LETHAL AND SUB - LETHAL EFFECTS OF DDT, CARBOFURAN, TRIFENMORPH AND NICLOSAMIDE ON *Oreochromis niger* GUNTHER (1898)

by

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DECLARATION

a. This Thesis is my original work and has not been presented for a degree in any other University.

H B Menigma

b. This Thesis has been submitted for examination with my approval as University Supervisor.

Malcolm Litchfield 29th January 1987

DEDICATION

Dedicated to my family; Gideon, Christian, Bradley, Arelene, Hector and Annette for their patience and moral support.

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ABSTRACT

Oreochromis niger fry were exposed to various concentrations of DDT, Carbofuran, Trifenmorph and Niclosamide to determine the toxicity of these chemicals to the fish. The concentration lethal to 50% of the fish population in a given time range 24 hrs - 96 hrs, for each of the chemicals, was determined. The growth and swimming patterns of these fish in sub - lethal concentrations of the biocides were also studied.

DDT was found to be the most poisonous to fish with a 24 hr LC_{50} of 0.042mg/litre. Carbofuran was the least toxic with an LC_{50} of 0.225 mg/litre. The toxicity of all the four chemicals increased with concentration and time.

The swimming activity of the fish decreased with time in all the experiments except in Niclosamide. There was a negative linear relationship between fish activity and time.

The fish increased weight very slowly from the first to the fifth week. Growth or increase in fish weight had a positive linear relationship with time in all the experiments. Increase in weight was however

slower in the treatments than in the control except in sub - lethal levels of Carbofuran. The experiment shows that fish cannot be grown in water containing sub - lethal amounts of DDT, Trifenmorph and Niclosamide as they are highly toxic. The fish safe levels would be too low for the control of snails.

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CHAPTER I

I N T R O D U C T I O N

Rice-fish culture is an ancient practice that started, as with all agriculture, with hunting. Wild fish, naturally occurring in rice fields were collected and used as food to supplement the rice diet (Khoo and Tan 1980, Grist 1965). Later, deliberate stocking of rice fields with required fish species was started (Hickling, 1972).

Rice is the most dominant cereal crop in Asia. It is the staple diet of over 1.4 billion people in the world, mostly in Asia, where 90% of all rice is grown and eaten (Khoo and Tan, 1980). Rice belongs to the genus Oryza, family Gramineae and sub-phylum Monocotyledonea (De Datta ,1981). The two cultivated species are Oryza sativa (Lin), commonly grown in the tropical and temperate zones and Oryza glaberrina (steud) grown mostly in West Africa (Morishima, 1984). [Classification based on Roscheviez 1931] (Tsunodoa, 1984).

Rice - fish culture consists of stocking rice fields with fish fingerlings of a selected size and

species to obtain a fish crop in addition to rice, as the main crop (Singh et al, 1980). The fish yield is then used as either a subsistence crop or cash crop (Hickling, 1972).

While rice - fish culture has been practiced for centuries, in the far east, there is little record of it in the rest of the world. In Europe, concurrent rice - fish culture, with gold fish and Common carp (Cyprinus carpio), is practiced in Italy (Hickling, 1972). Trials with common carp are being carried on in Hungary and Bulgaria. In the United States, alternate rice - fish culture is practiced with various fish species, such as Ictiobus spp, Cyprinellus spp, Ictalurus spp, and Punctatus spp.

In Japan, fish culturing in rice fields has been practiced for over a century (Kuronuma, 1980). The fish were kept in rice field water and regarded as a by-product of secondary importance to the rice crop. Fish culture was intensified during the second world war and over 4,400 tons of fish were produced in rice fields in 1943, compared to 401.8 tons in 1909 (Hickling, 1972).

In Indonesia, rice - fish culture started in the middle of the 19th Century (Khoo and Tan citing Ardiwinata, 1957). Fresh water fish culture is,

a well established rural activity and an important source of animal protein. It is widely practiced in Indonesia with Carp as the main fish. (Djajadiredja *et al*, 1980).

In China, fish culture became a popular practice around the year 1000 B.C. with the common Carp, (Ling, 1977), and was introduced in Southeast Asia, from India about 1,500 years ago (Khoo and Tan citing Tamura, 1961).

In Africa rice - fish culture is practiced in Madagascar with mixed species of carp and tilapia [*Cyprinus spp* and *Sarotherodon spp*] respectively (Huet, 1970). In Sierra Leone there are over 250 fish ponds integrated into existing rice paddy schemes.

In Kenya rice is grown on irrigated fields at Mwea, Ahero and Bunyala. Fish - rice culture is not practiced on these farms but farmers collect fish brought into the fields with the incoming water. Fish culture, however started in 1924 with the culture of *Oreochromis niger* (Trewavas, 1981), at the Sagana fish culture farm. In 1948 another fish farm was started at Kiganjo and the Fisheries Department was established in 1954 with the purpose of rearing fish for stocking rivers and dams (Balarin, 1985).

Fish culture in rice fields is one of the best ways of using available aquatic resources in that additional protein is obtained from irrigated areas (Coche, 1967). The rice field soil is very fertile, allowing the growth of both Zooplankton and phytoplankton. These provide food for fish, which in turn is converted into food for humans (Vincke, 1986).

After World War II the expansion of knowledge, expecially in science, technology and medicine has resulted in a rapid and continuing increase in world population. Consequently, there was a proportionate need for greatly increased food production (Coche, 1967). This could only be achieved by the modernisation of rice - cultivation methods based on new high yield varieties of rice and increased usage of fertilizers and pesticides. Introduction of the various pesticides and fertilizers, which are harmful to fish, led to a drastic reduction in fish catches from rice paddies. (Hickling, 1972 Koesoemadinata, 1980). In Japan the weight of fish produced in rice fields fell from 4,436 tons a year to 937 tons a year in 1944 - 1949 and in 1958, it fell to 462 tons a year (Hickling, 1972).

A renewal of interest in rice - fish culture has come about, with the introduction of insect - resistant

rice varieties and the discovery of pesticides which provided adequate protection to the rice plant and were less toxic to fish (Estores et al , 1980).

Pesticides are used in agriculture to increase rice yield. This results mainly in increased carbohydrates without meat. All over the world, especially in developing countries, there is need for increased meat production, to reduce malnutrition due to protein deficiency (Vincke, 1986). Rice - fish culture in irrigated fields would help solve the meat problem.

There was need for a study in Kenya of the effect of biocides used in irrigation schemes on fish. The chemicals used in this study are those in use, or being phased out, in the rice fields in Mwea. This work is a laboratory test without simulation of the field conditions in Mwea. It is a preliminary study to determine the toxicity of DDT, Carbofuran (Furadan ®), Trifenmorph (Frescon ®) and Niclosamide (Bayluscide ®) to Oreochromis niger. It can be a basis for later field studies to determine whether rice - fish culture is possible in Kenya. No attempt was made to determine the toxicity levels of these chemicals in the soil or water of rice - fields or in the tissues of the fish.

® - Trade name of the Chemicals

At Mwea, DDT and Furadan are used as insecticides to control mosquitos and the rice stalk borer. Bayluscide and Frescon are used to control the water snails, Bulinus sp., and the semi - aquatic snails, Biomphalaria sp., which are the vectors of bilharziasis and fascioloasis respectively.

The aim of the project is to make a preliminary study of the toxicity of DDT, Carbofuran, Trifenmorph and Niclosamide on Oreochromis niger.

Objectives

1. To determine the LC_{50} (lethal concentration) of DDT, Carbofuran, Trifenmorph and Niclosamide on Oreochromis niger.
2. To study and show the changes in swimming behaviour of O. niger, exposed to various concentrations of DDT, Carbofuran, Trifenmorph and Niclosamide.
3. To show the effect on growth pattern of O. niger exposed to sub-lethal concentrations of DDT, Carbofuran, Trifenmorph and Niclosamide for 35 days.

CHAPTER II

LITERATURE REVIEW

1. Rice Cultivation

World wide there are several systems of growing rice (Grist, 1965). The classification of rice-culture may be based on the source of water, whether rain or irrigation, type of land, lowland or highland, dryland or wetland (De Datta, 1981). Two methods of growing wet rice are described below.

Rice on river flood plains

Rice as a subsistence crop is grown on river flood plains as a rain fed crop until floods set in. The rice plants then grow with heads above the water and by the time the floods recede, the rice is ready for harvest. Fish brought in by floods is caught and is either sold or used by the farmer but no attempt is made to grow, maintain or protect them (Hickling, 1972). Farmers farming under these circumstances use few or no agrochemicals.

Rice cultivation in paddies

Rice being tolerant of standing water is grown mostly in paddies.

In Japan, the individual fields vary in area from 3.3 m^2 to 0.3 ha. and in shape according to land levels, irrigation and drainage systems (Kuronuma, 1980). Dikes are constructed around the areas to be cultivated to form water tight enclosures. The land within the enclosure is then cultivated and levelled to ensure an even depth of flooding which is critical for good rice yields (Hickling, 1972). Irrigation water from dams, reserviors and rivers is channelled into the irrigation canals and through inlets or ducts into the fields. The area around the inlet is screened with split bamboo, or mulberry or other local wood, to a height of 45 - 60 cm to keep out debris and small fish (Kuronuma, 1980).

The water depth in the fields vary according to the temperature, the strain of rice and the amount of pesticides used (Kuronuma, 1980). Both the maximum level of flooding and the speed at which it rises are controlled to match the growth of the rice plant. The fields remain flooded and puddled until rice seedlings are ready for transplanting, when they are drained to leave wet mud or a thin film of water. Fertilizers, insecticides, herbicides and other chemicals are applied, on the advice of agricultural and fisheries officers. Weeding is normally done by hand 2 - 3

times during the growing season. Pre - germinated seeds (under soil or water, NOT in muddy water) (De Datta, 1981) are grown in nurseries for 3 - 4 weeks or until they are 20 cm high, then transplanted by hand or machine at a regular spacing of 10 cm.

Immediately after transplanting the fields are flooded to a depth of 12 cm. to inhibit the growth of terrestial weeds (Choudhry, 1974). As the rice grows, the water depth is slowly increased to 15 - 18 cm.

The fields are drained about three months after transplanting and three weeks before harvesting, to mature the rice crop.

The aquatic weeds include:

i. Potamogeton richardi
ii. P. senegalense
iii. Polygonum salicifolium
iv. P. serulatum (Choudhry, 1974)

These are removed by hand about 42 days after transplanting, when the rice plant is well established and cannot be uprooted.

2. Techniques for growing fish with rice

There are two main systems of exploiting the rice field fisheries. Table 1 shows the techniques employed, the countries, and the fish cultured in various countries

a. Captural system

b. Cultural system

TABLE 1

RICE GROWING AREAS, FISHERY TECHNIQUE, PERIOD AVAILABLE FOR FISH GROWTH AND YIELD PER YEAR

RICE GROWING AREA	TYPE OF RICE FIELD FISHERIES	FISH SPECIES CAPTURAL/ CULTURED	GROWTH PERIOD (MONTHS)	FISH YIELD (KG) HA/YR
Malaysia	Captural	Trichogste r pectoralis Anabas testudineus Clarias Ophiocephalus	6 - 10	135
Indo-China (Thailand)	Captural rice - fish concurrent	Cyprinus carpio	3	80-100
			6 - 7	100 - 150
Vietnam	Capture	Clarias ophiocephallus	5 - 10	-
India	Capture Rice - fish concurrent	Lates, Mugil, Mystus spp	-	100 - 200
Taiwan	Culture	I mossambica	7	220
Java	Rice - fish concurrent	C. carpio, T. mossambica Helostoma tummincki	1 - 3	1.5
	Rice - fish alternate	Puntius javanicus	5 - 6	
Japan	Culture	carp	24 - 36	1,000
Italy	Concurrent	Carp tench & goldfish, carassius auratus		

(Huet, 1970)

a. Captural System

Here there is no special preparation of land for fish retention, except for digging sumps about 40 - 50 m^2 in area and 2 m deep, in the lowest region of a group of fields. Wild fish populate and reproduce in the flooded rice fields and are harvested at the end of the rice growing season (Khoo and Tan, 1980). This practice is popular and important in southeast Asia. In the Malaysian Peninsular the major fish harvested is Trichogaster pectoralis. Others, such as, Clarias macrocephalus, Ophicephalus striatus and Anabas testudineus, are also caught (Khoo and Tan, 1980).

b. Cultural System

In this system, the rice field is deliberately stocked with fish as in a fish pond. There are three main techniques of growing fish in rice fields;

i. Rice and fish concurrent
ii. Fish in rotation with rice
iii. Fish culture in the irrigation drainage canals or ponds in the irrigation scheme.

i. Rice and Fish Concurrent

This has been practiced for sometime in Japan, Taiwan, Indonesia, Italy, Thailand and Madagascar (Huet, 1970). The following is a description of rice - fish

culture in Japan, Taiwan and Indonesia.

Certain physical modifications must be made to rice fields for fish culture. In Japan, the land is prepared by digging a deep and wide ditch around the entire field set for rice cultivation. The soil from the ditch is used to build a bund around the field. The ditch may be 1.2 - 1.8 metres wide and 0.6 - 0.9 metres deep while the bund may be up to 30 cm wide at the top and 40 - 45 cm high and strengthened by stones and turfs (Hickling, 1972). The bunds are raised to give an adequate depth of water, which depends on the size and type of fish cultured. Javanese farmers maintain depths from 4 - 20 cm, while in India depths of 10 - 60 cm are maintained. For the culture of Tilapia spp a depth of 7.5 cm of water is sufficient (Khoo and Tan, 1980).

Fish drains or channels are dug across the centre of the field, diagonal or circular, depending on the area of the field. A sump, approximately 1 m^2 in area is dug at points where the channels meet (Hora and Pillay, 1962). The channels and fish drains provide a retreat for fish during periods of temperature extremes, or when insecticides and fertilizers are being applied and aids harvesting. The water inlets and outlets have screening devices to prevent loss of fish and

the entry of undesirable species. The ditches also provide space for spawning, where the fish will not uproot the rice plants (Hickling, 1972) and passage ways for easy movement of fish in rice fields (De la Cruz 1980).

In Japan many farmers purchase their fry from professional suppliers. The fields are usually stocked with 15 - 30 mm fry. The densities vary with the expected natural productivity of the water and the planned amount of supplemental feeding. A 990 m^2 field may be stocked with 300 fry when relying on natural feeding alone and 1,000 to 1,500 fry when planning substantial supplemental feeding (Kuronuma, 1980). Stocking practices vary from directly after flooding the field, to 10 - 14 days after transplanting. Fish yields are higher with supplemental feeding of the fry. Feeding on silkworm larvae with or without added cereal bran, starts 10 days after stocking, at a time when the plankton is depleted (Hickling, 1972; Kuronuma, 1980). Feeding frequency and amounts given, are adjusted according to the appetite of the fish.

Advantages of Growing Fish with Rice

Rice - fish culture results in the most economical and efficient utilisation of land (Huet, 1970). On

Irrigation schemes, the all year irrigation water may be more efficiently used by growing fish in the rice paddies. The fish crop adds animal protein and essential amino acids, particularly in the developing countries (Pantulu, 1980; Vincke, 1986) where protein - calorie malnutrition is wide spread.

Irrigated rice fields consist of several different ecological environments and by raising species with different feeding habits in association with one another, this environment can be fully used (Vincke, 1986).

Feeding of fish on algae and aquatic weeds reduce competition (of these weeds) with rice plants for nutrition and space (Huet, 1970) leading to better yields. Herbivorous fish include; Puntius gonionotus, T. rendali , Oewoxheomia mossambicus, Trichogaster pectoralis and Cyprinus carpio.

Vector borne diseases such as malaria and bilharzia are a serious problem in wet - rice cultivation areas. This problem can be alleviated by the presence of fish which prey on the vectors of these diseases. In the Sudan for example, grass carp, (Ctenopharyngodon idelta) is cultured in irrigation channels to clear them of macrophytes. As the fish eat the leaves and stems of the weeds, they also eat the young snails attached to

them. These are the vectors of bilharzias Bulinus sp and Biomphalaria spp. Biological control of snails has been achieved by such fish as Astatoreo-chromis alluandi, Chrysichthys mabusi, Haplochromis bimaculatus, H. mellandi and protopterus aethiopicus Coche, 1967).

The growing of fish in rice has increased economic returns. The lands where fish is grown are under water. This softens the ground and reduces the growth of terrestial weeds. Reports show a 5 - 15% increase in rice yield. This is believed to be due to fertilisation of the rice field by fish waste and artificial fish food, better tilling and mineralization as fish dig in mud, releasing oxygen and weed control (Huet, 1970).

The sale of fish provides additional income [trout KSh 71/98/kg, Cyprinus carpio KSh 9/50/kg and Tilapia KSh 13/=/kg (Dadzie, 1986)]

Negative Impacts of Rice Cultivation Practices on Fish Production

1. Biocides: biocides used to improve rice yields may kill the fish. Fish mortalities are commonly seen directly or shortly after pesticide application. Dead fish do not always surface, however, and pesticide

toxicity, particularly chronic effects, may pass undetected, together with indirect harmful effects to other biota in the rice fields which are important to the fish (Koesoemadinata, 1980). The prediction of pesticide hazards in irrigated rice fields requires knowledge of:

1. the pesticide formulation used
2. environmental persistence and bioaccumulation
3. the frequency of applications
4. the dosage rate and
5. the toxicity of the pesticide to fish and other aquatic biota

2. Water depth: the optimal depth for rice is 15 cm while the minimal depth for fish is 20 cm. In shallow water fish suffer adversely because of low dissolved oxygen concentration, high temperatures, waste accumulation, pH changes, poaching and predation by birds (Coche, 1967).

3. Draining: the water level in rice paddies has to be lowered during weeding and harvesting of rice and fish can be lost. This is overcome, (in fish - rice growing countries) by digging ditches around or in the paddy in which the fish take refuge during times of danger (Khoo and Tan 1980).

4. Shortness of the growing season: the time required

to grow fish is longer than that needed to grow rice depending on species used and temperature (6 months or more for fish and 4 - 5 months for rice) and so by the time the rice is ready for harvest and the fields are drained completely, the fish may be too small to be sold. In Japan bigger fingerlings are introduced into paddies instead of fry, if the intention is to harvest good sized fish (**Kuronuma, 1980**). Fish with a high growth rate, such as Oreochromis niger and Oreochromis mossambicus should be stocked.

Negative Impacts of Fish Production on Rice Cultivation

1. More water and greater depth is required for the growth of fish. Deep water may completely cover the rice plant and so prevent its ripening, and may also cause stem rot and lead to various plant diseases (**Coche, 1967**). On most irrigation schemes in Kenya, the irrigation water is expensive and the idea is to use as little water as possible. There are deep water rice varieties and so it is the water that limits the geographical area in which rice - fish culture can be practised. Building dams and reservoirs to catch all possible water and re-circulating used water are some of the methods used to ease this restriction. The sale of fish also offsets the expense of water

(Hora and Pillay, 1962).

2. Loss of cultivation area due to fish refuge ditches. Growing fish with rice diminishes the area of land on which rice can be grown, as part of the field may be used for fish refuge ditches (Hickling, 1972). It also limits the use of modern agricultural techniques such as mechanization, chemical fertilizers, herbicides and insecticides (Huet, 1970). Grover, (1975) conducted yield trials at the fresh water aquaculture centre in the Philippines using newly developed insect - resistant, high - yielding rice varieties. He found out that good rice production can be obtained without insecticide applications. Oreochromis mossambica and Cyprinus carpio gave yields of 69 - 208 kg/ha at harvest time.

3. Uprooting young rice plants. Fish activities, as they search for food or nesting ground can uproot the young rice plants. This is overcome by introducing fry into the fields two weeks after transplanting rice, or when the young plants are well established and cannot be uprooted by the young fish (Khoo and Tan, 1980; Kuronuma, 1980).

Negative Impacts of Fish - Rice Culture on the Farmer

1. Rice - fish culture is more involving than growing

the two crops separately. There is need for strict water control and movement of fish from paddies into the ditches and vice versa. The farmer has to carefully control the use of biocides, when to apply them; preferably when the fish are out of the paddies. There is also need for qualified and experienced extension workers to advise the farmers.

2. There may be a lack of fry of desirable species (Heut, 1970). Farmers need to have their own hatcheries to reduce costs. They may need knowledge of methods of artificially inducing spawning. They also need to know the details of feeding and growth of their choice of fish. In Tilapia culture, for example, over production may lead to stunting (Fryer and Iles, 1972).

3. Rice - fish culture is capital intensive. In Southeast Asia most of the labour needed is provided by the farmer's family. Money would be needed otherwise for the construction of ditches, outlets and inlets and more land is used leading to reduction in the area of land available for rice growing (Coche, 1967). More employees may be needed to handspray biocides so that they do not affect fish in the ditches.

ii. Rotational Rice And Fish Culture

In Arkansas and Indonesia common carp (Cyprinus carpio), channel fish (Ictalurus punctatus), and tilapia (Oreochromis mossambicus) are grown alternately with rice, one crop per season (Hickling, 1972). One or two weeks after harvesting rice, irrigation canals are cleared of weeds and the bunds raised and strengthened to give a greater depth of water (Khoo and Tan, 1980). The rice straw, husks and bi-products are raked into heaps in the paddy to give fish living space and to regulate the rate of decay, and to prevent anoxia. This trash acts as green manure which stimulates the growth of phytoplankton, zooplankton and aufwuchs, on which the fish feed (Hickling, 1972; Khoo and Tan 1980).

The fields must have a slight slope so as to drain off water for harvest. The shallow parts of the field may be only 45 cm deep but the deepest part may be 90 cm or more. After the rice harvest a dry period is allowed when the pond is treated with lime and copper sulphate to kill fungal spores, bacteria and protozoa which would infect fish.

The ponds are then flooded and after about 7 days, fish are introduced (Huet, 1970; Hickling, 1972). The fish are allowed a maximum period of growth (up to 3

years in Japan). They may be fed on a supplementary diet for high yield (Kuronuma, 1980), or fed naturally since the field is fertile (Table 1). When fish mature the ponds are drained and the fish harvested and sold. The field is left fallow and allowed to dry for 3 - 4 weeks. It is then ploughed and treated with molluscicides, herbicides and insecticides before rice seedlings are transplanted.

Fish may be rotated with rice and beans. For example, fish grown for two years or fish followed by beans then fish again as in polyculture (Djajadiredja et al, 1980). Other crops such as vegetables, coconuts and bananas, may be grown on the dikes.

Advantages of Rotational Fish and Rice Culture

Rotating fish and rice allows each crop to be given the optimum management it requires for producing a maximum yield. Under these circumstances, agrochemicals such as fertilizers, herbicides and insecticides can be freely applied to the rice crops so long as they do not persist into the fish cultivation season. There is better pest control since the life cycles of the pests are disrupted (De la Cruz, 1980). Rice production costs are reduced since the paddy bottom is soft and clean after fish harvest and allows immediate seeding or transplanting (De la Cruz,1980).

iii Fish Culture In The Irrigation And Drainage Canals

Fish may also be cultured in the water supply and drainage canals where the water is moving sufficiently slowly (less than 0.5 m sec $^{-1}$). The fish are usually held in cages constructed from a variety of materials. Their environment provides insufficient food and therefore artificial feeding is necessary. A feeding device which provides food pellets on demand is usually fitted to the cage.

Advantages of Fish Culture in the Canals

Fish production in running water is often greater than in stagnant water because of the higher concentration of dissolved oxygen and more efficient removal of metabolic waste products and surplus food which might inhibit growth (Hickling, 1972).

Fish in cages are easy to harvest and monitor in terms of yield, growth rate and general health. It is possible to control the breeding of fish in cages and so avoid stunting, for example in tilapia. When over stocked, the fishes (tilapia) attain only a small size and breed precociously (Fryer and Iles, 1972). If the number is reduced, the fish grow to a bigger size before breeding. Monosex cages can also be set up and only males with a fast growth rate cultured (Fram and Pagan Font, 1980). It is easier to sort out a cage or

move the fish into a pond of clean water during the spraying of insecticides than when they are in the rice paddy. Cages are also used where the substrate bed is bad (Milne, 1976).

Negative Effects of Fish culture in the Irrigation and Drainage Canals

Fish in these canals are still subject to molluscicides, insecticides and herbicides especially on highly mechanised fields. Herbicides are necessary to prevent aquatic weeds blocking the canals and providing a habitat for snails. Oreochromis melanopleura and T. rendali which feed on aquatic vegetation, could be used for biological weed control instead of herbicides (Philippart & Ruwet, 1982; Trewavas, 1981). Niclosamide is also usually applied in the water supply and drift of insecticides sprayed by aeroplanes and water run-off from the paddies would affect fish in the channels just as much as those in the field (Koesoemadinata, 1980).

Cage culture in canals is only possible where there is plenty of water and reliable management. Losses due to poachers and feeding expenses can be high where management is poor. Fish can also be grown in ponds in the irrigation scheme area, where water is available. Surface run off from rice fields has to be controlled

to avoid contamination of pond water with chemicals.

3. The Characteristics of Fish - Rice Cultivars

Rice paddy is a difficult environment for fish. Because of the shallow water, there can be oxygen and temperature stress. The water is usually turbid and hot in the daytime and may be cold in the night. Almost all the biocides used to improve the rice crop are either directly or indirectly harmful to fish. There are therefore only a few fish species that are suitable for introduction into rice fields (Table 2). Fish - rice cultivars need the following attributes (Coche, 1969);

A. It should be able to reproduce while in the rice field. This will enable the farmer to have fingerlings for sale or as seed for his next crop. Some fish need special places for laying eggs, such as holes in the ground while others may be affected by the water current. A fish species to be grown with rice should reproduce well in the rice paddies.

B. It should not be destructive to rice. The fish should not be one that eats rice or uproots the rice plants when foraging for invertebrates. All Oreochromis species are micro-herbivorous and are therefore suitable. In polyculture predators/prey

TABLE 2

TILAPIA SPECIES THAT ARE CULTURED (TREWAVAS, 1980)

GENUS	SUB GENUS	SPECIES	OTHER EXAMPLES
Tilapia (A. Smith 1840)	3 - 6 groups	T.Sparrmanni (A. Smith, 1840)	T rendali (Boulenger,1896) T zilli (Gervais)
Sarotherodon (Ruppell, 1952)		S. melanotherodon (Ruppell,1852)	S. galilaeus (Linn) S linnellii (Lonnbergy)
Oreochromis (Gunther, 1889)	Oreochromis (Gunther, 1889)	O huntery (Gunther 1889)	O niloticus (Linn) O mossambicus (Peters,1852) O aureus (Steindachner) O niger (Gunther,1889)
	Nyasalapia (Thys,1968)	O squamipinnis (Gunther,1889)	O macrochir (Boulenger,1896) O variabilis (Boulenger,1896) O angolensis (Trewavas,1982)
	Alcolapia (Thys,1968)	O. grahami	O alcalicus (Hilngendorf)
Danakilia (Thys, 1968)	Neotilapia	O tanganicae (Boulenger, 1896)	None
		O franchetti (Vinciguerra)	None

relationships between the choosen species should be considered (Philippart and Ruwet, 1982).

C. It should tolerate the prevailing conditions. The fish should tolerate conditions found in shallow waters of the paddies where high temperatures 10 - 34° C, oxygen shortage and high turbidity prevail. In the paddies the water levels are lowered periodically for weeding and application of biocides and the chosen fish should be capable of tolerating the ensuing stress (Coche, 1967).

D. It should have a good growth rate and the fish should grow to a marketable size within the given time. This depends on the size of fish that the consumer will accept and the number of rice crops grown per year. The fish should be such that it can command an appropriate selling price in relation to production costs (Reay, 1979).

4. Review of Yields

The yield of fish croped from concurrent fish and rice fields varies considerably and depends on a number of factors. These include the quantity and quality of the stocked fry, the amount of feeding, the weather conditions, especially water temperature the amount of fertilizers applied and the design of the field (Kuronuma, 1980). The stocking rate, survival and

growth, which are determined by the carring capacity of the field determines yield. The carring capacity in turn is influenced by soil type, fertilization of the field and supplemental feeding of the fish

A. Table 3 shows that feeding increases yield. Yields in Japan range from 70 - 100kg for 0.1 ha field stocked with 1,000 fry and given adequate supplemental feeding.

B. The species of fish, its growth rate, adaptability to the rice field and stocking density.

If the fish species stocked in the field has a natural growth check and stops growing after a certain size and/ or weight, the maximum yield in kilograms will only reach a certain point, however favourable the field environmental conditions may be (Hickling, 1972). The yield is higher if the fish is a herbivore. Being low in the food chain, it gets most of the manufactured plant food while a carnivore, higher in the food chain gets only approximately 10%. This is due to food chain transfer inefficiency, where some of the manufactured food is lost and some used by the plant. Most tilapia when densily stocked, grow very fast and attain sexual maturity when still very small in body. Although the bulk produced is heavy, the fish may be too small to be sold. Moderate stocking, preferably

TABLE 3

CARP AND RICE YIELDS OBTAINED IN 1942 OVER 90 DAY GROWOUT, IN 300M FIELDS, WITH AND WITHOUT SUPPLEMENTAL FEEDING (Kuronuma, 1980)

FIELD NUMBER	NO OF FISH STOCKED	WT OF FISH STOCKED (g)	SUPPLEMENTAL FEED (KG)	FERTILIZER	FISH YIELD WT (KG)	MEAN WT (g)	RICE YIELD WT KG
1	1,000	94	13	397.5	13.9	22.5	302.3
2	1,000	94	13	397.5	13.9	24.0	300.0
3	1,000	94	13	397.5	13.2	21.0	304.0
AVERAGE					13.6	22.5	303.1
4	1,000	94	0	401.3	8.2	18.8	300.0
5	1,000	94	0	397.5	6.3	18.8	337.8
6	1,000	94	0	26.3	6.8	18.8	277.9
AVERAGE					7.1	18.8	305.2

with monosex male populations with a fast growth rate is used.

C. Polyculture: Yield will be improved if two or more species of fish with complementary feeding habits are stocked, because a wider range of food in the field is utilised. In Israel, when Sarotherodon spp were stocked with carp (Cyprinus spp), there was a higher increase in the weight of the whole population of fish than with carp only, at the same stocking rate (Hickling, 1972). It was found that a mixed population (mixed species) of fish increased weight by 1.4 kg per day per pond while a population of carp alone increased by 1.3 kg per day per pond. Stocking should be based on the total weight of fish rather than the total number, to maximize production. Over stocked fish tend to inhibit the growth of the younger fish.

D. Temperature and Light Effects; Yield varies with climate. In temperate countries fish lose weight in Winter and the actual growing period is limited to the Spring and Summer (5 - 6 months). In Japan, fish are put in over wintering ponds (November to April) without feeding when temperatures are below 10° C. The pond either has a warm water stream passing through it or has a deep hole which is covered with boards and straw (Kuronuma, 1980).

Light is necessary for algae and other plants to photosynthesize. Some fish feed on algae. The average yield per hectare per unit time is lower in the temperates than in the tropics which have a higher yield. E. Management; This includes use of fertilizers, supplemental feeding and predator control. Fertilizers may be organic, such as husks, rice bi-products or artificial fertilizers applied to the field to improve the rice crop. Fertilization results in a richer growth of plankton on which the fish feed. It has been shown that feeding even without fertilizing may increase the yield sixfold (Hickling, 1972). Since the water in the paddies is shallow, poachers and predators (birds) need to be watched and controlled.

F. Water Depth; Generally deeper water will support more fish than shallow water because there is more living space (Hickling, 1972). This is not true of some tilapia species. Oreochromis niger grows better in shallow water (30 cm) than in deeper water (60 cm). A field will support a greater total weight of small fish because the small fish mean many mouths seeking food and can occupy small pools of water.

In some cases the growing of fish in rice paddies has led to a decrease in the yield of rice due to

decrease in land and low stocking density. Hickling, (1972) quotes a case where there was a 9% reduction per hectare of rice yield. The average yield of rice was 6,819 kg/ha without fish and 6,218 with fish. The crop of fish however was 303 kg of a value twice that of the rice lost. It is however, easier to grow and sell rice than fish which need plenty of water and a ready market nearby. With rice, less water is needed and storage is easier. A study was carried out on the economic prospects for rice - fish culture in the Philippines by De la Cruz,(1980). It showed that the return from the fish was lower than that from a rice enterprise, assuming favourable weather conditions. The margin however was small and can be changed in the wet season. It was also shown that rice culture involves higher expenses than fish culture. Fish is also a nutritionally richer food than rice (De la Cruz, 1980).

5. Fish Toxicology

Toxicology is the "qualitative and especially the quantitative study of the injurious effects of chemical and physical agents, as observed alterations of structure and response in living systems" (Hayes, 1975). Poisonous substances are used to control insect pests

of crops and vectors of human and animal diseases. Powerful biocidal chemicals when applied indiscriminately have undesirable effects on the environment and non-target organisms (Muirhead - Thomson, 1971). Insights and knowledge acquired from the study of the nature and behaviour of toxins contributes to the development of better drugs, safer pesticides and safer levels of toxins.

Environmental toxicology is becoming increasingly important because:

1. of the extensive use of industrial chemicals, pesticides and natural resources
2. more intense utilization of urban, agricultural, recreational and marine environments
3. the heightened awareness of the hazards of chemicals to wildlife, domestic animals and people (Matsumura, 1980).

Pesticides are the largest group of poisonous substances that are widely broadcast today. In testing the effectiveness of a biocide, the results are relative and are compared with known effects of conventional biocides to assess the potential toxicity of a given chemical (Matsumura, 1980).

A test aims at maintaining a test population under controlled environmental conditions for the duration

of the test. The abiotic environment, including dissolved oxygen concentration, pH, temperature, salinity, turbidity and light, play a major role in the reactivity of biocides and must be monitored. Tests are set up with replicates and controls. The replicate is exactly the same as the experiment while the control lacks only the variable being tested but is otherwise treated as the experiment and maintained under the same conditions (Apha, 1985).

A given test concentration of a biocide may kill the organism in the given experimental time and may be lethal or acutely toxic or it may adversely affect the organism so that though the organism is not killed immediately it suffers long term effects. Tests may therefore be set up to determine the lethal concentration of the biocide for a given duration of exposure or to determine the chronic effects of the biocide on an individual and the population as a whole; slow and prolonged effects as compared to acute, that rapidly develop to a crisis (Pimentel, 1971).

In determining acute toxicity, one is interested in knowing the threshhold lethal concentration of the toxin, that if slightly exceeded, would kill the organism after a given period of time. This is the

incipient lethal level and if it is not exceeded, the organism will not be directly killed by the biocides no matter how long the duration of exposure. The test organism however suffers some handicap either behavioural, physiological or reproductive which could eventually kill the individual or affect the population (Laws, 1981). The tolerance of a particular species to a particular pollutant varies from one individual to another and under varying experimental conditions.

Organisms to be studied may be divided into groups, typically of ten individuals. Ten or more fish are recommended per test since solitary fish behave in an odd manner (Apha, 1985). Each group is exposed to a different level of stress for a fixed but arbitrarily standardized time (24 hours, 48 hours and 96 hours). The percentage dead in each group is then plotted against the stress level (biocide concentration). The stress level corresponding to 50% survival is then estimated usually by fitting a smooth curve to the data. This 50% survival stress level is called the LC_{50}. LC_{50} (lethal concentration) of toxicant is the concentration of that toxicant which kills half the test population in a specified time usually 24, 48 or

96 hours (Matsumura, 1980; Apha, 1985).

Sub - lethal or chronic effects are produced by biocide concentrations below those producing mortality. Chronic stresses may in the long run be just as effective in eliminating an organism or a population as are acutely toxic stress factors. Chronic stresses generally modify or interfere with reproduction, development, growth and behaviour (Laws, 1981).

Any biocide that interferes with or prevents the reproduction of individuals could completely eliminate the species from a system without having any apparent effect on the individual members of the population. For example, high levels of DDE (a metabolite of DDT) in the diet of predatory birds resulted in the production of thin shelled eggs that cracked when the adult bird sat on them. Such breakage if repeated can cause recruitment failure and could eventually wipe out the bird species (Bitman et al, 1973; Laws,1981).

Any stress that interferes with an organism's ability to develop and grow normally, may severely impair the organisms' chances of survival (Laws, 1981). A stress that effects respiration or movement may alter the rate of efficiency of growth.

Predator avoidance, migratory behaviour, learning

and feeding behaviour may also be affected by biocides. DDT treated salmon avoid cold water, which could affect their upstream migration for spawning and therefore affect the whole population (Laws, 1981).

Some biocides tend to build up in the biosphere with resulting undesirable effects. A concentration of 14 - 20 ppm DDT was used in three applications to control gnats on Clear Lake during the period 1946 - 1957. In 1957 an analysis of the fat viscera of Western grebes, Aechmophorus occidentalis (Moriarty, 1975), (a bird species feeding on the lake) gave a concentration of 1600 ppm DDT; 80 times higher than the DDT concentration applied to the lake (Laws 1981 citing Hunt and Bischoff, 1960). This concentration killed the birds while the initial spraying did not.

Bioassay methods for conducting fish toxicity test may be classified as follows (Apha, 1985):

a. According to the test conditions as:
   i. Static
   ii. Static renewal
   iii. Continuous flow through

b. According to their duration as:
   iv. Short term - less than 15 days

v. Intermediate term - 15 - 90 days

vi. Long term - more than 90 days

i. STATIC BIOASSAYS

This is the simplest of the bioassay methods used in aquatic toxicological research in which the test organisms remain in the same test solutions for the duration of the test. This method has the disadvantages that important changes in the test conditions are likely to occur during the test period. Such changes may involve deterioration in water quality due to contamination by excretory products or the reduction in toxicant concentration by various means e.g. volatization, biological uptake and biodegradation (Apha, 1985).

The volume of the water used in static bioassays should be large in proportion to the biomass of the fish being tested. A ratio of three litres of water per gram of test organism is given as a guide (Sprague, 1969). This is necessary to provide adequate oxygen to avoid respiratory depletion and consequent stress, and to lessen the effects of waste products and carbon dioxide concentration on pH. Aeration of all tanks is usually recommended to ensure the concentration of

dissolved oxygen remains high. Static tests are generally regarded as being suitable for detecting and evaluating the toxicity of poisons in fairly clean water where there is no oxygen demand from oxidisable pollutants such as organic matter. In water with high biological oxygen demand or high chemical oxygen demand alternative methods are recommended.

ii. STATIC RENEWAL BIOASSAY

In static renewal bioassays, the test organisms are kept in fresh toxicant solution which is changed at periodic intervals. All the tanks are usually aerated. This type of test has the advantage of keeping the toxicant concentration and pH fairly constant by avoiding accumulation of waste. Controls (without toxicants) are treated in the same way as the test tanks to standardize the handling and disturbance effects on the fish (Apha, 1985).

iii. CONTINUOUS FLOW-THROUGH BIOASSAYS

Continous flow-through bioassays allow the test conditions to be constant and so the fish are not subjected to water change procedures or handling stress (Sprague, 1969; Apha, 1985).

In these bioassays measured quantities of dilution water and the concentrated toxicant solution are carefully mixed to the required test concentration and delivered at a constant rate to the test chambers, to give a continuous flow-through of the diluted toxicant. Dilution water without the toxicant provides the flow-through in the controls. The disadvantage of this method is the great complexicity and cost of the test apparatus needed to ensure adequate mixing and control of the test concentration and the very large volumes of dilution water required (Apha, 1985).

IV. SHORT TERM BIOASSAYS

Two complementary short term bioassays are recognised.

Range Bioassays.

These are preliminary bioassays used to establish the concentrations of the toxicant under investigation, which will later be used in short-term definitive bioassays. These bioassays involve a concentration of toxicant solution strong enough to bring about a speedy response by the test organism, usually within a few hours. Batches of test organisms are exposed to a wide range of

concentrations usually in logarithmic series such as 0.01, 0.10, 1.0, 10 percent (Sprague, 1969). Such tests may be conducted in static, renewal or continuous flow-through conditions depending on the characteristics of the test organisms, the objectives of the study, the availability of equipment, water supply and particularly the nature of the toxicant. The series should include an upper concentration which kills all the test organisms in the required time period and another of sufficiently low concentration for all the test organisms to survive for the selected duration of the test. The range is selected, either from past experience or by trial and error over a number of tests.

Definitive Bioassays.

Short term definitive bioassays may be conducted using static, static renewal or flow-through systems but must use the same system as that used in the range finding assays which determined the range of concentration to be used. Tests are usually set up with five or more concentrations and duplicate controls without biocides. The most commonly used short term bioassays are acute

lethality tests. These tests are used to obtain an estimate of the toxicity of the materials being tested to specific aquatic organisms and the results are usually expressed in terms of the lethal concentration LC_{50} or incipient LC_{50}. The 24 hour LC_{50} is the concentration which causes death of fifty percent (50%) of the population in 24 hours. The incipient LC_{50} is the concentration of which fifty percent (50%) of the population survives and tolerates indefinitely. (Apha, 1985). The duration of short-term tests is influenced by the toxicant under study and the precision desired but is arbitrarily standardized to 24, 48 or 96 hours. It is believed that the longer the test is carried on, the more reliable are the results so long as the environmental conditions in the test tank remain constant and favourable. (Apha, 1985)

In test procedures, the test organisms are starved for two days prior to and during the test period. This helps to standardize conditions in the test and control tanks as the quantities of food ingested by the fish in each would be unknown. This would introduce a new variable

since it could not be assumed that both test and control animals fed to an equal extent. If, however, the tests take more than 96 hours, feeding is usually unavoidable. The absence of food also prevents contamination of water by surplus food and fish excretory products.

V. INTERMEDIATE BIOASSAYS

Intermediate bioassays are used when the determination of the incipient LC_{50} requires more time than that given for short term tests, for studies of selected life stages and to indicate toxicant concentrations to be used in studies involving the life cycle of the organism. Intermediate length tests may be static, renewal or flow-through bioassays. They are also used to determine how the effects of a stressing agent decrease with time.

VI. LONG TERM BIOASSAYS

Long term bioassays are carried out as continuous flow-through bioassay type, with exposure to the toxicant extending over as much of the life cycle of the test organism as possible (Apha, 1985). Long term bioassays are used to quantify the sub-lethal

effects of toxicants on the reproduction, development and growth of the test organism to assess their influence on behaviour. They are further used to monitor the bioaccumulation of the biocides in the tissues of the test organisms. This is especially important if the test organism is a human or livestock food resource. Bioaccumulation of the biocide may reach levels toxic to man or/and his domestic animals. In these long term tests, the five or more concentrations used are selected on the basis of short or intermediate term bioassays.

6. The Influence Of Water Quality On The Toxicity Of Toxicants To Fish. Water quality is the single most important factor in fish production (Ray, 1978).

The hardness, alkalinity, pH, dissolved oxygen concentration and the temperature of the dilution water affect the toxicity of biocides used in all toxicology tests and these parameters need to be monitored throughout the test period.

High temperatures decrease the concentration of dissolved oxygen, increase the metabolic rates of most poikilotherms and solubility of pesticides into

the fatty tissues (Matsumura, 1980). This may put the test organism under stress even prior to exposure to the toxicant. Temperature may be both a lethal factor or a controlling factor (Brown, 1978). With few exceptions, almost all aquatic organisms are ectothermic, thus their body temperature is almost the same as that of the environment. This also means that the rate of metabolism of aquatic animals undergoes an approximate two fold increase with every 10° C rise in temperature within species specific limits. Acclimation tends to moderate the effect to an intermediate level (Sprague & Dixon 1981). Temperature also influences the toxicity of DDT. DDT was more toxic to Salmo salar , at 13° C than at 19° C (Brown, 1978). The median tolerance limit (TLM) for DDT in rainbow trout, Salmo gairderii was increased by 50% by raising the temperature from 7° C to 13° C. Over the same temperature range, the 96 hour TLM for Endrin was halved. Young Atlantic salmon, Salmo salar, when exposed to sub-lethal concentrations (5 ppm of DDT), select cooler temperatures in a thermal gradient but when DDT concentration is increased, they selected a high temperature (Brown, 1978).

Dissolved oxygen concentration is considered by Hickling, (1972) to be even more important than temperature or the chemical composition of the test water. Low dissolved oxygen concentrations increase the toxicity of poisons to fish. Fish react to a decrease in dissolved oxygen by increasing the volume of water passing over the gills and this may increase the rate at which poisons reach the gills (sites of absorption). A reduction in the dissolved oxygen concentration increases the toxicity of zinc, lead and copper salts to rainbow trout (Pickering, 1968). Blue gills showed an increased mortality to zinc as a result of stress due to low concentrations of dissolved oxygen.

Biocides are more toxic in soft water than in hard water at any pH. In hard water, at low pH , most of the ammonia stays in the less poisonous ionic state (Ray, 1978). At high calcium concentrations, the toxicity of copper is related to alkalinity rather than hardness. This is based on the ability of many ions to complex copper and therefore reduce the concentration of ionic copper (Beamish and Waiwood, 1978).

At a given pH less copper was required to reduce growth by a given amount in soft water than in hard water (Beamish and Waiwood, 1978). Niclosamide and

Trifenmorph are more toxic in soft water than in hard water. A concentration of 0.2 ppm Niclosamide sprayed on hard water streams in South Africa for the control of water snails killed fish after two hours of spraying while at 0.10 ppm Niclosamide sprayed on soft water streams killed fish within 5 - 10 minutes. Water hardness, however, appears to have no major effect on the toxicity of chlorinated hydrocarbons to fish (Muirhead - Thomson, 1971 citing Henerson et al, 1960).

At a given hardness of water, copper induced depressions in growth rate were more pronounced and recovery was slower at a lower pH than at a higher pH. The solubility of Niclosamide increases in alkaline water and falls sharply below pH 7. With Trifenmorph molluscicidal activity was greater at pH 6.5 than at pH 5 or 6. (Beamish and Waiwood, 1978)

Water chemistry may therefore indirectly affect the toxicity of biocides by affecting the general activity, metabolism and behaviour of fish, in such a way that they are under stress and are more readily susceptible to biocide effects. Water chemistry may also affect the availability of the toxic substances to fish.

Water chemistry may also effect the toxicity of biocides by affecting the chemical and physical state

of the biocide, the extent to which it remains in solution, its persistence in the environment and the extent to which it is broken down. The rate of bio-accumulation in aquatic environments generally appears to be higher than that in terrestrial environments. This is because persistent insecticides are more soluble in fat than in water, so they are taken up into the fatty tissues of the organisms. Water also acts as a transport medium. In general, biocides are more toxic in warm, acidic, soft water than in cold, alkaline, hard water.

CHAPTER III

M A T E R I A L S

The materials used in this study were as follows:

1. Fish-fry: Fry of *Oreochromis niger*
2. Biocides: DDT, Carbofuran, Niclosamide and Trifenmorph
3. Storage and Experimental Tanks
4. Dilution Water

1. Fish

Introduction

Most tilapia, generally have a herbivorous diet unlike other fish which feed predominantly on small invertebrates or on young or small fish. They are therefore close to the producers (plant life) and grow, fast, to a good size. They are a valuable source of food to man. The prefered diet of the various species varies from coarse vegetation (grasses, young shoots and leaves of water weeds) to unicellular algae and even bacteria (Trewavas, 1981).

All tilapia exhibit a high degree of parental care, where by they are sharply divided into substrate spawners and guarders of the brood on one hand and mouth brooders on the other (Lowe - McConnel, 1975).

The substrate spawners constitute the genus Tilapia Smith (1840), while the mouthbrooders constitute Sarotherodon spp, Ruppell (1952). Tilapia of West Africa are either paternal mouth brooders or biparental mouth brooders such as Sarotherodon melanotheron and S. galilaeus (Trewavas, 1981). The males of these species do not congregate in breeding places or have distinctive breeding colouration.

The East and Central African mouth brooders have now the generic name Oreochromis Gunther (1889). Here the males are sexually dimorphic and at breeding time gather in breeding places, where they form a territory. The females move freely in the territories. They lay eggs and the male fertilizes them either on the ground or in her mouth as she picks them up. The female then moves away to special nursery areas. The male is excluded from brood care (Trewavas, 1981).

The mouth brooders used in rice - fish culture are in Table 2.

1. History and Characteristics of Oreochromis niger Gunther, 1889

Approximately fourteen species of tilapia are currently cultured under widely differing conditions all over the world because of their good cultivar qualities (Fryer & Iles, 1972).

Oreochromis niger is essentially a riverine species indigenous to the rivers flowing eastward to the East African Coast (Fryer & Iles, 1972). It is a euryhaline fish found in both fresh and brackish water. It is cultured at the Tilapia culture section, Baobab Farm Limited, Bamburi, Mombasa. Some other tilapia species such as Oreochromis niloticus are cultured at the Fisheries Department Research Station, Sagana.

O. niger is a mouth brooder which reproduces in all types of water, moving or stagnant. The young, at the free swimming stage are protected by the female, which picks them up in the mouth at signs of danger. The fish is largely micro-herbivorous though it can be omnivorous. The fry feed on plankton. Only algae crushed by the gill rakers can be digested since the fish have no enzyme, cellulase, for digesting the cell wall (Fryer & Iles 1972; Balarin 1979; Bowen 1982).

In lightly stocked ponds the fish will breed at 18 cm and the age of two years but in a heavily stocked pond it will grow to 8 - 10 cm in four months and breed. This is too small for the market. In these ponds the fish grow very fast in the first four months giving a steep growth curve, thereafter the growth rate gradually decreases. Within this period, there is a

maximum growth rate of about 4 - 5 cm per month at 24° C - 26° C. It breeds throughout the year (in the tropics) at four month intervals (Fryer & Iles, 1972).

Male O. niger show a growth rate superior to that of the females (by 33 - 50%). This greater growth rate is still marked even after maturation. Isolated males grow faster than isolated females (Van Somereen, 1961). Length for length, isolated males also show a greater weight gain per fish than where males and females were mixed in a pond. This superior growth is believed to be genetically controlled.

When the population density is increased experimentally or naturally at a relatively rapid rate, at short-term intervals, there is a depression in the growth rate almost in direct proportion to the increase in density. This is due to a shortage in food supply, changes in shoaling patterns which introduce competition for feeding space, build up of waste products and presence of growth inhibiting substances. (Van Someren, 1961 and Whitehead 1959)

Under certain conditions, such as over crowding in ponds, cichlids produce a large number of off-spring at the age of a few months, when they are still very small in size. The fish are said to be runting.

Runting fish grow faster and reach sexual maturity earlier than 'normal' fish (Fryer & Iles, 1972). The percentage of body weight of the female, especially devoted to reproduction, is higher and the number of eggs produced per unit body weight is also increased. This shows increased fecundity with many small eggs being produced. Runting is not permanent, since when overcrowding is reduced the fish revert to normal growth rates and reproductive patterns. The transfer of mature, breeding males of various sizes from an over-stocked pond in which their growth rate had slowed or ceased, to another pond, at a much reduced stocking rate, resulted in a marked increase in their subsequent growth (Van Someren, 1961). Runting therefore involves miniaturization and a marked speeding up of growth and reproduction.

Runting has evolutionary and survival value to the fish. It helps to compensate for mortality (in this fish) in adverse environments. During the dry weather when lakes break up into many small isolated pools, the small sized fish can live in these pools of water where larger fish would perish. The ability to reproduce while still young is a form of neoteny while the ability to modify the size of the body according to environmental conditions helps this fish to survive

adverse conditions.

2. BIOCIDES

Since World War II, biocides have been increasingly used to control pests and vectors of human and domestic animal diseases. Biocides may be classified in a variety of ways based on the physical state, target species, and chemical nature. When classified according to the target species, they may be insecticides, herbicides, fungicides, molluscicides, rodenticides and others, depending on whether they are designed to kill insects, weeds, fungi, molluscs or rodents respectively (Matsumura, 1980). Classified according to their chemical nature, they may be organo-phosphates, chlorinated hydro-carbons and carbamates.

The widespread use of biocides is due to the ease and convenience of using them, their effectiveness in removing the pest and increased yield (Lim and Heong, 1984). Biocides are used to improve public health, increase agricultural production, improve wildlife and forestry management.

Ecological Effects of Biocides

a. Biocides may cause a reduction in abundance of individuals and species. They tend to significantly reduce the numbers of some species in a

biotic environment. The reduced species may be the prey of another that cannot switch to another food type. The predator species would be wiped out or migrate. Some species are also more susceptible to a particular biocide than others. After spraying, such a species may be so reduced that predators and parasites wipe it out (Pimentel, 1971).

b. The reduction in the number of species in a community may lead to instability within that community. This in turn may lead to a resurgence of some species which without competition for food and space, may multiply very fast. The increased species may not be a desired one. An example of this was shown by Dempster (1975). DDT was used to control a white butterfly Pieris rapae whose larvae feed on leaves of cabbages.
Other arthropods such as spiders, plant bugs and ground beetles prey on these caterpillars. When DDT was sprayed, both the pest and their predators were killed. As the plant formed new leaves (which had no DDT), the butterfly laid its eggs which hatched into caterpillars. The infestation was worse than before spraying since there were predators. Resurgence here was because the pest lives

in the growing part of the plant which quickly looses the pesticide while its predators live where the chemical persists.

c. Biocides may cause a selective reduction of predators and parasites. After pesticide application, species lower in the food chain (plant feeders) tend to increase due to removal of parasites and predators which are more affected by the biocide. Selective removal of parasites and predators may result in abundance of the prey species. Top predators and parasites may also be removed if there is a bioaccumulation of the biocide in the food chain.

d. Biocides may cause fluctuations in the stability of the populations and community. Large increases in the numbers of certain species may disrupt the structure and finally the stability of a natural community. A species may therefore not be killed directly by a given biocide but through the effects of the biocide on the food organisms, shelter plants or other aspects of the biological and physical environment, which may then have adverse effects on the species. The general health of the organisms may be affected such that it does not

feed, grow or reproduce effectively and eventually dies. The population may therefore die out through breeding failure rather than mortality of the individuals.

The biocides used in this study are:

a. DDT)
) Insecticides
b. Carbofuran)

c. Trifenmorph)
) Molluscicides
d. Niclosamide)

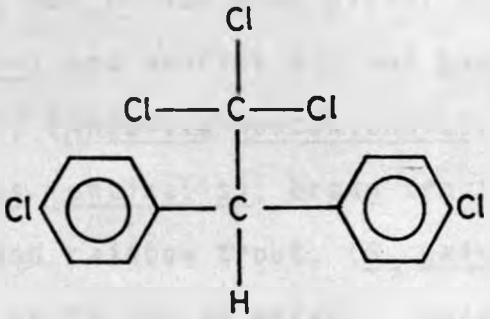
a. DDT (Dichlorodiphenyl trichloroethane)

DDT is a persistent broad spectrum chlorinated hydrocarbon, first synthesized and described by Zeider (1874). Its insecticidal properties were discovered by Muller (1939) (Moriarty, 1975) It was widely used in World War II to eradicate (malaria) mosquitoes * and (typhus fever) fleas* . It has been extensively used to eradicate diseases such as trypanosomiasis (tsetsefly*) , plague (fleas*) , yellow fever and river blindness (Simulium sp*) by killing the vector insects. It has also been used in agriculture to control arthropod pests on many crops including cotton, tobacco, soyabeans, potatoes, maize and rice.

* Vectors

Today however, the use of DDT has been banned for this purpose in the U.S.A., Canada and Europe. It is only used to a limited extent in other developed countries due to its effects on non-target species, persistance and bio accumulation (Sherman, 1977).

Structural formula: DDT



Mode of Action

The mode of action of chlorinated hydrocarbons is not well established (Laws 1981). DDT is known to affect the central nervous system at high doses leading to severe tremors, muscular contractions and convulsions (Bahr & Ball, 1973.) Fish are very sensitive to chlorinated hydrocarbons but death comes from suffocation due to interference with oxygen uptake at the gills. DDT interferes with calcium transport at the surface of the nerve. It inhibits oxidative phosphorylation in

the nerve. It also inhibits oxidative phosphorylation of the mitochondria, so affecting respiration. (Moriarty, 1975).

Toxicity

DDT is differentially toxic to fish. The short term LC_{50} for salmonids is about 5 ppb (Brown 1978). When applied weekly at 0.112 kg/hectare in Tennessee, DDT killed blue gills, (Lepomis macrochirus) and sunfish but not Gambusia affinis or the eel, (Anguilla bostoniensis). Brook trout, (Salvelinus fontinalis), brown trout, (Salmo trutta), and rainbow trout, (S. gairdenerri) succumbed to 14 ppb in water. Goldfish (Carassius auratus) and fathead minnows (Pimephales promelas) are fairly tolerant to DDT (Brown, 1978).

Fish previously exposed to sub-lethal concentrations of DDT prefer warmer water. Pre- exposure to 20 ppb for 24 hours, made brackish water Gambusia spp prefer warmer water. Fingerling salmon exposed to 5 ppb for 24 hours violently avoid entering cooler water where the temperature is less than 5° C. DDT also impairs osmoregulation and inhibits Na - K - ATPase activity responsible for active transport of ions (Laws, 1981).

This leads to a rise in sodium ion concentration in the blood and impairs the transmission of impulses. In brook trout, sub-lethal levels of DDT in the diet of yearlings resulted in their laying fewer eggs and those that hatched died young (Laws, 1981). Though the number and volume of eggs produced by trout, after exposure to DDT concentrations above the threshold toxicity, were unaffected by DDT poisoning, there was mortality among fry (Laws, 1981). This indicates a higher susceptibility of fry to toxin than adults. LC_{50} of various organisms to DDT are shown in Table 4.

Persistance

The rate of degradation of DDT varies in different parts of the biosphere. The half life of DDT sprayed on citrus crops is estimated to be about 50 days but only 11 - 15 days in peaches and 7 days on alfalfa. DDT in the soil has a half life of 2.2 years (Laws, 1981). Clear Lake (a shallow lake north of San Francisco, U.S.A.) has a large number of different species of fish. It is also infested by a large number of gnats. DDD, a metabolite of DDT, was sprayed on this lake to control the gnats. Fish that hatched

TABLE 4

LD_{50} / LC_{50} For Various Organisms to DDT

ORGANISM	EXPOSURE TIME (HOUR)	LC_{50}PPM	LC_{50}MG/KG
Rat	24	-	420
Mouse	24	-	200
Dog	24	-	60-75
Mallards	120	3,300-3,600	-
Pheasants	120	750-9,500	-
Rainbow Trout	96	0.007	-
Blue Gill	96	0.008	-
Carp	96	0.010	-
Fowler's Toad Tadpoles	24	2.4	-
Hermit Crab	24	0.007	-
Grass Shrimp	24	0.012	-
Water Flea	48	0.00036	-

(Pimentel, 1971)

7 - 9 months after the last DDD application to the lake, were found to contain 7 - 25 ppm DDD and died. This implied that DDD had persisted in the fish for 7 - 9 months. DDT accumlates in fatty tissues and egg yolk. Brown pelican eggs from birds on the Southern Californian Coast U.S.A. were found to contain 853 ppm DDE, another DDT metabolite, (Brown, 1978). Since there had been no DDT spraying in that area, the DDT must have entered the ocean from land run-off and rainfall and been taken up by pelicans in their fish food. DDT in the fat when metabolised during starvation leads to deferred poisoning (Brown, 1978). Dale et al, (1962) demonstrated that rats fed on sub-lethal levels of DDT when partially starved mobilised DDT from their fat deposits into the blood stream and brain resulting in death.

Bioaccumulation

According to Matsumura, (1980) bioaccumulation of a pesticide depends on:

1. The physiochemical characteristics of a pesticide. These include:
   a. water and lipid solubility and stability
   b. the lipid content of the animals or plants absorbing the insecticide and the concen-

tration of the insecticide in the environment

2. Competition; availability of other materials and/or organisms which can absorb the insecticide
3. The rate of food consumption and body size of the organism. Smaller organisms eat more per body weight and can therefore ingest more pesticide. They also have a large surface area for absorption. However, experiments show that big animals have a larger proportion of pesticides. This is probably due to food chain transfer, lipid content, food habits and and a longer life span while the small ones are killed off.
4. The pesticide level in any organism is dependent on the uptake and elimination of the chemical.

Fish absorb and concentrate DDT (Brown, 1978). Brown trout exposed to 2 ppb DDT for 3 weeks concentrated it about 500 times in the gill tissues and about 3,000 times in the muscle. DDT is stored primarily in the fatty tissues where they are metabolised or excreted slowly. Ecologists believe that such pesticides may

become greatly concentrated at higher trophic levels in the food chain. Laws (1981) however suggests that higher trophic level organisms simply absorb more DDT residues from the environment and that DDT is not magnified in the food chain. He suggests that biological magnification of pesticides may be due to different mechanisms, like exchange of pesticide residues between the organisms and the water. Biological magnification of biocides may therefore be due to direct exchange between the orgnism and the environment in addition to food chain transfer. It is however probable that the transfer of pesticides through food chains has been a major factor in some cases of poisoning of raptorial and fish eating birds (Laws 1981). A concentration of 20 ppm DDT was used in 1954 and 1957 to clear gnats on Clear Lake in California, U. S. A. In the same years, 100 and 75, respectively, Western Grebes,(Aechmophorus Occidentalis) were found dead. Analysis of specimens of these birds showed no evidence of disease but sections of the visceral fat taken from them were found to contain 1600 ppm, 80 times the DDT applied on the lake. Visceral fat in the fish taken from

from the lake contained hundreds to several thousand parts per million DDD. Analysis of a wide variety of samples of organisms from Clear Lake showed that DDT concentration increased in the order:

plankton --- small fish --- big fish --- grebes.

The average concentration of DDT in plankton was 5.3 ppm, that in the edible flesh of various plankton eating or plant eating fish was 5 - 80 ppm while that of predatory fish was 1 - 196 ppm and 1600 ppm in predatory birds. This works out a maximum magnification of:

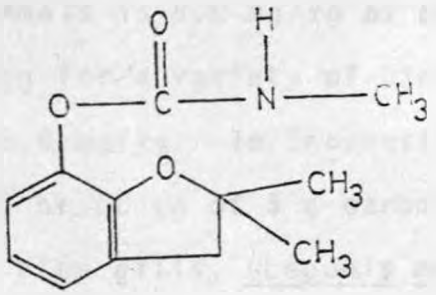
Plankton X 16 in small fish
Plankton X 39 in big fish
Plankton X 333 in grebes.

DDT is persistant, bioaccumulated and biomagnified. Repeated spraying as in rice paddies makes the paddies unfit for rice/fish culture as deferred toxicity may kill the fish even months later.

b. CARBOFURAN; [2,3 - dihydro - 2,2 - dimethyl - 7 - benzofuranyl - N - methylcarbamate] (FURADAN®)
Carbofuran is a non-persistant, non-accumulative broad spectrum insecticide and nematicide effective

by contact, stomach and systemic action. Carbamate insecticides originate from physostigmine found in the Calabar bean, Physostigma venosum (Ishii, 1984).

Structural Formula: Carbofuran



Mode of Action

In mammals, carbamates inhibit the nerve enzyme acetyl - cholinesterase so preventing the release of acetyl - choline after nerve transmission at the synapse. This affects muscle contraction and death is caused by asyphyxiation. Blockage of nerve acetyl - cholinesterse results in a failure of the nervous system due to the accumulation of acetylcholine (Kuhr and Dorough, 1976). This results in the continuous "firing" of the nervous impulses across the synapse. Symptoms of poisoning are muscular tremors, excessive salivation and in-

creased mobility of the gastro-intestinal tract (Estores et al, 1980).

Toxicity

Furadan® has a high oral toxicity if swallowed or inhaled by mammals but has a low dermal toxicity. It has no effect on non-target species. The oral LD_{50} for mammals is 8.0 mg/kg of body weight (rat) while the LD_{50} for a variety of birds varies between 0.24 - 5.0 mg/kg. In Indonesia, Swata (1973) showed the 96 hr LC_{50} of 3 g carbofuran for carp was 1.3 ppm, blue gills, (Lepomis macroechirus) and catfish, (Letaturus punctatus) were equally sensitive with 96 hr LC_{50} ranging from 0.21 - 0.28. In Japan, the 48 hr LC_{50} of 94.2% carbofuran for Cyprinus carpio was 1.4 ppm and Oryzias latipes 1.3 ppm (Estores et al, 1980).

Unlike with organophosphate poisoning which has the same symptoms, carbonfuran effects are reversible. The carbamylated cholinesterase is easily hydrolyzed to free cholinesterase and so the animal recovers. In human beings, the symptoms of carbamate poisoning are seen when only small amounts of the lethal does is taken in (1/20 - 1/30) and so the user is pre-warned (Estores et al, 1980).

Persistence and Bioaccumulation

Carbofuran is non-persistant and non-accumulative. Except for large scale spills, most methyl carbamates and their phenols persist in water only for a few days, or at most a few weeks. Labelled carbofuran applied at a rate of 3.0 ppm to a model ecosystem, released a steady flow of radiocarbon (^{14}C.) into the water up to a maximum level 35 - 38 ppm at day 34. About 60% of the radioactivity of the insecticide was tightly bound to the soil and never became available to the water (Kuhr and Dorough, 1976). In another experiment, blue gill fingerlings were continuously exposed to radioactive carbofuran at 0.02 and 0.01 ppm for 28 days. All the fish remained healthy. About 0.4 ppm was found in the flesh after 1 - 3 days at 0.01 ppm and 10 - 14 at 0.02 ppm. When put in clean water radioactivity declined to undetectable proportions after 14 days (Estores et al , 1980). The metabolites of carbofuran found in water, microbes, invertebrates and fish included N - hydroxy - methyl carbofuran, 3 - hydroxy carbofuran, 3 keto carbofuran, 3 - hydroxy carbofuran phenol (Kuhr and Dorough, 1976). The rate of

hydrolysis depends on temperature, being higher at higher temperatures than at low. The half life of Furadan at pH 7.0 - 8.5 varies from 40 days - 2 days at 25° C. It is broken down to non-toxic compounds which are soluble in water.

Several field studies have demonstrated that carbofuran is safe to fish when correctly applied. In the Philippines, the results of joint studies conducted at the International Rice Research Institute and the fresh water aquaculture systems showed no mortality (Estores et al, 1980) among fish placed in paddies, seven days after broadcasting. Broadcasting carbofuran in paddies with fish gave 100% mortality.

Carbofuran in the soil is degraded in 60 days (Venkatramesh et al, 1987). Thin layer chromatography in soil samples showed that carbofuran is degraded faster in alkaline, flooded, undisturbed soils rather than acidic, dry, disturbed ones. A bacterium isolated from flooded soil decomposed carbofuran in a mineral salts medium (Sethunathan et al, 1980).

Carbofuran, whether in liquid or granular form is the only pesticide now recommended in rice - fish

culture in the Philippines (Estores et al, 1980 citing Guerrero 1977; Anon 1979). It protects the rice crop from insect pests and does not affect fish growth. The fish should be introduced into the paddies 5 - 7 days after application. For late insect infestation, spraying with a 0.01% solution of Carbofuran is recommended (Estores et al ,1980).

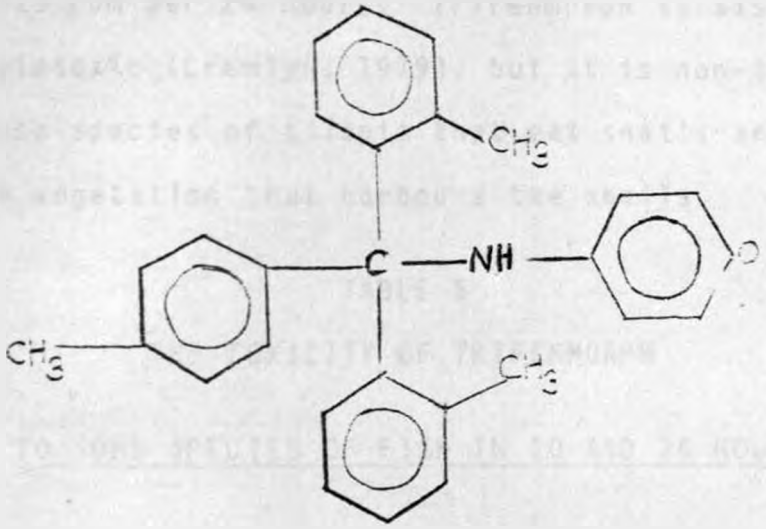
c. Trifenmorph (Frescon ®)

The control of aquatic snails which act as intermediate hosts of the parasites causing human bilharziasis (schistosomiasis) and fascioliasis in sheep, has been characterised over the last 20 - 25 years, by a succession of molluscicides. Trifenmorph is the most recent and highly effective molluscicide, although it has been withdrawn from use because it is expensive and non-ovicidal.

Commercial Trifenmorph is applied, as a 16.5% W/V emulsifiable concentrate, to snail infested water bodies, to achieve a concentration of 0.025mg active ingredient for several days (Matthiessen, 1976).

Trifenmorph is hydrolysed at acid pH to relatively non - toxic compounds; - triphenyl - methanol and morpholine.

Structural Formula: Trifenmorph



Mode of Action

The mode of action of Trifenmorph is not known.

Toxicity

Trifenmorph is lethal to snails at 1 - 2 ppm in 24 hours (Cremlyn, 1979). It is however not toxic to snail eggs. According to Matthiessen, (1976) however molluscicidal concentrations of Trifenmorph range from 0.1 - 0.5 ppm/hr and 0.01 - 0.05 ppm for 24 nours. The acute toxicities of Trifenmorph to fish vary depending on the fish species, temperature, pH and other factors, such as presence of organic matter (Matthiessen, 1976). Some of the common food fish species such as tilapia,

Sarotherodon, Barbus and Clarias succumb to 0.025 ppm per 24 hours. Trifenmorph is also highly phytotoxic (Cremlyn, 1979), but it is non-toxic to those species of tilapia that eat snails and to the vegetation that harbours the snails.

TABLE 5

THE TOXICITY OF TRIFENMORPH

TO SOME SPECIES OF FISH IN 10 AND 24 HOURS

FISH SPECIES	TOXICITY mg L^{-1}			
	24 HOUR LC_{50}	24 HOUR LC_{100}	10 HOUR LC_{100}	10 HOUR LC_{50}
Carassius auratus	0.07	0.5	-	-
Lebistes reticulatus	0.25	0.5	-	-
Cyprinus carpio	-	-	1.0	-
Tilapia melanopleura	-	-	-	0.03
Sarotherodon mossambicus	-	-	-	0.11

(After Matthiessen, 1976)

Persistance and Bioaccumulation

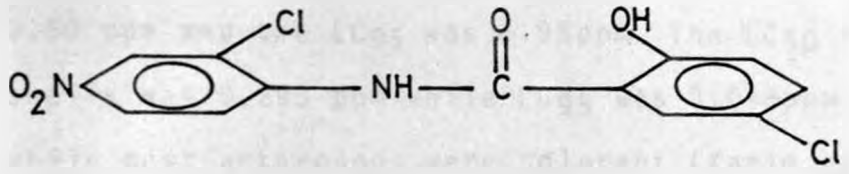
Trifenmorph is not persistant. Although it has been

shown that Oreochromis mossambicus can absorb and concentrate Trifenmorph up to a thousand times in 55 hours (Matthiessen 1976), 50% of this is excreted in the same length of time and the rest is slowly removed. Absorption is believed to occur mainly through the gills while it is metabolised in the liver and removed through bile and urine.

d. NICLOSAMIDE (Bayluscide®)

Niclosamide is the most commonly used molluscicide. It is toxic to snails, their juveniles and eggs and it is non-phytotoxic. It was first manufactured by Bayer, (1959). It is weakly acidic and is formulated as a water soluble ethanolamine salt. It is extensively used in Egypt in the control of aquatic snails which are vectors of bilharziasis and fascioliasis. It is lethal to snails at 0.3 - 1 ppm/ 24 hours but has low mammalian toxicity (Cremlyn, 1979). Niclosamide is marketed as a 70% wetable powder and 25% emulsifiable concentrate.

Structural Formula: Niclosamide



(Harthoorn et al, 1985)

Mode of Action

Niclosamide is a respiratory poison (WHO 1965). It is believed to paralyse the respiratory muscles and to block the uptake of oxygen and so kills by asyphixiation.

Toxicity

Niclosamide is highly toxic to fish and snails but has a low mammalian toxicity. Rats tolerated a single dose of 5g/kg niclosamide in their food and 1% in food continuous for one year had no effect. In man, 2 g. per 60 kg body weight of the pure chemical for 24 hours had no side effects. Toxic levels for most species of fish are below 0.5 ppm.

It is toxic to all fish in a concentration of 0.3 - 1.5 ppm for 24 hours. In an experiment done to show the effect of niclosamide on non-target species, the LC_{50} for Gambusia was found to be 0.50 ppm and the LC_{95} was 0.95ppm. The LC_{50} for snails was 0.295 ppm while LC_{95} was 0.048ppm while most arthropods were tolerant (Karim et al, 1986).

Some fish species however are more tolerant than others, as shown by the table following.

TABLE 6

THE VARIATIONS IN NICLOSAMIDE LC_{50} FOR DIFFERENT SPP OF FISH AFTER 24 AND 96 HOURS EXPOSURE TO CONTAMINATED WATER (Paflitschek 1976)

SPECIES	LC_{50} (PPM) 24 HOURS	LC_{50} (PPM) 96 HOURS
Large mouth bass	0.111	0.062
Blue gills	0.082	0.068
Carp	0.245	0.082
Channel catfish	0.084	0.082
Goldfish	0.279	0.230
Yellow perch	0.082	0.081
Brook trout	0.061	0.061
Rainbow trout	0.052	0.050

Concentrations as low as 0.2ppm controlled young

snails after exposure to Niclosamide for 24 hours, while this concentration killed newly laid eggs after a 6 hour exposure. In laboratory tests, 100% mortality of snails was obtained at concentration of 5 ppm for one hour. Niclosamide is also toxic to the larval stages (miracidia and cercariae) of schistosomes within a few minutes of exposure at 0.3 ppm (Paflitschek, 1976). Niclosamide is more toxic in soft water than in hard water. In soft water, fish began to jump out of the water within minutes of spraying and most fish died within 5 - 10 minutes at 0.1 ppm. In hard water, the fish kill was slower at 0.2 ppm and no fish mortality was observed until after 24 hours (Brown, 1978).

Bioaccumulation, Biomagnification and Persistance

Niclosamide is neither persistent nor accumulative in organisms or plants (WHO 1965). Sunlight degrades it and adsorption on mud and other colloidal particles, remove it from the water. This might affect fish that feed on detritus and algae growing on the mud. It is not accumulated in the organism or its food chain. However the effects of pollutants on fish and wild life need

to be considered in ecological terms of population dynamics and genetics instead of being taken as a matter of cause and effect (Oglesby, 1977).

The rice paddy can be seen as an ecosystem in a complete environment with its associated populations which are a result of certain inter-relationships between the abiotic factors and the species present. Physical factors in the ecosystem relating to the water and substrate and factors relating to food chains cannot be separated (Verneaus and Laynaud, 1977). The toxicant water and tissue concentration may therefore be different (McCarty, 1986).

3. Storage and Experimental Tanks

Fish storage tanks were made of cement. They had a capacity of 300 litres, Photograph 1. While the experimental containers were plastic packets containing 15 litres of water, Photograph 4.

4, Dilution Water

Tap water was left standing for 4 days to dechlorinate before being used to dilute the biocide, in a 700 litre tin tank, Photograph 2. This dechlorinated water was kept in a 230 litre tin tank, Photograph 3, for immediate use in the experimentals.

PHOTOGRAPH 1

Fish Storage Tank

300 litres of dechlorinated tap water to which 10 grams of sodium chloride has been added to maintain the fish.

PHOTOGRAPH 3

Dechlorinated Water Tank

230 litre tank containing dechlorinated dilution water.

PHOTOGRAPH 4

Experimental Tanks

15 litre plastic tank containing ten (10) fish.

CHAPTER IV

METHODS

1. Determination of LC_{50} of Pesticides on O.niger Fry

1.1 Preparation of tanks and dilution water.

The experiments were carried out in a temperature controlled room and 24 hour lighting. The experimental tanks were washed out and disinfected with Dettol® and allowed to dry. They were then rinsed out with dechlorinated dilution water before filling them to the required level. The dilution water was left standing for three days before use in a 700 litre tank (59cm X 98cm X 122cm) [Photograph 2] to dechlorinate and reach the ambient temperature of 22°C - 23°C. This water was then run into a smaller tank for immediate use (Photograph 3). The dechlorinating tank was refilled from the main tap water supply each time water was removed. The following parameters were also carried out on the dilution water:

i. pH Value

The pH was measured with an electronic meter. Measurements were made before each experiment in the short term bioassays and after every solution renewal in the growth bioassays. The pH of the dechlorinated water ranged from 6.9 - 7.1.

ii. Temperature

A thermometer was used to measure the temperature of the dilution water before changing the solution. The dilution water was left standing until it reached the ambient temperature of 22°C - 23°C before introducing it into the experimental tanks.

iii. Dissolved Oxygen

The concentration of dissolved oxygen of the dechlorinated dilution water was monitored before transfer to the experimental tanks. A 250ml glass stoppered bottle was carefully filled with dechlorinated water from the smaller tank (without fish), by a siphon. The bottle was allowed to overflow three times to ensure there were no air bubbles in the bottle. The siphon was slowly withdrawn

and the bottle carefully stoppered. The standard Wrinkler method was then used to determine the oxygen concentration of the water. Determination of dissolved oxygen concentration was carried out on the dilution water before the water was added to the experimental tanks, and found to be 9mg/litre. This was due to the continuous aeration of water in all the tanks. It was assumed that the dissolved oxygen concentration in the experimental tanks was at saturation point and did not vary much due to the continuous aeration of the solutions.

iv. Total Hardness

50 mls of the dechlorinated dilution water, to which 2 ml of buffer solution and 1 - 2 drops of of indicator solution (Eriochrome Black T) had been added were titrated against the Standard EDTA titrant. Total hardness was calculated by the Apha method and found as 20mg/L $CaCO_3$. This showed that the water used in these experiments was soft.

v. Free Chlorine Using DPD Tablets

A 10 ml cell containing 10ml of distilled water was put in the left hand compartment of a chlorine comparator (Nairobi City Council Water Treatment Laboratories, Kabete). To the other cell with a few drops of the sample water (tap water) one DPD No. 4 tablet was added and crushed. Sample water was added to reach the 10 ml mark, rapidly mixed by shaking and put in the right hand compartment of the comparator. After two minutes, the colours were matched and a reading recorded as residual chlorine. This was carried out using first tap water as sample water and then the dechlorated water that had been left standing for three days. Tap water contained 2 ppm/litre chlorine while the dechlorinated water give a zero reading.

1.2 Collection and Maintenance of Fish

A sample of Oreochromis niger fingerlings of about 0.2gms wet weight and 1.5 cm total length were obtained from the Baobab Farm at Bamburi, (Mombasa). 100 fish fry were transported in oxygen filled polythene bags each containing 1 - 2 litres of the slightly brackish

water in which they had been reared. A few drops of benzocaine were added to the water prior to transportation to make the fish less active and thereby reduce their oxygen demand, on their one hour flight to Nairobi (Taylor and Solomon, 1979). To reduce contamination of the water by fish faeces during transportation to Nairobi, the fry were starved for 48 hours before hand. On arrival in Nairobi, the fish were taken directly to the laboratory and immediately transferred into 250 litres of continuously aerated and dechlorinated tap water at 22° - 23° C, in which 10 grammes of table salt had been dissolved. This gave a concentration of 0.005g NaCl/Litre and was necessary to avoid loss of too much fluid due to the transfer of fish from the slightly saline water to fresh water. High mortality resulted if the fry were transferred directly into fresh water. The bag containing fish was opened with its mouth just under the water in the tank, so that the fish could swim out freely. This allowed for a gradual adjustment to the differences in temperature and salinity.

The fish in the holding tank were fed on tilapia pellets whose approximate composition was as follows:

TABLE 7

COMPONENT	PERCENTAGE
Yellow Maize	10
Maize Bran	20
Pollard	39
Meat Bone Meal	15
Herring Meal	8
Sunflower Cake	5
Breeders Premix *	3
TOTAL	100

*Mixture of vitamins and minerals

Tilapia pellets are manufactured by Unga Ltd., Nakuru. Fish were fed only during the daytime; waste products and surplus food were removed by siphoning at the end of each day.

After a two week acclimatisation period, in the holding tank, (Photograph 1), groups of ten fish were chosen arbitrarily and transferred to each of the eight experimental containers holding 10 litres of dechlorinated water (Photograph 4). Fish were taken from the holding tank into an intermediate bucket and from here one at a time, transferred sequentially to the first, second, third, ... nth tank until each of the eight containers had ten fish and a control. The fish

were again acclimatised in the experimental containers for a further 24 hours before the addition of the biocide.

1.3 Test Procedure

a. General Considerations

Problems of quantitative inference in biological and technological research concern the relation between a stimulus and the response. Where the response is not exactly determined by knowledge of the stimulus and repetitions of experiments or observations do not all give the same magnitude of response, quantitative assessment of the grade response is possible. In a quantal, the response is an "all or nothing" type; the animal is dead or it is not dead (Finner, 1971).

A bioassay is a set of techniques relevant to comparisons between the strengths of alternative but similar biological stimuli. It mostly refers to the assessment of the potency of vitamins, hormones, toxicants and drugs by means of the responses produced when doses are given to experimental animals (Apha, 1985).

In a quantal response, occurrence or non-occurrence depends on the intensity of the stimulus. There

will be a certain level of intensity below which the response, such as death, does not occur. This stimulus intensity, is the tolerance value and varies slightly from one subject to another in the population used. The distribution of the tolerance concentration of the toxic agent is seldom symmetrical because a few animals with very high tolerances can provide an 'extended tail' (Finner, 1971).

The tests in the experiments required a quantal type of response where the fish died or did not die. The tolerance concentration was taken as the LC_{50}. The concentration above which fifty percent of the population was killed within 24 hours, 48 hours, 72 hours or 96 hours. Probit analysis methods were used, where logarithmic concentrations were used to show the distribution of tolerance to avoid the extended tail. In plotting the Figures IXa - XIIb (Appendix 1). Log. concentrations were corrected by adding a number (1 or 2) to give a positive line when plotted against mortality. In determination of LC_{50} however, this was re-converted to normal concentration.

Due to the expense involved in getting fish of the same age and species from the hatchery, only 10 fish were used in each concentration and each control. The fish were also not sexed but were all from the same hatchery, of the same age (four weeks) and the experimental conditions were standard at water temperature 22°C - 23° C, continuous aeration of water for oxygen saturation of dechlorinated dilution water.

b. Short - term definitive test

Stock solutions of each biocide were made, kept in the refrigerator and used throughout the tests. One gram of 70% wettable powder of Niclosamide, 1 ml of 16.5% Trifenmorph, 1ml of 25% DDT and 5gm of 5% Carbofuran were each separately dissolved in 1 litre of water at 30°C. The warm water was necessary to ensure the chemicals completely dissolved especially Carbofuran which is deposited on sand grains (for ease of application). The purple colour slowly fades into the water and the appeareance of white sand grains is an indication of complete solution. The other biocides, DDT, Niclosamide and Trifenmorph at concentrations used mix well with water, leaving no residue.

Each test consisted of nine concentrations including a control. The highest concentration killed all the fish in 24 hours and fish in the lowest concentration survived for more than 96 hours. Each of the containers with ten litres of dilution water at 23°C were randomly arranged in the experimental room. Fish were randomly collected using a small net from the holding tank into an intermediate tank, and then into the experimental tanks one by one. The number of fish dead was counted hourly for the first 6 hours and then every 12 hours for a total of 96 hours. These were recorded as fish dead in 24, 48, 72 and 96 hrs.

2. Behaviour studies

One 0.1 ml stock solution for each biocide in 10 litres of water provided the sub-lethal concentrations which were used in the growth and behaviour studies. This gave the following concentrations, which were used as sub-lethal concentrations:

TABLE 8

SUB-LETHAL CONCENTRATIONS OF DDT, CARBOFURAN TRIFENMORPH AND NICLOSAMIDE USED IN THE EXPERIMENTS

BIOCIDE	FORMULATION	CONCENTRATION USED mg/L
DDT	25 % EC	0.0025
Carbofuran	5 % Granules	0.005
Trifenmorph	16.5 % W/V	0.00165
Niclosamide	70.0 W/P	0.007

Twenty fish were sequentially transferred from the holding tank into an intermediate tank and then into experimental tanks and one control. During the experiment, the fish in these tests were fed daily and any surplus removed by siphoning, each evening and the solution made up to the 10 litre mark. This reduced contamination of the water which would adversely affect the fish. Contamination of the water would change the water chemistry and so influence the toxicity of the biocide being tested.

Fish activity in sub-lethal concentrations were recorded as the mean number of times a fish entered the square drawn on the bottom of the tank. This

was calculated as the mean number of entries of a fish in three ten minute counts, three days a week.

3. Growth Studies

Ten fish were put into growth tanks as described in 2 above. The fish were weighed in a known volume of water and the initial weight recorded. Subsequently, the ten fish were weighed every 7th day until the 35th day. A mean increase in weight was recorded for each week.
During the experiments, the tanks were continously aerated and the biocide mixture changed every six days by siphoning it, leaving just enough to keep the fish (0.5 litres) and siphoning in fresh toxicant solution. One fish died in Frescon and was removed but not replaced. This was necessary to standardize the experimental tanks and control. To account for this loss, the mean weight increase was calculated based on the number of fish surviving at each weighing. In the controls the water was changed as in the experiments but no biocide was added.

CHAPTER V

R E S U L T S and D I S C U S S I O N S

GENERAL OBSERVATIONS OF THE EXPERIMENTAL FISH (*Oreochromis niger*)

Fish in the holding tank swam together as a shoal, up and down and across the tank. Movement was started by one fish, followed by one or two more until all the ten fish were involved. They came to the water surface for air at an average rate of eleven times per population per minute. Most of the time the fish swam about in the water just above the floor of the tank and near the air bubbles. They also made definite movements toward food which they nibbled at and toward each other, the bigger fish chasing the smaller ones. This would indicate that *O. niger* live in groups interacting with each other and exhibit territorial behaviour but it was not followed up. It was also noticed that a single fish in the experimental tank remained inactive most of the time unless disturbed.

The fish made regular respiratory movements, opening and closing the operculum at an average rate of

3 - 4 times per minute. If fed, as in the growth tests, the fish immediately came to the surface and picked at the food pellet, following it as it descended to the bottom. They continued feeding and swimming around till the food was removed. The fish were a dull brown colour, which blends well with the colour of their natural background substrate.

i Experimental Bioassays: Observations on Behaviour of Fish in Various Concentrations of the Biocides

A DDT

Three hours after the addition of 0.06 mg/litre active ingredient DDT solution to the experimental tank, the first fish to react did so by swimming rapidly to the water surface. The other fish followed immediately after, one by one. They made rapid uncoordinated movements round the surface of the water either on their sides or right way up. This swimming movement was not controlled by the fins and the fish twisted their bodies as they moved. After three minutes, the fish returned to the bottom of the tank, where the affected ones alternated between tetanic shivering in one spot for anytime between 20 - 60 seconds and rapid uncoordinated swimming movements. As it moved, the

fish collided with each other and with the sides of the tank. Difficulty in breathing was shown by exaggerated and fast opercular movements of the fish, 8 - 9 times per minute. Fifteen minutes after reacting to the biocide, the first fish died.

In lower concentrations the fish swam around apparently normal until after 6 , 24 and 48 hours, when they reacted to the biocide. Again one fish was affected at a time and went through the above described motions before dying. The affected fish became black and immediately came to the surface.

In sub-lethal concentrations of 0.0025 mg/L, the fish swam around but not as a shoal as in holding tanks and control. They fed poorly and stayed mostly at the side of the tank. They came to the square drawn at the bottom of the tank at the average rate of 10 - 46 counts per given time • Table 9 shows a decrease in activity in the experimental tanks from 46 units in the first week to 10 units in the fifth week. There was a sharp drop in activity in the third week and a gradual decrease in the fourth and fifth week, (Fig. 1). The controls show a slight variation

in activity with maximum movement in the third week and less in the fifth week.

TABLE 9

AVERAGE NUMBER OF ENTRIES A FISH MADE INTO A SQUARE DRAWN ON THE BASE OF THE TEST TANK

WEEK	CONTROL	DDT	CARBO-FURAN	TRIFEN-MORPH	NICLOSAMIDE
1	46	46	26	22	18
2	49	40	20	16	26
3	52	20	16	12	16
4	44	16	14	10	12
5	40	10	10	8	6
r^2*	0.341	0.934	0.970	0.938	0.650

* co-efficient of determination

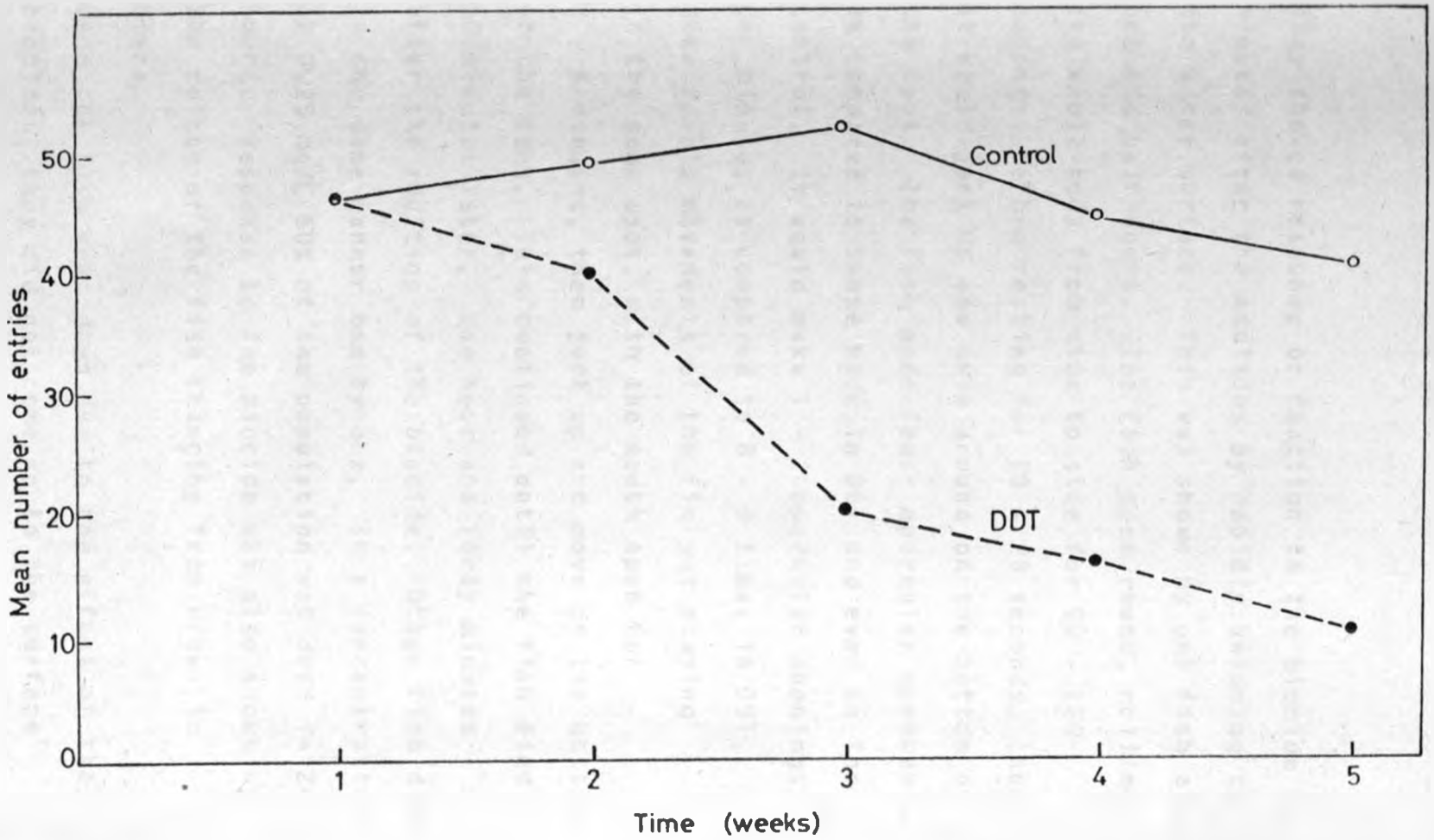
There was a linear relationship between activity and time (in weeks). The fish were more active in the first weeks than in the last, activity reduced with time (Fig. 1 and Table 9)

B CARBOFURAN

For one hour after the addition of 0.3 mg/Litre solution of Carbofuran to the test container, the fish swam around as normal (as for control).

FIG.I AVERAGE NUMBER OF ENTRIES PER FISH/WEEK INTO A SQUARE DRAWN ON THE BOTTOM OF THE TANK

(ACTIVITY OF FISH IN 0.0025 mg/l DDT)



They showed response or reaction to the biocide 90 minutes after the addition by rapidly swimming to the water surface. This was shown by one fish after one and half hours. The fish spun round, rolling the whole body from side to side for 90 - 120 seconds, before resting for 10 - 20 seconds, then it would jerk up and swim around on the bottom of the tank. The fish made fewer opercular movements as compared to those made in DDT and even in the control. It would make 1 - 2 opercular openings per minute, as compared to 8 - 9 times in DDT, make feeble movements of the fin yet staying in the same spot, with the mouth open for 2 - 4 minutes, then jerk up and move on the bottom of the tank. This continued until the fish died 20 minutes later, one hour and forty minutes after the addition of the biocide. Other fish died in the same manner one by one. In a concentration of 0.25 mg/L 50% of the population was dead in 24 hours. Response to the biocide was also shown by the colour of the fish changing from brown to black.

Once the fish went down due to the effect of the biocides, they did not come up to the surface

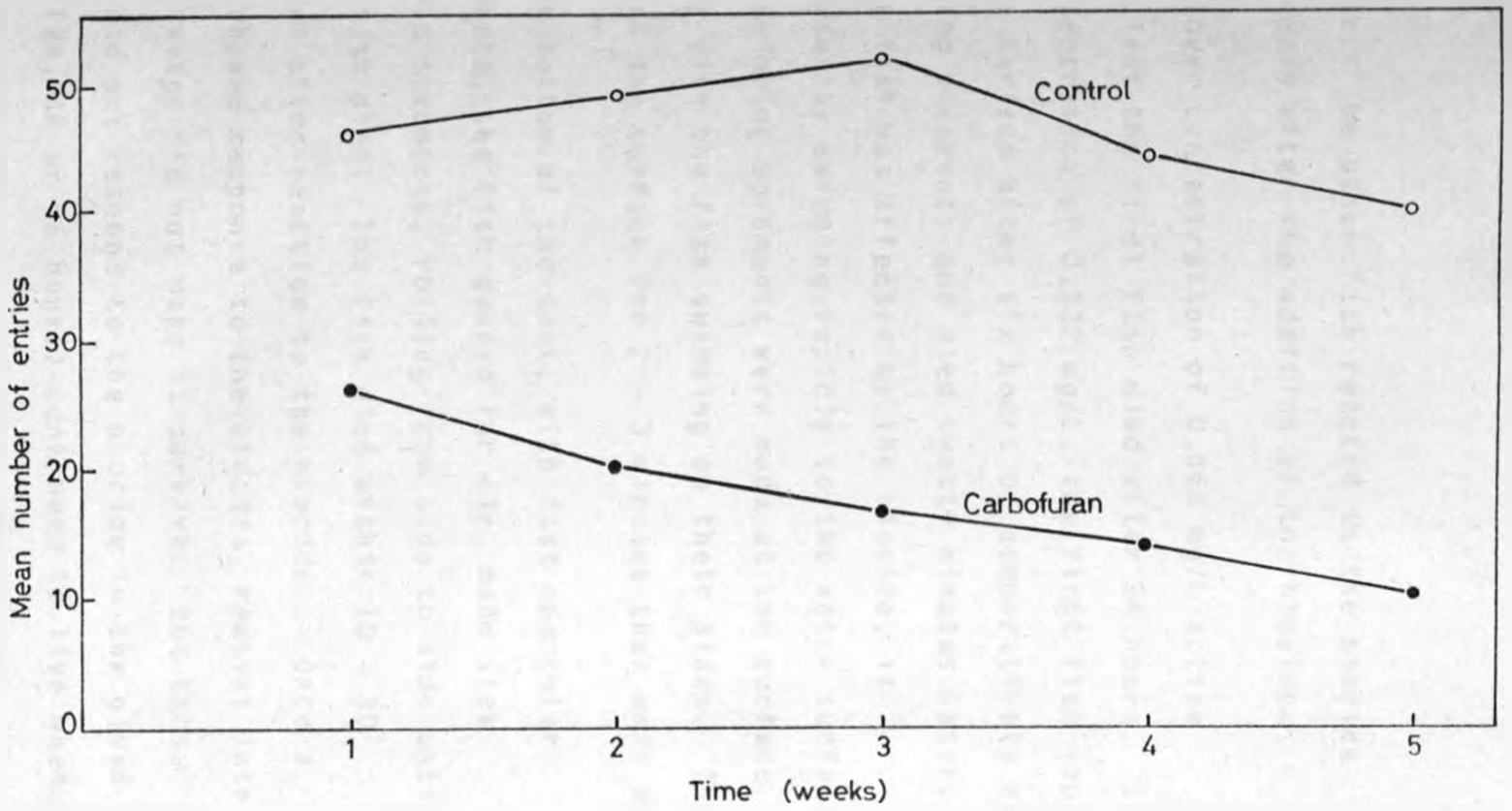
again. While making feeble swimming movements, they came into the square fewer times once or twice as compared to the control entering 5 - 6 times per minute.

In sub-lethal concentrations of Carbofuran (0.005 mg active ingredient), the fish swam with slow controlled movements. They kept to the edges of the tank most of the time and made fewer entries into the square. There was a gradual decrease in numbers of entries made into the square from the first week to the fifth (Figure II). Entries decreased from a weekly average of 26 units to 10 units (TABLE 9).

C TRIFENMORPH

Two hours after the addition of 0.182 mg/L Trifenmorph solution, the fish swam around individually but in a controlled manner. They stayed mostly just above the bottom of the tank. The first fish swam rapidly to the surface after two and a quarter hours. The fish made fast movements at the water surface, showing the silver side. Five minutes after the fish had reacted it went down to the bottom of the container where it continued its uncoordinated swimming movements, darting from side to side, twitching and gasping

FIG. II AVERAGE NUMBER OF ENTRIES PER FISH/WEEK INTO A SQUARE DRAWN ON THE BOTTOM OF THE TANK (ACTIVITY OF FISH IN 0.005 mg/L CARBOFURAN)



for air. The other fish reacted to the biocide five hours after the addition of the biocide.

At a lower concentration of 0.066 mg/L active ingredient the first fish died after 24 hours. In a concentration of 0.132 mg/L, the first fish shot to the surface after six hours of comparatively normal swimming movements and died twenty minutes later. Once a fish was affected by the biocide, it responded by swimming rapidly to the water surface. Fast swimming movements were made at the surface mainly with the fish swimming on their sides. They swam at the surface for 2 - 3 minutes then went down.

On the bottom of the tank, with fast opercular movements, the fish gasped for air, made slow tetanic movements, rolling from side to side until each fish died. The fish died within 10 - 30 minutes after reaction to the biocide. Once a fish showed response to the biocide, removal into fresh water did not make it survive. But those that did not respond to the biocide in the given time (24, 48 or 96 hours) continued to live when put in fresh water. The above behavioural description was true in 24 hour, 48 hour and 96 hour acute lethality tests. The difference was

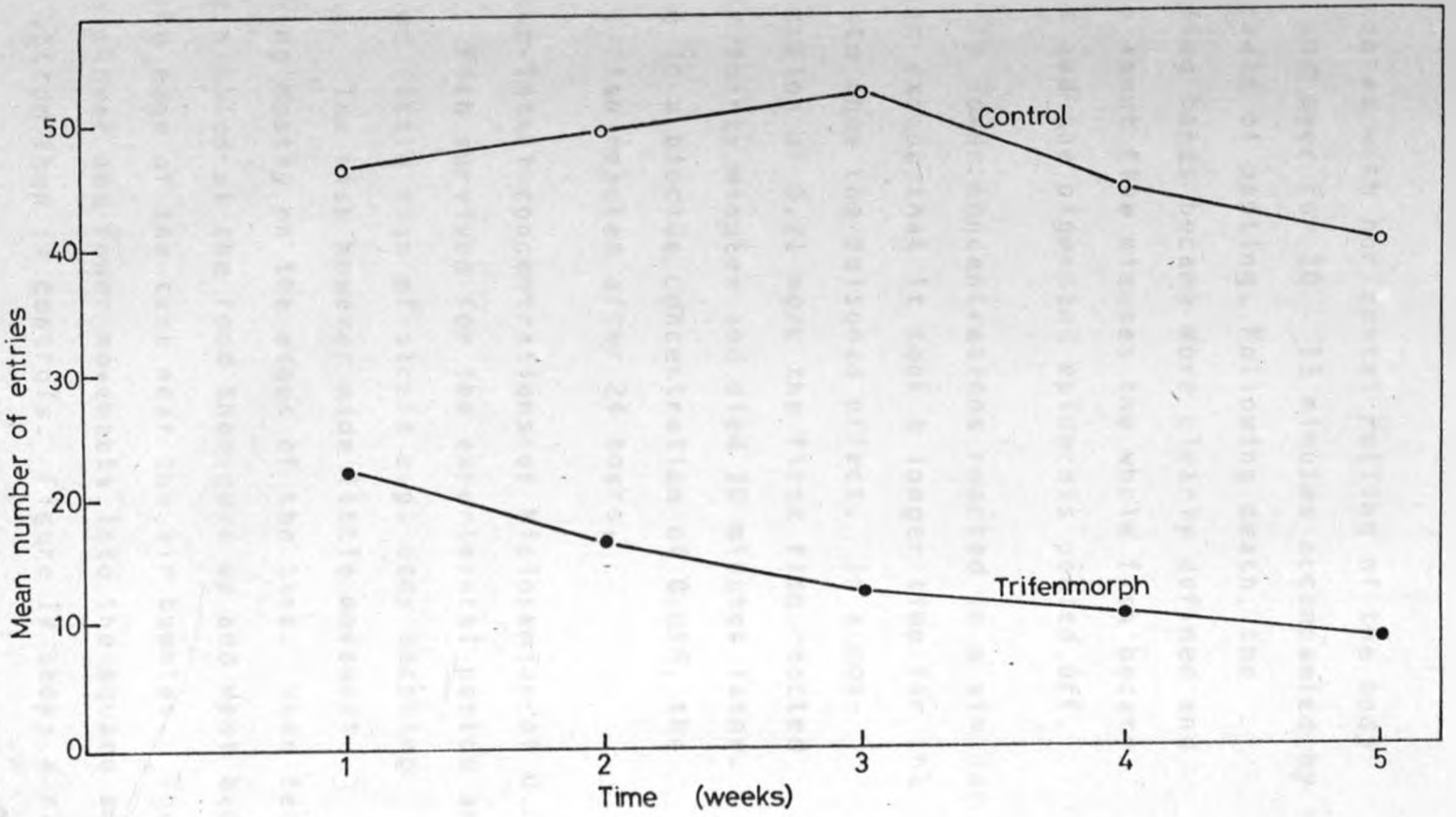
only noted in the time taken for the fish to respond to the biocide presence which lengthened at low concentration.

Figure III shows reduced swimming in sub-lethal concentrations of Trifenmorph 0.00165 mg/L. There was a sharp decrease in the swimming activity of the fish from the first to the third week and then a gradual decrease in the fourth and fifth week. Entries into the square decreased from a weekly average of 22 to 8 units.

D NICLOSAMIDE

For thirty minutes after the addition of the biocide 0.21 mg/L, the fish swam around normally as described for the control. After this, the first fish swam rapidly to the surface of the solution, one by one. They swam very fast colliding with each other and the sides of the experimental tank. They all swam at the water surface for about one minute before going down to the bottom of the tank. On the floor, the fish were observed to spin for 60 - 90 seconds, rest for 10 - 30 seconds and then spin again. Spinning behaviour consisted of the fish standing vertically with the head down while rapidly revolving around their long axis. It was

FIG.III AVERAGE NUMBER OF ENTRIES PER FISH/WEEK INTO A SQUARE DRAWN ON THE BOTTOM OF THE TANK (ACTIVITY OF FISH IN 0.00165 mg/l TRIFENMORPH)

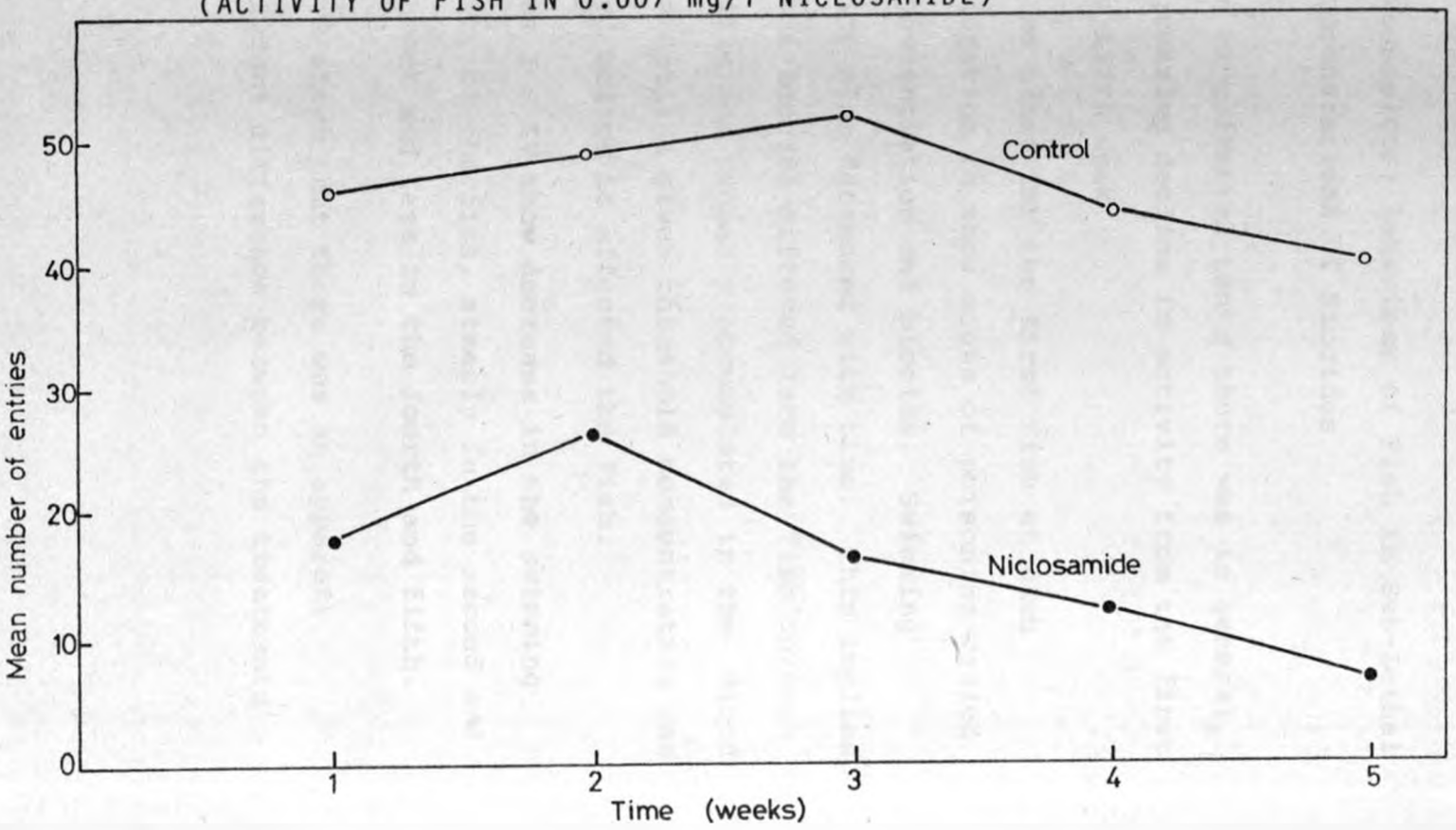


alternated with horizontal rolling of the body over and over for 10 - 15 minutes accompanied by intervals of panting. Following death, the shoaling bands became more clearly defined and after about five minutes the whole fish became black and the pigmented epidermis peeled off.

Fish in lower concentrations reacted in a similar manner except that it took a longer time for the fish to show the poisoned effect. In a concentration of 0.21 mg/L the first fish reacted after thirty minutes and died 20 minutes later. While in a biocide concentration of 0.098, the first fish reacted after 24 hours.

In sub-lethal concentrations of Niclosamide of 0.007 mg/L, fish survived for the experimental period and showed little sign of stress e.g. body becoming black. The fish however made little movement staying mostly on the edges of the tank. When fed, they nibbled at the food then gave up and went back to the edge of the tank near the air bubbles. They made slower and fewer movements into the square on the bottom than in controls. Figure IV shows a rise in fish activity in the second week, then a steep

FIG.IV AVERAGE NUMBER OF ENTRIES PER FISH/WEEK INTO A SQUARE DRAWN ON THE BOTTOM OF THE TANK (ACTIVITY OF FISH IN 0.007 mg/l NICLOSAMIDE)



drop in the third week and a gradual drop in the fourth and fifth weeks.

E Discussions; Behaviour of Fish in Sub-Lethal Concentrations of Biocides

In the experimental tanks there was in general, a progressive decline in activity from the first to the fifth week.

The time taken for the first fish at each concentration to show signs of poisoning varied with concentration and biocide. Swimming activity also decreased with time. This implies that the biocide diffused into the fish through the gills and probably accumulated in the blood stream until a given threshold concentration was reached before it effected the fish.

Figures I - IV show decrease in the swimming activity of the fish, steeply in the second and third week and less in the fourth and fifth.

Table 9 shows that there was an apparent significant difference between the treatments

and controls. The co-efficient of determination (r^2) was significant for all the treatments except Niclosamide. Fish activity in the experiments decreased significantly with time as compared to controls. Since the difference between the two are the chemicals, we can say that the pesticides increasingly reduced fish activity with time.

The darkening of the body colour seemed to indicate stress. This was a personal observation (1986). This is also indicated by the fish in all the biocides. It was also noticed when fish were crowded in a little water in a beaker and weighed, the skin darkened. Change in behaviour involving rapid, uncontrolled swimming and blackening of the body (in this fish) can be used by the farmer as an indication of stress. The farmer could then remove the fish to clear water before they were adversely affected. Sub-lethal biocide concentrations can therefore reduce the growth rate of the fish by affecting its feeding mechanism. It could also be that the biocides affected the food demand of the fish such that a different type of food was needed. This however was not investigated.

ii Experimental Bioassays: Mortality of Fish in Various Biocide Concentrations

TABLE 10

Computed 24 - 96 hr LC_{50} for Niclosamide, Trifenmorph, Carbofuran and DDT

BIOCIDE	24 H	48 H	72H	96H
Niclosamide	0.103	0.074	0.066	0.059
Trifenmorph	0.118	0.097	0.092	0.087
Carbofuran	0.225	0.213	0.162	0.121
DDT	0.042	0.029	0.020	0.017

A DDT

DDT is toxic to fish. There was zero mortality at 0.02 mg/litre in 24 hours and 50% mortality in 96 hours (Table 11). At a concentration of 0,06mg/litre there was a 10% mortality rate in three hours and 100% mortality rate in 24 hours. DDT is persistant and will stay in the environment being absorbed by the fish until it reaches a lethal level. It was the most toxic of the four biocides used. Fifty percent mortality occured at 0.042mg/litre in 24 hours, 0.029 mg/litre in 48 hours and 0.020 mg/litre in 72 hours and

Table 11

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF DDT TO *O. niger* FROM 1 HOUR - 96 HOURS

CONC. IN m/s	ACTIVE INGREDIENTS mg/l	NUMBER OF TEST FISH	NUMBER OF TEST FISH DEAD AT							
			1 h	2 h	3 h	6 h	24 h	48 h	72 h	96 h
2.5	0.06	10	0	0	1	2	10	10	10	10
2.0	0.05	10	0	0	0	2	5	8	9	9
1.5	0.0375	10	0	0	0	1	4	7	8	9
1.4	0.035	10	0	0	0	1	3	6	7	8
1.2	0.03	10	0	0	0	0	2	5	7	7
1.0	0.025	10	0	0	0	0	1	4	6	7
0.8	0.02	10	0	0	0	0	0	2	5	6
0.6	0.015	10	0	0	0	0	0	1	4	5
0.4	0.01	10	0	0	0	0	0	0	0	0
0.2	0.005	10	0	0	0	0	0	0	0	0
LC_{50} estimated by probit analysis (mg/Litre)							0.042	0.029	0.020	0.0165
X^2 at 6 DF (Chi square value at 6° freedom)							4.222	1.300	1.26	1.308
Standard Deviation (mg/Litre)							1.0659	1.0804	1.158	1.225

0.017 mg/litre in 96 hours. Mortality increased with concentration and time.

B CARBOFURAN

Carbofuran was the least toxic of the four biocides used in the experiments. The 24 hour LC_{50} was 0.225 mg/litre (Table 10) while the 96 hour LC_{50} was 0.12 mg/litre. Mortality increased with concentration and time. In a concentration of 0.3 mg/litre there was a 10% mortality in two hours and 100% in 24 hours, while at a concentration of 0.106 mg/litre there was 10% mortality in 48 hours and 30% in 96 hours (Table 12). Here again fish in low concentrations took longer to react.

C TRIFENMORPH

The 24 hour LC_{50} was 0.118 mg/litre while the 96 hour LC_{50} was 0.087 mg/l (Table 10). This indicates that Trifenmorph is slightly less toxic to O. niger than Niclosamide. Fifty percent mortality occured at a concentration of 0.118 mg/litre in 24 hours compared to 0.103 mg/l Niclosamide 24 hours. These two are toxic to fish at very low concentrations. As the concentration increases, so does the percentage mortality. At lower concentrations, the fish took a longer time to

Table 12

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF CARBOFURAN TO O. niger FROM 1 HOUR - 96 HOURS

CONC. mls	ACTIVE INGREDIENTS (MG) PER LITRE mg/l	NUMBER OF TEST FISH	NUMBER OF TEST FISH DEAD AT							
			1 h	2 h	3 h	6 h	24 h	48 h	72 h	96 h
12	0.3	10	0	1	2	2	10	10	10	10
10	0.25	10	0	0	2	2	5	7	7	10
9	0.225	10	0	0	0	0	4	4	7	9
8	0.20	10	0	0	0	0	3	3	6	8
7	0.175	10	0	0	0	0	2	2	5	8
6	0.15	10	0	0	0	0	1	2	4	7
5	0.125	10	0	0	0	0	1	2	3	6
4	0.106	10	0	0	0	0	0	1	3	3
0	0.0	10	0	0	0	0	0	0	0	0
LC_{50} estimated by probit analysis (mg/Litre)							0.225	0.213	0.162	0.121
X^2 at 6 DF (Chi square at 6° freedom)							5.132	6.889	2.946	1.622
Standard Deviation (mg/Litre)							1.059	1.067	1.093	1.099

react. At a concentration of 0.185 mg/litre there was 10% mortality in 1 hour and 100% mortality in 24 hours, while at the lowest concentration used 0.066 mg/litre there was 10% mortality after 24 hours and 40% mortality after 96 hours (Table 13)• The fish took a longer time to react to the biocide at low concentrations.

D NICLOSAMIDE

Niclosamide is slightly more toxic to fish than Trifenmorph (24 hour LC_{50} 0.103 mg/l and 0.118 mg/l respectively). The 96 hour LC_{50} was 0.059 mg/l, shows that Niclosamide is toxic to fish even at very low concentration. Mortality increased by 90% from the lowest lethal concentration 0.07mg/l giving a 10% mortality, to 100% in the highest concentration 0.21 mg/litre. 50% mortality occurred in 48 hours in a concentration of 0.074 mg/litre, and increased to 60% after 72 and 96 hours. Average percentage mortality decreased with concentrations. The higher the concentration the faster the fish died (Table 14).

E DISCUSSIONS; Toxicity of the Biocides

The four biocides used in these experiments are

TABLE 13

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF TRIFENMORPH TO O. niger FROM 1 HOUR - 96 HOURS

CONC(IN ml)Stock Solution	ACTIVE INGREDIENTS (MG) PER LITRE mg/l	NUMBER OF TEST FISH	NUMBER OF TEST FISH DEAD AT							
			1 h	2 h	3 h	6 h	24 h	48 h	72 h	96 h
1.5	0.2475	10	1	1	2	3	10	10	10	10
1.1	0.1815	10	0	0	1	2	8	9	10	10
0.9	0.1485	10	0	0	1	1	7	7	9	9
0.8	0.132	10	0	0	0	1	5	7	8	8
0.7	0.116	10	0	0	0	0	4	6	6	7
0.6	0.099	10	0	0	0	0	4	4	5	6
0.5	0.084	10	0	0	0	0	3	3	4	5
0.4	0.066	10	0	0	0	0	1	2	3	4
0.1	0.005	10	0	0	0	0	0	0	0	0
LC_{50} estimated by probit analysis (mg/Litre)							0.118	0.0967	0.092	0.868
X^2 at 6 DF (Chi square value at 6 ° of freedom)							1.653	1.157	1.966	0.4775
Standard Deviation (mg/Litre)							1.075	1.099	1.0797	1.11

TABLE 14

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF NICLOSAMIDE TO O. niger FROM 1 HOUR - 96 HOURS

STOCK SOLN. CONC. (mls)	ACTIVE INGREDIENTS (MG) PER LITRE mg/l	NUMBER OF TEST FISH	NUMBER OF TEST FISH DEAD AT							
			1 h	2 h	3 h	6 h	24 h	48 h	72 h	96 h
3.0	0.21	10	2	4	10	10	10	10	10	10
2.0	0.14	10	0	2	2	5	6	10	10	10
1.8	0.126	10	0	0	1	1	6	9	9	9
1.4	0.098	10	0	0	0	0	5	8	8	8
1.2	0.084	10	0	0	0	0	3	7	8	8
1.1	0.077	10	0	0	0	0	2	6	7	6
1.0	0.070	10	0	0	0	0	1	5	6	5
0.9	0.063	10	0	0	0	0	0	4	4	4
0.1	0.007	10	0	0	0	0	0	0	0	0
LC_{50} estimated by probit analysis (mg/Litre)							0.103	0.074	0.066	0.059
X^2 at 6 DF (Chi square value at 6° of freedom)							2.4637	1.1313	1.4649	2.4085
Standard Deviation (mg/Litre)							1.0556	1.1537	1.1026	1.1073

toxic to Oreochromis niger fingerlings in low concentrations. (DDT was the most toxic and Carbofuran the least). Trifenmorph is slightly less toxic than Niclosamide. Mortality in all the experiments decreased with decrease in concentration of the biocide. Use of DDT on irrigation schemes in Kenya is very much discouraged and is only used in cases of extreme insect infestation. (Personal communication, National Irrigation Board 1986) The other three biocides are non - persistant and nonaccumulative but the concentrations used or needed to kill molluscs and the stem borer are such that they would kill the fish. The recommended field application rate of Carbofuran is 750 - 900 g active ingredient per hectare 2 - 3 weeks after transplanting and if necessary repeated at 3 - 4 week intervals (Personal communication, Murphy Chemicals, 1986). In flowing waters, such as in the irrigation canals, a concentration of 0.3-1 ppm Niclosamide is recommended for snail control to be maintained for 24 hours. In this experiment a concentration of 0.21 mg/l Niclosamide active ingredient had 100% fish mortality in 24 hours. Trifenmorph is lethal to most fish at 1 - 2 ppm in 24 hours

(Cremlyn, 1979) and 0.025 mg/litre for several days. In this experiment a concentration of 0.2475 mg/L Trifenmorph active ingredient had 100% mortality in 24 hours. This indicates that O. niger and probably most Tilapia are susceptable to lower concentrations of these pesticides - DDT, Carbofuran, Trifenmorph, Niclosamide.

Though Niclosamide is slightly less toxic to adult snails than Trifenmorph it kills the eggs and juveniles of snails and is also effective on the larvae of the schistosomes. This compensates for its lower toxicity to adult snails. Niclosamide is used on the irrigation schemes in Kenya because it is cheaper than Trifenmorph. Since both Niclosamide and Trifenmorph are toxic to fish in low concentrations, one cannot grow fish in water that has either of the biocides.

From the Tables 11 - 14, it can be seen that mortality increased with time. Not all the fish died at once but each had a slightly different tolerance level and succumbed to the biocides when this level was reached. This indicates that exposure of the fish even to apparently low concentrations would kill fish of low tolerance.

Careful field research is needed before sub - lethal concentrations can be considered safe.

DDT is persistant and bio-accumulative. Even if a low concentration of it does not kill the fish, it is accumulated in the fat deposits of the fish. In the cold weather, when the fish stop feeding, DDT can be mobilized from the tissues and cause deferred poisoning. Since Carbofuran is non-accumulative and non-persistant, it should be the insecticide of choice.

iii GROWTH

Controls

On average, the fish in the controls grew faster than those in the biocides except Carbofuran and Niclosamide in the first 8 days. Table 15 shows that fish gradually increased in weight from day one to day eight. The growth rate then increased in the second and third week but was much faster in the last two weeks i.e. from day 22. Fish in the controls had a 13% increase in weight after the 35 days with a mean weekly weight increase of 0.026 g. They had increased their initial weight by 0.13g. A regressive test shows a positive linear relationship between growth and time.

TABLE 15

MEAN WEIGHT PER FISH IN GRAMS DURING A 35 DAY

GROWTH PERIOD IN VARIOUS BIOCIDES

DAY	CONTROL	DDT	TRIFEN-MORPH	NICLO-SAMIDE	CARBO-FURAN
1	0.02	0.02	0.02	0.02	0.02
8	0.028	0.024	0.028	0.028	0.030
15	0.049	0.030	0.035	0.034	0.055
22	0.060	0.042	0.051	0.050	0.067
29	0.100	0.075	0.092	0.091	0.120
35	0.150	0.090	0.120	0.131	0.175
TOTAL INCREMENT	0.13gm	0.07 gm	0.10 gm	0.11 gm	0.155 gm
MEAN WEEKLY INCREMENT $\bar{X}$	0.026gm	0.014gm	0.020gm	0.022gm	0.031 gm
r^2	0.909	0.906	0.904	0.878	0.913

iii BIOASSAYS: Observations on the Growth of Fish in Sub-lethal Concentrations of Biocides

A DDT

DDT inhibits growth by approximately 6% as compared to the controls. The rate of weight increase paralleled that of the controls but was slower. It slowly increased in the first eight days, then

fairly in the last two weeks (Fig. V). Growth increment was only 7% as compared to 13% in the controls, increasing by a mean weight of 0.014 g/week compared to 0.026 g/week in the controls.

B CARBOFURAN

Fish in Carbofuran showed a faster weight increment than in the other biocides and the controls. Increase in weight was by a mean of 0.031 g/week compared to 0.026 g/week in the controls. Percentage weight increment was 15.5% in Furadan as compared to 13% in the controls 7% in DDT, 10% in Trifenmorph and 11% in Niclosamide. The pattern of growth was however comparable in that it increased slowly in the first eight days, fairly steeply in the middle two weeks and steeply in the last two weeks. The weekly weight increase was also high, 0.031 as compared to 0.026 in the controls (Table 15, Fig. VI)

C TRIFENMORPH AND NICLOSAMIDE

The two molluscicides affected growth only very slightly. The weight increased by 10% and 11% respectively as compared to 13% in the controls. There was a weekly weight increase of 0.02 g and 0.022 g respectively as compared to 0.026 g in the

FIG. V MEAN WEIGHT (g) PER FISH

GROWTH: in 0.0025 mg/L ACTIVE INGREDIENT DDT

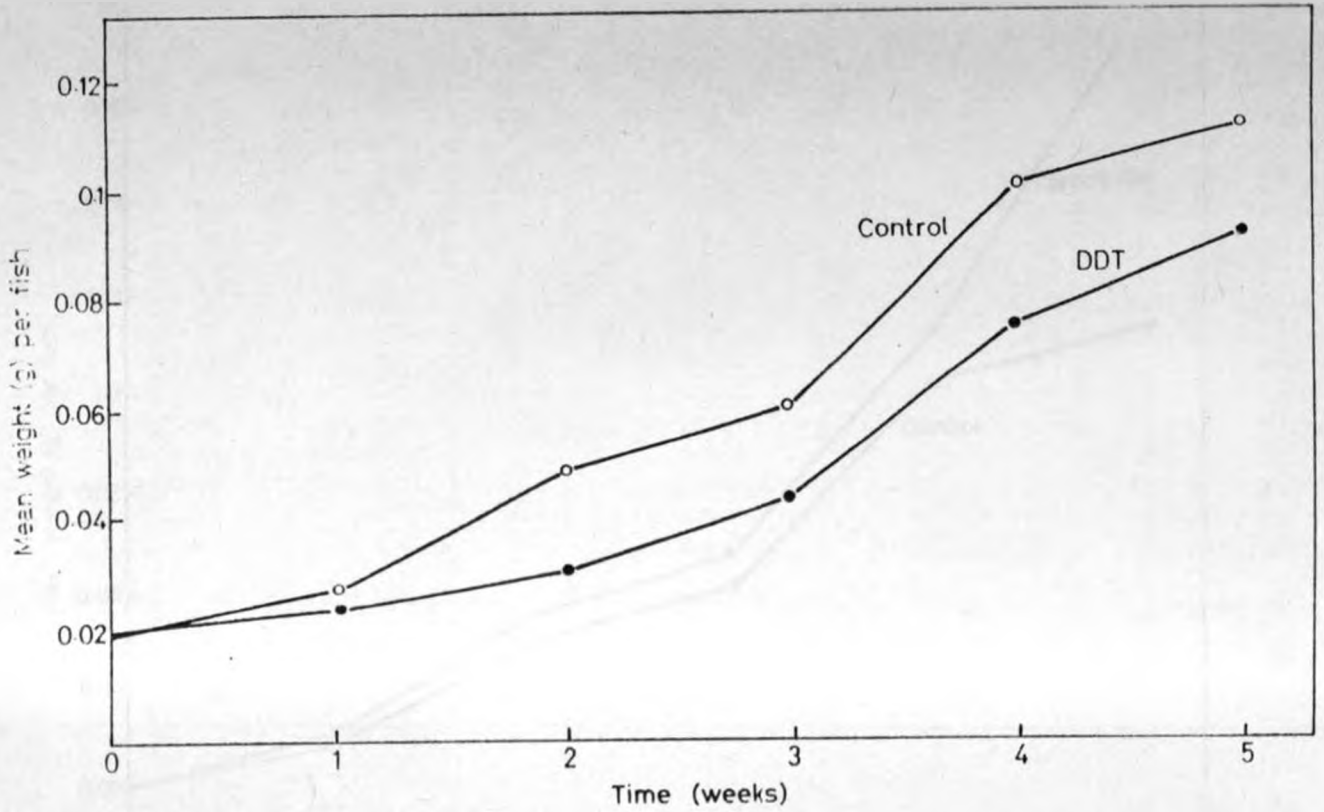
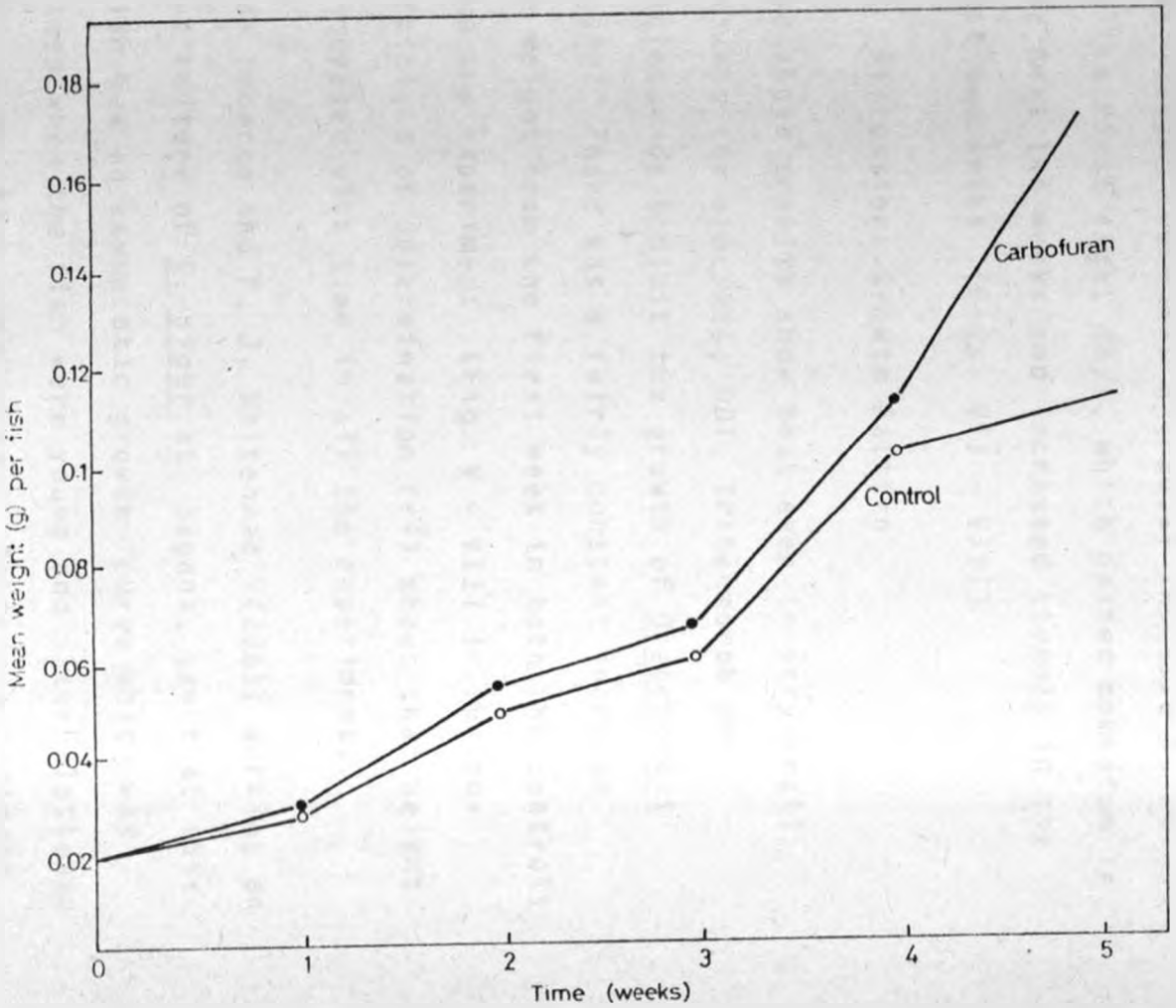


FIG. VI MEAN WEIGHT (g) PER FISH

GROWTH: in 0.005 mg/L ACTIVE INGREDIENT CARBOFURAN



controls. The rate was also parallel to that in the controls only that it was slower at the beginning. There was a gradual increase in weight in the first eight days, which gained momentum in the next two weeks and increased steeply in the last two weeks (Figs. VII - VIII).

E Discussion: Growth Pattern

The above results show that even in very small amounts the biocides; DDT, Trifenmorph and Niclosamide inhibit the growth of Oreochromis niger. There was a fairly constant increase in weight from the first week in both the controls and the experiment (Fig. V - VIII). The coefficient of determination (r^2) shows that weight increased with time in all the experiments.

Van Someren and P. J. Whitehead (1961) working on the culture of O. niger at Sagana, saw that this fish had an asymptotic growth curve which was steep when the fish were young and later flattened out as the fish grew larger. In this experiment, there was slow growth which steadily increased to a steep growth curve in the last two weeks. This indicates that the fish used were very small and

FIG. VII MEAN WEIGHT (g) PER FISH

GROWTH: in 0.00165 mg/L ACTIVE INGREDIENT TRIFENMORPH

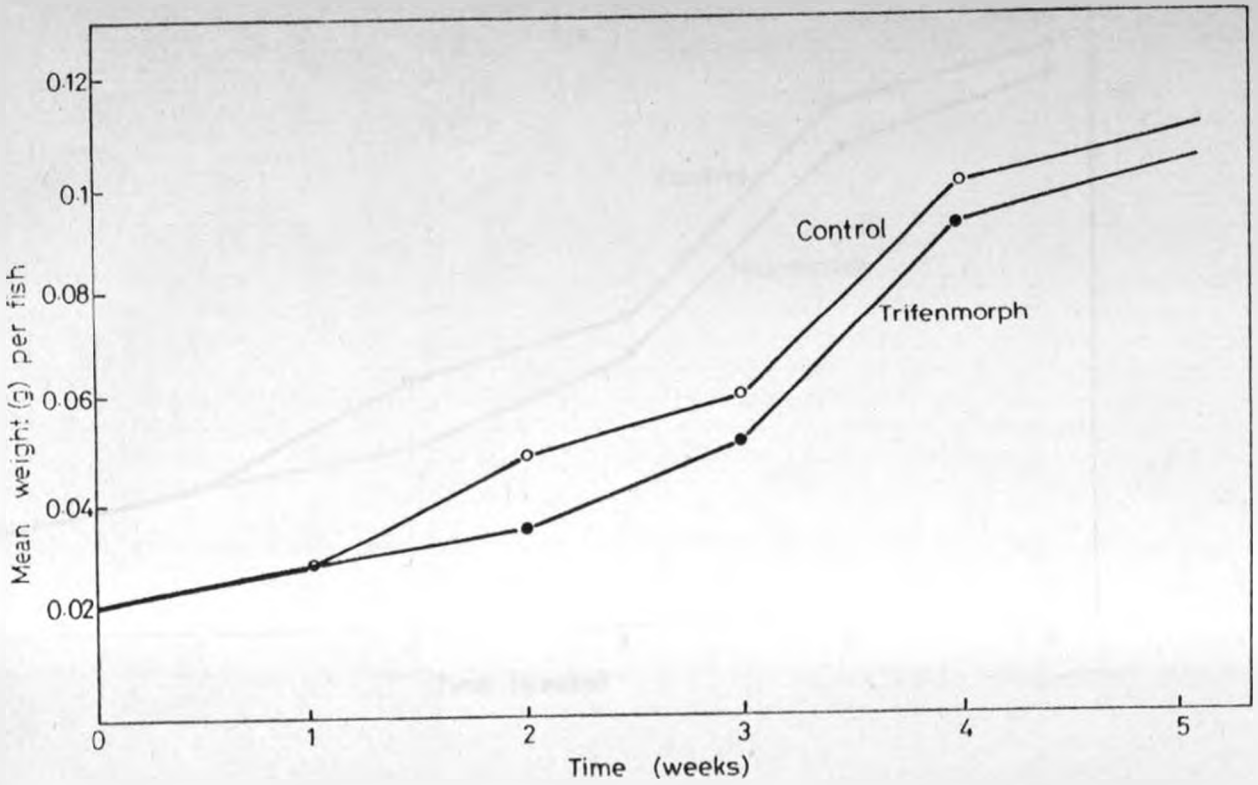
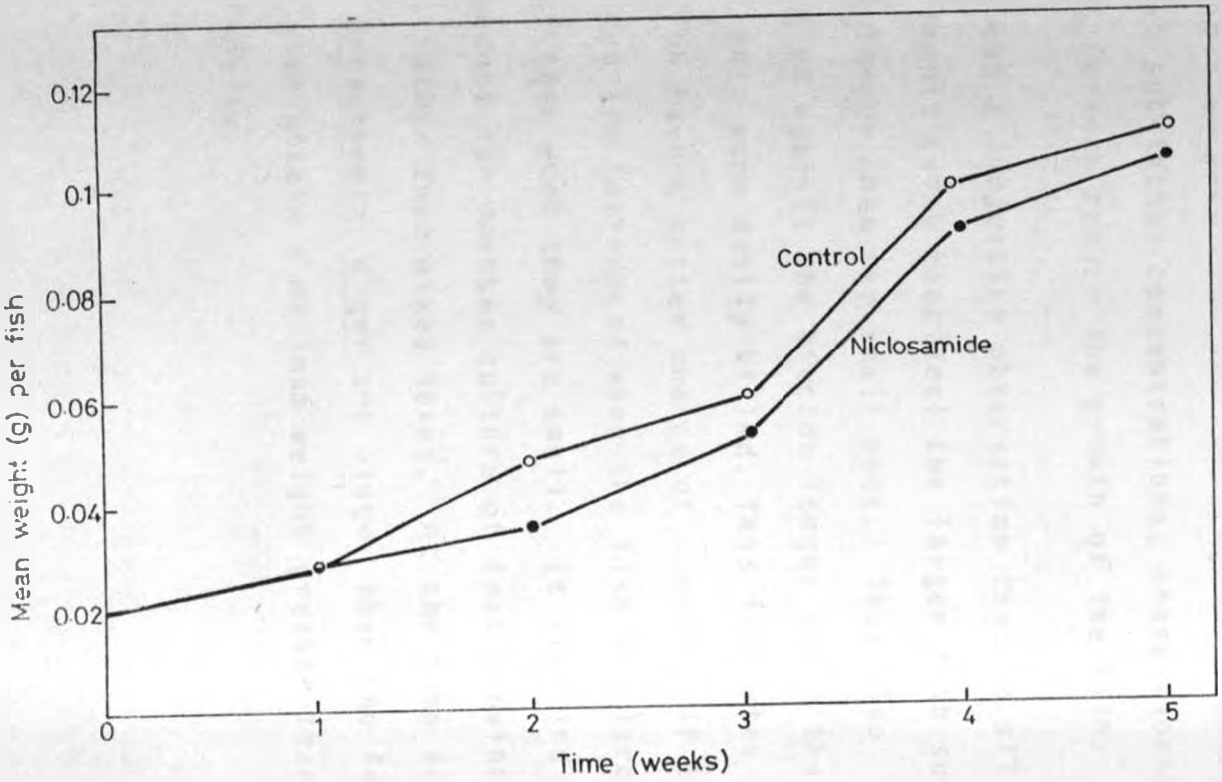


FIG. VIII MEAN WEIGHT (g) PER FISH

GROWTH: in 0.007 mg/L ACTIVE INGREDIENT NICLOSAMIDE



the experiments were discontinued before the peak growth rate was achieved.

From this experiment it would look like it is not advisable to grow fish and rice together, since even at sub-lethal concentrations, these biocides except Furadan reduce the growth of the fish.

There was a subjective observation that in all the experiments (with biocides) the larger fish survived longer than the small ones. They also struggled against the biocide longer while the small ones were easily killed. This indicates that the fish have a better chance of survival if the biocides are introduced when the fish are larger rather than when they are small. It also indicates preference for monosex culture of fast growing males rather than mixed sexes. At the same age, male Oreochromis niger are bigger than the females. They also attain a maximum weight greater than the females.

CHAPTER VI

C O N C L U S I O N S

At the concentrations used in the fields, DDT, Carbofuran, Trifenmorph and Niclosamide are lethal to fish. Very low concentrations may not kill the fish but they adversely affect their growth rates. There have been no reports of human deaths due to biocide poisoning of fish at Mwea, although fish killed at times of spraying are collected by farmers and eaten. It is however inadvisable to use accumulative biocides such as DDT since this would affect the ecosystem and indirectly man.

The non-accumulative biocides such as carbofuran (Furadan ®) and Niclosamide should be used. Carbofuran though expensive, is non-accumulative and not persistant and so once it has done its part, is quickly removed from the environment and does not continuously exert its toxic effects. Being a systemic biocide, most of it is absorbed by the plants and some is adsorbed on the soil particles and so is removed from the water. It is also very much less toxic than DDT and so even if

it gets into the drainage canals, it will not kill as many fish as DDT. The people who live on the schemes and the immediate neighbourhood should be informed of the spraying times and advised not to drink the water from the irrigation channels immediately after spraying, or eat the dead fish. The effects of these biocides on human life in Kenya have not been studied.

Niclosamide, though less toxic to adult snails than Trifenmorph, is cheaper and also has ovicidal effects. Trifenmorph is only used in very heavy infestation of the snails. Niclosamide is non-accumulative and non-phytotoxic. It will therefore not affect the rice plants. (There are few weeds at most, therefore herbicides are not needed.) According to Matthiessen (1976) Trifenmorph is accumulated in the tissues and organs of Oreochromis mossambicus. This was not studied in this work, but it is reasonable to assume that Oreochromis niger could accumulate Trifenmorph in its tissues and organs. The effects of such accumulation in predators, including man, have not yet been studied. (The use of Trifenmorph has been discontinued at Mwea.)

Fish in various biocide concentrations lost their shoaling ability, fed very poorly and were not very

active. This resulted in lower growth rates. Inactivity of the fish in the field would lead to easy predation by birds and poachers, so affecting yield.

From this work, it is possible to suggest that growing fish and rice concurrent may not be economically viable. This is especially so when rice is the major crop and repeated sprayings are necessary for a good yield. The few that survive one spraying would succumb at the next. Concentration of the biocide sprayed may also be increased to more effectively remove the pest. Alternate growing of fish and rice may be much more possible so that fish are grown without biocide spraying. This however involves a decision as to which crop is more valuable and which one can be spared for awhile, as also the availability of water. Fish should be grown in ponds and introduced into paddies after rice harvest. At Mwea, however, some indigenous fish are found in the rice paddies and reach marketable size. Experiments are needed to determine the growth rate of these fish in the paddies and their economic value.

The commercial biocides which were used in this work are applied in a mixture of solvents (Niclosamide and Trifenmorph), sand (Carbofuran) and dusts (DDT).

The molluscicides form colloidal suspensions when dissolved in water may adsorb on mud and other organic matter which may make them unavailable to the fish. (Cremlyn, 1979). Trifenmorph is known to readily adsorb on fine particulate matter (Mattiessen, 1976). Concentrations larger than the ones used in this study may probably be safely used on the fields.

Different species of fish react differently to these biocides. Carp (Cyprinus carpio) are more resistant to Niclosamide (96 hr LC_{50} = 0.082 ppm) than to DDT (96 hr LC_{50} = 0.010 ppm). A case study in the Philippines in 1980 showed that carbofuran is safe to use in rice - fish culture if applied at the roots - 14 days prior to introduction of fish (Estores et al, 1980). A detritus feeding fish could pick up a lot of adsorbed biocides which would increase their toxicity. Already there are fish, indigenous to Mwea Scheme, in the irrigation and sewage canals. It would therefore be appropriate to study the different species and choose one for culture rather than introduce new species. The indigenous species would be more resistant to the biocides used, and so breed better. This may lead to genetic resistance. Studies on the biology of the local species,

their growth rates, economic value, and consumer acceptability are needed.

Experiments on bioaccumulation and sub-lethal effects of these biocides on reproduction and development of the fish and on the best choice of fish are needed before proper recommendation on the possibility of a rice-fish culture. Field experiments to determine LC_{50} and growth at Mwea are also needed. Further work is also needed on synergistic effects of these biocides to fish. The insecticides and molluscicides are applied at the same time and although Niclosamide is sprayed in the drainage canals, the spray goes into the fields with Carbofuran or DDT.

The results of this work suggest that it is possible to grow rice and fish alternately so as to avoid poisoning of fish by repeated spraying of the fields and drainage systems when the fish are growing. The fish could be grown in one season and alternate with rice in the following season. One could conceivably get two crops of fish in one year. The paddies would be fertilised by the rice growing procedures and rice bi-products leading to growth of plankton on which the fish could feed. The fish in turn would fertilise the paddies by their wastes.

It would also be easier for the farmer to manage two crops separately. Fish that are too small to be sold can be used in the manufacture of cattle, pig and chicken feeds or to seed rivers and ponds.

Ponds can also be made in the less arable parts of the scheme area and fish cultured here. One would need to control the water in the drainage canals to ensure the surface run-of from the rice fields does not enter the culture ponds.

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Fig. IXa

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF DDT TO *O. niger* IN 24 and 48 HRS

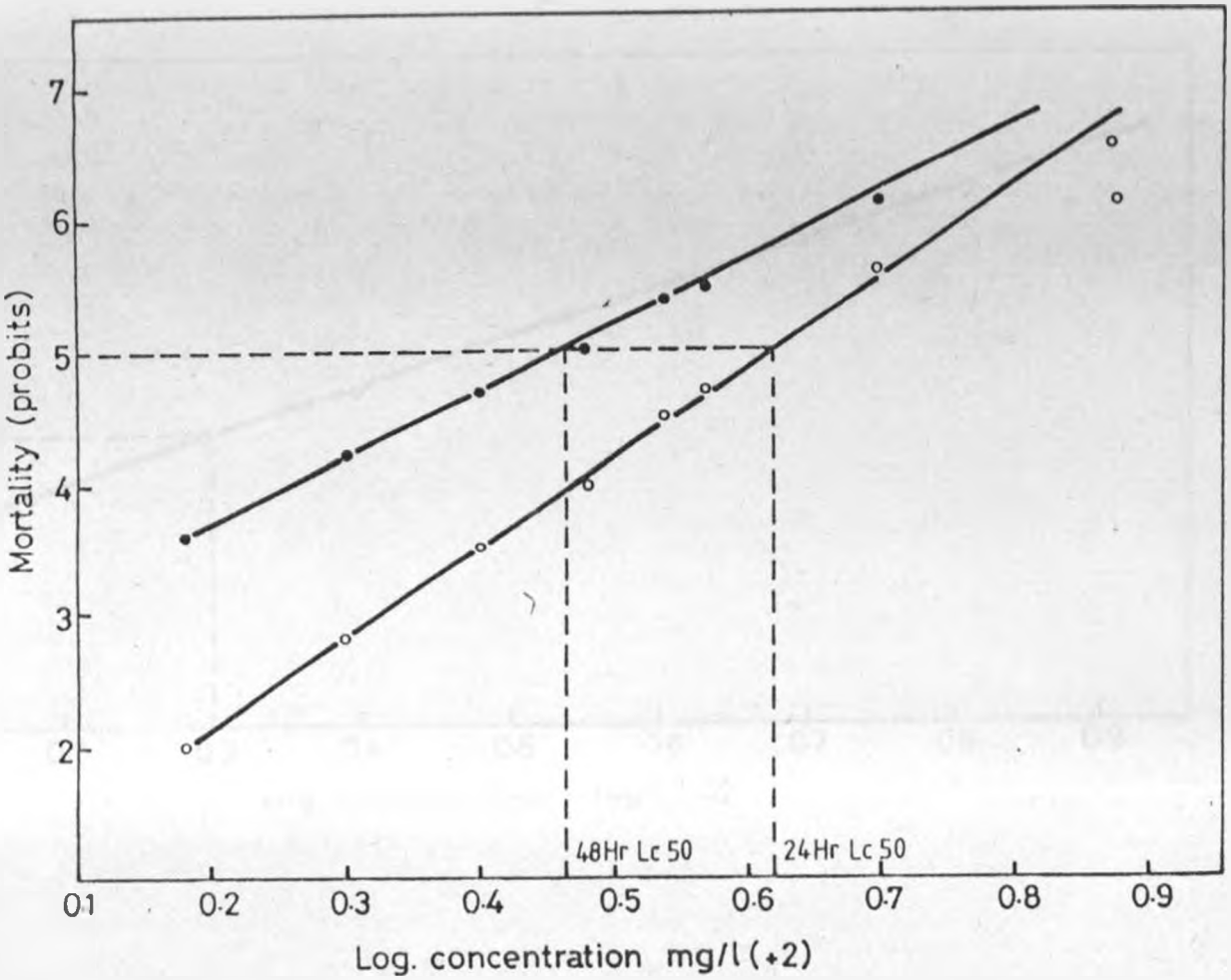


Fig. IXb

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF DDT TO *O. niger* IN 72 HRS

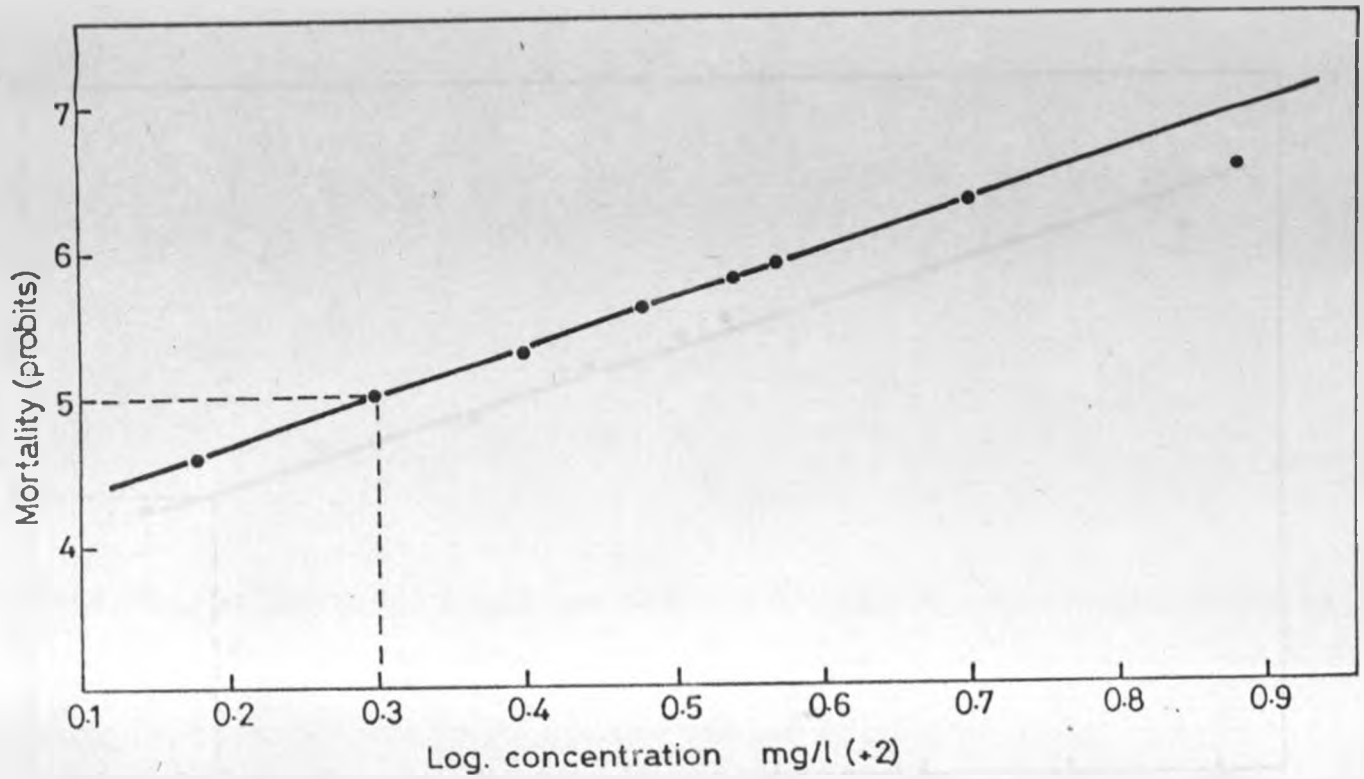


Fig. IXc

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF DDT TO *O. niger* IN 96 HRS

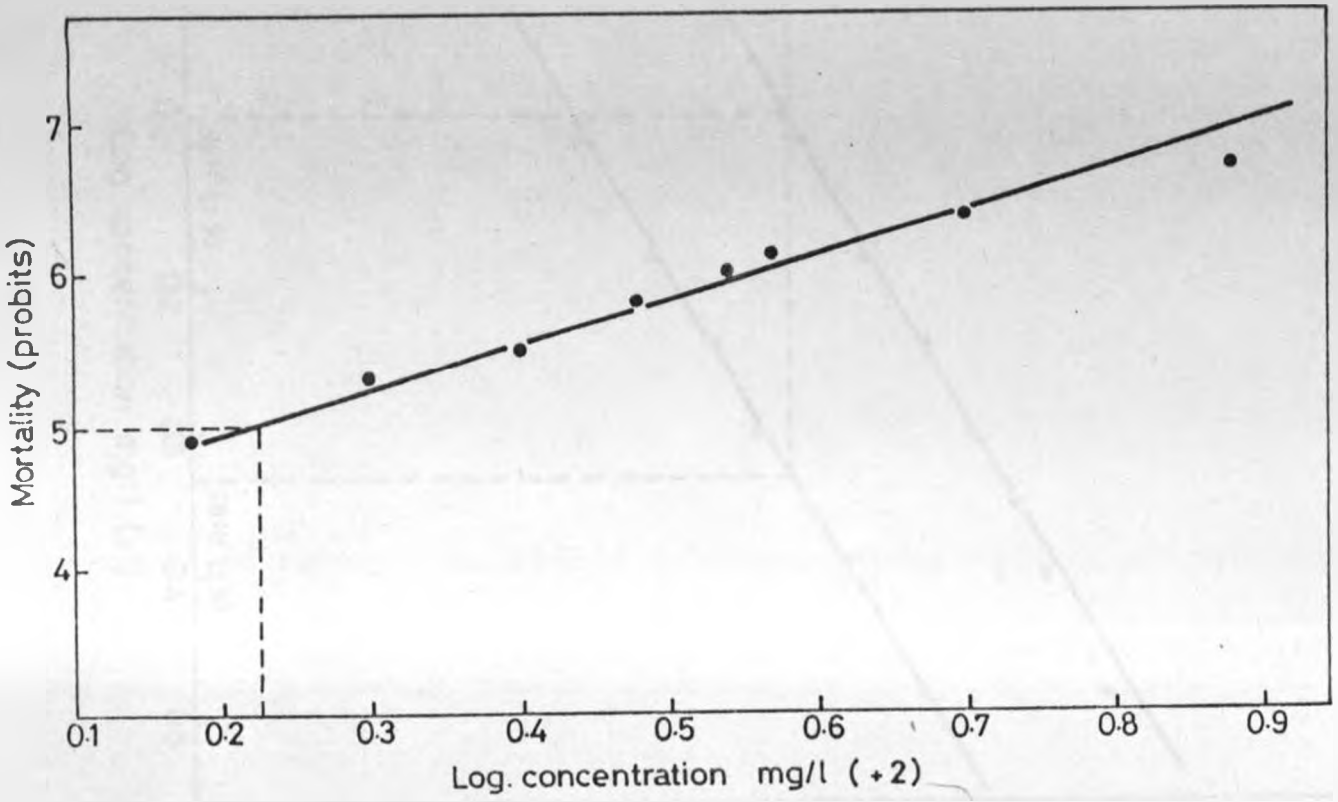


Fig. Xa

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF CARBOFURAN TO *O. niger* IN 48 and 96 HRS

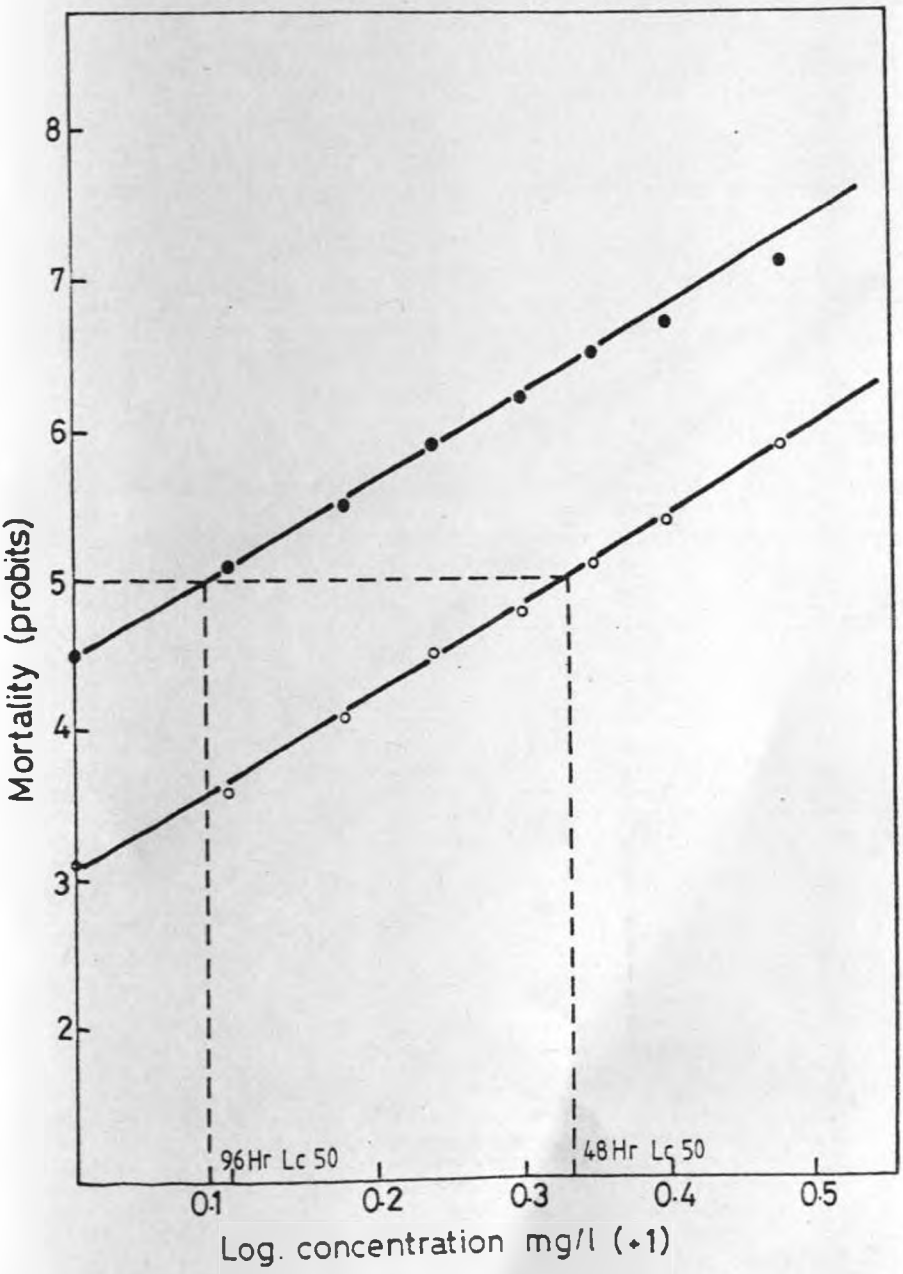


Fig. Xb

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF CARBOFURAN TO *O. niger* IN 24 and 72 HRS

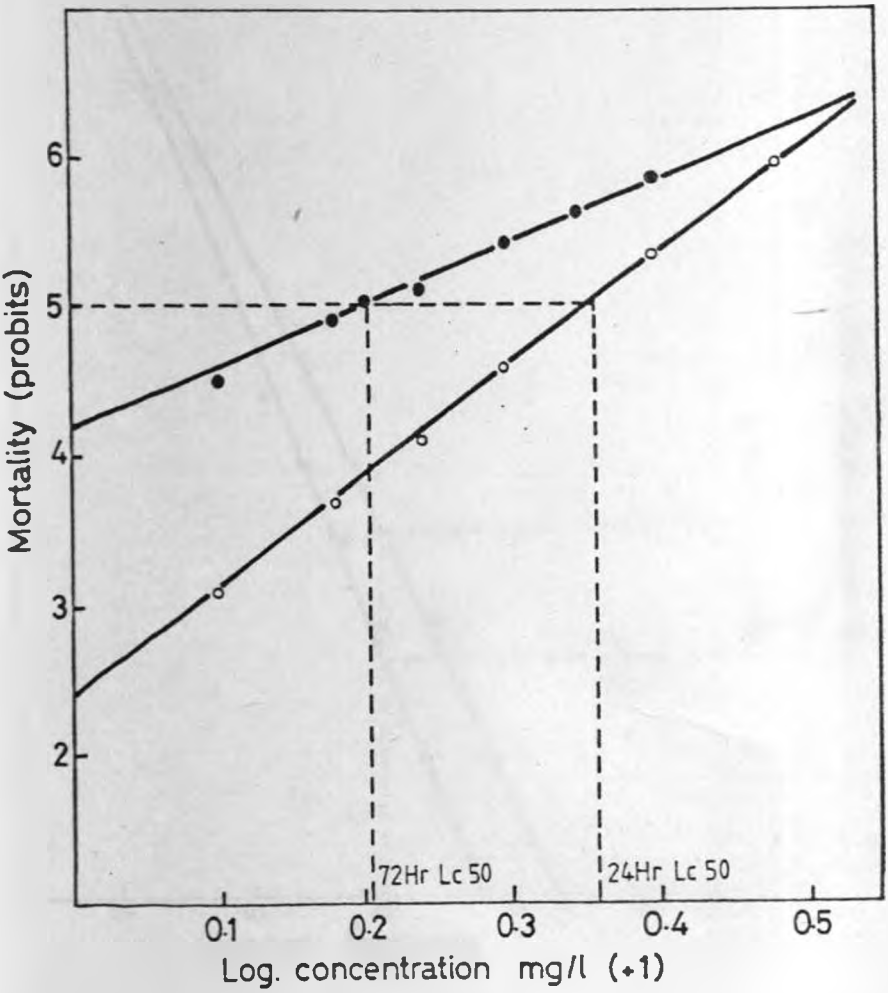


Fig. XIa

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF TRIFENMORPH TO *O. niger* IN 24 and 48 HRS

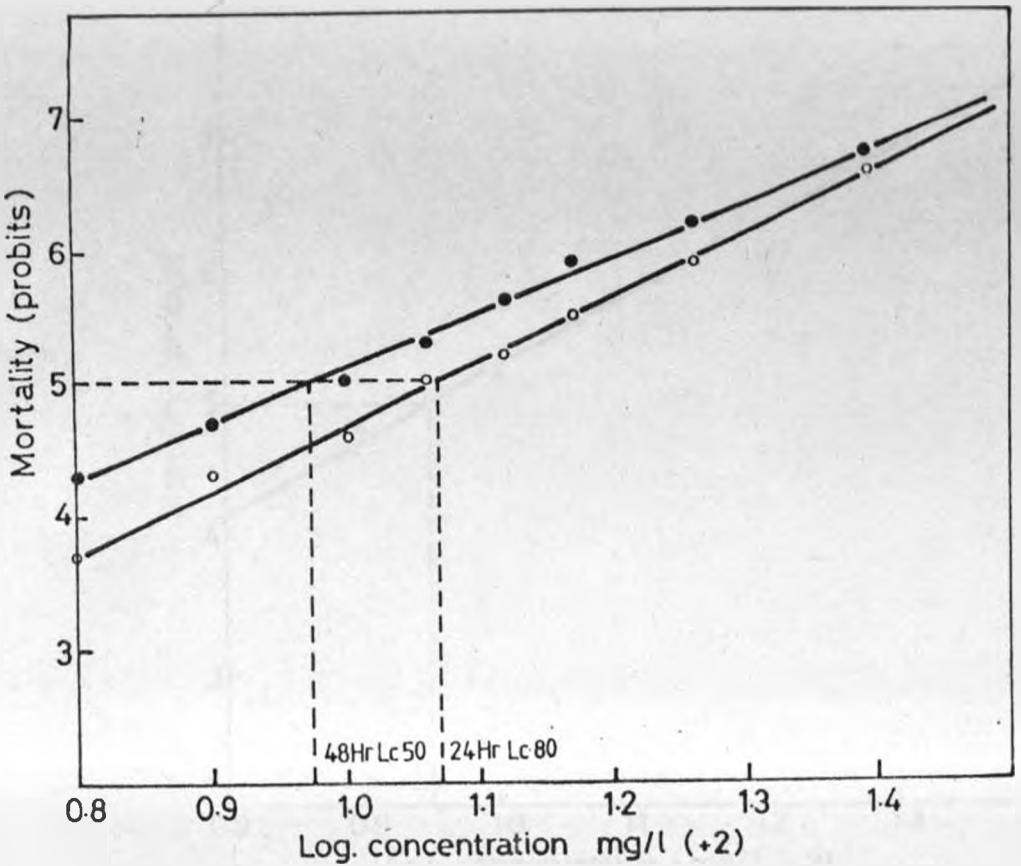


Fig. XIb

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF TRIFENMORPH TO *O. niger* IN 72 HRS

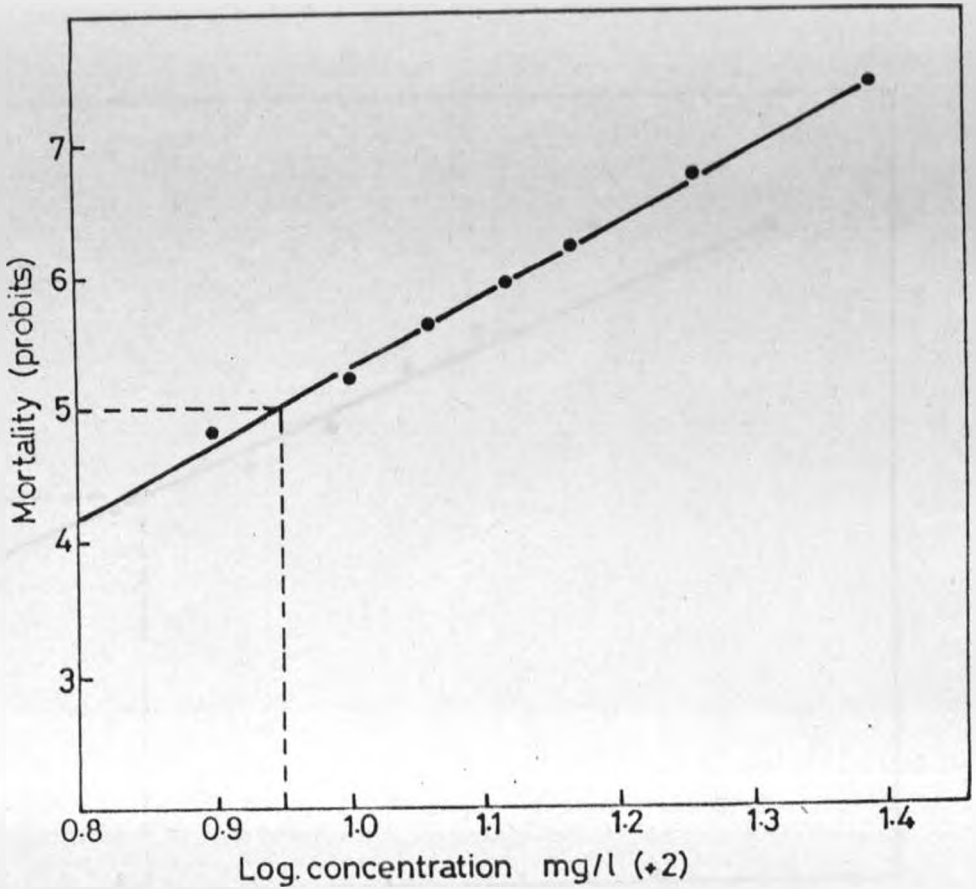


Fig. XIc

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF TRIFENMORPH TO *O. niger* IN 96 HRS

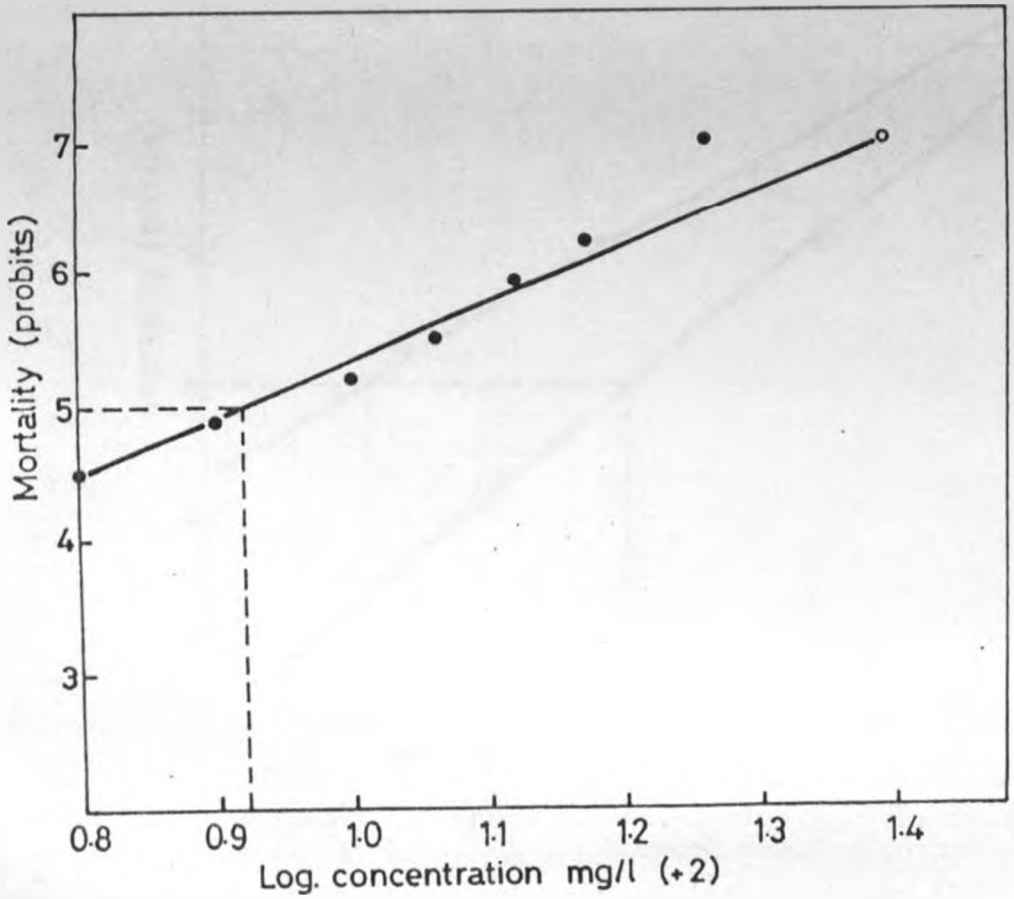


Fig. XIIa

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF NICLOSAMIDE TO *O. niger* IN 24 and 96 HRS

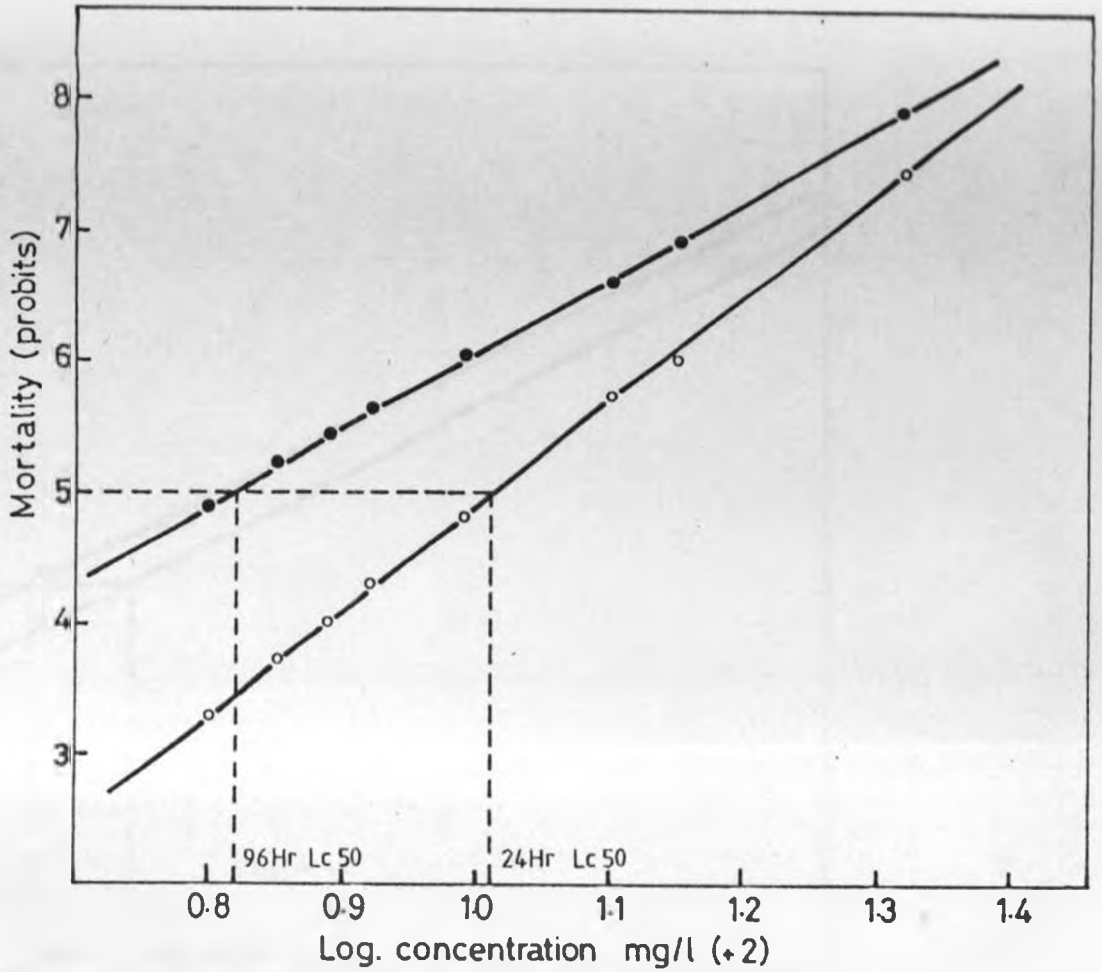
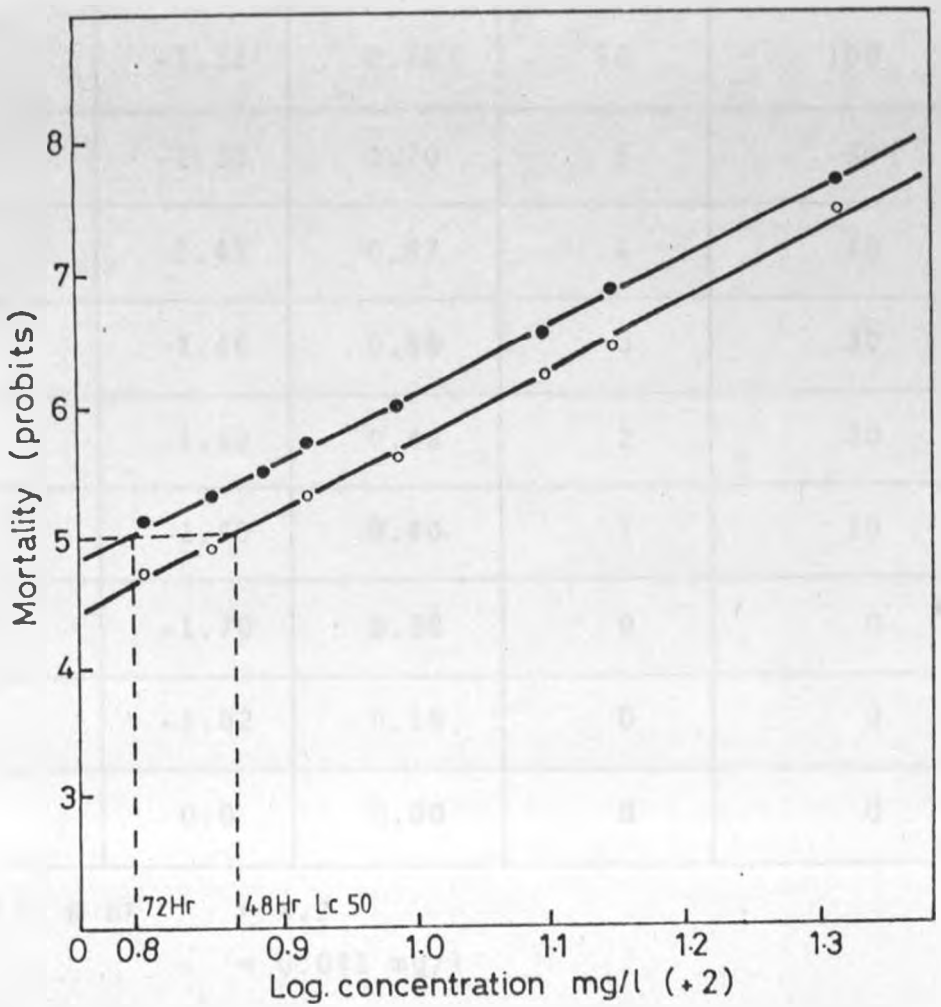


Fig. XIIb

COMPUTATION OF LC_{50} FOR THE TOXICITY OF VARIOUS CONCENTRATIONS OF NICLOSAMIDE TO *O. niger* IN 48 and 72 HRS



NUMBER OF FISH	CONC. OF D D T (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.06	-1.22	0.78	10	100	6.1
10	0.05	-1.30	0.70	5	50	5.6
10	0.038	-1.43	0.57	4	40	4.7
10	0.035	-1.46	0.54	3	30	4.5
10	0.030	-1.52	0.48	2	20	4.0
10	0.025	-1.60	0.40	1	10	3.5
10	0.020	-1.70	0.30	0	0	2.8
10	0.015	-1.82	0.18	0	0	2.0
10	0.00	0.0	0.00	0	0	0.0

Computed Chi square with 6 DF = 4.2

LC_{50} Computed = 0.041 mg/l

Standard Deviation = 1.1

NUMBER OF FISH	CONC. OF D D T (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.06	-1.22	0.88	10	100	6.5
10	0.05	-1.30	0.70	8	80	6.1
10	0.038	-1.43	0.57	7	70	5.5
10	0.035	-1.46	0.54	6	60	5.4
10	0.030	-1.52	0.48	5	50	5.0
10	0.025	-1.60	0.40	4	40	4.7
10	0.020	-1.70	0.30	2	20	4.2
10	0.015	-1.82	0.18	1	10	3.6
10	0.00	0.0	0.00	0	0	0.0

Computed Chi square with 6 DF = 1.3

LC_{50} Computed = 0.029 mg/l

Standard Deviation = 1.1

CONCENTRATIONS RANGING FROM 0.00 - 0.6 mg/l FOR 72 HOURS

NUMBER OF FISH	CONC. OF D D T (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.06	-1.22	0.88	10	100	6.5
10	0.05	-1.30	0.70	9	90	6.3
10	0.038	-1.43	0.57	8	80	5.9
10	0.035	-1.46	0.54	7	70	5.8
10	0.030	-1.52	0.48	7	70	5.6
10	0.025	-1.60	0.40	6	60	5.3
10	0.020	-1.70	0.30	5	50	5.0
10	0.015	-1.82	0.18	4	40	4.6
10	0.00	0.0	0.00	0	0	0.0

Computed Chi square with 6 DF = 1.3

LC_{50} Computed = 0.02 mg/l

Standard Deviation = 1.2

THE COMPUTATION OF LC50 [illegible] TREATED IN BIOASSAY
CONCENTRATIONS RANGING FROM 0.00 - 0.6 mg/l FOR 96 HOURS

NUMBER OF FISH	CONC. OF D D T (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.06	-1.22	0.88	10	100	6.7
10	0.05	-1.30	0.70	9	90	6.4
10	0.038	-1.43	0.57	9	90	6.1
10	0.035	-1.46	0.54	8	80	6.0
10	0.030	-1.52	0.48	7	70	5.8
10	0.025	-1.60	0.40	7	70	5.5
10	0.020	-1.70	0.30	6	60	5.3
10	0.015	-1.82	0.18	5	50	4.9
10	0.00	0.0	0.00	0	0	0.0

Computed Chi square with 6 DF = 1.3

LC_{50} Computed = 0.016 mg/l

Standard Deviation = 1.2

THE COMPUTATION OF LC_{50} FOR CARBOFURAN AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.3 mg/l FOR 24 HOURS

NUMBER OF FISH	CONC. OF CARBOFURAN (mg/l)	LOG CONC.	LOG CONC +1	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.3	-0.52	0.48	10	100	5.9
10	0.25	-0.60	0.40	5	50	5.3
10	0.225	-0.65	0.35	4	40	5.0
10	0.20	-0.70	0.30	3	30	4.6
10	0.175	-0.76	0.24	2	20	4.2
10	0.15	-0.82	0.18	1	10	3.7
10	0.125	-0.90	0.10	1	10	3.1
10	0.106	-1.00	0.00	0	00	2.4

Computed Chi square with 6 DF = 5.1

LC_{50} Computed = 0.225 mg/l

Standard Deviation = 1.059

THE COMPUTATION OF LC_{50} FOR CARBOFURAN AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.3 mg/l FOR 48 HOURS

NUMBER OF FISH	CONC. OF CARBOFURAN (mg/l)	LOG CONC.	LOG CONC +1	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.30	-0.52	0.48	10	100	5.9
10	0.25	-0.60	0.40	7	70	5.4
10	0.225	-0.65	0.35	4	40	5.1
10	0.20	-0.70	0.30	3	30	4.8
10	0.175	-0.76	0.24	2	20	4.5
10	0.15	-0.82	0.18	2	20	4.1
10	0.125	-0.90	0.10	1	10	3.6
10	0.106	-1.00	0.00	1	10	3.1
10	0.00	0.0	0.00	0	0	0.0

Computed Chi square with 6 DF = 6.9

LC_{50} Computed = 0.2 mg/l

Standard Deviation = 1.1

THE COMPUTATION OF LC_{50} FOR CARBOFURAN AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.30 mg/l FOR 72 HOURS

NUMBER OF FISH	CONC. OF CARBOFURAN (mg/l)	LOG CONC.	LOG CONC +1	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.30	-0.52	0.48	10	100	6.1
10	0.25	-0.60	0.40	7	70	5.8
10	0.225	-0.65	0.35	7	70	5.6
10	0.20	-0.70	0.30	6	60	5.4
10	0.175	-0.76	0.24	5	50	5.1
10	0.15	-0.82	0.18	4	40	4.9
10	0.125	-0.90	0.10	3	30	4.5
10	0.106	-1.00	0.00	3	30	4.2
10	0.00	0.0	0.00	0	0	0.0

Computed Chi square with 6 DF = 2.95

LC_{50} Computed = 0.16 mg/l

Standard Deviation = 1.1

THE COMPUTATION OF LC_{50} FOR CARBOFURAN AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.30 mg/l in 96 HOURS

NUMBER OF FISH	CONC. OF CARBOFURAN (mg/l)	LOG CONC.	LOG CONC +1	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.30	-0.52	0.48	10	100	7.1
10	0.25	-0.60	0.40	10	100	6.7
10	0.225	-0.65	0.35	9	90	6.5
10	0.20	-0.70	0.30	8	80	6.2
10	0.175	-0.76	0.24	8	80	5.9
10	0.15	-0.82	0.18	7	70	5.5
10	0.125	-0.90	0.10	6	60	5.1
10	0.106	-1.00	0.00	3	30	4.5
10	0.00	0.0	0.00	0	0	0.0

Computed Chi square with 6 DF = 1.6

LC_{50} Computed = 0.12 mg/l

Standard Deviation = 1.1

THE COMPUTATION OF LC_{50} FOR TRIFENMORPH AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.25 mg/l for 24 HOURS

NUMBER OF FISH	CONC. OF TRIFENMORPH (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.25	-0.61	1.39	10	100	6.6
10	0.18	-0.74	1.26	8	80	5.9
10	0.15	-0.83	1.17	7	70	5.5
10	0.13	-0.88	1.12	5	50	5.2
10	0.12	-0.94	1.06	4	40	5.0
10	0.099	-1.0	1.0	4	40	4.6
10	0.084	-1.1	0.9	3	30	4.3
10	0.066	-1.2	0.8	1	10	3.7
10	0.00	0.0	0.0	0	0	0.0

Computed Chi square with 6 D F = 1.65

LC_{50} Computed = 0.12

Standard Deviation = 1.1

THE COMPUTATION OF LC_{50} FOR TRIFENMORPH AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.25 mg/l in 48 HOURS

NUMBER OF FISH	CONC. OF TRIFENMORPH (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.25	-0.61	1.39	10	100	6.7
10	0.18	-0.74	1.26	9	90	6.2
10	0.15	-0.83	1.17	7	70	5.9
10	0.13	-0.88	1.12	7	70	5.6
10	0.12	-0.94	1.06	6	60	5.3
10	0.099	-1.0	1.0	5	50	5.0
10	0.084	-1.1	0.9	4	40	4.7
10	0.066	-1.2	0.8	3	30	4.3
10	0.00	0.0	0.0	0	0	0.0

Computed Chi square with 6 D F = 1.16

LC_{50} Computed = 0.097

Standard Deviation = 1.1

THE COMPUTATION OF LC 50 FOR TRIFENMORPH AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.25 mg/l for 72 HOURS

NUMBER OF FISH	CONC. OF TRIFENMORPH (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.25	-0.61	1.39	10	100	7.4
10	0.18	-0.74	1.26	10	100	6.7
10	0.15	-0.83	1.17	9	90	6.2
10	0.13	-0.88	1.12	8	80	5.9
10	0.12	-0.94	1.06	6	60	5.6
10	0.099	-1.0	1.0	5	50	5.2
10	0.084	-1.1	0.9	4	40	4.8
10	0.066	-1.2	0.8	3	30	4.2
10	0.00	0.0	0.0	0	0	0.0

Computed Chi square with 6 D F = 2

LC_{50} Computed = 0.092

Standard Deviation = 1.1

THE COMPUTATION OF LC_{50} FOR TRIFENMORPH AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.25 mg/l FOR 96 HOURS

NUMBER OF FISH	CONC. OF TRIFENMORPH (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.25	-0.61	1.39	10	100	7.0
10	0.18	-0.74	1.26	10	100	7.0
10	0.15	-0.83	1.17	9	90	6.2
10	0.13	-0.88	1.12	8	80	5.9
10	0.12	-0.94	1.06	7	70	5.5
10	0.099	-1.0	1.0	6	60	5.2
10	0.084	-1.1	0.9	5	50	4.9
10	0.066	-1.2	0.8	4	40	4.5
10	0.00	0.0	0.0	0	0	0.0

Computed Chi square with 6 D F = 0.5

LC_{50} Computed = 0.087

Standard Deviation = 1.1

THE COMPUTATION OF LC_{50} FOR NICLOSAMIDE AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.21 mg/l for 24 HOURS

NUMBER OF FISH	CONC. OF NICLOSAMIDE (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.21	-0.68	1.32	10	100	7.4
10	0.14	-0.85	1.15	6	90	6.0
10	0.126	-0.90	1.1	6	60	5.7
10	0.098	-1.009	0.99	5	50	4.8
10	0.084	-1.076	0.92	3	30	4.3
10	0.077	-1.11	0.89	2	20	4.0
10	0.070	-1.15	0.85	1	10	3.7
10	0.063	-1.2	0.80	0	0	3.3
10	0.00	0.0	0.0	0	0	0.0

Computed Chi square with 6 D - F = 2.5

LC_{50} Computed = 0.103

Standard Deviation = 1.06

THE COMPUTATION OF LC_{50} FOR NICLOSAMIDE AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.21 mg/l FOR 48 HOURS

NUMBER OF FISH	CONC. OF NICLOSAMIDE (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.21	-0.68	1.32	10	100	7.4
10	0.14	-0.85	1.15	10	100	6.4
10	0.126	-0.90	1.1	9	90	6.2
10	0.098	-1.009	0.99	8	80	5.6
10	0.084	-1.076	0.92	7	70	5.3
10	0.077	-1.11	0.89	6	60	5.1
10	0.070	-1.15	0.85	5	50	4.9
10	0.063	-1.2	0.80	4	40	4.7
10	0.00	0.0	0.0	0	0	0.0

Computed Chi square with 6 D F = 8.4

LC_{50} Computed = 0.074

Standard Deviation = 1.1

THE COMPUTATION OF LC_{50} FOR NICLOSAMIDE AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.21 mg/l FOR 72 HOURS

NUMBER OF FISH	CONC. OF NICLOSAMIDE	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.21	-0.68	1.32	10	100	7.9
10	0.14	-0.85	1.15	10	100	6.9
10	0.126	-0.90	1.1	9	90	6.6
10	0.098	-1.009	0.99	8	80	6.0
10	0.084	-1.076	0.92	8	80	5.6
10	0.077	-1.11	0.89	7	70	5.4
10	0.070	-1.15	0.85	6	60	5.2
10	0.063	-1.2	0.80	4	40	4.9
10	0.00	0.0	0.0	0	0	0.0

Computed Chi square with 6 D F = 1.46

LC_{50} Computed = 0.065

Standard Deviation = 1.1

THE COMPUTATION OF LC_{50} FOR NICLOSAMIDE AFTER THE FISH SAMPLES WERE TREATED IN SERIAL CONCENTRATIONS RANGING FROM 0.00 - 0.21 mg/l for 96 HOURS

NUMBER OF FISH	CONC. OF NICLOSAMIDE (mg/l)	LOG CONC.	LOG CONC +2	MORTALITY	PERCENTAGE MORTALITY	PROBITS COMPUTER ANALYSED
10	0.21	-0.68	1.32	10	100	7.6
10	0.14	-0.85	1.15	10	100	6.8
10	0.126	-0.90	1.1	9	90	6.5
10	0.098	-1.009	0.99	8	80	6.0
10	0.084	-1.076	0.92	8	80	5.7
10	0.077	-1.11	0.89	6	60	5.5
10	0.070	-1.15	0.85	5	50	5.3
10	0.063	-1.2	0.80	4	40	5.1
10	0.00	0.0	0.0	0	0	0.0

Computed Chi square with 6 D F = 1.13

LC_{50} Computed = 0.059

Standard Deviation = 1.15