An Acad Bras Cienc (2022) 94(4): e20201234 DOI 10.1590/0001-3765202220201234
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ECOSYSTEMS

Toxic tests show the sensitivity of the *Daphnia similis* (Crustacea, Cladocera) and *Pseudokirchneriella subcapitata* (Chlorophyceae) to commercial pesticides

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Abstract: Pesticides have reached aquatic ecosystems and have caused numerous impacts on organisms. The present study aimed to assess the sensitivity of *Daphnia similis* Straus 1820 and *Pseudokirchneriella subcapitata* (Korshikov) F.Hindák to three commercial pesticides with different active ingredients: Siptran 500SC® (atrazine), Dimilin® (diflubenzuron), and Aproach Prima® (picoxystrobin + cyproconazole). For this purpose, we performed acute toxicity tests on these organisms. The compound most toxic to *D. similis* was the insecticide Dimilin® (toxic up to 5 µg/L of the active ingredient), followed by the fungicide Aproach Prima® (48h-EC50: 47.33 µg of the active ingredients/L) and the herbicide Siptran 500SC® (48h-EC50: 534.69 mg of the active ingredient/L). In contrast, Siptran 500SC® was the most toxic compound (96h-IC50: 52.61 µg/L) to *P. subcapitata*, followed by Aproach Prima® (96h-IC50: 164.73 µg/L) and Dimilin® (non-toxic up to 1 g/L). The toxicity of the fungicide to algae and microcrustaceans demonstrates that compounds developed for certain organisms are able to affect others, indicating the relevance of conducting ecotoxicological tests on different organisms.

Key words: Commercial formulations, pesticides, microcrustaceans, phytoplankton algae.

INTRODUCTION

The increasing use of pesticides in agriculture for the purpose of controlling weeds has led to contamination of surface and groundwater, altering the chemical and physical factors of the environment and is considered an imminent risk to freshwater ecosystems (Archambault et al. 2015). Pesticides can have different destinations in the environment depending on the physical and chemical properties of the products, quantity and frequency of use, application methods, biotic and abiotic conditions of the environment, and meteorological conditions (Mahmood et al. 2016, Ramakrishnan et al. 2019, Barizon et al. 2020). They can reach aquatic environments through runoff derived from the areas where they are applied; disposal of the remnants of formulation in water; washing of pulverization equipment; accidental splashes in wells, tanks, water tanks, fountains, streams, rivers, and lakes; direct application of pesticides into water for the control of larvae and insects, snails, and excess aquatic vegetation; disposal of municipal and industrial sewage; inappropriate disposal of used packages; and dust deposition or precipitation of lost pesticides into the atmosphere during application (Silva & Santos 2007, Albuquerque et al. 2016, Kim et al. 2017).

Atrazine is an herbicide belonging to the chemical group triazines, members of which inhibit photosynthesis by blocking the electron transfer early in the process (Singh et al. 2018, Takeshita et al. 2018). It is one of the most extensively applied pesticides, worldwide (Ralston-Hooper et al. 2009, Sharma et al. 2019) and is the main herbicide detected in samples of groundwater and surface waters (Pinheiro et al. 2010, Coelho et al. 2012, Dias et al. 2018, Caldas et al. 2019, de Albuquerque et al. 2020). Diflubenzuron is an insecticide belonging to the benzoylurea chemical group, which acts by interfering in chitin synthesis (Merzendorfer 2013, Grigoraki et al. 2017), is highly toxic to insects and crustaceans, affecting the growth of these organisms (Grigoraki et al 2017, Sun et al. 2015).

Picoxystrobin and cyproconazole are fungicides belonging to the chemical groups, strobilurins, and triazoles, respectively. They act by inhibiting cellular respiration and synthesis of ergosterol, a steroid required for the synthesis of fungal cell walls (Farm Chemicals International 2011, Cao et al. 2019, Zhang et al. 2020). Cyproconazole also may affect non-target organisms because the sterol is expressed in several groups, from bacteria to mammals (Zarn et al. 2003). Picoxystrobin has only been in the market since 2001 and has been commercialized in formulations with other fungicides, such as picoxystrobin, cyproconazole, cyprodinil, and chlorothalonil. Therefore, few toxicological studies on picoxystrobin or its mixtures have been reported in the scientific literature (Hume 2001, Farm Chemicals International 2011, Domingues et al. 2017, Li et al. 2018).

Freshwater microcrustaceans such as *Daphnia* and *Ceriodaphnia* (Cladocera) are organisms that are mostly used in experimental analysis because of their high sensitivity to several types of contaminants, easy cultivation and manipulation, short life cycle, high fertility, and parthenogenetic reproduction (Knie & Lopes 2004, Ferreira et al. 2008, Bownik 2017). The microcrustaceans also function as important links in the food chain, in this work they are represented by the *Daphnia* genus, that can be found in the pelagic zones of lakes (Rizo et al. 2017), which results in the organisms being exposed to toxic substances present in both the water column and the substrate (Munawar et al. 1999, Leoni 2017).

Additionally, algae have proved to be sensitive to several contaminants, such as herbicides, insecticides, fungicides, metals, and industrial organic substances (Guida et al. 2008, Pereira et al. 2009, Sáenz et al. 2012, Baker et al. 2016, Bi et al. 2018). *Pseudokirchneriella subcapitata* (Korshikov) F.Hindák (Guiry 2021) is a unicellular freshwater green alga, which belongs to the class Chlorophyceae, family Selenastraceae, and is most often used in ecotoxicological tests (Aruoja et al. 2011). Generally, it is employed in routine assays because it reproduces asexually by self-spores, producing small replicas of the mother cell; it undergoes uniform division and does not bind to surfaces (advantageous characteristics for the tests), has moderate sensitivity to several substances, and is easy to cultivate (Ansilago et al. 2016, Yamagishi et al. 2017). *Daphnia similis* (Claus 1876), is a Cladocera species, which belongs to the class Branchiopoda, family Daphniidae, and is most often used in ecotoxicological tests (Maziero et al. 2020). This family is often used to evaluate the acute and chronic toxicity of chemical agents, because it presents high sensitivity to aquatic contaminants, in addition to its easy laboratory cultivation and its parthenogenetic reproduction, which allows obtaining organisms for the tests, with constant sensitivity (Buratini et al. 2004).

Considering the progressive increase in the application and the impacts that of pesticides in ecosystems this study aimed to evaluate the sensitivity of *Daphnia similis* and

Pseudokirchneriella subcapitata to atrazine, diflubenzuron, and picoxystrobin + cyproconazole commercial by performing acute through toxicity tests.

MATERIALS AND METHODS

Pesticides

Three commercial formulations were used for the toxicity tests: Aproach Prima®, Siptran 500SC®, and Dimilin®. Aproach Prima® is a fungicide in concentrated suspension, containing 20% picoxystrobin and 8% cyproconazole. Siptran 500SC® is an herbicide in concentrated suspension, containing 50% atrazine, and Dimilin® is a wettable powder insecticide, containing 25% diflubenzuron in its formulation. Other characteristics of each of the pesticides and their toxicological and environmental hazard classifications according to Brazilian regulations (ANVISA 2014, IBAMA 2014) are presented in Table I.

Culture and tests with microcrustaceans

The individuals used in the tests (*D. similis*) were cultivated in the Laboratory of Ecophysiology of Aquatic Organisms of the Centre of Hidric Resources and Applied Ecology (CRHEA) at Escola de Engenharia de São Carlos (Engineering School of São Carlos), Universidade de São Paulo - USP (University of São Paulo), according to Brazilian Association of Technical Standards (ABNT 2004).

The organisms were cultivated in glass vessels (2L) in an acclimatized room at 20±2 °C with a 16-h light/8-h dark photoperiod. ASTM medium was used in the culture water, with a pH ranging 7.0–7.6, total hardness ranging from 40–48 mg calcium carbonate (CaCO3)/L, and conductivity of up to 180 µS/cm. The individuals were fed daily with an algal suspension (*P.*

Table I. General characteristics of the pesticides tested, indicated crops, mode of application and toxicological and environmental hazard classifications.

Trade name	Aproach Prima®	Siptran 500SC®	Dimilin®
Class	Fungicide	Herbicide	Insecticide
Type of formulation	Concentrated suspension	Concentrated suspension	Wettable powder
Active Ingredients	Picoxystrobin and Cyproconazole	Atrazine	Diflubenzuron
Chemical group	Strobilurin and Triazole	Triazine	Benzoylurea
Active ingredients concentration	200g/L and 80g/L	500g/L	250g/Kg
% of active ingredients in the formulation	20% and 8% (28%)	50%	25%
Crops indicated	Soy	Corn and Sugar cane	Cotton, citrus, corn, soy, tomato and wheat
Mode of application	Terrestrial/aerial	Terrestrial/aerial	Terrestrial/aerial
Toxicological classification (ANVISA 2014)	Moderately toxic (Class III)	Moderately toxic (Class III)	Extremely toxic (Class I)
Environmental Hazard classification (IBAMA 2014)	Very dangerous to the environment (Class II)	´Dangerous to the environment (Class III)	Dangerous to the environment (Class III)

subcapitata) at a concentration of 106 cells/mL and a suspension compounded by a fermented ration of fish and yeast at a concentration of 1 mL/L of water culture (ABNT 2004).

The algae used as food were cultivated in an LC Oligo culture medium at 25 ± 2°C, with luminous intensity greater than 4500 lux, constant light photoperiod, and constant aeration; on reaching the exponential growth phase, the algae were stored under refrigeration (ABNT 2004).

Preliminary tests with *D. similis* were conducted using decimal dilutions to obtain the concentration range of substance to which the species was sensitive. Based on the preliminary results, definitive assays were carried out using 5-8 dilutions per pesticide, with five replicates for each dilution series. The immobility was observed in the dilutions and controls, and the 48h-EC50 (average effective concentration that caused immobility in 50% of the organisms after a 48-h exposure under the testing conditions) and 95% confidence intervals were calculated. In addition, the average sensitivity, its standard deviation and coefficient of variation, as well as the sensitivity range of the five replicates of each dilution series were calculated.

Both the preliminary and definitive tests were carried out with neonates aged 6-24 h. Five organisms were added to each beaker containing 10 mL of the dilution, and four replicates were established for each dilution and control. The tests were conducted under the same conditions of temperature and photoperiod as the cultures and lasted 48 h, during which the organisms were not fed. The control was set using culture water (ABNT 2004).

The conductivity, pH, dissolved oxygen (YSI oximeter model 55), and hardness were measured at the beginning and end of the tests in order to verify whether they were within the acceptable limits (ABNT 2004). For this purpose, an Orion conductivity meter model 145, a Micronal potentiometer model B374, and a YSY oximeter model 55 were used, and titrations were conducted using EDTA.

Culture and tests with algae

Pseudokirchneriella subcapitata was also obtained through culture belonging to University of São Paulo (USP), according to the (ABNT 2005).

The algae were cultivated in an LC Oligo culture medium in 250-mL Erlenmeyer flasks at 25 ± 2°C, with a constant light photoperiod, luminous intensity greater than 4500 lux, and agitation ranging from 100 to 175 rpm. On reaching the exponential growth phase, the algae inoculum was stored under refrigeration (4-10°C) in stock culture (ABNT 2005).

Pseudokirchneriella subcapitata was subjected to preliminary and definitive tests using organisms in the exponential growth phase obtained from stock culture. On the day of the tests, a stock culture was taken and counted to set the concentration of cells to be added to each trial solution. An algal suspension was prepared with a density of 10,000 cells/mL, established by counting in a Neubauer chamber (ABNT 2005).

Three replicates for each dilution and control were carried out with the analyzed products, and the final volumes of the solutions were 100 mL. The control consisted exclusively of the LC Oligo culture medium. The 96-h tests were conducted under the same conditions of temperature, luminous intensity, photoperiod, and agitation as the cultures (ABNT 2005). To ensure that the test conditions were homogenous, daily rotation of the positions of Erlenmeyer flask on the shaking table was done.

Algal reproduction was ascertained by counting the number of cells in the Neubauer chamber. Additionally, the pH was measured for each treatment and the inhibitory concentration

of 50% algal growth during 96-h exposure (96 h-IC50) was calculated. The pH was obtained using a Micronal potentiometer model B374.

Sensitivity tests

Daphnia similis and *P. subcapitata* were subjected to sensitivity testing using potassium chloride (KCl) and sodium chloride (NaCl), respectively, as the reference substances, to verify the culture quality and homogeneity along the generations as well as to test the repeatability and validation of the definitive tests. For these purposes, the immobility of microcrustaceans and inhibition of algal reproduction at each concentration and the control were observed, with four replicates of the former and three of the latter. The tests were carried out under the same conditions as the culture. The control for *D. similis* consisted of the water used in the cultures, and, for *P. subcapitata*, it consisted of the LC Oligo culture medium (ABNT 2004, 2005).

The 48h-EC50 and 96h-IC50 values for the microcrustaceans and algae, respectively, were calculated and compared with the mean values presented in laboratory control charts (ABNT 2004, 2005).

Data analysis

The 48h-EC50 of the treatments and sensitivity test for *D. similis* were calculated according to the trimmed Spearman-Karber method ($p < 0.05$) using the Trimmed Spearman-Karber Method program, version 1.5 (Hamilton et al. 1977). The sensitivity range was obtained by adding or subtracting twice the value of the standard deviation from the average (± 2SD; Zagatto 2006). The 96h-IC50 of *P. subcapitata* was calculated by linear interpolation using the ICp program, version 2.0 (Norberg-King 1993).

RESULTS

The sensitivity of *D. similis* to KCl and *P. subcapitata* to NaCl during the test period was determined to range from 440.09 to 639.72 mg/L and from 4.65 to 5.22 g/L, respectively. Because these ranges were within the interval of ± 2 standard deviations of the values presented in the laboratory control charts, all the definitive tests were validated.

The fungicide Aproach Prima® was more toxic to *D. similis* than the herbicide Siptran 500SC® (Tables II, III, and IV). The average 48h-EC50 for the cladocerans was 47.33 µg of the active ingredients/L or 168.98 nL of the commercial fungicide (based on the nominal concentration). The sensitivity range of Aproach Prima® was established as 29.12-65.54 µg/L, with a coefficient of variation of 19.23%, which is a satisfactory result (at ≤ 30%; Environment Canada 1990) (Table II). For the herbicide Siptran 500SC®, the 48h-EC50 average value for *D. similis* was 534.79 mg of the active ingredient/L or 1.06 mL of the formulation (based on the nominal concentration) (Tables III and IV). The herbicide sensitivity was 305.52-763.87 mg/L, with a coefficient of variation of 21.43%. (Table III).

The microcrustaceans were even more sensitive to the insecticide Dimilin®; immobility was observed with all the tested concentrations of the commercial formulation used in the study (up to 5 ppb of the active ingredient). Thus, the 48h-EC50 of the product could not be calculated because of the absence of a linear relationship between the substance concentration and the response of the organisms.

The values of 96h-IC50 for *P. subcapitata* were 52.61 µg/L (or 63.15 nL of the formulation) and 164.73 µg/L (or 329.47 nL) for the herbicide Siptran 500SC® and fungicide Aproach Prima®, respectively. The insecticide Dimilin® was not toxic to the algae up to 1 g/L (Table IV).

Table II. 48h-EC50 values for *D. similis* obtained in the tests with the fungicide Aproach Prima®.

Assays	48h-EC50 (µg/L)	Confidence Interval (95%)
1	57.21	47.87 – 68.39
2	43.58	38.19 – 49.73
3	56.02	48.85 – 64.27
4	44.08	34.35 – 56.57
5	35.77	28.87 – 44.16
Average	47.33	-
Standard-Deviation	9.10	-
Coefficient of Variation	19.23%	-

Table III. 48h-EC50 values for *D. similis* obtained in the tests with the herbicide Siptran 500SC®.

Assays	48h-EC50 (mg/L)	Confidence Interval (95%)
1	499.99	315.09 – 793.41
2	546.25	435.11 – 685.78
3	358.41	249.33 – 515.22
4	644.8	548.12 – 758.53
5	624.04	532.96 – 730.68
Average	534.69	-
Standard-Deviation	114.58	-
Coefficient of Variation	21.43%	-
Sensitivity Range	305.52 – 763.87	-

Table IV. 48h-EC50 and 96h-IC50 of three commercial pesticides to *D. similis* and *P. subcapitata*.

		Aproach Prima®	Siptran 500SC®	Dimilin®
Daphnia similis	48h-EC50 (Average) Active ingredients	47.33 µg/L	534.69 mg/L	-
	48h-EC50 (Average) Formulation	168.98 nL	1.06 ml	-
Pseudokirchneriella subcapitata	96h-IC50 Active ingredients	164.73 µg/L (130.97 – 179.39)	52.61 µg/L (45.11 – 59.33)	No toxic up to 1 g/L
	96h-IC50 Formulation	329.47 nL (274.87 – 364.13)	63.15 nL (55.59 – 74.65)	-

DISCUSSION

Among the studies on the effects of fungicides on ecosystems, changes in the structure of the zooplankton and algae community are the most frequently found responses. These changes in fungicide-treated ecosystems have been associated with both direct toxicity and side effects, such as interspecific interactions (Elskus 2014). Studies on the active ingredients of the fungicide Aproach Prima® (picoxystrobin and cyproconazole) have not been conducted, but several studies with *D. magna* and substances belonging to the same chemical groups were reported, like Azoxystrobin, Pyraclostrobin, Propiconazole, Atrazine and others (NCBI 2013, Kunz et al. 2017, Lu et al. 2017, 2019, Wang et al. 2020). A great variation can be observed in the sensitivity of cladocerans to the compounds tested, with values comparable to those obtained in this study and those reported for strobilurins. It is largely recognized that substances from the same chemical group act similarly. In this context, studies on similar compounds investigating sub-lethal effects can indicate the physiological attributes that could be affected by the product. Studies on azoxystrobin, for instance, reported an increase in *D. magna* respiratory activity after a 25-min exposure to 0.026 µg/L of the compound (Warming et al. 2009). Friberg-Jensen et al. (2010) noted decreased activities of heart, mandible, and filtering limb due to an exposure to 0.5-2 mg/L azoxystrobin for 1440 minutes or 24 h. Elskus (2014) found that the fungicide Azoxystrobin has some physiological effects on aquatic invertebrates, such as changes in the structure of the zooplankton community, swimming daphnids, fecundity, respiration and heart rate.

Although cyproconazole is present in the study at a lower concentration (80 g/L) than picoxystrobin (200 g/L) in the Aproach Prima® formulation, greater emphasis has been given to the compounds that inhibit ergosterol biosynthesis. This can because of their supposed ability to affect the activity of the enzymes responsible for the oxidative detoxification of a series of pesticides in vertebrates, invertebrates and algae (Thies et al. 1996, Thompson 1996, Saraiva et al. 2018). Even knowing that the concentration of cyproconazole found in this work was low, we cannot rule out the ecological risk that this fungicide presents due to its use close to aquatic environments, so the evaluation of toxicity in aquatic invertebrates is of great importance (Saraiva et al. 2018).

For atrazine the most effective concentrations are from the tests on analytical-grade substances (Perez et al. 2011). However, a wide variation in the results in studies on commercial formulations of the mentioned substances was found. It is important to consider that formulations contain other herbicides in addition to atrazine, such as metolachlor and cyanazine, with the exception of Atrazine 500®. A comparison between the value obtained for Siptran 500SC® and the others studies indicates a great variation in the results, which is probably associated with different grades of purity of the active ingredient and other substances present in the commercial formulations.

It is also important to emphasize that during the tests conducted with *D. similis* and the pesticide Siptran 500SC®, at higher concentrations, a film was observed around the antennae and filtering limbs of the test organisms. Slow movements of the filtering limbs indicated that the microcrustaceans were alive but were unable to move actively inside the trial container. At the highest concentration, the pesticide was deposited over the organisms and covered them, as a result of which visualization was conducted exclusively through active search. Thus, the exposure of these organisms to the

studied formulation demonstrates an evident physical effect on them.

Despite the known toxic effects of the isolated atrazine on non-target species (He et al. 2012, Khan et al. 2016), other factors should be considered when dealing with commercial products. These substances are known as adjuvants and can function as solubilizers and emulsifiers in the product mixture. These components can make the mixture more homogenous and adherent to the surface of the plant of interest, allowing the active ingredient to be more soluble, facilitating access to its target location, or even increasing its performance (Calow 1993). In this context, other substances in Siptran 500SC® might have contributed to the formation of the film around the antennae and filtering limbs and the consequent immobility of *D. similis* at higher concentrations because there are no reports of such occurrences in studies on pure atrazine. Therefore, it becomes clearly important to consider these effects, because, once in the environment, these products could interfere with the survival of organisms by inhibiting their movements, food filtration (zooplankton), and ability to escape their predators.

The 48h-EC50 values of 6.7 µg/L (Brock et al. 2006) and 7.1 µg/L (Tomlin 1994) were reported for the exposure of *D. magna* to analytical grade diflubenzuron, the active ingredient of the insecticide Dimilin® (Supplementary Material tables SI and SII). The concentrations tested in this study, up to 5 ppb or 5 µg/L, were inconclusive with respect to the immobility of the organisms and the treatments employed.

The fungicide Aproach Prima® (picoxystrobin + cyproconazole) was able to significantly affect the growth of *P. subcapitata*, despite being designed to inhibit fungal growth in several crops. A relevant point about pesticides is their ability to affect non-target species (i.e., those that should not be killed or damaged). An ideal pesticide should exclusively affect its target species and should immediately be degraded into non-toxic, elementary constituents (Calow 1993). However, other coexistent species are also affected in the environment due to the non-selective nature of these compounds (Calow 1993, Vieira 2010, Galic et al. 2019).

Several studies have already demonstrated the effects of fungicides on freshwater biodiversity (measured as taxonomic richness), resulting in the mortality of zooplankton, algae and macrophytes. Normally the zooplankton and algae present short generation time, which allows a quick recovery due to the use of fungicides, so it is important to consider the temporal dynamics and the recovery process in response to anthropogenic stresses. In addition to reducing biodiversity, fungicides can cause changes in ecosystem functions, such as changes in primary productivity, dissolved oxygen level, water clarity and light availability (Cuppen et al. 2000, McMahon et al. 2012).

When considering experimental studies that seek to evaluate the effects of atrazine on *P. subcapitata*, there is still no consensus in the literature. Fairchild et al. (1998) and Heever & Grobbelaar (1996) reported 96h-IC50 and 72h-IC50 values of 117 and 359 µg/L, respectively, for *P. subcapitata*. On the other hand, Caux et al. (1996) and Weiner et al. (2004) reported 96h-IC50 values of 26 and 48.77 µg/L for *P. subcapitata*, respectively, which is within the range observed in this study (96h-IC50: 52.61 µg/L). However, we must consider that atrazine presents toxic effects in several groups of aquatic organisms, from algae, affecting photosynthesis and development, to reptiles, affecting the immune system and development. For more details, see De Albuquerque et al. 2020.

For diflubenzuron (Dimilin®), no data are available on *P. subcapitata*; however, its

effects on other species of Chlorophyceae have been reported. Yu-yun et al. (1993) reported no significant differences in the growth of *Scenedesmus subspicatus* Chodat relative to the control when it was exposed to 200 µg/L of diflubenzuron. The results of the study reveal that diflubenzuron has no apparent effect on the growth of *P. subcapitata* up to a concentration of 1 g/L, despite its extreme toxicity (Toxicological Class I) (ANVISA 2014). Although analytical-grade diflubenzuron is almost water-insoluble (EPA 1997), the tested formulation contains other substances in its composition that are responsible for improving the commercial product by making it more soluble or by increasing the performance of the active ingredient. Therefore, the specificity could explain the low toxicity observed, because it acts by inhibiting the chitin polysaccharide synthesis, unlike other insecticides that act by modulating and blocking the ion flow through cell membranes (Gallo 2002, Larini 1999).

The inability of the insecticide to interfere with the growth of *P. subcapitata* and its extreme toxicity to *D. similis*, as observed in the present study, reveal that organisms exhibit differential sensitivity to compounds. Under a more realistic perspective (i.e., the presence of Dimilin® in an aquatic ecosystem), it could affect the survival of organisms (short-term effect) and/or lead to an imbalance in the growth and development of zooplankton and phytoplankton communities (long-term effect). In an experimental study developed by Farlow (1976), a highly significant reduction was observed in the aquatic organism populations after applications of Dimilin with a rate of 28 gm Al/ha when compared with untreated population. These findings indicate the relevance of conducting tests with species and distinct trophic levels to establish appropriate concentrations of pesticides for use in the field, because organisms respond differently when exposed to the pesticides.

The results obtained in the present study demonstrate that even compounds developed for certain species can affect others, such as in the case of the fungicide Aproach Prima®. The toxicity of the compound to *P. subcapitata* and *D. similis* can be attributed to the presence of picoxystrobin in its formulation, which acts as an inhibitor of cellular respiration, because the other active ingredient (cyproconazole) inhibits the ergosterol biosynthesis in fungi. Presumably, similar mechanisms of action and consequent toxicity of pesticides to species other than those of interest are caused by the metabolic pathways and processes that they share with other organisms, such as those involved in the transport of substances through membranes and cellular respiration, in addition to enzymes and hormones performing similar functions. Nevertheless, many mechanisms of action remain unknown, mainly for the compounds that somehow operate during a specific life stage of an organism. Therefore, we recommend that more studies be carried out with different aquatic organisms, mainly for phytoplankton algae that are part of the base of the aquatic food web.

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SUPPLEMENTARY MATERIAL

Table SI. 48h-EC50 values reported in the scientific literature to *D. magna* and other species.

Table SII. 96h-IC50 and 72h- IC50 values reported in the scientific literature to *P. subcapitata* and other species.

How to cite

KÜHL AM, ESPÍNDOLA ELG, MANTOVANO T, DEOSTI S & LANSAC-TÔHA FA. 2022. Toxic tests show the sensitivity of the *Daphnia similis* (Crustacea, Cladocera) and *Pseudokirchneriella subcapitata* (Chlorophyceae) to commercial pesticides. An Acad Bras Cienc 94: e20201234. DOI 10.1590/0001-3765202220201234.

Manuscript received on July 22, 2020;
accepted for publication on June 04, 2021

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