

The Enrichment and Distribution of Trifloxystrobin in Medaka Fish

Xiyuan Chang^{1,2}, Jiangfei Wang³, Xiaoguang Feng³, Xiangning Chen^{1,2}, Yuqi Zhou^{1,2}, Huijun Liu^{1,2*}

¹Food Science and Engineering College, Beijing University of Agriculture, Beijing 102206, China

²Beijing Key Laboratory of Agricultural Product Detection and Control for Spoilage Organisms and Pesticides, Beijing 102206, China

³Beijing Yunong High Quality Cultivation of Agricultural Products Company, Beijing 101400, China

Abstract. In order to evaluate the safety of trifloxystrobin in environment and non-target organisms, the enrichment and distribution of trifloxystrobin in Medaka (*Oryzias latipes*) were studied in this experiment. Medaka were exposed in the water containing 0.1 µg/L, 1 µg/L, 10 µg/L and 100 µg/L trifloxystrobin continuously for 21 days. The results indicated that in female, the concentration order was as follows: fat > liver > intestine > gonad > flesh; in male, the concentration order was as follows: fat > intestine > liver > flesh in the low concentration exposure group with 0.1 µg/L and 1 µg/L, while the concentration order was as follows: fat > liver > intestine > flesh in the high concentration exposure group with 10 µg/L and 100 µg/L. This study provides important data support and theoretical basis for predicting the behavior of trifloxystrobin in the environment, evaluating the environmental safety of trifloxystrobin and guiding the use of drugs.

1 Introduction

Pesticides enter the natural water body through direct spraying, cleaning of spraying equipment, surface runoff and other ways to pollute the water source. As the top layer of the aquatic food chain, fish can enrich the concentration of pollutants through water and plankton ingestion, so as to indicate the pollution status of harmful substances in the surrounding waters. Trifloxystrobin is a kind of strobilurins, which is widely used in fruits, vegetables, wheat, rice and other crops, and has good control effect on many kinds of fungal diseases. The results of environmental toxicology study showed that trifloxystrobin was highly toxic to a variety of non-target aquatic organisms. The 96 h-LC₅₀ of trifloxystrobin to zebrafish (*Brachydanio rerio*) was 5.40×10^{-2} mg a.i.·L⁻¹, and the 96 h-LC₅₀ of *Xenopus laevis* tadpole was 8.95×10^{-2} mg a.i.·L⁻¹. In the investigation of pesticide pollution in surface water, researchers frequently detected trifloxystrobin residues, with the highest concentration up to 0.73 µg/L [1]. Although trifloxystrobin at this concentration will not directly cause fish death, it can be enriched and metabolized in fish, distributed in different parts of fish, damaging muscle, liver, gonad and other tissues and organs, affecting biological growth, development and reproduction.

2 Materials and Reagent

2.1 Materials

Medaka (*Oryzias latipes*) belongs to d-rR strain. The water for aquaculture and exposure is tap water filtered by activated carbon and treated by chlorine exposure, with pH of 7.2-7.6, water hardness of 44.0-61.0 mg CaCO₃/L, water temperature of 25±1 °C, light dark ratio of 16:8, and dissolved oxygen content of not less than 7 mg/L. Feed small-sized commercial bait for once a day, twice for newly hatched larvae of *Artemia*, and regularly clean up fish manure and food residues.

2.2 Reagents

The trifloxystrobin standard (99.0%) was provided by Jiangsu Changqing Agrochemical Co., Ltd. Analysis of pure acetone, acetonitrile, ethyl acetate and petroleum ether were purchased from Beijing Chemical Works.

3 Experimental Methods

3.1 Biological enrichment and distribution

Dynamic test method was adopted. Healthy and active adult Medaka fish with body length (4.0±1.0) cm were selected, and four groups with exposure concentration gradients of 0.1 µg/L, 1 µg/L, 10 µg/L and 100 µg/L trifloxystrobin were set. Each group cultured 15 pairs of Medaka (15 males and 15 females respectively). Each group was treated with two repetitions. At the same time, the control treatment without pesticide was set to remove the background value. Using a running water device, the volume of aquaculture water was 15 L, and the daily flow water volume was 15 L during the test, 100 ml of

*Corresponding author's e-mail: huijunliu78@163.com

aquaculture water was taken from each test group once a week, so as to detect the actual exposure concentration of trifloxystrobin. After 21 days of exposure, the fish for the experiment were taken, the water on the surface of the fish was dried with absorbent paper, then dissected, the fish intestines, fat, liver, gonad and the remaining flesh were taken and weighed respectively, and the residues of trifloxystrobin in different organs and tissues were detected.

3.2 Test for the determination of Trifloxystrobin in aqueous solution

20 ml of trifloxystrobin solution was taken and put into a 150 ml triangular flask, added 40 ml of ethyl acetate, extracted it by ultrasound for 20 min, transferred it into a separating funnel, added saturated sodium chloride solution, stood for layering, taken 2 ml of organic layer, dried it with nitrogen, fixed volume with 2 ml of acetonitrile, filtered through 0.22 µm membrane, and tested by LC-MS/MS.

3.3 Test for the determination of Trifloxystrobin in fish

All kinds of fish organ and tissue samples was taken, homogenized them, transferred them into 50 ml polypropylene centrifuge tube, added 5 ml water, 20 ml petroleum ether : ethyl acetate = 1:1, extracted it by ultrasound for 20 min, centrifugation at 6000 rpm for 5 min. Taken 2 ml of supernatant, dried it with nitrogen, fixed volume with 2 ml of acetonitrile, added 10 mg of PSA, shaken for 30 s, stood for 5 min, filtered through 0.22 µm membrane, and tested by LC-MS/MS.

4 Data analysis

The content of pesticide in the water of the control group was used to correct the content of pesticide in the water of the fish culture group, and the actual value of pesticide intake was calculated. At the end of the test, the change of pesticide content in water and fish has reached equilibrium,

then the enrichment coefficient of pesticide in fish is calculated as follows:

$$BCF = \frac{C_{fs}}{C_{ws}} \dots\dots\dots(1)$$

In the formula:

BCF—Bioconcentration factor

C_{fs}—The pesticide content of fish in equilibrium, with unit for mg/kg

C_{ws}—The pesticide content in the water at equilibrium, with unit for mg/L

5 Results and Discussion

5.1 The distribution of gliostatin in different tissues of medaka fish

During the exposure period, the measured concentrations of trifloxystrobin were 0.13±0.06 (0.1 µg/L), 1.15±0.16 (1 µg/L), 10.06±1.38 (10 µg/L) and 90.21±3.45 (100 µg/L). Trifloxystrobin enters into the body of Medaka through respiration, feeding and body surface penetration, and accumulates in the body of Medaka. After the exposure test, the fish for the test was taken, the fat, intestines, liver, flesh and gonad tissues of Medaka was taken respectively after dissection, and measure the residual amount of trifloxystrobin was measured. The test results are listed in Table 1. It was found that the residues of trifloxystrobin in different tissues were different, with the highest in fat and the lowest in fish. The distribution of trifloxystrobin in different tissues was different with gender and exposure concentration. In female, the order of concentration of trifloxystrobin in different tissues was as follows: fat > liver > intestine > gonad > flesh. In male, the concentration of trifloxystrobin in lower 0.1 µg/L and 1 µg/L exposure groups was as follows: fat > intestine > liver > flesh. In higher 10 µg/L and 100 µg/L exposure groups, the concentration of trifloxystrobin was as follows: fat > liver > intestine > flesh.

Table 1. Residue of trifloxystrobin in different tissues of Medaka

Organ / Tissue	Exposure concentration (µg/L)	Residual amount of female fish (mg/kg)	Residual amount of male fish (mg/kg)
Fat	0.1	0.106±0.009	0.116±0.010
	1	0.173±0.011	0.183±0.012
	10	0.567±0.035	1.991±0.101
	100	0.990±0.072	2.672±0.134
Intestine	0.1	0.024±0.003	0.049±0.005
	1	0.039±0.005	0.084±0.006
	10	0.293±0.014	0.487±0.037
	100	0.515±0.035	0.675±0.056
Liver	0.1	0.064±0.005	0.019±0.003
	1	0.087±0.008	0.063±0.005
	10	0.486±0.051	0.508±0.044
	100	0.763±0.052	1.313±0.117
Flesh	0.1	0.012±0.002	0.009±0.001
	1	0.027±0.002	0.026±0.003
	10	0.177±0.018	0.161±0.012
	100	0.391±0.025	0.199±0.014
Gonad	0.1	0.019±0.003	/

After the pollutants are intaken into the fish, they can be transported to different tissues of the body through the lymphatic system and blood circulation system, and can be metabolized and biotransformed in different parts. The physiological characteristics of target tissue, such as the difference of fat content and metabolic potential, result in the different absorption and removal rates of pollutants, and become the key factor for the different distribution of pollutants in different tissues.

Trifloxystrobins are lipophilic organic compounds. In fish, lipophilic organic pollutants diffuse from water to gill and are absorbed by the fish, then transfer from viscera to body, preferentially accumulate in fat[2]. The concentration enrichment of fish intestine comes from diet and liver transport[3]. The longitudinal extension of the liver surrounds the whole intestine and stomach, and the Omni-faceted connection between the liver and the outside of the intestine and stomach increases the ability of the liver to absorb the pollutant metabolites through ingestion, resulting in the liver becoming the largest target tissue except fat. Liver is the detoxification and storage organ of fish, which may participate in the metabolism of pollutants and enrich a large number of pollutants, and help to distribute toxic substances and their metabolites to other tissues and organs[4]. In addition, the liver provides a large number of non-specific enzymes, which have a wide range of metabolic effects on organic matter[5-6]. The liver and intestine have obvious metabolic effects on trifloxystrobin at a lower concentration, but when the male fish ingests a higher dose of trifloxystrobin, due to the similar metabolic rate, the concentration of trifloxystrobin that needs to be metabolized is higher, and the metabolic effect appears to be weakened, leading to the liver of the male fish at a lower concentration. The concentration in viscera was lower than that in fish intestine, while that in high concentration was higher than that in fish intestine. Gonadal tissue is related to the growth and reproduction of fish, and its concentration is lower than that of fish

intestine and higher than that of flesh. In the acute exposure of lipophilic substances, muscle is not the target organ, with the lowest concentration. Ballesteros et al.[7] hold that the lowest concentration of endosulfan in flesh may be due to the low fat content in flesh.

In fat and intestines, the concentration of trifloxystrobin in male was higher than that in female, while in flesh, the concentration in male was lower than that in female. In the liver tissue, the concentration of trifloxystrobin in females was higher than that in males in the 0.1 µg/L and 1 µg/L exposure groups, while in the 10 µg/L and 100 µg/L exposure groups was the opposite. Lee et al.[8] believed that the gender difference in the absorption and excretion of pollutants in the fathead minnow was due to the change of organic anion transfer channels induced by sex hormones, which led to the gender difference in the absorption and excretion of pollutants in the fish, including renal tubules, so that the removal rate of pollutants in the female was much higher than that in the male. Ankley et al.[9] also believed that the gender difference of chemical excretion rate resulted in the difference of pollutant concentration in male and female fish.

5.2 Bioenrichment coefficients of medlfish in different tissues

After 21 days of exposure, BCF (*Bio-concentration factor*) for different organs and tissues of Medaka is shown in Table 2. BCF of Medaka is 4.3-815 in female and 2.2-894 in male. Because of the same exposure concentration, BCF of trifloxystrobin in various tissues was the same as that of enrichment concentration, and the BCF was the highest in fat and the lowest in fish. BCF of Medaka in different tissues was higher than that in high concentrations at low trifloxystrobin concentration, which was consistent with the results of Yu Yanyan et al.

Table 2. BCF of trifloxystrobin in different organs/tissues of medaka

Tissue	Exposure concentration(µg/L)	Female BCF	Male BCF
Fat	0.1	815.4±69.2	894.7±76.9
	1	150.7±9.6	159.6±10.4
	10	56.3±3.5	197.9±10.0
	100	11.0±0.8	29.6±1.5
Intestine	0.1	184.5±23.1	380.0±38.5
	1	33.7±4.3	72.9±5.2
	10	29.2±1.4	48.4±3.7
	100	5.7±0.4	7.5±0.6
Liver	0.1	489.7±38.5	148.0±23.1
	1	75.5±7.0	55.0±4.3
	10	48.4±5.1	50.5±4.4
	100	8.5±0.6	14.6±1.3
Flesh	0.1	91.9±15.4	66.7±7.7
	1	23.9±1.7	22.5±2.6
	10	17.6±1.8	16.0±1.2
	100	4.3±0.3	2.2±0.2
Gonad	0.1	146.2±23.1	/

There was a good exponential correlation between the logarithm value of trifloxystrobin exposure concentration and BCF (Tables 2-3). Yu Yanyan et al. found that the BCF of PFTriDA in the same organ of Medaka decreased

with the increase of exposure concentration, and there was a significant logarithmic linear correlation between the exposure concentration and BCF. Similar phenomena were found in BCP, 2, 4-BCP, TBT and 4-NP, which is an

important factor affecting the evaluation of field bioaccumulation and ecological effect.

Table 3. Correlation between exposure concentration of trifloxystrobin and BCFs in different

Tissue	Female		Male	
Fat	$y = 207.88e^{-0.641x}$	$R^2 = 0.9930$	$y = 303.24e^{-0.463x}$	$R^2 = 0.8664$
Intestine	$y = 58.704e^{-0.489x}$	$R^2 = 0.9303$	$y = 113.63e^{-0.563x}$	$R^2 = 0.9566$
Liver	$y = 129.11e^{-0.583x}$	$R^2 = 0.9607$	$y = 74.235e^{-0.325x}$	$R^2 = 0.9213$
Flesh	$y = 34.904e^{-0.437x}$	$R^2 = 0.9568$	$y = 27.864e^{-0.487x}$	$R^2 = 0.9227$

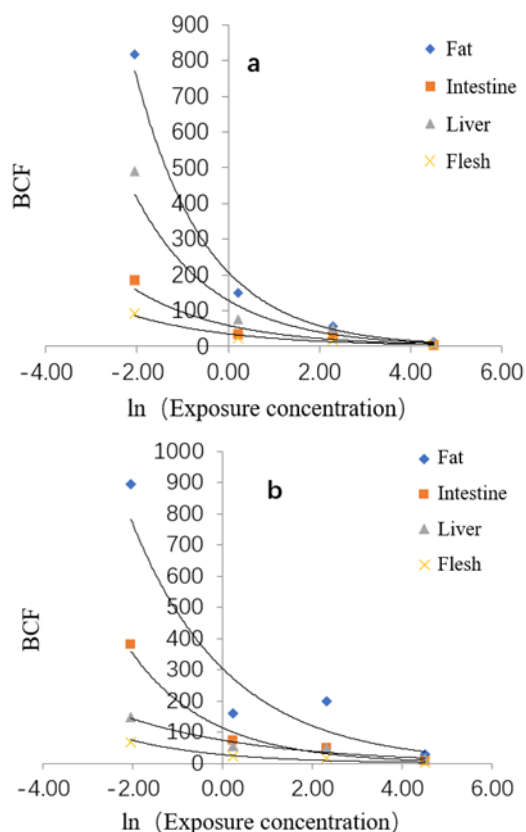


Figure 1. Correlation between exposure concentration of trifloxystrobin and BCFs in different tissues of medaka (a. female; b. male)

6 Conclusions

Bioaccumulation and distribution is very important for the evaluation of pesticide environmental behavior and its subsequent chronic harm. The results showed that the concentration order of trifloxystrobin in different tissues of Medaka was different. In female, the concentration order was as follows: fat > liver > intestine > gonad > flesh. In male, the concentration order was as follows: fat > fish intestine > liver > flesh in 0.1 µg/L and 1 µg/L exposure concentration, and fat > liver > intestine > gonad > flesh in 10 µg/L and 100 µg/L exposure concentration. There are gender differences in the distribution in different tissues of Medaka. In fat and intestines, the concentration

of trifloxystrobin in male was higher than that in female, while in flesh, the concentration in male was lower than that in female. In the liver tissue, the concentration of trifloxystrobin in female was higher than that in male in the 0.1 µg/L and 1 µg/L exposure groups, while that in the 10 µg/L and 100 µg/L exposure groups was the opposite. In the tissues of Medaka, the value of BCF is the same as that of the concentration. In the same tissue, BCF decreased with the increase of exposure concentration, and there was a good exponential correlation between the exposure concentration and BCF. In order to evaluate the environmental safety of trifloxystrobin more comprehensively, systematically and accurately, toxicity tests and environmental behavior of other non-target organisms are needed.

Acknowledgments

This work was supported by the Research Plan Program of Educational Commission of Beijing under Grant SQKM201710020014 and National Natural Science Foundation of China under Grant 31601658 and “Undergraduate graduation design (scientific research) project funding under the cross-cultivation and training program for high-level talents in Beijing institutions of higher learning” (PXM2020_014207_000009).

References

1. Wightwick A M, Bui A D, Zhang P, et al. Environmental fate of fungicides in surface waters of a horticultural-production catchment in southeastern Australia [J]. Archives of Environmental Contamination and Toxicology, 2012, 62:380-390.
2. McIntyre J K, Beauchamp D A. Age and Trophic Position Dominate Bioaccumulation of Mercury and Organochlorines in the Food Web of Lake Washington [J]. Science of the Total Environment, 2007, 372:571-584.
3. Rao D M R, Priyamvada Devi A, Murty A S. Relative toxicity of endosulfan, its isomers, and formulated products to the freshwater fish *Labeo rohita* [J]. Journal of Toxicology and Environmental Health, 1980, (6):825-834.
4. Gonzalez P, Dominique Y, Massabuau J C, et al. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebra fish (*Danio rerio*) [J]. Environmental Science & Technology, 2005, 39 (11) : 3972-3980.
5. Fanta E, Sant’ Anna Rios F, Romão S, et al. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food [J]. Ecotoxicology and Environmental Safety, 2003, 54:119-130.
6. Landis W G, Yu M H. Introduction to Environmental Toxicology: Impacts of Chemicals

upon Ecological Systems, third ed [M]. USA :
CRC Press, 2003.

7. Ballesteros M L, Gonzalez M, Wunderlin M, et al. Uptake, tissue distribution and metabolism of the insecticide endosulfan in *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes) [J]. *Environmental Pollution*, 2011, 159:1709-1714.
8. Lee J J, Schultz I R. Sex differences in the uptake and disposition of perfluorooctanoic acid in fathead minnows after oral dosing [J]. *Environmental Science and Technology*, 2010, 44(1):491-496.
9. Ankley G T, Kuehl D W, Kahl M D, et al. Reproductive and developmental toxicity and bioconcentration of perfluorooctane sulfonate in a partial life-cycle test with the fathead minnow (*Pimephales promelas*) [J]. *Environmental Toxicology and Chemistry*, 2005, 24(9):2316-2324.