# A Tale of Two Metrics: The EPA Risk Quotient Approach versus the Delay in Population Growth Index for Determination of Pesticide Risk to Aquatic Species

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# Abstract

The risk that two closely related insecticides, spinetoram and spinosad, posed to three Cladoceran species, *Ceriodaphnia dubia*, *Daphnia pulex*, and *D. magna* was determined using two approaches, the USEPA Risk Quotient method and the Delay in Population Growth Index (DPGI). Results of the RQ method showed that spinetoram posed a risk to all three species, but spinosad posed a risk only to *C. dubia*. The DPGI analysis showed that exposure to spinetoram resulted in populations of all three species being delayed $\geq$ 3 generation times. Exposure to the $LC_{50}$ and the lower 95% CL resulted in delayed populations while exposure to the upper 95% CL concentration of spinetoram resulted in no recovery of any of the three species over the course of the modeling exercise (88 d). Exposure to the lower and upper 95% Cl and the $LC_{50}$ of spinosad resulted in *C. dubia* populations being delayed $\geq$ 3 generations. *D. pulex* populations were not negatively affected after exposure to spinosad. *D. magna* populations were delayed $\geq$ 3 generations, but only after exposure to the upper 95% Cl of spinosad. These results illustrate that although the EPA risk quotient method indicated that spinetoram posed a risk to all three species and that spinosad only posed a risk to *C. dubia*, the DPGI showed that *D. magna* would be negatively affected by spinosad and none of the three species would recover after exposure to the upper 95% CL of spinetoram. Because the DPGI uses the 95% Cl as well as the $LC_{50}$ in its calculation and produces a measure of population growth and recovery or lack thereof, it provides more detailed information in terms of the potential risk of pesticides to populations than the RQ method.


# Introduction

A central concern in risk assessment is accurately characterizing the effects of toxicants on different organisms – and using this characterization to effectively and accurately predict how different species will react to different toxicants. Historically, risk assessment has relied heavily on static point-estimates of toxicity such as $LC_{50}$ or $LD_{50}$. In recent decades, a large number of studies have highlighted the importance of moving beyond this static-metric approach to consider population-endpoints (Sibly 1999; Banks and Stark 1998; Forbes and Calow 2002; Stark and Banks 2001, 2003; Stark et al. 2004; Billoir et al. 2008; Banks et al. 2008; Dalkvist et al. 2009; Banks and Stark 2009; Grimm et al. 2009; Forbes et al. 2011; Van den Brink 2013; Stark and Banks 2016). Simply speaking, point estimates such as the $LC_{50}$ cannot capture population-level effects that consider differences in demographic vital rates. Differences exist among species in various demographic vital rates such that some species reach sexual maturity quickly, have many broods and offspring, and have a short life span (e.g. rats) and other species take a long time to reach sexual maturity, have long life spans, and few offspring (e.g. elephants). These differences in vital rates make some species less susceptible to stress than others (Stark et al 2004). Several studies have now shown that differences in vital rates among even closely related species can result in different population outcomes after exposure to toxicants (Stark et al. 2004; Banks et al. 2010, 2011, 2014, 2017).

The United States Environmental Protection Agency (USEPA) uses a tiered ecological risk assessment approach to estimate potential risk of pesticides to various species by comparing species susceptibility to an expected environmental concentration (EEC) (USEPA 2004). A ratio called a risk quotient (RQ) is compared to a level of concern (LOC) for pesticides. If the ratio exceeds the LOC, then the pesticide poses an environmental risk. The EPA approach uses a point estimate for toxicity, the $LC_{50}$.

In contrast, Wennergren and Stark (2000) developed an approach to pesticide risk assessment called the Delay in Population Growth Index (DPGI) that takes into account population-level effects by evaluating the recovery of a population after pesticide exposure. The DPGI involves the development of matrix population models for control populations and populations exposed to pesticides. These pesticide-exposed populations are compared to the control, and the time to recovery is the endpoint of interest. Stark et al. (2015) used the DPGI to evaluate the effects of pesticides on pest and beneficial species.

In this study, we directly compare the effectiveness of two risk assessment approaches using lab-derived data for several closely-related important environmental indicator species subjected to two different pesticides. In particular, we developed risk assessments for three Cladoceran species, *Ceriodaphnia dubia*, *Daphnia pulex*, and *D. magna* exposed to the insecticides spinosad and spinetoram, using the EPA and DPGI methods. We then compared the results of the EPA Risk Quotient method and the DPGI in order to quantify differences in these two methods in terms of the risk of pesticides to aquatic organisms exposed to pesticides.

# Materials And Methods

### Insecticides evaluated

Spinosad (Dow AgroSciences LLC, Indianapolis, IN) is a natural insecticide derived from the fermentation of a strain of *Saccharo-polyspora spinosa*. A mixture of spinosyns A and D, which make up the active ingredients of spinosad, are extracted from the fermentation broth. Spinetoram is a mixture of chemically modified spinosyns J and L. Spinosyns J and L are obtained after fermentation of a mutant strain of *Saccharopolyspora spinosa* that produces primarily spinosyns J and L, instead of spinosyns A and D. Spinosyns J and L then undergo two chemical synthesis steps to produce the final product, spinetoram. Both spinosad and spinetoram are neurotoxic and the mode of action is to disrupt the nicotinic and GABA-gated chloride channels (Galm and Sparks 2016).

The USEPA considers spinosad and spinetoram to be “toxicologically equivalent” and that the major risk they pose is to freshwater invertebrates, but only after chronic exposure (USEPA 2009).

### Acute mortality data used in population models

Acute mortality data for *C. dubia*, *D. pulex* and *D. magna* exposed to Spinosad and spinetoram have been previously published (Deardorff and Stark 2009; Stark and Banks 2019). The $LC_{50}$ and their respective 95% CLs for each species and pesticide were used to parameterize matrix population models (Table 1).

Expected environmental concentrations (EEC) of spinetoram and spinosad in freshwater systems have been developed. The EECs for spinetoram (EPA 2007) and spinosad (Federal Register 2005) are 14.4 and 2.3 µg/l, respectively.

## EPA RQ development

RQs for Spinosad and the three Daphniid species have been previously published (Deardorff and Stark 2009). RQs for spinetoram and the three Daphniid species were developed by dividing the EEC/$LC_{50}$ for each insecticide and species. RQs developed for each species and insecticide were compared to a level of concern of 0.5. RQs that exceeded 0.5 indicated that the insecticide posed a risk to that species.

## Population model construction and development of the DPGI

We define the delay in population growth index as the number of days it takes for a pesticide-exposed population to grow from 100 to 1,000 individuals minus the number of days it takes the control population to grow from 100 to 1,000 individuals (Wennergren and Stark 2000; Stark et al. 2004; Stark et al. 2015). Percent mortalities corresponding to exposure to the EECs for spinosad (2.3 µg/l) and spinetoram (14.4 µg/l) were calculated by reading mortality values from the concentration response curves for the daphniid species, *Ceriodaphnia dubia, Daphnia pulex* and *D. magna* (Figs 1 & 2). Percent mortalities were read from the mean, lower, and upper 95% CL curves (Table 2).

The predicted mortality estimates read from the LC curve, upper, and lower 95% CL curves (Table 2) were incorporated into matrix population models to develop the Delay in Population Growth Index (DPGI) (Wennergren and Stark 2000; Stark et al 2004; Stark et al. 2015). Thus, three models were developed for each mortality estimate, one model for each control population for each of the three species for each insecticide (24 models in total).

Stochastic matrix population models were developed using RAMAS Metapop© software (Akçakaya 2005). Models were developed for *C. dubia, D. magna,* and *D. pulex* based on the approach outlined in Stark et al. (2015). The models were stage structured and consisted of vital rates for a neonate, juvenile 1, juvenile 2, and adult stage (Table 3). Populations were started as 100 individuals in the stable age distribution for each species. Ceiling density dependence was incorporated into each matrix model with 1,000 individuals as the final population size. Each model was run 1,000 times by resampling using a Monte Carlo method in RAMAS. The time step of each matrix multiplication was four days, which corresponded to the approximate generation time for each species (Banks et al. 2019). The model was run for 22-time steps which corresponded to 88 days, which was supposed to approximate a summer season in a temperate zone. Model runs resulted in population trajectories for each species exposed to the two insecticides over an 88d period.

The DPGI was also converted from delays in recovery time (in terms of days) to the number of generations (based on a 4-d generation time) that could have been produced during the delay time

interval. A population delay was considered to cause significant damage if the pesticide-exposed population was delayed ≥ three generations over the 88 d of the study timeframe.

# Results

### EPA RQ

Results of the EPA RQ method showed that spinetoram posed a risk to all three species while spinosad only posed a risk to *C. dubia* (Table 4).

### Spinetoram DPGI

Results of the DIPG modeling study showed that population growth of all three species was delayed after exposure to spinetoram compared to the control after exposure to mortality levels obtained from the lower and upper 95% CL and the $LC_{50}$ curves (Figs. 3-5, Table 5). Population recovery occurred after exposure to mortality levels obtained from the lower 95% CL and $LC_{50}$ curves. However, none of the three species recovered after being subjected to mortality obtained from the spinetoram upper 95% over the time frame of this study (Fig. 3-5, Table 5).

Population delays were also examined in terms of the number of generations that could have been completed during the delay time interval. All three species were delayed for 3 or more generations after exposure to mortality derived from the lower 95% Cl and LC curve at the EEC (Table 5).

*D. pulex* was the most negatively affected species in terms of days of delay and number of generations that could have been completed followed by *D. magna* and *C. dubia* after exposure to mortality from the LC curve (Table 5). In contrast, according to the RQ method, *D. magna* had the highest quotient indicating it was most at risk, followed by *D. pulex* and then *C. dubia* (Table 4).

### Spinosad DPGI

Results of the DIPG modeling study showed that populations of *C. dubia* and *D. magna* were delayed after exposure to spinosad, but *D. pulex* populations were unaffected after exposure to the Spinosad EEC (Figs. 6-8, Table 5).

Population delays in terms of the number of generations that could have been completed during the delay time interval showed that *C. dubia* populations were delayed for 3 or more generations after exposure to mortality derived from the lower and upper 95% Cl and the LC curve at the EEC (Table 5). *D. magna* populations were delayed for >3 generations after exposure only to mortality from the upper 95% Cl (Table 5).

The RQ method indicated that Spinosad posed a risk only to *C. dubia*, but the RQ for *D. magna* (0.48) was close to the 0.5 level of concern (Table 4).

# Discussion

In this study, both the EPA RQ method and the DPGI yielded similar results for the insecticide spinetoram and three daphniid species. However, the DPGI provided more information with regard to population-level impacts. The RQ method for Spinosad showed that this insecticide only posed a risk to *C. dubia*. However, the RQ for *D. magna* was borderline (0.48) and could be rounded to 0.5 indicating that Spinosad at the EEC would also pose a risk to *D. magna*. The DPGI for Spinosad provided more detailed information about population-level effects, highlighting that *D. magna* populations would only be negatively affected (>3 generation delay) after mortality levels obtained from the upper 95% CL.

Risk assessment methodologies are often refined in order to optimize tradeoffs between cost and accuracy. For instance, in human health risk assessment, toxicity has traditionally been measured by applying uncertainty factors to standard point-estimates of risk to calculate reference doses or concentrations (Price et al. 1999). Recently the World Health Organization has been exploring moving away from using these point estimates, instead using probability distributions for reference doses/concentrations, citing the need for a more holistic understanding of the risk to human health posed by environmental toxicants (Bhat et al. 2017). In risk assessment for animal populations, point-estimates such as $LC/LD_{50}$ measures remain the standard – they are simple to develop and easy to generate across a multitude of species, thereby rendering them ideal for large-scale comparisons and policy guidance. In contrast, population endpoints require more extensive time and resources to develop than static measures, requiring partial life tables at a minimum (Stark and Banks 2016). However, these latter metrics provide more insight into the nuances of species' responses to toxicants. In some cases, population level analyses and population recovery can lead to major insights into the nature of toxicant risks, revealing risks not detected by static metrics (Stark and Banks 2003, Stark et al. 2004, Barnthouse 2009, Knillmann et al. 2012, Baveco et al. 2014).

In the current study, we illustrated that the major advantage for using the RQ method is that it requires less work to develop than the DPGI. In contrast, however, using the DPGI allows for the incorporation of the $LC_{50}$ 95% CLs as well as providing a population-level measure of effects. We anticipate that differences in output/accuracy of these two methods will vary with species and toxicants. We evaluated only two insecticides and three species in this study, but to gain a better understanding of the value of the RQ and DPGI methods, additional studies should be conducted in the future.

# Declarations


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### Conflicts of interest/Competing interests

Not applicable

### Availability of data and material

All data and models are available upon request to the authors

### Code availability

Not applicable

### Authors' contributions

Both authors contributed equally to the development of this study, gathering of data, statistical analysis of the data and writing of the manuscript.

### Ethics approval

Not applicable

### Consent to participate

Not applicable

### Consent for publication

Not applicable

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# Tables

**Table 1. Acute mortality estimates of spinetoram and spinosad to three Daphniid species**

| | Spinetoram (Delegate)[1] | Spinosad (Success)[2] |
|---|---|---|
| Species | $LC_{50}$ (95% CL) (µg ai/L water) | $LC_{50}$ (95% CL (µg ai/L water) |
| *C. dubia* | 12.6 (4.0-25.2) | 1.8 (1.3-2.5) |
| *D. pulex* | 3.4 (1.0-9.5) | 129 (77-181) |
| *D. magna* | 10.5 (2.5-36.8) | 4.8 (1.9-10) |

1/Data from Stark and Banks 2019.

2/Data from Deardorff and Stark 2009.

**Table 2. Predicted acute mortality for three Cladoceran species exposed to 14.4 µg/l spinetoram or 2.3 µg/l spinosad derived from concentration-mortality curves.**

| | Spinetoram | Spinosad |
|---|---|---|
| *C. dubia* | 52 (40-64)[1] | 55 (49-59)[1] |
| *D. pulex* | 61 (52-70) | 4 (1-15) |
| *D. magna* | 53 (42-64) | 40 (28-52) |

1/ first value is mortality derived from the lethal concentration curve. Values in parentheses are mortality derived from the **lower and upper 95% CL curves (Figs. 1 & 2).**

**Table 3. Four stage vital rates for the three daphniid species calculated on a four-day timestep (from Banks et al. 2018).**

| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ |
|---|---|---|---|---|---|
| *C. dubia* | 1 | 1 | 1 | 0.81 | 18.69 |
| *D. pulex* | 1 | 1 | 1 | 0.71 | 25.13 |
| *D. magna* | 1 | 1 | 1 | 0.80 | 17.53 |

$S_1$ = survival in the neonate stage, $S_2$ = survival in the juvenile 1 stage, S3 = survival in **the juvenile 2 stage, S4 survival in the adult stage, f = fecundity**

**Table 4. Risk Quotients (RQ) for spinetoram and Spinosad and three Daphniid species.**

| Species | RQ for spinetoram[1] | RQ for spinosad[1,2] |
|---|---|---|
| *C. dubia* | 1.15 | 1.29 |
| *D. magna* | 4.19 | 0.48 |
| *D. pulex* | 1.37 | 0.02 |

1/ Risk assessment values were calculated with the equation:

Risk = $EEC/LC_{50}$.

Values greater than 0.5 indicate that the chemical poses a risk to the

tested species.

2/from Deardorff and Stark 2009.

**Table 5. Delay in population growth after exposure to the expected environmental concentration of spinetoram or spinosad.**

| | Spinetoram | | |
|---|---|---|---|
| | Lower 95% CL | $LC_{50}$ | Upper 95% CL |
| Delay (d)[1] [generations][2] | | | |
| *C. dubia* | 12 [3] | 24 [6] | No recovery[3] |
| *D. pulex* | 16 [4] | 40 [10] | No recovery |
| *D. magna* | 12 [3] | 28 [7] | No recovery |
| | Spinosad | | |
| | Lower 95% CL | $LC_{50}$ | Upper 95% CL |
| *C. dubia* | 16 [4] | 24 [6] | 44 [11] |
| *D. pulex* | 0 [0] | 0 [0] | 0 [0] |
| *D. magna* | 4 [1] | 8 [2] | 24 [6] |

1/ Number of days it takes for a pesticide-exposed population to grow from 100 to 1,000

individuals minus the number of days it takes the control population to grow from 100 to 1,000 individuals (Stark et al. 2004).

2/ number of generations (based on a 4-d generation time) that could have been produced during the delay time interval.

3/population does not recover (reach 1,000 individuals) over the length of the study

# Figures

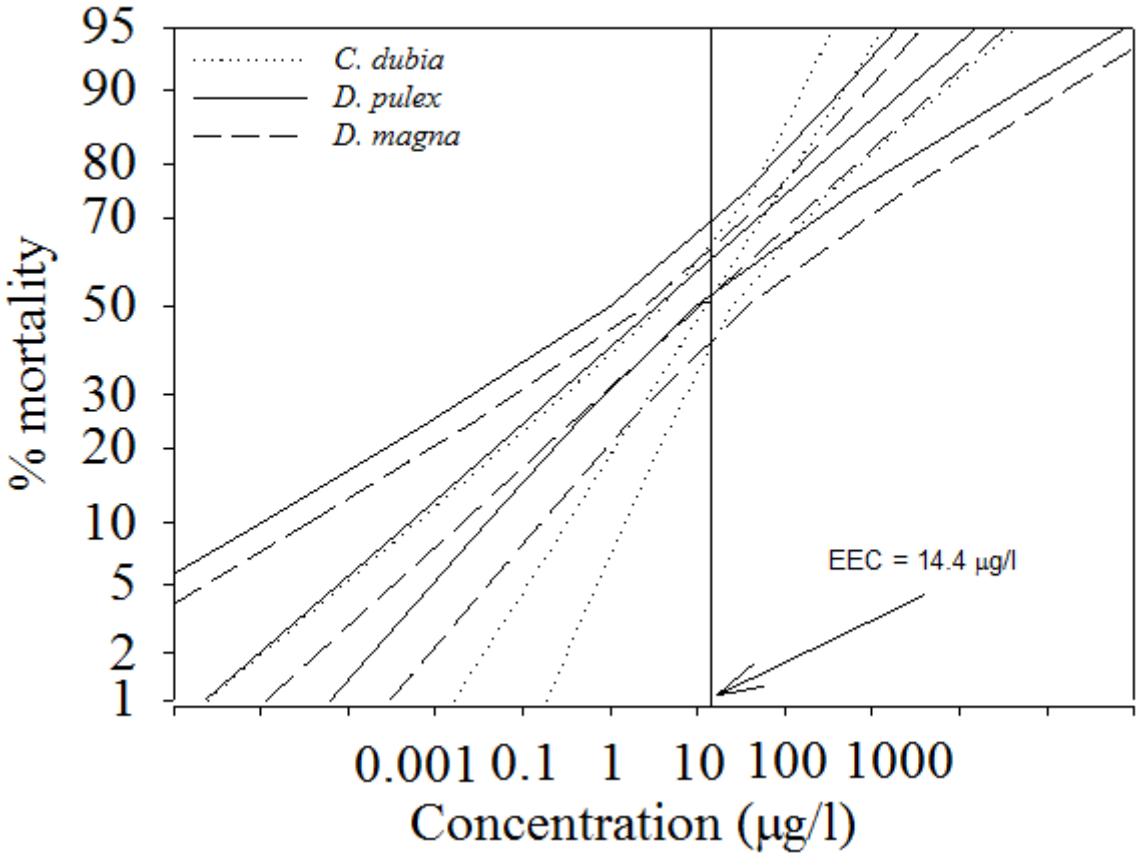



**Figure 1**

Acute concentration-response curves for three Cladoceran species exposed to Spinetoram

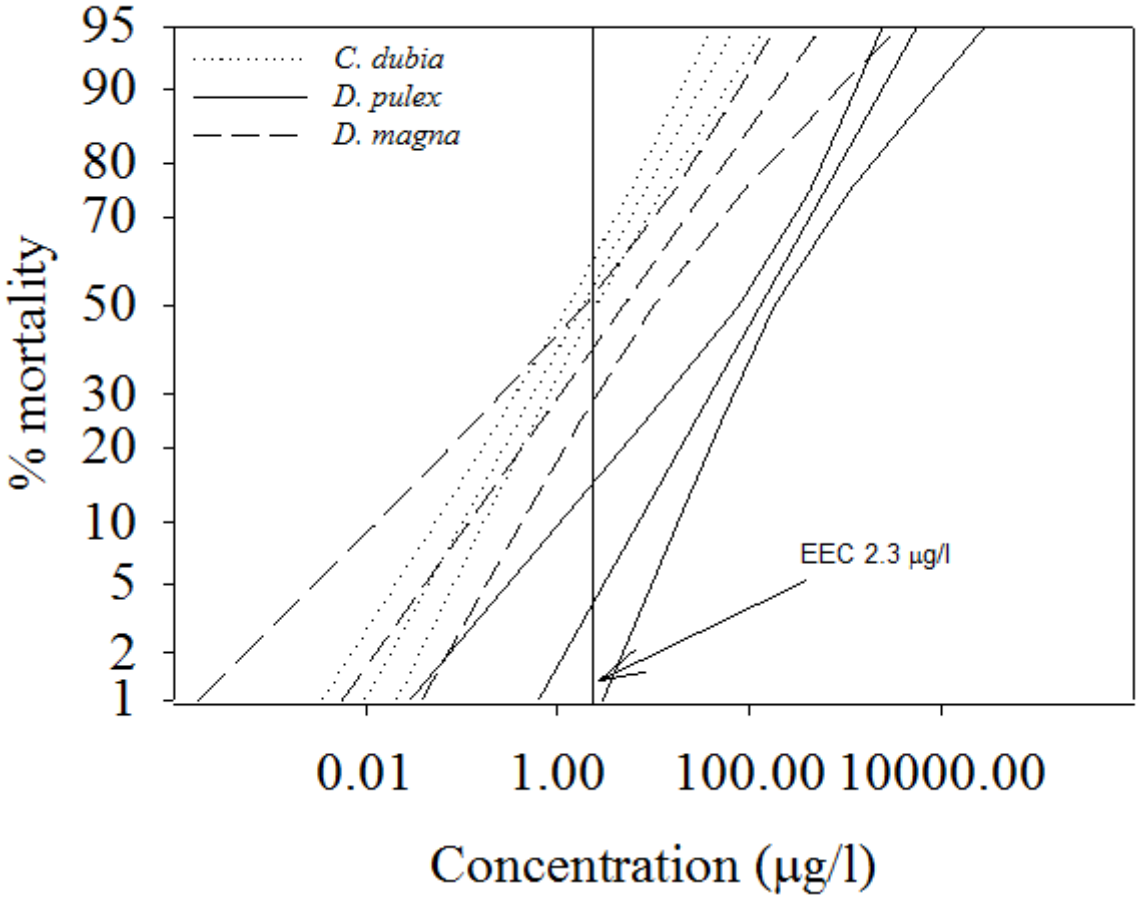
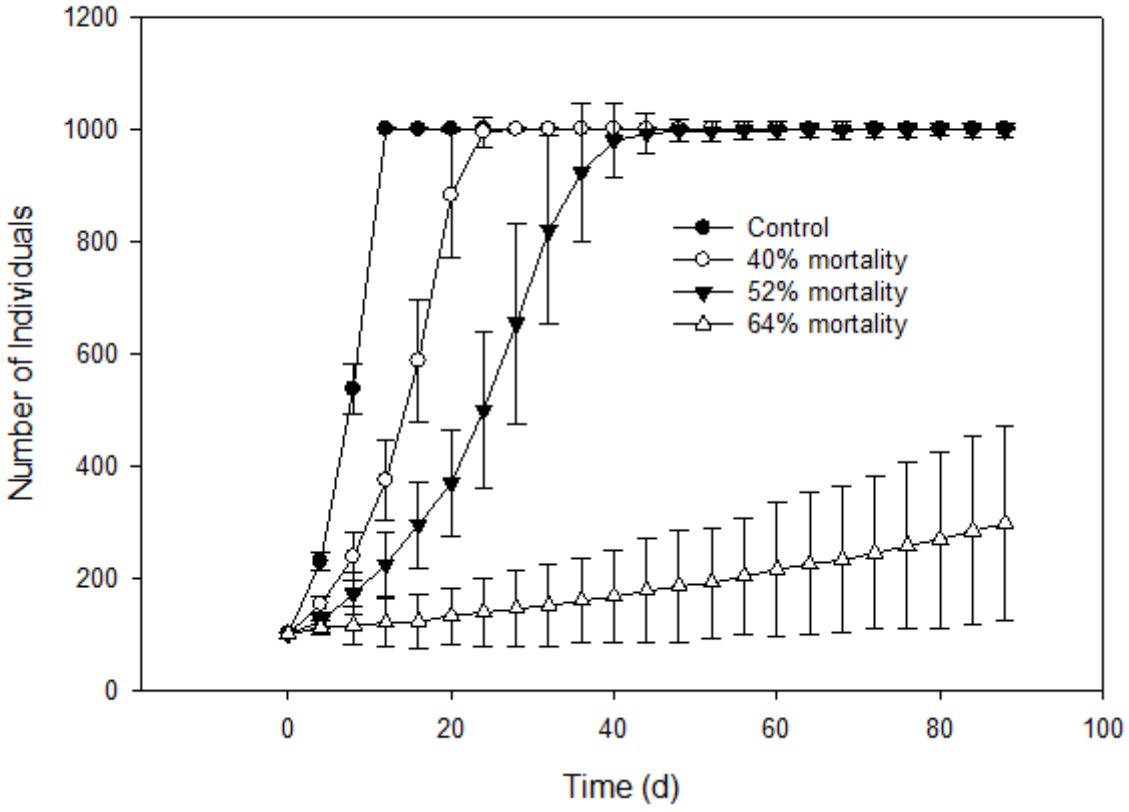


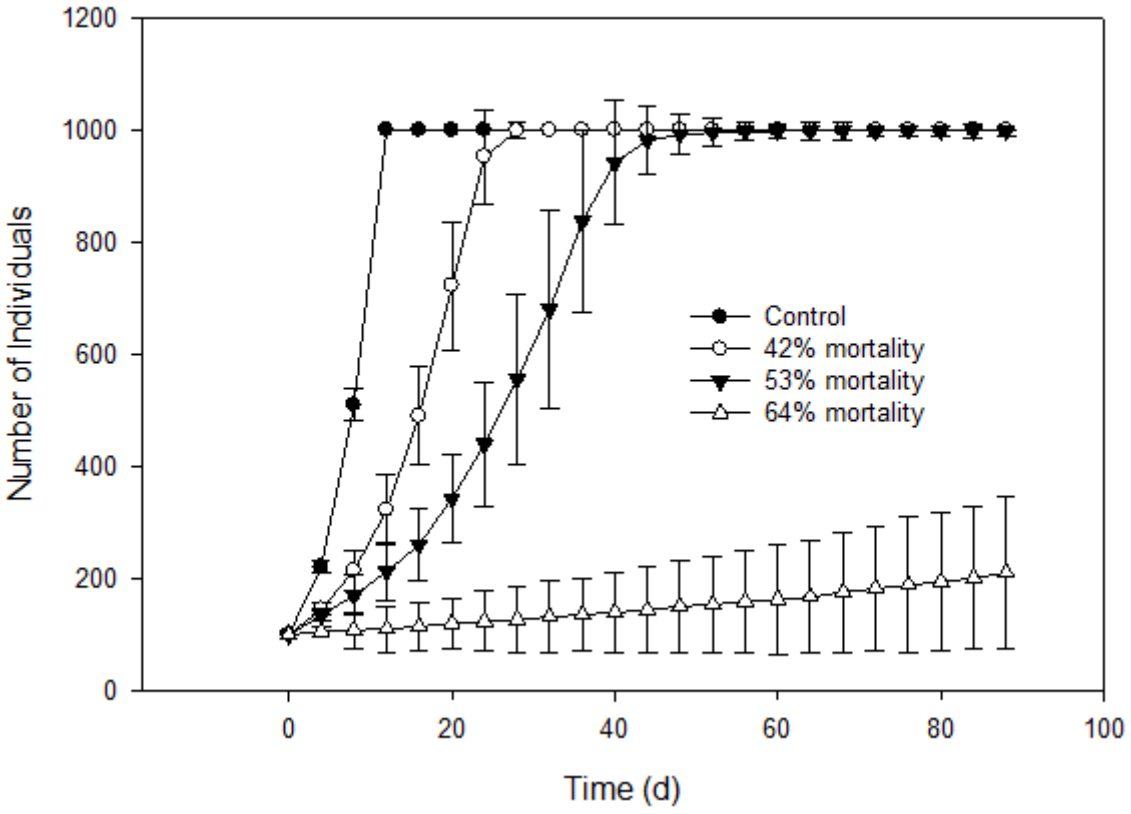

**Figure 2**

Acute concentration-response curves for three Cladoceran species exposed to Spinosad.



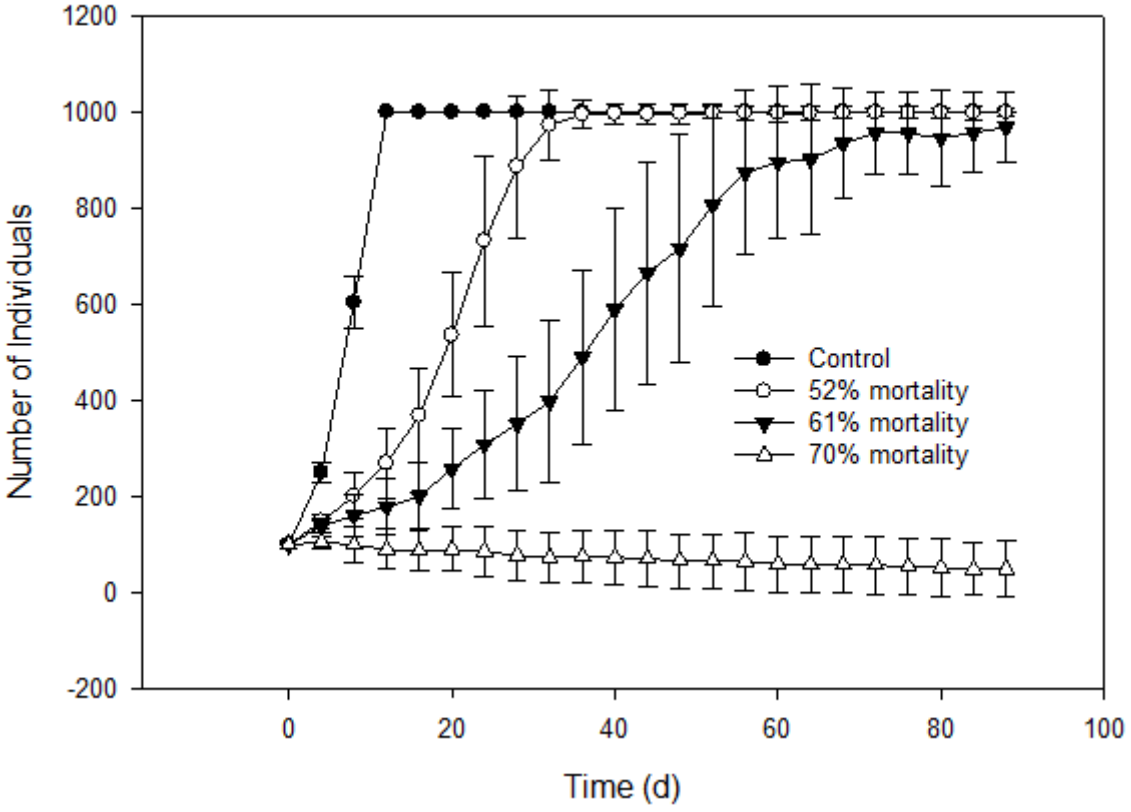

## Figure 3

Population size of C. dubia after imposing mortality values from the spinetoram LC curve, lower and upper 95% CL curves that correspond to exposure to the EEC.



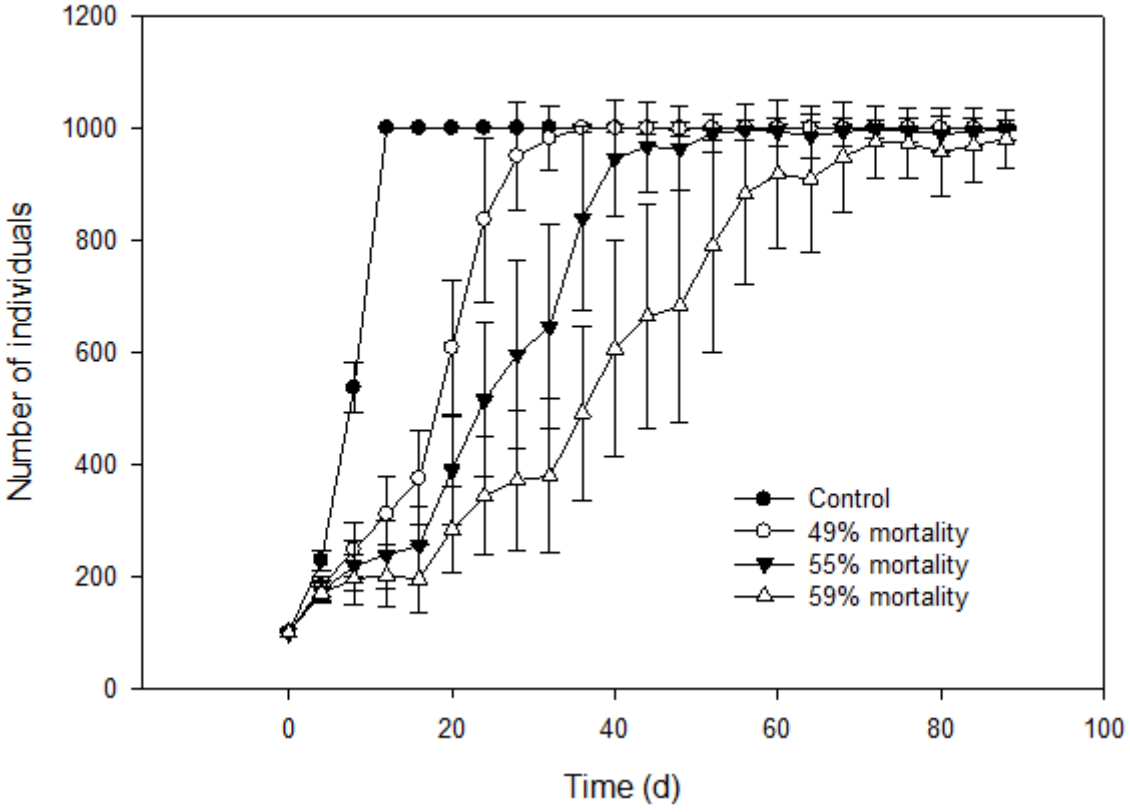

## Figure 4

Population size of D. magna after imposing mortality values from the spinetoram LC curve and the lower and upper 95% CL curves that correspond to exposure to the EEC.



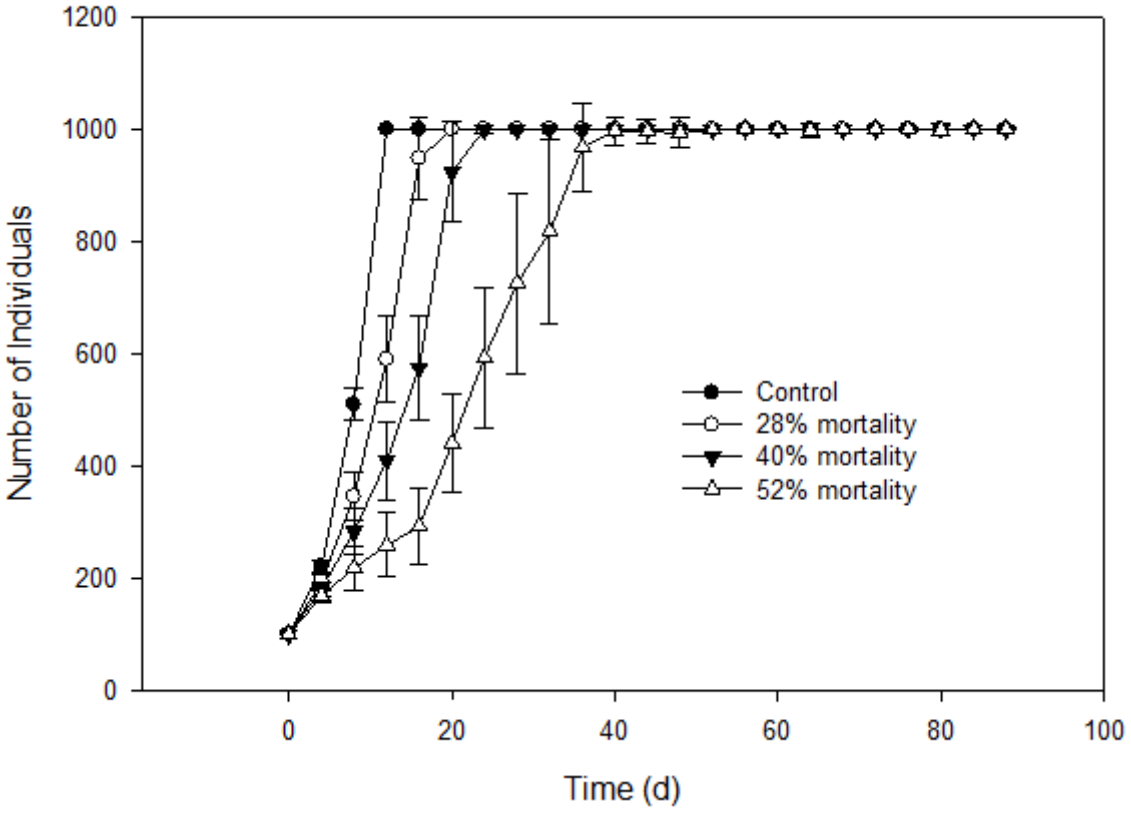

## Figure 5

Population size of D. pulex after imposing mortality values from the spinetoram LC curve, lower and upper 95% CL curves that correspond to exposure to the EEC.




## Figure 6

Population size of C. dubia after imposing mortality values from the spinosad LC curve, lower and upper 95% CL curves that correspond to exposure to the EEC.




## Figure 7

Population size of D. magna after imposing mortality values from the spinosad LC curve, lower and upper 95% CL curves that correspond to exposure to the EEC.

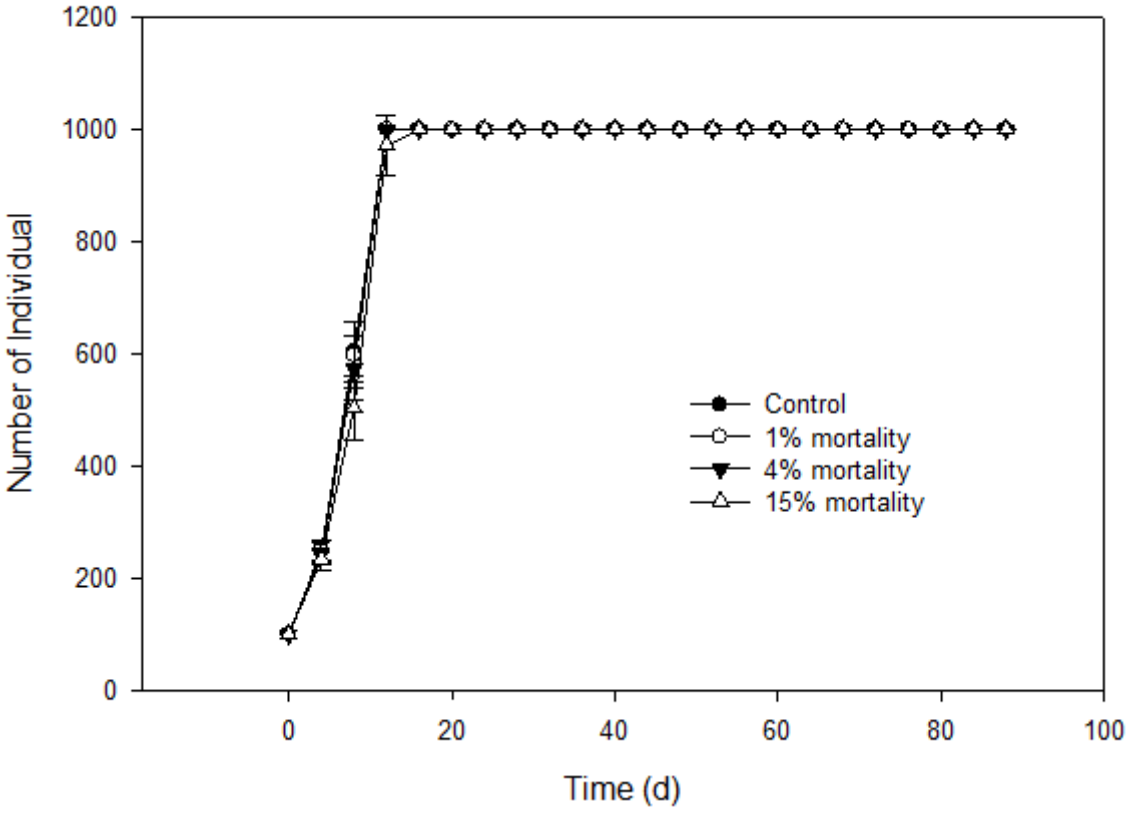



**Figure 8**

Population size of D. pulex after imposing mortality values from the spinosad LC curve, lower and upper 95% CL curves that correspond to exposure to the EEC.