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Predicted no-effect concentration (PNEC) and assessment of risk for the fungicide, triadimefon based on reproductive fitness of aquatic organisms

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Abstract: Triadimefon, a broad-spectrum, systemic fungicide used to protect agricultural crops is popular in China. However, sub-lethal effects of triadimefon on aquatic organisms remained poorly understood, and its risks to aquatic organisms were unclear. In the current study, thresholds for chronic toxicity to five aquatic organisms were determined and a PNEC based on reproductive fitness of nine aquatic organisms was derived through use of a species sensitivity distribution (SSD). NOECs, based on reproduction or inhabitation of growth, for *Oryzias latipes*, *Daphnia magna*, *Brachionus calyciflorus*, *Heterocypris incongruens* and *Soirodela polyrhiza* were 5, 25, 80, 320 and 500 μg L^{-1}, respectively, and the final PNEC derived was 3.66 μg L^{-1}. A screening-level hazard assessment of surface water based on both measured environment concentrations (ND~5.22 μg L^{-1}) in 3 lakes, 2 reservoirs and 1 river and predicted environment concentrations (0.36~65μg L^{-1}) in a simulated river and pond, identified unacceptable hazard to aquatic organisms posed by triadimefon, with maximum hazard quotients (HQs) of 1.43 and 17.8, respectively. Potential deleterious effects and hazards or risks of exposure of aquatic organisms from current patterns of use of triadimefon in surface water if of concern. Since HQs were relatively small and the benefits large, it is suggested that mitigations be applied to allow use while minimizing potential for adverse effects on aqutic organisms.

Key words: Pesticides; Reproduction; Species sensitivity distribution; Ecological risk assessment; Water Quality Criteria

1. Introduction

Triadimefon (1-(4-chlorophenoxy)-3, 3-dimethyl-1-(1, 2, 4-triazol-1-l) butan-2-one; CAS 43121-43-3) is a broad-spectrum, systemic fungicide, belonging to the triazole family, that is used to control rust and mildew on fruits, row crops and ornamental plants. Triadimefon has a solubility of 64 mg L^{-1} in water at 20 □, but the limit of solubility of its transformation product, 1,2,4-triazole is 1250 g L^{-1}. Residues of triadimefon can be desorbed from soil and transported by rain to surface waters or groundwater. This potential mobility coupled with possible detrimental effects on aquatic organisms following spray drift or runoff from surfaces has led to concerns about widespread use of this fungicide. In water, triadimefon is degraded by photolysis with a half-life of 7.6 hours, but in anaerobic aquatic environments, it is relatively stable to hydrolysis with a half-life of 217 d (USEPA, 2006). Triadimefon has been previously detected in water in the USA at concentrations as great as 922 μg L^{-1} (Watschke et al., 1999). By use of the exposure analysis model, PRZM-EXAMS (the Pesticide Root Zone Model and Exposure Analysis Modeling System), an environmental concentration of triadimefon in surface waters was predicted to be 4.1~100.8 μg L^{-1} with the use scenario of 2 applications of 2.7 lbs ai A^{-1} for golf courses and sod farms (USEPA, 2006). With adjustment of the national policy and agricultural planting structure, usage of triadimefon in China has been increasing annually (Wang, 2015). Concentrations of triadimefon in rivers in China ranged from 0.00152 to 5.22 μg L^{-1} (Liu et al, 2017; Wei et al, 2016; Liu et al, 2015; Lu, 2016). Considering increasing use of triadimefon as a fungicide in agriculture, and potential for it to reach surface waters, further assessment of risks of triadimefon to aquatic ecosystems of China was deemed necessary.

Acording to the Chinese pesticide toxicity classification standard, triadimefon

exhibits little acute toxicity to vertebrates, and therefore has been classed as a fungicide of lower toxic potency (Shao et al, 2002), and concentrations of triadimefon are restricted to 5000 μg L^{-1} by Chinese wastewater effluent standards (GB 21523-2008) (SBTS, 2008). During a 96-hr test, with *Lemna minor*, triadimefon inhibited development of roots and vegetative reproduction with an IC_{50} of 5470 μg L^{-1} (Liu, 2005). Sub-lethal effects of triadimefon have been suggested particularly endocrine disrupting effects, by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO et al, 1986), the World Wildlife Fund (WWF, 2005), and the State of California Environmental Protection Agency Office of Environmental Health Hazard Assessment (OEHHA, 2015). In the Endocrine Disruptor Screening Program (EDSP) Tier l screening assays for 52 pesticides implemented by the US EPA, potential effects of triadimefon on estrogen, androgen and thyroid hormone systems of mammals were evaluated using *in-vitro* and *in-vivo* tests. Observed effects were due to increased metabolic activity in the liver, and generally observed in connection with systemic/overt toxicity (i.e., body weight decreases, skeletal malformations) in wildlife. However, no direct actions on the hypothalamic-pituitary-gonadal (HPG) or hypothalamic-pituitary-thyroidal (HPT) axes were observed (USEPA, 2015), so triadimefon it thought to have no direct interactions with estrogen-, androgen- or thyroid-mediated pathways. Triazole fungicides have been reported to adversely affect early development of freshwater fish (Zhu et al, 2014; Hermsen, 2011). In a 120-day toxicity test with *Danio rerio*, 250 μg triadimefon L^{-1}, adversely affected the number of individuals and caused masculinization of females exposed to 500 μg triadimefon L^{-1} (Liu, 2011). Overall, it can be concluded that long-term exposure of aquatic organisms to triadimefon has potential to disrupt development or reproduction and can cause malformations that

adversely affect fitness and survival of juveniles although the mechanisms are unclear.

Despite data on effects of triadimefon on several fishes, the African clawed frog (*Xenopus laevis*) and duckweed, potential effects of triadimefon on other aquatic organisms remained poorly understood, particularly with regard to reproductive fitness, which most accurately represents variations among populations and species diversity for modulation of endocrine function in aquatic organisms (Jin et al, 2014; Liu et al, 2016). Also, a predicted no-effect concentration (PNEC) had not been derived for triadimefon so that hazard to aquatic organisms was unclear, especially in surface waters of China.

The 1st objective of this study was to investigate potential for sub-lethal effects of triadimefon on various species and then to derive a PNEC based on reproductive fitness of aquatic organisms. Chronic toxicity tests were conducted with five aquatic organisms, including the small fish, Japanese medaka (*Oryzias latipes*), cladoceran water flea (*Daphnia magna*), rotifer (*Brachionus calyciflorus*), ostracod (*Heterocypris incongruens*) and duckweed (*Soirodela polyrhiza*). Then, empirical information on chronic toxicity of triadimefon determined during this study was combined with ecotoxicity data reported in the literature and used to derive a PNEC, by use of species sensitivity distribution (SSD). The 2nd objective was to use the derived PNEC in conjunction with both measured and predicted concentrations in surface waters of China to calculate hazard quotients (HQs) and assess risks based on probabilities of exceedances of concentrations and probabilities of effects on species.

2. Materials and methods

2.1. Test substances and media

Triadimefon of 99.8% purity was purchased from Aladdin®, Shanghai, China.

Acetone was purchased from KangLin Science & Technology Co. Ltd., Beijing, China. Solutions of triadimefon were prepared by mixing the appropriate amount of triadimefon into acetone (<0.1 ml L^{-1}) to form a stock solution. During tests, concentrations of triadimefon in control and test samples were performed on Agilent Series 1290UHPLC-6495QQQ MS.

The test medium for studies with rotifers and ostracoda were prepared with deionized water and reagent grade chemicals in a synthetic water, of moderate hardness, with the following water quality characteristics: hardness = 80 to 100 mg L^{-1} (as $CaCO_3$), pH = 7.6 ± 0.3, total organic carbon (TOC) = 0.017 mg L^{-1}, and saturation dissolution oxygen >80%. The test medium for duckweed was prepared according to Swedish Standards Institute (SIS), with a pH of 7.24±0.16.

2.2. Species and test methods

(1) *O. latipes* reproductive toxicity test

O. latipes, which had been maintained in our laboratory for more than two years were allowed to acclimate under controlled laboratory conditions for two weeks. During the 28-day study of reproduction adult fish were exposed to 1, 5, 10, 25, or 50 μg triadimefon L^{-1}. In addition, controls of dilution water only and solvent controls containing 0.01% acetone were included in the experimental design. Based on OECD method 236 (OECD, 2013a), breeding pairs of medaka (approximately 4 months old) were kept in a flow-through 5 L, glass aquaria, under a photoperiod of 16: 8 h (light: dark) at a constant temperature (25±1 °C), and fed with newly hatched brine shrimp (*Artemia sp.*) twice daily. During the last 4 days, newly-spawned eggs and rates of fertility were determined daily, and then fertilized eggs were placed into dilution medium, which was renewed daily. Rates and times of hatching were recorded at the end of test.

(2) *D. magna* chronic toxicity test

Stock individuals were cloned in the laboratory by raising a single parthenogenic female. Offspring to be used in tests were cultured more than 3 generations in conditions identical to those to be used in tests. The experimental design was based on that of a previous study (Hassold et al, 2009) and contained five test concentrations in a log-bisected scale: 0 (control), 25, 50, 100, 200 and 400 μg L^{-1}. To allow for enumeration of neonates produced from each adult female and to avoid pseudo replication, 10 females were exposed individually to each test concentrations in cups. Forty milliliters of individual test solutions containing various concentrations of triadimefon were prepared with Elendt M4, including green algae (*Scenedesmus obliquus*) with a density of 2.0×10^5~3.0×10^5 cells mL^{-1}. A single juvenile *D. magna*, less than 24 hours old was placed into a 50 mL cup. Based on the OECD 211 (OECD, 2013b) method, all test vessels were kept in a climate controlled chamber at 22±1□, with a photoperiod of 16:8 hours (light: dark), and 16 hours light at an intensity not exceeding 1110 lx ~ 1480 lx. The exposure was a semi-static renewal test, during which test solutions were renewed daily over the duration of 21 days. Frequencies of molting of adults and numbers of *D. magna* in each cup were measured during the 21-d test.

(3) *B. calyciflorus* reproductive toxicity test

Based on procedure 20666 (ISO, 2008), dormant eggs of *B. calyciflorus* were purchased from MicroBioTests Inc. and hatched at (25±1) □ for 16-18 hours with continuous lighting of intensity 3000 lx to 4000 lx. Individual female neonates were subsequently transferred into test wells within 2 hours after hatching from the cyst. Methods for the tests were adapted from those reported previously (Xu, 2015). Test concentrations, based on a log-bisected scale were: 0 (control), 20, 40, 80, 160 and

320 μg L^{-1}. One milliliter of test solution and 10 *B. calyciflorus* neonates were placed into rinsing wells. Individual *B. calyciflorus* were then transferred to 48-well polystyrene plates. The volume used to transfer each *B. calyciflorus* was less than 20 μL) along with 0.9 ml test solution, 0.1 ml *S. obliquus* of 3×10^7~4×10^6 cell ml^{-1}. Plates were kept at 25±1 □ in darkness for 96 hours. Each treatment consisted of eight replicates. After 96 hours, numbers of sexual females carrying resting eggs, asexual females carrying female eggs and all female *B. calyciflorus* (offspring and mothers) for each batch were recorded separately and the proportion of *B. calyciflorus* carrying resting eggs or female eggs was calculated.

(4) *H. incongruens* growth inhibition test

Dormant eggs of *H. incongruens* were purchased from MicroBio Tests Inc., and hatched at 25±1 □ with continuous lighting of intensity of 3000 lx to 4000 lx. The nauplii within 4 hours after cyst hatching were used as test animals. Tests of inhibition of growth were carried out in accordance with the ISO protocol (ISO, 2012). Based on results of pre-experiment pilot studies, the test concentrations used in the definitive tests were: 0 (control), 40, 80, 160, 320 and 640 μg L^{-1}, with a solvent control (0.0064% acetone) conducted at the same time. Tests were conducted in 6-well plates. Each well was filled with 2 mL test solutions, 2 ml *S. obliquus* of 1.5×10^7 cell ml^{-1} and 10 nauplii. Each batch consisted of three replicates. Test plates, which were covered with a piece of parafilm, were placed in an incubator at 25±1□, in darkness, for 6 days. Body lengths of *H. incongruens* were measured under a dissection microscope, with an accuracy of 10 μm inhibition of growth (I) calculated at the end of test. In this assay, two criteria were provided to assess validity of test results: Mean mortality of *H. incongruens* in the control test did not exceed 20% and mean length increments of *H. incongruens* in the control batch were at least 400 μm (Belgis et al, 2003).

(5) *S. polyrhiza* population growth inhibition test

This assay was a 10-day chronic test, conducted with the duckweed *Soirodela polyrhiza*, which is indigenous to China. Specimens of *S. polyrhiza* were obtained from the Chinese Academy of Sciences, and maintained under controlled laboratory conditions for two weeks. The test was conducted according to guideline 221 (OECD, 2006). Test solutions were renewed every three days. Based on previously published results (Liu, 2005) and results of the pilot study, test concentrations were: 0 (control), 0.5, 1.0, 2.0, 4.0, 8.0 mg L^{-1}, with a solvent control (0.10 ml L^{-1}) performed simultaneously. The test was conducted using crystallizing dishes with a diameter of 90 mm in 200 ml of test solutions. Five individual *S. polyrhiza* each with two leaves were added to each dish. Covered dishes were exposed to continuous light (6600 lux) at 24±2□ for 10 d. During the trial, the numbers of leaves were counted every day, and fitted with exponential functions for each concentration. At the end of test, the mean special growth rate (μ) and the percentage growth inhibition (I) calculated (Equations 1 and 2).

$$\mu = \frac{\ln(N_{end}) - \ln(N_{start})}{t} \quad (1)$$

$$I = \frac{(\mu_{control} - \mu_{test})}{\mu_{control}} \times 100\% \quad (2)$$

Valid tests had at least mean, specific growth rate in the control batch of 0.275 (OECD, 2006). The experiment was replicated three times.

2.3. Statistical analysis and generation of SSDs

Results of chronic toxicity tests were tested using SPSS 16. Normality for each concentration was confirmed by use of the Kolmogorov-Smirnov test and homogeneity of variance was confirmed by use of Levine's test. One-way analysis of

variance (ANOVA) followed by Dunnett's multiple comparison tests to determine which concentration produced responses that were different from the control. The NOEC was defined as the greatest test concentration that did not result in a significant effect ($P>0.05$) compared with the control.

The SSD approach is based on the assumption that toxicity data obtained is a subsample of a larger dataset, and single-species data for many species fit to a distribution such as the log-normal or log-logistic. In this study, a log-normal distribution model was fitted to data for effects of triadimefon on aquatic organisms, and the fit of the model was evaluated using the Anderson-Darling test and homogeneity of variance was confirmed by use of Levine's test. The HC_5 (hazardous concentration for 5% species affected) value with 50% confidence was then estimated by use of ETX software packages (ETX 2.0, RIVM) based on methods of Aldenberg (2000). Final PNECs were calculated as the derived HC_5 divided by a factor of 2, which was a qualitatively chosen factor depending on the amount of supporting evidence, such as non-native species data and multi-species data available (Jin et al, 2011; Jin et al, 2015).

2.4. Aquatic environmental exposure and risk assessment

To assess the overall status of triadimefon in aquatic environments of China, data on exposure to triadimefon, expressed as concentrations in surface waters including rivers, lakes, reservoirs and urban rivers were collected from literature published in China, with master's theses and doctoral dissertations included. For statistical analyses, values that were less than the method detection limits (MDL) were replaced with a surrogate value equal to half the MDL. Distributions of concentrations of triadimefon in surface waters of China determined in this study were tested for normality by use of the Kolmogorov-Smirnov test in SPSS Version 22 software

(SPSS Inc., Chicago, Illinois). The statistical summary of the exposure distributions are reported in the Supporting Information (Table S1).

Because measurements of triadimefon in waters of China were limited, predicted environmental concentrations (PECs) of triadimefon in surface waters under agricultural conditions in China were also simulated by use of the PRZM-EXAMS simulation model module within the Pesticide Risk Assessment Exposure Simulation Shell (PRAESS) (Zhao et al, 2012; Zhou et al, 2015), which has been developed by the Nanjing Institute of Environmental Sciences (NIES). PRZM and EXAMS simulate transport, fate, and behavior of triadimefon in soil and rivers or ponds respectively. The models can be parameterized with relevant weather conditions, crop planting areas, amounts of triadimefon applied, soil texture, annual average rainfall, and chemical characteristics of triadimefon (Table S2). The PRZM-EXAMS model was used to simulate four scenarios of combinations of crops, regions of China and receiving aquatic systems. These scenarios were: maize-river and maize-pond in Zhumadian, Henan province, maize-river and maize-pond in Nantong, Jiangsu province. Predicted exposure concentrations (PEC) were estimated as running averages and output for durations of 1, 4, 21, 60, 90 and 365 days (Table S3).

Based on both existing measured environmental concentration (MEC) and PEC in conjunction with sub-lethal toxicities of triadimefon to aquatic organisms, semi-probabilistic assessments based on the hazard quotients (HQs) were conducted and compared.

3. Results and discussion

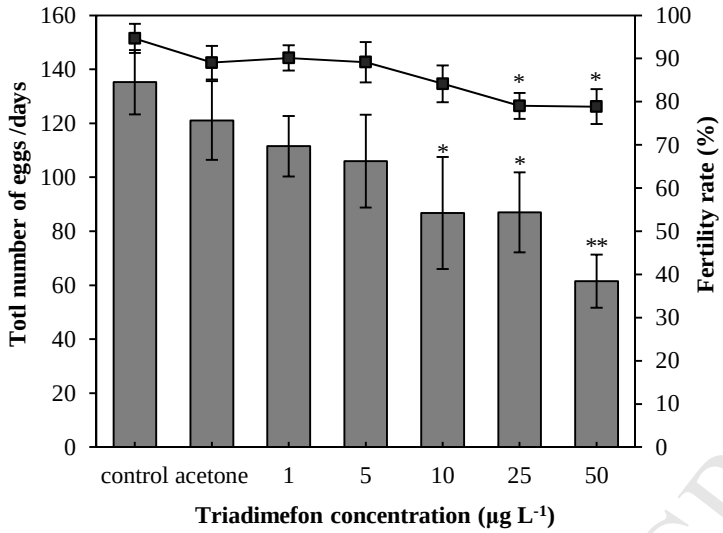
During tests, the measured concentrations of triadimefon were 85%~97% (with means of 89.2%) of nominal concentrations, and no triadimefon was detected in controls. Therefore, results were expressed based on nominal concentrations.

3.1. Sub-lethal toxicity test results of triadimefon

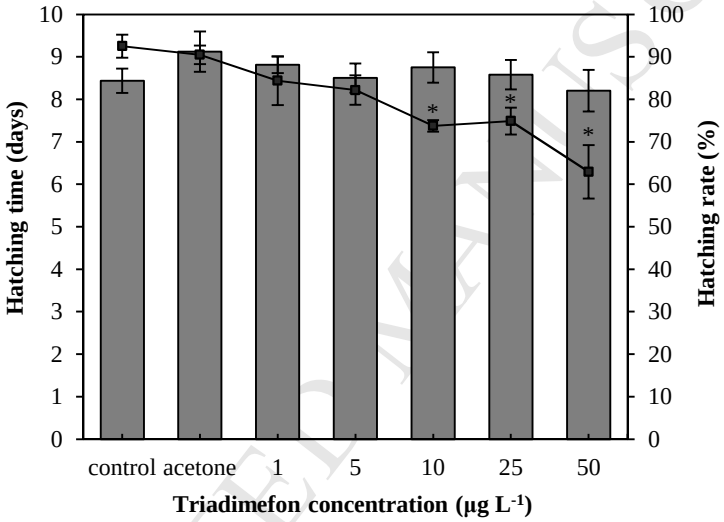
3.1.1 *O. latipes* 28-day reproductive toxicity test

Triadimefon caused statistically significant, dose-dependent, decreases in numbers of eggs produced by medaka. Adult, *O. latipes*, not exposed to triadimefon (controls) produced 135.3 eggs d^{-1} (Fig. 1). For *O. latipes* exposed to 1 or 5 μg triadimefon L^{-1}, no statistically significant effects on numbers of eggs were observed. Exposure to 10 μg or 25 μg L^{-1}, resulted in fewer eggs produced, with means of 86.8 and 87, respectively, which were significantly ($P<0.05$) fewer than the number produced by the controls. Mean number of eggs produced by adults exposed to 50 μg triadimefon L^{-1} was 61.5. Thus, at the greatest concentration tested, fecundity was significantly ($P<0.01$) inhibited by 54.5%. In contrast, fertility was less affected and decreased from 94.6% in eggs from control adults to 79.0% for those exposed to of 25 μg triadimefon L^{-1}. Similarly, significant (Jonckheere-Terpstra; $P<0.05$) lesser 39% at 250 μg triadimefon L^{-1} was observed in a 21-day short-term reproduction assay of triadimefon with *Pimephales promelas* (USEPA, 2015); spawning frequency and fertility rate was also significantly less in individuals exposed to 250 μg triadimefon L^{-1} in a 120-day toxicity test with *Danio rerio* (Liu, 2011) .

Mean proportion of eggs that hatched was also significantly ($P<0.05$) less for adults exposed to 10 μg triadimefon L^{-1}. However, the time taken to hatch was not affected even at 50 μg L^{-1}. Overall fecundity, which included the number of eggs laid as well as the hatching rate, was the most sensitive endpoint, from which to derive the NOEC, which was determined to be 5 μg triadimefon L^{-1}. To shorten processing time and make toxicity testing easier, fecundity is suggested to be a suitable endpoint to assess reproductive toxicity of medaka.



(a)



(b)

Fig. 1 Effect of 28-d exposure to triadimefon on reproduction fitness of Japanese medaka *Oryzias latipes.* Data are shown as the mean ± SD. *and **indicate statistical significant differences set at P≤0.05 and P≤0.01 respectively.

3.1.2 *D. magna* 21-day chronic toxicity test

Triadimefon significantly affected reproduction of *D. magna* during the 21-day test (Fig. 2). Molting frequencies of *D. magna* were inversely proportional to concentration of triadimefon. Relative to negative controls, there was little effect on frequency of molting when *D. magna* exposed to 25 μg triadimefon L^{-1}, while a significant (P<0.05) but statistically lesser frequencies were observed at concentrations of 50 or 100 μg triadimefon L^{-1}. Effects on frequencies of molting

were more significant (P<0.01) and less for *D. magna* exposed to 200 or 400 μg triadimefon L^{-1}, which were 3.02 and 2.53 times, respectively. These results were similar to data collected from the ECOTOX data base, where the LOEC value based on reproduction was 283μg L^{-1}, and NOEC values, based on various endpoints ranged from 52 to 87μg L^{-1} in 21-day chronic toxicity tests with *D. magna*. *D. magna* was less sensitive to effects of triadimefon on reproduction than was medaka with a significant (P<0.05) decreases in numbers of neonates produced only at 400 μg L^{-1}. Results reported herein support the hypothesis that some chemicals, which disrupt endocrine processes in vertebrates, can also interfere with molting of arthropods through acting as antagonists of endogenous ecdysteroid by inhibiting the activity of P450 enzymes (Kenneke et al, 2009).

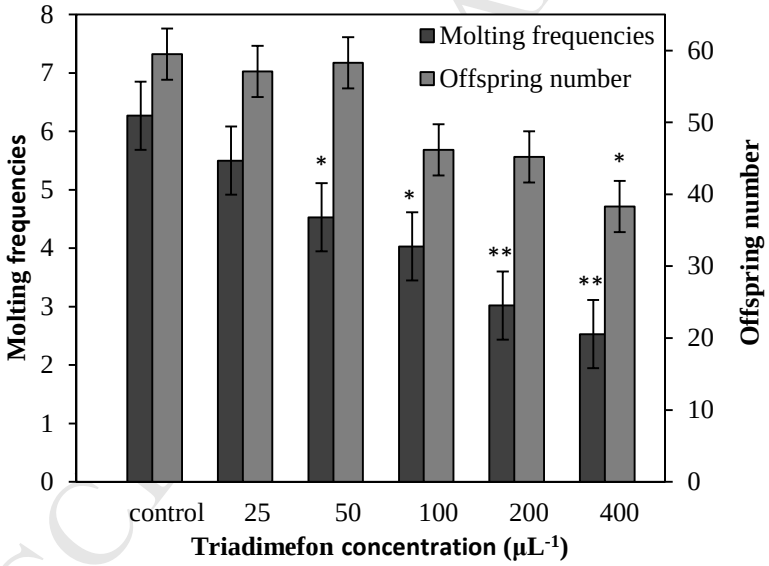


Fig. 2 Effect of 21-d exposure to triadimefon on molting frequencies and offspring numbers of *Daphnia magna*. Data are shown as the mean ± SD. *and **indicate statistical significant differences set at P≤0.05 and P≤ 0.01 respectively.

3.1.3 *B. calyciflorus* 96-hour reproduction test

Mean duration before young, adult, asexual female *B. calyciflorus* produced eggs young was 21.34 hours, and duration of reproduction was 77.77 hours (Chen et al,

2012). Normally, asexual, female rotifers produce eggs mitotically that develop into female rotifers. Given appropriate environmental conditions, including population density, temperature, food availability and quality, chemical toxicity, sexual reproduction can occur in rotifers, and asexual female rotifers produce sexual female rotifers. Sexual females subsequently produce eggs meiotically that develop into haploid males, or resting eggs if fertilized by males (Chen, 2005). The test was designed to determine effects on reproduction of *B. calyciflorus* from F_0 to F_2. During the experiment, there were no *B. calyciflorus* died and the proportion for *B. calyciflorus* carrying two kinds of eggs for each batch is shown (Fig. 3).

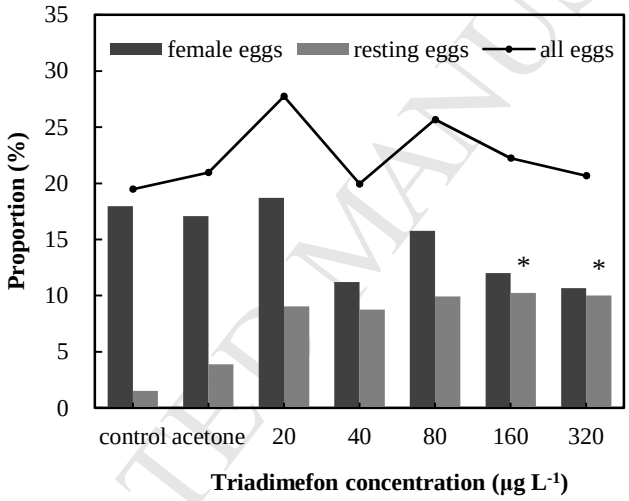


Fig. 3 Effect of 96-h exposure to triadimefon on reproduction of rotifer *Brachionus calyciflorus*. Data are shown as the mean ± SD. * indicate statistical significant differences set at P≤0.05.

Effects of triadimefon on proportions of *B. calyciflorus* carrying resting eggs and female eggs were variable. Exposure concentrations of 20, 80 and 160 μg L^{-1} triadimefon resulted in a greater proportion than the control. However, after exposure to 40 or 320 μg triadimefon L^{-1}, proportions of *B. calyciflorus* carrying resting eggs were similar to that of unexposed controls. Carrying capacities of females were slightly less in adults exposed to greater concentrations, but these effects were not statistically significant (P>0.05). Proportions of *B. calyciflorus* carrying resting eggs

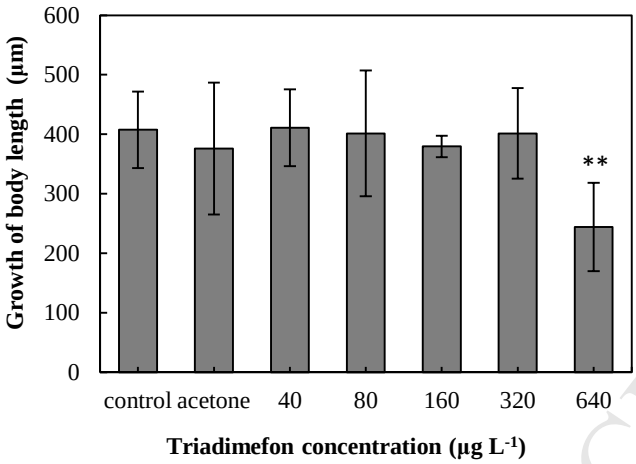
346 were 1.5 and 3.9% for the control and acetone controls, while the proportion of *B.*
347 *calyciflorus* carrying resting eggs was 9% at 20 μg L^{-1}, which was not statistically
348 significant ($P>0.05$) but was considered to potentially be biologically relevant relative
349 to the negative control. Proportions of females carrying resting eggs were inversely
350 proportional to concentrations of triadimefon. At 160 μg triadimefon L^{-1}, carrying
351 capacity for resting eggs, was significantly greater than the control ($P<0.05$) which
352 resulted in a NOEC of 80 μg triadimefon L^{-1}.

353 Results of previous studies have revealed that sexual reproduction in rotifers is a
354 more sensitive indicator of toxic stress than is asexual reproduction (Chen et al, 2012;
355 Preston et al, 2001). Sexual reproduction of the rotifer *B. plicalitis* was adversely
356 affected in the presence of small concentrations of diazinon, while amictic females
357 reproduced normally. The 96-hour toxicity study with *B. calyciflorus* indicated that
358 types of reproduction offspring of *B. calyciflorus* exposed to triadimefon was different
359 from those not exposed to triadimefon. This provides another line of evidence that
360 sub-lethal exposures to triadimefon would disturb reproduction of invertebrates like *B.*
361 *calyciflorus*.

362 3.1.4 *H. incongruens* 6-day body growth inhibition test

363 Size of 10 randomly selected *H. incongruens* neonates measured microscopically
364 ranged from 150 to 250 μm, with a mean of 206 μm. At the end of the experiment,
365 survival of *H. incongruens* in controls was greater than 80% in every treatment and
366 mean length was 410 μm, which was sufficient to meet the requirements of the test
367 (Belgis et al., 2003). Length increased for each batch of *H. incongruens* (Fig. 4). For
368 concentrations from 40 to 320 μg triadimefon L^{-1}, no inhibition of growth of *H.*
369 *incongruens* was observed. Inhibition of growth compared to the control was 33.4%
370 when exposed to 640 μg triadimefon L^{-1}, and lengths of bodies of *H. incongruens* was

371 significantly ($P<0.01$) shorter, with a NOEC of 320 μg triadimefon L^{-1}.



372

373 **Fig. 4** Effect of 6-day exposure to triadimefon on growth of Ostracoda *Heterocypris incongruens*
374 larval body length. Data are shown as the mean ± SD. **indicate statistical significant differences
375 set at $P\leq0.01$.

376 3.1.5 *S. polyrhiza* 10-day population growth inhibition test

377 There was an exponential increase in individuals either not exposed or exposed
378 to 0.5 or 1 mg triadimefon L^{-1} ($R^2>0.99$) (Fig. 5). Compared to controls, there was no
379 effect of triadimefon on growth during the first 2 days. On day 3, growth of *S.*
380 *polyrhiza* exposed to triadimefon slowed then the deficit in growth relative to the
381 controls remained constant at greater concentrations. Effects of triadimefon to *S.*
382 *polyrhiza* were time- and concentration-dependent. After four days of exposure, there
383 was a significant reduction of growth relative to that of the control by exposure to
384 either 4 or 8 mg triadimefon L^{-1}, with 53.3% and 86% inhibition, respectively. Leaves
385 gradually turned yellow and eventually died. These results are consistent with those of
386 a previous study (Liu, 2005), where exposure of *S. polyrhiza* to triadimefon for 96
387 hours resulted in an IC_{50} based in inhibition of growth of 5.47 mg L^{-1}.

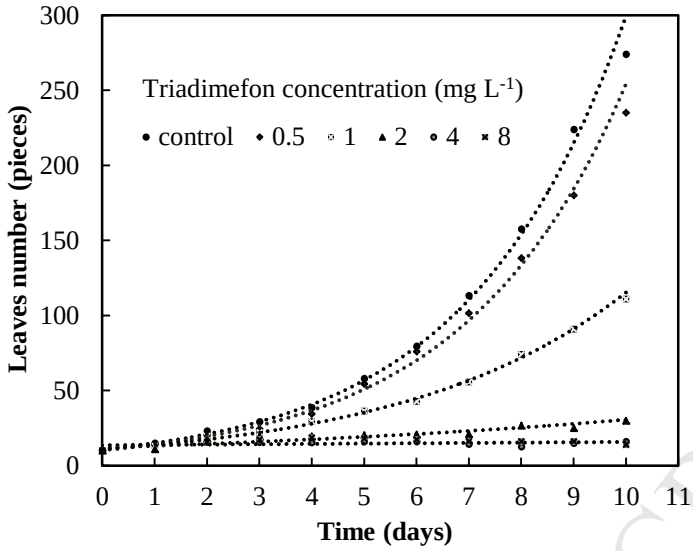


Fig. 5 Growth curves of *Soirodela polyrhiza* exposed to triadimefon at various concentration.

During the 10-day test, specific growth rate of plants in the control was 0.33, which met the requirements of the OECD test guidelines (Table 1). As concentrations of triadimefon increased, growth decreased gradually. Growth was significantly ($P < 0.01$) less than that of controls at concentrations greater than 1 mg triadimefon L^{-1}, with growth inhibited by 27.3%. The LOEC, based on reduced growth, was 1 mg triadimefon L^{-1}, and the NOEC was 0.5 mg triadimefon L^{-1}.

Table 1 Analysis of inhibition of growth of *Soirodela polyrhiza* by triadimefon.

Concentration (mg/L)	Control	0.5	1**	2**	4**	8**
1st replicate (leaves)	292	213	120	31	15	13
2nd replicate (leaves)	240	260	101	29	17	11
3rd replicate (leaves)	289	231	113	29	16	18
Mean	273.67	234.67	111.33	29.67	16.00	14.00

(leaves)						
SD	29.19	23.71	9.61	1.15	1.00	3.61
μ	0.33	0.32	0.24	0.11	0.05	0.03
I (%)	--	3.0	27.3	66.7	84.8	90.9

Note: **Significantly difference (ANOVA) to control ($P \leq 0.01$).

3.2. Predicted no effect concentration of triadimefon based on sub-lethal effects

A total of 9 (five from this study and four obtained from literature) chronic toxicity values, based on sub-lethal effects, especially for reproductive fitness, were used for derivation of species sensitivity distributions (SSD) (Table 2). These included three fishes, three invertebrates, one amphibian, one planktonic algae and one hygrophyte. Values of NOECs ranged from 5 to 500 with a mean of 150.22±161.78 μg triadimefon L^{-1}. Toxicity data for triadimefon, based on various endpoints, were investigated by use of the Anderson-Darling test ($p<0.05$) to determine if they met the assumption of log-normality for application of parametric statistics. The median HC_5 value (with 50% confidence intervals) of 7.32 (1.16~19.93) μg triadimefon L^{-1} was slightly greater than the measured NOEC for fecundity of the Japanese medaka (*Oryzias latipes*). According to the RIVM (Dutch National Institute for Public Health and the Environment) report (Vlaardingen et al, 2007), species for which cumulative probabilities were less than 5% in SSDs are regarded to be sensitive species. The PNEC of 3.66 μg triadimefon L^{-1} was derived as the HC_5 divided by a factor of 2 because it was derived by use of species not endemic to China (Jin et al, 2011; Jin et al,2015). However, this result is less by a factor of 1,366 than the Chinese effluent standards of pollutants from heterocyclic pesticides industries (GB 21523-2008) (SBTS, 2008) allowed for wastewater treatment plant effluents, which is 5 mg triadimefon L^{-1}. Compared with guidelines for deriving water quality criteria

418 developed by the US EPA (USEPA, 1984), a toxicity datum for an insect was lacking.
419 However, the 48-hour, LC_{50} for black fly larvae was 6.1 mg triadimefon L^{-1} (Kenneke
420 et al, 2009), which was similar to the 48-hour EC_{50} for *D. magna* of 7.16 mg
421 triadimefon L^{-1} (USEPA, 1992) and the 96-hour IC_{50} of *S. polyrhiza* of 5.47 mg
422 triadimefon L^{-1} (Liu, 2005). So it was concluded that inclusion of chronic toxicity for
423 an insect would probably not dramatically change the result of the assessment. The
424 final PNEC of 3.66 μg L^{-1} was recommended to protect aquatic organisms in surface
425 waters of China.

426 **Table 2** Summary of results obtained with various bioassays carried out with triadimefon.

Class/family	Species	Measurement	Duration (days)	NOEC (μg L^{-1})	Ref.
Osteichthyes Salmonidae	*Oncorhynchus mykiss*	Growth	60	40	(USEPA, 1992)
Osteichthyes Fathead Minnow	*Pimephales promelas*	Growth	35	170	(USEPA, 1992)
Osteichthyes Medaka	*Oryzias latipes*	Fecundity	28	5	this study
Amphibian	*Xenopus laevis*	Growth	21	112	(Li et al,2016)
Zooplankton Crustacean	*Daphnia magna*	Molting	21	25	this study
Zoobenthos Crustacean	*Heterocypris incongruens*	Growth	6	320	this study
Zooplankton Rotifera	*Brachionus calyciflorus*	Sexual reproduction	4	80	this study
Vascular plant	*Soirodela polyrhiza*	Population growth	10	500	this study
Alga	*Scenedesmus*	Population	4	100	(USEPA, 1992)

	subspicatus	growth

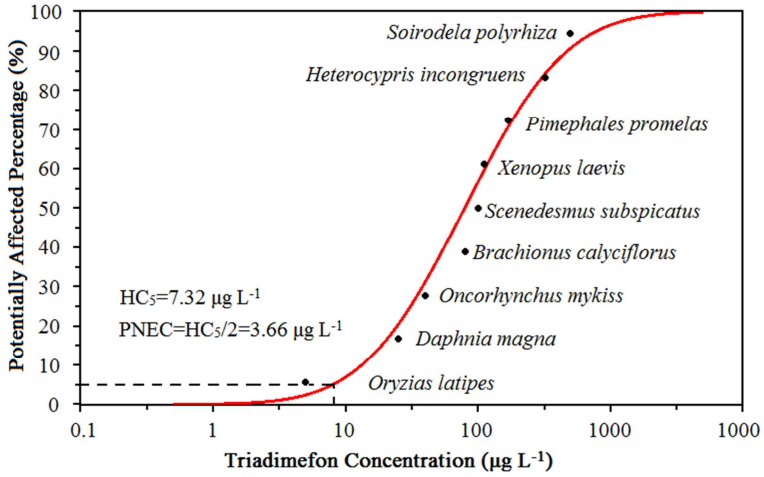


Fig. 6 Species sensitivity distributions (SSDs) of triadimefon based on sub-lethal endpoint for nine aquatic species.

3.3. Exposure assessment of triadimefon in surface waters of China

Concentrations of triadimefon for 6 surface waters were collected, with concentrations for the various sites ranging from less than the limit of quantification to 5.22 μg L^{-1} (Table S1). Concentrations varied among uses of surface waters. Concentrations of triadimefon in Tai Lake (Ch: *Taihu*) ranged from 0.00152 to 0.00727 μg L^{-1}, which were less than concentrations in the Jiulong River (Ch: *Jiulongjiang*) and lakes in Guizhou province, in the far, southwest of China.

Predicted environmental concentrations (PEC) of triadimefon used in cotton (Nantong, Jiangsu) and maize (Zhumadian, Henan) were predicted by use of a combination of simulation models, parameterized for specific crops and regions, including the PRZM-EXAMS model in PRAESS (Table S2). For the two row crops, triadimefon used in cotton, resulted in greater PECs in surface waters. After application to cotton, the greatest PEC in an adjacent river and pond were predicted to be 36 and 65 μg triadimefon L^{-1} respectively, while the PEC for maize in the

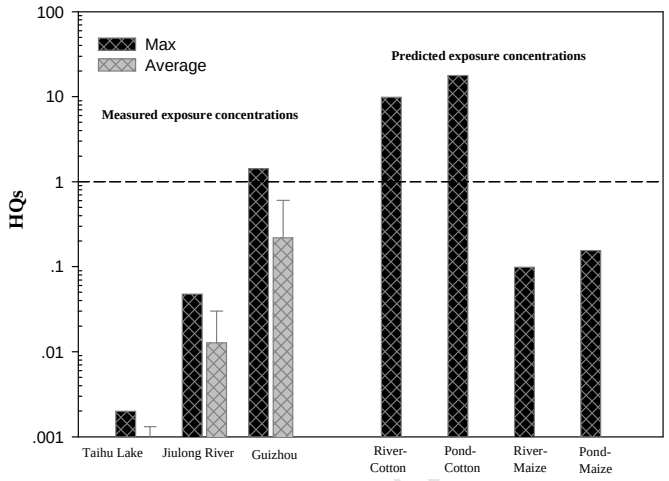
simulated river and pond were 0.36 and 0.569 μg triadimefon L^{-1} respectively, which were 100- and 114-fold less than the former. That difference was due to the greater rate applied to cotton (0.6 kg ha^{-1}) and method of spray application, which presented a large surface area to triadimefon for diffusion in soil. Alternatively, the amount of triadimefon used in maize was 0.018 kg ha^{-1}, and it was less easy to mobilize because it was applied as a seed dressing.

3.4. Assessment of risks posed by triadimefon

Assessments of potential for adverse effects of triadimefon on aquatic organisms were achieved by applying semi-probabilistic, hazard assessment methods by calculation of a hazard quotient (HQ). Based on the PNEC derived here from nine species' sub-lethal data in conjunction with both the MECs and the PECs from simulations of runoff from several scenarios, the chronic, sublethal HQs for effects of triadimefon on aquatic organisms were calculated (Fig. 7).

Hazards were assessed using either the mean or maximum concentration for measured triadimefon in each surface water. Although HQs for triadimefon were less than 0.1 in Tai Lake and the Jiulong River, overall results indicated a potential ecological risk from concentrations of triadimefon in lakes of Guizhou province, for which the HQ, based on maximum concentrations, was 1.43. Alternatively, for the maize-river scenario and maize-pond scenario, greatest HQs for triadimefon were 0.1 and 0.16, which indicated *de minimis* risk to aquatic organisms. For the cotton-river scenario, the HQ was 9.8 and then decreased rapidly to 2.2 in four days, then decreasing to less than 1.0 after approximately 21 days (HQ=0.6). For the cotton-pond scenario, the greatest HQ for triadimefon was 17.8, which was greater than the HQ for the river. Due to the small rate of decrease of the HQ, the pond exhibited a greater annual average risk, with an annual mean HQ of 1.8 (Fig. S1).

Triadimefon posed a hazard for damage to reproductive fitness of aquatic organisms in parts of Chinese surface water based on both MECs and PECs. The HQs based on the MECs were about one order of magnitude lower than the HQs based on the PECs might be due to the interaction of pollutants in actual water environment and the conservatism of the predict models when choosing simulation parameters.



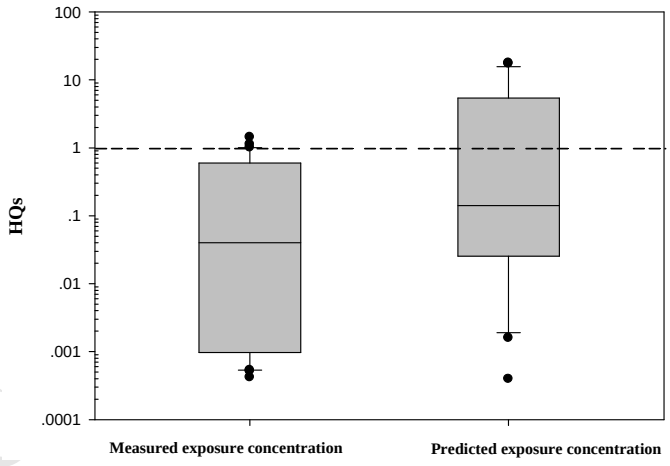


Fig. 7 Ecological risk assessment of triadimefon based on both the measured environmental concentrations and predicted environmental concentrations in Chinese surface water.

4. Conclusions

Since data based on sub-lethal effects are limited, assessments of effects of triadimefon on aquatic environments determined here was not definitive. Triadimefon

was predicted to cause different toxicities to various organisms, especially affecting reproductive fitness. The final PNEC of 3.66 μg L^{-1} was recommended to protect aquatic organisms in surface waters. Although there are limited reports of concentrations of triadimefon in surface waters of China, it was predicted to have potential ecological risk based on both MECs and PECs in surface waters under agricultural and climatic conditions of China. Considering that large amounts of triadimefon are used as a fungicide in agricultural crops, it is likely that triadimefon will contaminate the surface waters of China, which should be a concern given the effects discussed here.

Measures should be taken to minimize the ecological risk posed by triadimefon. Clearly, given the limitations and uncertainties of the HQ, a higher-tier, quantitative probabilistic risk assessment using the joint probability curve (JPC) method that accounted for variability in exposure and toxicity profiles to quantify risk would be helpful. In addition, when assessing the risk of environmental endocrine disruptors, data on chronic effects especially subtle effects on reproduction should be primarily considered. However, there is limited information of this type available, especially for Chinese native species. So, data on effects of triadimefon on reproduction of site-specific species are critically needed in order to produce more accurate ecological risk assessments.

The main sources of uncertainty in the present study are the limited measured surface water concentrations, PECs based PRZM-EXAMS simulation model also have not been corroborated against measured concentrations. To more accurately describe exposure and ecological risk, measured concentrations of triadimefon at various spatial and temporal scales in Chinese waters are required.

Acknowledgements

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Supporting Information Available

Data on exposure to triadimefon, expressed as concentrations in surface waters including rivers, lakes, reservoirs and urban rivers were collected from literature published in China. Considering measurements of triadimefon in waters of china were limited, predicted environmental concentrations (PECs) of triadimefon in surface waters under the agricultural conditions of China were also simulated by use of the PRZM-EXAMS simulation model module within the Pesticide Risk Assessment Exposure Simulation Shell (PRAESS).

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Highlights

(1) Sub-lethal effects of triadimefon on reproduction and growth of five aquatic species were determined.

(2) Triadimefon caused significant effects on endocrine functions of aquatic organisms.

(3) A final, Predicted No Effect Concentration (PNEC) of 3.66 μg L^{-1}, to protect aquatic organisms in surface waters, was recommended.

(4) Triadimefon was found to have a potential ecological risk to aquatic organisms in some surface waters of China.