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Bioconcentration and Metabolism of Diclofop-Methyl in Freshwater Fish (*Oreochromis niloticus*)

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Diclofop-methyl is being increasingly used as an herbicide under intensive cultivation to control weeds in wheat. The bioconcentration of diclofop-methyl in two different groups of freshwater fish (*Oreochromis niloticus*) was investigated after 28 days of exposure. Low and high concentrations applied to two different fish groups were 1/10 (0.19 mg L^{-1}) and 1/3 (0.63 mg L^{-1}) of the determined 96 h-LC_{50}, respectively. The bioconcentration factors for whole fish were 7.05 and 9.21 for fish exposed to lower and higher concentrations, respectively; while the respective bioconcentration factors for muscle tissue were 1.61 and 2.15 LC for fish exposed to lower and higher concentrations, respectively. Diclofop-acid was the main metabolite during the exposure period, ranging concentrations from 5 to 8% of the parent diclofop-methyl concentration.

Keywords: Diclofop-methyl, Herbicide, Bioaccumulation, Fish.

INTRODUCTION

Diclofop-methyl, (methyl 2-[4-(2,4-dichlorophenoxy)-phenoxy]propanoate) is a selective post-emergence phenoxy-herbicide developed for use in control of wild oats, wild millet and other annual grass weeds[1]. It has been newly registered in Egypt as an herbicide. Limited data are available on environmental behaviour of diclofop-methyl. Among the many properties available for describing distributions and environmental behaviour of pesticides, the bioconcentration factor has proven very important as far as the behaviour and fate of water-born chemicals in the aquatic environment is concerned[2]. Aquatic organisms can accumulate chemicals present in the aquatic media. Some chemicals may be found only at low levels in various tissues, whereas others may build up to significant concentrations[3].

Fish are widely used to evaluate the health of aquatic ecosystems and biochemical changes among fishes serve as biomarkers of environmental pollution[4]. The tendency of the organism to bioaccumulate is measured by the bioconcentration factor, which is formally defined as the equilibrium ratio of the concentration of the substance in the exposed organism to the concentration of the dissolved substance bioavailable in the surrounding aquatic environment[5,6]. Fishes with an average lipid content (4.8 %) are good model animals for bioconcentration studies[7]. The objectives of this study were to first determine the 96 h LC_{50} value for diclofop-methyl in tilapia (*Oreochromis niloticus*) and then to assess the bioconcentration and metabolism of the herbicide following 28 days of exposure to two sublethal concentrations.

EXPERIMENTAL

Diclofop-methyl (Fig. 1) (Iloxan 36.0 % EC) was used in this study. The formulation and active ingredient of diclofop-methyl and diclofop-acid were obtained from Bayer (Bayer Cropscience, Gujarat-India). Diclofop-methyl has boiling boint ranged from 370-395 °C, solubility in water found to be 0.39 mg L^{-1} and log P_{ow} = 4.8.

Tested species: A total of 150 specimens of fish, *Oreochromis niloticus* (90 ± 10 g in weight) were obtained

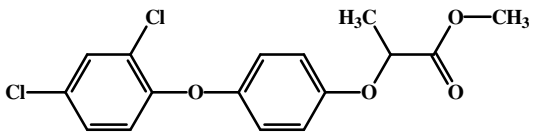

Fig. 1. Structural formula of diclofop-methyl

from Al-Abasa fish farm (El-Sharkia, Egypt). They were randomly placed into glass aquaria (100 L for each 10 fish) and allowed to acclimatize under laboratory conditions for 14 days. The physico-chemical characteristics of water were measured[8] and maintained at optimal mean levels: pH (7.1 ± 0.2 mg/L), salinity (0), dissolved oxygen (8-8.6 mg/L), total alkalinity (256 ± 10.8 mg/L), total hardness as $CaCO_3$ (98.6 ± 2.4 mg/L), calcium (68 ± 6.5 mg/L) and magnesium (18 ±1.2 mg/L). During the experiment, fish were maintained under constant laboratory conditions. Constant and continuous aeration, 12:12 h light/dark cycle, 40-70 % humidity at 27 ± 2 °C. The fish were fed daily with commercial pellets.

Acute toxicity study: The 96 h-LC_{50} value for diclofop-methyl were determined according to Organization for Economic Cooperation and Development (OECD) Guidelines[9] using 60 fish. The LC_{50} value was calculated according to Will (1952)[10]. After 96 h of exposure, the LC_{50} was determined to be 1.89 mg/L.

Bioaccumulation study: Bioaccumulation of diclofop-methyl in various tissues of fish was monitored during exposure period 28-days under flow-through conditions. Ninety fish were divided into three groups of 30 fish each for use in studying bioconcentration and metabolism. Two groups were exposed to diclofop-methyl with high concentration (HC) 1/3 of 96 LC_{50} and low concentration (LC) 1/10 of 96 LC50, (0.63 and 0.19 mg/L), respectively, respectively and the third an unexposed group under flow-through conditions. This study was patterned after the steady-state approach presented by OECD[11].

Residual analysis

Sample preparation: Water samples (500 mL) were collected from low and high concentrations at 1, 7, 14, 28 days during the exposure period. Water samples were extracted according the method described by Madsen *et al.*[12] (2003). Four fish were collected from each group on days 1, 7, 14 and 28 for residual analysis of herbicide and metabolites in both whole fish and muscle tissue. Homogenized samples (10 g) of either whole fish or muscle tissue were placed in 50 mL polypropylene centrifuge tubes to which 20 mL of acetonitrile containing 1 % acetic acid was added. This was centrifuged at 4000 rpm for 10 min. After which 5 mL of supernatant was transferred to a clean 15 mL polypropylene centrifuge tube. Anhydrous $MgSO_4$ (1 g), sodium acetate dihydrate (1 g) and NaCl (1 g) were added, followed by centrifugation for 5 min at 5000 rpm. Three mL of supernatant was evaporated to 1 mL for analysis.

Calibration curve and assay validation: The series of diclofop-methyl and diclofop-acid standard solutions (0.5, 2.5, 5, 10, 50, 100 and 200 mg/L) were prepared in acetonitrile for linearity. Calibration curves were generated by plotting peak area *versus* concentration. All the samples were prepared as previously described. The standard calibration curve presented excellent linearity, with regression coefficient $r^2 > 0.998$. Good separation and repeatability were achieved. The limits of detection were 0.01 and 0.05 ppm for diclofop-methyl and diclofop-acid, respectively. The lowest possible standard on the calibration curve was accepted as the limit of quantification (LOQ). The calibration curve and recovery validation study were all repeated three times (n = 3).

Chromatographic analysis: Samples were analyzed by gas chromatography, using an Agilent 7890 GC (Agilent, USA), coupled with an electron capture detector (ECD) (Agilent, USA) and a capillary column HP-5 (30 m × 0.25 mm × 0.25 µm) (Agilent Technologies, USA). Nitrogen gas was used as mobile phase at flow rate 2 mL/min. The temperature program was started at 180 ºC hold on 1 min and increasing to 220 in rate in 25 ºC min^{-1}, hold on 2 min and increasing to reach 245 ºC in rate 3 ºC min^{-1}. The mean recovery values from spiked samples with standard ranged from 90-93 % for water and 85-91 % for fish samples.

RESULTS AND DISCUSSION

The accumulation of diclofop-methyl residues in two exposed *Oreochromis niloticus* fish groups as well as the concentration of diclofop-methyl in water, muscle tissue and whole fish are shown in Tables 1 and 2. The average measured diclofop-methyl concentrations in water were ranged from 0.19 to 0.23 and 0.6 to 0.68 µg L^{-1} for group1 and group 2, respectively. This is similar to the nominal concentration 0.19 µg L^{-1} for group1and 0.63 µg L^{-1} for group 2. Diclofop-methyl was not detected in control water. Concentration of diclofop-methyl in muscle tissue in both of exposed *Oreochromis niloticus* fish groups significantly increased during the exposure period. The average of diclofop-methyl concentrations in muscle tissue were ranged from 0.006 to 0.34 and 0.008 to 0.43 µg g^{-1} for group 1 and group 2, respectively. However, whole fish concentrations of diclofop-methyl were ranged from 0.12 to 1.36 and 0.36 to 1.8 µg g^{-1} for group 1 and group 2, respectively.

TABLE-1
WATER, MUSCLE TISSUE AND WHOLE FISH CONCENTRATIONS OF DICLOFOP-METHYL IN *Oreochromis niloticus* FISH GROUP 1 (HC, 0.63 mg/L)

Time period	Water concentration (µg L^{-1})	Concentrations (µg g^{-1})			
		Muscle tissue		Whole fish	
		ppm	BCF	ppm	BCF
Initial	0	0.006 ± 0.01	0.02	0.123 ± 0.02	0.63
7	0.19 ± 0.03	0.09 ± 0.05	0.42	0.35 ± 0.04	1.84
14	0.22 ± 0.02	0.21 ± 0.02	0.98	0.98 ± 0.09	5.11
28	0.23 ± 0.05	0.34 ± 0.04	1.61	1.36 ± 0.13	7.05

Data are mean ± standard deviation, n = 3 samples

TABLE-2
WATER, MUSCLE TISSUE AND WHOLE FISH CONCENTRATIONS OF DICLOFOP-METHYL IN *Oreochromis niloticus* FISH GROUP 2 (LC, 0.19 mg/L)

Time period	Water concentration (µg L^{-1})	Concentrations (µg g^{-1})			
		Muscle tissue		Whole fish	
		ppm	BCF	ppm	BCF
Initial	0	0.008 ± 0.01	0.03	0.36 ± 0.07	1.78
7	0.6 ± 0.03	0.16 ± 0.03	0.79	0.52 ± 0.07	2.79
14	0.65 ± 0.04	0.29 ± 0.04	1.46	1.26 ± 0.11	6.42
28	0.68 ± 0.03	0.43 ± 0.08	2.15	1.80 ± 0.15	9.21

Data are mean ± standard deviation, n = 3 samples

The bioconcentration factor of a chemical is the ratio of its concentrations in the organism and in water during steady state or equilibrium. For substances with log K_{ow}-3, the bioconcentration factor calculation shall use equation 74 of the EU technical guidance document on risk assessment (TGD), Part II. Log bioconcentration factor = 0.85 log K_{ow}-0.70[13]. The accumulation of diclofop-methyl residues in two exposed fish groups are shown in Tables 1 and 2 and Fig. 2.

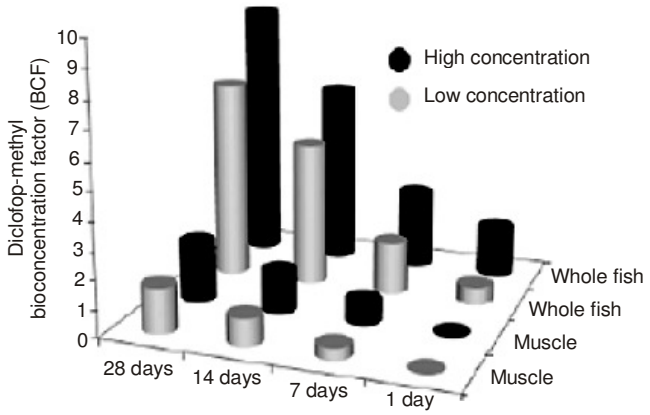

Fig. 2. Diclofop-methyl bioconcentration factor in muscle tissue and whole fish treated with low concentration (0.19 mg L^{-1}) and high concentration (0.63 mg L^{-1})

Results demonstrate that, under the experimental conditions, the concentration of diclofop-methyl in the exposure media remained constant (variations in their nominal concentrations were always below 20 %) within the exposure period 28 days, at the two concentrations evaluated. This type of behaviour has been previously observed in some studies involving atrazine[13].

The accumulation profiles obtained for the two diclofop-methyl concentrations investigated in *Oreochromis niloticus* through the bioaccumulation experiments are shown in Fig. 2. Bioaccumulation results demonstrated that the two diclofop-methyl concentrations were accumulated by *Oreochromis niloticus* fish, a result that would support the potential suitability of this organism to study bioconcentration processes. For a given pesticide, the accumulation rate depended on both the exposure concentration and the exposure time. The bioconcentration factors obtained for diclofop-methyl in the present study showed a good agreement with results found in the literature. bioconcentration factor values of 8.8, using a concentration of diclofop-methyl in the exposure medium of 0.2 µg L^{-1} have been reported for bluegill fish *Lepomis marcochirus*[12].

It could be seen from the results that the bioaccumulation of diclofop-methyl was in order whole fish > muscle. Further, the accumulation in group 2 which exposed to one-third of LC_{50} was higher than group 1 which exposed to one-tenth of LC_{50}, indicating that the positive relation between pesticides concentration and bioconcentration factor in fish. The highest level of accumulation for diclofop-methyl (bioconcentration factor = 7.05 and 9.21) was found in the whole fish for group 1 and group 2, respectively. However, the muscle recorded the low values (bioconcentration factor = 1.61 and 2.15) for group 1 and group 2, respectively. Diclofop-acid was detected in fish samples which collected after 14 and 28 days in both of low and high concentrations treated groups and has been defined as a main metabolite of diclofop-methyl. It was ranged from 4 to 8 % of parent diclofop-methyl in fish tissue samples in both treatment groups. Our results are in line with those of many experiments have been done on herbicides which were showed that the herbicides did not has a strong potential for tissue accumulation in fish. Negligible bioconcentration factors in fish were obtained for 2,4-dichlorophenoxy acetic acid[14], atrazine[8]. However, trifluralin concentrated in fish approximately 1,000 times the water exposure level[8]. Recently more attention has been given to tissue-specific contaminant distribution[15,16]. Variability in the bioconcentration factor can, in some cases, be explained by differences in whole body lipid contents among the test animal or species investigated[14,17,18]. The low bioaccumulation of diclofop-methyl in fish muscle might be due to the low amount of lipids in muscle tissue of *Oreochromis niloticus* fish.

Conclusion

Over a long period, the pollutants present in the environment at very low levels may accumulate within the body of aquatic species by various mechanisms to the extent that they exert toxic effects. Therefore, it is of great importance to know the bioaccumulation potential of a pollutant. To our best of knowledge, diclofop-methyl has few studies which have clearly accounted for the cause and effect regarding suspected diclofop-methyl herbicide contamination and bioaccumulation of the aquatic environment. So in future research, we suggests to consider diclofop-methyl level in the environment, not only direct effects of single parent diclofop-methyl, but also indirect effects caused by its mixtures with other pesticides, including possible pesticide transformation products and numerous other biomarker responses have been widely used in field bio-monitoring studies as well as in laboratory investigations.

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