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Bioconcentration of Fenitrothion in Freshwater Fish (*Oreochromis niloticus*)

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Abstract: Fenitrothion is being increasingly used as an insecticide under intensive cultivation to control vegetables and fruits pests. The bioconcentration of fenitrothion 2 different groups of freshwater fish (*Oreochromis niloticus*) was investigated after 28 days of exposure. One tenth of the determined 96 h-LC_{50} concentrations applied to fish. Fenitrothion concentration was 3.85 mg kg^{-1} and the Bioconcentration Factors (BCFs) after the experimental period 28 days 675.43 for whole fish body while the concentration was 1.51 mg kg^{-1} with respective BCFs was reached 264.91 for muscle tissue were 1.61 after daily exposure during the experimental time 28 days. Results concluded that the fenitrothion has ability to fenitrothion bioconcentration factor was high in whole fish compared to muscle tissue.

Key words: Fenitrothion, insecticide, bioaccumulation, fish, fresh water

INTRODUCTION

The Organophosphorus Pesticides (OPs) are an important group of pesticides used extensively all over the world for >60 years. They have been widely used as an alternative to organochlorine compounds for pest control. About 100 OPS account for w38% of the total pesticide usage. Organophosphorus insecticides represent >80% of total insecticides used in Egypt (Badawy, 1998). However, most of them are highly toxic can exhibit chemical stability and resistance to biodegradation (Chiron and Barcelo, 1993; Harada *et al*., 1987, 1990), due to the probability of their being discharged into aquatic systems, great attention has to be paid to their degradation, to diminish their harmful effects on the environment. The principal degradation pathway for these pesticides involves photolysis, hydrolysis, dehalogenation and oxidation. Photochemical degradation is one of the major transformation processes and one of the factor controlling the fate of pesticides and other chemicals in the environment (Herrmann *et al*., 1999).

Fenitrothion [O,O-dimethyl-(3-methyl-4-nitro phenyl) phosphorothioate an organophosphorus pesticide] is mainly used against spruce bud worms and cotton pests. It is widely used in tropical countries against malaria. Fenitrothion shows low toxicity against mammals (Nishizawa *et al*., 1961). It is one of the most representative and commonly used organophosphorus pesticides (Derbalah *et al*., 2004). Production of fenitrothion is estimated around 15,000-20,000 tons $year^{-1}$ (IPCS, 1992). It is marketed under different trade names, e.g., Sumithion, Novathion and Metathion. It is considered to be a common river water pollutant and its residues in natural water undergo photodegradation,

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resulting in the release of many toxic metabolites some being more toxic than the parent compound to aquatic organisms (Derbalah *et al*., 2004; Amoros *et al*., 2000; Amoroso *et al*., 2000).

Fish are widely used to evaluate the health of aquatic ecosystemsand biochemical changes among fishes serve as biomarkers of environmental pollution (Schlenk and Di-Giulio, 2002). The tendency of the organism to bioaccumulate is measured by the BCF 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 (Mackay and Fraser, 2000; Meylan *et al*., 1999). Fishes with an average lipid content (4.8%) are good model animals for bioconcentration studies (Barron, 1990). The objectives of this study were to first determine the 96 h-LC_{50} value for fenitrothion in tilapia (*Oreochromis niloticus*) and then to assess the bioconcentration and metabolism of the insecticide following 28 days of exposure to two sublethal concentrations.

MATERIALS AND METHODS

Tested pesticide: Fenitrothion certified analytical standard (purity>98%) was purchased from Dr. Ehrenstorfer GmbH, general structure of fenitrothion (Fig. 1) fenitrothion has boiling point ranged from 370-395°C, solubility in water found to be 0.39 mg L^{-1} and $logP_{ow}$ = 4.8.

Chemicals: All organic solvent were HPLC grade and supplied by Merck Ltd. Deionized water was prepared by a Milli-Q water purification system. Anhydrous magnesium sulfate and sodium chloride were of analytical grade and purchased from Merck Ltd. Anhydrous magnesium sulfate was activated by heating at 150°C for 4 h in the oven before use and kept in desiccators.

Tested species: A total of 150 specimens of fish, *Oreochromis niloticus* (90±10 g in weight) were obtained 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 (APHA, 2005) and maintained at optimal mean levels: pH (7.1±0.2 mg L^{-1}), salinity (0), dissolved oxygen (8-8.6 mg L^{-1}), total alkalinity (256±10.8 mg L^{-1}), total hardness as $CaCO_3$ (98.6±2.4 mg L^{-1}), calcium (68±6.5 mg L^{-1}) and magnesium (18±1.2 mg L^{-1}). 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.

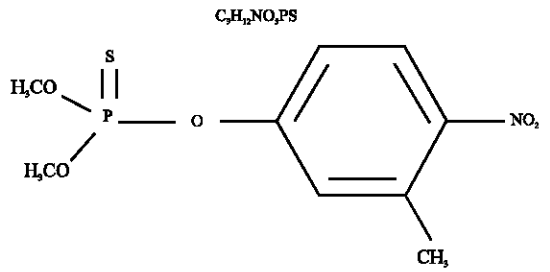

Fig. 1: Chemical structure and chemical formula of fenitrothion

Acute toxicity study: The 96 h-LC_{50} value for Fenitrothion were determined according to Organization for Economic Cooperation and Development (OECD) Guidelines, (OCED, 1992) using 60 fish. The LC_{50} value was calculated according to Weil (1952) . After 96 h of exposure, the LC_{50} was determined to be 1.89 mg L^{-1}.

Bioaccumulation study: Bioaccumulation of fenitrothion in various tissues of fish was monitored during exposure period 28 days under flow-through conditions. Ninety fish were divided into 3 groups of 30 fish each for use in studying bioconcentration and metabolism. Two groups were exposed to fenitrothion with High Concentrations (HC) 1/10 of 96-LC_{50} 0.57 mg L^{-1} and an unexposed group (control group) under flow-through conditions. This study was patterned after the steady-state approach presented by OECD (1996). During experiment whole and muscle fish were taken after 1 (0-time or initial), 3, 7, 14, 21 and 28 days of treatments for residual analysis to investigate the fenitrothion BCF in fish during the experimental period 28 days.

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. Four fish were collected from each group on days 1, 7, 14 and 28 for residual analysis of insecticide 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. The 3 mL of supernatant was evaporated to 1 mL for analysis.

Calibration curve and assay validation: The stock solution of fenitrothion was prepared by dissolving 50 mg of the analyte (accurate weight) in 50 mL n-hexane to obtain concentration 1 mg mL^{-1}. Working standard solutions of 0.05, 0.1, 0.25, 0.5 and 1.0 μg mL^{-1} were prepared by appropriately diluting the stock solution with n-hexane. Stock solution was stored at -20±2°C and working standard solutions were stored in ≤4°C when not in use. Calibration curves were generated by plotting peak area versus concentration.

The series of fenitrothion standard solution (0.5, 2.5, 5, 10, 50, 100 and 200 mg L^{-1}) were prepared in acetonitrile for linearity. Calibration curves were generated by plotting peak area versus concentration. All the samples were prepared as earlierly 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 fenitrothion 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 analysed by gas chromatography using an Agilent 7890 GC (Agilent, USA), coupled with an Flame Photometric Detector (FPD) (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^{-1}. The temperature program was started at 180°C hold on 1 min and increasing to 220 in rate in 25°C min^{-1}, hold on 2 min and increasing to reach 245°C in rate 3°C min^{-1}. The mean recovery values from spiked samples with standard ranged from 90-93% for water and 85-91% for fish samples.

Statistical analysis procedures: In evaluating the data obtained from the bioconcentration test, a steady-state approach was used. This consists of a two compartment model (water and fish) which was used to describe the movement of the test substance into and out of the test fish. This approach was used to determine the steady-state Bioconcentration Factor (BCF), the uptake rate constant (K1) and the depuration rate constant (K2). To exemplify this approach, the following reaction is presented.

In additional, all data from biochemical analysis were subjected to statistical analysis by one-way Analysis of Variance (ANOVA) test (Gad, 1999, 2001) using SPSS Software for Windows Version16.

RESULTS AND DISCUSSION

The accumulation of fenitrothion residues in the exposed *Oreochromis niloticus* fish as well as the concentration of fenitrothion in water, muscle tissue and whole fish are shown in Table 1. The concentration of fenitrothion in muscle tissue of the exposed *Oreochromis niloticus* fish significantly increased during the exposure period. The Bioconcentration Factor of fenitrothion (BCF) in the muscle tissue were reached 28.94, 53.68, 77.36, 159.64 and 264.91 times as the initial amount of pesticide exposure after 3, 7, 14, 21 and 28 days, comparing to the whole fish body concentration which reached to 69.82, 120, 196.49, 391.22 and 675.4 times compared with the initial amount at the same intervals. Fenitrothion was not detected in control water.

The Bioconcentration Factor (BCF) 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 BCF calculation shall use equation 74 of the EU Technical Guidance Document on risk assessment (TGD), Part II. Log BCF = 0.85 log K_{ow} - 0.70 (Veith *et al*., 1979). Results demonstrate that under the experimental conditions, the concentration of Fenitrothion in the exposure media remained constant (variations in their nominal concentrations were always below 20%) within the exposure period 28 days. This type of behavior has been earlierly observed in some studies involving atrazine (Veith *et al*., 1979).

The accumulation profiles of the fenitrothion residue was investigated in *Oreochromis niloticus* through the bioaccumulation experiments are shown in Fig. 2. Bioaccumulation results demonstrated that the fenitrothion residues were accumulated in *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

Table 1: Muscle tissue, whole fish and bioconcentration factor of fenitrothion in *Oreochromis niloticus* fish

		Concentration (μg kg^{-1})			
		Whole fish		Muscle tissue	
Time period	Applied water concentration (mg L^{-1})	PPM	BCF	PPM	BCFm
Initial (1 day)		0.04	-	0.070	-
3	1/10 of 96 h LC50:	0.40	69.82	0.165	28.94
7	0.57 ppm	0.69	120.00	0.306	53.68
14		1.12	196.50	0.441	77.36
21		2.23	391.20	0.910	159.64
28		3.85	675.40	1.510	264.91

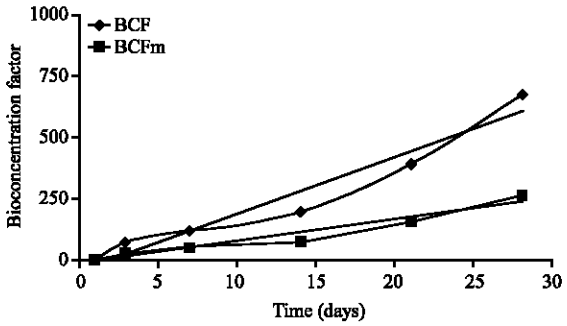

Fig. 2: Bioconcentration Factor of fenitrothion in muscle tissue (BCFm) and whole fish (BCF)

depended on both the exposure concentration and the exposure time. The BCFs obtained for fenitrothion in the present study showed a good agreement with results found in the literature. BCF values of 8.8 using a concentration of fenitrothion in the exposure medium of 0.2 μg L^{-1} have been reported for bluegill fish *Lepomis marcochirus* (European Commission, 2003).

The highest level of fenitrothion was found in whole fish after 28 days of exposure to 1/10 of 96-LC_{50} was 3.85 mg kg^{-1} with BCF was reached 675.4. However, the muscle recorded the low level of fenitrothion concentration was 1.51 mg kg^{-1} with BCF was reached to 264.9 at the same time period 28 days (Fig. 2). Results were indicated that the bioaccumulation of fenitrothion in fish was exposed to one-tenth of LC_{50} was in order whole fish>muscle indicating that the positive relation between pesticides Bioconcentration Factor (BCF) and fish exposure period to the pesticide. The results are in line with those of many experiments have been done on insecticides which were showed that the insecticides did not has a strong potential for tissue accumulation in fish. Negligible bioconcentration factors in fish were obtained for 2,4-D (Sikka *et al*., 1977), atrazine (APHA, 2005). However, trifluralin concentrated in fish approximately 1,000 times the water exposure level (APHA, 2005). Recently more attention has been given to tissue-specific contaminant distribution (Monosson *et al*., 2003; Sapozhnikova *et al*., 2005). Variability in the BCF can in some cases be explained by differences in whole body lipid contents among the test animal or species investigated (Sikka *et al*., 1977; Ferrando *et al*., 1991; (Larsson *et al*., 1991). The low bioaccumulation of fenitrothion in fish muscle might be due to the low amount of lipids in muscle tissue of *Oreochromis niloticus* fish.

CONCLUSION

Generally, from the results researchers are conclude that 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. Finally, because of fenitrothion is common insecticide used in many pests control in Egypt there are few studies have clearly accounted for the cause and effect regarding suspected fenitrothion insecticide contamination and bioaccumulation of the aquatic environment.

So, further research was suggested to consider of this compound in the Egyptian environment not only direct effects of single parent fenitrothion 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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REFERENCES

APHA., 2005. American public health association 133rd annual meeting and exposition. December 10-14, 2005, Philadelphia, PA.

Amoros, I., R. Connon, H. Garelick, J.L. Alonso and J.M. Carrasco, 2000. An assessment of the toxicity of some pesticides and their metabolites affecting a natural aquatic environment using the Microtox™ system. Water Sci. Technol., 42: 19-24.

Amoroso, P.J., G.S. Smith and N.S. Bell, 2000. Qualitative assessment of cause-of-injury coding in U.S. military hospitals: NATO standardization agreement (STANAG) 2050. Am. J. Preventive Med., 18: 174-187.

Badawy, M.I., 1998. Use and impact of pesticides in Egypt. Int. Environ. Health Res., 8: 223-239.

Barron, M.G., 1990. Bioconcentration. Environ. Sci. Technol., 24: 1612-1618.

Chiron, S. and D. Barcelo, 1993. Determination of pesticides in drinking water by on-line solid-phase disk extraction followed by various liquid chromatographic systems. J. Chromatography A, 645: 125 -134.

Derbalah, A.S., N. Nakatani and H. Sakugawa, 2004. Photocatalytic removal of fenitrothion in pure and natural waters by photo-Fenton reaction. Chemosphere, 57: 635-644.

European Commission, 2003. Technical guidance document on risk assessment in support of commission directive 93/67/EEC on risk assessment for new notified substances, commission regulation (EC) No 1488/94 on risk assessment for existing substances, directive 98/8/EC of the European parliament and of the council concerning the placing of biocidal products on the market, Part II. EUR 20418 EN/2, European Commission-Joint Research Centre, Institute for Health and Consumer Protection, European Chemicals Bureau (ECB), Luxembourg. http://ihcp.jrc.ec.europa.eu/our_activities/public-health/risk_assessment_of_Biocides/doc/tgd/tgdpart2_2e.

Ferrando, M.D., E. Sancho and E. Andreu-Moliner, 1991. Comparative acute toxicities of selected pesticides to *Anguilla anguilla*. J. Environ. Sci. Health B, 26: 491-498.

Gad, S.C., 1999. Statistics and Experimental Design for Toxicologists. 3rd Edn., CRC Press, New York, USA., ISBN-13: 9780849331381, pp: 175-200.

Gad, S.C., 2001. Statistics for Toxicologists. In: Principle and Method of Toxicology. Hayes, AW (Eds.). Taylor and Francis, USA., pp: 285-364.

Harada, K., T. Hisanaga and K. Tanaka, 1987. Photocatalytic degradation of organophosphorus compounds in semiconductor suspension. New J. Chem., 11: 597-600.

Harada, K., T. Hisanaga and K. Tanaka, 1990. Photocatalytic degradation of organophosphorous insecticides in aqueous semiconductor suspensions. Water Res., 24: 1415-1417.

Herrmann, J.M., C. Guillard, M. Arguello, A. Aguera, A. Jejedor, L. Piedra and A. Fernandez-Alba, 1999. Photocatalytic degradation of pesticde pirimiphos-methyl determination of the reaction pathway and inentification of intermediate products by various analytical methods. Catalysis Today, 54: 353-367.

IPCS, 1992. Fenitrothion (Environmental Health Criteria 133). World Health Organization, Geneva, Switzerland, ISBN-13: 9789241571333, Pages: 184.

Larsson, P., S. Hamrin and L. Okla, 1991. Factors determining the uptake of persistent pollutants in an eel population (*Anguila anguilla* L.). Environ. Pollut., 69: 39-50.

Mackay, D. and A. Fraser, 2000. Bioaccumulation of persistent organic chemicals: Mechanisms and models. Environ. Pollut., 111: 375-391.

Meylan, W.M., P.H. Howard, R.S. Boethling, D. Aronson, H. Printup and S. Gouchie, 1999. Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coefficient. Environ. Toxicol. Chem., 18: 664-672.

Monosson, E., J.T. Ashley, A.E. McElroy, D. Woltering and A.A. Elskus, 2003. PCB congener distributions in muscle, liver and gonad of *Fundulus heteroclitus* from the lower hudson river estuary and newark bay. Chemosphere, 52: 777-787.

Nishizawa, N., J. Konishike, N. Moroki, J. Okada, Y. Takayama, M. Watanabe and K. Oda, 1961. [Drug-resistance of tubercle bacilli in patients with pulmonary tuberculosis at the time of entering hospital]. Iryo, 15: 212-216.

OCED, 1992. Guidelines for testing of chemicals, No. 203. Organization for Economic Cooperation and Development.

OECD, 1996. Guidelines for testing of chemicals. Proposal for Updating Guideline 305, Bioconcentration: Flow-Through Fish Test, Organization for Economic Cooperation and Development.

Sapozhnikova, Y., N. Zubcov, S. Hungerford, L.A. Roy, N. Boicenco, E. Zubcov and D. Schlenk, 2005. Evaluation of pesticides and metals in fish of the Dniester River, Moldova. Chemosphere, 60: 196-205.

Schlenk, D. and R. Di-Giulio, 2002. Biochemical Responses as Indicators of Aquatic Ecosystem Health. In: Biological Indicators of Aquatic Ecosystem Stress, Adams, S.M. (Ed.). American Fisheries Society, Bethesda, Maryland, pp: 13-42.

Sikka, H.C., H.T. Appleton, E.O. Gangstad, 1977. Uptake and metabolism of dimethylamine salt of 2,4-dichlorophenoxyacetic acid by fish. J. Agric. Food Chem., 25: 1030-1033.

Veith, G., D. DeFoe and B. Bergstedt, 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Canada, 36: 1040-1048.

Weil, C.S., 1952. Tables for convenient calculation of median effective dose (LD_{50} of ED_{50}) and instructions in their use. Biometrics, 8: 249-263.