Research Article

Residue Levels of a Sub Lethal Dose of Organochlorine Endosulfan in Whole Fish of Tilapia Species *Oreochromis niloticus*.

Alim, Dina I.M¹; Abugideiri, Yousif, B²

¹Environment and Natural Resources Research Institute.

²Faculty of Science, University of Khartoum.

Abstract

Residual analysis of a sub lethal dose (0.02mg/l) of the insecticide endosulfan was studied in Nile tilapia species (*Oreochromis niloticus*). The species showed an ability to accumulate both alpha and beta endosulfan reaching maximum after 48 hours' exposure. Beyond which there was a steady elimination of the pesticide until reaching undetectable levels at day 7 for alpha endosulfan and day 9 for beta endosulfan. At day 11 none of the two isomers were detected. Results revealed higher levels of beta isomer relative to alpha isomer. Bioaccumulation Factor (BAF) of Total Endosulfan reached its maximum of 13,860 folds after 48 hours of exposure and minimum of 400 after seven days. At day 11 Endosulfan level was not detected. Following that BAF increased at day 13 to 11,420 folds and then decreased to 8,970 folds at day 15.

Key words: residues, bioaccumulation factor, endosulfan, *Oreochromis niloticus*.

Background

Endosulfan is a chlorinated hydrocarbon insecticide, that belongs to the chemical group of cyclodienes. Technical grade of endosulfan is a 7:3 mixtures of the two isomers alpha and beta endosulfan.

In Sudan endosulfan is used in cotton fields by aerial spraying. This type of application unintentionally introduces this pesticide in the aquatic ecosystems. Although endosulfan is less persistent in the environment, yet it is more toxic to fish when compared to other organochlorine insecticides (Henry et al., 2004). Many investigators worked in tissue residues of pesticides, as indicator to the presence of toxicological actions to aquatic organisms

(Landrum et al., 2003 Henry and Kishimba 2006).

Bioaccumulation of a pesticide is the net accumulation of the pesticide by an aquatic organism as a result of uptake from water through gill membrane and ingestion of contaminated food (Burkhard et al., 2003).

This piece of work aimed at determine residues of alpha and beta endosulfan in the body of tilapia species *Oreochromis niloticus* at different exposure times to a sub lethal dose. It also aimed at interpreting the bioaccumulation factor of the insecticide at different exposure times.

Materials And Methods

Fish in this study were collected from fish ponds at El Shagara Fisheries Research

Station, 10 km south of Khartoum. Fish caught from ponds were transported in large plastic barrels, at early morning to the wet laboratory of the Department of Studies and Development of Natural Resources. In the laboratory fish were kept in glass aquaria, measuring 80×60×60 cm and 60×40×40 cm. Each aquarium was continuously aerated using an air pump and filled with 50 liters of dechlorinated water. Fish were fed once daily at a ratio of 3% of their body weight taking into consideration that no food is given more than could be quickly consumed.

Prior to starting the experiments fish were left to acclimate to dechlorinated tap water, through mixing tap and pond water at ratio of 0:4, 1:3, 2:2, 3:1 leading ultimately to tap water. In each mixture fish were kept for two days. During acclimation period sick fish and ones showing deformation were removed.

The acclimated fish were exposed to a daily sub lethal dose of Endosulfan with active ingredient of 1 µg/L for 15 days. Fish were kept under natural light conditions with continuous aeration of water and left at room temperature. At each time interval of 0,1,2,3,7,9,11,13, and 15 days three fish were scarified. A ten gm amount of each fish tissue was weighed into a beaker containing 50 gms of anhydrous sodium sulphate and mixed thoroughly. The sample mixture was transferred to an extraction thimble and placed in a soxhlet extractor as described by Therdetepitak and Yammeng

(2003). The mixture was extracted for four hours with 150 ml of acetone - normal hexane solvent. The ratio of acetone: n-hexane was 10:90 (v/v). The extracts were then concentrated to about 0.25 ml in a water bath at a temperature of 60 °c.

The 0.25 ml of concentrated extract was applied to a column of 6 cm height of silica gel topped with 1 cm of anhydrous sodium sulphate, which was pre washed with n-hexane.

The column was eluted with diethyl ether: n-hexane (6:94 (v: v), F₁) and diethyl ether: n-hexane (50:50 (v: v), F₂) respectively. Each of the two fractions collected (F₁ and F₂) were kept at a water bath until dryness. After which 5ml of n-hexane was added to each fraction. The two fractions were used for analysis.

A gas chromatography equipped with an electron capture detector (ECD) was used for analysis. Identification of α and β Endosulphan was made on the bases of matching the mass spectrum and retention time of the compound to that of a known standard.

The bioaccumulation factor (BAF) was calculated according to Haque *et al* (1977):
BAF= concentration of chemical in fish/
concentration of chemical in water

Results and discussion

Chromatographic analysis of fish samples at different exposure times, indicated levels of alpha and beta Endosulfan in varying concentrations as shown in table (1)

Table 1. Residues of Endosulfan in whole body of *Oreochromis niloticus* exposed to 0.02mg/L Endosulfan.

Time of Exposure in days	Sample1 (R ₁)		Sample2 (R ₂)		Sample 3 (R ₃)	
	α -Endo (mg/kg)	β -Endo (mg/kg)	α -Endo (mg/kg)	β -Endo (mg/kg)	α -Endo (mg/kg)	β -Endo (mg/kg)
0	0.00	0.00	0.00	0.00	0.00	0.00
1	1.905	4.160	2.670	11.390	0.853	1.895
2	3.450	9.530	2.570	10.429	-	15.59
3	0.790	0.380	1.250	1.410	0.77	2.790
7	-	-	-	1.240	-	-
9	2.350	-	1.760	-	-	-
11	-	-	-	-	-	-
13	0.470	2.46	13.900	14.090	1.80	1.150
15	6.280	3.860	7.974	5.400	0.750	2.640

Fig 1. Mean alpha and beta Endosulfan in *Oreochromis niloticus* exposed to 0.02mg /L in 50% EC.

There were no mortalities observed throughout the whole period of 15 days of exposure. Accumulation of both alpha and beta Endosulfan at the first 24 hours and 48 hours respectively was noted. Beyond which there was a decrease in accumulation until reaching undetectable levels at day 7 for alpha Endosulfan and day 9 for beta Endosulfan (Fig 1). At day 11 none of the two isomers were detected. Results revealed higher levels of beta isomer relative to alpha isomer. Bioaccumulation factor (BAF of total Endosulfan) reached maximum after 48 hours. At day 11 none of the two isomers accumulated as shown in Figure (2).

Fig 2. Variation of bioaccumulation factor values with time in *Oreochromis niloticus* Exposed to 0.002mg/L

Oreochromis niloticus exposed to 0.02 mg /L Endosulfan of technical grade of 50 % EC, exhibited rapid accumulation of the pesticide in the first 24 hours for alpha isomer and 48 hours for beta isomer.

Following that there was a decrease in accumulation until reaching non detectable levels at day 7 for alpha isomer and day 9 for beta isomer. These findings agreed with those of Henry et al. (2003) who worked with Endosulfan and its accumulation in *Tilapia rendalli*. They found that Endosulfan reached undetectable levels after 6 days of exposure after which levels started to increase. In this study similar results were found.

These findings suggest that *Oreochromis niloticus* have a maximum capacity of holding the chemical (Saturation point) beyond which more elimination occurs rather than absorption. This saturation point depends on factors such as species, size, sex and time of exposure. (Henry et al ,2003).

Results showed an unexpectedly higher accumulation levels of beta isomer than alpha isomer at the different exposure times. This higher rate of beta to alpha accumulation could be attributed to the faster conversion of alpha isomer to Endosulfan sulfate Lichtenstein (1980) and Singh et al (1991) stated that the more water solubility and more volatility of alpha Endosulfan has a great impact on its disappearance when compared to beta Endosulfan.

Antonious and Byers (1996) confirmed that beta isomer is more persistent than alpha isomer. Angle et al. (1991) reported that volatility of alpha Endosulfan is twice that of beta isomer.

Bioaccumulation Factor (BAF) of Total Endosulfan reached its maximum of 13,860 folds after 48 hours of exposure and minimum of 400 after seven days. At day 11 Endosulfan level was not detected. Following that BAF increased at day 13 to 11,420 folds and then decreased to 8,970 folds at day 15. Fig (20). These findings indicated that Endosulfan has a moderate

potential for bioaccumulation. Supporting this, Shimmel et al. (1977) found that bioaccumulation factor for fish exposed to 0.014mg/L Endosulfan was between 1000 and 2400; (Henry *et al.* (2003) found that BAF of Endosulfan in *Tilapia renal* was 1,900 folds. They showed that bioaccumulation of Endosulfan depends on the exposure concentration). Veith (1979) found that fish bioaccumulate chlordane 37,800 folds greater than water concentration. Leist and Kramer (1987) showed that alpha and beta Endosulfan are temporary stored in the body of rats.

References:

- Angle**, J. S.; McInTo, M. S. and Hill, R. L. (1991). Tension lysimeters for collecting soil percolate. In Nash, R. S. ed. Groundwater Residue sampling Desing. *American Chemical Society Symposium Series, Washington, DC.*: 290-299.
- Antonious**, G. F. and Byers, M. (1996). Fate and movement of Endosulfan under field conditions. *Environmental Toxicology and Chemistry*. 16: 644-649.
- Burkhard**, L. P., Cook, P. M. and Mount, D. R. (2003). The Relationship of Bioaccumulative Chemicals in Water and Sediment to Residues in Fish: A Visualization Approach. *Environ. Toxicol. and Chem.* 22: 2822-2830.
- Henry**, L and Kishimba, M.A. (2006). Pesticide residues in Nile tilapia (*Oreochromis niloticus*) and Nile Rerch (*Lates niloticus*) from southern Lake Victoria, Tanzania. *Environmental Pollution* .140(2):348-354.
- Henry**, L.; Kishimba, M. A.; Norrgrein, L. and Wandiga, S. O. (2003). Chemodynamics of pure and formulated Endosulfan in *Tilapia* species under laboratory conditions. ANCAP report of the second regional symposium.
- Landrum**, P.F.; Lotufo, G.R.; Gossiaux, D.C.; Gedeon, M.L. and Lee, J.H. (2003). Bioaccumulation and critical body residue of PAHs in the amphipod, *Diporeia* spp: Additional evidence to support toxicity additivity for PAH mixtures. *Chemosphere*.51:481-489.
- Leist**, K. H. and Kramer, P. (1987). Endosulfan active ingredient technical. 30 days feeding study in adult male Wistar rats. *Report No. A30776 Pharma Forschung Toxileologie. WHO.*

- Lichtenstein**, E. P. (1980). Bound residues in soils and transfer of soil residues in crops. *Residue Rev.* 76: 147-154.
- Shimmel**, S. C.; Patrick, Jr. and Wilson, A. J. (1977). Acute toxicity and bioconcentration Endosulfan by estuarine animals in: Aquatic Toxicology and Hazard Evaluation. Mayer, FL and Hamelink J.L. (Eds.). *American Society for Testing and Materials*. pp: 241-252.
- Singh**, N. C.; Dasgupta, T. P.; Roberts, E. V.; Mansingh, A. (1991). Kinetic studies of volatilization and hydrolysis of dieldrin and α - and β - Endosulfan. *J. Agric. Food Chem.* 39: 575-579.
- Therdteppitak**, A. and Yammeng, K. (2003). Determination of Organochlorine Pesticides in Commercial Fish by Gas Chromatography with Electron Capture Detector and Confirmation by Gas Chromatography – Mass Spectrometry. *Science Asia*, 29: 127-134.
- Veith**, E. (1979). In Environmental Protection Agency (1980). Ambient water quality criteria for Chlordane. *EPA*, 440/5-80-02