

## APPLICATION OF THE PERPEST MODEL IN THE PREDICTION OF ENVIRONMENTAL RISKS OF ENDOSULFAN ON *OREOCHROMIS LEOCOSTICTUS*. A CASE STUDY OF LAKE NAIVASHA, KENYA

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### Abstract

The occurrence, concentration and spatial distributions of Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide) and endosulfan sulfate were studied in the Lake Naivasha basin during the months of May to December 2010. The aim of the study was to determine the concentration of endosulfan in the watercourse. Endosulfan sulfate was the most predominant with a range of 16.2 - 345 ng/L and a mean of  $131 \pm 110.2$  ng/L followed by endosulfan II 41.7 - 92.8 ng/L and a mean of  $60.6 \pm 20.3$  ng/L, and endosulfan I 20.1 - 57.9 ng/L and a mean of  $34.3 \pm 14.7$  ng/L respectively. The measured exposure concentrations were translated into environmental risks factors using the PERPEST model Version 3.0. The model was calibrated using laboratory experimental data on exposure of *O. leocostictus* to endosulfan in aquariums simulating Lake Naivasha. The environmental risks posed by the measured exposure concentrations of endosulfan on *O. leocostictus* were predicted as total endosulfan ( $\Sigma$ Endosulfan).  $\Sigma$ Endosulfan ranged from 80.9 - 450 ng/L within the basin with a mean of  $225.8 \pm 129.1$  ng/L. Results of the prediction were compared those obtained from microcosm laboratory experiments simulating the Lake Naivasha ecosystem to assess the accuracy of the model. The study shows that though the insecticide is not targeted to kill fish it has an adverse effect on the population of *O. Leocostictus*. The measured exposure concentrations can cause reduction in population of *O. Leocostictus* by between 0 - 6% in *O. Leocostictus*. Comparison of the results of the prediction shows that there is no significant difference between the results obtained from the microcosm experiment at  $p = 0.05$ . The study shows that the model can be applied in environmental and toxicity studies of chemicals without the use of laboratory specimens. The study also explains the variability of aquatic organisms' populations in the lake can explain the current decline in populations of aquatic life in Lake Naivasha. Increased monitoring is thus recommended to detect inflow of toxic chemicals to safeguard aquatic life.

**Key words:** Lake Naivasha, organochlorine, PERPEST, POPs, aquatic

## 1 Introduction

Lake Naivasha is a freshwater lake found at the foot of the Eastern Rift Valley in Kenya, with an approximate surface area of 140 km<sup>2</sup> with no surface outlet (Mugacia et al. 1992, Gitahi et al. 2002). The neighboring Naivasha municipality covers approximately 941 km<sup>2</sup> and is one of the Kenya's fastest growing municipalities with a population of about 250,000 people within a catchment of approximately 3,000 km<sup>2</sup> (Becht and Harper, 2002).

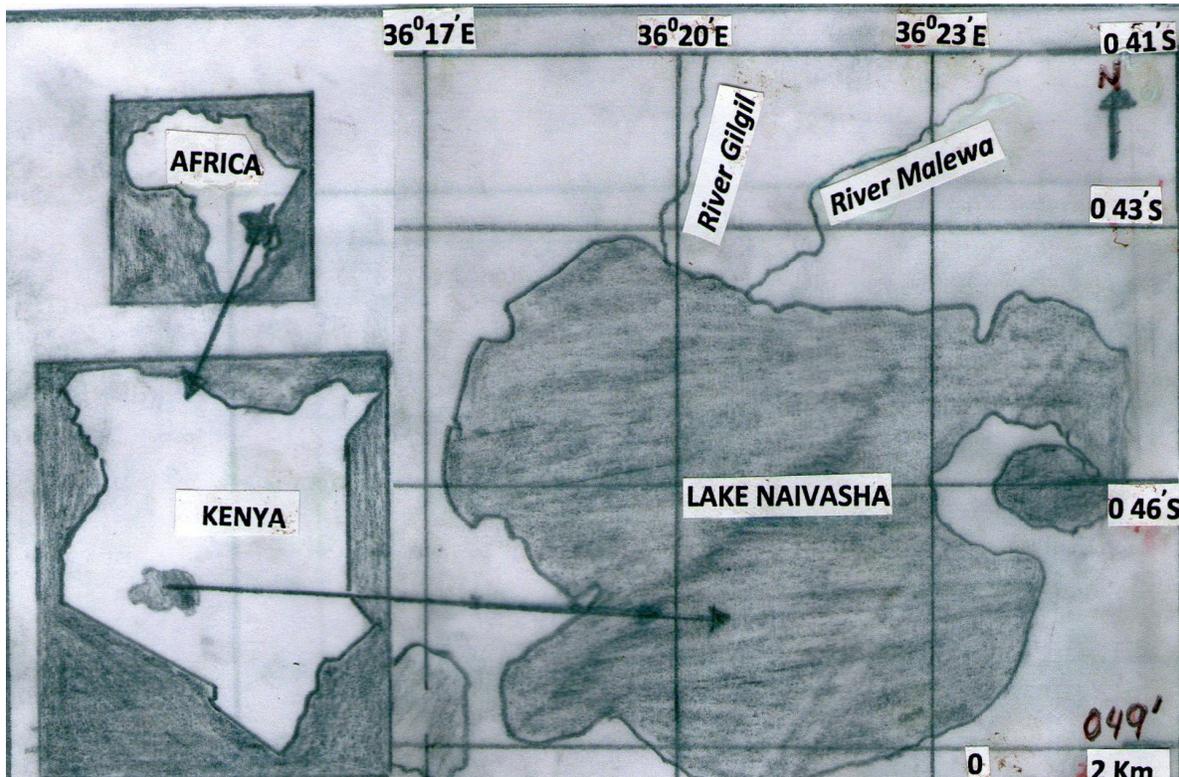


Figure 1: Location of the Lake Naivasha basin

The introduction of intensive flower farming and geothermal power generation in the Lake Naivasha basin has led to increases in human-induced chemical pollution, excessive water abstraction and encroachment of the lake's catchment area (Njogu *et al.*, 2010a). Intensive use of agrochemicals has led to frequent episodes of chemical pollution, killing thousands of aquatic organisms (Food and Waterwatch 2008; Gitonga, 2010). The lake is a Ramsar site due to its significance as a bird breeding and feeding site (Ramsar, 2010).

Organochlorine pesticides have been identified for priority action at the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR, 2002) as potential endocrine disrupters. Endosulfan, a chlorine based insecticide, is widely used in Kenya due to its high toxicity and persistence. It retails under trade names such as Thiodane, Thiofanex, Phase Plus, Callsulfan among others (Njogu, 2011). The US EPA (2007) described endosulfan as very highly toxic to all aquatic organisms, as demonstrated by the low concentration of endosulfan that kills 50% of a species (LC<sub>50</sub>) after 96 hours of exposure and the low NOEACs. LC<sub>50</sub> values of 0.8, 1.7 and 0.45 µg/L were reported for rainbow trout, bluegill sunfish and eastern oyster, respectively. It is reported that endosulfan metabolizes moderately

rapidly in fish. Whilst its acute toxicity to mammals and birds is low, it is an endocrine disrupting chemical (Cummings, 1997).

Organochlorine pesticides have been reported in other inland Lake in Kenya (Mugacia et al. 1992; Kairu, 1992; Gitahi *et al.*, 2002; Wandiga *et al.*, 2002; Mavura and Wangila, 2004; Getanga *et al.*, 2004; Njogu et al. 2010b). This study assessed the environmental risks of the measured concentrations of endosulfan to fish using a modified version of the PERPEST (Prediction of Ecological Risks of Pesticides) model. The model has been applied in various ecological risk assessment studies (Van den Brink et al. 2002, 2006; Van Nes and Van den Brink, 2003; Ansara *et al.*, 2006; Van den Brink 2008).

## **2 Materials and Methods**

### **2.1 Field and Laboratory Work**

Water samples were collected from seven sampling sites in the lake (Figure 1). Limnology parameters, including transparency, temperature and dissolved oxygen were measured for all sampling sites on site (Table 1). Transparency was measured in triplicate, using a 20 cm Secchi disc with white and black quadrants. Mean depth was calculated as the depth at which the disc was no longer visible. Dissolved oxygen concentrations and temperature were measured using a Clark model ion selective oxygen electrode.

Water samples of one liter aliquots were sampled in triplicates from seven sampling sites in the lake to assess the occurrence and spatial distribution of endosulfan and heptachlor pesticide residues. The samples were stored in pre-cleaned brown amber bottles and preserved with mercuric chloride to inhibit microbial activity on the insecticides. Samples were transported under ice in cooler boxes to a laboratory in Nairobi.

Extraction of the insecticides fraction in water samples were carried out through solvent extraction as described by Aufbau and Afghan (1990) and APHA (1995). One liter water samples were transferred to 2 L separatory funnel and treated with 50 mL phosphate buffer to maintain neutral pH. Analytical grade sodium chloride was added to the water and mixed thoroughly to salt out pesticides (Herlich, 1990). Extraction was effected by shaking the samples with three portions of HPLC grade dichloromethane and decanting the organic layer. The three extracts were combined and concentrated to 1 mL at 40 °C using a rotary evaporator and cleaned by passing through a 25 cm and 1.5 i.d. chromatographic column packed with analytical grade florisil (Floridin Co., Berkeley Springs, West Virginia) and anhydrous sodium sulfate (at a flow rate of 2 mL/min).

Samples were analyzed using a Varian CP 3800 Gas Chromatograph equipped with Electron Capture Detector. Separation was done using BPX 5 capillary column of dimensions 30 m x 0.25 mm x 0.25 µm film thickness. Confirmatory analysis was done using BPX35 capillary column of dimensions 50 m x 0.25 mm x 0.25 µm film thickness.

A temperature programme was used, starting from 90°C with a hold time of 3 minutes, increased to 215°C at 8°C /min with a hold time of 25 min., then increased to 270°C at 5°C /min with a hold time of 5.37 min., and finally ramped to 275°C at 5°C /min with a hold time of 18.63 min. The carrier gas was high purity helium (99.9%) with white spot nitrogen as the makeup gas. Quantification followed external calibration method using high purity pesticide reference standard (Ultra Scientific, North Kingstown, USA).

## 2.2 PERPEST

The PERPEST model ([www.perpest.wur.nl](http://www.perpest.wur.nl)) is based on case-based reasoning (Van den Brink *et al.*, 2002) which is based on the idea that similar problems have similar outcomes/solutions (Ani *et al.*, 2009). Case-based reasoning is a problem-solving technique that imitates human thinking in trying to make a decision based on earlier experiences (Avramenko and Kraslawski, 2008). In this model the prediction of the effect of a certain concentration of a pesticide on a defined aquatic ecosystem is based on published information on the effects of other pesticides with similar toxicity modes of action (TMOA) on the structure and function of aquatic ecosystems, as observed in semi field experiments. This case-based reasoning system consists of the database containing this information and a weighted analogies prediction search routine (Van Nes and Van den Brink, 2003). The rationale behind weighted analogies prediction is that based on the characteristics of the question case (such as pesticide characteristics, exposure concentration, and exposure regime) analogous cases in the inbuilt database are identified. These analogous cases are weighted and summarized in a prediction. This means that, although for certain pesticides no microcosm or mesocosm experiment has been published, one is able to predict its effect on a semi-field scale using the results of experiments performed with other pesticides that have a similar toxicological mode of action and fate characteristics. One of the main advantages of the PERPEST model over approaches using results of single species studies, like the species sensitivity distribution concept is that, since the results of ecosystem level experiments are used, indirect effects are also included.

A modified version of the PERPEST model Version 2.0 was used to predict the ecological risks of endosulfan on fish from Lake Naivasha. Aquarium tanks were set up using sediments and water from Lake Naivasha. Juvenile fish were exposed to endosulfan at various concentrations and the mortality of fish recorded with time for each concentration as described by Henry *et al.* (2003).

## 3 Results

### 3.1 Field and Laboratory Work

The limnology parameters of the sampling sites were determined and are presented in Table 1 and Figure 2. The transparency of the water varied widely from 30 to 110 cm in the basin. The data show higher transparency, temperature and dissolved oxygen levels compared to the ones reported by Campbell *et al.*, (2003). The high dissolved oxygen levels indicate improved water quality. The increase in temperature could be attributed to the season at sampling.

Table 1: Limnology parameters as measured in water samples in Lake Naivasha in 2009

Site No.	Temperature (°C)	Dissolved Oxygen (mgL <sup>-1</sup> )	Secchi depth (cm)
S1	24 ± 2.5	7.9 ± 1.1	55 ± 1.5
S2	24 ± 1.5	8.45 ± 1.1	65 ± 2.0
S3	23 ± 1.0	7.9 ± 1.1	30 ± 1.5
S4	26 ± 1.2	6.9 ± 1.2	45 ± 2.5
S5	22 ± 1.3	7.9 ± 1.0	110 ± 2.5
S6	25 ± 1.2	8.5 ± 1.1	53 ± 1.1

(Mean ± standard deviation, n = 9, ND – Not Determined)

There was a wide variation in dissolved oxygen levels (6.9 - 8.5 mgL<sup>-1</sup>), temperature (22 - 26°C) and transparency (30 - 110 cm). The highest clarity was observed at S5, which is the deepest point in the lake

receiving no inflows. S3, where most inflowing rivers enter the lake, had the lowest transparency of 30 cm, indicating inflow of suspended matter from the upper catchment.

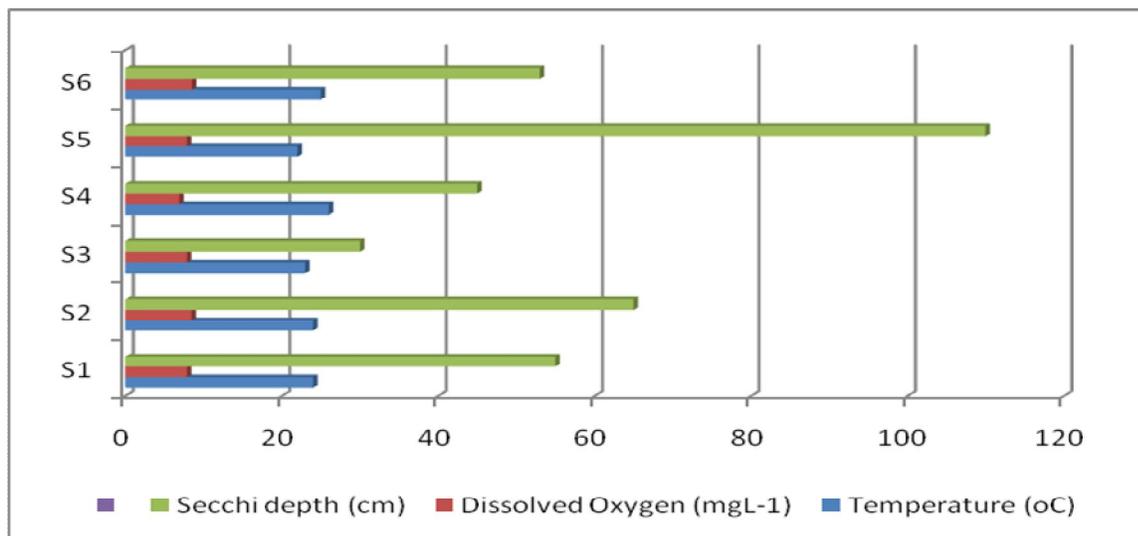


Figure 2: Bar graph for the limnology parameters in Lake Naivasha

Endosulfan isomers ( $\beta$  and  $\alpha$ ) and its metabolite, endosulfan sulfate were detected in samples from all sites in the basin. Results are presented in Table 2 and Figure 3.

Table 2: Concentrations (ng/L) of Endosulfan and Metabolites in Lake Naivasha (n = 6)

Sites	Endosulfan $\alpha$ - Isomer	Endosulfan $\beta$ - Isomer	Endosulfan sulfate	$\Sigma$ Endosulfan	Mean $\pm$ Std dev
<b>S1</b>	23.0 $\pm$ 2.3	41.7 $\pm$ 2.4	16.2 $\pm$ 1.2	80.9	26.9 $\pm$ 13.2
<b>S2</b>	20.1 $\pm$ 2.1	44.2 $\pm$ 3.1	78.4 $\pm$ 4.3	142.7	47.7 $\pm$ 29.3
<b>S3</b>	46.1 $\pm$ 3.4	51.1 $\pm$ 4.3	133.0 $\pm$ 4.2	230.2	76.7 $\pm$ 48.8
<b>S4</b>	57.9 $\pm$ 4.5	92.8 $\pm$ 5.1	195.5 $\pm$ 9.2	346.2	115.4 $\pm$ 71.5
<b>S5</b>	21.2 $\pm$ 1.9	84.3 $\pm$ 6.5	60.2 $\pm$ 3.2	165.7	55.2 $\pm$ 31.8
<b>S6</b>	42.9 $\pm$ 3.1	61.9 $\pm$ 5.5	345.2 $\pm$ 6.7	450	150 $\pm$ 169.3

The highest and lowest concentrations of  $\Sigma$ Endosulfan were found at S4 and S1 respectively. Endosulfan sulfate was predominant in most sites with a range of 16.2 – 345.2 ng/L followed by endosulfan  $\beta$  (41.7 – 92.8 ng/L), and endosulfan  $\alpha$  (20.1 – 57.9 ng/L), respectively. There were wide variation between samples with sites S6, S4, S3 and S2 showing the highest concentrations, site S3 is at a point of entry of several rivers while S2 is along discharge canals, discharging from the flower farms, site S6 is at a wastewater discharge from the Naivasha Municipality treatment site at the Lake.

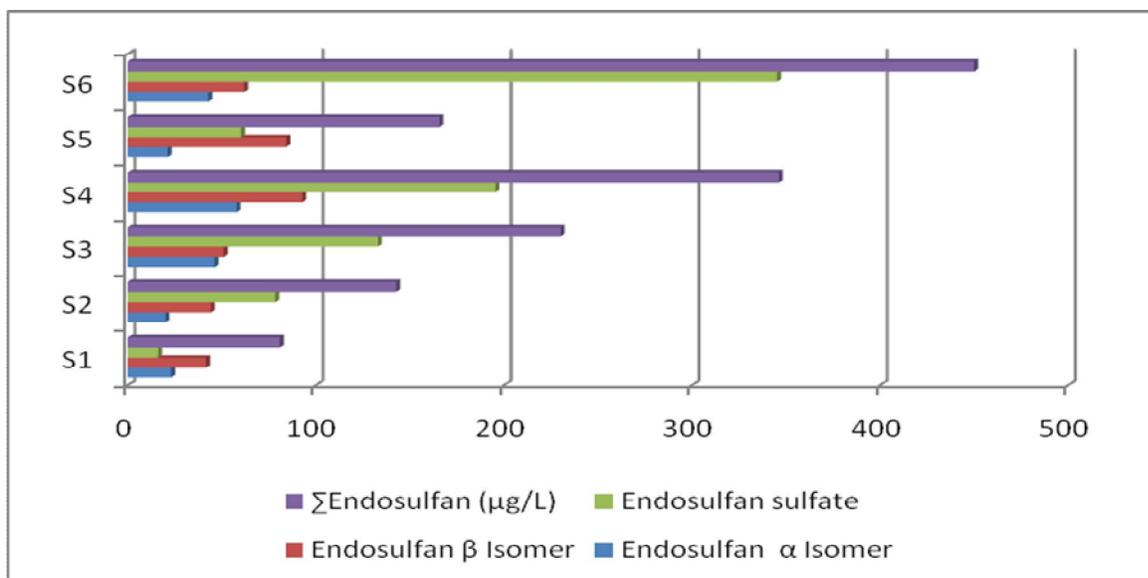


Figure 3: Bar Graph for the Endosulfan Pesticides Residues in Lake Naivasha

The high concentrations of endosulfan found in these sites indicate that flower farms, River Malewa and the Naivasha Municipal Council are important sources of endosulfan and its metabolites. The results of water analysis agree with findings made on the endosulfan usage in the catchment. A survey on insecticides used in the catchment indicated that Thionex, Thiofanex, Phaser plus, Callisulfan and Thiodane, pesticides formulated with endosulfan as the active ingredient are widely used. Endosulfan is also added to other insecticides to increase their efficacy.

The concentrations of endosulfan sulfate were generally higher than those of isomers β and α in most of the sites indicating rapid transformation of the insecticide to its metabolite. The study shows that endosulfan is rapidly oxidized to endosulfan sulphate. The detection of endosulfan implies contamination due to previous use in the basin which could be the main source of the compounds in the lake.

### 3.2 PERPEST Prediction

Predictions of the effects of endosulfan on fish as a result of exposure to endosulfan are presented in Table 3. The data reveal a progressive decrease in the probability of no and slight effects occurring with increase in concentration and an increase in clear effects occurring for 0.25 to 0.5 µg/L.

The results show positive correlation between probability of effect and concentration. An increased probability of adverse effect of endosulfan on fish was observed with increase in exposure concentrations in water. The observable effect concentration for endosulfan was 0.25 µg/L-1, which present a 4.5% probability of adverse effects occurring with a confidence limit of 0.141 at 95%.

Table 3: PERPEST prediction for the endosulfan concentrations ( $\mu\text{g/L}$ ) in Lake Naivasha

Conc.	No effect	CL 95%	Slight effect	CL95%	Clear effect	CL95%
0.05	1	1	0	0	0	0
0.1	1	1	0	0	0	0
0.15	1	1	0	0	0	0
0.2	1	1	0	0	0	0
0.25	0.919	1	0.039	0.125	0.041	0.141
0.3	0.852	1	0.073	0.182	0.074	0.188
0.35	0.839	0.956	0.095	0.208	0.065	0.177
0.4	0.816	0.932	0.121	0.256	0.061	0.159
0.45	0.822	0.948	0.117	0.229	0.059	0.162
0.5	0.816	0.949	0.121	0.227	0.061	0.166

This rises to 6.1 % at  $0.5 \mu\text{gL}^{-1}$  with a confidence limit of 0.166 at 95%, indicating that fish are not adversely affected by presence of endosulfan in water. The observable effect exposure concentration was higher than the Toxicity Reference Value (TRV) for testing aquatic biota in water, illustrating the accuracy of the prediction (Suter and Tsao, 1996). The study shows that, although the pesticide is not targeted to fish, it has a potential of altering the fish population.

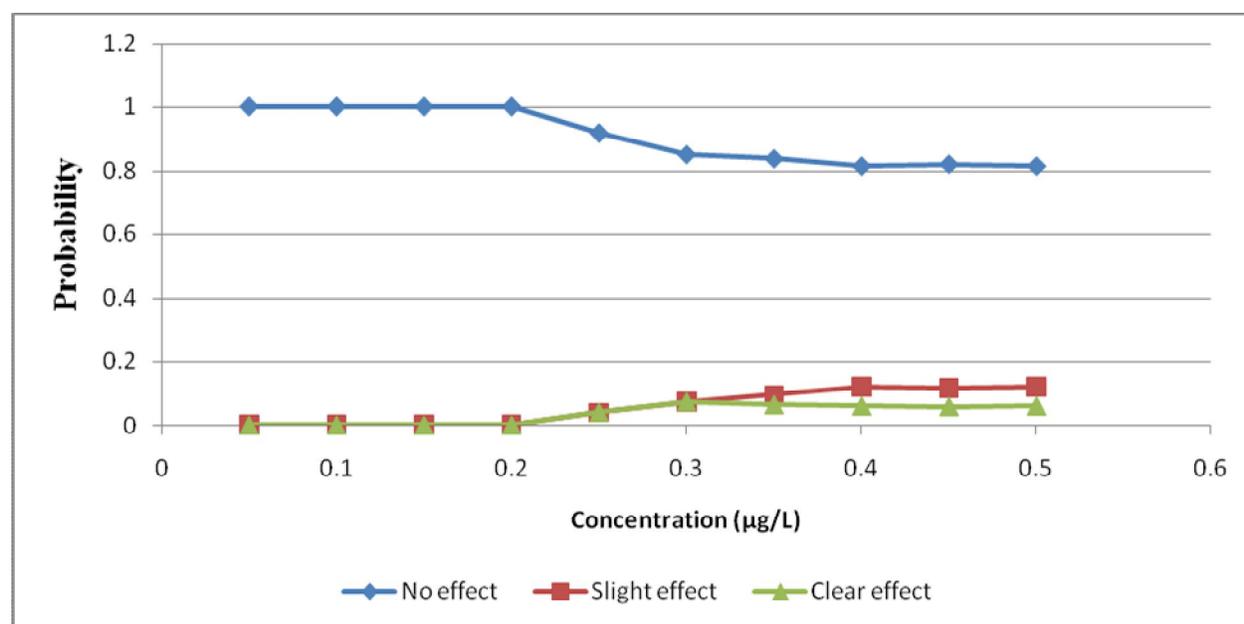


Figure 4: A plot of probability of effect versus concentration

#### 4.0 Discussion and Conclusions

The study shows that there is increased sensitivity of endosulfan to fish as the concentration increases. The measured concentrations of endosulfan in the lake have a potential to alter the biodiversity of the lake, changing important inter-relationships of different organisms. This is in agreement with the findings of other organochlorine pesticides like with similar TMOA (Hose *et al.*, 2003; Van Wijngaarden *et al.* 2005; Van den Brink *et al.*, 2009). A loss of biodiversity is likely to occur even at low concentrations because some aquatic organisms will be eliminated long before the concentration can

reach concentrations that will affect fish. Since each organism has a specific role (niche) in the ecosystem, discriminate elimination of species will lead to loss of functionality and changes in the ecological niches. It is thus important that use of toxic insecticides such as endosulfan be controlled in order to reduce episodes of washdown into the lake.

Current decline in the aquatic organisms' populations in Lake Naivasha can be explained through similar studies. Other studies have also indicated agrochemicals as the cause of the problem (Food and Waterwatch, 2008).

Table 4: Correlation coefficient values

	Temperature	Dissolved oxygen	Secchi depth	ΣEndosulfan	Endosulfan Sulfate	α - Endosulfan	β - Endosulfan
Temperature	1	-0.344	-0.555	0.54	0.574	0.624	0.184
Dissolved Oxygen	-0.344	1	0.173	-0.083	0.099	-0.576	-0.661
Secchi Depth	-0.555	0.173	1	0.328	-0.354	-0.661	0.341
ΣEndosulfan	0.54	0.54	0.328	1	0.98	0.761	0.45
Endosulfan Sulfate	0.574	0.574	-0.354	0.98	1	0.668	0.283
α - Endosulfan	0.624	0.624	-0.661	0.761	0.668	1	0.463

Correlation coefficients between limnology parameters and pesticide residue values are indicated in Table 4. The data shows positive correlation between temperature and pesticide residues indicating that temperature could be a factor in their transformation. The negative correlation between sechi depth and pesticide residue levels indicate that the concentrations of the pesticide decrease with increase with water clarity, which can lead to the conclusion that most of the insecticides are adsorbed on suspended particulate matter. There is also positive correlation between the various pesticide, this can be interpreted to mean that the pesticides have similar chemical sources or reactivity.

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