Acute Toxicity and Lethal Body Burden of Endosulfan in Tilapia (*Oreochromis niloticus (L)*)

Werimo Kenneth[1,*] and Seinen Willem[2]

[1]*Kenya Marine & Fisheries Research Institute, Kisumu Research Centre, P.O. Box 1881, Kisumu, Kenya*

[2]*Institute of Risk Assessment Sciences (IRAS), Utrecht University, The Netherlands*

Abstract: Acute (96-hr) semi-static tests with endosulfan were conducted with Tilapia (O*reochromis niloticus*) by determining LC50 values and their 95 confidence interval end points for 24,48,72 and 96 hr exposure. The LC50 (96-hr) was found to be 10.20μg/L. The behavioral toxicity syndromes corresponded to effects of chemicals that act by specific mode of neurotoxin action.

The lethal body burden/Critical body residue (LBB) in Tilapia was found to be 4.6 ng/g fish and 0.096 μg/g lipid. The LBB values were found to be dependent on time of exposure and concentration. The LBB values found in tilapia may probably not pose a serious health risk, to humans who may consume fish dying from endosulfan poisoning as they are within the range of the tolerable daily intake (TDI) level of endosulfan.

The estimated bioconcentration factor for endosulfan in tilapia was calculated to be 187.

Keywords: Endosulfan Tilapia Acute toxicity lethal body burden, Lake Victoria.

INTRODUCTION

One of the properties which describe the (acute) toxicity to fish is the LC50. In aquatic toxicology LC50 may be defined as the concentration of a compound that causes lethality of 50% of the exposed individuals [1]. The LC50 is a function of the intrinsic toxicity of the substance and of its distribution equilibrium between the organism and its surroundings [2]. LC50 values reflect both bioconcentration potential of a compound and its intrinsic toxicity, i.e. the toxicological potency of the chemical once inside the organism [3-5]. LC50 values for different fishes species may vary widely [6]. LC50 values of endosulfan to Tilapia has been reported to range from 1.42μg/L-10.3μg /L [7], 13.0μg/L [8] to, 1492 μg/L [9]. According to Mc Carty [3] LBB and LC50 are related according to the equation:

$$LBB=BCF*LC50 \quad (1)$$

Where LBB is the concentration of the compound within the organism at the time of death and bioconcentration factor (BCF) is the relation of concentration of the chemical in fish tissue and water at equilibrium.

LC50 tests are however, of limited value since the results are valid only for the species that is tested and the specific conditions used [3]. A major shortcoming is that it does not give information on the concentration of the chemical in the body or more precisely at the site of the toxic action. Several important aspects such as the kinetic behavior, bioavailability and biotransformation can influence the results of the LC50 tests. In short-term experiments with relatively hydrophobic chemicals, steady-state is not achieved and the toxicity may be severely underestimated [10].

A better description of the intrinsic toxicity of chemicals to fish can be obtained by measuring the lethal body burden (LBB)/critical body residue (CBR), i.e. the concentration of the chemical inside the fish at the time of death [11]. Compared to LC50 which relates the acute effect to ambient exposure concentration, LBB/CBR includes bioavailabilty, bioaccumulation and biotransformation [12]. LBB/CBR is independent of exposure concentration and time, if at the moment of death equilibrium is attained in distribution between organism and its surroundings.

During the last 20 years fish from Lake Victoria has found markets in Europe, America, Asia, Japan, Australia and Middle East. This has therefore resulted in a rise in the prices of the fish and the subsequent rise in the demand for the fish by both fishermen and processors. Driven by selfish motives some fishermen were reported to have been using illegal methods of catching fish especially by use of pesticides [13]). One of the pesticides that were identified then as being used to catch fish was endosulfan [13]. The quantities of pesticide used were not always quantified, but volumes of five (5) litre were used by pouring the pesticide in a localized area within the water surface of the lake, and by water currents the lethal compound spreads, thus killing any organism within its contact. The sizes of fish caught were not specific as the compound was discriminately killing any organism it came in contact with. The fat content also varies considerably with respect to species of fish, size and sex .The normal fat content of Nile perch may vary up to 20% with that for tilapia upto 19 % depending on size, sex and diet, etc. Re-

*Address correspondence to this author at the Kenya marine & Fisheries research Institute, Kisumu Research Centre, P.O. Box 1881, Kisumu, Tel: +254 733 914 615/=254 728 570 728; E-mail: werimok@yahoo.com; w.seinen1@uu.nl

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ports of human deaths along the Lake Victoria shores were being attributed to consumption of fish poisoned by endosulfan [14]. However, it was not possible then to quantify the possible amounts of endosulfan that could have been ingested from the poisoned fish. The environmental and health risks associated with this practice calls for proper information on the effects and fate of this chemical on fish, the aquatic system and the consumers, while the environmental fate and toxicity of endosulfan is well described and its specific effects on Lake Victoria fish such as tilapiaare not well documented.

Lake Victoria is the largest tropical lake in the world and comprises of some territory of Tanzania, Uganda and Kenya. It is situated 1134m above sea level and has a surface area of 68,800 km^2. The lake is roughly square in shape, its greatest length and width being about 400 and 320 km respectively. Much of the lake is less than 40 m deep and the deepest part, 60-90m, is in the northeast. The bottom is mainly covered by a thick layer of organic mud, but with parches of hard substrate, sand, shingle or rock. The coastline is indented with many bays and gulfs. The Kagera and Nzoia Rivers are the principal influents, while the only outflow occurs to the River Nile is via Lake Kyoga [15].

Environmental changes have been resulting in various contaminants reaching the aquatic systems and finally to the fish that is harvested. This leads to public health problems especially when the contaminants exceed the minimum residue levels (MRLs). The environmental and health risks associated with there changes require proper information on the types of contaminants, sources and levels, their effects especially on consumers of the aquatic products.

The purpose of the present study was to experimentally determine the LC50 and LBB/CBR of endosulfan in Tilapia *(oreochromis niloticus)* and derive the BCF of the chemical based on equation 1 above and apply the toxicokinetic parameters obtained to assess the potential risk of ingesting fish killed by endosulfan. Tilapia was chosen to give a representative view of Lake Victoria fishes being the most preferred choice by local consumers.

MATERIALS AND METHODS

Test Fish

The fish used in the present study were Nile tilapia (*oreochromis niloticus*) with an average length of 8.36cm (±0.78) and weight of 10.26g (±2.84) and an average lipid content of 4.4% (±1.97). The size of fish used for the experiment is smaller compared to the size caught for consumption, however, the results may be extrapolated to relate with the size normally consumed. They were obtained from commercial grocery in the Netherlands and were acclimatized to the laboratory conditions for two weeks prior to the experiment. During the acclimatization the fish were fed on mosquito larva.

Chemicals

Analytical grade endosulfan, (Thiodan, 99% purity). The compound was a 2:1 mixture of α- and β-isomers. C_{18} part# 1221-3012, phase C_{18}, 40μm, lot No. 070420. All the chemicals were obtained from Riedel-dehean (Ridh laborchemikalien GmbH & Co.KG).

Experiment

A semi-static system was used to expose the fish to the test chemical. 10-L glass aquaria were filled with Utrecht tap water (copper free). The water was continuously aerated. Stock solution of the insecticide was prepared in acetone. This was prepared by dissolving endosulfan (5mg) in acetone (50ml) and the desired concentrations of endosulfan in test water were prepared by pipetting appropriate volumes of this stock solution into test aquarium. Fishes were exposed to five different concentrations i.e. (9.4μg/L, 10.3μg/L,11.4 μg/L, 12.5μg/L and 13.8μg/L) using ten specimens in each tank The test solution was replaced after every 24hrs. Median lethal concentrations (LC50) values were based on nominal (calculated initial) endosulfan concentrations in test solution. Every day, pH, temperature, and oxygen content of the solutions were measured.The physical-chemical parameters of the experimental water were: temperature 24°C(1±°C), conductivity 25S/cm2, pH 7.5, and dissolved oxygen was maintained at more than 8 mg/L.The test organisms were not fed during the test period. Throughout the experiment control was maintained simultaneously.

During the exposure in different concentrations of insecticide, the behavioral changes of the fish were recorded. Number of dead fish were recorded after 24, 48, 72 and 96 hrs. Those fishes which did not show any tactile response were considered dead. The dead fishes were removed from the aquaria immediately after death to avoid depletion of oxygen. They were rinsed with distilled water, weighed and extracted by MSPD method and used for determination of lethal body burden. No death was recorded in the controls.

EXTRACTION, CLEAN-UP AND GAS CHROMATOGRAPHY

Preparation of Sample Extracts

Fish Sample Extracts

Sample extraction was carried out according to the procedure as described by Long *et al.* [16]. Briefly the method employed the Matrix Solid Phase Dispersion (MSPD). In the MSPD approach, 2g of C18 packing was placed in a glass mortar and 0.5g fish sample added on the C18. The fish sample was then gently blended into the C18 with a glass pestle until a homogeneous mixture was obtained.

The resultant homogeneous matrix blend was transferred into a previously prepared 10ml syringe barrel that contained 2g activated Florisil. Two Whatman No. 1.5 filter paper discs with 1.5cm in diameter were placed on the column head and the column was compressed to 7.5ml with a syringe plunger from which the rubber end and pointed plastic portion had been removed. The tip of a 100μL plastic pipette was placed on the column outlet to increase the residence time of the eluting solvents on the column.

Pesticides were eluted with 8ml of acetonitrile into a 10ml conical screwthread disposable glass centrifuge tube (Kimble, Vineland, NJ). A final volume between 3.8-4.4 ml was obtained. The average extraction relative percentage recoveries of 87.4±5.69% (n=4) was obtained. The tube was

Table 1. Mortality Readings at 24,48,72 and 96 hrs Exposure

Concentration/Time	Mortality				
	24 hrs	48 hrs	72 hrs	96 hrs	Total
9.4μg/L	0	0	0	2	2
10.3μg/L	0	2	1	5	8
11.4μg/L	1	0	2	3	6
12.5μg/L	0	3	4	3	10
13.8μg/L	1	3	6	0	10

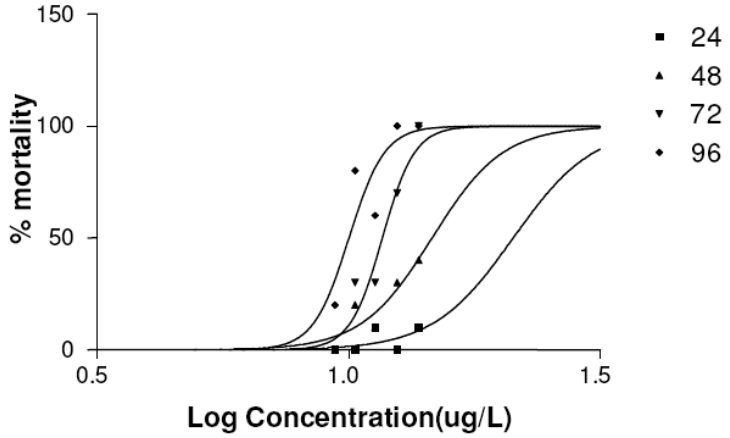

Fig. (1). Dose-response curve for endosulfan to tilapia.

tightly capped, and the contents thoroughly mixed by inverting the tube 3 times. A 2μL portion of the extract was then directly analyzed by gas chromatography with electron capture detector. Detection limit was <0.1μg/L. Fat was determined by soxhlet extraction.

Gas-Liquid Chromatography

Endosulfan residues analysis were carried out using a Carlo Erba GC 8000 Fisons gas chromatogram(GC) equipped with an on-column injector, and electron capture detector (ECD) in a constant current mode, and a capillary fused silica J & W (brand) type DB-5.625 column, (30 m long, 0.25 mm i.d., film thickness(df) 0.25 μm. Detectorbase and detector temperatures were 325 and 365°C , respectively. The oven temperature was held at 80°C for 1 min,subsequently increased by 15°C/min to 275°C,and kept at this temperature for 2 mins. Helium (5.0 pure with pressure of 140kpa) was used as carrier-gas. Detector gas was argon/methane (90:10V/V 5.0 purity) and pressure of 150kpa. Injection was via an autosampler AS 800. Injector volume of 2μl was used. Data system was Fisons Chromcard 3.1 version.

Statistical Analysis

LC50 values for 24, 48, 72 and 96 hr and 95% confidence interval end points were estimated according to OECD test guideline 203. Mean values and standard deviations for LBB/CBR were calculated based on the individual fish LBB/CBR from each group. A linear regression was used to evaluate the influence of exposure time and concentrations on LBB/CBR.

RESULTS

Toxicity: Effects on Behavior

The effects on behavior of the fish in the experiments with endosulfan were similar to and corresponded well to the pattern seen in fathead minnows (*Pimephales promelas*) to neurotic mode of action as described by l [17]. The fishes showed restlessness/hyperactivity, irritation/rapid body movement, and difficulty in respiration displayed by fish moving to the surface to gulp air, intense opercula movement, darkening of the color and loss of equilibrium by swimming sideways, finally fish collapsed and died.

LC50 VALUES

From the results of mortality readings at 24,48,72 and 96 hr exposure (Table **1**) LC50 values and 95 % confidence limits for endosulfan were calculated to be 16.03μg/L, 14.49μg/L, 11.74μg/L, and 10.20 μg/L, respectively (Table **2**). The dose-response curve for endosulfan in Tilapia is shown in Fig. (**1**).

Lethal Body Burden

Lethal body burdens of all test fishes in relation to the duration of exposure before death are given in Table **3** and Figs. (**2** & **3**). Lethal body burdens in relation to aqueous concentrations are given in Table **4** and Figs. (**4 & 5**). The LBB in tilapia was expressed both on the basis of fish weight

Table 2. LC50 Values, (μg/L) and their 95% Confidence Limits

Time (hours)	Endosulfan LC50 (μg/L)	95 % Confidence Limits
24 hr	16.03	13.79-18.63
48 hr	14.49	13.16-15.94
72 hr	11.74	11.09-12.42
96 hr	10.20	9.58-10.86

and on the basis of extrea table lipid weight corrected for percent recoveries. The mean LBB/CBR was found to be 0.0046μmol/g fish (±0.0025) and 0.096 μmol/g lipid (±0.057). The data on exposure time was separated into four groups, group that died within ≤24 hours hrs, ≥ 24 ≤ 48 hours, ≥ 48 ≤ 72 hours, and ≥ 72≤ 96 hours. The LBBs of these groups differ, with the highest value at the last two longest survival period (Table **1**). There is a notable increase in the LBB from 24 hrs to 48 hrs and then a constant value at 72 and 96 hrs (Figs. **1 & 2**). The LBB is observed to be lowest in fish exposed to low endosulfan concentration and highest and constant to those exposed to last two highest concentration (Figs. **3 & 4**).

The present study shows that the LBB/CBR may be dependent on exposure time during the initial period of exposure and on lower exposure concentration i.e. before steady state is attained. However, as equilibrium is reached the LBB remains constant and becomes independent of exposure time and concentration. The explanation for this phenomenon may be due to faster distribution of the chemical in highly perfused organs like the nervous system (brain) during the early periods of exposure, but as the equilibrium is attained there is a uniform distribution to other parts of the body, like muscle and fat tissues and thus a constant value is maintained.

Estimation of Bioconcentration Factors of Endosulfan in Tilapia

The relationship between LC50 value, LBB and bioconcentration factor(BCF) is described by Mc Carty [3], (equation: 1)

The present study has found the LC50 value of endosulfan in tilapia to be 10.2μg/L and the LBB to be 0.0046μmol/g fish (equivalent to 1.87mg/kg). Therefore, the BCF of endosulfan is estimated to be 187.

Table 3. Effect of Time on the Lethal Body Burden of Endosulfan in Tilapia Based on Lipid Weight and Fish Weight

Exposure Time	Average Lethal Body Burden (±Standard Deviation)		
	μmol/g Fish	μmol/g Lipid	n
≤24 hours	0.0007(±0.0004)	0.0143(0.0.0074)	2
≥ 24 ≤ 48 hours	0.0029(0.0022)	0.065(0.0481)	6
≥ 48 ≤ 72 hours	0.0054(0.0024)	0.1124(0.0597)	13
≥ 72≤ 96 hours	0.0052(0.0021)	0.1045(0.0529)	10

Table 4. Effect of Concentration on the Lethal Body Burden of Endosulfan in Tilapia Based on Lipid Weight and Fish Weight

Concentration (μg/L)	Average Lethal Body Burden (±Standard Deviation)		
	μmol/g Fish	μmol/g Fish	n
9.4	0.0032(±0.0011)	0.068(±0.02)	2
10.3	0.0043(±0.0017)	0.095(±0.04)	7
11.3	0.0033(±0.0022)	0.051(±0.05)	4
12.5	0.0050(±0.00.7)	0.10(±0.07)	8
13.8	0.0054(±0.0033)	0.12(±0.07)	10

DISCUSSION

Acute Toxicity

In the present study the (96-hr) LC50 value for endosulfan in tilapia was found to be 10.2μg/L (Table **1**). This value is in agreement with the 10.3μg/L found by Loing *et al.* [8]. The 1492μg/L found by Li and Chen appears to be an overestimation of the LC50 value of endosulfan in tilapia.

The fishes showed restlessness/ hyperactivity, irritation/ rapid body movement, difficulty in respiration displayed by fish moving to the surface to gulp air, intense opercula movement, darkening of the color and loss of equilibrium by swimming sideways, finally fish collapses. In a pilot experiment the range of exposure concentrations was found to be very narrow from no effect to 100% effect i.e. 8.2ug/L to 12.5ug/L. These observed characteristics suggest a specific mode of action at the nervous system.

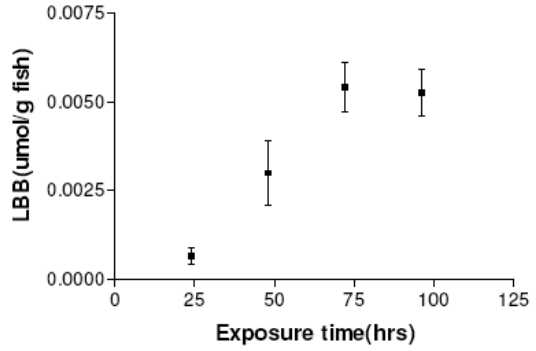

Fig. (2). Lethal body burden in tilapia exposed to endosulfan,plotted against concentration.

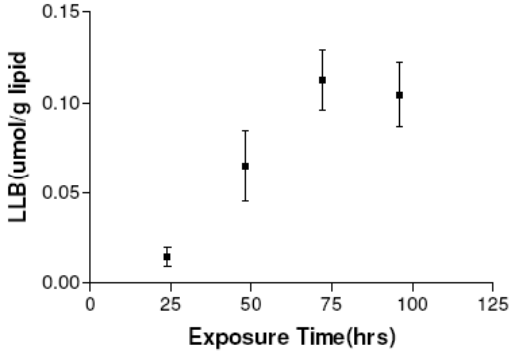

Fig. (3). Lethal body burden in tilapia exposed to endosulfan, plotted against concentration.

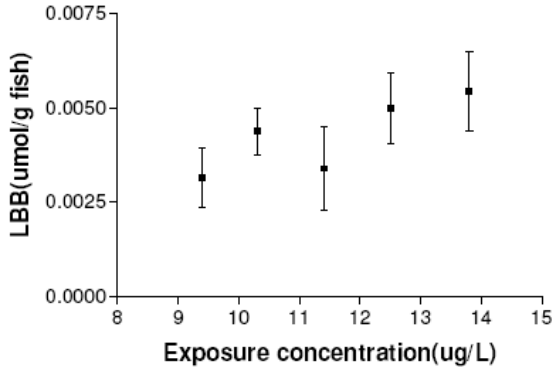

Fig. (4). Lethal body burden in tilapia exposed to endosulfan, plotted against concentration.

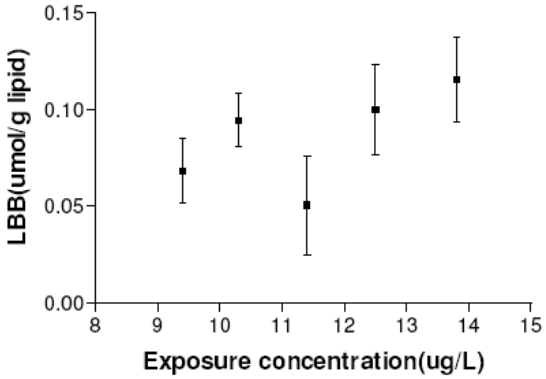

Fig. (5). Lethal body burden in tilapia exposed to endosulfan,plotted against concentration.

Lethal Body Burden

Lethal body burden of endosulfan (organochlorine) in tilapia is found to be 0.0046μmol/g fish and is much lower than e.g. the LBB for organophosphates, such as chlorothion and methidathion as previously determined by [11]. This demonstrates the applicability of lethal body burden for the assessment of the toxic potency of endosulfan. The low LBB value of endosulfan also indicates a specific mode of action.

Influence of Exposure Time and Concentration before Death on LBB

[10] it is indicated that LBB within a class of chemical may be constant i.e. independent of exposure time and exposure concentration.The present study shows that the LBB/CBR may not be constant with exposure time and concentration during the initial period of exposure i.e. before steady state is attained. However, as equilibrium is reached the LBB remains constant and is independent of exposure time and concentration.This may be explained by distribution effects. At lower concentration, it might take some more time before the compound reaches its primary site of action, the nervous system, than at higher concentrations. When equilibrium is attained there is a uniform distribution to all sites of the body and therefore a constant LBB value is maintained.

Estimated Bioconcentration Factor (BCF) for Endosulfan in Tilapia

Bioconcentration factor is the ratio between the concentration of the chemical in fish (Cf) and in water (Cw) at steady state i.e. the situation where the uptake rate of a compound equals to the elimination rate:

$$BCF=Cf/Cw \quad (2)$$

The bioconcentration factor of endosulfan in tilapia derived from results of this study was 187. This BCF value is much lower than that in yellow tetra (*Hyphessobrycon bifasciatus*) of 11,583(±2361) as previously determined experimentally by [18, 19] Found BCF values of endosulfan in *crassostrea madrasensis* and *katelysia opima* are 49 and 31 respectively.

The quantitative structure activity relationship (QSAR) relating BCF with the octanol/water partition coefficient(Kow) have shown that this physical-chemical parameter can give a fair approximation of the BCF for many chemicals with log Kow between 3 and 7 [20-24]. This equation correlating BCF and Kow of various organic compounds is:

$$\text{Log BCF}=0.85\log \text{Kow}-0.70 \quad (3)$$

The log Kow of endosulfan is 3.5 [25], therefore the BCF of endosulfan is 188.This value is in agreement with 187 derived from results of the present study [26]. found that the BCF of endosulfan for estuarine animals such as striped mullet(*mugil cephalus*) was 2239 for edible part and 2755 for whole body. Results from this study therefore suggests that the bioaccumulative potential of endosulfan in tilapia in Lake Victoria may be low compared to that of estuarine animals.

LBB/CBR of Endosulfan in Tilapia and Safety to Consumers

Reports of human deaths along the shores of Lake Victoria were being attributed to consumption of fish poisoned by endosulfan. However, it was not possible then to quantify the possible amounts of endosulfan that could have been ingested from the poisoned fish. From the results of LBB of 0.0046μmol/g fish, determined from this study, and assuming that an average man of 70 kg consumes 300g of fish per meal, then the amount of endosulfan ingested will be 1.38 μmol i.e. 0.02μmol/kg body weight. The ADI for endosulfan is 0.02μmol/kg (0.006mg/kg body weight) [27]. It's therefore unlikely that the consumption of fish killed by endosulfan may be harmful to the consumer.

CONCLUSIONS

These observed toxic characteristic and low LBB value of endosulfan indicate a specific mode of action at the nervous system. The toxic potency of endosulfan may be assessed by using lethal body burden. The BCF of endosulfan for tilapia may be estimated to be 188. The bioaccumulative potential of endosulfan in tilapia in Lake Victoria may be low compared to that of estuarine animals. It's therefore unlikely that consumption of fish killed by endosulfan will be harmful to the consumer.

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REFERENCES

[1] Wolf WD. Influence of biotransformation on the bioconcentration of chemicals in fish. Ph.D. Thesis Utrecht University 1992.

[2] Ferguson J. The use of chemical potential as indices of toxicity. Proc R Soc Lond (Series B) 1939; 387-404.

[3] McCarty LS. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. Environ Toxicol Chem 1986; 5: 1071-80.

[4] McCarty LS, Mackay D. Enhancing ecotoxicological modelling and assessment,body residues and modes of action. Environ Sci Technol 1993a; 27: 1719-28.

[5] McKim JM, Schmieder PK. Bioaccumulation :does it reflect toxicity? In: Nagel R, Loskill R, Eds. Bioaccumulation in aquatic systems; contributions to the assessment. VCH Verlargesellschaft, Weinheim: New York 1991; pp. 161-88.

[6] Sijm D. Influence of biotransformation on bioaccumulation and toxicity of chlorinated aromatic compounds in fish. Ph. D. Thesis, Utrecht University: Dutch 1992.

[7] Gurure RM. Influence of two organochlorides pesticides, thiodan and lindane on survival of fingerlings of oreochromis niloticus and Tilapia zilli. UNDP/FAO/Nigerian Institute for oceanography and marine Research, Port Harcourt, Nigeria. ARAC/WP/6/87, 1987; p. 14.

[8] Loing PC, Hamzah WP, Murugan V. Toxicity of some pesticides towards freshwater fishes. Malaysian Agric J 1988; 54: 147-56.

[9] Li GC, Chen CY. Study on the acute toxicity of commonly used pesticides to two kinds of fish. K.O. Hsueh Fa Chan Yueh Kan 1981; 9(2): 146-52 (CHI)(ENG ABS).

[10] Van Hoogen G, Opperhuizen A. Toxicokinetics of chlorobenzenes in fish. Environ Toxicol Chem 1989; 7: 213-9.

[11] De Bruijn J, Yedema E, Seinen W, Hermens J. Lethal Body Burden of four organophosphorus pesticides in the guppy (*Poecilia reticulata)*. Aqua Toxicol 1991; 20: 111-22.

[12] De Maagd PGJ. Polycyclic aromatic hydrocarbons: fate and effects in the aquatic environment. Ph.D Thesis, Utrecht University: Dutch 1996.

[13] Mrosso HD, WerimoK. A study of fish poisoning in Lake Victoria. Seventh FAO Expert Consultation on Fish technology in Africa. Saly-Mbour, Republic of Senegal,10-13 December 2001.

[14] http://www.fas.org/promed/ahead

[15] Frans W, Wim LT. van Densen Fish Stocks and Fisheries of Lake Victoria. A handbook for field observations, Haplochromis Ecology Survey Team(HEST), Leiden University,Tanzania Fisheries Research Institute(TAFIRI), Department of Fish Culture and Fisheries,Wageningen University, Samara Publishers Limited1995.

[16] Long AR, Barker SA. Matrix solid phase dispersion(MSPD) extraction and gas chromatographic screening of nine pesticides in beef fat. J Assoc Off Anal Chem 1991; 74(3): 493-6.

[17] Drummond RA, Russon CL, Geiger DL, Defoe DL. Behavioural and morphological changes in fathead minnow (*pimephales promelas*) as diagnostic endpoint for screening chemicals according to mode of action. In: Position TM, Purdy R, Eds. Aquatic Toxicology and Environmental fate: STP 921. American Society for Testing and Materials, Philadelphia 1986; vol. 9: pp. 415-35.

[18] Claudio MJ, Cecilia MFT. Bioaccumulation and elimination of endosulfan in the fish yellow tetra (Hyphessobrycon bifasciatus). Bull Environ Contam Toxicol 1993; 50: 572-7.

[19] Narasimmalu R, Venugopalan VK. Bioconcentration of endosulfan in different parts of estuarine organisms under sublethal exposure. Bull Environ Contam Toxicol 1991; 46:151-8.

[20] Veith GD, Defoe DC, Bergestedl BV. Measuring and estimating the bioconcentration factor of chemicals in fish. J Fish Res Board Can 1979; 36:1040-8

[21] Mackay D. Correlation of bioconcentration factors. Environ Technol 1981 16: 274-278. Measurement of the Bioconcentration Factors of pesticides by freshwater fish and their correlation with physico-chemical properties or Acute toxicity. Pest Sci 1982; 12: 417-24.

[22] Oliver BG, Niimi AJ. Bioconcentration of chlorobenzene from water by rainbow trout:correlations with partition coefficients and environmental residues. Environ Sci Technol 1983; 17: 287-91.

[23] Davies RP, Dobbs AJ. The prediction of Bioconcentration in fish. Water Res 1984; 10: 1253-62.

[24] McCarty LS. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. Environ Toxicol Chem 1986; 5: 1071-80.

[25] Azawa J. Measurement of the bioconcentration factors of pesticides by freshwater fish and their correlation with physico-chemical properties or acute toxicity. Pest Sci 1981; 12: 417-24.

[26] Hansch C, Leo A, Hoekman D. In: Exploring QSAR. Fundamentals and applications in chemistry and biology; Hydrophobis, electronic and steric constants. American Chemical Society: Washington D.C. 1995.

[27] Schimmel SC, Patrick Jr. JM, Wilson AJ. Acute toxicity to and bioconcentration of endosulfan by estuarine animals. In: Mayer FL, Hamelink JL, Eds. Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. American Society for Testing and Materials, Philadelphia, PAP 1977; vol. 2: pp. 41-52. (ERL,BG 289).

[28] Worthing CR, Hance RJ. Pesticide Manual, 9th ed. British Crop Protection Council: UK 1991.

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