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Aquatic risk assessment of a novel strobilurin fungicide: A microcosm study compared with the species sensitivity distribution approach

Lang Chen [a,b,c], Yufang Song [a,*], Baohua Tang [c], Xueying Song [d], Hairong Yang [c], Baiyu Li [c], Yu Zhao [c], Chengtian Huang [c], Xue Han [c], Shaoye Wang [c], Zhaoli Li [c]

[a] Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang 110016, China
[b] College of Resources and Environment, University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049, China
[c] Safety Evaluation Center, Shenyang Research Institute of Chemical Industry, 8 East Shenliao Road, Shenyang 110016, China
[d] College of Environment, Shenyang University, No. 21 Wanghua South Street Dadong District, Shenyang 110044, China

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ABSTRACT

The ecotoxicological effects of pyraoxystrobin, a novel strobilurin fungicide, were studied using outdoor freshwater microcosms and the species sensitivity distribution approach. The microcosms were treated with pyraoxystrobin at concentrations of 0, 1.0, 3.0, 10, 30 and 100 μg/L. Species sensitivity distribution (SSD) curves were constructed by means of acute toxicity data using the BurrliOZ model for fourteen representatives of sensitive invertebrates, algae and fish and eleven taxa of invertebrates and algae, respectively.

The responses of zooplankton, phytoplankton and physical and chemical endpoints in microcosms were studied. Zooplankton, especially *Sinodiaptomus sarsi* was the most sensitive to pyraoxystrobin exposure in the microcosms. Short-term toxic effects (<8 weeks) on zooplankton occurred in 1 μg/L treatment group. The duration of toxic effects on *S. sarsi* could not be evaluated within the initial 56 days. Significant long-term toxic effects were observed at 10, 30 and 100 μg/L (>281 days) for *S. sarsi* and the zooplankton community. Based on the results obtained from the organisms in the microcosm system, 1 μg/L was recommended as the NOEAEC (no observed ecologically adverse effect concentration). Also, 0.33 μg/L was derived as the Regulatory Acceptable Concentration based on the ecological recovery option (ERO-RAC) of pyraoxystrobin. For all fourteen tested species, the median HC_5 (hazardous concentration affecting 5% of species) was 0.86 μg/L, and the lower limit HC_5 (LL-HC_5) was 0.39 μg/L. For the eleven taxa of invertebrates and algae tested, the median HC_5 was 1.1 μg/L, and the LL-HC_5 was 0.26 μg/L. The present study positively contributes to the suggestion of adequately using acute L(E)C_{50}-based HC_5/ LL-HC_5 for deriving protective concentrations for strobilurin fungicides, and it should be valuable for full comprehension of the potential toxicity of pyraoxystrobin in aquatic ecosystems.

1. Introduction

The intensive use of pesticides has caused many environmental problems worldwide, and it has become one of the most severe stressors of pollutants in aquatic systems (Aydinalp and Porca, 2004; Ippolito et al., 2012). In aquatic systems the ecological risk assessment for pesticides usually follows a tiered procedure: the first tier is based on standardised single-species toxicity studies; the higher tier is based on species sensitivity distributions (SSD) and semi-field microcosm/mesocosm studies (EU, 1997; US EPA 1998; EC (European Commission), 2002, 2009). Microcosm/mesocosm test systems are a useful tool for higher-tier risk assessments since these test systems provide more ecological realism than laboratory toxicity tests. Microcosm/mesocosm test systems allow to study direct and indirect effects at more realistic exposure conditions while recovery of affected endpoints and delayed effects can also be studied (Van den Brink et al., 2006).

However, microcosms and/or mesocosm studies have the disadvantages of high cost and long test time. In Europe, it is common practice to use the SSD approach for regulatory decision making (Posthuma et al., 2002; EFSA (European Food Safety Authority), 2005; Van Wijngaarden et al., 2010). The endpoint of the SSD approach used by regulatory authorities is usually HC_5 (hazardous concentration affecting 5% of species) based on acute or chronic toxicity data. However, problems persist and debates occur. Whether acute HC_5 is an adequate estimator of safe concentrations

* Corresponding author.
E-mail addresses: songyufang@iae.ac.cn (Y. Song), baiguanqi@163.com (B. Li).

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in the field is still in discussion (Van Wijngaarden et al., 2010). For insecticides and herbicides, comparisons of the SSD results and the effects on freshwater ecosystems have been made (Maltby et al., 2005; Van den Brink et al., 2006). In contrast to herbicides and insecticides, comparisons of acute HC_5 values and safe threshold concentrations given in microcosms/mesocosm have seldom been published for fungicides (Maltby et al., 2009; Van Wijngaarden et al., 2010).

Strobilurin fungicides are relatively new crop protection products used worldwide. However, fairly scarce eco-toxicology information is available on them (Balba, 2007). According to our knowledge, no SSD study is available for strobilurin fungicides, and no information other than that published in Gustafsson et al. (2010), Zafar et al. (2012) and Van Wijngaarden et al. (2014) is available on the toxic effects of strobilurin fungicides in microcosms and/or mesocosm. Pyraoxystrobin is a strobilurin fungicide, and it has demonstrated an excellent antifungal effect. Developed by the Shenyang Research Institute of Chemical Industry (SRICI) of China, it has been patented in the People's Republic of China and the U.S. for preventing and curing terrestrial crop and vegetable infections. Recently, an excellent antifungal effect of pyraoxystrobin on rice has been proven (Chen et al., 2011).

In this paper an ecological risk assessment study was performed with the novel fungicide pyraoxystrobin using an outdoor freshwater microcosm system and the species sensitivity distribution approach by means of a series of laboratory aquatic acute toxicity tests. The aims of the present paper were: (a) to provide data on pyraoxystrobin toxicity in aquatic ecosystems for its safe application under extensive conditions in paddy fields; and (b) to compare the acute HC_5 of pyraoxystrobin with the safe threshold level derived from the microcosm study, contributing to the discussion whether SSD approach would be an adequate estimator of safe concentrations in the field for strobilurin fungicides.

2. Materials and methods

2.1. Chemicals

Acetone (AR), aqueous solution of formaldehyde, potassium iodide (AR), iodine (AR) and glycerol (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. Dikma Technologies Inc. Dichloromethane (HPLC purity grade) and acetonitrile (HPLC purity grade) were provided by Dikma Technologies, Inc.

Pyraoxystrobin ($C_{22}H_{21}ClN_2O_4$, purity, 96%) was supplied by SRICI. The solubility of pyraoxystrobin in water is 0.13 mg L^{-1} (20 °C). Pyraoxystrobin is stable in water at pH 4 and pH 7, with a half-life of 577.5 days at pH 9 (25 °C). The photolysisi rate in water is 27.2 min (25 °C). The octanol/water partitioning coefficient (Kow) of pyraoxystrobin is 2512 (log Kow: 3.4). The sorption partition coefficients between water and organic matrix (Kom) range from 20,081 to 30,605 L kg^{-1} (depending on soil characteristics). In laboratory aquatic sediment systems (50 μg/L pyraoxystrobin treatment level), the DT_{50} in water was 3.3 days (mean value), while the DT50 in sediment was 165 days (mean value). All the above data were measured in SRICI, however, have not yet been published.

2.2. Microcosms experiment

2.2.1. Description

This experiment was performed at SYRICI, Shenyang, China. The microcosm facility consisted of fifteen outdoor circular concrete tanks (diameter 1.5 m, total depth 1.0 m, water depth 0.8 m, water volume ca. 1410 L). Tanks were sunken into the ground, and their tops were elevated 10 cm above the ground to avoid run-off into the microcosms. Each microcosm bottom was covered with a 10 cm layer of sediment collected from the Hun River (Shenyang) two years before test. Because the sediments contained a variety of spores and/or eggs and/or seeds of aquatic organisms, including phytoplankton, zooplankton, bacteria, snails, floating herb (Lemna minor), submerged macrophytes (Potamogeton cristatus, Vallisneria spiralis), etc, after the 24-month aging period they have been naturally grown up and widely distributed in each microcosm tank. Water was circulated twice to achieve similar biocoenoses in the test systems two months before the start of the test. Proper amounts of essential nutrients (KNO_3, NaH_2PO_4, $NaHCO_3$ and soluble starch) were added according to the measured total organic carbon (TOC), total nitrogen (TN) and phosphate content in the microcosms before test. There were no statistically significant differences between the control and potential treated microcosms prior to treatment for any biotic (zooplankton community, phytoplankton community, etc.) or non-biotic endpoint (day-7, $p_{mean} > 0.5$). The microcosm study was conducted according to OECD (2006).

2.2.2. Experimental design

The treatment regime was in duplicate: 1.0, 3.0, 10, 30, 100 μg/L nominal levels and controls (acetone at 0.001%, v/v as co-solvent). Pyraoxystrobin/acetone solutions (15 mL) were slowly injected into three locations of each microcosm and mixed with a low power paint stirrer (Sanderson et al., 2007). Control microcosms were injected with 15 mL of acetone using the same mixing technique. This study was triggered by the acute test on *Daphnia magna* (48 h EC_{50}, 8.28 μg/L). A single application of the fungicide started on August 19th, 2013 in accordance with the normal agricultural use of pyraoxystrobin in late rice paddies. The end of the study was on 5 May 2014. The test duration was 84 days for all endpoints except zooplankton, for which the sampling continued to day 247 and day 281. Microcosms were cultured under the condition of natural light. The light intensities were identical among different tanks because they were located in an open area without shadow. Zooplankton, phytoplankton and physical and chemical endpoints were monitored to determine the effects of pyraoxystrobin.

2.2.2.1. Zooplankton and phytoplankton investigation. Zooplankton and phytoplankton were sampled weekly. A depth-integrated water sample of 6 L was collected into a bucket from three spots in each microcosm. From this mixed sample, a sub-sample of 1 L was stained by Lugol's iodine solution (15‰) for the future analysis of rotifer and phytoplankton (Daam et al., 2009); the remaining 5 L water was passed through a 55 μm mesh net, and the collected microcrustaceans were fixed with formalin solutions (final concentration: 4%, v/v) for investigation (Daam et al., 2009). The remaining excess water was then returned to the sampled microcosm.

To investigate the species composition of zooplankton, the total number of cladocerans, copepods and ostracods were identified and counted with a stereo microscope (NIKON Eclipse E200; magnification 10–40 ×) according to Jiang and Du (1979), Shen (1979) and Shi et al. (2012). Rotifers and copepod nauplii were quantified and identified under a binocular microscope (magnification 100–400 ×) according to Wang (1961) and Zhou and Chen (2005). Identifications of the phytoplankton species were made according to Hu and Wei 2006 and Shi et al. (2012) under a binocular microscope (magnification 100–400 ×). The abundance of zooplankton and phytoplankton was expressed as the number of individuals per litre.

2.2.2.2. Physical and chemical measurements. A single depth-integrated sample (2 L) was taken from each tank for biweekly water