Acute and Chronic Toxicity of Prothioconazole and its Metabolite Prothioconazole-Desthio To *Daphnia Magna*

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Abstract

Prothioconazole (PTC) is a broad-spectrum triazole fungicide. Current research has mainly focused on its efficacy and residues, with few studies on its toxicological effects. This study assessed the effects of PTC, and its metabolite prothioconazole-desthio (PTCd), on the inhibition of activity, growth, and reproduction of *Daphnia magna* using acute and chronic toxicity tests. Additionally, the dose-response relationship was established to determine sensitive biological indicators. The acute toxicity test shows that the 48 h EC₅₀ of PTC and PTCd to *D. magna* were 2.82 and 5.19 mg/L, respectively. The chronic toxicity of PTC and PTCd to *D. magna* were 0.00860 and 0.132 mg/L, respectively, with the parent compound being 15.3 times more toxic than its metabolite. The acute to chronic toxicity ratio (ACR) was calculated using chronic toxicity data, with ACR values of 227 and 27.5 for PTC and PTCd, respectively. These results indicate that both PTC and PTCd affect the growth and reproduction of *D. magna*, and the toxicity of the parent compound is greater than that of its metabolite. In conclusion, the metabolites of this pesticide have sufficient toxicity to harm *D. magna* at relevant environmental concentrations, and their environmental risk should not be neglected.

1. Introduction

Pesticides, and other aquatic ecosystem pollutants, widely exist in water, causing serious environmental problems. Pesticides in the aquatic environment can directly harm *Daphnia*, fish, algae, and other aquatic organisms, invariably affecting human health through consumption of the polluted water or via the aquatic food chain. Therefore, it is necessary to evaluate the effects of pesticides on aquatic organisms.

Daphnia magna is a representative aquatic zooplankton and an important intermediate connecting the aquatic biological chain. It has a critical role in purifying water, and its primary production directly affects the structure and function of aquatic ecosystems. Growth and activity inhibitions are the initial responses of *D. magna* to environmental perturbations and can be used as a sensitive toxicity indicator. Hence, *D. magna* has gained global attention due to its significant role in aquatic ecosystems and is an important research subject, particularly in aquatic toxicology (Hu et al. 2018; Liu et al. 2018).

Prothioconazole (PTC), a triazole fungicide that inhibits demethylation of sterol precursors 14-lanosterol or 24-methylene, is an effective agent in controlling various plant diseases. Launched in 2004, PTC is currently registered and sold in over 60 countries (Sjerps et al. 2019). It has become one of the world's best-selling fungicides (Zhang et al. 2018), with global sales exceeding US\$800 million in 2014 (Zhang et al. 2020). PTC usually transforms gradually into prothioconazole-desthio (PTCd) (Xie et al. 2019; Haas and Justus 2004; Hellpointner and Borchers 2004), which is a major metabolite in photolysis and plant metabolism and has certain teratogenicity (Hoaip et al. 2011; Hasan et al. 2019). Therefore, it is critical to evaluate the effects of both PTC and PTCd. Most existing studies on PTC are related to PTC residues in soil, fruits, vegetables, wheat, and animal products (Deb et al. 2010; Paul et al. 2019), with few studies reporting PTC toxicity on aquatic organisms. In this study, acute and chronic toxicity tests of PTC and PTCd on *D. magna* were conducted to provide a theoretical basis for the evaluation of their effects. The results can also provide fundamental data for the assessment of the aquatic environmental risk of PTC and offer a scientific basis for the protection of aquatic ecosystems.

2. Materials And Methods

2.1 Reagents and instruments

D. magna was fed *Pseudokirchneriella subcapitata* and was cultured and propagated in Elendt M4 base in the laboratory (Zhejiang Academy of Agricultural Sciences). Healthy, non-first-born *Daphnia* with parthenogenetic reproduction of more than three generations, and individuals aged < 24 h were selected for the toxicity tests.

PTC (98.0%, CAS 178928-70-6) and PTCd (98.0%, CAS 120983-64-4) were provided by Dr. Ehrenstorfer GmbH and Sigma-Aldrich, respectively. The following reagents were used: Acetonitrile (Merck & Co.), formic acid (Anaqua™ Chemicals Supply), and acetone (Yonghua Chemical Technology [Jiangsu] Co. Ltd.). The following instruments were used for the analysis: Exion LCAD-Triple Quad™ 5500 (ABSciex Co. U.S.), an ACQUITY UPLCTM BEH C₁₈ column (2.1 mm × 100 mm × 1.7 μm) (Waters™ Corporation, U.S.), and a light incubator (Sanyo MLR-350HT, SANYO Electric CO. Japan).

2.2. Test method

2.2.1 Acute toxicity test

The acute toxicity test was conducted according to the updated OECD guideline 202 for *Daphnia* sp. Acute Immobilization Test (OECD 2006). Test solutions were prepared with Elendt M4 base based on the pre-test. In this study, PTC test concentrations were 2.21, 2.65, 3.1, 3.82, 4.58, and 5.50 mg/L, and those of PTCd were 3.23, 3.88, 4.64, 5.56, 6.67, and 8.00 mg/L. In addition, the blank and solvent control groups were set.

The "semi-static method" was employed for the PTC test, and the test solution was changed 24 h after the test. The "static method" was employed for the PTCd test, and the test solution remained the same throughout the test period. The test solution was first filtered using 0.45 μm membrane filters, and a 50 mL solution was added to the crystallizing dish (60 mm diameter). Five test *Daphnia* species were added to each dish and then placed into the light incubator. Each treatment group and the control group had four replicates, with one dish for each replicate. The experimental period was 48 h, and no bait was added during this period. The condition for PTC was darkness throughout, and 16 h light and 8 h darkness for PTCd.

During the acute toxicity test, PTC samples were subjected to concentration analysis at the beginning of the test (0 h) and 24 h before the test solution was changed. PTCd was sampled at the beginning and end of the experiment for concentration analysis.

2.2.2 Chronic toxicity test

The 21-day chronic toxicity test was conducted based on the updated OECD guideline 211 for *Daphnia* sp. (OECD 1998). According to the acute toxicity test results of PTC and PTCd to *D. magna*, PTC test concentrations were 0.0125, 0.0250, 0.0500, 0.100, 0.200, and 0.400 mg/L, and those of PTCd were 0.0315, 0.0630, 0.126, 0.250, 0.500, 0.500, and 1.00 mg/L. The blank and solvent control groups were set. Thirteen replicates (crystallizing dishes) were set in each test group, each containing 50 mL test solution and one parent *Daphnia*. The light condition was 16 h light and 8 h darkness, and the test period was 21 days. The PTC test solution was changed every 24 h, whereas that of PTCd was changed every three days. Samples were taken irregularly throughout the experiment (see Table 4 for sampling times) to estimate the actual concentrations of PTC and PTCd in the test solution.

During the experiment, the appearance and survival of the parent *Daphnia* were observed daily, newborn *Daphnia* species were removed on time, and the number of living offspring was calculated. Furthermore, the mortality of parent *Daphnia* and the number of broods per parent *Daphnia* were recorded. At the end of the experiment, the body length of all surviving parent *Daphnia* was also measured.

2.3 Concentration analysis

2.3.1 Sample pretreatment

Approximately 5 mL of the test solution sample was obtained and diluted to 10 mL with acetonitrile (constant volume) and vortexed for 30 s. Then, it was filtered with a 0.22 µm membrane filter or diluted with acetonitrile:water (1:1, v/v) and filtered with a 0.22 µm membrane filter for analysis.

2.3.2 Test conditions

The Exion LCAD-Triple Quad™ 5500 was used for high-performance liquid chromatography (HPLC), with the following conditions: ACQUITY UPLCTM BEH C₁₈ column (2.1 mm × 100 mm × 1.7 µm), gradient elution with 0.2% acetic acid + 5 mmol/L ammonium acetate solution and acetonitrile, and flow velocity: 30 mL/min. The retention times of PTC and PTCd were approximately 3.2 and 2.8 min, respectively.

The mass spectrum conditions were as follows: capillary voltage, 5.5 kV; air curtain gas, 35 psi; collision gas, 7 psi; spray gas, 50 psi; auxiliary heating gas, 50 psi; ion source temperature, 550°C; collision cell entrance voltage, 10 V; and collision cell outlet voltage, 16 V. The qualitative and quantitative modes involved multiple reaction monitoring, and an electrospray ionization source operated in the positive mode. The other mass spectrometric analysis parameters are shown in Table 1.

Table 1 Mass spectrometric analysis parameters for prothioconazole (PTC) and prothioconazole-desthio (PTCd).					
Replicate	Parent ion	Daughter ion	Dwell time (ms)	Declustering potential (V)	Collision energy (V)
PTC	343.7 (343.7)	326.0 (154.2)	100 (100)	100 (100)	12 (30)
PTCd	312.2 (312.2)	70.0 (125.2)	100 (100)	140 (140)	38 (47)
Note: The numbers in () are qualitative ions.					

2.4 Statistical analysis

2.4.1 Acute toxicity test

SPSS 16.0 (IBM, USA) was used for regression analysis to establish a “dose-response” linear relationship and calculate EC₅₀ (concentration for 50% of maximal effect) and 95% confidence intervals (CI), based on the measured concentration of the test solution and inhibition rate of *D. magna* activity for each treatment group.

2.4.2 Chronic toxicity test

SPSS was used to perform one way ANOVA and Williams tests, based on the measured concentration of test solution and various indicators (toxicity data of the time of the first presenting offspring, number of broods per parent *Daphnia*, total number of living offspring produced by each parent, and body length of each surviving *Daphnia*). *P* < 0.05 indicated a significant difference.

3. Results And Discussion

3.1 Pesticide stability and method accuracy

The stability of PTC and PTCd in the Elendt M4 medium is shown in Table 2. The results show that within a certain concentration range (0.0005–100 mg/L), the quantitative ion peak area of PTC and PTCd in the blank medium (Elendt M4) had a good linear relationship with the injection volume and concentration, and the correlation coefficients were > 0.9990.

The accuracy of the method was evaluated by adding PTC and PTCd in the blank medium (Elendt M4). Consequently, the average recoveries of PTC and PTCd were 93.8–97.8% and 97.8–102%, respectively. Hence, this test met the average recovery requirement of 70–120% (OECD 2006).

Table 2
Regression equation limits of quantity and average recovery of prothioconazole (PTC) and prothioconazole-desthio (PTCd) in the Elendt M4 medium.

Replicate	Regression equation	Correlation coefficient	Quantity limit (mg/L)	Added concentration (mg/L)	Average recovery (%)
PTC	$y = 29113876x - 11345$	0.9999	0.00200	0.00200, 20.0	93.8–97.8
PTCd	$y = 9004111x - 450$	0.9999	0.00200	0.00200, 20.0	97.8–102

3.2 Concentration analysis of test solution

During the acute toxicity test of PTC and PTCd, water samples were collected at the beginning of the test (0 h) and 24 h before the test solution was changed at the end of the test (48 h) for concentration analysis. The corresponding results are presented in Table 3. The measured concentration of PTC at 24 h before the test solution replacement was 53.3–72.4% of the measured concentration at 0 h, indicating that degradation occurred in this study environment (Xie et al. 2019). However, the measured concentration of PTCd at 48 h was 95.9–102% of the measured concentration at 0 h, and the concentration variation was < 20%, indicating a relatively stable test period. The geometric mean values of the measured concentration of PTC at 0 h and 24 h (before test solution replacement) were calculated based on the test guidelines of environmental safety assessment for chemical pesticides (OECD 2006). In addition, the geometric mean values of the measured concentrations of PTCd at 0 h and 48 h were also calculated. These mean values were used to measure the acute toxicity of PTC and PTCd to *D. magna*.

Table 3
Analysis of prothioconazole (PTC) and prothioconazole-desthio (PTCd) concentrations in the acute toxicity test.

Replicate	Theoretical concentration (mg/L)	Measured concentration at 0 h (mg/L)	Measured concentration at 24 h (mg/L)	Measured concentration (mg/L)	Ratio of 24 h concentration by 0 h concentration (%)
PTC	5.50	4.89	3.22	3.97	65.8
	4.58	3.52	2.21	2.79	62.8
	3.82	3.60	1.92	2.63	53.3
	3.18	2.88	1.58	2.13	54.9
	2.65	2.21	1.60	1.88	72.4
	2.21	2.05	1.23	1.59	60.0
Replicate	Theoretical concentration (mg/L)	Measured concentration at 0 h (mg/L)	Measured concentration at 48 h (mg/L)	Measured concentration (mg/L)	Ratio of 48 h concentration by 0 h concentration (%)
PTCd	8.00	7.00	7.10	7.05	101
	6.67	6.23	6.33	6.28	102
	5.56	5.44	5.34	5.39	98.2
	4.64	4.31	4.21	4.26	97.7
	3.88	3.66	3.54	3.60	96.7
	3.23	3.18	3.05	3.11	95.9
Note: The measured concentration is the geometric mean value of the measured concentrations at 0 h and 24 h/48 h before the replacement of test solutions.					

The PTC and PTCd concentrations during the chronic toxicity test were analyzed, and the results are presented in Table 4. Table 4 shows that the variation between the PTC concentration in the test solution and the initial determined concentration is $> \pm 20\%$ throughout the test period. According to OECD 211 (OECD 1998), considering the long-term effect of PTC on *D. magna*, the reproductive toxicity test concentration of PTC can be represented as the time-weighted arithmetic mean value of measured concentration during the test. Table 4 indicates that the PTCd concentration in the test solution remained at $> \pm 20\%$ of the initial determined concentration throughout the test period. Therefore, the reproductive toxicity test concentration of PTCd can be represented as the geometric mean value of the measured concentration (OECD 1998).

Table 4
Chronic toxicity test results of prothioconazole (PTC) and prothioconazole-desthio (PTCd).

Chronic toxicity test results of protonoconazole (PTC) and protonoconazole deslino (PTCd).													
Theoretical concentration	PTC measured concentration (mg/L)												
(mg/L)	0 d	1 d old	1 d new	2 d old	2 d new	3 d old	3 d new	4 d old	9 d new	10 d old	15 d new	16 d old	20 d new
0.400	0.462	0.352	0.348	0.331	0.383	0.266	0.414	0.273	0.337	0.288	0.400	0.273	0.37
0.200	0.220	0.147	0.163	0.125	0.179	0.1019	0.206	0.125	0.160	0.107	0.171	0.113	0.16
0.100	0.111	0.0747	0.0845	0.0503	0.1065	0.0568	0.103	0.0626	0.0894	0.0626	0.099	0.0546	0.09
0.0500	0.0576	0.0353	0.0470	0.0279	0.0628	0.0295	0.0558	0.0305	0.0457	0.0277	0.0514	0.0279	0.05
0.0250	0.0267	0.0161	0.0211	0.0116	0.0264	0.0113	0.0250	0.0154	0.0215	0.0123	0.0221	0.0117	0.02
0.0125	0.0127	0.00591	0.00970	0.00470	0.0133	0.00559	0.0120	0.00677	0.00981	0.00592	0.0101	0.00609	0.01
Theoretical concentration	PTCd measured concentration (mg/L)												
(mg/L)	0 d	3 d old		9 d new		12 d old		18 d new		21 d			
1.00	0.923	0.916		0.951		1.020		1.150		0.951			
0.500	0.486	0.492		0.502		0.512		0.513		0.492			
0.250	0.266	0.356		0.256		0.230		0.263		0.256			
0.126	0.129	0.123		0.130		0.155		0.130		0.125			
0.0630	0.0629	0.0588		0.0631		0.0582		0.0645		0.0644			
0.0315	0.0320	0.0312		0.0315		0.0296		0.0333		0.0326			
Note: ^a The measured concentration is the time-weighted average concentration of PTC/PTCd in the test solution.													

3.3 Toxicity to *D. magna*

3.3.1 Acute toxicity

Pesticide metabolites are critical in evaluating the ecological risk of pesticides (Wang et al. 2016). *D. magna* activity inhibition test can indicate the degree of triazole fungicide pollution in the aquatic ecosystem (Cassani et al. 2013).

In the acute toxicity activity inhibition test, *D. magna* in the blank and solvent control groups grew normally without any mortality. At 48 h exposure to PTC concentrations of 1.59, 1.88, 2.13, 2.63, 2.79, and 3.97 mg/L, *D. magna* activity was inhibited by 10, 20, 30, 40, 55, and 75%, respectively. At PTCd concentrations of 3.11, 3.60, 4.26, 5.39, 6.28, and 7.05 mg/L, *D. magna* activity was inhibited by 15, 25, 30, 50, 60, and 80%, respectively. Therefore, there was a dose-response relationship, with increasing exposure concentrations causing increased inhibition of *D. magna* activity. Table 5 shows that the 24 h and 48 h EC₅₀ of PTC to *D. magna* were 3.11 mg/L (95% CI 2.91–3.32 mg/L) and 2.82 mg/L (95% CI 2.69–2.95 mg/L), respectively, while those of PTCd to *D. magna* were 5.93 mg/L (95% CI 5.47–6.44 mg/L) and 5.19 mg/L (95% CI 4.89–5.51 mg/L), respectively. Zhai et al. (2019) revealed that the 48 h EC₅₀ of PTC to *D. magna* was 2.68 mg/L, which is consistent with the results of the present study. Sinclair and Boxall (2003) observed that the toxicity of 70% of degradation products was almost equal to, or less than, that of the parent compound. In this study, PTC, and its metabolite PTCd, were found to have similar toxicities. Although triazole fungicides have similar structures, they have significantly different acute inhibition of *D. magna* activity (Guo et al. 2009). Additionally, their residues in the environment may have harmful effects on humans (Tröskén et al. 2006; Tröskén et al. 2004); therefore, it is necessary to assess the chronic toxicity of triazole fungicides to *D. magna*.

Table 5
The 24 h and 48 h EC₅₀ values of prothioconazole (PTC) and its metabolite prothioconazole-desthio (PTCd) to *Daphnia magna*.

Replicate	Exposure (h)	Toxicity regression equations	Correlation coefficient	48 h-EC ₅₀ (mg/L)	95% confidence intervals (mg/L)
PTC	24	$y = 3.0717 + 4.0225x$	0.9859	3.11	2.91–3.32
	48	$y = 2.8012 + 4.8901x$	0.9906	2.82	2.69–2.95
PTCd	24	$y = 1.4294 + 4.6176x$	0.9755	5.93	5.47–6.44
	48	$y = 1.5509 + 4.8208x$	0.9808	5.19	4.89–5.51
Note: EC ₅₀ is the concentration for 50% of maximal effect.					

3.3.2 Chronic toxicity

Growth and development status, as well as reproductive capacity, are sensitive indicators to evaluate the chronic toxicity of pesticides to *D. magna*. Furthermore, these indicators are key parameters to evaluate the population's growth capacity (Kim et al. 2012). The chronic toxicity test showed that the mortality of *Daphnia* in the blank and solvent control groups was 0. Moreover, no significant differences were observed in the toxicity data of the time of the first presenting offspring, the number of broods per parent *Daphnia*, the total number of living offspring produced by each parent, or the body length of each surviving *Daphnia* ($P > 0.05$). Therefore, the blank and solvent control groups were combined (combination control) and used to analyze the difference between each treatment group. The results are presented in Table 6.

There was no significant difference between the 0.00860 mg/L PTC treatment and the combination control groups (Table 6). However, with increasing PTC concentrations, the total number of juveniles produced by each surviving parent *Daphnia* decreased significantly. Moreover, the number of broods per parent *Daphnia*, and the body length of each surviving parent were inhibited to varying degrees. Particularly, in the maximum concentration group (0.341 mg/L), the time of the first presenting offspring was significantly inhibited. Additionally, the number of broods per parent *Daphnia*, total number of living offspring produced by each surviving parent, and body length of each surviving parent were significantly reduced, and the reproductive capacity was seriously inhibited. Using the body length of each surviving parent as the toxicity index, the no observed effect concentration (NOEC) of PTC to *D. magna* at 21 days was 0.00860 mg/L, and the lowest observed effect concentration (LOEC) was 0.0180 mg/L.

Table 6 shows that there was no significant difference between PTCd treatments at concentrations of 0.0317–0.132 mg/L and the combination control group. However, at a PTCd concentration of 0.982 mg/L, a significant difference was observed in the time of the first presenting offspring ($P < 0.05$). The number of broods per parent *Daphnia* and the body length of each surviving parent were significantly inhibited when the concentration was 0.499–0.982 mg/L ($P < 0.05$). Moreover, when the concentration was 0.269–0.982 mg/L, the total number of living offspring produced by each surviving *Daphnia* was significantly reduced ($P < 0.05$). Using the total number of living offspring produced by each surviving *Daphnia* as the toxicity index, the NOEC of PTC to *D. magna* at 21 days was 0.132 mg/L, and the LOEC was 0.269 mg/L.

There was a 15.3-fold difference between the NOEC of PTC and PTCd at 21 days in *D. magna*, and the parent compound was more toxic than its metabolites. Therefore, the long-term chronic threat of PTC to aquatic organisms should not be ignored. Furthermore, Cassani et al. (2013) proposed that triadimenol could lead to a higher abnormality rate in the *D. magna* offspring, resulting in a decreased and rare population of *D. magna*. Therefore, the environmental risks caused by widely used triazole fungicides should be assessed.

3.4 Acute-chronic toxicity ratio

Chronic toxicity tests require a long time period, complex test conditions, and significant manpower and material resources. Hence, fixed factor methods have been developed to evaluate the chronic effects of harmful substances in the environment, such as the acute-chronic toxicity ratio (ACR). According to previous studies (Rodriguez et al. 2006; Yuan 2012), the ACR can predict the safety concentration when chronic toxicity data regarding environmental risk assessments are lacking. Table 7 presents the NOEC and LOEC of PTC and PTCd to *D. magna* based on four biological indexes, including the time of the first presenting offspring, number of broods per parent *Daphnia*, total number of living offspring, and body length of the living parent. The application factors (AFs) and ACR of PTC and PTCd to *D. magna* were determined in combination with the acute and chronic toxicity test results. In Table 7, $AF = ChV/48 \text{ h EC}_{50}$, where ChV represents the chronic value, which is the geometric mean of NOEC and LOEC. ACR_1 was calculated by $48 \text{ h EC}_{50}/ChV$. Meanwhile, ACR_2 ($ACR_2 = 48 \text{ h EC}_{50}/48 \text{ h EC}_{10}$), calculated using acute toxicity EC_{10} , was compared with ACR_1 . The AFs of PTC and PTCd were 0.00441 and 0.0363, respectively, which had one order of magnitude difference. Kuhn et al. (1989) measured the NOEC and 24 h EC_{50} of 73 environmental pollutants to *D. magna* and compared their AF values. Some AF values varied by three orders of magnitude, whereas some values varied by 5–9 fold.

Rodriguez et al. (2006) calculated the ACR by dividing the acute toxicity 48 h EC_{50} by chronic toxicity data. Yuan (2012) calculated the ACR by replacing chronic toxicity data with acute toxicity EC_{10} . The ACR_1 values obtained from chronic toxicity data for PTC and PTCd in this study were 227 and 27.5, respectively. The ACR_2 values calculated from the acute toxicity data EC_{10} for PTC and PTCd were 1.83 and 1.84, respectively. Thus, ACR_1 is closer to the recommended value of 100 (Ahlers et al. 2006), thereby indicating that ACR_1 is a more suitable ACR. In this study, the ACR_1 of PTC was 124 times higher than ACR_2 , and ACR_1 of PTCd was 15 times higher than ACR_2 . This establishes that replacing chronic toxicity data NOEC with acute toxicity data EC_{10} might have

a significant impact on ACR in environmental risk assessment. Therefore, when chronic toxicity data are lacking, ACR should be cautiously used to predict the safe concentration.

Table 6
Summary of all endpoints observed in the chronic test of prothioconazole (PTC) and prothioconazole-desthio (PTCd) to *Daphnia magna*.

Replicate	Measured concentration (mg/L)	Number of parent <i>Daphnia</i>	Mortality of parent <i>Daphnia</i>	Time of the first presenting offspring (d)	Number of brood per parent <i>Daphnia</i>	Total number of living offspring	Body length of living parent <i>Daphnia</i> (mm)
PTC	0.341	13	0	9.8 ± 1.8*	6.08 ± 1.04*	127 ± 23.5*	3.034 ± 0.091*
	0.155	13	1	6.5 ± 0.5	7.15 ± 1.41*	172 ± 26.2*	3.165 ± 0.129*
	0.0807	13	1	6.5 ± 0.9	6.85 ± 2.15*	212 ± 43.4*	3.306 ± 0.132*
	0.0405	13	0	6.0 ± 0	8.00 ± 0.58*	355 ± 29.4*	3.351 ± 0.104*
	0.0180	13	1	6.0 ± 0	8.31 ± 2.53	337 ± 38.9	3.393 ± 0.100*
	0.00860	13	0	6.1 ± 0.1	8.85 ± 0.55	382 ± 31.3	3.477 ± 0.186
	Combination control	26	0	6.3 ± 0.5	9.08 ± 0.74	374 ± 23.9	3.505 ± 0.098
	21d-LOEC	–	–	0.341	0.0405	0.0405	0.0180
	21d-NOEC	–	–	0.155	0.0180	0.0180	0.00860
PTCd	0.982	13	0	7.1 ± 1.2*	7.0 ± 2.1*	250 ± 30.4*	3.751 ± 0.131*
	0.499	13	0	6.4 ± 0.3	7.8 ± 1.8*	300 ± 40.9*	3.998 ± 0.267*
	0.269	13	0	6.6 ± 0.9	8.8 ± 0.8	339 ± 61.3*	4.155 ± 0.114
	0.132	13	0	6.1 ± 0.1	8.2 ± 1.2	366 ± 47.6	4.170 ± 0.112
	0.0619	13	0	6.3 ± 0.5	8.5 ± 1.0	380 ± 37.5	4.231 ± 0.068
	0.0317	13	0	6.0 ± 0	9.1 ± 1.8	374 ± 36.8	4.123 ± 0.212
	Combination control	26	0	6.1 ± 0.1	8.7 ± 0.6	382 ± 46.2	4.240 ± 0.110
	21 d-LOEC	–	–	0.982	0.499	0.269	0.499
	21 d-NOEC	–	–	0.499	0.269	0.132	0.269
Note: The measured concentration is the time-weighted average concentration of PTC/PTCd in the test solution. Combination control represents the combination of the blank and solvent controls. * The difference between the combined control group and the treatment group was significant at 5%. Parent <i>Daphnia</i> that died accidentally were excluded from the results. LOEC: lowest observed effect concentration; NOEC: no observed effect concentration.							

Table 7
Acute and chronic ratio of prothioconazole (PTC) and prothioconazole-desthio (PTCd) to *Daphnia magna*.

Replicate	NOEC	LOEC	48 h-EC ₅₀	48 h-EC ₁₀	ChV	AF	ACR ₁	ACR ₂
PTC	0.00860	0.0180	2.82	1.54	0.0124	0.00441	227	1.83
PTCd	0.132	0.269	5.19	2.82	0.188	0.0363	27.5	1.84
Note: NOEC: no observed effect concentration; LOEC: lowest observed effect concentration; ChV: the geometric mean of NOEC and LOEC; EC ₁₀ : concentration for 10% of maximal effect; EC ₅₀ : concentration for 50% of maximal effect; ACR: acute and chronic ratio.								

In conclusion, these results show that PTC, and its metabolite PTCd, have acute and chronic toxicity to *D. magna*. In the natural environment, the exposure of *D. magna* to the concentrations analyzed in this study resulted in a decreasing and rare population of *D. magna*. Therefore, the reproductive and developmental toxicity of PTC to *D. magna* should be further studied. Moreover, the environmental risk caused by other triazole fungicides should also be assessed.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

in this section

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lui Xinju, Jiang Jinhua, Wang Feidi, Lv Lu, Li Gang, Wu Shenggan, and Zhao Xueping. The first draft of the manuscript was written by An Xuehua and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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