Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2020, 12(9): 07-16

Research Article

ISSN: 0975-7384
CODEN(USA): JCPRC5

Investigation of Acute Fish Toxicity of Oxadiazon 25% EC Herbicide in Freshwater Fish (*Poecilia reticulata*)

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ABSTRACT

A Static exposure was performed as per OECD Guidelines for the Testing of Chemicals, Number 203 and EC Directive 92/69/EEC C.1 Acute Toxicity for Fish with oxadiazon 25% EC. Limit test was conducted with two groups, each group containing ten freshwater fish, Poecilia reticulata, in the treatment group fish was exposed to oxadiazon 25% EC at 100 mg (active ingredient)/L (2005.2 mg/L) and concurrently a control was maintained.

Fish were observed daily during the acclimatization period for mortality. After the exposure on day 0 the fish were observed for mortality and toxicity signs approximately at 3rd hour and 6th hour and then once daily at 24, 48, 72 and 96 hour. Based on the results of stability analysis of oxadiazon 25% EC in the exposure medium under test conditions, the test item was stable upto 96 hour exposure period. At 96 hour the recovery percentage was greater than 80%. During the main experiment physic chemical parameters such as pH, temperature, dissolved oxygen, hardness and conductivity were analysed in the exposure water. The temperature of the control and test item concentrations maintained in the range of 21.0°C-21.1°C during the 96 hour exposure period. All fish were normal during the acclimatization period. No mortality and toxicity sign were observed in fish exposed to 100 mg (active ingredient)/L and in control group. Length and body weight of ten fish were recorded prior to acclimatization and on completion of the experiment all surviving fish weight and length was recorded. Analytical dose verification of the test item in the exposure medium samples was carried out in the test item concentration of 100 mg a.i./L. The samples at 0 hour and 96 hour were analysed by HPLC. The mean measured concentration ranged between 90.76% and 90.72% of the nominal concentrations at 0 hour is 89.75% and 89.80% at 96h exposure period.

Keywords: Oxadiazon 25% EC; *Poecilia reticulate*; Acute fish toxicity; HPLC; LC50

INTRODUCTION

Aquatic toxicity has come to be a vital part for the assessment of environmentally unsafe pollutants. Generally, the capability effect of pollution is more for aquatic organisms [1]. The synthetic chemical compounds along with pesticides, insecticides, herbicides are used to control pests or weeds in contemporary agriculture generation for the production of greater food and control of public health. Dependence on pesticides for the pest manipulate has been increasing for the reason that onset of inexperienced revolution in agriculture. This phenomenon is particularly visible in tropical regions, where agriculture has increased dramatically over the previous couple of many years [2]. More than a thousand insecticides presently utilized in maximum of the countries accidentally attain the aquatic ecosystems [3]. These insecticides while applied even in restricted areas are washed off and over excited by using floods and rain to the nearby aquatic system, thereby, posing risk to aquatic biota, specifically fish, which is critical due to its excessive nutritive fee for human intake [4].

(Guppy) Poecilia reticulata, a small benthopelagic, non-migratory fish, occupies a wide range of aquatic habitats, such as estuaries, lakes, ponds, weedy ditches and canals. This species is widely studied as a model species ecology and evolutionary biology, and has had a long and popular history as an ornamental fish. A wide variety of strains differing in color and fin shape have been developed by aquarists (Figure 1) [5,6].

Figure 1. *Poecilia reticulate,* a small benthopelagic, non-migratory fish

Oxadiazon,5-tert-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)- l,3,4-oxadiazol-2(3H)-one is an effective herbicide for control of obnoxious grasses and broad leaf weeds in a wide variety of crops, e.g., citrus fruit, vines, cotton, rice, soya beans and onions [7,8]. Environmental fate studies indicate that oxadiazon persists in the environment bound to organic matter [9,10]. In clear, shallow bodies of water, oxadiazon not bound to organic matter may be degraded by sunlight. Alternatively, oxadiazon is defined as a Light-Dependent Peroxidizing Herbicide (LDPH), which suggests that toxicity is greater in the presence of light. Studies indicate that after application to soil, oxadiazon remains near the surface, and can be transported *via* runoff to nearby surface water bodies. Leaching from surface soils to groundwater is expected to be low or negligible, unless the soil is very porous. Since this stable compound can bind to particulate and organic matter, oxadiazon residues can accumulate in sediments at the bottom of bodies of water.

The current research work was to evaluate the acute toxicity of oxadiazon 25% EC to fish by static exposure, followed by an observation period of 96 hour and to estimate LC50 of the test item to fish (as per OECD 203 and OECD ENV/JM/MONO) [11].

MATERIALS AND METHODS

Materials

Oxadiazon standard was procured from Sigmaaldrich, oxadiazon 25% EC were purchased from market having expiry date of 2 years from the date of manufacturing.

Test System

Freshwater Fish (*Poecilia reticulata*) was procured from Rajamallay Hatchery, Kerala, India, having length of 2 ± 1 cm. The fish supplied was quarantined for 12 days in the laboratory, before they were used for testing and the fish was fed with commercially available aquarium fish feed daily. The fishes are kept under acclimatization for 8 days under laboratory condition. Only fishes without any visible signs of illness were used for the study. Light cycle of 12 hours light and 12 hours dark were maintained in the laboratory. Feed was withdrawn approximately 24 hour before starting the test. During the test the fish was not fed.

Test Conditions

Controlled environment room maintained the range of 21.0-21.4°C for a period of 96 hours in Glass Aquaria. Test conditions for the water (Ground water and Reverse osmosis water was blended in the ratio of 1: 1 v/v) and aerated before use. pH was maintained between 7.53-7.59 and dissolved oxygen was maintained in between 66-67 % throughout the experiment and checked with suitable calibrated instruments and recorded.

Vehicle

Based on the solubility test with distilled water, exposure water was used for the dose formulation.

Preparation of Dose Formulation

The dose formulations were prepared shortly before each exposure. In range finding experiment five different test item concentrations of 1, 25, 50, 75, 100 mg/L were chosen and for 10 L of exposure water 10, 250, 500, 750, 1000 mg were individually weighed and formulated with 10 ml of exposure water and mixed in 10 L of aerated exposure water stirred well with glass rod and for 100 mg (active ingredient)/L, 2005.2 mg of the test item was weighed and it was formulated with 10 ml of exposure water before mixing into the 10 L aerated exposure water.

In the limit test 100 mg (active ingredient)/L (2005.2 mg/L) was weighed and made up to 20 ml with aerated exposure water and mixed in 10 L of aerated exposure water stirred well with glass rod.

EXPERIMENTAL

Control

Control group was maintained along with the treatment group.

Range Finding Experiment

Static exposure was performed and ten fish per group was exposed to the test item. In range finding experiment fish were exposed to a range of concentrations of 1, 25, 50, 75, 100 mg and 100 mg (active ingredient)/L.

Limit Test

Based on the range finding experiment limit test was performed at 100 mg (active ingredient)/L.

Physicochemical Parameters of Exposure Water

Physicochemical parameter such as pH, temperature, dissolved oxygen, hardness and conductivity were analysed in the exposure water. Hardness and conductivity were analyzed at the start of the experiment in control. pH, temperature and dissolved oxygen were monitored daily in control and test concentration in limit test .

Dose Verification Analysis

Prior to the conduct of the main experiment sample was checked for stability analysis for 0 hour and 96 hours. During the conduct of the main experiment, Sample was collected on the 0 hour of exposure and on completion at 96 hour. 100 mL of control, Solvent control, and test (100 mg/L) concentration samples were collected from the aquaria and checked the dose verification with validated analytical HPLC method.

Method Validation, Stability and a.i. Analysis of Oxadiazon HPLC Conditions

Waters Alliance Series with e2695 Separations Module and 2998 Photodiode Array Detector using Empower3 software. The chromatographic separation was achieved using Phenomenex-C18 (250.0 × 4.6 mm, 5 µm) at 30ºC. The mobile phase consists of 0.1% v/v ortho phosphoric acid in water and acetonitrile in the ratio 10:90 v/v. Flow rate was 1.2 mL/min was consistent throughout the analysis and the wavelength was observed at 230 nm. The retention time of oxadiazon is approximately 5.0 min.

Preparation of Oxadiazon Standard Stock Solution

Weighed accurately 5.02 mg of oxadiazon reference standard (purity 99.6 %) in a 25 ml volumetric flask, dissolved, diluted and the volume made up to the mark using acetonitrile. The concentration of the solution was 200 ppm. The linearity solutions were prepared from 200 ppm standard stock solution. The details were given in Table 1.

Table 1. Preparation of linearity solutions

Concentration of stock solution taken (ppm)	Aliquot taken (mL)	Volume made up (mL)	Concentration of diluted solution (ppm)
200	0.5	10	10
200	0.25	10	5
10	1.0	10	1
10	0.5	10	0.5
10	0.1	10	0.1
1	0.5	10	0.05

Preparation of Oxadiazon Sample Stock Solution

Weighed accurately 19.99 mg of oxadiazon sample (purity 25%) in a 25 ml volumetric flask, dissolved, diluted and the volume made up to the mark using acetonitrile. The concentration of the solution was 200 ppm.

Each of these Standard solutions (0.05, 0.1, 0.5, 1.0, 5.0 and 10 ppm) were injected in to HPLC under the given conditions and the peak area for each injection was recorded and a graph of detector response (peak area) *versus* concentration in ppm (µg/mL) was plotted. The values for intercept (a), slope (b), and the linear regression coefficient (r^2) were calculated.

The detector response to varying concentrations of oxadiazon was found to be linear (r2=0.999) in the range of 0.05 to 10 ppm. The plot of concentrations versus detector response along with the regression parameters is attached (Figure 2 and Table 2).

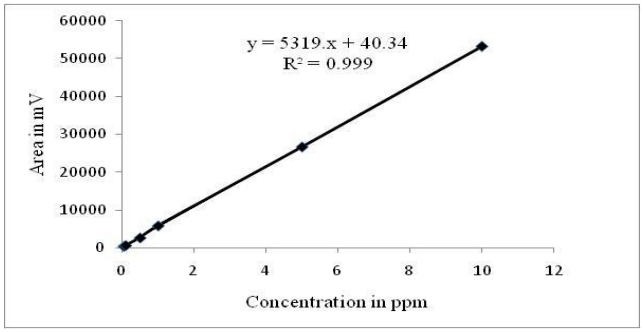

Figure 2. Calibration Curve of oxadiazon Standard

Table 2. Detector Linearity Check for oxadiazon

Concentration (µg/mL)	Peak area counts	Variables
0.05	269	
0.1	506	Intercept=40.34
0.5	2514	Slope=5319
1.0	5694	Linear regression
5.0	26598	coefficient (R^2)=0.999
10.0	54236	

Assay Accuracy and Precision

Fortification was carried out in the control samples at two levels (at 0.05 ppm and 0.5 ppm level). 0.5 mL of 1 ppm standard solution was taken into a 10 mL volumetric flask and made up with solvent control and shaken well for homogenization. 0.5 ml of 10 ppm standard was taken into a 10 mL volumetric flask and made up with solvent control and shaken well for homogenization. The above solution was prepared in five replicates. (Coded as AC1R1, AC1R2, AC1R3, AC1R4, AC1R5 and AC2R1, A2CR2, AC2R3, AC2R4, AC2R5).

The above solutions were injected onto the HPLC along with standard solution (5 ppm). The results are mentioned in Table 3 (Figures 3 and 4).

Table 3. Precision and accuracy

Sample code	Actual concentration in ppm	Recovered concentration in ppm	% Recovery	Mean recovery (%)	SD	RSD
AC1R1	0.05	0.0471	94.20			
AC1R2	0.05	0.0469	93.80			
AC1R3	0.05	0.0474	94.80			
AC1R4	0.05	0.0465	93.00			
AC1R5	0.05	0.0479	95.80	94.32	1.05	1.12

AC2R1	0.5	0.489	97.80			
AC2R2	0.5	0.481	96.20			
AC2R3	0.5	0.485	97.00			
AC2R4	0.5	0.483	96.60			
AC2R5	0.5	0.492	98.40	97.20	0.89	0.92

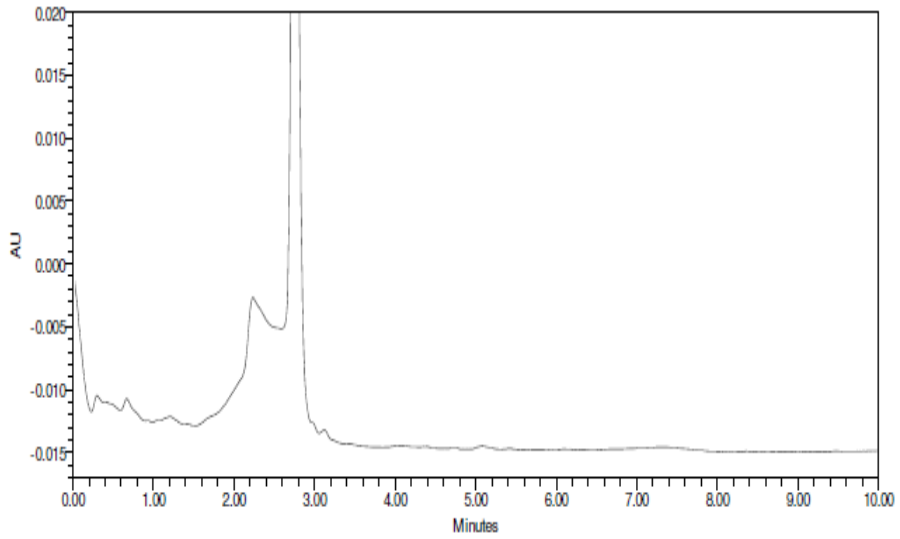

Figure 3. Typical Chromatogram of oxadiazon Control

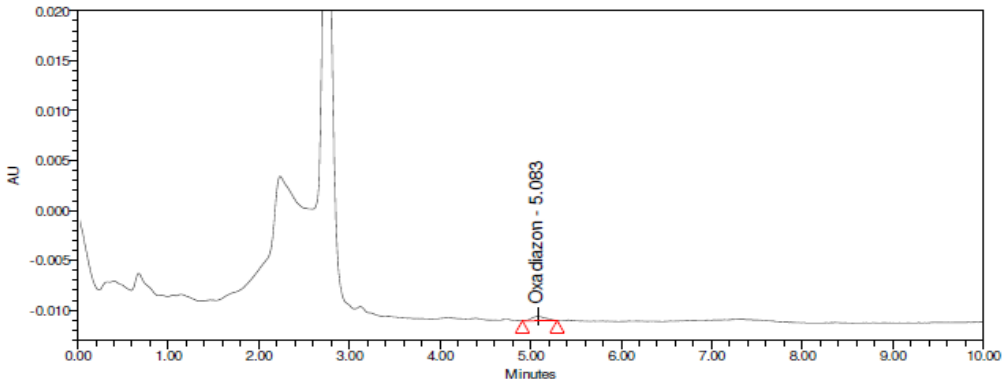

Figure 4. Typical Chromatogram of Oxadiazon 80% WP-recovery-AC1R1

LOQ (Limit of Quantification)

Based on the recovery from Reddy et al. [7] (for 1 ppm fortification), which is 94.32%, 0.05 ppm is considered as LOQ for this method of analysis.

Stability Analysis

'0' hour: Accurately 0.25 mL of sample stock solution was pipette out into a 10 mL volumetric flask and made up to the mark with acetonitrile. The diluted sample was then injected on to the HPLC along with standard solution (5

ppm), control and solvent control (control and solvent control was injected directly), under the stated HPLC conditions.

After 96 hours: Accurately 0.25 mL of sample stock solution was pipette out into a 10 mL volumetric flask and made up to the mark with acetonitrile. The diluted sample was then injected on to the HPLC along with standard solution (5 ppm), control and solvent control (control and solvent control was injected directly) under the stated HPLC conditions. The results are mentioned in Table 4.

Table 4. Stability analysis

Sample Details		**A.I. Content in the sample (ppm)**	**%Deviation from the nominal Concentration**
0 hour	Control	ND	ND
	Sample (5 ppm) R1	4.965	0.70
	Sample (5 ppm) R2	4.959	0.86
	Mean	**4.962**	**0.76**
	Solvent control	ND	ND
Sample Details		**A.I. Content in the sample (ppm)**	**% Deviation from 0 Hour Concentration**
96 hour	Control	ND	ND
	Sample (5.0 ppm) R1	4.912	1.00
	Sample (5.0 ppm) R2	4.898	1.28
	Mean	**4.905**	**1.14**
	Solvent control	ND	ND

A.I. Analysis (Limit Test)

0-hour: The samples were diluted as follows. Accurately 0.5 mL of sample was pipetted out into 10 mL volumetric flask and made up to the mark with acetonitrile. The diluted samples were then injected on to the HPLC under given conditions along with standard solution (5 ppm) under the stated HPLC conditions. The results are mentioned in Table 5.

Table 5. Active ingredient content analysis-0 hour

Sample Code	**Nominal concentration (mg a.i/L)**	**Obtained concentration (mg a.i/L)**	**%Deviation from the nominal Concentration**	**Mean concentration (mg a.i/L)**	**%Deviation from the nominal Concentration**
Control	NIL	ND	ND	ND	ND
Sample R1	100	90.76	-9.24	**90.74**	**-9.26**
Sample R2	100	90.72	-9.28		

ND-Not detected.

96-hour: The samples were diluted as follows. Accurately 0.5 mL of sample was pipetted out into separate 10 mL volumetric flasks and made up to the mark with acetonitrile. The diluted samples were then injected on to the HPLC

under given conditions along with standard solution (5 ppm) under the stated HPLC conditions. The results are mentioned in Table 6.

Table 6. Active ingredient content analysis-96 hour

Sample Code	Nominal concentration (mg a.i/L)	Obtained concentration (mg a.i/L)	% Deviation from 0 Hour Concentration	Mean concentration (mg a.i/L)	% Deviation from 0 Hour Concentration
Control	NIL	ND	ND	ND	ND
Sample R1	100	89.75	-1.11	**89.77**	**-1.07**
Sample R2	100	89.80	-1.02		

ND- Not detected.

RESULTS AND DISCUSSION

Mortality

Range finding experiment was performed with ten freshwater fish in each group.

No mortality was observed in control, 1, 25, 50, 75, 100 mg /L and 100 mg (active ingredient)/L. Limit test 100 mg (active ingredient)/L was performed with ten freshwater fish in each group. No mortality was observed in control and 100 mg (active ingredient)/L (Table 7).

Table 7. Mortality data-limit test

Concentration mg (active ingredient) /L	No.of Fish	Day						% mortality
		0		1	2	3	4	
		3 hour	6 hour	24 hour	48 hour	72 hour	96 hour	
Control	10	0	0	0	0	0	0	0
100	10	0	0	0	0	0	0	0

mg/L: milligram/Litre; No: Number; %: Percentage.

Toxicity Signs

Range finding experiment: No toxicity signs or abnormal behavior were observed in control, 1, 25, 50, 75, 100 mg/L and 100 mg (active ingredient)/L.

Limit test: No toxicity signs or abnormal behaviour were observed in control and 100 mg (active ingredient)/L (Table 8).

Table 8. Toxicity sign-limit test

Concentration mg (active ingredient) /L	No.of Fish	Day					
		0		1	2	3	4
		3 hour	6 hour	24 hour	48 hour	72 hour	96 hour

Control	10	10/10N	10/10N	10/10N	10/10N	10/10N	10/10N
100	10	10/10N	10/10N	10/10N	10/10N	10/10N	10/10N

N: Normal; mg/L: milligram/Litre; No: Number

Physicochemical Parameters

Hardness, conductivity were analyzed at the start of the experiment in control. pH, temperature and dissolved oxygen were monitored daily in control, test concentration during the conduct of the limit test and all were within the limits (Table 9).

Table 9. Physicochemical parameter-limit test

Parameters	**Concentration mg (active ingredient) /L**	**Day**				
		0	**1**	**2**	**3**	**4**
		0 hour	**24 hour**	**48 hour**	**72 hour**	**96 hour**
pH	Control	7.53	7.53	7.55	7.58	7.58
	100	7.53	7.55	7.57	7.59	7.59
Temperature (°C)	Control	21.1	21.1	21.1	21.0	21.0
	100	21.4	21.2	21.1	21.0	21.0
Dissolved oxygen (%)	Control	66.4	66.3	66.2	66.1	66.0
	100	66.8	66.2	66.1	66.1	66.0
Hardness (mg/L)	Control	145	-	-	-	-
Conductivity (µsiemens)	Control	921	-	-	-	-

Key words: - Denotes the parameter was not observed, mg/L: milligram/Litre;°C: centigrade; %: percentage

Dose verification analysis

For the main study the active ingredient content in the samples were found to be 90.76 ppm and 90.72 ppm for replication 1 and 2 respectively at 0 hr and 89.75 ppm and 89.80 ppm for replication 1 and 2 respectively at 96 hr.

CONCLUSIONS

Based on the results, the LC_{50} of Oxadiazon 25%EC observed over a period of 96 hour was found to be greater than 100 mg (active ingredient)/L. The concentration of the test item being tested was satisfactorily maintained and it was above 80 percent of the nominal concentration throughout the test.

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