivalent concentration can result in a non-existent or very small

effect. This is due to the organism's metabolism that happens between exposures, or the adaptation of the organism to the compound (Rand, 1995). Susceptibility, as mention earlier, affects the toxicity of a compound in an organism, so that different species have different susceptibilities, depending on their eating habits, morphology, metabolism, and biology.

A young individual is generally more susceptible to the toxic effects of a toxic than an adult individual of the same species, as their level of development and metabolism are quite different. Organisms that have already been subjected to other compounds or environmental stressors (e.g. conditions near their tolerance limits) and therefore are stressed also have their susceptibility increased (Rand, 1995). This is likely to occur in the natural environment where organisms are usually exposed to more than one stressor.

The toxicity of a compound is influenced by the characteristics of the compound, such as its purity or composition, as there may exist impurities or metabolites (degradation products) that may be more toxic than the parent compound itself (Klaassen, 2003). Thus, it is important to account for the purity and identity of the test compounds and their metabolites in toxicity tests.

Chemical and physical properties of compounds are important factors in toxicity tests. Solubility and pH affect the bioavailability, persistence and transformation, and the fate of the compound in the environment.

Environmental factors, defined by biotic and abiotic characteristics, can also alter the toxicity of a compound in the aquatic environment. Biotic factors, including size, development stage, larval stage, juvenile or adult, the type of organism, e.g., algae or fish, and seasonal changes in the physiological state influence the organism's response to the compound. Abiotic factors, which include physical and chemical characteristics of the water in which the aquatic organisms live, such as pH, hardness, salinity, temperature, dissolved oxygen content, organic matter content and suspended matter and the speed of water flow, also modify the toxicity of a compound.

There are, compounds that have negative effects on various types of cells and tissues in organisms. On the contrary, there are also compounds that only affects one type of cell or tissue. Thus the mode of action of the compound can influence their toxicity and ultimately the organism that will be most affected by the compound (Rand, 1995).

Several test approaches have been developed and applied to evaluate the toxicity of chemicals, ranging from laboratory bioassays to field studies (figure 3). The advantage of laboratory bioassays is that they are relatively cheap, fast and highly protocolized and, therefore, have a high reproducibility (section 2.4). however, there are ecological realism evidently rather low as compared to field studies, which on the other hand are difficult to interpret due to the high number of potential confounding factors occurring under field conditions (section 2.5). An alternative, frequently used approach in prospective chemical ERA is the use of man-made experimental ecosystems: model ecosystems or microcosms and mesocosms (section 2.6). The use of microcosms or mesocosms has been considered to provide a bridge between laboratory

(bioassays) and the field trials for being manageable and allowing replication and hence an experimental set-up on the one side and providing realism in terms of ecological processes and exposure to the chemical on the other hand (figure 3, Brock et al., 2000).

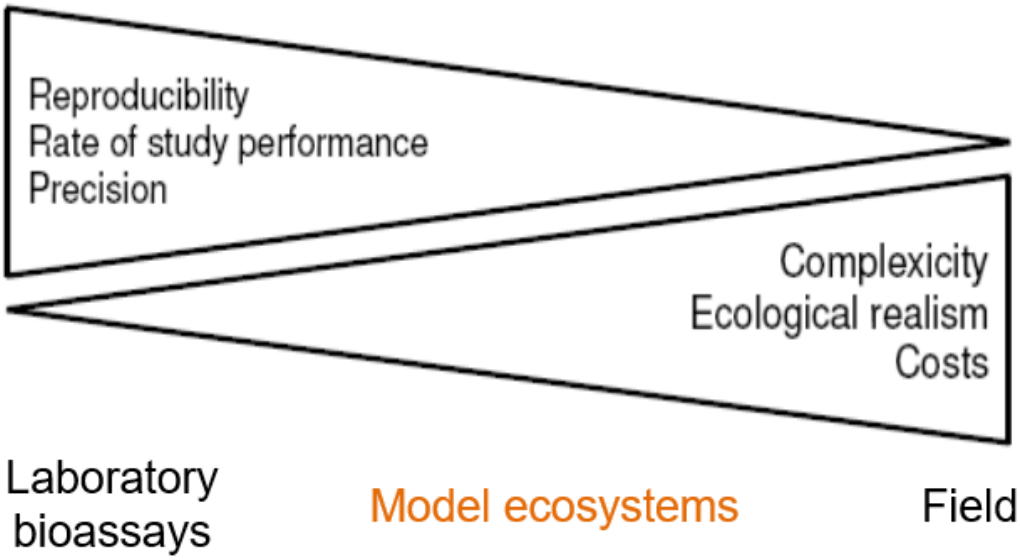

Figure 3 – Illustration on the bridge that model ecosystems provide between laboratory and the field (Source: Brock et al., 2000)

3.4. Effect assessment: Laboratory bioassays

A pollutant is a contaminant that manifests an adverse response in a biological system (Chapman, 2007). These manifestations can cause negative changes in the structures and functions of an organism, and can, in some cases, lead to their death. The aquatic ecosystem is adapted to innumerable physiochemical and biological mechanisms, ways in which contaminants can be absorbed without major implications for the biome, however high levels of contaminations can largely affect growth, development, survival and reproduction of organisms (Dias, 2002).

Due to variability and complexity of organic and inorganic compounds that are or may be present in wastewater effluent, and because chemical analysis alone do not portray or show the impact that the sub-selection of all compounds potentially present in wastewater can have on the environment or the ecosystem, toxicity tests were developed in which live organisms are used as indicators of toxic effects in effluents, the so called whole effect tests (Williams et al., 2002).

The increased need to obtain information not solely related with physiochemical characteristics of wastewaters has been encouraging the choice of toxicity tests. They are now considered indispensable for the comprehensive control of wastewater pollution sources. Toxicity tests

determine the toxic potential of a compound or a mixture of compounds, with the response of living organisms measuring the effects of these compounds (Tisler & Zargorc-Koncan, 1999).

The idea that the quality of the environment is indicated by living organisms is widespread. The quality of the environment can be indicated both by mortality of the indicator organism and by the physiological stress of the environment. According to Reginatto (1998) physiological stress can be disclosed by decreasing the rate of growth, loss of reproductive capacity and/or change in behaviour of the organism. In both Europe and United States of America, the use of toxicity tests to assess water and effluent quality began in the 1970s and was regulated in the 1980s. The first bioassays used algae and bacteria as indicators and were limited to account for the mortality of the species used. Although they are still used today, new tests have been developed.

The choice of the organism to be used in ecotoxicity testes depends on selection criteria. One must take into consideration the availability and abundance of the species, ecological representativeness, native species are preferred, knowledge of biology, physiology and eating habits, genetic stability and uniformity of organisms, sensitivity to various substances, dimensions of the organisms and preferably with short life cycles, as well as low seasonality and easy maintenance on the laboratory (Van Leeuwen et al., 1995).

Aquatic organisms such as algae and fish complete their life cycle in the water and are therefore often used to monitor the quality of the ecosystems in which they live. A change in water quality is possible during their lifetime and depending on the length of their lifetime, different organisms are used as indicators to different pollutants. Since different organism have different sensitivities to different compounds, it is beneficial to use different species from different taxonomic groups (Klaassen & Watkins III, 2003). For example, both aquatic and terrestrial non-targets arthropods may be expected to be especially sensitive to insecticides, but relatively insensitive to herbicides (Daam et al., 2011; Rico & Van Den Brink, 2015). On the other hand, beneficial primary producers (algae, macrophytes, terrestrial plants) may be expected to be tolerant to insecticides and sensitive to herbicides (Van Wijngaarden & Arts, 2018). For other pesticide groups such as fungicides, the most sensitive taxonomic group appears to depend on the fungicide of concern (e.g. Rico et al, 2019).

Traditionally, ecotoxicity tests have focussed on the bioassay approach, where the toxicity of a compound is tested on a single species at a time (Van Den Brink, 2008). In these tests, the response of the studied species is compared between a concentration series of the test compound and the population variation in the controls. Concentration-response measures are easily quantified using basic statistical techniques (Klaassen & Watkins III, 2003). As discussed above, these tests are easy to conduct and highly protocolized and, hence, reproducible (Brock et al., 2000).

Therefore, bioassays are an important tool for assessing the sensitivity of aquatic organisms to different chemicals and may be divided in acute and chronic toxicity tests (Walker et al., 1997).

In acute toxicity tests, a rapid and severe response of a test organism is evaluated following a stimulus that manifests itself in an interval of 0 to 96 hours, in general, and can go up to 7 days.

In these tests, the evaluated effects include mortality and immobility. Mortality effects are usually evaluated in fish species, while immobilization effects are usually measured in invertebrate species (e.g., EFSA, 2013).

Thus, acute toxicity tests are short-term tests that show rapid responses in estimating the lethal effects of compounds and pollutants. As acute mortality is easily observed, these tests were widely used for the first assessments of pure toxins and complex effluents (EPA, 2002).

The toxicity endpoint that is normally calculated from these tests, is the EC_{50}, which corresponds to the concentration that affects 50% of the organisms tested, or LC_{50} which corresponds to the median lethal concentration if the endpoint is mortality. Thus, these tests provide basic information, and also serve as a basis for defining the conditions and test concentrations used for chronic toxicity tests, if necessary, and toxicological risk assessment.

Chronic toxicity test are long-term trials that aim to study non-lethal effects, given the prolonged exposure of organisms to sub-lethal concentrations that may occur in edge of field surface waters. These tests last between weeks up to 3 or more months.

In these tests, the evaluated effects pass thought the biochemical, physiological, and behavioural effects, as well as the growth and reproduction of the organisms.

Thus, chronic toxicity tests are long-terms tests that evaluate rigorous and direct responses to the lower limit sublethal concentrations of organisms. These tests include a significant part, or sometimes even the entire life cycle of the organism under study.

Chronic toxicity tests should be studied when information from acute toxicity tests is not sufficient to characterize the toxic effect of a compound and when chronic concentrations in the environment may be expected (EFSA, 2013b).

These tests make it possible to implement legal measures to normalize the quality of natural water sources, as well as emissions of wastewater.

3.5. Effect assessment: Field studies

Field monitoring of the effects of actual chemical use on aquatic ecosystem structures and functioning is not frequently conducted. This is due to the difficulty of established cause-effect relationships though this type of studies as well as their high costs and labour intensity (figure 3). In addition, the field monitoring studies can evidently only be conducted in retrospective ERA since in prospective ERA the use the chemicals has not been allowed yet.

Despite and increase in field studies in recent years, the availability of such studies evaluating, e.g., the field effects of pesticides remain meagre. Nevertheless, available studies generally indicate high pesticide risks to aquatic organisms under current agricultural practices, especially in freshwater systems (Schäfer, 2019; Schepker et al., 2020; Zubrod et al., 2019).

3.6. Effect assessment: Model ecosystems (microcosms and mesocosms)

It is important in an ecotoxicity test to have a model very close to the natural ecosystem, because not only do we have interactions between organisms that live in the natural environment, but it is easier to understand what the real effect of a compound is on the ecosystem.

Regarding this ecological realism, laboratory toxicity tests are very limited, not only in term of the number of species tested, but also because it only studies a single animal species in the water environment, not counting with species interaction nor the substrate or sediment of these systems.

In view of this problem, the study of ecological risk of pesticides has used model ecosystem in their assessments. Model ecosystems allows replication an experimental design in the laboratory or outdoors that replicate ecosystems in the natural environment. Thus, they provide a robust experimental design as well as realism in terms of ecologic processes and exposure to the chemical(s) being studied (Brock et al., 2000; figure 3).

Depending on the dimension of the model ecosystems, and hence their complexity, are referred as microcosms, relatively small test systems, and mesocosms, relatively large test systems. For aquatic model ecosystems, microcosms are man-made test systems with water volume of less than 15 m^3 or experimental streams with less than 15 m in length and mesocosms are defined as model ecosystems containing more than 15 m^3 of water or experimental streams longer than 15m (Van Den Brink & Daam, 2014).

Microcosms and mesocosms are confined test systems that are constructed artificially form parts of natural ecosystems or consist of enclosed parts of natural ecosystems. These experimental ecosystems may be used as an ecological research tool for hypothesis testing and hypothesis generation, and in environmental effect assessment of chemicals (Brock et al., 2014).

According to Brock et al. (2014) the advantages of using model ecosystems in ERA, when compared to laboratory bioassays, are:

- Better control over confounding factors, making it easier to demonstrate causality between exposure and ecological effects;
- The ability to replicate microcosm/mesocosm allowing the derivation of concentration-effect relationship and statistical interpretation of the treatment-related responses;

- The possibility to integrate more or less realistic exposure regimes of toxicants with the assessment of endpoints at a higher level of biological integration, for example having responses at population or even community levels;
- The possibility to study intra- and inter-species interactions and indirect effects within a community;
- The chance to perform medium to long-term observations so that latency of effects and population and community recovery can be assessed.

In order to interpret the often complex ecological information and the concentration-response relationship that results from the model ecosystem studies, it is common practice to use a combination of univariate and multivariate statistical techniques to calculate the no observed effect concentrations (NOECs) and lowest observed effect concentrations (LOECs) at the population and community levels (Brock et al., 2014). The NOEC is the highest concentration in the concentration series tested that did not lead to a statistically significant effect, whereas the LOEC is the lowest concentration in this series that led to a statistically significant effect.

3.7. Tiered pesticide risk assessment

In ERA, it is necessary to divide complex tasks into smaller and simpler components that can be easily done, managed and distributed. This is specially applied to ERA, where the interactions and relationships between the components of the ecosystem can be very complex. One method to reduce the complexity of the risk assessments is the use of tiers or steps, this allows to narrow the focus of the risk assessment to key issues. The use of tiers in ERA has several advantages, for the risk assessor and for those proposing the activity that caused the risk. (Posthuma et al., 2001).

The use of tiers or steps in the ERA process of criteria settings has therefore frequently been recommended (e.g. Campell et al., 1999). The initial use of a lower of first tier with conservative assessment criteria allows substances that do not present a risk to be eliminated from the risk assessment early, thus allowing the focus of resources and expertise (i.e. in a higher tier evaluation) on more problematic substances (Daam & Van Den Brink, 2011). From lower to higher tiers, the exposure and effects estimates become more realistic and hence the uncertainty in the extrapolation of effects is reduced (Solomon et al., 2006).

The lower tier tests in the toxicity or effect assessment component of ERA consist of concentration-effect responses studied in laboratory toxicity with a limited number of standard species (see section 2.4). These test species are regarded as convenient surrogates for sensitive indigenous species of aquatic ecosystems, despite the uncertainty associated with the extrapolation from one species to another. These single-species tests have been the source of biological data for ERA. This history explains why only a very limited amount of ecological theory has become integrated into the field of ecotoxicology and ERA (Van den Brink & Daam, 2014).

Only multispecies testing designs allow for considering species interactions, and so the evaluation of indirect effects and functional redundancy, and to evaluate the recovery potential of impaired ecosystems. Alternative testing to single species toxicity includes a wide range of experimental designs, from simple indoor multispecies assemblages to large model ecosystems experiments (see section 2.6) field studies (see section 2.5). Great progress in the development of such designs had been made on the experimental side and also on the modelling side (Van Den Brink & Daam, 2014).

Both the estimate of exposure (more complex model scenarios) and effects becomes more realistic as higher tiers, as uncertainty is reduced through the acquisition of more and better quality, as well as realistic, data. Tiers are designed so that lower tiers in risk assessment are more conservative, meaning it is less likely to pass a hazardous chemical, and higher tiers are more realistic with assumptions approaching the reality (Posthuma et al., 2001).

Subsequently, in the context of the registration of pesticides (prospective ERA) on the European market, it is a common practice to use microcosm and mesocosm experiments as a higher tier test approach to derive regulatory acceptable concentrations (RACs) for edge-of-field surface waters (EFSA, 2013b). They can also be used to achieve environmental quality standards (EQS), i.e. retrospective ERA, within the European Union's Water Framework Directive (EC, 2011).

A problem that has frequently been disputed with the model ecosystem experiment is their statistical power, i.e., their ability/sensibility to demonstrate statistically significant effects at the population and community levels. To date, few practical guides are available for dealing with the statistical power of a microcosm and mesocosm experiments. One of the most promising tool for this end is the minimum detectable difference (MDD) outlined by Brock et al., (2014), and currently requested in the EU for model ecosystem experiments conducted in the scope of the prospective ERA of pesticides (EFSA, 2013).

3.8. Minimum Detectable Difference

The minimum detectable difference (MDD) is a measure of the difference between the means of a treatment and the control that must exist to detect a statistically significant effects, at a defined level of probability and a given variability of the data (Duquesne et al., 2020).

The MDD concept was first introduced as an extension of the least significant difference (LSD) in 1967 by Snedecor and Cochran and it has been further discussed and developed in several publications afterwards.

Thus, the MDD analysis provides an indication of the robustness of the ecotoxicological thresholds such as the LOEC and NOEC, when analysing the endpoints at a given time after

treatment, for example, a MDD with a β-value of 0.2 indicates that the effect level that will not be overlooked is 80% of the cases, that is, a probability of 80% (Duquesne et al., 2020).

Duquesne et al., (2020) indicated that although MDD reports the sensitivity of the system to a toxic substance and thus the suitability for studying treatment-related effects, it is not designed or meant to define the degree of acceptability of model ecosystem studies.

These authors further indicated that conclusions on the acceptability of risks are usually drawn from statistical analysis of complex results of these studies and since these studies often have high variability and low replicate number, it is important to analyse and communicate the degree of certainty on which decisions are based off (Duquesne et al., 2020).

In the environmental risk assessment of pesticides in the European Union (EU), the MDD was first proposed in the Aquatic Guidance Document published by EFSA in 2013, in order to support the interpretation of the results and outcomes from complex microcosms/mesocosm studies. It is important and decisive to determinate whether an effect has a high probability of being detected, this is if an endpoint deviation from controls at another concentration of toxic substance can be identified as statistically significant effect or not (Duquesne et al., 2020).

Subsequently, this is to assure that eventual effects not detected in model ecosystems are in fact because the pesticide of concern may be expected not to have side effects at the concentrations tested, or whether this is due to intrinsic conditions of the test design and hence statistical power of the experiment.

Therefore, it is important to determinate the statistical reliability of the conclusions drawn from microcosm/mesocosms experiments, which depends on the power of the experiment conducted (Brock et al., 2014). This power is the probability that will find a given difference between the control and the means of treatment is statistically significant. Power analysis can be used “a priori” to calculate the minimum number of replicants per treatment required so that it can be reasonably likely to detect a relevant effect (Brock et al., 2014).

In microcosm and mesocosm experiments, however, it is difficult to perform a power analysis “a priori”. This is because of the inherent variability of these community level tests (Brock et al., 2014). With that in mind, higher tier studies, such as microcosms and mesocosms experiments, use the specific “a posteriori” MDD method. It is important to mention that MDD is calculated for each sampling moment by species.

The first equation to calculate the MDD was adapted from Lee and Gurland, 1975:

$$MDD = (x_1 - x_2) = t\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} \quad (1)$$

where x_1 is the arithmetic mean of control, that is the number of individuals for each species found in the control, x_2 is the arithmetic mean of treatment, that is, the number of individual found for

each species per mean of treatment, s_1^2 and s_2^2 are the variance of control and treatment, n_1 and n_2 are the numbers of control and treatment samples and t is the tabulated t value for t test. The percentage of control means is usually expressed by:

$$\%MDD = \frac{MDD \times 100}{x_1} \quad (2)$$

The general formula of the student's t value can be expressed as:

$$t = t_{\alpha,n_0+n-2} + t_{\beta,n_0+n-2} \quad (3)$$

where t_{α,n_0+n-2} is the student's t value with $(n_0 + n - 2)$ degrees of freedom corresponding to α and t_{β,n_0+n-2} is the student's t value with $(n_0 + n - 2)$ degrees of freedom corresponding to β, n_0 corresponds to the number of replicates in control and n corresponds to the number of replicates for treatment. The formula for t can also be expressed as:

$$t = t_{1-\alpha,df} + t_{1-\beta,df} \quad (4)$$

where df corresponds to the degree of freedom for α and β. That way and taking in account equations 1 and 4, the MDD can be calculated with the follow:

$$MDD = (x_1 - x_2) = (t_{1-\alpha,df} + t_{1-\beta,df})\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} \quad (5)$$

With the use of equation 5, it is possible to control the parameters α and β. In practice, this equation allows to detect minimal changes between the treatments and control.

The use of MDD and its equation has been discussed various times and in various studies. Wang et al. (2000) recommended in their study to apply a β-value of 0,05 which would guarantee a statistical power of 95% for the test to detect a difference and consequently a higher degree of certainty.

When MDD was further discussed a new approach was proposed by EFSA in 2013, to apply the MDD calculation for the evaluation of aquatic microcosm and mesocosm studies. With that the MDD formula was introduced with a different variant of the t-formula.

According to Brock et al. (2014), who explain this new method adapted for model ecosystems in detail, for the two sample and multitude test, the MDD can be calculated by rearranged formula of the t test using equation 6 or 7. In equation 6, s_0^2 and s^2 corresponds to the treatment and control variances, respectively. As for equation 7 s corresponds to the residual standard error.

$$MDD = (\overline{x_0} - \bar{x})^* = t_{1-\alpha,df,k}\sqrt{\frac{s_0^2}{n_0} + \frac{s^2}{n}} \quad (6)$$

$$MDD = (\overline{x_0} - \bar{x})^* = t_{1-\alpha,df,k}\, s\sqrt{\frac{s_0^2}{n_0} + \frac{s^2}{n}} \quad (7)$$

where $t_{1-\alpha,df,k}$ is the quantile of the t-distribution, df is the degree of freedom, k corresponds to the number of comparisons, $(\overline{x_0} - \bar{x})^*$ is the difference between the control and the treatments and n_0 and n are the sample sizes. This MDD can only be derived from results of parametric tests, i.e., variants of the t test. Still according to Brock et al. (2014), it is convenient to give the MDD as a percentage of the control mean, what is illustrated in equation 8.

$$\%MDD = \frac{MDD}{\overline{x_0}} \times 100 \quad (8)$$

Abundance data is usually log-transformed for statistical testing, so the MDD is also related to the transformed data. Because the percentage effects on a log-scale are difficult to interpret, Brock et al. (2014) suggested back-transforming the MDD to the abundance scale and using that MDD for evaluation. For the present study, equations 7 and 8 were applied and the abundance scale MDD were used (see section 4).

If the transformation, y(x) = ln(Ax + 1), suggested be Van den Brink et al (2000) is used, which is often the case for biological population and community datasets from model ecosystem experiments, the MDD_{abu} (MDD for abundance) can be calculated from the MDD_{ln}, which represents the MDD given for the transformed data, with the following formula and using the back-transformation, x = (exp(y(x)) – 1)/a and the arithmetic mean for the transformed value, $mean_{co,ln}$.

$$MDD_{abu} = \frac{(\exp(mean_{co,ln})-1)}{a} - \frac{\exp(mean_{co,ln} - MDD_{ln})-1}{a} \quad (9)$$

This formula is simplified to

$$MDD_{abu} = \frac{(\exp(mean_{co,ln})-1)-\exp(mean_{co,ln} - MDD_{ln})-1}{a} \quad (10)$$

$$\%MDD_{abu} = 100MDD_{abu} / \left(\frac{\exp(mean_{co,ln})-1}{a}\right) \quad (11)$$

The $\%MDD_{abu}$ is the MDD_{abu} related to the transformed means of control. In this means that the back-transformed means of the control corresponds to the geometric mean of the controls.

4. Method

4.1. Overview and rationale of the study methodology

In this thesis work, the MDD was calculated for the zooplankton populations in three model ecosystem experiments that had been previously conducted by the supervisor of the MSc candidate during his MSc and PhD thesis work. Evidently, these MDDs had never been calculated before and at the time these model ecosystem experiments were conducted, the calculation of an MDD was not common practice yet.

Below, a general description of the methodology of the three microcosm experiments is provided as well as some of the main results relevant for this dissertation. All experiments evaluated the same pesticide: the organophosphorus insecticide chlorpyrifos. This pesticide was and still is widely used worldwide, although its use was recently prohibited (10 January 2020) in the European Union due to possible genotoxic and neurological effects during development based on epidemiological data indicating effects in children (EC, 2020). However, for years, chlorpyrifos has been one of the most commonly applied insecticides in southern Europe due to its efficacy to combat coleopteran, dipteran, and orthopteran pests in vine, citrus and other fruit trees (Rico et al., 2020). In 2016, CPF was one of the 15 pesticides that was most frequently detected in food samples in Europe (out of 791 different pesticide residues analysed), with a large contribution coming from fruit and vegetable samples taken in South European countries (EFSA, 2019).

Since chlorpyrifos is an insecticide, arthropods may be expected to be especially sensitive to this compound (e.g. Rico et al., 2020; Rico & Van Den Brink, 2015). For aquatic communities, cladocerans and, often to a lesser extent, some rotifer and copepod taxa appear to be especially sensitive, which was confirmed in the three studies considered in this dissertation(Daam & Van den Brink, 2007; Daam et al., 2008a, b). Therefore, the MDDs were calculated for the zooplankton populations identified and counted throughout the course of the experimental period of the three microcosm studies.

4.2. General description of the experimental design of the microcosm studies

All the studies described below, were made, and published before this dissertation, and as so have not been made by the author of this dissertation. The next table provides a resume of the experimental design pf the three studies.

Table 1 – Summary of the experimental design.

Study	Test system	V(water)	Location	Water origin	Added Nutrients	Times added	Country
1	Microcosm	8,5 L	Indoor	Pond next to the research center	N as NaNO3; P as KH2PO4; HCO3 as NaHCO3	Twice in pre-treatment; Twice a week	The Netherlands
2	Microcosm	250 L	Outdoor	Canal surrounding Asian Institute of Technology	N as urea; P as Triple superphosphate	Twice a week	Thailand
3	Microcosm	1000 L	Outdoor	Canal surrounding Asian Institute of Technology	N as urea; P as Triple superphosphate	Twice a week	Thailand

Microcosm study 1 (Daam and Van den Brink, 2007)

In the first study, twelve microcosms were situated in a laboratory room devoid of daylight and maintained at 21ºC ± 1ºC (figure 4). Each test system consisted of a glass chamber, with a diameter of 24.5 cm and a height of 36 cm, filled with 8.5 L pond water. Additional plankton was introduced into the microcosms along with the pond water during the preparatory phase of the experiment. The water and additional plankton were obtained from a pond next to the building where the research was made (Alterra, Wageningen, the Netherlands). The water was sieved through a 0.75 mm mesh size before adding it to the microcosms to exclude the addition of (predatory) macroinvertebrates and fish.

Figure 4 – Model Ecosystems used and predesposition (Source: Michiel Daam).

The systems were stirred for 5 min every 30 min at a velocity of 20 rpm to prevent setting of planktonic algae (figure 4). To provide light to the systems, a fluorescent lamp was placed around the microcosms, which resulted in a light intensity of 45 µE/m^{2}s in the middle and 60 µE/m^{2}s at the edges of the chambers. The photoperiod was 14 hours daily. Nutrient addition of 0.0115 mg of N as $NaNO_3$, 0.014 mg of P as KH_2PO_4 and 0.7 mg of HCO_3 as $NaHCO_3$ was applied twice

during the pre-treatment period and twice time a week during the treatment period. The pre-treatment period consisted in the stabilization of the microcosms which took 1 week.

Other than the stirrer opening, the microcosms had five smaller openings, of which four were closed with air-tight screw caps and one was connected to an air compressor. To take water samples, one of the screw caps was replaced by a cap with a rubber ring through which a glass pipette was inserted to ± 10 cm bellow the water surface. The sample was extracted by adding compressed air into the system which forced water through the pipette into a sampling cup.

Chlorpyrifos was applied as Dursban 4E (nominal concentrations: 0.005, 0.05, 0.5, and 5 µg active ingredient/L) to two microcosms for each concentration, while four other systems were untreated to serve as controls. Just after application, as well as 3 and 14 days post application, water samples were taken for chemical analysis of chlorpyrifos (for detailed method, please refer to Daam and Van den Brink, 2007). Given the small water volume of the microcosms, samples for zooplankton species identification and counting were only taken at the end of the experiment (day 14).

Microcosm study 2 (Daam et al., 2008a)

The second microcosm study was conducted using ten circular outdoor experimental microcosms, with a diameter of 0.76 m and a water depth of 0.56 m, which corresponds to a water volume of ± 250 L (figure 5). The concrete tanks were coated with a watertight non-toxic epoxy paint and set up outdoors at the hatchery of the Asian Institute of Technology (AIT), approximately 40 km North of Bangkok, Thailand.

Figure 5 – Circular tanks used to set the microcoms (Source: Michiel Daam).

The test systems were filled with water from the canal surrounding AIT after being filtered through a net with a mesh size of 0.1 mm to avoid fish and prawns entering the test systems. No sediment was added. One week prior to chlorpyrifos application, zooplankton was collected from the AIT canal and homogeneously distributed over the test systems.

During the week prior to chlorpyrifos application, the water was circulated between the tanks two times, by manually exchanging 100 L between the microcosms using a Perspex tube, in order to achieve similarity between the communities in the systems. A nutrient addition of 1.4 mg N/L (as urea) and 0.35 mg P/L (as triple superphosphate) was made twice a week during the entire experiment. These nutrients concentrations were based on the concentrations used in growth ponds in the AIT hatchery.

Chlorpyrifos was applied as an aqueous solution of Dursban (active ingredient chlorpyrifos: 40%) to six microcosms at a concentration of 1 µg active ingredient/L. This concentration was chosen since it matched the LC_{50} of the temperate standard test species *Daphnia magna* and corresponded as well with the LC_{50} of two others temperate cladocerans and the tropical cladoceran *Moina micrura* (Daam et al., 2008a). Four other systems were used as controls and were therefore untreated. Two weeks after the application, three of the six microcosms received a second dosage of chlorpyrifos at 1 µg/L. Subsequently, three different treatment were considered in this study: 1) control ((n = 4); 2) single chlorpyrifos application of 1 µg a.i./L (n = 3); 3) repeated chlorpyrifos application of 1 µg a.i./L (n = 3). Samples for the determination of chlorpyrifos concentrations were made at several moments throughout the experimental period (for details: see Daam et al., 2008a). In addition, the zooplankton community was sampled half a week and a few hours before chlorpyrifos application, and weekly after the application of the insecticide.

Microcosm study 3 (Daam et al., 2008b)

The third microcosm study was also conducted in Thailand but in 2002/2003, whereas the study described above was conducted in early 2005. This third study was also conducted at AIT (Thailand) using twelve outdoor microcosms, but different tanks than those used in the second study. Each microcosm consisted of a concrete tank with 1 m length, 1 m width and 1.15 m height, coated with a non-toxic epoxy paint.

Figure 6 – Tanks used in the experiment of the second study (Source: Micheil Daam).

The tanks were filled a 10-cm sediment layer and ± 1000 L water obtained from the AIT canal. Besides plankton, these microcosms were also seeded with macroinvertebrates, but fish and prawns were excluded, by passing the pond water through a net, with a mesh size of 0.1 mm. Macrophytes were not allowed to grow in the tanks (i.e., roots were manually removed from the sediment) since canals in agricultural areas, which were intended to be simulated, were noted to be devoid of macrophytes.

The pre-treatment period lasted six weeks, during which a biocoenosis was allowed to develop in the microcosms. During that period, the water was circulated twice a week by manually exchanging 100 L between the microcosms to achieve similarity between the communities. An addition of 1.4 mg N/L (as urea) and 0.35 mg P/L (as triple superphosphate) was made twice a week during the entire experiment.

The application of chlorpyrifos (as Dursban 40 EC) was made once to eight microcosms in four duplicate treatments, with nominal concentrations of 0.1, 1, 10, and 100 µg active ingredient/L, whereas four other test systems served as controls. Samples to determine chlorpyrifos concentrations in water and sediment were taken throughout the course of the experiment (See Daam et al., 2008 for details). The zooplankton community was sampled at weekly intervals.

Zooplankton identification and statistical analysis

Depth-integrated water samples of 5 L (or 6 L in Daam and Van den Brink, 2007) were taken at sampling days for zooplankton. This water sample was passed through a zooplankton net (mesh size, 60 µm or 40 µm in Daam and Van den Brink, 2007) and the concentrated zooplankton samples were fixed with formalin to a final concentration of 4%. Zooplankton in subsamples were identified and counted with an inverted microscope, and numbers were converted to numbers per litre microcosm water.

In all studies, no observed effects concentrations (NOECs) were calculated for all parameters using the parametric Williams test. The Williams test is an analysis of variance (ANOVA) test that assumes increasing effect for increased dose. Abundance data were ln (Ax +1) transformed, where x stands for the abundance value, and Ax makes 2 when taking the lowest abundance value higher than zero for x. This was done to down-weight high abundance values and approximate a normal distribution of the data. Statistical significance was accepted at $p < 0,05$. In the study where a second application was made (Daam et al., 2008a), before that application the p value was accepted at $p < 0,005$, and after the second application the statistical significance between the treatments and the control were calculated using the Dunnett's test and expressed as p-values.

Main results on zooplankton in the three studies

In the study by Van den Brink and Daam (2007) 21 invertebrate taxa were identified in the chlorpyrifos experiment, and their abundances determined. The most important taxonomic groups were (in decreasing order) Rotifera, Cladocera, Copepoda, Insecta and Ostracoda (not identified at a species level). It was verified that Cladocera increased in time in the controls and that they were eliminated in the test systems with the higher chlorpyrifos concentrations, which was in line with the expectations (see above).

In the study by Daam et al. (2008a), the dominant zooplankton species before application belonged to the Rotifera and Copepoda groups, while Cladocera and Ostracoda were less dominant. Before the first chlorpyrifos application, the dominant zooplankton species in all microcosms belonged to the Rotifera and Copepoda groups, whereas Cladocera and Ostracoda occurred in low numbers. During the course of the experiment, Cladocera and Ostracoda increased in numbers in the control systems while Copepoda showed the opposite trend. By the end of the experiment, Cladocera and Ostracoda, and to a lesser extent the Rotifera, dominated the control zooplankton community. Also, in this experiment, cladocerans were the most sensitive zooplankton taxa, although the most sensitive cladoceran species was different after the first and second chlorpyrifos application: *Ceriodaphnia cornuta* was the most responding cladoceran after the first treatment, while *Moina micrura* responded most to the second. This was explained by differences in the growth phase of the *M. micrura* at the time of the two applications and an increase in the cyanobacteria *Microcystis* spp. abundance over the course of the experiment (Daam et al., 2008a).

Like in the previous study, the pre-treatment zooplankton community was dominated by Rotifera and Copepoda, followed by Cladocera and Ostracoda, with Rotifera as the most diverse group.

The cladoceran *M. micrura* decreased in numbers because of the chlorpyrifos treatment, whereas the rotifer *Filinia longiseta*, the cladoceran *C. cornuta*, and Calanoid copepods increased in numbers (Daam et al., 2008b). Contrarily to the other two studies, the zooplankton communities in the 1 µg chlorpyrifos/L treated microcosms returned to a state resembling that of the controls within the experimental period (8 weeks), indicating recovery. The table 2 synthesizes the results of zooplankton for the three studies.

Table 2 – Species identifies on the three studies.

Taxa	Study 1	Study 2	Study 3	Group
Alona rectangula	X	-	-	Cladocera
Alona sp.	-	X	-	Cladocera
Asplanchna sp.	-	-	X	Rotifera
Bosmina spp.	X	-	-	Cladocera
Brachionus angularis	-	X	X	Rotifera
Brachionus calyciflorus	-	X	X	Rotifera
Brachionus falcatus	-	X	X	Rotifera

Table 2 (cont.) – Species identifies on the three studies.

Taxa	Study 1	Study 2	Study 3	Group
Brachionus quadridentatus	-	X	-	Rotifera
Brachionus urceolaris	-	X	-	Rotifera
Calanoid copepod	-	X	X	Copepoda
Cephalodella gibba	X	-	-	Rotifera
Ceriodaphnia cornuta	-	X	X	Cladocera
Chydorus sphaerica	X	-	-	Cladocera
Colurella sp.	-	X	-	Rotifera
Colurella uncinata	X	-	-	Rotifera
Cyclopoid copepod	-	X	X	Copepoda
Cyclops cyclopoidae	X	-	-	Copepoda
Daphnia galeata	X	-	-	Cladocera
Daphnia magna	X	-	-	Cladocera
Diffugia sp.	-	-	X	Rotifera
Dunhevedia crassa	-	X	X	Cladocera
Ephemeroptera	X	-	-	-
Ephippia	X	-	-	Cladocera
Filinia longiseta	-	X	X	Rotifera
Filinia opoliensis	-	X	-	Rotifera
Hexarthra mira	-	X	X	Rotifera
Keratella coclearis	X	-	-	Rotifera
Keratella quadrata	X	-	-	Rotifera
Keratella tropica	-	X	X	Rotifera
Lecane bulla	X	X	-	Rotifera
Lecane closterocerca	-	X	-	Rotifera
Lecane luna	X	X	-	Rotifera
Lecane lunaris	X	-	-	Rotifera
Lecane quadridentata	X	-	-	Rotifera
Lepadella patella	X	X	-	Rotifera
Moina micrura	-	X	-	Cladocera
Mytilina ventralis	X	-	-	Rotifera
Nauplii	-	X	-	Copepoda
Ostracoda	X	X	X	Ostracoda
Simocephalus vetulus	X	-	-	Cladocera
Streblocerus pygmaues	-	X	-	Cladocera
Trichocerca	X	-	-	Rotifera

4.3. MDD calculations

The MDD was calculated based on the method detailed in Brock et al. (2014), which is in accordance with the official EU Requirements outlined in the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters

(EFSA, 2013). To this end, equations 7, 8, 10 and 11 provided in section 2.8 were applied, which are available in a windows Excel file provided by Brock et al. (2014) as Supplementary Material to their paper. Brock et al. (2014) suggested that the MDD should be reported together with the NOEC table for each taxon and time period, along with tables with means of the transformed data, the retransformation of the means and the two MDDs related to the transformed and non-transformed abundance data.

If the MDD_{abu} is inferior to 100% for a specific taxon, a treatment related effect and a decline in abundance can be demonstrated in theory. On another hand, if the MDD_{abu} value is above 100%, the power of the test is too low to demonstrate a treatment related effect. With this in mind, EFSA (2013) categorized the %MDD values in five classes (table 3). These classes can be used to categorize taxa in microcosms and mesocosms experiments on the basis on their calculated MDD for each individual sampling day.

Table 3 – Proposal on classes of minimal detectable differences (MDD) due to treatment-related declines in abundance/biomass (Source: EFSA, 2013)

Class	MDD	Comment
0	> 100%	No effect can be determined
I	90 - 100%	Only large effects can be determined
II	70 - 90%	Large to medium effects can be determined
III	50 - 70%	Medium effects can be determined
IV	< 50%	Small effects can be determined

Since zooplankton samples are taken throughout the course of model ecosystem experiments, (see e.g. section 4.2), multiple MDDs are calculated for a single taxon. Subsequently, it is necessary to categorize these MDDs calculated for a single taxon on these different sampling moments. In Brock et al. (2014), three categories of taxa on the basis of their MDD_{abu} values measured throughout the experimental period of a model ecosystem experiment were distinguished. Category 1 taxa are characterized by a sufficient statistical power to potentially demonstrate treatment related responses and a no adverse effect concentration, following the MDD criteria outlined in table 1. There are four ways that taxa may fall into this category, depending on the MDD_{abu} value and the number of sampling days within the post-exposure period that this value is achieved:

- MDD_{abu} < 100% at no less than five samplings, or
- MDD_{abu} < 90% at no less than four samplings, or
- MDD_{abu} < 70% at no less than three samplings, or
- MDD_{abu} < 50% at no less than two samplings.

Is worth noting that category 1 is relevant to all taxa that show consistent treatment related declines in population abundance. If the requirements for category 1 are not meet, they belong to category 2 taxa if a LOEC could be calculated on at least one sampling day. This category

comprises taxa that show statistically significant treatment-related effects on samplings when the MDD_{abu} values are < 100%. Category 2 taxa also comprise those taxa that are characterised by statistically significant increases for which (i) MDD_{abu} values are < 100% but do not meet conditions for category 1, (ii) MDD_{abu} values are higher than 100% and (iii) MDD_{abu} values cannot be determined due to the absence of the taxon in controls. The third and final category comprises the taxa that do not meet the category 1 criterion and for which no significant differences with the control were found on any sampling moment. The following flowchart helps synthesize and represent the criteria and how to achieve it.

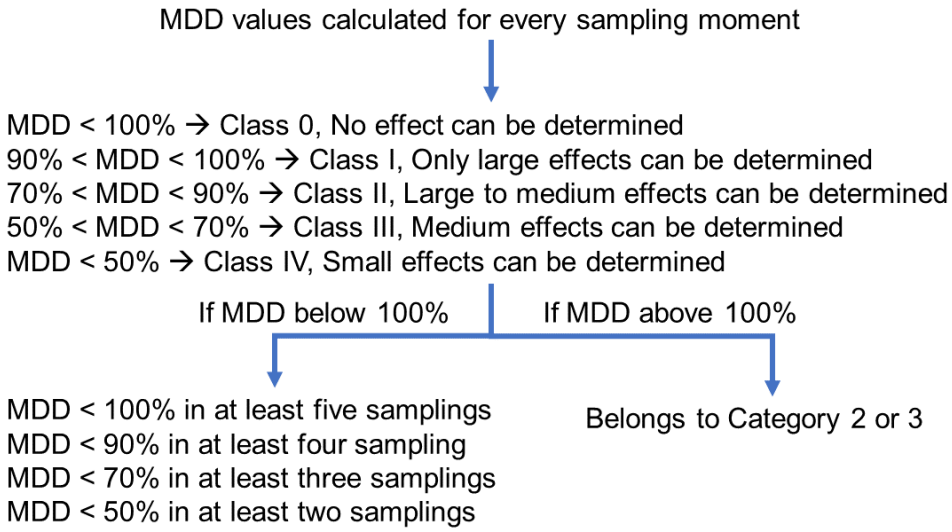

Figure 7 – Flowchart with MDD classes and Category 1 criteria.

For each taxon belonging to a specific organism group and characterised by the sampling methods, the statistical findings are used to create summary tables on the basis of the above categories. These tables include the NOECs and the related MDD_{abu} for each taxon, and for each sampling date.

Category 1 taxa are and can be used to evaluate the reliability of a microcosm/mesocosm experiment to demonstrate treatment related effects. For the effect classification of treatment related effects, category 1 and 2 can be used. As for category 3, it cannot be used in the evaluation of the treatment related effects.

It is evidently important that a model ecosystem study is reliable so that conclusions and decisions can be taken with based on it. Given the above, Brock et al. (2014) and EFSA (2013) concluded that at least eight taxa of a potentially sensitive taxonomic group should be present. In practical terms, this means that a model ecosystem study is considered to have a reliable statistical power based on the MDD analyses if at least eight category 1 taxa are present.

5. Results and Discussion

5.1. Data availability

In the present study, MDDs were calculated for each zooplankton taxon and taxon group (Cladocera, Copepoda, Ostracoda and Rotifera) of all the three studies (see section 4.2). In total, MDDs could be calculated for 21(Daam & Van Den Brink, 2007) , 27 (Michiel A. Daam et al., 2008a), and 18 (Daam et al., 2008b) zooplankton taxa. Thus, were used in total, 66 zooplankton taxa from all the three studies.

Of all these 66 zooplankton taxa, only 12 were category 1 taxa. There were cases in which none of the individual species met the criteria but when grouped where able to meet the criteria, as such it was 22 MDDs that follow the criteria to fall on category 1. Some of the species that showed significant effect did not fall in category 1 based on the calculated MDD value. Of all the 66 used species, only 14 had a MDD below 100% and showed significant effect. Also, 21 species were only sampled once and therefore they do not meet the criteria.

Tables 16 to 21 are expressed in the Annexes and there are expressed the raw data for the three studies (tables 16, 17 and 18) and the results from the calculation of MDD, meaning, the values of $\%MDD_{abu}$ used (tables 19, 20 and 21)

5.2. Microcosm study 1

The zooplankton species of the first model ecosystem study, that used the 8.5 L laboratory microcosms in the Netherlands (Daam & Van Den Brink, 2007), are presented in table 4. None of the zooplankton species of this study could fall under category 1 for the simple fact that there was only one zooplankton sampling moment (i.e. at the end of the experiment), so it is impossible to fulfil the criteria with only one sample. Even by grouping the species in taxonomic groups, it was not possible to meet the criteria, as seen in table 5. Even when only considering the last sampling day, only three species showed a negative treatment-related effect: the rotifer *Lepadella patella* and the cladocerans *Chydorus sphaerica* and *Simocephalus vetulus* (table 4).

During the course of this model ecosystem experiment (3, 7 and 14 days post application), the total numbers of cladocerans were determined in a 250-mL water sample. After counting, the water samples were returned to the corresponding microcosm. The MDD_{abu} that were calculated from these samples were: 63-66% (day 3); 67-79% (day 7) and 55-56% (day 14). Subsequently, since these MDD_{abu} were < 70% at no less than three samplings, this parameter may be categorized as category 1, the more since the respective NOECs indicating decreased cladoceran abundances relative to controls were 0.1 µg/L (day 3) and 0.01 µg/L (days 7 and 14).

Table 4 – Species used in study mande in laboratory (8.5 L glass chambers), taxa, statistically significant effect (positive for increased effect and negative for decreased effect) and if catefory 1 criteria was met (N for no and Y for yes)

Taxa	Group	Category 1	Statistically significant effect
Alona rectangula	Cladocera	N	-
Bosmina spp.	Cladocera	N	-
Cephalodella gibba	Rotifera	N	Positive
Chydorus sphaerica	Cladocera	N	Negative
Colurella uncicanata	Rotifera	N	-
Cyclops cyclopoidae	Copepoda	N	-
Daphnia galeata	Cladocera	N	-
Daphnia magna	Cladocera	N	-
Ephemeroptera	-	N	-
Ephippia	Cladocera	N	-
Keratella cochlearis	Rotifera	N	-
Keratella quadrata	Rotifera	N	-
Lecane bulla	Rotifera	N	Positive
Lecane luna	Rotifera	N	-
Lecane lunaris	Rotifera	N	-
Lecane quadridentata	Rotifera	N	-
Lepadella patella	Rotifera	N	Negative
Mytilina ventralis	Rotifera	N	-
Ostracoda	Ostracoda	N	-
Simocephalus vetulus	Cladocera	N	Negative
Trichocerca	Rotifera	N	Positive
Small Cladocerans	-	N	-
Big Cladocerans	-	N	-

Table 5 – Species grouped by taxonomic group, number of species in each taxonomic group whether the group met the category 1 criteria and if statiscal significant effects were encountered (and if so, its direction: positive i.e. an increase relarive to controls, or negative, i.e., a decrease relative to the controls).

Taxa	Total of Species	Category 1 total	Category 1 with statistically significant effect
Rotifera	11	N	Negative
Copepoda	1	N	Negative
Cladocera	7	N	Negative
Ostracoda	1	N	Negative

5.3. Microcosms study 2

In the second study, whose data are expressed in table 6, there were two zooplankton species out of a total of 23 (8.7%) with MDDs that met the category 1 criteria. For three taxonomic groups,

the MDDs also met the category 1 criteria; only for Copepoda they did not. Both species in category 1 also showed significant negative treatments effects on the application of chlorpyrifos. Regarding the taxonomically grouped MDDs, the Cladocera taxa showed significant negative treatment effect, and Rotifera and Ostracoda both showed significant positive treatment effects of chlorpyrifos. Through table 7 it is possible to see that, while no individual rotifer species met the category 1 criteria, when the abundance data of all the rotifer species were grouped together, they were able to meet these criteria.

Table 6 – Species used in study made in Thailand (250 L in concrete tanks; chlorpyrifos), taxa, statistically significant effect (positive for increased effect and negative for decreased effect) and if catefory 1 criteria was met (N for no and Y for yes)

Taxa	**Group**	**Category 1**	**Statistically significant effect**
Ceriodaphnia cornuta	Cladocera	Y	Negative
Moina micrura	Cladocera	Y	Negative
Alona sp.	Cladocera	N	-
Brachionus angularis	Rotifera	N	-
Brachionus calyciflorus	Rotifera	N	Positive
Brachionus falcatus	Rotifera	N	-
Brachionus quadridentatus	Rotifera	N	-
Brachionus urceolaris	Rotifera	N	Negative
Calanoid copepod	Copepoda	N	Negative
Colurella sp.	Rotifera	N	Positive
Cyclopoid copepod	Copepoda	N	Negative
Dunhevedia crassa	Cladocera	N	-
Filinia longiseta	Rotifera	N	-
Filinia opoliensis	Rotifera	N	-
Hexarthra mira	Rotifera	N	Positive
Keratella tropica	Rotifera	N	Positive
Lecane bulla	Rotifera	N	-
Lecane closterocerca	Rotifera	N	-
Lecane luna	Rotifera	N	-
Lepadella patella	Rotifera	N	-
Nauplii	Copepoda	N	-
Streblocerus pygmaues	Cladocera	N	Positive
Trichocerca sp.	Rotifera	N	Positive
Ostracoda	Ostracoda	Y	Positive
Rotifera	-	Y	Positive
Copepoda	-	N	No Effect
Cladocera	-	Y	Negative

Table 7 – Species grouped by taxa, number of species in each taxa and if the group met the category 1 criteria.

Taxa	Total of Species	Category 1 total	Category 1 with statistically significant effect
Rotifera	15	Y	8/11 samples <100
Copepoda	3	N	3/11 samples <100
Cladocera	5	Y	11/11 samples <100
Ostracoda	1	Y	8/11 samples <100

5.4. Microcosms study 3

In the third study using the 1000 L outdoor square tanks (Daam et al., 2008b), the MDDs of four out of fifteen species (27%) MDDs met the category 1 criteria (table 8). One of the species presented a positive significant effect, what means that during the experiment it had a treatment-related increase that was statistically significant, and the three other species had a statistically significant decrease. When it comes to the species grouped in the taxonomic groups Rotifera, Copepoda and Cladocera. All MDDs met the category 1 criteria, and all showed a statistically significant treatment related decrease (table 9).

Table 8 – Species used in study made in Thailand (1 000 L in concrete tanks), taxa, statistically significant effect (positive for increased effect and negative for decreased effect) and if catefory 1 criteria was met (N for no and Y for yes)

Taxa	Group	Category 1	Statistically significant effect
Cyclopoid copepod	Copepods	Y	Positive
Keratella tropica	Rotifera	Y	Negative
Moina micrura	Cladocera	Y	Negative
Nauplii	Copepods	Y	Negative
Asplanchna sp.	Rotifera	N	Positive
Brachionus angularis	Rotifera	N	-
Brachionus calyciflorus	Rotifera	N	-
Brachionus falcatus	Rotifera	N	-
Calanoid copepod	Copepods	N	Positive
Ceriodaphnia cornuta	Cladocera	N	Negative
Diffugia sp.	Rotifera	N	-
Dunhevedia crassa	Cladocera	N	-
Filinia longiseta	Rotifera	N	Positive
Hexarthra mira	Rotifera	N	Positive
Ostracoda	Ostracoda	N	Negative
Rotifera	-	Y	Negative
Copepoda	-	Y	Negative
Cladocera	-	Y	Negative

Table 9 – Species grouped by taxa, number of species in each taxa and if the group met the category 1 criteria.

Taxa	Total of Species	Category 1 total	Category 1 with statistically significant effect
Rotifera	8	Y	9 samples <100
Copepoda	3	Y	9 samples <100
Cladocera	3	Y	9 samples <100
Ostracoda	1	N	0 samples <100

5.5. Comparison of the MDD values of the three microcosm studies evaluating chlorpyrifos

Overall, there were species that showed statistically significant effects, either positive or negative, but did not meet the category 1 MDD criteria or did not have enough data for the MDD calculation, such as low control data, or large differences and discrepancy between the data. There were also species that were assent in the control and therefore it was not possible to calculate the MDD.

There were also species that either showed no statistically significant effect but presented a MDD class that met the criteria for MDD category 1. These species belong to another category that was not considered in this study (category 3; Brock et al., 2014).

The three studies evaluating chlorpyrifos did not meet the MDD category 1 criteria. There can be multiple reasons for this. Brock et al. (2014) provides some reasons why the criteria may not be met, but for the most cases, it was verified that this was due to the fact that there were only abundance values for control, i.e., the species was absent in all chlorpyrifos treatments, leading to elevated MDD values. In other cases, it was impossible to calculate MDD values due to the absence of individuals in control microcosms since the MDD formula needs control data > 0 to enable MDD to be calculated.

Another reason for the high MDD in general can be that, mainly in the smaller tanks, there were few species since the tank space is limited and this may have resulted in high intra- and inter species competition, in addition to mechanical interference (Daam and Van den Brink, 2008a). Many rare species that thus occurred in low abundances and/or on few sampling moments only, are also factors that are likely to have affected the MDD values of such species.

5.6. Time trend in MDD

The MDD evaluation above considered the MDD values calculated throughout the experimental periods and as to whether these adhered to the MDD category 1 criteria set in Brock et al. (2014). To also evaluate the time trend in MDD values calculated over the course of the experiments, the

MDD values for the taxonomic groups (Ostracoda, Rotifera, Copepoda and Cladocera are visualized is figure 7. To allow a comparison of the results, only the treatment of 1 µg/L (Daam et al., 2008a, b) or 0.05 µg/L (Daam & Van Den Brink, 2007) were considered.

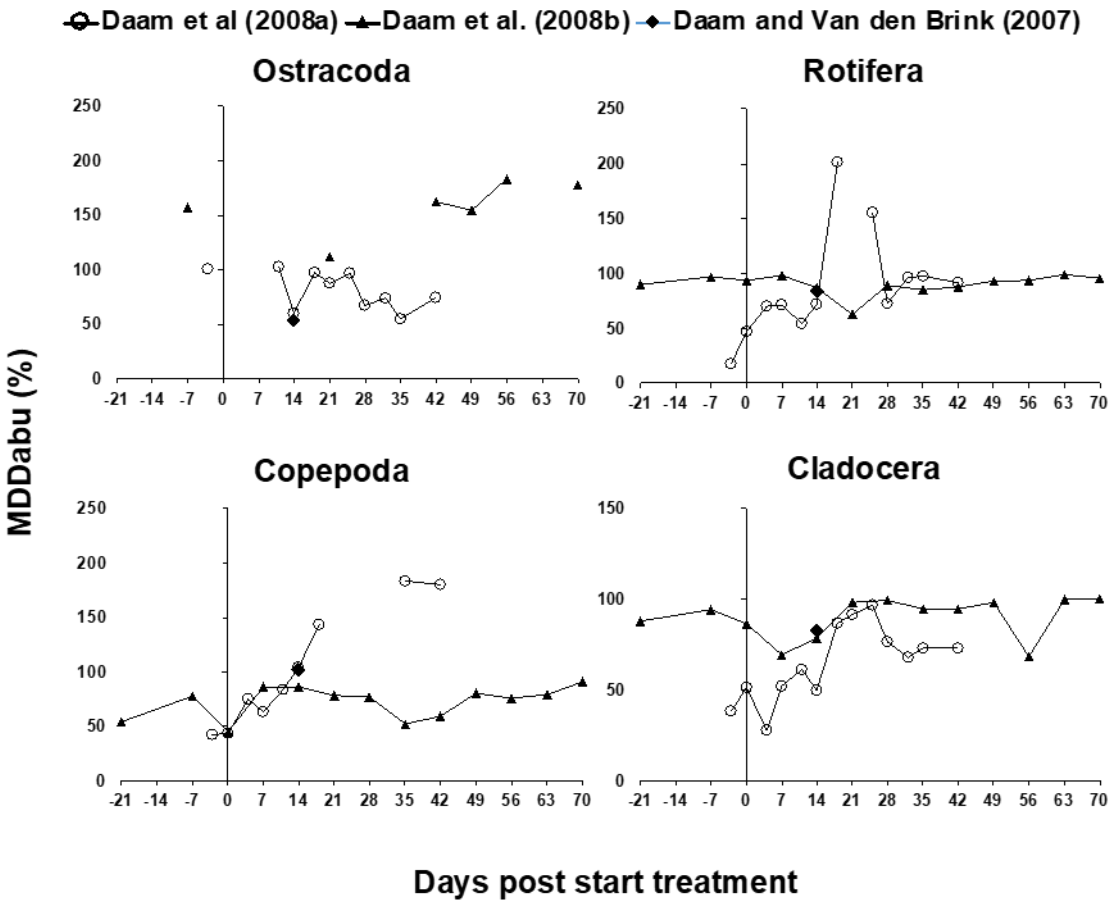

Figure 8 – Time trend in calculated MDD values for the taxonomic zooplankton groups Ostraconda, Rotifera, Copepoda and Cladocera over the course of the threemodel ecosystem experiments evaluating the insecticide chlorpyrifos.

As may be deducted from figure 8, MDD could not be calculated for many sampling days (section 5.5), which explains why lines are interrupted at several moments throughout the course of the experiments. Overall, the MDD values calculated on day 14 of all four groups for the laboratory study (Daam & Van Den Brink, 2007) appears to be similar to those calculated in the two outdoor microcosm studies conducted in Thailand. Interestingly, the MDD values of rotifers and cladocerans for the circular outdoor microcosms were lower at the beginning of the experiment and increased over time. This indicates that the statistical power lowered during this experiment. Although not discussed in Daam et al. (2008a), this may have been an additional reason why the second chlorpyrifos application of 1 µg/L made 14 days after the first application led to different treatment effects than the first application. The fact that this was not observed in the larger 1000 L microcosms may have been due to the fact that in the latter experiment a longer pre-treatment

period was used. Climatic conditions in tropical freshwater ecosystems are characterized by overall high temperatures, light conditions, and generally non-limited nutrient conditions, like occurred in the microcosms with the nutrient additions made (see section 4.2). Therefore, tropical freshwater communities are known to dominated by fact growing, *r*-selected species with high levels of competition (Daam & Van Den Brink, 2011). For these reasons, populations and communities in tropical freshwaters are likely to show large fluctuations over time, as observed in the Thai model ecosystem studies, which increases the MDD values and thus decreases their statistical power. In the initial stage of the pre-treatment, after seeding the tanks with plankton (see section 4.2), many species were probably still present in sufficient numbers to achieve low MDD values. However, these high competition and population turnover levels are likely to have resulted in large fluctuations in abundances, as well as appearances and disappearances of rare species in subsequent sampling moments.

5.7. Additional study: phytoplankton and zooplankton in a model ecosystem study evaluating the herbicide linuron

An additional microcosm study conducted in Thailand evaluating the herbicide linuron was also evaluated (Daam et al., 2009). For this study, both the zooplankton and phytoplankton datasets were available, enabling a comparison in MDD values between these communities. This study was conducted with the same circular tanks as those used in the Daam et al. (2008a) study and followed a similar experimental set-up.

It was used 12 outdoor microcosms consisting of circular concrete tanks, with a diameter of 0,75 m and 0,65 m of height. The tanks were painted with watertight and non-toxic epoxy paint to make sure there was no influence from previous experiments. The test systems contained a water volume of 250 l which represented a water layer of 0,55 m, the water was collected from a canal surrounding AIT and was filtered thought a net with a mesh size of 0,1 mm to avoid fish and prawns entering in the system. To keep the system as simple as possible and to facilitate the interpretation of the treatment results, no sediment was added to the system.

Additional zooplankton was collected at the same canal and introduced in the microcosms in the preparatory phase of the experiment. There was an acclimatization period of 5 weeks, in which a biocoenosis developed in the microcosms. During this period, the water was circulated 2 times a week by collecting 100 l from each microcosm into a container, mixing and pumping it back to each of the microcosms, that was made to achieve similarity between the communities. There was also an addition of nutrients, mainly N (1,4 mg/l as urea) and P (0,18 mg/l as TSP) twice a week during the entire experimental period. For details on sampling frequency and plankton identification methods, please refer to Daam et al. (2009).

As discussed in section 2.4, beneficial primary producers like algae (and macrophytes, but these were not present in the microcosm) may be expected to be the aquatic organisms that are especially sensitive to herbicides (Van Wijngaarden and Arts, 2018). Indeed, linuron is a photosynthesis-inhibiting herbicide and existing laboratory toxicity data indicated that no severe direct toxic effects were anticipated at the linuron concentrations tested (0, 15, 50, 150, and 500 µg/L; Daam et al., 2009).

As can be deducted from table 10, six out of a total of 77 (7.7%) identified species over the course of the experiment had MDDs met the criteria for category 1 and showed a negative treatment-related effect. Two species families also met the criteria for category 1 and has treatment related effects of linuron (Chlorophyta and Bacillariophyta; table 11). Interestingly, none of the individual diatom taxa (Bacillariophyta) showed a statistically significant treatment-related effect (table 10), whereas as a sum of the taxonomic group (table 11) it did. This shows the importance of also evaluating the taxonomic groups in univariate statistical analyses of populations besides individual species.

Regarding zooplankton, 7 out of 19 (37%) species MDDs met the criteria for category 1 (table 12). However, all grouped zooplankton taxa met the category 1 MDD criteria (table 13). Mature copepods and the cladoceran *Moina micrura* decreased in abundances after linuron exposure (tables 12 and 13), probably as an indirect effect from the disappearance of edible phytoplankters (tables 10 and 11). The increase in immature copepod life stages (nauplii) was explained by Daam et al. (2009) with their possible diet of microorganisms, which may have increased due to the death of sensitive phytoplankton and zooplankton species.

The phytoplankton had 6 sensitive taxa out of 101 taxa and the zooplankton had 7 sensitive taxa in 23 taxa. Thus, it is possible to affirm that the fourth study was the one that had the highest level of sensitive taxa with MDD < 100% in at least five samplings, although the criterium of having at least eight taxa with MDD < 100% in at least five samplings (Brock et al., 2014) was not achieved. This overall higher level of MDD in this fourth study is probably related with the fact that this study was conducted in the cool/hot season (January-April 2005), whereas the two Thai model ecosystem studies evaluating chlorpyrifos were simultaneously in the rainy season of 2003. In the rainy season, the water stratification leads to dominance of the cyanobacterium *Microcystis aeruginosa*, which is not a preferred food source for zooplankters (Daam & Van Den Brink, 2011).

Table 10 – Species used in study made in Thailand (250 L in concrete tanks; linuron), taxa, statistically significant effect (positive for increased effect and negative for decreased effect) and if catefory 1 criteria was met (N for no and Y for yes) – phytoplankton.

Taxa	Group	Category 1	Statistically significant effect
Coelastrum cambricum	Chlorophyta	Y	Negative
Coelastrum spp.	Chlorophyta	Y	-
Oocystis spp.	Chlorophyta	Y	-

Table 10 (cont.) – Species used in study made in Thailand (250 L in concrete tanks; linuron), taxa, statistically significant effect (positive for increased effect and negative for decreased effect) and if catefory 1 criteria was met (N for no and Y for yes) – phytoplankton.

Taxa	Group	Category 1	Statistically significant effect
Pediastrum spp.	Chlorophyta	Y	-
Pediastrum tetras	Chlorophyta	Y	Negative
Scenedesmus aristatus	Chlorophyta	Y	Negative
Scenedesmus bicaudatus	Chlorophyta	Y	Negative
Scenedesmus dispar	Chlorophyta	Y	Negative
Scenedesmus maximus	Chlorophyta	Y	Negative
Scenedesmus spp.	Chlorophyta	Y	-
Amphora sp.	Bacillariophyta	N	-
Ankistrodesmus falcatus	Chlorophyta	N	No effect
Ankistrodesmus nannoselene	Chlorophyta	N	No effect
Ankistrodesmus spp.	Chlorophyta	N	-
Botryococcus braunnii	Chlorophyta	N	No effect
Campilomonas spp.	Cryptophyta	N	-
Chilomonas paramecium	Cryptophyta	N	No effect
Chroococcus dispersus	Cyanobacteria	N	-
Chroococcus dispersus var minor	Cyanobacteria	N	-
Chroococcus limneticus	Cyanobacteria	N	-
Chroococcus spp.	Cyanobacteria	N	-
Chroomonas acuta	Cryptophyta	N	-
Cocconeis sp.	Bacillariophyta	N	No effect
Coelastrum astroideum	Chlorophyta	N	-
Coelastrum microporum	Chlorophyta	N	-
Coelastrum reticulatum	Chlorophyta	N	No effect
Coelastrum sphaericum	Chlorophyta	N	-
Coelosphaerium sp.	Cyanobacteria	N	No effect
Cosmarium sp.	Charophyta	N	-
Cryptomonas ovata	Cryptophyta	N	-
Cryptomonas pyrenoidifera	Cryptophyta	N	No effect
Cryptomonas spp.	Cryptophyta	N	-
Cyclotella sp.	Bacillariophyta	N	No effect
Elakatothrix gelatinosa	Charophyta	N	-
Frustulia sp.	Bacillariophyta	N	-
Golenkinia radiata	Chlorophyta	N	-
Gomphonema parvulum	Bacillariophyta	N	-
Gomphosphaeria sp.	Cyanobacteria	N	-
Kirchneriella obesa	Chlorophyta	N	-
Mallomonas caudata	Ochrophyta	N	-
Mallomonas spp.	Ochrophyta	N	-
Merismopedia minima	Cyanobacteria	N	-
Merismopedia spp.	Cyanobacteria	N	-
Merismopedia tenuissima	Cyanobacteria	N	Negative

Table 10 (cont.) – Species used in study made in Thailand (250 L in concrete tanks; linuron), taxa, statistically significant effect (positive for increased effect and negative for decreased effect) and if catefory 1 criteria was met (N for no and Y for yes) – phytoplankton.

Taxa	Group	Category 1	Statistically significant effect
Micractinium pusillum	Chlorophyta	N	-
Microcystis aeruginosa	Cyanobacteria	N	-
Microcystis incerta	Cyanobacteria	N	-
Microcystis spp.	Cyanobacteria	N	-
Monoraphidium sp.	Chlorophyta	N	No effect
Nitzschia amphibia	Bacillariophyta	N	-
Nitzschia palea	Bacillariophyta	N	No effect
Nitzschia spp.	Bacillariophyta	N	-
Oocystis borgei	Chlorophyta	N	-
Oocystis elliptica	Chlorophyta	N	-
Oocystis lacustris	Chlorophyta	N	No effect
Oocystis pusilla	Chlorophyta	N	No effect
Oocystis rupestris	Chlorophyta	N	-
Oscillatoria limnetica	Cyanobacteria	N	-
Oscillatoria spp.	Cyanobacteria	N	-
Oscillatoria tenuis	Cyanobacteria	N	No effect
Pediastrum duplex	Chlorophyta	N	Negative
Pediastrum simplex	Chlorophyta	N	No effect
Phacus longispina	Euglenozoa	N	-
Phormidium mucicola	Cyanobacteria	N	-
Pseudanabaena limnetica	Cyanobacteria	N	-
Scenedesmus bernardii	Chlorophyta	N	-
Scenedesmus bijuga	Chlorophyta	N	-
Scenedesmus denticulatus	Chlorophyta	N	Negative
Scenedesmus denticulatus var linearis	Chlorophyta	N	-
Scenedesmus dimorphus	Chlorophyta	N	No effect
Scenedesmus longispina	Chlorophyta	N	-
Scenedesmus obliquus	Chlorophyta	N	-
Scenedesmus opoliensis	Chlorophyta	N	No effect
Scenedesmus perforatus	Chlorophyta	N	-
Scenedesmus quadricauda	Chlorophyta	N	Negative
Scenedesmus quadrispina	Chlorophyta	N	-
Scenedesmus tropicus	Chlorophyta	N	Negative
Schizochlamys sp.	Chlorophyta	N	-
Schroederia sp.	Chlorophyta	N	-
Sphaerocystis schoeteri	Chlorophyta	N	-
Sphaerocystis shroeteri	Chlorophyta	N	-
Spirulina laxissima	Cyanobacteria	N	-
Staurastrum sexangulare	Charophyta	N	-
Staurastrum sp.	Charophyta	N	-
Staurastrum spp.	Charophyta	N	-

Table 10 (cont.) – Species used in study made in Thailand (250 L in concrete tanks; linuron), taxa, statistically significant effect (positive for increased effect and negative for decreased effect) and if catefory 1 criteria was met (N for no and Y for yes) – phytoplankton.

Taxa	Group	Category 1	Statistically significant effect
Surirella tenera	Bacillariophyta	N	No effect
Tetraedron caudatum	Chlorophyta	N	No effect
Tetraedron minimum	Chlorophyta	N	-
Tetraedron spp.	Chlorophyta	N	-
Tetraedron trigonium	Chlorophyta	N	-
Chlorophyta	-	Y	-
Charophyta	-	N	-
Cyanoacteria	-	N	-
Bacillariophyta	-	Y	-
Cryptophyta	-	N	-
Ochrophyta	-	N	-
Euglenozoa	-	N	-
Charophyta	-	N	-
Other (Ochrophyta + Miscellaneous + Euglenozoa + Charophyta)	-	N	-

Table 11 – Species grouped by taxa, number of species in each taxa and if the group met the category 1 criteria – phytoplankton.

	Total of Species	Category 1 total	Category 1 with Statistically Significant Effect
Chlorophyta	49	Y	8 samples <100
Charophyta	4	N	0 samples <100
Cyanobacteria	18	N	4 samples <100
Bacillariophyta	9	Y	5 samples <100
Cryptophytas	5	N	1 samples <100
Ochorophyta	3	N	0 samples <100
Euglenozoa	1	N	0 samples <100
Charophyta	1	N	0 samples <100

Table 12 – Species used in study made in Thailand (250 L in concrete tanks; linuron), taxa, statistically significant effect (positive for increased effect and negative for decreased effect) and if catefory 1 criteria was met (N for no and Y for yes) – zooplankton.

Taxa	Group	Category 1	Statistically Significant Effect
Ceriodaphnia cornuta	Cladocera	Y	-
Cyclopoid copepod	Copepoda	Y	Negative
Diaphanosoma sp.	Cladocera	Y	-
Keratella tropica	Rotifera	Y	-
Moina micrura	Cladocera	Y	Negative
Nauplii	Copepoda	Y	Positive
Ostracoda	Ostracoda	Y	-

Table 12 (cont.) – Species used in study made in Thailand (250 L in concrete tanks; linuron), taxa, statistically significant effect (positive for increased effect and negative for decreased effect) and if catefory 1 criteria was met (N for no and Y for yes) – zooplankton.

Species	Group	Category 1	Statistically Significant Effect
Brachionus angularis	Rotifera	N	-
Brachionus calyciflorus	Rotifera	N	Negative
Brachionus falcatus	Rotifera	N	-
Brachionus urceolaris	Rotifera	N	-
Calanoid copepod	Copepoda	N	Negative
Filinia longiseta	Rotifera	N	-
Lecane bulla	Rotifera	N	-
Lecane closterocerca	Rotifera	N	Positive
Lecane luna	Rotifera	N	-
Lepadella patella	Rotifera	N	-
Streblocerus pygmaues	Cladocera	N	-
Trichocerca sp.	Rotifera	N	Positive
Rotifera	-	Y	-
Copepoda	-	Y	-
Copepoda mature stages	-	Y	Negative
Cladocera	-	Y	-

Table 13 – Species grouped by taxa, number of species in each taxa and if the group met the category 1 criteria – zooplankton.

Taxa	Total of Species	Category 1 total	Category 1 with Statistically Significant Effect
Rotifera	11	Y	8 samples <100
Copepoda	4	Y	8 samples <100
Cladocera	4	Y	8 samples <100
Ostracoda	1	Y	8 samples <100

5.8. Comparison of MDD values in published model ecosystem studies

A comparison was made between the studies used in this work and other studies that involved MDD calculations. In table 14 it is possible to notice that zooplankton groups fulfilled in general, the MDD criteria in most published studies. On species level, a very low percentage of the species identified in the experimental periods fulfilled the criteria. For example, in the study by Lobson et al., (2018) none of the species met the criteria set in Brock et al., (2014), and low percentages, up to a maximum of ± 20%, were calculated based on the data presented in Finnegan et al., (2018), Nys et al., (2019) and Yin et al., (2018).

An exception to the above was the study by Van Regenmortel et al. (2018), for which 72% of the zooplankton species met and fulfilled the MDD criteria. However, upon further analysis it may be

questioned if the MDDs in this study were calculated correctly. Many rare species, that only occurred on a few sampling dates or in few treatments were indicated as presenting low MDD levels, what means high statistical power. As an example, the abundance for *Trichocerca* group *similis* on day 28 of the experiment was only 1.66/L in two of the control replicates and one replicate of two metal mixture treatment on this day. Recalculation of the MDD using the raw data in the Supplemental Material of the paper indicated MDD values above 100% for the different mixture treatments, which is more in line with what may be expected from the abundance values.

Subsequently, the MDD values and interpretations presented in Van Regenmorter et al., (2018), should be interpretated with caution. The manual MDD calculation is not very complicated and need to be conducted with care, something that was also learned in the trial-and-error way at the initial state of this MSc dissertation work.

As indicated above, none of the zooplankton species in the study by Lobson et al., (2018), fulfilled the MDD criteria. This study used the largest test systems (4 000 L) from the studies included in table 15. Therefore, it appears that larger test systems do not necessarily lead to a greater statistical power and hence a higher probability of fulfillment of the MDD criteria. Similarly, a very large number of replicates do not necessarily increase the number of category 1 species, as is reflected by the study by Yin et al., (2018). Despite using 12 replicates for both untreated control and treated microcosms, only 5 out of 45 species (11%) and 1 out of 16 cladoceran species (6%) the MDD criteria was fulfilled (Yin et al., 2018).

What can and may be deduced from table 15 is that the number of sampling moments, especially post start treatment, may have a large influence. Given that this criterion requires a %MDD on a certain number of sampling moments, and not a percentage of the total of sampling moments, this influence is imperative. For example, a study with four post exposure sampling moments will not easily attain MDD category 1, since the first criteria is %MDD < 100% at no less than five sampling, and, if this is not verified, the %MDD < 90% at no less than four samplings becomes harder to obtain (Brock et al., 2014).

Evidently, more detailed characteristics of the study design and methods may also influence the number of category 1 species. This includes the duration of the pre-treatment period, homogeneous seeding of the microcosms with species in the pre-treatment period, mixing of water during that pre-treatment period and methods and accuracy of species identification.

Overall, it appears from tables 14 and 15 that achieving the criterion of eight sensitive MDD category 1 species in model ecosystem experiments is a daunting task. Several indications have been provided of factors that influence the MDD of taxa in model ecosystem experiments. These could thus be used as the bases for adapting model ecosystem studies and MDD calculations to increase the robustness of model ecosystem MDDs (mostly based on Brock et al., 2014 and Duquesne et al., 2020):

- The calculation of the MDD could differ considerably depending on the chosen statistical parameters. This difference is linked to the formula used and the value of its parameters. Analysing equation 7 suggests that the MDD is mainly affected by three factors: (i) the number of replicates n_0 and n, so increasing the number of replicates reduces the square root term, but also increases the degree of freedom of the test and, consequently, the critical t-value; (ii) the variance s^2 to which the MDD is directly proportional to the variance of the measurement endpoints, that can be separated into the inherent variability between the replicates and the variability caused by the sampling methods; and (iii) the selected error level α, the critical t-value also depends on the error level α, the decision of the value of α also affects the MDD.
- The current Aquatic Guidance Document published by EFSA (2013), recommends five or more test concentrations with at least two replicates per treatment level. It is also advised to use a higher number of replicates for the control than that used for the treatments. There are some practical limitations of increasing the number of test units that must be taken into consideration, such as, costs in constructing and managing replicate test systems, manpower for sampling, identification and counting, as such it is better to reduce other sources of variation. Therefore, microcosms/mesocosms studies often have a total number of test systems below 20.
- In line with this, in Brock et all. (2014) it is possible to see that the $\%MDD_{abu}$ is affected by the coefficient of variation in the data and by the increasing of the number of replicates. It is also possible to observe that the data variation has a stronger effect on the $\%MDD_{abu}$ than the replicates numbers. Still for a specific endpoint, a given coefficient of variation, the increase in the number of control and treatment replicates reduces de $\%MDD_{abu}$. The increasing of the number of replicates for the control also results in an increased statistical power.
- Another factor that can affect the MDD, is the variance caused by the differences between the replicants. This variance can be minimized when constructing and preparing the test systems and by measures taken during the pre-experimental period, for example by mixing water of the different test systems and mixing the organisms in order to have similarities between the systems.
- Sampling errors that can also influence the MDD, can be reduced by increasing the number of individuals sampled or counted, thus this can help the reduction of the MDD_{abu}, mainly because of the reduction of variability between samples. With this in mind, the MDD_{abu} can be reduced with the improving of the sampling techniques that allow the increase of the number of sampled individuals. This is possible in most organic groups, by either increasing the sample volume or the number of sampling devices.
- A way to increase the number of sampled individuals, is to adapt the water volume collected to determine the zooplankton and the number of subsamples evaluated in phytoplankton quantification. Another improvement method could include habitat-specific sampling,

additional types of sampling devices, mostly those with a higher trapping rate. The increasing in the number of sampled individuals significantly increases the statistical power by reducing the %MDD.

- It is also possible to group low-abundance taxa based on their taxonomy, for example, in order to obtain taxa with higher number of counted individuals, as done in the present study. It is worth noting that the evaluation of treatment related effects in microcosms/mesocosms experiments should be performed with sufficient representative and potential sensitive biological populations.
- On the other hand, the aggregation of taxa could result in the aggregation of sensitive and non-sensitive species, and therefore, should only be considered when the MDDs of the non-aggregated taxa are too high for a conclusion to be drawn. It should be mentioned that it is impossible to predict which species will be present in appropriate densities, when designing an outdoor microcosm/mesocosm experiment, since they are influenced by the weather and environmental conditions and events.

Table 14 – Summary table with the studies used on the dissertation. General information of the experimental design and fulfillement of the MDD crtiteria.

Test system	Test compound	Country	# replicates	# post exposure sampling moments	MDD	Reference
8,5 L indoor microcosms	Chlorpyrifos, and linuron	The Netherlands	Control: n = 4 Treatments: n = 2	1	Criteria not achieved or meet. Only one sampling moment, so not possible to meet criteria, since the minimum, according to EFSA, is the at least two sampleing moments with MDD <50%	Van den Brink and Daam (2007)
250 L outdoor microcosms	Chlorpyrifos	Thailand	Control: n = 4 Treatments: n = 2	11	Criteria was met for three species and on the group Rotifera, Cladocera and Ostracado groups. Overall it was needed 8 species for the study to meet the criteria, which was not achieved	Daam et al. (2008a)
1 000 L outdoor microcosms	Chlorpyrifos (two applications)	Thailand	Control: n = 4 Treatments: n = 2	10	Criteria were met for four species and for the taxonomic groups, Rotifera, Copepod and Cladocera. Overall it was needed 8 species for the study to meet the criteria, which was not achieved	Daam et al. (2008b)
250 L outdoor microcosms	Linuron	Thailand	Control: n = 4 Treatments: n = 2	8	Criteria was met for seven zooplankton species and six phytoplankton species, along with that 3 species families that also met the criteria. All zooplankton groups (Rotifera, Copepod, Cladocera and Ostracado) met the criteria and te Chlorophyta and Bacillariophyta phytoplankton groups met the criteria. Although the closest of the model ecosystem studies analyzed in this thesis, the criterium of 8 species was not achieved.	Daam et al. (2009a)

Table 15 – Selected model ecosystem studies that calculated MDD values for zooplankton. General information of the experimental design and fulfillment of the MDD criteria listed in Brock et al., 2014 are also indicated.

Test system	Test compound	Country	# replicates	# post exposure sampling moments	MDD	Reference
1000 L indoor microcosms	Nickel	Germany	Control: n = 4 Nickel treatments: n = 2	11	Criteria achieved for almost all taxa (inc. groups; n = 13) except cyclopoid and calanoid copepods and the cladoceran *Chydorus sphaericus*, probably due to overall low numbers in the microcosms.	Hommen et al. (2016)
18 L indoor microcosms	Zinc	The Netherlands	Control: n = 4 Zinc treatments: n = 3	5	Criteria achieved for the negative effects on the total sum of cladocerans and the cladoceran species *Chydorus sphaericus* and *Daphnia longispina*, but not for the cladoceran *Simocephalus vetulus.* Increased species and total abundances of rotifers and copepods did not adhere to the MDD criteria.	Van der Perre et al. (2016)
± 1267 L outdoor microcosms	Thiamethoxam (neonicotinoid insecticide)	UK	Control: n = 4 Treatments: n = 2, 3 or 4	7	Criteria achieved for all zooplankton species groups, but only for 1 cladoceran, copepod and rotifer species, besides cyclopoid copepods and copepod Nauplii out of 26 zooplankton taxa (19%).	Finnegan et al. (2018)
± 4000 L outdoor microcosms	Thiamethoxam (neonicotinoid insecticide)	Canada	Control: n = 3 Pesticide treatments: n = 3	6	Criteria only achieved for the total sum of rotifers, copepod Nauplii and cyclopoid copepods, but for none of the individual species.	Lobson et al. (2018)

Table 15 (cont.) – Selected model ecosystem studies that calculated MDD values for zooplankton. General information of the experimental design and fulfillment of the MDD criteria listed in Brock et al., 2014 are also indicated.

Test system	Test compound	Country	# replicates	# post exposure sampling moments	MDD	Reference
5 L indoor microcosms	Copper, nickel and zinc mixtures	Belgium	Control: n = 4 6 mixture treatments: n = 3	5	Criteria achieved for 30 out of 42 zooplankton taxa (72%) and for all zooplankton species groups.	Van Regenmortel et al. (2018)
160 L outdoor microcosms	Sediment-spiked fludioxonil (fungicide)	The Netherlands	Control: n = 12 Pesticide treatments: n = 12	6	Criteria achieved for sum of rotifers, cladocerans and copepods (as well as nauplii, calanoid and cyclopoid copepods; not identified to species level). On species-level, however, only for 5 out of 45 rotifer species (11%) and 1 out of 16 cladoceran species (6%).	Yin et al. (2018)
10 L indoor microcosms	Nickel	Belgium	Control: n = 4 Treatments: n = 3	4	All zooplankton groups fulfilled the MDD criteria, except for the Ostracoda group. 5 out of 35 species (14%) fulfilled the MDD criteria.	Nys et al. (2019)

6. Conclusions

The present study had as objective to evaluate a method of MDD calculation using the datasets of previously conducted model ecosystem studies. Overall, this method could successfully be applied and allowed to calculate the MDD for zooplankton and phytoplankton species and taxonomic groups. The calculation of the MDD on its own it is not conclusive of the sensitivity of a species to a substance. To get to such a conclusion it is also necessary to calculate the NOECs and LOECs for each species.

It was also possible to identify the aspects that influence and increase and/or decrease the MDD. It was concluded that, the number of post-treatment sampling moments is likely to be positively related to the probability of a species belonging to Category 1, which determinates if a study is well designed and if the species used represents sensitive taxa. In other words, the higher the number and the frequency of sampling, the higher the probability to meet the MDD criteria in a model ecosystem study.

Therefore, it is concluded that the experimental design of microcosms and mesocosms greatly influence the MDD values. This means that the designs of the experimental test systems must be adapted to take into consideration the factors, such as samplings moments and sampling methods, that affect the MDD.

It was also possible to notice that the MDD values in the evaluated and those reported in pulished model ecosystem studies were almost always high. Values close to 100% or higher mean that no statistically significant treatment-related effects may be demonstrated or that only large effects can be seen.

The number of sampling moments, the raw data in itself, and the sampling method and the MDD calculation method, are all factors that can influence the MDD results. If a study had in mind the calculation of the MDD values, it should pay special attention to the homogeneously and similarities between the microcosms in the pre-treatment period and give priority to the use of species that are common and less rare and more abundant.

A promising way forward could also be the use of species traits to aggregate data, rather than using taxonomic groups like used in the present study. Species traits are morphological (e.g. mode of respiration), biological (e.g. life cycle duration) and ecological (e.g. substrate) characteristics that have been shown in recent years to be related with the intrinsic sensitivity of species to chemicals like pesticides (e.g. Rico and Van den Brink, 2015). Subsequently, this may be a promising way forward to improve grouping of sensitive taxa, rather than using taxonomic grouping.

7. Future Perspectives

The criteria of evaluation of the MDD calculations should be more studied and justified, as well as refined, so that the criteria and category presented by EFSA, 2013 become easier to apply and to meet. The study that only presented one sample in all duration of the experiment, does not meet the criteria for category 1. It is possible to deduce from table 13, that the studies used did not had into consideration the MDD calculations, this is, they were not designed to meet the criteria nor did the factors that affect the MDD taken into consideration.

The data of the sampling was also a factor that influence the MDD results. Species whose sampling concluded that they only existed in the control, had MDD, usually, above 100%. On another hand, for the species that did not existed in the control at a certain time it was not possible to calculate the MDD.

For future reference, it is important that when designing and experiment with microcosms to have attention at the taxon level. The taxon level must be the lowest possible. It is also important to have a duration of experiment long enough and with a high sampling frequency- this allows to increase the probability of meeting the asked criteria. For example, a experiment with 4 weeks of duration and with a sampling frequency of once a week, it will be hard to meet the criteria for category 1, since all the MDD had to be below 90% and there had to be a sampled data very complete, what it is not always possible to control.

Another factor that could influence the result of the studies, are the species used. Rarer and less abundant species may have high MDDs since they could be only present on the control, that has the most conditions for them to develop. Therefore, it is important to be careful on the chosen species and opt by very abundant species that are not so rare and can be found in many habitats and ecosystems.

Based on the erroneous MDD calculations by Van Regenmortel, 2018, and almost happened in the initial stage of this dissertation, MDD values should be calculated in a more exhaustive future evaluation, including more model of ecosystems studies. If possible, the MDD values should be recalculated based on the original data.

8. Bibliographic references

Adriaanse, P., Allen, R., Gouy, V., Hollis, J., Hosang, J., Jarvis, N., Jarvis, T., Klein, M., Layton, R., Linders, J., Schäfer, H., Smeets, L., Yon, D. (2001). Multispecies Environmental Testing Designs. In *Encyclopedia of Toxicology* (3rd ed., pp. 399–402). Academic Press.

Brock, T. C. M., Van Wijngaarden, R. P. A., Van Geest, G. J. (2000b). Ecological risks of pesticides in freshwater ecosystems; part 2: Insecticides. Report 089, Alterra, Green World Research, Wageningen, The Netherlands.

Brock, T. C. M., Hammers-Wirtz, M., Hommen, U., Preuss, T. G., Ratte, H-T., Roessink, I., Strauss, T., Van den Brink, P. J. (2014). The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. *Environmental Science and Pollution Research*, *22*(2), 1160–1174. https://doi.org/10.1007/s11356-014-3398-2

Brock, T. C. M., Bas, D. A., Belgers, J. D. M., Bibbe, L., Boerwinkel, M-C., Crum, S. J. H., Diepens, N. J., Kraak, M. H. S., Vonk, J. A., Roessink, I. (2016). Effects of sediment-spiked lufenuron on benthic macroinvertebrates in outdoor microcosms and single-species toxicity tests. *Aquatic Toxicology*, *177*, 464–475. https://doi.org/10.1016/j.aquatox.2016.06.021

Campbell, P.J., Arnold, D.J.S., Brock, T.C.M., Grandy, N.J., Heger, W., Heimbach, F., Maund, S.J. and Streloke, M. (eds.) (1999). Guidance Document on higher-tier aquatic risk assessment for pesticides (HARAP). Brussels, Belgium, SETAC- Europe.

Chapman, P. M. (2007). Determining when contamination is pollution — Weight of evidence determinations for sediments and effluents. *Environment International*, *33*, 492–501. https://doi.org/10.1016/j.envint.2006.09.001

Daam, M. A., & Van den Brink, P. J. (2007). Effects of chlorpyrifos, carbendazim, and linuron on the ecology of a small indoor aquatic microcosm. *Archives of Environmental Contamination and Toxicology*, *53*(1), 22–35. https://doi.org/10.1007/s00244-006-0001-y

Daam, M. A., Crum, S. J. H., Van den Brink, P. J., & Nogueira, A. J. A. (2008). Fate and effects of the Insecticide Chlorpyrifos in outdoor Plankton-dominated Microcosms in Thailand. *Environmental Toxicology and Chemistry*, *27*(12), 2530–2538.

Daam, M. A., Van den Brink, P. J., & Nogueira, A. J. A. (2008). Impact of single and repeated applications of the insecticide chlorpyrifos on tropical freshwater plankton communities. *Ecotoxicology*, *17*(8), 756–771. https://doi.org/10.1007/s10646-008-0227-8

Daam, M. A., Leitão, S., Cerejeira, M. J., & Sousa, J. P. (2011). Comparing the sensitivity of soil invertebrates to pesticides with that of *Eisenia fetida*. *Chemosphere: Environmental Toxicology and Risk Assessment*, *85*(6), 1040–1047. https://doi.org/10.1016/j.chemosphere.2011.07.032

Daam, M. A., & Van den Brink, P. J. (2011). Conducting model ecosystem studies in tropical climate zones: Lessons learned from Thailand and way forward. *Environmental Pollution*, *159*, 940–946. https://doi.org/10.1016/j.envpol.2010.12.018

Dias, A. (2002). Testes de Toxicidade em *Armetia salina:* Contaminante (K2Cr2O7) e Efluentes Químicos (Tratados e Não Tratados). Relatório de final de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve.

Duquesne, S., Alalouni, U., Gräff, T., Frische, T., Pieper, S., Egerer, S., Gergs, R., Wogram, J. (2020). Better define beta–optimizing MDD (minimum detectable difference) when interpreting treatment-related effects of pesticides in semi-field and field studies. *Environmental Science and Pollution Research*, *27*(8), 8814–8821. https://doi.org/10.1007/s11356-020-07761-0

EC, European Commission. (2011). *Common Implementation Strategy for the Water Framework Directive (2000 / 60 / EC). Guidance Document No. 27. Technical Guidance for Deriving Environmental Quality Standards*. 204pp. https://doi.org/10.2779/43816

EC, European Commission. (2020). Commission Implementing Regulation (EU) 2020/17 of 10 January 2020 concerning the non-renewal of the approval of the active substance chlorpyrifos-methyl, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. *Official Journal of the European Union*, *2016*(68), 48–119.

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

EFSA PPR Panel, EFSA Panel on Plant Protection Products and their Residues. (2015). Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water. *EFSA Journal 2015, 13*(7):4176, 145 pp. https://doi.org/10.2903/j.efsa.2015.4176

EFSA, European Food Safety Authority (2018). The 2016 European Union report on pesticide residues in food. *EFSA Journal 2018*; *16*(7):5348, 139 pp. EFSA (European Food Safety Authority), 2018. https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2018.5348

EFSA, European Food Safety Authority, (2019). Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. *EFSA Supporting publication 2019*:EN-1673. 117 pp. https://doi.org/10.2903/sp.efsa.2019.EN-1673

Finnegan, M. C., Emburey, S., Hommen, U., Baxter, L. R., Hoekstra, P. F., Hanson, M. L., Thompson, H., Hamer, M. (2018). A freshwater mesocosm study into the effects of the neonicotinoid insecticide thiamethoxam at multiple trophic levels. *Environmental Pollution*, *242*, 1444–1457. https://doi.org/10.1016/j.envpol.2018.07.096

Pickford, D. B., Finnegan, M. C., Baxter, L. R., Böhmer, W., Hanson, M. L., Stegger, P., Hommen, U., Hoesktra, P. F., Hamer, M. (2018). Response of the Mayfly (*Cloeon dipterum*) to Chronic Exposure to Thiamethoxam in Outdoor Mesocosms. *Environmental Toxicology and Chemistry*, *37*(4), 1040–1050. https://doi.org/10.1002/etc.4028

Homme, U., Knopf, B., Rüdel, H., Schäfers, C., De Schamphelaere, K., Schlekat, C., & Garman, E. R. (2016). A Microcosm Study to Support Aquatic Risk Assessment of Nickle: Community-level Effects and Comparison with Bioavailability-Normalized Species Sensitivity Distribution. *Environmental Toxicology and Chemistry*, *35*(5), 1172–1182. https://doi.org/10.1002/etc.3255

Klaassen, C. D., & Watkins III, J. B. (2003). *Essentials of Toxicology* (07–2015th ed.). Europe: Mcgraw-Hill Education.

Lobson, C., Luong, K., Seburn, D., White, M., Hann, B., Prosser, R. S., Wong, C. S., Hanson, M. L. (2018). Fate of thiamethoxam in mesocosms and response of the zooplankton community. *Science of the Total Environment*, *637–638*, 1150–1157. https://doi.org/10.1016/j.scitotenv.2018.05.087

Mair, M. M., Kattwinkel, M., Jakoby, O., & Hartig, F. (2020). The Minimum Detectable Difference (MDD) Concept for Establishing Trust in Nonsignificant Results: A Critical Review.

Environmental Toxicology and Chemistry, *39*(11), 2109–2123. https://doi.org/10.1002/etc.4847

Nys, C., Van Regenmortel, T., & De Schamphelaere, K. (2019). The Effects of Nickel on the Structure and Functioning of a Freshwater Plankton Community under High Dissolved Organic Carbon Conditions: A Microcosm Experiment. *Environmental Toxicology and Chemistry*, *38*(9), 1923–1939. https://doi.org/10.1002/etc.4504

Pereira, A. S., Daam, M. A., & Cerejeira, M. J. (2017). Evaluation of FOCUS surface water pesticide concentration predictions and risk assessment of field-measured pesticide mixtures—a crop-based approach under Mediterranean conditions. *Environmental Science and Pollution Research*, *24*(21), 17394–17406. https://doi.org/10.1007/s11356-017-9393-7

Posthuma, L., Suter II, G. W., & Traas, T. P. (2001). *Species Sensitivity Distributions in Ecotoxicology* (Vol. 4). Lewis Publisher.

Ramos, C., Carbonell, G., Jma G. B., & Tarazona, J. V. (2000). Ecological risk assessment of pesticides in the Mediterranean region. The need for crop-specific scenarios. *Science of The Total Environment, 247*(2–3), 269–278. https://doi.org/10.1016/S0048-9697(99)00496-9

Rand, G. M. (1995). *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment* (Second edition; G. M. Rand, ed.). CRC Press.

Rico, A., Arenas-Sánchez, A., Pasqualini, J., García-Astillero, A., Gherta, L., Nozal, L., & Vighi, M. (2018). Effects of imidacloprid and a neonicotinoid mixture on aquatic invertebrate communities under Mediterranean conditions. *Aquatic Toxicology*, *204*(August), 130–143. https://doi.org/10.1016/j.aquatox.2018.09.004

Rico, A., Brock, T. C. M., & Daam, M. A. (2019). Is the Effect Assessment Approach for Fungicides as Laid Down in the European Food Safety Authority Aquatic Guidance Document Sufficiently Protective for Freshwater Ecosystems? *Environmental Toxicology and Chemistry*, *38*(10), 2279–2293. https://doi.org/10.1002/etc.4520

Rico, A., Dafouz, R., Vighi, M., Rodrígues-Gil, J. L., & Daam, M. A. (2020). *Use of post-registration monitoring data to evaluate the ecotoxicological risks of pesticides to surface waters: a case study with chlorpyrifos in the Iberian Peninsula*. 0–3. https://doi.org/10.1002/etc.4927

Rico, A., & Van Den Brink, P. J. (2015). Evaluating Aquatic Invertebrate Vulnerability to Insecticides based in Intrinsic Sensitivity, Biological Traits and Toxic Mode-of-Action. *Environmental Toxicology and Chemistry*. https://doi.org/10.1002/etc.3008

Riedl, V., Agatz, A., Benstead, R., & Ashauer, R. (2018). A Standardized Tritrophic Small-Scale System (TriCosm) for the Assessment of Stressor-Induced Effects on Aquatic Community Dynamics. *Environmental Toxicology and Chemistry*, *37*(4), 1051–1060. https://doi.org/10.1002/etc.4032

Sanderson, H., Laird, B., Brain, I., Wilson, C. J., & Solomon, K. R. (2009). Detectability of fifteen aquatic micro/mesocosms. *Ecotoxicology*, *18*, 838–845. https://doi.org/10.1007/s10646-009-0327-0

Schäfer, R. B. (2019). Responses of freshwater macroinvertebrates to pesticides: insights from field studies. *Current Opinion in Environmental Science & Health*, *11*, 1–7. https://doi.org/10.1016/j.coesh.2019.06.001

Schepker, T. J., Webb, E. B., Tillitt, D., & LaGrange, T. (2020). Neonicotinoid insecticide concentrations in agricultural wetlands and associations with aquatic invertebrate communities. *Agriculture, Ecosystems and Environment*, *287*(March 2019), 106678. https://doi.org/10.1016/j.agee.2019.106678

Solomon, K. R., & Takacs, P. (2001). Probabilistic Risk Assessment Using Species Sensitivity Distributions. In *Species Sensitivity Distributions in Ecotoxicology* (pp. 285–313). Retrieved from http://library1.nida.ac.th/termpaper6/sd/2554/19755.pdf

Szöes, E., Van den Brink, P. J., Lagadic, L., Caquet, T., Roucaure, M., Auber, A., Bayona, Y., Liess, M., Ebke, P., Ippolito, A., ter Braak, C. J. F., Brock, T. C. M., Schäfer, R. B. (2015). Analyzing chemical-induced changes in macroinvertebrate communities in aquatic mesocosm experiments: a comparison of methods. *Ecotoxicology*, *24*, 760–769. https://doi.org/10.1007/s10646-015-1421-0

Tisler, T., & Zargorc-Koncan, J. (1999). Toxicity evaluation of wastewater from the pharmaceutical industry to aquatic organisms. *Water Science Technology*, *39*(10–11), 71–76. https://doi.org/10.1016/S0273-1223(99)00255-3

Van de Perre, D., Roessink, I., Janssen, C. R., Smolders, E., Van Regenmorter, T., Van Wichelen, J., Van den Brink, P. J., De Schamphelaere, K. (2016). The effects of zinc on the structure

and functioning of a freshwater community: a microcosm experiment. *Environmental Toxicology and Chemistry*, *35*(11), 2698–2712. https://doi.org/10.1002/etc.3435

Van de Perre, D., Roessink, I., Janssen, C. R., Smolders, E., De Laender, F., & Van den Brink, P. J. (2018). The Combined and Interactive Effects of Zinc, Temperature, and Phosphorus on the Structure and Functioning of a Freshwater Community. *Environmental Toxicology and Chemistry*, *37*(9), 2413–2427. https://doi.org/10.1002/etc.4201

Van Den Brink, P. J. (2008). Ecological Risk Assessment: From Book-Keeping to Chemical Stress Ecology Ecological Risk Assessment: From Book-Keeping to Chemical Stress Ecology. *Environmental Science and Technology*, *42*(24), 8999–9004. https://doi.org/10.1021/es801991c

Van den Brink, P. J., & Daam, M. A. (2014). Multispecies Environmental Testing Designs. In *Encyclopedia of Toxicology: Third Edition* (Vol. 3, pp. 399–402). https://doi.org/10.1016/B978-0-12-386454-3.00569-8

Van Regenmorter, T., Van de Perre, D., Janssen, C. R., & De Schamphelaere, K. (2018). The Effects of a Mixture of Copper, Nickel, and Zinc on the Structure and Function of a Freshwater Planktonic Community. *Environmental Toxicology and Chemistry*, *37*(9), 2380–2400. https://doi.org/10.1002/etc.4185

Van Wijngaarden, P. A., & Arts, G. H. P. (2018). Is the Tier-1 Effect Assessment for Herbicides Protective for Aquatic Algae and Vascular Plant Communities? *Environmental Toxicology and Chemistry*, *37*(1), 175–183. https://doi.org/10.1002/etc.3936

Walker, C. H., Hopkins, S. P., Sibly, R. M., & Peakall, D. B. (1997). *Principles of Ecotoxicology*. Taylor & Francis.

Wieczorek, M. V, Bakanov, N., Stang, C., Bilancia, D., Lagadic, L., Bruns, E., & Schulz, R. (2016). Reference scenarios for exposure to plant protection products and invertebrate communities in stream mesocosms. *Science of the Total Environment*, *545–546*, 308–319. https://doi.org/10.1016/j.scitotenv.2015.12.048

Williams, P. L., James, R. C., & Roberts, S. M. (2002). *Principles of Toxicology: Environmental and Industrial Applications*. New York: John Wiley and Sons.

Yin, X. H., Brock, T. C. M., Barone, L. E., Belgers, J. D. M., Boerwinkel, M., Buijse, L., Van Wijngaarden, R. P. A., Hamer, M., Roessink, I. (2018). Exposure and effects of sediment-

spiked fludioxonil on macroinvertebrates and zooplankton in outdoor aquatic microcosms. *Science of the Total Environment*, *610–611*, 1222–1238. https://doi.org/10.1016/j.scitotenv.2017.08.155

Zubrod, J. P., Bundschuh, M., Arts, G., Brühl, C. A., Imfled, G., Knäbel, A., Payraudeau, S., Rasmussen, J. J., Rohr, J., Scharmüller, A., Smalling, K., Schulz, R., Schäfer, R. B. (2019). Fungicides: An Overlooked Pesticide Class? *Environmental Science and Pollution Research*, *53*, 3347–3365. https://doi.org/10.1021/acs.est.8b04392

Annexes

Table A1 – Data base for study 1; number of individuals of each treatment and for each species; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,005 µg/L Pond 1	0,005 µg/L Pond 2	0,05 µg/L Pond 1	0,05 µg/L Pond 2	0,5 µg/L Pond 1	0,5 µg/L Pond 2	5 µg/L Pond 1	5 µg/L Pond 2
Alona rectangula	1	0	0	0	0	0	0	0	0	0	0	0
Bosmina spp.	0	0	0	0	5	0	0	0	0	0	0	0
Cephalodella gibba	0	0	0	0	0	8	47	17	46	102	0	8
Chydorus sphaerica	4	9	8	52	122	5	0	0	0	0	0	0
Colurella uncicanata	3	11	8	0	5	0	0	17	5	0	0	0
Cyclops cyclopoidae	1	0	8	23	0	4	0	3	3	4	0	0
Daphnia galeata	0	0	0	3	0	0	0	0	0	0	0	0
Daphnia magna	0	0	0	3	0	0	0	0	0	0	0	0
Ephemeroptera	1	3	6	2	3	10	4	3	3	3	5	2
Ephippia	0	0	17	11	0	0	0	0	0	0	0	1
Keratella coclearis	411	68	92	158	164	186	527	219	384	141	234	328
Keratella quadrata	157	48	28	35	57	67	141	87	157	53	75	100
Lecane bulla	0	9	0	0	24	0	4	22	91	4	226	189
Lecane luna	0	0	0	0	3	0	4	0	0	0	0	0
Lecane lunaris	0	278	0	0	105	0	4	0	10	13	79	0
Lecane quadridentata	0	11	0	9	22	0	4	4	0	4	0	0
Lepadella patella	391	168	80	22	94	186	56	144	455	13	0	23
Mytilina ventralis	0	0	0	0	8	0	0	0	0	0	0	0
Ostracoda	60	26	40	105	119	91	56	57	91	35	38	42
Simocephalus vetulus	0	9	73	53	18	27	0	0	0	0	0	0
Trichocerca	0	6	4	0	35	0	0	0	15	4	125	42

Table A2 – Data base for study 2; number of individuals of each treatment and for each species on for sampling day 0; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	14	12	12	13	0
Brachionus calyciflorus	616	517	344	907	761	636	463	1049	358	982
Brachionus falcatus	170	154	57	26	658	338	46	250	0	97
Brachionus quadridentatus	0	0	0	0	0	0	0	0	0	0
Brachionus urceolaris	26	28	23	53	26	41	23	12	40	19
Calanoid copepod	197	237	206	132	155	271	81	287	80	88
Ceriodaphnia cornuta	26	70	46	66	0	108	12	87	27	29
Colurella sp.	0	0	0	0	0	0	0	0	0	0
Cyclopoid copepod	157	140	161	105	103	189	69	187	80	88
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	26	0	0	12	13	0
Filinia opoliensis	0	0	0	0	0	0	0	12	0	0
Hexarthra mira	0	14	0	0	0	0	0	0	0	0
Keratella tropica	13	0	0	13	0	14	0	0	0	0
Lecane bulla	144	112	69	26	64	41	35	12	27	39
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	13	0	0	0	0	0	0
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	315	363	516	565	129	447	174	625	226	272
Nauplii	118	126	126	39	116	135	58	12	40	68
Streblocerus pygmaues	0	0	0	0	0	0	0	0	0	0
Trichocerca	0	0	0	0	0	0	0	0	0	0

Table A3 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 4; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	10	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	0	0	0	0	0
Brachionus calyciflorus	10	22	20	6	86	579	254	566	0	112
Brachionus falcatus	0	0	0	0	0	20	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	0	0	0	0
Brachionus urceolaris	41	15	27	65	6	27	6	10	24	0
Calanoid copepod	102	132	20	65	23	54	6	21	8	6
Ceriodaphnia cornuta	133	59	0	0	0	0	0	0	0	0
Colurella sp.	0	0	0	0	0	0	0	0	0	0
Cyclopoid copepod	102	155	46	19	12	0	6	0	0	6
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	0	0	0	0
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	0	0	0	0	0	0	0	0	0	0
Keratella tropica	0	0	0	0	0	0	0	0	0	0
Lecane bulla	0	7	13	0	6	48	12	21	0	44
Lecane closterocerca	0	0	0	0	6	0	0	0	0	0
Lecane luna	0	0	0	0	0	7	6	31	0	19
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	675	640	651	668	391	599	551	339	185	355
Nauplii	573	861	53	285	270	157	67	401	28	267
Streblocerus pygmaues	0	0	0	0	0	0	0	0	0	0
Trichocerca	0	0	0	0	0	0	12	0	20	6

Table A4 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 7; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	0	0	0	0	0
Brachionus calyciflorus	37	164	141	74	146	285	56	217	0	258
Brachionus falcatus	0	0	0	0	0	5	0	0	0	0
Brachionus quadridentatus	0	4	0	0	11	5	33	0	0	4
Brachionus urceolaris	106	86	448	252	54	88	17	26	9	0
Calanoid copepod	51	141	98	34	86	99	39	58	17	16
Ceriodaphnia cornuta	14	31	18	17	0	0	0	0	0	0
Colurella sp.	0	0	0	0	0	0	0	0	0	0
Cyclopoid copepod	37	106	117	51	38	121	39	69	17	39
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	0	0	0	0
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	0	0	0	0	0	0	0	0	0	0
Keratella tropica	0	4	0	0	0	0	0	5	0	4
Lecane bulla	0	4	0	0	5	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	0	5	0	61	0	4	94
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	226	199	792	589	561	351	301	561	301	184
Nauplii	568	399	233	132	335	170	28	164	13	35
Streblocerus pygmaues	0	0	0	0	0	0	0	0	0	0
Trichocerca	9	0	6	6	0	5	50	11	9	0

Table A5 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 11; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	0	0	0	0	0
Brachionus calyciflorus	0	92	89	54	219	11	0	38	4	0
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	0	0	4	0
Brachionus urceolaris	27	38	0	0	12	0	0	4	15	6
Calanoid copepod	11	32	0	6	41	22	13	11	0	0
Ceriodaphnia cornuta	16	43	11	12	0	0	0	0	0	0
Colurella sp.	11	32	22	0	94	105	144	412	108	102
Cyclopoid copepod	5	22	78	54	65	17	0	8	0	6
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	0	0	0	0
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	0	0	0	0	0	0	7	0	0	0
Keratella tropica	0	0	0	0	0	0	0	0	0	6
Lecane bulla	0	0	0	0	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	0	0	0	46	0	0	232
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	455	552	156	137	450	94	202	317	97	187
Nauplii	5	38	0	0	8	0	0	11	0	0
Streblocerus pygmaues	0	0	0	0	0	0	0	0	0	0
Trichocerca	27	27	0	0	24	0	39	26	23	34

Table A6 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 14; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	0	0	0	0	0
Brachionus calyciflorus	7	34	10	5	3	11	0	27	0	6
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	0	0	0	0
Brachionus urceolaris	0	0	10	0	0	0	0	0	0	0
Calanoid copepod	0	11	0	0	0	0	0	0	0	0
Ceriodaphnia cornuta	0	0	26	5	0	0	0	0	0	0
Colurella sp.	7	17	0	52	3	11	55	5	12	0
Cyclopoid copepod	0	28	5	0	3	0	0	0	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	0	0	0	0
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	0	0	0	5	0	11	0	5	0	0
Keratella tropica	0	0	0	0	0	0	0	0	0	0
Lecane bulla	0	0	0	0	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	0	0	0	0	0	0	17
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	87	152	168	182	177	232	420	341	86	199
Nauplii	0	11	5	0	0	0	0	0	0	0
Streblocerus pygmaues	0	0	5	0	0	0	0	0	0	6
Trichocerca	0	118	16	31	23	17	49	65	6	34

Table A7 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 18; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	0	0	0	0	0
Brachionus calyciflorus	0	0	0	0	6	0	0	43	246	66
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	0	0	0	0
Brachionus urceolaris	0	0	0	0	0	0	0	0	1979	6
Calanoid copepod	6	0	0	0	0	0	0	0	0	0
Ceriodaphnia cornuta	0	0	0	0	0	0	0	0	31	0
Colurella sp.	0	0	0	0	0	0	0	0	0	0
Cyclopoid copepod	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	0	0	0	11	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	0	0	0	0
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	0	0	0	5	0	0	36	0	359	0
Keratella tropica	0	0	0	0	0	0	0	0	10	0
Lecane bulla	0	0	0	0	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	0	0	0	0	0	0	0
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	115	211	41	74	294	37	210	0	0	0
Nauplii	0	0	0	0	0	0	0	0	0	0
Streblocerus pygmaues	0	0	0	0	0	0	0	0	0	0
Trichocerca	0	0	0	0	13	0	0	5	0	11

Table A8 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 21; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	0	0	0	0	0
Brachionus calyciflorus	0	0	0	0	49	0	0	215	1694	198
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	5	0	11	0
Brachionus urceolaris	0	0	0	0	0	0	0	46	645	188
Calanoid copepod	0	0	0	0	0	0	0	0	0	0
Ceriodaphnia cornuta	0	0	0	0	0	0	0	0	33	0
Colurella sp.	0	0	0	0	0	0	0	0	0	5
Cyclopoid copepod	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	23	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	0	0	0	0
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	0	0	0	0	0	12	53	5	1792	31
Keratella tropica	0	0	0	0	0	0	0	5	0	5
Lecane bulla	0	0	0	0	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	0	0	0	0	0	0	0
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	51	116	68	31	592	69	501	0	33	0
Nauplii	0	0	0	0	0	0	0	0	0	0
Streblocerus pygmaues	0	0	0	0	0	0	0	0	22	0
Trichocerca	0	0	0	0	0	0	0	5	11	55

Table A9 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 25; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	0	0	0	71	0
Brachionus calyciflorus	0	0	0	0	30	95	65	154	218	114
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	11	0	0	0
Brachionus urceolaris	0	0	0	0	65	0	60	0	5	258
Calanoid copepod	0	0	0	0	0	0	0	0	0	0
Ceriodaphnia cornuta	0	0	9	0	0	0	97	0	60	0
Colurella sp.	0	0	0	0	0	0	70	0	0	57
Cyclopoid copepod	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	6	20	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	0	0	0	0
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	0	10	0	0	0	10	157	30	1069	1631
Keratella tropica	0	0	0	0	0	10	0	0	0	0
Lecane bulla	0	0	0	0	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	0	0	0	0	0	0	0
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	11	507	216	143	126	0	790	6	333	57
Nauplii	0	0	0	0	0	0	0	0	0	0
Streblocerus pygmaues	0	0	0	10	0	0	5	12	49	258
Trichocerca	0	0	0	0	0	0	76	0	0	0

Table A10 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 28; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	118	0	0	66	0
Brachionus calyciflorus	0	24	0	16	50	128	133	316	996	105
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	32	0	0	8
Brachionus urceolaris	0	0	83	0	113	0	56	8	0	0
Calanoid copepod	0	0	0	0	0	0	0	0	0	0
Ceriodaphnia cornuta	0	0	0	0	7	73	216	0	72	8
Colurella sp.	9	0	21	15	0	13	0	8	17	0
Cyclopoid copepod	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	9	0	0	8	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	0	0	0	15
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	0	24	72	23	0	126	136	79	385	1501
Keratella tropica	0	0	0	0	0	0	0	0	0	0
Lecane bulla	0	0	0	0	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	0	0	0	0	0	0	0
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	344	1699	2088	969	1217	33	1041	31	550	0
Nauplii	0	0	0	0	0	0	40	0	0	0
Streblocerus pygmaues	9	0	0	23	0	0	72	94	72	113
Trichocerca	0	0	0	0	7	0	72	0	6	0

Table A11 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 32; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	439	19	0	61	0
Brachionus calyciflorus	8	0	0	1660	0	285	126	270	3618	37
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	10	0	0	9
Brachionus urceolaris	0	0	12	0	213	0	0	0	0	0
Calanoid copepod	0	0	0	0	0	0	0	0	0	0
Ceriodaphnia cornuta	0	0	0	0	0	132	126	0	30	0
Colurella sp.	23	0	6	0	0	0	10	0	0	0
Cyclopoid copepod	0	0	0	0	0	0	39	0	0	0
Dunhevedia crassa	8	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	7	10	0	0	407
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	8	0	70	31	87	315	319	1904	213	1823
Keratella tropica	0	0	0	0	0	0	0	0	0	0
Lecane bulla	0	0	0	0	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	0	0	0	0	0	0	19
Lepadella patella	0	0	0	0	0	0	0	0	0	0
Moina micrura	297	423	1249	1066	923	22	367	304	894	9
Nauplii	0	0	0	0	0	0	10	0	0	0
Streblocerus pygmaues	15	0	0	113	0	0	0	17	183	167
Trichocerca	0	0	0	0	0	0	29	0	0	0

Table A12 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 35; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	2611	0	0	9	9
Brachionus calyciflorus	90	0	0	1002	20	140	0	1935	1529	90
Brachionus falcatus	0	0	0	0	0	7	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	0	0	0	0
Brachionus urceolaris	0	0	0	0	0	0	0	0	0	0
Calanoid copepod	9	0	0	0	0	0	0	0	0	0
Ceriodaphnia cornuta	0	0	0	12	10	840	764	0	0	0
Colurella sp.	0	0	0	0	0	0	0	0	0	0
Cyclopoid copepod	0	0	0	0	0	0	165	0	0	0
Dunhevedia crassa	18	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	140	0	0	0	2059
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	81	0	423	495	167	252	104	2855	110	343
Keratella tropica	0	0	0	0	0	0	0	0	0	0
Lecane bulla	0	0	0	0	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	9
Lecane luna	0	0	0	0	0	0	0	0	0	0
Lepadella patella	36	0	0	0	0	0	0	0	0	0
Moina micrura	1033	198	2186	2789	1798	49	9	1635	203	0
Nauplii	0	0	0	0	0	0	78	7	0	0
Streblocerus pygmaues	99	10	0	60	0	0	9	102	37	840
Trichocerca	0	0	0	0	0	0	0	0	0	0

Table A13 – Data base for study 2; number of individuals of each treatment and for each species for sampling day 42; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	1 µg/L Pond 1	1 µg/L Pond 2	1 µg/L Pond 3	1 µg/L * 2 Pond 1	1 µg/L * 2 Pond 2	1 µg/L * 2 Pond 3
Alona sp.	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	11	0	2065	11	0	7	77
Brachionus calyciflorus	99	0	0	65	0	136	0	702	966	4228
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0	0	0	0	0
Brachionus urceolaris	0	0	0	0	0	0	0	0	0	0
Calanoid copepod	0	0	0	0	0	0	0	0	0	0
Ceriodaphnia cornuta	0	0	0	22	11	1794	897	10	0	0
Colurella sp.	7	0	0	0	0	0	0	0	0	0
Cyclopoid copepod	0	0	0	0	11	0	389	0	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	292	0	0	0	1274
Filinia opoliensis	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	53	25	64	313	195	699	0	454	76	22
Keratella tropica	0	0	0	0	0	0	0	0	0	0
Lecane bulla	0	0	0	0	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0	0	0	0	0
Lecane luna	0	0	0	0	0	0	0	0	0	0
Lepadella patella	7	0	0	0	0	0	0	0	0	0
Moina micrura	598	692	652	820	770	292	0	1899	83	11
Nauplii	7	0	0	0	0	21	497	0	0	0
Streblocerus pygmaues	59	0	0	11	0	0	0	485	0	242
Trichocerca	0	0	0	0	0	0	0	0	0	0

Table A14 – Data base for study 3; number of individuals of each treatment and for each species for sampling day 0; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	11	0	0	7	254	17	0	0	0	43	0	0
Brachionus calyciflorus	0	1389	7	0	172	0	18	24	135	319	827	0
Brachionus falcatus	0	0	0	50	0	0	0	0	0	0	0	0
Calanoid copepod	79	24	0	150	18	0	18	0	37	7	0	23
Ceriodaphnia cornuta	113	0	170	0	1153	51	107	56	0	0	178	0
Cyclopoid copepod	11	0	92	43	109	77	330	73	257	43	25	58
Diffugia sp.	1375	0	0	0	0	9	0	0	49	0	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	8	0	0	154	0	9	0	61	0	0	0
Hexarthra mira	0	8	0	0	0	0	0	8	0	0	0	0
Keratella tropica	1228	73	28	200	82	179	107	0	245	268	51	12
Moina micrura	45	57	21	14	54	43	63	0	330	36	25	267
Nauplii	113	284	411	236	917	666	491	403	159	210	674	337
Ostracoda	0	0	0	0	0	0	0	0	0	0	13	0

Table A15 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 1; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	256	12	0	24	0	23	0	0
Brachionus calyciflorus	0	0	26	0	537	0	37	645	665	125	0	1519
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0	0	0
Calanoid copepod	67	0	0	99	98	12	67	33	13	0	0	22
Ceriodaphnia cornuta	40	29	31	0	305	83	156	33	0	0	0	0
Cyclopoid copepod	13	0	63	7	293	12	15	16	7	23	0	0
Diffugia sp.	657	0	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	29	0	0	110	0	67	0	1096	125	0	0
Hexarthra mira	0	0	0	0	0	0	0	8	0	202	0	0
Keratella tropica	657	233	31	33	159	236	30	24	170	8	0	22
Moina micrura	121	44	42	20	24	59	0	0	0	0	0	0
Nauplii	121	117	52	26	1146	118	44	98	150	78	0	0
Ostracoda	0	0	0	0	0	0	0	0	13	8	0	0

Table A16 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 2; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	15	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	640	0	0	673	0	65	0	22
Brachionus calyciflorus	0	558	1054	0	62	15	0	0	730	26	0	14
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0	0	0
Calanoid copepod	30	12	0	150	86	0	53	0	143	52	14	86
Ceriodaphnia cornuta	15	0	1078	15	541	30	578	1395	0	0	0	0
Cyclopoid copepod	120	24	235	22	185	60	0	299	86	98	57	50
Diffugia sp.	540	0	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	49	0	0	861	0	70	0	4510	151	0	720
Hexarthra mira	8	24	0	0	0	0	0	8	100	13	397	281
Keratella tropica	1223	109	559	187	283	502	35	249	1339	26	0	7
Moina micrura	308	133	478	60	0	120	0	0	0	0	0	0
Nauplii	53	24	997	172	1132	52	35	573	465	465	283	151
Ostracoda	0	0	0	0	0	0	0	0	0	7	0	0

Table A17 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 3; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	9	0	0	0	0	0	0	0	0	0	8
Brachionus angularis	0	831	127	0	1133	0	0	201	0	23	359	191
Brachionus calyciflorus	0	1046	1243	0	357	0	8	9	0	8	0	0
Brachionus falcatus	0	0	0	0	52	0	0	0	0	0	0	0
Calanoid copepod	0	0	0	388	35	0	361	0	190	318	0	66
Ceriodaphnia cornuta	68	0	181	0	0	31	0	1490	8	0	0	0
Cyclopoid copepod	29	111	45	8	1037	233	336	210	281	682	1119	273
Diffugia sp.	429	0	0	0	0	0	0	0	8	0	0	0
Dunhevedia crassa	10	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	86	0	0	2483	47	587	0	220	212	0	671
Hexarthra mira	0	111	136	0	17	0	0	0	0	0	531	0
Keratella tropica	1003	26	526	355	993	2515	25	91	99	0	57	8
Moina micrura	185	163	662	25	105	279	0	0	0	0	0	0
Nauplii	107	574	2721	66	906	124	428	439	205	326	144	688
Ostracoda	19	0	9	0	9	0	0	0	0	0	0	0

Table A18 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 4; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	14	998	159	11	2787	65	44	695	0	5	454	97
Brachionus calyciflorus	0	0	1270	11	12	0	0	0	0	0	0	0
Brachionus falcatus	14	0	0	0	12	0	0	0	0	0	0	0
Calanoid copepod	55	0	0	134	12	39	177	0	244	34	0	136
Ceriodaphnia cornuta	137	0	331	0	0	13	388	2680	135	0	0	0
Cyclopoid copepod	219	209	66	0	569	740	698	142	212	4	1334	340
Diffugia sp.	14	0	0	0	0	0	0	0	0	1	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	36	26	155	0	77	1	88	165
Hexarthra mira	0	0	119	0	0	0	0	0	0	0	0	0
Keratella tropica	1752	338	146	67	1245	2427	66	14	187	1	410	126
Moina micrura	274	80	185	0	296	324	0	0	0	0	0	0
Nauplii	424	515	1204	0	451	376	221	269	71	16	15	1097
Ostracoda	0	0	0	0	0	0	0	14	0	1	0	0

Table A19 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 5; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	0	0	0	0	0	0	0	9	0
Brachionus angularis	0	38	0	0	128	0	0	155	0	20	340	27
Brachionus calyciflorus	0	0	1853	21	0	0	0	0	0	0	0	0
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0	0	0
Calanoid copepod	48	0	12	356	7	0	77	0	94	199	0	107
Ceriodaphnia cornuta	0	0	108	0	14	0	1887	13	668	10	0	0
Cyclopoid copepod	96	280	36	21	342	269	286	246	187	80	1011	228
Diffugia sp.	0	0	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	39	0	33	30	28	1155
Hexarthra mira	0	0	289	0	0	0	0	0	0	0	0	0
Keratella tropica	241	1249	60	105	356	1898	31	0	414	0	4126	497
Moina micrura	161	38	156	115	235	208	23	0	0	0	0	0
Nauplii	185	38	1107	293	862	612	263	563	180	149	37	1853
Ostracoda	0	0	0	0	0	0	0	0	0	10	0	0

Table A20 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 6; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	104	0	0	106	0	59	101	0
Brachionus calyciflorus	0	0	173	6	0	0	0	0	0	0	0	0
Brachionus falcatus	0	0	0	0	16	0	0	0	0	0	0	0
Calanoid copepod	221	65	0	215	8	24	165	0	78	106	29	476
Ceriodaphnia cornuta	0	0	12	0	0	0	1055	7	1726	70	0	0
Cyclopoid copepod	88	195	74	0	199	193	228	156	123	188	623	119
Diffugia sp.	0	0	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	11	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	11	11	0	0	0	0	0	0	0	0	14	9165
Hexarthra mira	0	0	272	0	0	0	0	7	0	0	0	0
Keratella tropica	309	76	0	166	151	1077	10	7	448	12	362	132
Moina micrura	342	22	284	68	151	64	21	0	0	0	0	0
Nauplii	365	141	951	6	852	281	124	347	269	0	0	1177
Ostracoda	0	11	0	0	0	0	0	0	0	12	0	0

Table A21 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 7; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	7	0	0	234	0	0	4	0	123	15	0
Brachionus calyciflorus	0	0	3941	0	26	0	0	4	0	0	0	0
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0	0	0
Calanoid copepod	40	14	0	209	0	18	61	8	14	36	45	231
Ceriodaphnia cornuta	17	0	0	11	0	6	36	0	129	276	321	0
Cyclopoid copepod	6	1709	38	0	175	193	449	93	41	44	194	51
Diffugia sp.	0	0	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	14	49	39	6	0	213	0	0	152	634	5449
Hexarthra mira	0	0	390	0	0	0	0	0	0	0	0	0
Keratella tropica	102	538	148	4804	0	762	55	0	41	87	2878	45
Moina micrura	34	34	769	23	52	35	12	0	0	0	0	0
Nauplii	23	689	374	242	721	340	383	266	61	29	7	224
Ostracoda	0	7	0	0	0	0	0	0	0	0	0	0

Table A22 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 8; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	38	0	0	0	14	56	0	0
Brachionus calyciflorus	0	0	542	13	0	0	0	117	0	0	0	0
Brachionus falcatus	0	0	0	0	13	0	0	0	0	0	0	0
Calanoid copepod	9	0	0	59	0	0	15	0	7	45	68	287
Ceriodaphnia cornuta	104	0	0	33	6	0	212	968	311	765	601	165
Cyclopoid copepod	0	887	108	0	1321	801	446	539	173	45	53	22
Diffugia sp.	17	0	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	17	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	52	145	0	0	32	81	0	0	428	601	4771
Hexarthra mira	0	0	121	0	0	0	0	62	0	0	0	0
Keratella tropica	17	82	0	1068	64	360	22	0	0	11	38	201
Moina micrura	147	104	193	132	134	560	44	23	0	0	0	0
Nauplii	191	253	0	40	906	649	505	47	21	259	38	459
Ostracoda	0	7	0	0	0	0	15	0	0	11	0	0

Table A23 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 9; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	0	0	0	0	0	0	0	0	11
Brachionus angularis	0	0	0	0	0	0	0	0	21	113	0	0
Brachionus calyciflorus	11	0	322	0	0	0	0	0	0	0	0	0
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0	0	0
Calanoid copepod	0	0	0	25	0	0	0	0	21	0	179	272
Ceriodaphnia cornuta	1441	8	99	0	12	0	0	1380	2177	32	64	1240
Cyclopoid copepod	66	474	66	0	1147	742	885	460	268	338	201	0
Diffugia sp.	0	8	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	114	83	16	0	8	16	0	0	161	1060	5241
Hexarthra mira	0	0	207	0	0	0	0	89	0	0	0	0
Keratella tropica	11	0	25	16	0	734	8	0	10	16	50	250
Moina micrura	176	16	174	33	106	128	0	26	0	0	0	0
Nauplii	198	221	165	25	2494	224	509	128	330	193	322	130
Ostracoda	0	0	0	0	0	0	0	64	0	48	0	0

Table A24 – Data base for study 3; number of individuals of each treatment and for each species for sampling week 10; Concentrations in active product per litre.

Treatment / Taxa	Control Pond 1	Control Pond 2	Control Pond 3	Control Pond 4	0,1 µg/L Pond 1	0,1 µg/L Pond 2	1 µg/L Pond 1	1 µg/L Pond 2	10 µg/L Pond 1	10 µg/L Pond 2	100 µg/L Pond 1	100 µg/L Pond 2
Asplanchna sp.	0	0	0	0	0	0	0	0	0	0	0	0
Brachionus angularis	0	0	0	0	0	0	7	182	0	25	0	0
Brachionus calyciflorus	1275	0	366	7	0	12	0	11	0	0	0	6
Brachionus falcatus	0	0	0	0	0	0	0	0	0	0	0	0
Calanoid copepod	9	0	0	0	0	12	0	0	37	0	685	104
Ceriodaphnia cornuta	950	0	806	0	0	0	0	91	1418	17	1349	191
Cyclopoid copepod	394	455	63	0	1560	576	244	409	885	211	64	0
Diffugia sp.	0	0	0	0	0	0	0	0	0	0	0	0
Dunhevedia crassa	0	0	0	0	0	0	0	0	0	0	0	0
Filinia longiseta	0	200	188	15	14	0	299	0	0	42	493	6989
Hexarthra mira	0	0	481	0	0	0	7	307	0	0	0	0
Keratella tropica	17	0	52	0	0	81	0	0	0	0	118	0
Moina micrura	34	0	523	29	83	46	0	1352	22	0	0	0
Nauplii	231	176	1015	22	745	795	334	3227	439	84	749	12
Ostracoda	0	0	10	0	0	0	0	0	0	8	0	0

Table A25 – Values in percentage of MDD_{abu} to each moment of sampling and for each treatement studied on study 1; Concentration in active indredient by litre.

Treatment / Taxa	0,005 µg/L Day 14	0,05 µg/L Day 14	0,5 µg/L Day 14	5 µg/L Day 14
Alona rectangula	173%	178%	180%	180%
Bosmina spp.	-	-	-	-
Cephalodella gibba	-	-	-	-
Chydorus sphaerica	84%	85%	86%	86%
Colurella uncicanata	106%	107%	107%	107%
Cyclops cyclopoidae	101%	102%	103%	103%
Daphnia galeata	162%	166%	168%	168%
Daphnia magna	162%	166%	168%	168%
Ephemeroptera	101%	102%	103%	103%
Ephippia	107%	108%	109%	109%
Keratella coclearis	63%	64%	65%	65%
Keratella quadrata	61%	62%	63%	63%
Lecane bulla	179%	181%	181%	182%
Lecane luna	-	-	-	-
Lecane lunaris	125%	125%	125%	125%
Lecane quadridentata	118%	119%	119%	119%
Lepadella patella	92%	93%	93%	94%
Mytilina ventralis	-	-	-	-
Ostracoda	52%	54%	54%	54%
Simocephalus vetulus	94%	95%	95%	95%
Trichocerca	130%	132%	132%	132%

Table A26 – Values in percentage of MDD_{abu} for moment of sampling of day 0 and 4 and for each treatement studied on study 2 ; Concentration in active indredient by litre.

Treatment / Taxa	1 µg/L Day 0	1 µg/L * 2 Day 0	1 µg/L Day 4	1 µg/L * 2 Day 4
Alona sp.	-	-	139%	142%
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	45%	46%	98%	98%
Brachionus falcatus	94%	95%	-	-
Brachionus quadridentatus	-	-	-	-
Brachionus urceolaris	83%	85%	83%	84%
Calanoid copepod	51%	53%	69%	71%
Ceriodaphnia cornuta	88%	89%	101%	102%
Colurella sp.	-	-	-	-
Cyclopoid copepod	41%	43%	85%	86%
Dunhevedia crassa	-	-	-	-
Filinia longiseta	-	-	-	-
Filinia opoliensis	-	-	-	-
Hexarthra mira	137%	139%	-	-
Keratella tropica	110%	111%	-	-
Lecane bulla	56%	58%	117%	118%
Lecane closterocerca	-	-	-	-
Lecane luna	137%	140%	-	-
Lepadella patella	-	-	-	-
Moina micrura	48%	50%	27%	28%
Nauplii	58%	60%	80%	81%
Streblocerus pygmaues	-	-	-	-
Trichocerca	-	-	-	-

Table A27 – Values in percentage of MDD_{abu} for moment of sampling of day 7 and 11 and for each treatement studied on study 2 ; Concentration in active indredient by litre.

Treatment / Taxa	1 µg/L Day 7	1 µg/L * 2 Day 7	1 µg/L Day 11	1 µg/L * 2 Day 11
Alona sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	94%	95%	99%	100%
Brachionus falcatus	-	-	-	-
Brachionus quadridentatus	184%	188%	-	-
Brachionus urceolaris	106%	107%	139%	142%
Calanoid copepod	58%	59%	96%	97%
Ceriodaphnia cornuta	27%	28%	43%	45%
Colurella sp.	-	-	89%	90%
Cyclopoid copepod	58%	59%	92%	93%
Dunhevedia crassa	-	-	-	-
Filinia longiseta	-	-	-	-
Filinia opoliensis	-	-	-	-
Hexarthra mira	-	-	-	-
Keratella tropica	178%	182%	-	-
Lecane bulla	181%	184%	-	-
Lecane closterocerca	-	-	-	-
Lecane luna	-	-	-	-
Lepadella patella	-	-	-	-
Moina micrura	54%	55%	62%	63%
Nauplii	76%	78%	113%	114%
Streblocerus pygmaues	-	-	-	-
Trichocerca	105%	106%	108%	109%

Table A28 – Values in percentage of MDD_{abu} for moment of sampling of day 14 and 18 and for each treatement studied on study 2 ; Concentration in active indredient by litre.

Treatment / Taxa	1 µg/L Day 14	1 µg/L * 2 Day 14	1 µg/L Day 18	1 µg/L * 2 Day 18
Alona sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	91%	92%	-	-
Brachionus falcatus	-	-	-	-
Brachionus quadridentatus	-	-	-	-
Brachionus urceolaris	-	-	-	-
Calanoid copepod	138%	141%	144%	147%
Ceriodaphnia cornuta	103%	105%	-	-
Colurella sp.	97%	98%	-	-
Cyclopoid copepod	107%	109%	-	-
Dunhevedia crassa	-	-	139%	142%
Filinia longiseta	-	-	-	-
Filinia opoliensis	-	-	-	-
Hexarthra mira	190%	192%	211%	212%
Keratella tropica	-	-	-	-
Lecane bulla	-	-	-	-
Lecane closterocerca	-	-	-	-
Lecane luna	-	-	-	-
Lepadella patella	-	-	-	-
Moina micrura	49%	50%	64%	66%
Nauplii	102%	104%	-	-
Streblocerus pygmaues	171%	174%	-	-
Trichocerca	93%	94%	-	-

Table A29 – Values in percentage of MDD_{abu} for moment of sampling of day 21 and 25 and for each treatement studied on study 2 ; Concentration in active indredient by litre.

Treatment / Taxa	1 µg/L Day 21	1 µg/L * 2 Day 21	1 µg/L Day 25	1 µg/L * 2 Day 25
Alona sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	-	-	-	-
Brachionus falcatus	-	-	-	-
Brachionus quadridentatus	-	-	-	-
Brachionus urceolaris	-	-	134%	134%
Calanoid copepod	-	-	-	-
Ceriodaphnia cornuta	-	-	183%	184%
Colurella sp.	-	-	-	-
Cyclopoid copepod	-	-	-	-
Dunhevedia crassa	133%	135%	103%	104%
Filinia longiseta	-	-	-	-
Filinia opoliensis	-	-	-	-
Hexarthra mira	-	-	178%	179%
Keratella tropica	-	-	-	-
Lecane bulla	-	-	-	-
Lecane closterocerca	-	-	-	-
Lecane luna	-	-	-	-
Lepadella patella	-	-	-	-
Moina micrura	88%	89%	97%	98%
Nauplii	-	-	-	-
Streblocerus pygmaues	-	-	162%	165%
Trichocerca	-	-	-	-

Table A30 – Values in percentage of MDD_{abu} for moment of sampling of day 28 and 32 and for each treatement studied on study 2 ; Concentration in active indredient by litre.

Treatment / Taxa	1 µg/L Day 28	1 µg/L * 2 Day 28	1 µg/L Day 32	1 µg/L * 2 Day 32
Alona sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	104%	106%	106%	106%
Brachionus falcatus	-	-	-	-
Brachionus quadridentatus	-	-	-	-
Brachionus urceolaris	172%	173%	-	-
Calanoid copepod	-	-	-	-
Ceriodaphnia cornuta	-	-	-	-
Colurella sp.	100%	101%	110%	112%
Cyclopoid copepod	-	-	-	-
Dunhevedia crassa	101%	103%	142%	145%
Filinia longiseta	-	-	-	-
Filinia opoliensis	-	-	-	-
Hexarthra mira	100%	101%	94%	95%
Keratella tropica	-	-	-	-
Lecane bulla	-	-	-	-
Lecane closterocerca	-	-	-	-
Lecane luna	-	-	-	-
Lepadella patella	-	-	-	-
Moina micrura	95%	96%	90%	91%
Nauplii	-	-	-	-
Streblocerus pygmaues	115%	116%	104%	105%
Trichocerca	-	-	-	-

Table A31 – Values in percentage of MDD_{abu} for moment of sampling of day 35 and 42 and for each treatement studied on study 2 ; Concentration in active indredient by litre.

Treatment / Taxa	1 µg/L Day 35	1 µg/L * 2 Day 35	1 µg/L Day 42	1 µg/L * 2 Day 42
Alona sp.	-	-	-	-
Brachionus angularis	-	-	181%	181%
Brachionus calyciflorus	103%	103%	106%	106%
Brachionus falcatus	-	-	-	-
Brachionus quadridentatus	-	-	-	-
Brachionus urceolaris	-	-	-	-
Calanoid copepod	140%	143%	-	-
Ceriodaphnia cornuta	163%	165%	154%	156%
Colurella sp.	-	-	143%	146%
Cyclopoid copepod	-	-	-	-
Dunhevedia crassa	135%	137%	-	-
Filinia longiseta	-	-	-	-
Filinia opoliensis	-	-	-	-
Hexarthra mira	97%	97%	96%	97%
Keratella tropica	-	-	-	-
Lecane bulla	-	-	-	-
Lecane closterocerca	-	-	-	-
Lecane luna	-	-	-	-
Lepadella patella	130%	131%	143%	146%
Moina micrura	98%	98%	97%	97%
Nauplii	-	-	194%	195%
Streblocerus pygmaues	97%	98%	113%	113%
Trichocerca	-	-	-	-

Table A32 – Values in percentage of MDD_{abu} for moment of sampling in week 0 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 0	1 µg/L Week 0	10 µg/L Week 0	100 µg/L Week 0
Asplanchna sp.	-	-	-	-
Brachionus angularis	122%	122%	123%	123%
Brachionus calyciflorus	107%	107%	107%	107%
Brachionus falcatus	132%	133%	134%	134%
Calanoid copepod	100%	101%	101%	101%
Ceriodaphnia cornuta	105%	105%	105%	105%
Cyclopoid copepod	96%	97%	97%	97%
Diffugia sp.	115%	115%	115%	115%
Dunhevedia crassa	-	-	-	-
Filinia longiseta	191%	192%	192%	192%
Hexarthra mira	166%	168%	169%	169%
Keratella tropica	94%	95%	95%	95%
Moina micrura	93%	94%	94%	94%
Nauplii	48%	49%	50%	50%
Ostracoda	-	-	-	-

Table A33 – Values in percentage of MDD_{abu} for moment of sampling in week 1 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 1	1 µg/L Week 1	10 µg/L Week 1	100 µg/L Week 1
Asplanchna sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	158%	158%	158%	158%
Brachionus falcatus	-	-	-	-
Calanoid copepod	106%	106%	106%	106%
Ceriodaphnia cornuta	94%	95%	95%	95%
Cyclopoid copepod	98%	99%	99%	99%
Diffugia sp.	117%	117%	117%	117%
Dunhevedia crassa	-	-	-	-
Filinia longiseta	153%	153%	153%	153%
Hexarthra mira	-	-	-	-
Keratella tropica	92%	93%	93%	94%
Moina micrura	57%	58%	59%	59%
Nauplii	72%	74%	74%	74%
Ostracoda	-	-	-	-

Table A34 – Values in percentage of MDD_{abu} for moment of sampling in week 2 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 2	1 µg/L Week 2	10 µg/L Week 2	100 µg/L Week 2
Asplanchna sp.	143%	145%	146%	146%
Brachionus angularis	104%	104%	104%	104%
Brachionus calyciflorus	102%	102%	102%	102%
Brachionus falcatus	-	-	-	-
Calanoid copepod	102%	102%	102%	102%
Ceriodaphnia cornuta	99%	100%	100%	100%
Cyclopoid copepod	95%	96%	96%	96%
Diffugia sp.	118%	118%	118%	118%
Dunhevedia crassa	-	-	-	-
Filinia longiseta	146%	146%	146%	146%
Hexarthra mira	113%	114%	114%	114%
Keratella tropica	91%	92%	92%	92%
Moina micrura	92%	92%	93%	93%
Nauplii	91%	92%	92%	92%
Ostracoda	-	-	-	-

Table A35 – Values in percentage of MDD_{abu} for moment of sampling in week 3 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 3	1 µg/L Week 3	10 µg/L Week 3	100 µg/L Week 3
Asplanchna sp.	164%	167%	167%	168%
Brachionus angularis	96%	97%	97%	97%
Brachionus calyciflorus	102%	102%	102%	102%
Brachionus falcatus	-	-	-	-
Calanoid copepod	123%	123%	123%	123%
Ceriodaphnia cornuta	107%	107%	107%	107%
Cyclopoid copepod	77%	79%	79%	79%
Diffugia sp.	119%	120%	120%	120%
Dunhevedia crassa	148%	150%	151%	151%
Filinia longiseta	137%	137%	138%	138%
Hexarthra mira	106%	106%	106%	106%
Keratella tropica	95%	95%	96%	96%
Moina micrura	76%	77%	78%	78%
Nauplii	87%	88%	88%	88%
Ostracoda	111%	112%	113%	113%

Table A36 – Values in percentage of MDD_{abu} for moment of sampling in week 4 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 4	1 µg/L Week 4	10 µg/L Week 4	100 µg/L Week 4
Asplanchna sp.	-	-	-	-
Brachionus angularis	149%	149%	149%	149%
Brachionus calyciflorus	105%	105%	105%	105%
Brachionus falcatus	156%	158%	158%	158%
Calanoid copepod	107%	107%	107%	107%
Ceriodaphnia cornuta	104%	104%	104%	104%
Cyclopoid copepod	98%	99%	99%	99%
Diffugia sp.	146%	149%	149%	149%
Dunhevedia crassa	-	-	-	-
Filinia longiseta	-	-	-	-
Hexarthra mira	126%	127%	127%	127%
Keratella tropica	92%	93%	93%	93%
Moina micrura	96%	97%	97%	97%
Nauplii	99%	99%	99%	99%
Ostracoda	-	-	-	-

Table A37 – Values in percentage of MDD_{abu} for moment of sampling in week 5 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 5	1 µg/L Week 5	10 µg/L Week 5	100 µg/L Week 5
Asplanchna sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	103%	104%	104%	104%
Brachionus falcatus	-	-	-	-
Calanoid copepod	101%	102%	102%	102%
Ceriodaphnia cornuta	133%	133%	133%	133%
Cyclopoid copepod	75%	76%	77%	77%
Diffugia sp.	-	-	-	-
Dunhevedia crassa	-	-	-	-
Filinia longiseta	-	-	-	-
Hexarthra mira	121%	121%	121%	121%
Keratella tropica	98%	98%	98%	98%
Moina micrura	83%	84%	84%	84%
Nauplii	89%	90%	90%	90%
Ostracoda	-	-	-	-

Table A38 – Values in percentage of MDD_{abu} for moment of sampling in week 6 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 6	1 µg/L Week 6	10 µg/L Week 6	100 µg/L Week 6
Asplanchna sp.	-	-	-	-
Brachionus angularis	200%	201%	201%	201%
Brachionus calyciflorus	107%	108%	108%	108%
Brachionus falcatus	-	-	-	-
Calanoid copepod	100%	100%	100%	100%
Ceriodaphnia cornuta	170%	171%	172%	172%
Cyclopoid copepod	96%	97%	97%	97%
Diffugia sp.	-	-	-	-
Dunhevedia crassa	146%	149%	149%	150%
Filinia longiseta	121%	122%	122%	122%
Hexarthra mira	122%	122%	123%	123%
Keratella tropica	98%	99%	99%	99%
Moina micrura	88%	89%	89%	89%
Nauplii	99%	100%	100%	100%
Ostracoda	161%	163%	164%	164%

Table A39 – Values in percentage of MDD_{abu} for moment of sampling in week 7 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 7	1 µg/L Week 7	10 µg/L Week 7	100 µg/L Week 7
Asplanchna sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	111%	111%	111%	111%
Brachionus falcatus	-	-	-	-
Calanoid copepod	99%	100%	100%	100%
Ceriodaphnia cornuta	120%	121%	121%	121%
Cyclopoid copepod	100%	100%	100%	100%
Diffugia sp.	-	-	-	-
Dunhevedia crassa	-	-	-	-
Filinia longiseta	103%	104%	104%	104%
Hexarthra mira	119%	120%	120%	120%
Keratella tropica	99%	99%	99%	99%
Moina micrura	89%	90%	90%	90%
Nauplii	88%	89%	89%	89%
Ostracoda	152%	155%	156%	156%

Table A40 – Values in percentage of MDD_{abu} for moment of sampling in week 8 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 8	1 µg/L Week 8	10 µg/L Week 8	100 µg/L Week 8
Asplanchna sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	106%	107%	107%	107%
Brachionus falcatus	-	-	-	-
Calanoid copepod	111%	112%	112%	112%
Ceriodaphnia cornuta	105%	106%	106%	106%
Cyclopoid copepod	102%	102%	103%	103%
Diffugia sp.	141%	143%	144%	144%
Dunhevedia crassa	141%	143%	144%	144%
Filinia longiseta	107%	108%	108%	108%
Hexarthra mira	130%	130%	130%	130%
Keratella tropica	100%	100%	100%	100%
Moina micrura	50%	52%	52%	52%
Nauplii	98%	99%	99%	99%
Ostracoda	182%	183%	184%	184%

Table A41 – Values in percentage of MDD_{abu} for moment of sampling in week 9 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 9	1 µg/L Week 9	10 µg/L Week 9	100 µg/L Week 9
Asplanchna sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	105%	107%	108%	108%
Brachionus falcatus	-	-	-	-
Calanoid copepod	147%	152%	153%	153%
Ceriodaphnia cornuta	101%	102%	102%	102%
Cyclopoid copepod	99%	101%	101%	101%
Diffugia sp.	150%	161%	162%	163%
Dunhevedia crassa	-	-	-	-
Filinia longiseta	101%	102%	102%	102%
Hexarthra mira	125%	127%	127%	127%
Keratella tropica	105%	106%	107%	107%
Moina micrura	88%	92%	92%	92%
Nauplii	80%	86%	86%	86%
Ostracoda	-	-	-	-

Table A42 – Values in percentage of MDD_{abu} for moment of sampling in week 10 and for each treatement studied on study 3; Concentration in active indredient by litre.

Treatment / Taxa	0,1 µg/L Week 10	1 µg/L Week 10	10 µg/L Week 10	100 µg/L Week 10
Asplanchna sp.	-	-	-	-
Brachionus angularis	-	-	-	-
Brachionus calyciflorus	100%	110%	111%	111%
Brachionus falcatus	-	-	-	-
Calanoid copepod	182%	97%	98%	98%
Ceriodaphnia cornuta	102%	122%	122%	122%
Cyclopoid copepod	99%	101%	101%	101%
Diffugia sp.	-	-	-	-
Dunhevedia crassa	-	-	-	-
Filinia longiseta	101%	104%	104%	104%
Hexarthra mira	119%	121%	121%	121%
Keratella tropica	112%	98%	99%	99%
Moina micrura	101%	100%	100%	100%
Nauplii	93%	93%	94%	94%
Ostracoda	160%	178%	178%	179%