Species Sensitivity Distributions of Benthic Macroinvertebrates in Fludioxonil-Spiked Sediment Toxicity Tests

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Abstract

In China, the fungicide fludioxonil, that accumulates and persists in sediments, has a widespread agricultural use to control various fungal diseases. Its residues may cause toxic effects to benthic aquatic fauna, thereby impacting ecosystem service functions of aquatic ecosystems. To assess the potential environmental effects of fludioxonil in the sediment compartment of edge-of-field surface waters, sediment-spiked single-species toxicity tests with benthic macroinvertebrates were performed. In all experiments artificial sediment was used with an organic carbon content of 2.43% on dry weight basis. The single-species tests were conducted with 8 benthic macroinvertebrates covering different taxonomic groups typical for the Yangtze River Delta, China. The 28d-EC₁₀ and 28-LC₁₀ values thus obtained were used to construct species sensitivity distributions (SSDs). In addition, our data were supplemented with similar fludioxonil-spiked sediment toxicity data for benthic invertebrates from the Netherlands. Based on SSDs constructed with 28d-EC₁₀ values of 8 benthic species from our experiments in China, hazardous concentrations to 5% of the species tested (HC₅'s) of respectively 0.57 mg fludioxonil/kg dry weight sediment and 5.4 µg fludioxonil/L pore water were obtained. Supplementing our data from China with 8 similar toxicity data for other benthic species from the Netherlands, these HC₅ values became respectively 1.2 mg fludioxonil/kg dry weight sediment and 11 µg fludioxonil/L pore water.

1 Introduction

Sediments may act as sink and exposure route of lipophilic pesticides, potentially giving rise to adverse ecological effects on benthic populations and communities (Boyle et al. 2016; Brock et al. 2016; Mcknight et al. 2015; Nowell et al. 2016; Warren et al. 2003). To assess benthic organisms from potential exposure to pesticides that accumulate in sediments, prospective effect assessment procedures for sediment organisms have been proposed (Diepens et al. 2016; EFSA 2015). Nevertheless, information on effects of sediment-exposure of pesticides on benthic organisms others than standard test species is relatively scarce (Brock et al. 2020; Diepens et al. 2014; EFSA 2015; Yin et al. 2018). In China, an ERA approach for sediment-dwelling organisms is not yet implemented in the authorization procedure of pesticides if they are predicted to accumulate in the sediment compartment. Consequently, results of sediment-spiked toxicity tests with pesticides and benthic invertebrates are not often published in Chinese regulatory reports until now. In addition, compared to insecticides and herbicides, the potential environmental risks of fungicides in the sediment compartment of freshwater ecosystems received less attention in the open literature (Köhler and Triebkorn 2013; Wightwick et al. 2010; Zubrod et al. 2019). Particularly if the K_{ow} and lipophilicity of the fungicide is high, risks to sediment-dwelling organisms cannot be excluded (EFSA 2015). In addition, many fungicides may have biocidal properties, affecting different taxonomic groups and ecological processes in aquatic ecosystems (Maltby et al. 2009; Rico et al. 2019).

Fludioxonil was selected as benchmark compound in this study, since in China this fungicide is extensively used for reducing the disease caused by various fungi in crops like grapes, rice and wheat (Wang 2016). In addition, recent experimental experience on environmental fate and ecotoxicological effects of fludioxonil in the Netherlands could be used to design the Chinese experiments presented in this paper (Brock et al. 2020; Yin et al. 2018). Furthermore, this also offered the possibility for a geographical comparison of the sensitivity of benthic invertebrates between the Netherlands and China and to combine the datasets from both countries in the effect assessment procedure by applying the species sensitivity distribution (SSD) approach.

Fludioxonil is a non-systemic and broad-spectrum fungicide. It strongly interferes with mycelia growth and stimulates glycerol synthesis via the mitogen-activated protein kinase pathway (Kanetis et al. 2008). Fludioxonil is stable to hydrolysis but may rapidly photodegrade in water. It has a low solubility in water (1.8 mg/L) and a high mean partitioning coefficient between soil organic carbon and water ($K_{oc} = 145,600$ mL/g OC) (EFSA 2007), implying that, when present in surface water, fludioxonil will rapidly adsorb to sediment from the water phase. It was found that fludioxonil has a long persistence in sediments of edge-of-field surface waters since residues of this fungicide were present up to 2 years after its

use in grape vineyards (Bermúdez-Couso et al. 2007). Fludioxonil is persistent in aerobic aquatic water-sediment systems, with half-lives of 737 and 643 days in pond and river systems (Health Canada 2016). Several publications showed that fludioxonil has biocidal properties and that in aquatic ecosystems several taxonomic groups, including sediment-dwelling organisms, may be relatively sensitive (Brock et al. 2020; Höss et al. 2020; Yin et al. 2018).

In China the fungicide fludioxonil was identified as emerging chemical of concern because of its high or increasing use rates and frequent detection in surface waters (Zhang et al. 2010; Zhang et al. 2015; Zhang et al. 2018). For this reason, the studies presented in this paper focused the aquatic risks of fludioxonil, with reference to responses of benthic macroinvertebrates and sediment exposure. The purpose of this paper is threefold; (i) to generate chronic fludioxonil-spiked sediment toxicity data for 8 different benthic invertebrate species typical for the Yangtze River Delta in sediment-spiked single species toxicity tests, (ii) compare the SSDs based on these chronic toxicity with those of similar toxicity data for 8 different benthic invertebrates from the Netherlands, and (iii) to derive hazardous concentrations to 5% of the species (HC_5 's) for fludioxonil based on our Chinese dataset and the combined dataset from China and the Netherlands.

2 Materials And Methods

2.1 Test species in single species toxicity tests

8 different benthic species belonging to seven different taxonomic orders were selected to conduct sediment-spiked single species toxicity tests with fludioxonil. We selected two species of Oligochaeta worms, *Branchiura sowerbyi* and *Limnodrilus hoffmeisteri* both belonging to Tubificidae, one bivalve, *Corbicula fluminea* (Veneroida), one snail, *Physa acuta* (Basommatophora), one turbellarian worm, *Dugesia japonica* (Tricladida), one leech, *Hirudo nipponia* (Arhynchopdellida), one insect, *Dicrotendipes pelochloris* (Diptera), and one shrimp *Neocaridina denticulata* (Decapoda). Information about origin, size/life-stage and test conditions of these 8 benthic species is presented in Table 1.

Table 1

Species name, origin, life-stage and testing conditions for selected benthic species in the 28-d sediment-spiked toxicity tests with fludioxonil using artificial sediment.

Taxon	Origin test species	A: Mean length in mm	No of replicate test systems to study treatment related effects	Individuals per test vessel(% mortality in controls at day28)	Treatment: Geometric mean test concentrations	DO(mg/L) ± SD
		B: Mean wet weight in mg			A: mg a.s./kg dry weight sediment B: µg a.s./L pore water	PH ± SD Temp(°C) ± SD
<i>Branchiura sowerbyi</i> (Oligochaeta)	Outdoor ditches in Machege	A: not measured B: 52.4 ± 2.1	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-2.48-4.78-9-18.69-36.45 B:0-23.65-45.41-85.36-176.92-345.16	DO = 8.54±0.07 PH = 7.42±0.04 T = 19.88 ± 0.27
<i>Limnodrilus hoffmeisteri</i> (Oligochaeta)	Outdoor ditches in Machege	A: not measured B: 3.74 ± 0.47	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-5.4-10.42-20.44-40.11-80.54 B:0-49.58-95.44-187.12-370.29-736.66	DO = 8.53±0.01 PH = 7.41±0.04 T = 19.9 ± 0.30
<i>Corbicula fluminea</i> (Mollusca)	Purchased commercial culture	A: 2.47 ± 0.1 B: 4.81 ± 0.34	Controls(n = 5) Treatments(n = 5)	10 (8%)	A:0-2.89-5.41-10.42-20.45-40.45 B:0-26.62-49.59-95.44-193.51-383.1	DO = 8.53±0.04 PH = 7.5±0.04 T =19.84 ± 0.22
<i>Physa acuta</i> (Mollusca)	Outdoor ditches in Machege	A: embryo B:	Controls(n = 5) Treatments(n = 5)	20 (13%)	A:0-0.38-0.75-1.47-2.98-5.74 B:0-3.41-6.84-13.72-27.45-52.6	DO = 8.54±0.03 PH = 7.46±0.01 T =19.82 ± 0.23
<i>Dicrotendipes pelochloris</i> (Insecta)	Outdoor ditches in Machege	A: first instar larvae B:	Controls(n = 5) Treatments(n = 5)	20 (18%)	A:0-2.62-5.4-10.42-20.33-40.4 B:0-23.46-45.58-92.44-173.05-358.53	DO =8.55±0.05 PH = 7.44±0.04 T =20.1 ± 0.31
<i>Neocaridina denticulata</i> (Crustacea)	Purchased commercial culture	A:6.77 ±0.11 B: 2.37 ± 0.04	Controls(n = 5) Treatments(n = 5)	10 (2%)	A:0-2.68-5.15-10.36-20.32-40.63 B:0-27.52-51.27-98.69-193.49-383.13	DO = 8.54±0.06 PH = 7.4±0.03 T =19.86 ± 0.27

Taxon	Origin test species	A: Mean length in mm	No of replicate test systems to study treatment related effects	Individuals per test vessel(% mortality in controls at day28)	Treatment: Geometric mean test concentrations	DO(mg/L) ± SD
		B: Mean wet weight in mg			A: mg a.s./kg dry weight sediment B: µg a.s./L pore water	PH ± SD Temp(°C) ± SD
<i>Dugesia japonica</i> (Turbellaria)	Purchased commercial culture	A: 11.5 ±0.3 B: not measured	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-1.08-2.24-4.49-9.82-18.66 B:0-10.58-21.38-42.57-91.79-175.95	DO = 8.58±0.07 PH = 7.42±0.06 T =20.12 ± 0.12
<i>Hirudo nipponia</i> (Hirudinea)	Purchased commercial culture	A: not measured B: 271.71 ±10.27	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-1.09-2.33-4.94-9.26-19.42 B:0-10.59-21.88-47.57-88.79-189.25	DO = 8.56±0.06 PH = 7.45±0.06 T =19.76 ± 0.21

2.2 Sediment preparation and spiking

2.2.1 Single species toxicity tests

All 28-d single species toxicity tests were performed in artificial sediment according to OECD guidelines (OECD 2004a,2004b,2007,2010). The artificial sediment was composed of 5% finely ground sphagnum peat, 20% kaolin clay and 75% industrial sand. The wet artificial sediment moisture content was maintained at about 65.4%. These sediments contained, on average an organic carbon (OC) content of 2.43% w/w in dry sediment.

The spiking treatments included a solvent control (sediment spiked with 0.5 ml acetone/kg dry sediment), and five treatment-levels of fludioxonil. For each treatment (including controls), 5 test systems were used. The spiking was conducted from low to high concentrations and per treatment level the dosing solution was gently mixed with a hand-held electric mixer in wet artificial sediment for 5 minutes. For measured geometric mean sediment exposure concentrations of each treatment in each laboratory toxicity test see Table 1. Further details on fludioxonil analysis is presented in section 2.5 below.

2.2.2 Design of sediment-spiked laboratory single species-toxicity tests

The 28-d sediment-spiked toxicity tests were all conducted in the same type of test system in the laboratories of Zhejiang University Ecological and Environmental Toxicology Research Center (see Fig. 1). The test systems consisted of 500 mL glass beakers, containing 290 g wet artificial (representing approximately 100 g dry artificial sediment) and about 450 mL of aerated tap water (before introduction aerated for 72 h). Before adding overlying water the sediment was covered with a plastic sheet. The plastic sheet avoided that the sediment became suspended in the overlying water. The plastic sheet was gently removed from each test system after 10 minutes. The test systems were placed in a climate room of around 20 °C. Aeration of the water column was provided through a rubber tube and a bubble stone. pH, dissolved oxygen (DO) and

temperature were measured weekly in the overlying water of each test system. The test animals were introduced in the test systems 48 h after spiking the sediment with fludioxonil.

The test performed with the omnivorous benthic species *D. pelochloris*, *C. fluminea*, *N. denticulata* and *P. acuta* followed OECD test guideline 218 (OECD 2004a) as much as possible. At the start of the experiments with these species, 0.5 g spinach powder was blended in the artificial sediment of each test system as initial food source. In addition, feed was applied topically in the form of a ground paste of a 7.2 g/L algal-water mixture. Initially, 0.5 mL of this paste was supplied every three days to each test system but with the growth of the organisms the volume of the feed was appropriately increased to 1 ml on day 7 onwards.

Also the test with the carnivorous flatworm *D. japonicus* and carnivorous leech *H. nipponia* followed OECD test guideline 218 (OECD 2004a) as much as possible, except the feeding regime. *D. japonicus* was fed with 10 living 3rd instar *D. pelochloris* larvae every three days. In the tests with *H. nipponia* test, tofu of the blood of pig was fed once at the start of the experiment. No feeding occurred during the 28-day toxicity test.

The test design for the two aquatic oligochaetes was based on OECD test guideline 225 (OECD 2007). At the start of the experiment (before spiking) 0.5 g of spinach powder was mixed in the artificial sediment of each test system as a potential food source.

The endpoints measured for *C. fluminea*, *N. denticulata* and *P. acuta* were number of surviving individuals and relative rate in daily length increase. Measured endpoints for *H. nipponia* were number of surviving individuals and relative wet weight biomass decrease. The endpoints measured for *D. japonicus* were the number of surviving individuals on day 28 as well as increase in numbers due to asexual reproduction. The endpoint measured for the *D. pelochloris* was emergence of adults on day 28. In the tests with the Oligochaete worms *B. sowerbyi* and *L. hoffmeisteri* the measured endpoints were surviving individuals and the production of cocoons. In addition, for *B. sowerbyi*, which in most test systems decreased in biomass during the 28d test period, relative wet weight biomass decrease was selected as measurement endpoint. For *L. hoffmeisteri*, which increased in biomass in all test systems during the 28d toxicity test, the measurement endpoint relative rate in daily biomass increase was selected. For the test to be considered valid, the mortality in control test systems should not be higher than 20% at the end of the test (USEPA 2000).

2.3 Fludioxonil analysis

The concentrations of fludioxonil in the total sediment, sediment pore water and overlying water were measured at the start (day 0) and the end (day 28) of the laboratory toxicity tests. For this, six extra test systems for each treatment (including controls) were prepared for each test species. On each sampling date (days 0 and 28) approximately 200 mL of overlying water and 100 g well-mixed wet sediment were sampled from each test system (without organism). The samples were collected in 250 mL high density polyethylene (HDPE) bottles and stored at -20°C until qualitative analysis. The samples were collected and analyzed for fludioxonil as described in (Brock et al. 2020). For a description and analysis of quality control see (SI Appendix B Tables 1–4 and SI Appendix B Figures 1 and 2). The toxicity values of the fungicide were described in terms of (i) mg a.s./kg dry sediment, (ii) mg a.s./g OC in dry sediment and (iii) µg a. s./L sediment pore water. To calculate these toxicity values, geometric mean concentrations of fludioxonil in total sediment and sediment porewater of each laboratory toxicity test were used as calculated from measurements on days 0 and 28. For all basic data on fludioxonil concentrations in sediment and water samples is referred to Supporting Information Appendix A Tables 9 - 10

2.4 Data analysis

28-day LC_x and EC_x values (and 95% confidence bands) for the benthic test species were calculated by the MOSAIC web-interface for statistical analysis in ecotoxicology. Hazardous concentrations to 5% (HC₅) and 50% (HC₅₀) of the species tested were calculated from species sensitivity distribution (SSD) curves. These HC_p values, and their 95% confidence

bands, also were estimated using the MOSAIC tool, using the median $L(E)C_{10}$ values as input. MOSAIC is available at <http://pbil.univ-lyon1.fr/software/mosaic/>. The basic data underlying these toxicity estimates are presented in SI Appendix A Tables 1 - 8.

Table 2

28d-L(E)Cx values (and 95% confidence bands) for eight benthic invertebrates in laboratory toxicity tests using artificial sediment spiked with the fungicide fludioxonil. The 28d-L(E)Cx values are expressed in terms of geometric mean fludioxonil concentration during the test in the total dry sediment (mg a.s./kg DW) and in sediment pore water (µg a.s./L). The toxicity values were calculated with the MOSAIC computer program.

Taxon Endpoint	Toxicity value	Total sediment (mg a.s./kg DW)	Pore water (µg a.s./L)	Fludioxonil concentration in (mg/g organic carbon OC)
<i>Branchiura sowerbyi</i> (Oligochaeta)				
- Reproduction (number of cocoons)	28d-EC10	2.56 (1.47-4.14)	25.9 (15.3-40.8)	0.11 (0.06-0.17)
	28d-EC50	7.01 (5.46-8.96)	68.4 (54.0-86.4)	0.29 (0.23-0.37)
- Relative biomass decrease	28d-EC10	5.78 (5.02-6.59)	56.4 (49.5-64.0)	0.24 (0.21-0.27)
	28d-EC50	22.8 (21.2-24.6)	217 (202-233)	0.94 (0.87-1.01)
- Survival	28d-LC10	19.0 (12.7-26.8)	182 (122-253)	0.78 (0.52-1.10)
	28d-LC50	90.1 (59.0-166)	839 (550-1520)	3.71 (2.43-6.83)
<i>Limnodrilus hoffmeisteri</i> (Oligochaeta)				
- Reproduction (number of cocoons)	28d-EC10	11.9 (1.19-36.4)	98.8 (9.86-321)	0.49 (0.05-1.50)
	28d-EC50	35.9 (24.3-50.2)	313 (209-439)	1.48 (1.0-2.07)
- Relative rate in daily biomass increase	28d-EC10	3.64 (2.39 -5.22)	31.2 (20.6-44.8)	0.15 (0.10-0.22)
	28d-EC50	25.3 (22.3-29.1)	221 (194-253)	1.04 (0.92-1.20)
- Survival	28d-LC10	18.3 (13.1-23.7)	158 (112-206)	0.75 (0.54-0.98)
	28d-LC50	56.6 (43.1-87.3)	506 (384-786)	2.33 (1.77-3.59)
<i>Corbicula fluminea</i> (Mollusca)				
- Relative rate in daily length increase	28d-EC10	0.402 (0.296-0.534)	3.75 (2.75-4.97)	0.017 (0.012-0.022)
	28d-EC50	1.58 (1.37-1.82)	14.7 (12.7-16.9)	0.065 (0.056-0.075)
- Survival	28d-LC10	0.791 (0.390-1.42)	7.41 (3.54-13.3)	0.033 (0.016-0.058)
	28d-LC50	4.44 (3.30-6.49)	40.8 (30.5-60.4)	0.18 (0.14-0.27)
<i>Physa acuta</i> (Mollusca)				
- Relative rate in daily length increase	28d-EC10	11.6 (6.7-19.1)	109 (61.8-180)	0.48 (0.28-0.79)
	28d-EC50	143 (101-219)	1390 (972-2120)	5.89 (4.16-9.01)

Taxon Endpoint	Toxicity value	Total sediment (mg a.s./kg DW)	Pore water (µg a.s./L)	Fludioxonil concentration in (mg/g organic carbon OC)
- Survival	28d-LC10	35.9 (24.8-40.2)	341 (235-400)	1.48 (1.02-1.65)
	28d-LC50	49.4 (41.3-77.6)	465 (391-744)	2.03 (1.7-3.193)
<i>Dicrotendipes pelochloris</i> (Insecta)				
- Emergence	28d-EC10	67.2 (33.3-80.3)	617 (308-733)	2.77 (1.37-3.31)
	28d-EC50	100 (82.2-177)	912 (752-1620)	4.12 (3.83-7.28)
<i>Neocaridina denticulata</i> (Crustacea)				
- Relative rate in daily length increase	28d-EC10	17.5 (15.5-18.6)	166 (147-176)	0.72 (0.64-0.77)
	28d-EC50	19.6 (18.9-21.5)	186 (179-204)	0.81 (0.78-0.89)
- Survival	28d-LC10	4.14 (3.11-5.31)	39.5 (29.3-50.7)	0.17 (0.13-0.22)
	28d-LC50	9.24 (7.95-10.8)	87.5 (75.2-102)	0.38 (0.33-0.44)
<i>Dugesia japonica</i> (Turbellaria)				
- Asexual reproduction	28d-EC10	9.18 (7.02-13.6)	85.9 (65.3-129)	0.38 (0.29-0.56)
	28d-EC50	10.4 (9.71-14.4)	97.1 (90.9-136)	0.43 (0.40-0.59)
- Survival	28d-LC10	17.2 (13.9-18.5)	163 (132-174)	0.71 (0.57-0.76)
	28d-LC50	20.9 (19.0-28.5)	197 (179-267)	0.86 (0.78-1.17)
<i>Hirudo nipponia</i> (Hirudinea)				
- Relative biomass decrease	28d-EC10	3.24 (2.97-3.52)	31.0 (28.3-33.7)	0.13 (0.12-0.15)
	28d-EC50	7.17 (6.84-7.52)	68.8 (65.6-72.2)	0.30 (0.28-0.31)
- Survival	28d-LC10	11.8 (9.67-16.6)	114 (92.8-162)	0.49 (0.40-0.68)
	28d-LC50	12.6 (10.0-17.2)	122 (96.2-168)	0.52 (0.41-0.71)

3 Results

3.1.1 The Chinese dataset

All 28d-L(E)C₁₀ and 28d-L(E)C₅₀ values (and 95% confidence intervals) of the 8 benthic macroinvertebrates and the measured endpoints from the experiments conducted in China are shown in Table 2.

SSDs were constructed with 28-d EC₁₀ and 28-d LC₁₀ values from the fludioxonil-spiked sediment toxicity tests as input. For each species and the different sublethal endpoints measured we selected the lowest 28-d EC₁₀ value.

The SSD constructed with 28d-EC₁₀ estimates expressed in terms of fludioxonil concentration in total dry sediment and in pore water showed the following order: *C. fluminea* < *B. sowerbyi* < *H. nipponia* < *L. hoffmeisteri* < *D. japonica* < *P. acuta* < *N. denticulata* < *D. pelochloris* (Fig. 2A&B). The calculated HC₅ concentrations were 0.57 (0.15 – 3.1) mg fludioxonil/kg DW sediment (Fig. 2A; Table 3), 0.024 (0.0067 – 0.12) mg fludioxonil/g OC in dry sediment (Table 3) and 5.4 (1.5 – 28) µg fludioxonil/L pore water (Fig. 2B; Table 3).

In Fig. 2C&D the SSDs constructed with 28d-LC₁₀ values for the 8 invertebrates from China are presented. The 28-d EC₁₀ for the endpoint emergence of *D. pelochloris* was also considered to be the 28-d LC₁₀ value for this species, since after 28 days no surviving larvae could be found in the sediment anymore. The SSD with 28d-LC₁₀ estimates expressed in terms of total sediment concentration showed the following order: *C. fluminalis* < *N. denticulate* < *D. japonicus* < *H. nipponia* < *L. hoffmeisteri* < *B. sowerbyi* < *P. acuta* < *D. pelochloris*, while HC₅'s of 1.5 (0.48 – 6.8) mg fludioxonil/kg dry sediment (Fig. 2C; Table 3) and 0.062 (0.018 – 0.28) mg fludioxonil/g OC in dry sediment (Table 3) were calculated. In the SSD with 28d-LC₁₀'s expressed in terms of pore water concentration, the sensitivity order of species slightly shifted in that *H. nipponia* and *L. hoffmeisteri* were more sensitive than *D. japonicus*: the corresponding HC₅ was 14 (4.2 – 65) µg fludioxonil/L pore water (Fig. 2D; Table 3).

3.1.2 The dataset from the Netherlands

28d-L(E)C₁₀ and 28d-L(E)C₅₀ values derived from fludioxonil-spiked sediment toxicity test with the benthic species *Lumbriculus variegatus* (Oligochaeta; Lumbricidae), *Tubifex tubifex* (Oligochaeta; Tubificidae), *Corbicula fluminalis* (Mollusca; Cyrenidae), *Pisidium amnicum* (Mollusca; Sphaeriidae), *Asellus aquaticus* (Crustacea; Isopoda), *Hyaella azteca* (Crustacea; Amphipoda), *Chironomus riparius* (Insecta; Chironomidae) and *Caenis hoiraria* (Insecta; Ephemeroptera) are reported in detail by (Brock et al. 2020). The design of these toxicity test differed from those conducted in China in the type of sediment used, namely field-collected sediment with an OC content of 1.85%. To make the sediment-spiked toxicity tests between China and the Netherlands more comparable we normalized the toxicity values, that were expressed by (Brock et al. 2020) in terms mg a.s./kg DW sediment, to sediment with an OC content of 2.43% (similar to that of the Chinese toxicity tests) by applying a multiplication factor of 2.43/1.85 = 1.31. Alternatively, we also expressed the toxicity values in mg a.s./kg DW sediment (with an OC content of 1.85%) reported in (Brock et al. 2020) in terms of mg a.s./g OC in dry sediment.

SSDs constructed with (OC normalized) 28d-EC₁₀ or 28d-LC₁₀ values of the dataset from the Netherlands resulted in the HC₅ and HC₅₀ values reported in Table 3. These HC₅ and HC₅₀ values are consistently higher than the corresponding values from the Chinese dataset. For example, the median HC₅ and median HC₅₀ values derived from SSDs constructed with 28d-EC₁₀'s from tests performed in the Netherlands were, respectively, a factor of approximately 7 and 3 higher than corresponding values derived from tests performed in China. However, in all cases the 95 confidence bands of corresponding HC_p values overlapped between the datasets from China and the Netherlands (Table 3).

3.1.3 The combined datasets from China and the Netherlands

The SSDs constructed with (OC normalized) 28d-EC₁₀ or 28d-LC₁₀ estimates are presented in Figure 3. In all these SSDs the most sensitive species tested, *C. fluminea*, can always be found in the tail of the curve. The OECD standard benthic test specie *C. riparius* and *L. variegatus* are positioned relatively high in the SSD curves, but this is generally the case for all benthic invertebrates in the dataset from the Netherlands.

The calculated HC₅'s derived from SSDs constructed with 28d-EC₁₀ values for 16 different benthic invertebrates were 1.2 (0.51 – 3.5) mg fludioxonil/kg DW sediment (Fig. 3A; Table 3), 0.050 (0.021 – 0.13) mg fludioxonil/g OC in dry sediment (Table 3) and 11 (4.1 – 31) µg fludioxonil/L pore water (Fig. 3B; Table 3). Overall, the median HC₅ values from the combined datasets were a factor of 2 higher than the corresponding median HC₅ values from the Chinese dataset (Table 3).

The calculated HC₅ values derived from SSDs constructed with 28d-LC₁₀'s for 16 different benthic invertebrates were 2.0 (0.69 – 7.1) mg fludioxonil/kg DW sediment (Fig. 3C; Table 3), 0.083 (0.030 – 0.29) mg fludioxonil/g OC in dry sediment (Table 3) and 27 (11 – 78) µg fludioxonil/L pore water (Fig. 3D; Table 3). The median HC₅ values of the combined datasets based on lethal endpoints did not deviate substantially from those based on sublethal endpoints. These HC₅ values expressed in terms of total sediment concentration deviated less than a factor of 2, while that was slightly more than a factor of 2 for HC₅'s expressed in terms of pore water concentration (Table 3). Overall, differences between median HC₅'s from SSDs constructed with 28d-EC₁₀'s and 28d-LC₁₀'s of the Chinese dataset were slightly larger than for the combined datasets (Table 3).

4 Discussion

The coastal regions of China experienced an unprecedented rate of conversion to agricultural, urban, and industrial land uses (Wang et al. 2012; Zhang et al. 2010). It is reported that benthic macroinvertebrate assemblages are good indicators for assessing ecological quality in surface waters of river basins in China (Liu et al. 2016). For this reason we selected benthic test species for our sediment-spiked toxicity tests that are more or less typical for edge-of-field surface waters in the Yangtze river-delta. In our sediment-spiked single-species toxicity tests with fludioxonil, the Asian clam *C. fluminea* appeared to be the most sensitive benthic species tested (Fig. 2 and 3). *C. fluminea* also is an economically important aquatic species in China and has been used as a test organism in many environmental studies (Chen et al. 2013). It is a suspension feeder that filter-feeds on unicellular algae, bacteria and detrital particles.

The SSD curves presented in Figures 2 and 3 confirm the biocidal property of the fungicide fludioxonil in that the toxicity values of different taxonomic groups result in a nice fit of the SSD curves, while the position of different taxonomic groups is more or less randomly distributed in the SSD curves.

Comparing the SSD curves constructed with toxicity data performed in China and the OC normalized toxicity data from the Netherlands, it appears that median HC_p values derived from the Chinese dataset are overall lower, although the 95% confidence bands overlapped (Table 3). The fact that Chinese data overall resulted in lower median HC_p values might have several reasons. The most obvious reasons likely concern differences in species and life-stages tested, assessment endpoint selected and differences in sediment properties. The sublethal assessment endpoints selected for HC₅ calculation in the study from the Netherlands (Brock et al. 2020) were 'relative rate in daily dry weight biomass increase' for the six non-insect taxa and 'emergence' for the two insect taxa. In our study the selected sublethal assessment endpoints for HC_p calculation were more diverse, including reproduction, length increase, emergence and wet weight endpoints (see Table 2). Another difference is that in all the single-species tests conducted in China (this study) artificial sediment was used, while the sediment-spiked toxicity tests of (Brock et al. 2016) used field-collected sediment. This may have caused differences in bioavailability of fludioxonil in the sediments used and/or in environmental conditions (e.g. microbial community; different types of organic material) that affect growth and sensitivity of test organisms. Studies that compared the sensitivity of the standard benthic test species *Chironomus riparius* between tests that used artificial and field-collecting sediment reported a higher sensitivity in artificial sediment spiked with pyrethroids (Åkerblom et al. 2008; Fleming et al. 1998) but a lower toxicity in artificial sediment spiked with the benzoyl insecticide lufenuron (Brock et al. 2016). Results of sediment-spiked toxicity tests with the oligochaete *Lumbriculus variegatus* and fludioxonil revealed an 28d-EC₁₀ of 107 (53-225) µg/L pore water in artificial sediment (unpublished data Wageningen Environmental

Research) and of 208 (123-313) $\mu\text{g/L}$ pore water in field-collected sediment (Brock et al. 2020), suggesting a somewhat higher toxicity in artificial sediment although the 95% confidence bands overlapped. Another difference between our experiments performed in China and those of (Brock et al. 2020) in the Netherlands is that we used fludioxonil-spiked sediment characterized by an ageing period of 2 days before introducing test organisms, while the sediment-spiked toxicity tests of (Brock et al. 2020) adopted an ageing period of 7-8 days when the sediment was not in the freezer. (Brock et al. 2020) stored the fludioxonil-spiked sediment in a freezer for approximately a year before use in laboratory toxicity tests and it was assumed that ageing processes were stopped at temperatures of -20°C . The relatively short ageing period in our tests performed in China may have caused an initial higher bioavailability of fludioxonil in the total sediment and sediment pore water, compared to that of the sediment used in the tests conducted in the Netherlands. A decline in bioavailability of persistent organic chemicals due to ageing processes is reported in the scientific literature (Alexander 2000; Choi et al. 2012; Landrum et al. 1992; Nybom et al. 2015).

The data presented in this paper do not allow to conclude that Chinese benthic macroinvertebrates in general are more sensitive to sediment-exposure of fludioxonil than Dutch benthic macroinvertebrates. For an appropriate comparison similar external test conditions (e.g. artificial sediment; similar ageing periods and sediment storage practices) and effect assessment endpoints need to be adopted.

The HC_5 values obtained from the SSDs constructed with 28d-EC_{10} 's from fludioxonil-spiked sediment toxicity tests with benthic invertebrates might be used as effect estimate for sediment-dwelling invertebrates in the regulatory risk assessment procedure. As an estimate for the regulatory acceptable concentration (RAC) in the sediment compartment either the median HC_5 divided by small AF (e.g., an assessment factor (AF) of 3 was proposed by (EFSA, 2015) or the lower limit of the 95% confidence band of the HC_5 might be used. Note that the median HC_5 is the concentration that protects with 50% certainty, 95% of the benthic invertebrates tested, while the lower limit HC_5 is the concentration that protects with 95% certainty, 95% of the benthic species tested. Particularly in the case that a relatively large number of 28d-EC_{10} values for different benthic species are used in the SSD approach (e.g. the combined dataset from China and the Netherlands), selecting the lower limit HC_5 as RAC may be the best option. The RAC thus obtained (median HC_5/AF or lower limit HC_5) should be larger than the long-term predicted environmental concentration (PEC) in the upper layer of the sediment compartment. Note that in the risk assessment procedure the ecotoxicologically relevant concentration (e.g. freely dissolved concentration in sediment pore water or total concentration in sediment) as well as the relevant sediment depth (e.g. upper 1 cm of sediment layer) for the PEC estimation needs to be set a priori. In addition, the OC carbon content of the sediment layer for which the PEC is estimated, should be normalized to the OC content of the sediment used in the toxicity tests, or the other way around.

Declarations

CRediT authorship contribution statement

J Sun conducted most laboratory toxicity tests in China; XH Yin and J Sun designed these experiments; PF Xiao, GN Zhu and K Zhang assisted in the experiments and in raising funds; XH Yin and Theo Brock analyzed most of toxicity data; XH Yin and J Sun wrote earlier drafts of the manuscript and Theo Brock contributed substantially by editing these drafts.

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Tables

Table 1: Species name, origin, life-stage and testing conditions for selected benthic species in the 28-d sediment-spiked toxicity tests with fludioxonil using artificial sediment.

Taxon	Origin test species	A: Mean length in mm	No of replicate test systems to study treatment related effects	Individuals per test vessel(% mortality in controls at day28)	Treatment: Geometric mean test concentrations	DO(mg/L) ± SD
		B: Mean wet weight in mg			A: mg a.s./kg dry weight sediment B: µg a.s./L pore water	PH ± SD Temp(°C) ± SD
<i>Branchiura sowerbyi</i> (Oligochaeta)	Outdoor ditches in Machege	A: not measured B: 52.4 ± 2.1	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-2.48-4.78-9-18.69-36.45 B:0-23.65-45.41-85.36-176.92-345.16	DO = 8.54±0.07 PH = 7.42±0.04 T = 19.88 ± 0.27
<i>Limnodrilus hoffmeisteri</i> (Oligochaeta)	Outdoor ditches in Machege	A: not measured B: 3.74 ± 0.47	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-5.4-10.42-20.44-40.11-80.54 B:0-49.58-95.44-187.12-370.29-736.66	DO = 8.53±0.01 PH = 7.41±0.04 T = 19.9 ± 0.30
<i>Corbicula fluminea</i> (Mollusca)	Purchased commercial culture	A: 2.47 ± 0.1 B: 4.81 ± 0.34	Controls(n = 5) Treatments(n = 5)	10 (8%)	A:0-2.89-5.41-10.42-20.45-40.45 B:0-26.62-49.59-95.44-193.51-383.1	DO = 8.53±0.04 PH = 7.5±0.04 T =19.84 ± 0.22
<i>Physa acuta</i> (Mollusca)	Outdoor ditches in Machege	A: embryo B:	Controls(n = 5) Treatments(n = 5)	20 (13%)	A:0-0.38-0.75-1.47-2.98-5.74 B:0-3.41-6.84-13.72-27.45-52.6	DO = 8.54±0.03 PH = 7.46±0.01 T =19.82 ± 0.23
<i>Dicrotendipes pelochloris</i> (Insecta)	Outdoor ditches in Machege	A: first instar larvae B:	Controls(n = 5) Treatments(n = 5)	20 (18%)	A:0-2.62-5.4-10.42-20.33-40.4 B:0-23.46-45.58-92.44-173.05-358.53	DO =8.55±0.05 PH = 7.44±0.04 T =20.1 ± 0.31
<i>Neocaridina denticulata</i> (Crustacea)	Purchased commercial culture	A:6.77 ±0.11 B: 2.37 ± 0.04	Controls(n = 5) Treatments(n = 5)	10 (2%)	A:0-2.68-5.15-10.36-20.32-40.63 B:0-27.52-51.27-98.69-193.49-383.13	DO = 8.54±0.06 PH = 7.4±0.03 T =19.86 ± 0.27
<i>Dugesia japonica</i> (Turbellaria)	Purchased commercial culture	A: 11.5 ±0.3	Controls(n = 5)	10 (0%)	A:0-1.08-2.24-4.49-9.82-18.66	DO = 8.58±0.07

		B: not measured	Treatments(n = 5)		B:0-10.58-21.38-42.57-91.79-175.95	PH = 7.42±0.06 T =20.12 ± 0.12
<i>Hirudo nipponia</i> (Hirudinea)	Purchased commercial culture	A: not measured B: 271.71 ±10.27	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-1.09-2.33-4.94-9.26-19.42 B:0-10.59-21.88-47.57-88.79-189.25	DO = 8.56±0.06 PH = 7.45±0.06 T =19.76 ± 0.21

Table 2: 28d-L(E)Cx values (and 95% confidence bands) for eight benthic invertebrates in laboratory toxicity tests using artificial sediment spiked with the fungicide fludioxonil. The 28d-L(E)Cx values are expressed in terms of geometric mean fludioxonil concentration during the test in the total dry sediment (mg a.s./kg DW) and in sediment pore water (µg a.s./L). The toxicity values were calculated with the MOSAIC computer program.

Taxon Endpoint	Toxicity value	Total sediment (mg a.s./kg DW)	Pore water (µg a.s./L)	Fludioxonil concentration in (mg/g organic carbon OC)
<i>Branchiura sowerbyi</i> (Oligochaeta)				
• Reproduction (number of cocoons)	28d-EC10	2.56 (1.47-4.14)	25.9 (15.3-40.8)	0.11 (0.06-0.17)
	28d-EC50	7.01 (5.46-8.96)	68.4 (54.0-86.4)	0.29 (0.23-0.37)
• Relative biomass decrease	28d-EC10	5.78 (5.02-6.59)	56.4 (49.5-64.0)	0.24 (0.21-0.27)
	28d-EC50	22.8 (21.2-24.6)	217 (202-233)	0.94 (0.87-1.01)
• Survival	28d-LC10	19.0 (12.7-26.8)	182 (122-253)	0.78 (0.52-1.10)
	28d-LC50	90.1 (59.0-166)	839 (550-1520)	3.71 (2.43-6.83)
<i>Limnodrilus hoffmeisteri</i> (Oligochaeta)				
• Reproduction (number of cocoons)	28d-EC10	11.9 (1.19-36.4)	98.8 (9.86-321)	0.49 (0.05-1.50)
	28d-EC50	35.9 (24.3-50.2)	313 (209-439)	1.48 (1.0-2.07)
• Relative rate in daily biomass increase	28d-EC10	3.64 (2.39 -5.22)	31.2 (20.6-44.8)	0.15 (0.10-0.22)
	28d-EC50	25.3 (22.3-29.1)	221 (194-253)	1.04 (0.92-1.20)
• Survival	28d-LC10	18.3 (13.1-23.7)	158 (112-206)	0.75 (0.54-0.98)
	28d-LC50	56.6 (43.1-87.3)	506 (384-786)	2.33 (1.77-3.59)
<i>Corbicula fluminea</i> (Mollusca)				
• Relative rate in daily length increase	28d-EC10	0.402 (0.296-0.534)	3.75 (2.75-4.97)	0.017 (0.012-0.022)
	28d-EC50	1.58 (1.37-1.82)	14.7 (12.7-16.9)	0.065 (0.056-0.075)
• Survival	28d-LC10	0.791 (0.390-1.42)	7.41 (3.54-13.3)	0.033 (0.016-0.058)
	28d-LC50	4.44 (3.30-6.49)	40.8 (30.5-60.4)	0.18 (0.14-0.27)
<i>Physa acuta</i> (Mollusca)				
• Relative rate in daily length increase	28d-EC10	11.6 (6.7-19.1)	109 (61.8-180)	0.48 (0.28-0.79)
	28d-EC50	143 (101-219)	1390 (972-2120)	5.89 (4.16-9.01)
• Survival	28d-LC10	35.9 (24.8-40.2)	341 (235-400)	1.48 (1.02-1.65)
	28d-LC50	49.4 (41.3-77.6)	465 (391-744)	2.03 (1.7-3.193)

<i>Dicrotendipes pelochloris</i> (Insecta)				
• Emergence	28d-EC10	67.2 (33.3-80.3)	617 (308-733)	2.77 (1.37-3.31)
	28d-EC50	100 (82.2-177)	912 (752-1620)	4.12 (3.83-7.28)
<i>Neocaridina denticulata</i> (Crustacea)				
• Relative rate in daily length increase	28d-EC10	17.5 (15.5-18.6)	166 (147-176)	0.72 (0.64-0.77)
	28d-EC50	19.6 (18.9-21.5)	186 (179-204)	0.81 (0.78-0.89)
• Survival	28d-LC10	4.14 (3.11-5.31)	39.5 (29.3-50.7)	0.17 (0.13-0.22)
	28d-LC50	9.24 (7.95-10.8)	87.5 (75.2-102)	0.38 (0.33-0.44)
<i>Dugesia japonica</i> (Turbellaria)				
• Asexual reproduction	28d-EC10	9.18 (7.02-13.6)	85.9 (65.3-129)	0.38 (0.29-0.56)
	28d-EC50	10.4 (9.71-14.4)	97.1 (90.9-136)	0.43 (0.40-0.59)
• Survival	28d-LC10	17.2 (13.9-18.5)	163 (132-174)	0.71 (0.57-0.76)
	28d-LC50	20.9 (19.0-28.5)	197 (179-267)	0.86 (0.78-1.17)
<i>Hirudo nipponia</i> (Hirudinea)				
• Relative biomass decrease	28d-EC10	3.24 (2.97-3.52)	31.0 (28.3-33.7)	0.13 (0.12-0.15)
	28d-EC50	7.17 (6.84-7.52)	68.8 (65.6-72.2)	0.30 (0.28-0.31)
• Survival	28d-LC10	11.8 (9.67-16.6)	114 (92.8-162)	0.49 (0.40-0.68)
	28d-LC50	12.6 (10.0-17.2)	122 (96.2-168)	0.52 (0.41-0.71)

Table 3: Hazardous concentrations to 5% (HC5) and their 95% confidence bands for fludioxonil of the species tested in China (presented in Figs x and y), the Netherlands (presented in Brock et al. 2020) and China and the Netherlands (presented in Figs x and y). HC5 values were calculated with the computer program MOSAICSSD. The toxicity data of the benthic species tested in the Netherlands expressed in terms of total sediment concentration were normalised to an organic carbon content of

2.43%.

Figures

	Dataset China (this study)	Dataset NL (Brock et al. 2020)	Dataset China + NL (combined)
SSD constructed with 28d-EC₁₀'s			
HC ₅ (mg a.s./kg DW sed.; 2.43% OC)	0.57 (0.15 – 3.1)	4.1 (2.0 – 10.4)	1.2 (0.51 – 3.5)
HC ₅₀ (mg a.s./kg DW sed.; 2.43% OC)	5.9 (2.1 – 15)	17 (9.4 – 29)	9.9 (5.2 – 18)
HC ₅ (mg a.s./g OC)	0.024 (0.007 – 0.12)	0.17 (0.081 – 0.43)	0.050 (0.021 – 0.13)
HC ₅₀ (mg a.s./g OC)	0.25 (0.094 – 0.64)	0.70 (0.39 – 1.2)	0.41 (0.23 – 0.75)
HC ₅ (µg a.s./L pore water)	5.4 (1.5 – 28)	35 (14 – 110)	11 (4.1 – 31)
HC ₅₀ (µg a.s./L pore water)	55 (20 – 150)	170 (87 – 340)	97 (50 – 190)
SSD constructed with 28d-LC₁₀'s			
HC ₅ (mg a.s./kg DW sed.; 2.43% OC)	1.5 (0.48 – 6.8)	4.6 (1.2 – 25)	2.0 (0.69 – 7.1)
HC ₅₀ (mg a.s./kg DW sed.; 2.43% OC)	12 (5.2 – 30)	48 (18 – 127)	25 (12 – 52)
HC ₅ (mg a.s./g OC)	0.062 (0.018 – 0.28)	0.19 (0.048 – 1.03)	0.083 (0.030 – 0.29)
HC ₅₀ (mg a.s./g OC)	0.51 (0.21 – 1.2)	2.0 (0.76 – 5.2)	1.0 (0.49 – 2.1)
HC ₅ (µg a.s./L pore water)	14 (4.2 – 65)	98 (36 – 320)	27 (11 – 78)
HC ₅₀ (µg a.s./L pore water)	120 (48 – 280)	440 (220 – 800)	230 (120 – 430)

Figure 1

Left: A Schematic diagram of the experimental system used. (A, rotary scavenging valve; B, overlying water level; C, upside down plastic bowl used in tests with emerging insects and evaporating water; D, bubble stone; E, sediment; Arrow, direction of air flow.) Right: The experimental systems in incubator.

Figure 2

Species sensitivity distributions constructed with 28d-EC₁₀ values (panels A and B) and 28d-LC₁₀ values (panels C and D) from fludioxonil-spiked sediment toxicity tests with benthic invertebrates conducted in China. Panels A and C present the toxicity data expressed in terms of fludioxonil concentrations in total sediment and those of panels B and D expressed in terms of sediment pore water concentrations. The sediment-spiked toxicity tests were conducted using artificial sediment with an organic carbon content of 2.43%. Hazardous concentrations to 5% (HC₅) and 50% (HC₅₀) of the species and their 95% confidence bands calculated with MOSAICSSD are presented in each panel.

Figure 3

Species sensitivity distributions constructed with 28d-EC₁₀ values (panels A and B) and 28d-LC₁₀ values (panels C and D) from fludioxonil-spiked sediment toxicity tests with benthic invertebrates conducted in China the Netherlands. Panels A and C present the toxicity data expressed in terms of fludioxonil concentrations in total sediment and those of panels B and D expressed in terms of sediment pore water concentrations. In the panels the species tested in the Netherlands are indicated with an asterisk (*). The sediment-spiked toxicity tests from China were conducted using artificial sediment with an organic carbon content of 2.43%. Since the test from the Netherlands were conducted on field-collected sediment with an organic carbon content of 1.85% the toxicity values from the Netherlands in panels A and C were normalized to an organic carbon content of 2.43%. Background information on the sediment-spiked toxicity tests from the Netherlands can be found in Brock et al. (2020). Hazardous concentrations to 5% (HC₅) and 50% (HC₅₀) of the species and their 95% confidence bands calculated with MOSAICSSD are presented in each panel.

Supplementary Files

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- [FludioonilChinaSIAppendixB9feb2021.docx](#)
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