The tendency of linuron in microcosmic system and its response to hydrodynamic conditions

J C Liu[1], Y Sun[1], K K Hou[1], G H Lu[1,2,3] and W Chen[1]

[1]Key Laboratory for Integrated Regulation and Resources Development on Shallow Lakes of Ministry of Education, College of Environment, Hohai University, Nanjing 210098, P R China
[2]College of Hydraulic and Civil Engineering, Xizang Agricultural and Animal Husbandry College, Linzhi 860000, P R China

E-mail: ghlu@hhu.edu.cn

Abstract. The environmental fate and bioaccumulation of linuron in a laboratory microcosm were investigated at three flow velocity over a period of 10 days. Linuron concentrations were quantified in the environmental media water, sediment, algae and fish after 1, 2, 4, 7 and 10 days of exposure. The results showed that rapid decrease in water phase of the microcosm was occurred, linuron had decreased to 56.7%-67.9% of the initial concentrations on day 10, and reduction rates showed an obvious increase with increased flow velocity. The linuron concentrations in the sediment were 1.25-2.13 ng/g on day 10, which were much less than that in algae (387-477 ng/g). The linuron was widely detected in fish tissues, and the bioaccumulation concentrations were generally in the following order: liver > kidney > brain > skin > muscle > gill at an early stage of exposure. However, the ability of liver detoxification increased with the exposure time and exposure flow velocity, so that the bioaccumulation potential was highest in kidney at the end stage of exposure. This result indicates that a significant uptake potential of linuron in organisms, further studies are necessary to elucidate food chain bioaccumulation and toxicological effects of linuron.

1. Introduction

Linuron is a phenylurea herbicide widely used to control weeds by blocking the electron transfer in photosystem II [1]. Because of its good water solubility (75 mg/L), linuron is easily transferred by rainwater or irrigation water from the soil into the receiving water [2]. Therefore, linuron is frequently detected with concentrations blow the μg/L threshold in aquatic environments. For example, in Canada, linuron was detected in the surface water of Ontario [3] and the Lower Fraser Valley region of British Columbia [4] at concentrations of 12 μg/L and 1.05 μg/L, respectively. In the United States, the concentration of linuron in the source water of Missouri was detected at 4.42 μg/L in the irrigation water of southern Florida [5]. Due to the moderate long-lasting properties and sustained input, linuron may pose a threat to aquatic ecosystems. According to the Canadian Water Quality Guideline, linuron of 7 μg/L can cause acute toxicity for aquatic life, including fish and plants [6]. However, limited information exists regarding the bioaccumulation of linuron in fish.

Many studies have shown that linuron has potential toxicological hazards to organisms. For example, feeding linuron to rats resulted in histopathological damage in rat brain tissue [7], and male sexual organ dysplasia in the offspring of rats [8]. Feeding low concentrations of linuron to adult female Xenopus laevis resulted in changes in plasma hormone levels and toxic effects on the ovary [9].

Studies have also shown that linuron is an endocrine disruptor in fish, amphibians and mammals based on binding competitive on the androgen receptors [10]. Therefore, the potential risks of linuron to ecosystems are needed for further research. Laboratory microcosm is both realistic and reproducible. Many biochemical and ecological interactions occur simultaneously within microcosm, involving multiple biotic and abiotic compartments, thus microcosm can be used to predict the states of actual aquatic pollution [11].

The focus of this study was to investigate the fate of linuron in microcosm compartments, including freshwater and sediment, primary producer algae, and higher trophic organism fish. The main thrust of this paper is twofold. First, we investigated the distribution of linuron in environmental media. Second, we determined the bioaccumulation of linuron in fish tissues over a period of 10 days. Finally, the results of this study can provide a scientific basis for ecological risk assessment of linuron.

2. Materials and methods

2.1. Materials and test organism

Linuron (>98.5%) was obtained from Sigma-Aldrich (Flanders, New Jersey, USA). Methanol (>99.9%, HPLC grade) was purchased from Merck Serono Co., Ltd. (Darmstadt, Germany). Ultra-pure water used for LC-MS/MS analysis and extraction procedures was purified with a Milli-Q integral water purification system (Millipore, Milford, MA, USA). The freshwater algae (*Microcystis aeruginosa*) was purchased from Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB-collection, Wuhan, China), and inoculated in BG-11 medium. The specific BG11 medium can be found in previous study [12]. Crucian carp (30.5 ± 2.5 g, 10.0 ± 1.5 cm) were purchased from the Nanjing Institute of Fishery Science (Nanjing, China).

2.2. Microcosm design

A water-sediment-algae-fish microcosmic system mimicking a local lake field was established using a glass container (85×30×40 cm^3). Surface water and sediment were collected from two drinking water sources of Taihu Lake, as reference sites. 4.5-5.0 cm sediment was homogenously placed on the bottom, and then slowly filled with 100 L of freshwater. The microcosm systems were operated and maintained for 14 days at 20 ± 1°C before the experiment begins.

The system was automatically controlled by an electrical pump, which was used to lift water from reservoir to the microcosm systems. Three flow velocity gradients were designed as follows: V1= 0.01 m/s, V2 = 0.05 m/s and V3 = 0.10 m/s. The tanks were monitored every day for water quality parameters (pH and temperature). Small living organisms were protected by water filtration systems with 40 μm mesh. After the microcosm systems become stable, fish and algae were placed in the ecological system. During the experiment, the initial concentration of linuron in the aqueous phase was maintained at 10 μg/L (the methanol content was less than 0.001%). The experimental microcosm setups were exposed to linuron for 10 days, and samples of water, sediment, *M. aeruginosa* and crucian carps were collected on days 1, 2, 4 and 10, respectively. At the same time, 1 L of linuron solution at a concentration of 10 μg/L was placed in the same position as the microcosmic system for the control.

2.3. Sample extraction

Water sample and algal cell sample: The exposure liquid collected at each time point was divided into liquid supernatant and algal cell by centrifugation at 1000 ×g for 10 min. The supernatant was extracted using a solid-phase extraction (SPE). The procedure for SPE was as follows: water samples were passed through C18 SPE cartridges (500 mg, 6 mL, Waters), which were preconditioned with 6 mL methanol and 6 mL ultrapure water. Water sample flowed through extraction column at rate of 3-5 mL/min. After then the column was rinsed with 10 mL of ultrapure water, dried for 30 min under negative pressure. Finally, the target was eluted with 4 mL of acetonitrile/methanol (1:1, V/V) solution. The extracts were evaporated to dryness with nitrogen, and the dry residues were reconstituted with 1

mL methanol. The algae cell samples were resuspended by distilled water and separated, repeated twice. Then the algae samples were added 15 mL of 75% (v/v) acetone-water and extracted by ultrasonic for 20 min. The above steps were replicated three times. The collected supernatant was removed with acetone on a rotary evaporator at 40°C. The residues were extracted and purified using SPE. The detailed procedure is similar to the treatment of water samples.

Biological tissue sample: The crucian carps were anesthetized with 0.1% MS-222, and sacrificed by cervical section. The brain, liver, gill, kidney, muscle and skin tissues were excised, and washed with 0.15 M KCl solution. Tissue samples were extracted by an ASE 350 PLE system (Thermo Fisher, Germering, Germany). The tissue sample was mixed with 6 g of aluminum oxide. The mixed matrix was put into a 22 mL extraction cell. The cell was filled with hydromatrix, and positioned in the PLE system. The extraction solvent was 100% methanol. The operating conditions were as follows: extraction pressure, 1500 psi; temperature, 100°C; preheating time, 5 min; static extraction time, 5 min; washing volume, 60%; nitrogen purge, 90 s; and static cycle, 3 times. After extraction, the extract was concentrated to about 1 mL using a quantitative concentrator. The concentrated solution was stored in the freezer at -80°C for 4 h. After the frozen, the solution was centrifuged at 0°C and 12000 ×g for 15 min to remove lipids. The supernatant was transferred to a brown chromatographic flask and prepared for analysis.

Sediment sample: Sediment samples were centrifuged at speed of 4000 ×g for 5 min, and then freeze-dried, ground, placed in PP bags. Processed sample was positioned in the system of ASE 350, and the extraction method was same as the fish tissue sample.

2.4. Instrument analysis and quality control

The linuron was measured by Waters Acquity ultra-high performance liquid chromatograph (UPLC) coupled with a Waters Acquity Xevo TQ triple quadrupole mass spectrometer (MS/MS). A Waters BEH C18 column (2.1 mm × 100 mm, 1.7 μm) was used to chromatographic separation with the column temperature of 40°C and flow rate 0.4 mL/min. The injection volume was 5 μL. A binary gradient consisting of A (0.1% formic acid + 2% methanol in ultrapure water) and B (0.1% formic acid in methanol) was used for the separation. The gradient began with 90% A and 10% B for the first 0.25 min, increasing to 50% B in 1 min, and subsequently 50% B rose to 98% in 3.0 min and held for 1.0 min, returning to the initial chromatographic conditions within 6.0 min. Multiple reaction monitoring (MRM) mode was used to identify and quantify linuron. The collision voltage, collision energy, precursor, and production ions optimized using a standard compound linuron were 25, 18, 249.1 and 160.1/181.1, respectively.

The limit of detection (LOD) and the limit of the quantitation (LOQ) were used to assess the method sensitivity of linuron. The LOD and LOQ of linuron in water samples were 0.22 and 0.72 ng/L, respectively. For sediment samples, LOD and LOQ of linuron were 0.67 and 2.16 ng/g (dw), respectively. For biota samples, LOD and LOQ of linuron were 0.22-0.30 and 0.65-0.90 ng/g, respectively. The recovery rates of linuron in water, sediment and biota samples ranged from 75%-98%, and the relative standard deviation was less than 20%.

2.5. Data processing and statistical analysis

All data were normalized and covariance tested using Shapiro-Wilk and Levene test, respectively. Dunnett's analysis in the ANOVA method was used to compare the differences among the data of different groups. When $p < 0.05$, there was a significant difference between the two groups of data. Statistical analysis of the data was processed using SPSS software and SigmaPlot 12.5.

3. Results and discussion

3.1. Linuron level in water phase

Linuron concentrations in water phase of microcosm on days 1, 2, 4, 7, and 10 are presented in figure 1. During the whole exposure period, linuron concentrations had no obvious changes in control group,

ranging from 9.86-10.59 μg/L. According to the figure 1, linuron concentrations declined in all treatments in 1-2 days of exposure, and tended to stable after 4 days of exposure. In order to understand the fate of linuron in the microcosm, we measured the accumulation in biota and adsorption in sediment. The results on the concentrations of linuron in other median indicate the linuron in the microcosm had a rapid migration process from water phase to other media during the early stages of exposure. In addition, the results showed that the greater of relative velocity, the more conducive to the migration of linuron from the water phase into other media and/or degradation by organisms, which suggests that the hydrodynamic conditions affected the migration and transformation of linuron in the microcosmic system.

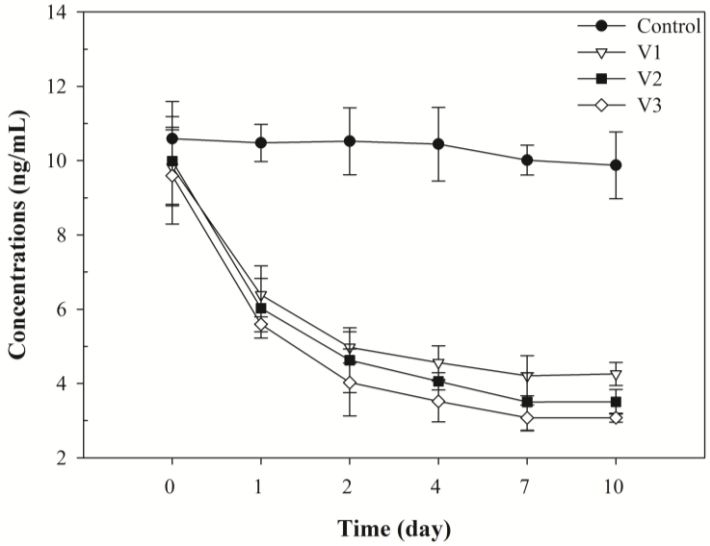

Figure 1. Changes of linuron concentration in the aqueous phase at flow rates V1, V2 and V3 during all exposure periods.

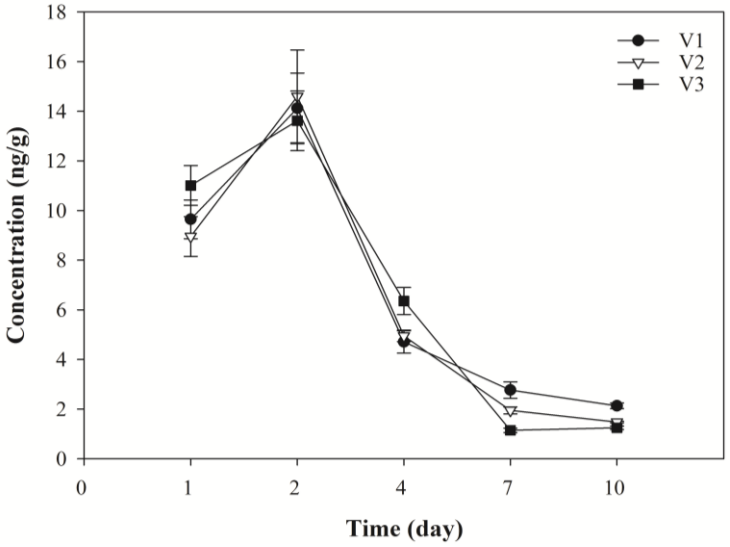

Figure 2. Changes of linuron concentration in sediments at flow rates V1, V2 and V3 during all exposure periods.

3.2. Linuron levels in sediment

The concentrations of linuron in sediment samples on days 1, 2, 4, 7, and 10 are presented in figure 2.

The concentrations of linuron increased gradually in sediment with exposure time to reach their maxima at day 2 for the three exposure groups. The decreased linuron concentrations in sediment after 4 days of exposure might arise from a rapid biodegradation and uptake of aquatic biota. To date, the few studies on the adsorption of linuron in sediment were published. A study on the mineralisation of linuron by *Variovorax sp.* using a microcosm found that linuron has strong adsorption property in sediment, about 30% of linuron migrated from water phase to sediment in 14 days [13]. The adsorbed amount increased with the increasing concentration of linuron in soil, and the distribution coefficient (K_d) of linuron (0.069) values was higher than diuron (0.016) [14].

3.3. Linuron levels in algae cells

The accumulation of linuron by the algae over 10 days is presented in figure 3. A determination of the amount of linuron residues in algae showed that by day 1 the cellular concentration had reached a maximum bioaccumulation potential (1004.6-1244.6 ng/g). However, by day 4, the amount of linuron in algae had decreased sharply, because of the larger cell population and a rapid migration process of linuron from water phase to other media. Up to now, however, very few studies have been reported on the bioaccumulation of linuron on algae. A similar result was reported by Guo *et al* [15], who showed that the concentration of bisphenol A that accumulated in the algae reached a maximum after 2 days of exposure and then decreased following exposure time. On the contrary, accumulation of isoproturon in first 2 days was rather lower, after 2 days, however, accumulation of isoproturon was substantially higher [16]. Anyhow, the results of present study suggest that a certain proportion of linuron in water phase was absorbed by algae, and the accumulation concentrations in algae were slightly negative correlation with flow rates.

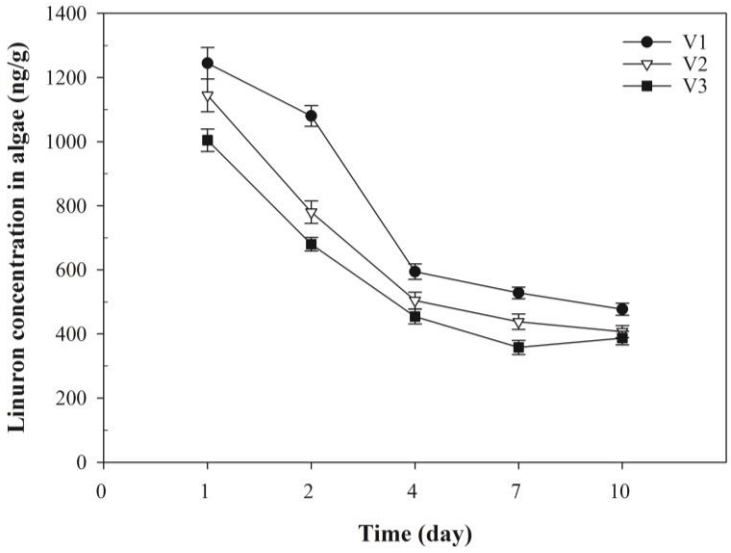

Figure 3. Changes of linuron concentration in algae at flow rates V1, V2 and V3 during all exposure periods.

3.4. Linuron levels in fish

To examine the distribution, figure 4 shows the concentrations of linuron in fish tissues (brain, liver, kidney, gill, muscle and skin) during the whole exposure period. In control group, no linuron was detected in any examined tissues. A similar bioaccumulation pattern of linuron was observed for three treatment group. On day 2, fish exposed to linuron had accumulated high levels, subsequently, the linuron concentrations decreased, and reached to accumulative balance after 7 days. In six fish tissues, the liver showed high bioaccumulative potential, followed by kidney, brain, skin, muscle and gill for the three treatments. The concentrations of linuron were in the range of 2.13 to 28.21 ng/g in the liver,

7.03 to 25.29 ng/g in the kidney, 2.11 to 19.16 ng/g in the brain, 4.20 to 15.68 ng/g in the skin, 3.42 to 11.04 ng/g in the muscle and 0.79 to 7.71 ng/g in the gill. The distribution pattern observed was similar with the accumulation of pharmaceuticals in fish, such as diphenhydramine [17] and roxithromycin [18] in crucian carp.

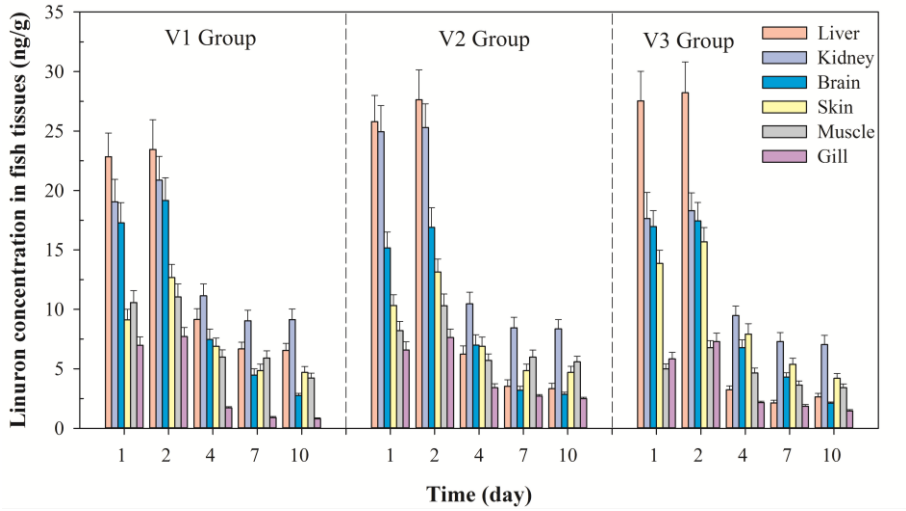

Figure 4. Changes of linuron concentration in fish tissues at flow rates V1, V2 and V3 during all exposure periods.

Similar accumulative potential in different velocity exposure groups indicates that the existing hydrodynamic conditions have few effects on the bioaccumulation of linuron in fish. However, it is important to note that the tissue distribution showed an obvious change with increased exposure time, i.e., the observed kidney concentrations of linuron were higher than other tissues in the accumulative balance period. The most likely explanation for the phenomenon is that the liver and kidney are important detoxifying organs, the metabolism and excretion functions of both tissues were activated by the accumulation of linuron. Thus, metabolites will be produced in the liver, and may be excreted through the kidney.

3.5. The fate of linuron in the microcosmic system

Based on the measured concentrations of linuron in water, sediment and biota samples, the bioaccumulation factor (BAF) of the biota phase and the K_d of the sediment were calculated during adsorption and accumulation equilibrium periods (figure 5). In the present study, the average K_d values of linuron in sediment were 0.58, 0.49 and 0.39 for V1, V2 and V3 treatment groups, respectively. For algae, the average BAFs of linuron were 118.80, 120.57 and 121.10 for V1, V2 and V3 treatment groups, respectively. In fish tissues, the BAFs of linuron were 0.20-2.15, 0.74-2.40 and 0.77-2.33 for V1, V2 and V3 treatment groups, respectively. The results showed that the strong bioaccumulation for linuron in algae, followed by fish and sediment. These BAF values of linuron in algae were lower than that reported by other study, where the BAF value of linuron was 758 in green algae *Chlorella fusca* [19]. Limited experimental data for bioaccumulation of linuron exists, the few studies conducted to date for linuron suggest low accumulative potential in fish, the BAFs were usually below 2400 for linuron [20]. The BAFs of linuron in biota were well below the current regulatory criteria (5000) for a chemical to be considered bioaccumulative [21]. These low BAFs in fish coincide with the low octanol-water partition coefficients and moderate-high water solubility for linuron. However, the field BAF is more complicated than that in the laboratory because of diet and potential biomagnification. For the same species and compound, the BAFs in the field are usually 100-1000 times larger than that in laboratory [22,23]. There is an apparent absence of studies reporting BAFs in field fish in the currently available scientific literature. In addition, a trend was found that as

the flow rate increases, the adsorption potential of sediment decreases, however, which was not observed in algae and fish.

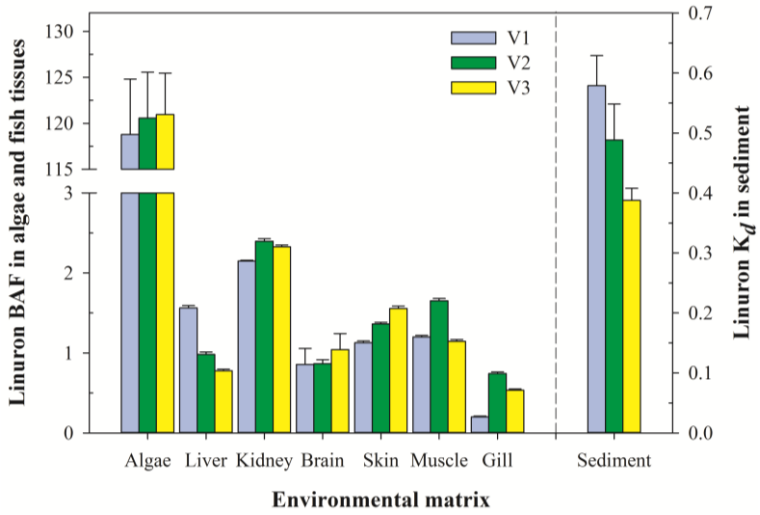

Figure 5. BAF and K_d values of linuron in multiple environmental matrix at flow rates V1, V2 and V3 during adsorption and accumulation equilibrium periods.

4. Conclusion

A water-sediment-algae-fish microcosmic system imitating a shallow lake was created to investigate the distribution and fate of linuron for the concentration of 10 μg/L over a period of 10 days. In the microcosmic system, linuron had a significant migration and transformation, and reached to the equilibrium state at day 7. A high amount of linuron was transferred from water phase to other environmental media, and the linuron level was the highest in the algae with the BAFs 118.8-121.1. The relatively high levels in fish liver at an early stage of exposure maybe suggest that metabolism is one of the major elimination routes. Compared to the V1 group, linuron concentrations in all environmental media showed lower levels in the V3 group, indicating that the hydrodynamic conditions (increased flow velocity) could promote rapid linuron degradation and fish detoxification.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant 51609066, 51769034), the National Science Funds for Creative Research Groups of China (Grant 51421006), the Fundamental Research Funds for the Central Universities (Grant 2018B14714), China Postdoctoral Science Foundation (Grant 2018M630507), and the PAPD.

References

[1] Pascal-Lorber S, *et al* 2010 *J. Agr. Food Chem.* **58** 10935-44
[2] Paule A, *et al* 2015 *Water Air Soil Pollut.* **226** 3
[3] Frank R, *et al* 1990 *B. Environ. Contam. Tox.* **44** 410-9
[4] Woudneh M B, *et al* 2009 *J. Environ. Qual.* **38** 940-7
[5] Schuler L J and Rand G M 2008 *Arch. Environ. Contam. Tox.* **54** 571-83
[6] Marlatt V L, *et al* 2013 *Comp. Biochem. Phys. C* **157** 24-32
[7] Topal A, *et al* 2017 *Environm. Toxicol. Phar.* **49** 14-20
[8] Ding H, *et al* 2017 *Toxicol. Lett.* **266** 49-55
[9] Orton F, *et al* 2009 *Environ. Sci. Technol.* **43** 2144-50

[10] Wilson V S, *et al* 2007 *Environ. Toxicol. Chem.* **26** 1793-802
[11] Al-Khazrajy O S A and Boxall A B A 2016 *J. Hazard. Mater.* **317** 198-209
[12] Ding J, *et al* 2017 *Ecotox. Environ. Safe.* **142** 102-9
[13] Satsuma K 2010 *Pest Manag. Sci.* **66** 847-52
[14] Wang D, *et al* 2015 J. *Environ. Sci. Heal. B* **50** 544-51
[15] Guo R, *et al* 2017 *Environ. Pollut.* **227** 460-7
[16] Bi Y F, *et al* 2012 *J. Hazard. Mater.* **243** 242-9
[17] Xie Z, *et al* 2016 *Sci. Total Environ.* **544** 400-9
[18] Liu J, *et al* 2014 *Chemosphere* **99** 102-8
[19] Manthey M, *et al* 1993 *Sci. Total Environ.* **134** 453-9
[20] Marlatt V L and Martyniuk C J 2017 *Comp. Biochem. Phys. C* **194** 9-21
[21] Arnot J A and Gobas F A P C 2006 *Environ. Rev.* **14** 257-97
[22] Liu J, *et al* 2015 *Sci. Total Environ.* **511** 54-62
[23] Liu J, *et al* 2018 *Ecotox. Environ. Safe.* **154** 214-20