STUDIES ON RIVER WATER FACTORS INFLUENCING THE EFFICACY OF VECTOBAC® (*BACILLUS THURINGIENSIS* (Serotype H-14) FORMULATION) AGAINST THE LARVAE OF *SIMULIUM DAMNOSUM* (THEOBALD) SPECIES COMPLEX

FELIX JERRY AKPABEY

(BSc. Biology; Dip. Ed.)

A thesis presented in partial fulfilment of the requirements for the Degree of M.Phil. Entomology of the University of Ghana.

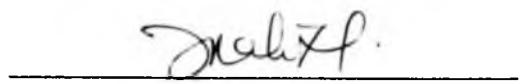
Insect Science Programme •
University of Ghana
Legon.

JUNE 2000

•Joint interfaculty International programme for the training of entomologists in West Africa
Collaborating Departments: Zoology (Faculty of Science) & Crop Science (Faculty of Agriculture).

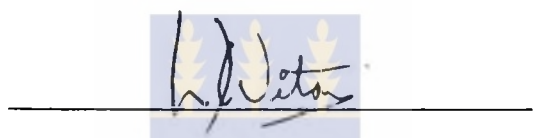
DECLARATION

THE EXPERIMENTAL WORK DESCRIBED IN THIS PROJECT WAS CARRIED OUT BY ME AND ALL REFERENCES CITED IN THIS WORK HAVE BEEN FULLY ACKNOWLEDGED



FELIX JERRY AKPABEY

(CANDIDATE)

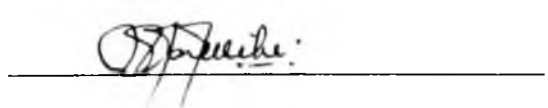


DR. MICHAEL.D WILSON

(SUPERVISOR)

DR. JOHN KPIKPI

(CO-SUPERVISOR)



MR. M. Y. OSEI – ATWENEBOANA

(CO-SUPERVISOR)

DEDICATION

To my wife, COMFORT AMA SEWA LEBI, and my children

SENANU & SEYRAM

ACKNOWLEDGEMENT

I wish to acknowledge with sincere thanks, my supervisors Dr. Michael D Wilson of Noguchi Memorial Institute for Medical Research (NMIMR) and Dr. John K Kpikpi of the Department of Zoology, University of Ghana, Legon.

I am also grateful to Dr. Michael Ocran of the Water Research Institute, CSIR and Mr Michael Osei-Atweneboana, also of the CSIR for their immense help and encouragement in the fieldwork and also to Mr Samson Otoo, Joseph Asaah and Samuel Aquandah of Daboase for their immense help in the field.

My sincere thanks also go to Professor J. N. A. Ayertey, the Co-ordinator of the ARPPIS programme, University of Ghana, for his fatherly love and to the Director and staff of the Ecological Laboratory of the University of Ghana for their financial support in this work. I also wish to thank Messrs Daniel Kurtak and Ernest Dankwa of Abbott Laboratories, North Chicago, USA. I am grateful to Dr. Daniel Adjei Boakye and Mr. Charles Brown of the Parasitology Unit and Mr. George Mensah of the Epidemiology Unit, all of Noguchi Memorial Institute for Medical Research for their technical support and to my mother, Madam Rebecca Adzo Ahortor, Comfort, my wife and Senanu and Seyram, my children for their prayers and support.

I finally thank Professor Ofori Adjei, the Director of the Noguchi Memorial Institute for Medical research, for allowing me to carry out this research work at NMIMR.

This research received financial support from Abbott laboratories Inc., North Chicago Illinois, USA.

ABSTRACT

The principal goal of this work was to identify specific conditions of river water in West Africa, that influence the efficacy of the microbial insecticide, *Bacillus thuringiensis* H-14 formulation (VectoBac®) against *Simulium damnosum* sensu lato larvae for onchocerciasis control. One hundred and thirty assays of a standard formulation using the mini-gutter system were carried out over a period of 15 months at a field station situated near Pra River in southern Ghana. To identify the river conditions influencing the performance of the VectoBac®, both univariate and multivariate statistical procedures were applied to the dataset of lethal concentrations (LC) values, river temperature, conductivity, turbidity and pH measured over the study period. River temperature, conductivity and turbidity (in that order) were the factors identified as having direct effect on the efficacy of VectoBac®. Water temperature and conductivity were negatively correlated, whilst turbidity and pH were positively correlated to LC values Analysis of representative water samples from the field revealed that, total solids, sodium and potassium cations and chloride concentrations differed significantly between wet and dry season and these were thought to be responsible for the observed variations in turbidity and conductivity of the River Pra. A simple method for rearing *S. damnosum* was adopted to study the effect of conductivity on potency of VectoBac® in the laboratory. This

showed that increasing the conductivity of either water or the insecticide, up to 3,000μS both improved potency by about a maximum of 42% and 36.7% respectively. The implications of these findings for the operational use of VectoBac® in blackfly control in West Africa, for the development of laboratory based assay system and improved formulations were discussed. Furthermore, the study provides the basis of determining the real potency of products tested under vagary conditions in the field.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Figure

LIST OF TABLES

TABLE

LIST OF APPENDICES

CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The Simuliidae, or blackflies, comprise a cosmopolitan family of biting flies of great importance, which in many parts of the world are bloodsuckers, and vectors of certain parasitic organisms (Kettle, 1992). Blackflies are usually small and stout, blood-sucking insects with a humped thorax and about 1,300 species have so far been named (Stone, 1965). These include 240 species from Neotropical Region (Vulcano, 1967), 140 species from Ethiopian Region (Crosskey, 1969), 400 species from Palearctic Region (Rubtzov, 1969), 190 species from the Oriental Region (Crosskey, 1973a), and 50 species from the Australian Region (Colless and McAlpine, 1970).

Blackflies in many regions are the most feared of all biting insects because of the relentless and intolerable nature of their attacks, not only on man, but also on livestock, poultry and wildlife. Severe attacks do not only provoke severe reactions in many bitten individuals, but often have serious social and economic effects, even if a "blackfly season" should last only a few weeks. The experience of being continually bitten, unable to step outside without soon oozing blood from countless bites, is a demoraliser with few equals and if the flies are not deterred with repellents or controlled by insecticides they can disrupt any commercial or recreational outdoor pursuit. Forestry, mining, agriculture, construction and tourism are some of the industries that can be adversely affected (Crosskey, 1990). Early

Christian missionaries to the American Indians, for example, regarded blackflies as a scourge from God. A priest travelling in the Ottawa valley in 1624 was reported to have said that:

> "If I had not kept my face wrapped, I am almost sure they would have blinded me, so pestiferous and poisonous are the bites of these little demons They make one look like a leper hideous to the sight. I confess that this is the worst martyrdom I suffered in this country" (cf Davies *et al*., 1962)

Simuliidae include the only insects responsible for transmitting the human blinding disease called onchocerciasis (Crosskey, 1990). Human onchocerciasis or river blindness is an important disease of man and although, it is not fatal, it is extremely debilitating and causes untold misery in tropical Africa, Central and South America, and the Arabian Peninsula. The disease is caused by the development in the human dermis of the filarial worm *Onchocerca volvulus*. The main symptoms of the disease are severe itching, and skin lesions resulting in irritating dermatitis, depigmentation of the skin, and eye lesions which leads to the impairment of sight and ultimately, total blindness (WHO, 1994).

It is estimated that the disease is endemic in 26 African countries, affecting about 293 million people, out of which 78 million are at risk and 1.7 million people actually affected by the parasite and 326,000 people totally blind. In the Americas, the disease affects 265 million people with 5 million at risk, 97,000 actually affected and 1,400 totally blind. In the Arabian Peninsula, it is estimated that 26 million are affected, 2 million at risk, 54,000 actually affected and 8,400 being totally blind (WHO, 1995).

Faced with the desperate situation of populations affected by onchocerciasis in West Africa, the USAID, in 1968, sponsored a meeting of 35 experts under the aegis of USAID/OCGE/WHO in Tunis to discuss the feasibility of onchocerciasis control. The meeting passed a resolution which strongly stimulated the setting up of the Onchocerciasis Control Programme (OCP) in the Volta River Basin (Walsh, 1990). The (OCP) was thus established in 1974 with the support of several international organisations with the WHO as the implementing agency.

The aim of the OCP was to eliminate the health and socio-economic problems posed by the disease in seven countries. Another objective of OCP is to ensure that there is no recrudescence of the disease thereafter. The Programme now covers 11 West African countries and is financed by 22 countries and Organisations from other regions of the World. From its inception until 1987, the tactical approach adopted by the OCP was the interruption of *O. volvulus* transmission through the control of its blackfly vector *S. damnosum*, using the organophosphorus insecticide, temephos (Abate) as choice larvicide in foci of blinding onchocerciasis.

The OCP's vector control activities was started in 1975, using temephos (Abate®). However, prolonged and intensive use of an insecticide encourages the development of resistance, therefore resistance to temephos soon developed in *S. sanctipauli* populations in Cote d' Ivoire (Guillet *et al.*, 1980) which soon spread to *S. sirbanum* (Kurtak *et al.*, 1982). To combat this threat to insecticide use OCP adopted a strategy of alternating insecticides with different modes of action to clear

resistant *S. damnosum* s.l. population and to forestall the appearance of new cases of resistance. This rotation of insecticides has actually given ample proof of its worth because today, few *Simulium* populations in the OCP area are truly resistant to organophosphorus compounds (WHO, 1994). It also added to its arsenal of insecticides, the biological insecticide, *Bacillus thuringiensis* H-14 formulations.

Vector control activities in OCP was achieved by weekly applications of formulations of selected insecticides, upstream of fast-flowing stretches of water courses demonstrated to be productive breeding sites for *S. damnosum.*

Originally, *B. thuringiensis* was introduced into the OCP as Teknar®WDC (Lacey *et al.,* 1982), a formulation which was found to be effective against *S. damnosum*, but at a relatively high dose. It could only be used economically at river discharges of $25m^3$/sec and below, and also it was found to have a low carry of between two to ten kilometres. Subsequently, Abbott Laboratories Inc. became the main supplier of *Bacillus thuringiensis* formulations, which is marketed as VectoBac®12AS. However, batches of *B. thuringiensis* H-14 that have been tried in the field so far against *S. damnosum* s.l. have produced variable results. The search for the best formulation has therefore become the priority of both OCP and Abbott Laboratories. It has long been suspected that its efficacy is affected by the river conditions in the OCP area (Ocran, personal observation), but for some unknown reasons, the environmental factors which influence its efficacy as a larvicide have not been investigated.

In 1996, Abbott Laboratories of the USA, in collaboration with Noguchi Memorial Institute for Medical Research (NMIMR), established a field station at Daboase in the Western Region of Ghana to assay different formulations of its product VectoBac®12AS. Thus, the opportunity was created to investigate into the factors likely to affect its efficacy in the field.

1.1.1 General Objectives

The principal goal of this research work is to investigate into factors that affect the efficacy of VectoBac®, a *Bacillus thuringiensis* serotype H-14 formulation as a larvicide against the larvae of *S. damnosum* s.l., and to conduct experiments in the laboratory to study in detail one of the most important factor that is identified.

The first part of the study involves the collection and analyses of physical and chemical parameters of the river water used for evaluating VectoBac® in the field. It also involves estimating the relative median potency of the standard formulation which would then reveal the most significant factor influencing the efficacy of VectoBac®.

The second part of the study involves testing the effect of conductivity on potency under controlled conditions in the laboratory.

1.1.2 Specific objectives

The specific objectives are as follows:

1. To conduct field-testing of a standard formulation of VectoBac®, using a mini-gutter system.

2. To calculate the lethal concentrations, LC_{50}, LC_{95} and LC_{99} for each test that is conducted.
3. To record and analyse data on the temperature, conductivity, pH, and turbidity of the river water during each test.
4. To conduct chemical analysis of representative water samples collected during the wet and dry seasons with the aim of studying seasonal variations.
5. To use statistical methods (both univariate and multivariate) to identify the factors that influence significantly, the potency of VectoBac® in the field.
6. To test the effect of the identified parameter in this case, conductivity in the laboratory, using a simple system for testing VectoBac® against blackfly larvae.

1.2 LITERATURE REVIEW

1.2.1The Symptomology of Onchocerciasis

The clinical manifestations of onchocerciasis are predominantly dermal nodule, ocular and lymphatic in character. Several other features of uncertain association, aetiology or pathogenesis have also been described, including low body weight, general debility, diffuse musculo-skeletal pain and in Africa, epilepsy and hyposexual dwarfism (WHO, 1995).

The extent and distribution of skin and lymphatic lesions permit classification of the disease into generalised and local forms. Generalised onchocerciasis is the usual presentation, characterised by fairly symmetrical lesions which may be more marked in the lower and less commonly, in the upper part of the body. The localised form is asymmetric and may be confined to one limb and the adjacent area or to a circumscribed part of the body. Acute manifestations of localised onchodermatitis occur in new residents and in people from outside the endemic areas. The chronic form of localised onchodermatitis is synonymous with hyperactive onchodermatitis or sowda and is characterised by frequent acute exacerbation.

Onchocercal nodules are subcutaneous tumours that harbour the adult *O volvulus* that are either visible or palpable on the surface and found mainly over bony prominences on the head, shoulder girdle, ribs, trochanter, knees and ankles. The nodules are rarely considered to have medical importance causing the patient

little or no discomfort, but when on the head, may erode the bone (Muller, 1975; Manson-Bahr and Apted, 1982; Duke, 1990).

Blindness is the most serious consequence of onchocerciasis and it results from microfilariae invading different parts of the eye. In the eye, a few microfilariae may invade the cornea in response to the local presence or local microfilariae death may produce fluffy opacities in early and late infections. Individual opacities, however, clear spontaneously but are succeeded by others. The symptoms at this stage include irritation of the eye, lacrimation and photophobia. Heavier and chronic infections lead to sclerosing keratitis in the cornea and this is largely responsible for the exceptionally high onchocercal blindness in the Sudan-Guinea savanna zones of West Africa (Anderson and Fuglsang, 1977; Duke, 1990). Inflammation of the iris and the ciliary body of the eye with secondary glaucoma is also a major cause of blindness. Iridocylitis can occur, often with microfilariae, flare and keratic precipitate cells in the anterior chamber. Pigment is deposited on the anterior capsule of the lens and the iris becomes bound down by posterior synechiae. The iris may display patches of atrophy. Progressive loss of vision takes place because the pupil becomes occluded with attendant secondary glaucoma or cataract (Nelson, 1970; Muller, 1975).

Disturbance of the retinal pigment epithelium due to inflammation leads to marked sclerosis of the choroid and the retinal vessels. As the retina and choroid degenerate, they result in choroidal and optic nerve atrophy. Optic nerve atrophy may also result when it is invaded directly leading to gross reduction of peripheral

are, however, differences in the situation of the lesions in African and Central American onchocerciasis foci. Generally, in the savanna area of West Africa, blindness is mainly due to sclerosing keratitis, whereas in the rain forest area, blindness is often due to posterior segment lesions. In the Central American foci in Guatemala and Mexico, blindness usually results from anterior uveitis (WHO, 1987).

In heavy microfilariae infection of the skin, the lymphatic system becomes involved when some of the parasites get to the lymph nodes where they produce lymphadenitis with subsequent fibrosis. Enlarged inguino-femoral nodes that hang in pockets of skin (hanging groin) can also predispose the patient to hernia. Lymphatic enlargement of the scrotum and hydrocoele and enlarged testes also result in scrotal elephantiasis (Nelson, 1970; Duke, 1990). The involvement of the central nervous system has also been noted (Mazzotti, 1959; Rodger, 1960). Raper and Ladkin (1950) claim that *O. volvulus* is the causative agent of the "Nakalanga syndrome" (the pygmy dwarfing resulting from damage to the pituitary gland) in Uganda.

1. 2. 2 The Socio-economic and Public Health Importance of Onchocerciasis

The serious eye lesions, which occur when the intensity of infection is high and where the strain of parasite is pathogenic, and the prominent skin lesions are responsible for the major public health impact of the disease. The consequences of onchocerciasis have repercussions beyond the individual and directly affect the family, community and country. In severely afflicted areas populations move to healthier local environments, away from the rivers where the vectors breed but where the soil is usually not so fertile. While onchocerciasis has not always been the only cause of depopulation of the valleys of tropical Africa, it is nonetheless, the main obstacle to their development and to the establishment of communities in those regions (WHO, 1994). Furthermore, blindness leads to a reduction of some 10 years in life expectancy; therefore the disease not only disables, but it is indirectly responsible for considerable premature mortality. Similar effects are seen in Amazonian communities, where eyesight is a fundamental requisite for hunting and tribal migration and thus, for nutrition and survival (WHO, 1995).

In persons with prolonged intense infections, the skin lesions and itching are responsible for much chronic misery and disfigurement and can lead to a degree of social isolation, with detrimental psychological effects.

Within Africa, the rate of blindness in hyperendemic communities that are not under control may rise to 15% and up to 40% of adults may show severe ocular impairment. When there are high rates of visual impairment, communities become unstable, their agricultural capacity declines, and eventually the villages are abandoned.

In Ghana for example, the disease was responsible for a mass desertion of people from fertile riverine valleys in the northern region, to poor lands which in short time became overcropped and overgrazed leading to poor agricultural production. In these villages, the mortality rate among the blind persons over 30 years of age was 3-4 times as high as among sighted persons of the same age groups (Prost and Vaugelade, 1981).

Studies carried out in Guinea have also revealed that if the impact of onchocerciasis-related blindness on the household is severe, it most often results in the family's inability to support itself. The disruption of family life is directly related to stress within the household, contributing to its destitution. The extra burdens placed on other members of the family once the main breadwinner is blind and can no longer continue with normal activities, have adverse effects on their physical, psychological and emotional health. The older children often choose to migrate, fearful of becoming blind themselves (WHO, 1995).

In the hypo- and meso-endemic areas in Africa and in the Arabian Peninsula, where blinding onchocerciasis is less prevalent, the socio-economic consequences of onchocerciasis are less striking and less well studied.

In the Americas, the socio-economic consequences of onchocerciasis has not been well studied, particularly with regard to the impact of blindness and skin diseases on productivity (WHO, 1995).

Knowledge of onchocerciasis, including its name, the method of transmission, and the complex signs and symptoms of the disease, is poor in endemic communities in Africa and the Americas. Nevertheless, the studies

conducted to date indicate that populations are aware of, and concerned about, the signs and symptoms of the disease, especially blindness and the skin lesions, as well as the stigmatisation associated with these conditions and the psychosocial consequences. The consequences of the skin lesions have been less well studied because research has focused mainly on the OCP area and on blindness as a public health problem. However, in Nigeria, there have been reports of social stigmatisation and rejection of those with skin disease because of the belief that it is caused by dirtiness (WHO, 1995).

The psychological repercussions of social marginalisation in some Amerindian cultures are known to cause serious difficulties for the affected individuals in their interactions with other members of the community. In the southern Venezuelan foci, cultural interpretations of the origin of some of disease signs and symptoms, as well as the mechanisms of onchocerciasis transmission, are sometimes a source of conflict between communities. These cultural factors also determine the social acceptance of the changes in physical appearance associated with the disease. Individuals with severe skin manifestations (e.g. hanging groin) are socially isolated (WHO, 1995).

1. 2. 3 The Epidemiology of Onchocerciasis

The factors influencing the epidemiology of onchocerciasis can be grouped into those relating to the host, the parasite and the vector, but behavioural and community factors also need to be taken into account.

The epidemiology of onchocerciasis is that of a vector-borne disease of which human beings are the only vertebrate host. Infection with *O. volvulus* like other filarial infections is also characterised by coincidence between the degree of human infection and the intensity of exposure to infected vectors.

However, the epidemiology of onchocerciasis is not uniform throughout its distribution because, different disease patterns are associated with different variants or strains of the parasite. Disease patterns are also associated with differences in the vector competence and feeding characteristics of local blackfly populations, with the abundance of the vector and with differences in the human host responses to the parasite. These factors, together with those related to environmental, geographical, social and demographic influences, increase the complexity of the epidemiology of onchocerciasis in different areas of its distribution.

Studies including risk-factor analysis provide vital information needed in deciding which control measures should be adopted. The recent identification of a relationship between geographical forms of the parasite, which are genetically distinct and patterns of blinding and non-blinding ocular disease is therefore a major finding (WHO, 1995)

With respect to host factors, there are no known sex differences in acquisition of infection and age merely determines cumulative exposure to infection. Variations in the immune response to infection are apparent in individuals with "sowda" lesions, but more detailed studies on different aspects of the status of the human immune system under different pathological conditions are needed.

Parasite factors such as genotype may also explain the pattern of disease in certain foci. For example, two different strains of *O. volvulus* (forest and savanna) that exist in West Africa are associated with milder and severe lesions and this is of importance in setting priorities for control measures.

Vector factors are important in as much as they affect the transmission of the parasite. Transmission rates may vary both seasonally and by geographical location. Vector abundance depends on hydrological conditions, which determine the number and productivity of blackfly larval habitats. Vector density is also determined by dispersal habits. Ecological factors, such as prevailing winds and humidity, also contribute to passive dispersal and migration. Some species of *S. damnosum* species complex in West Africa are known to migrate up to 400km from their breeding sites (Crosskey, 1990). There are also major differences between vector species in their feeding habits, for example, in the degree of preference for human as opposed to animal hosts. Furthermore, the intensity of microfilarial infection in the skin may play a critical role in determining the infection of the vector, since each species has an infection threshold (Crosskey, 1990).

Behavioural and community factors are most important in the planning, implementation and evaluation of control measures. In savanna areas, the intensity

of exposure to transmission is determined by the distance between a community and a fly breeding site and by the presence or absence of other human settlements in the intervening area (WHO, 1995). These considerations have led to the characterisation of villages as either first, second or third-line. Furthermore, individuals who frequently visit the breeding sites or whose work requires them to spend long periods on the riverbank (e.g. fishermen) tend to have very severe manifestations of onchocerciasis.

The density of the human population in relation to the vector population emerging from local breeding sites is also an important determinant of intensity of infection, as is the presence of cattle near rivers, since it reduces the contact of the human population with zoophilic vectors of *O. volvulus*. In addition, the regular inoculation of the human population with *Onchocerca* L_3 larvae of animal origin may provide an immunological stimulus to the host and thus help prevent infection with *O. volvulus* (WHO, 1995).

Geographic variation in the symptomology of the disease exists. In Latin America (except Venezuela) head nodules are common. It is believed that this phenomenon is related to the fact that the vector, *S. ochraceum* tends to bite about the head and neck regions. In contrast, in Africa and Venezuela, the nodules are found most commonly in the pelvic, knees, lateral chest and spine regions, less frequently in the shoulder girdle and least on the head. The African vectors, *S. damnosum* s.l. and *S. neavei* and *S. metallicum* in Venezuela tend to bite on the lower parts of the body.

Variations in the rates of blindness also exist. In Africa and America, blindness occurs more commonly in the savanna areas than in the forest zones. It is seldom over 1% in forest regions, but in some savanna regions of Africa and in Guatemala, over 10% of the population may be completely blind. There is evidence that in West Africa, this variation is related to differences between the strain(s) of *O. volvulus* found in the Sudano-Guinean savanna and those found in the forest zones further south (Duke *et al.*, 1966; Philippon, 1977). For example, the savanna form of the parasite has been shown to be more pathogenic than the forest forms when inoculated into the cornea of the rabbit's eye (Duke and Anderson, 1972). Furthermore, the main savanna vectors *S. sirbanum* and *S. damnosum* s. str., while acting as efficient vectors of the savanna form of *O. volvulus*, do not support equally well the forest form of the parasite (Duke, *et al.*, 1966). Thus, there appears to be two separate *O. volvulus*- *S. damnosum* s.l. complexes which correspond to the two vegetation zones in West Africa (Philippon, 1977).

1. 2. 4 The Life Cycle and Transmission of *Onchocerca volvulus*

In Africa and the southern Arabian Peninsula, onchocerciasis is associated mainly with members of the *S. damnosum* complex and to a lesser extent with the *S. neavei* complex. In East and Central Africa *S. albivirgulatum* the vector in the "Cuvette Centrale" focus of Zaire is the only vector species in Africa outside these two taxonomic groups.

Members of the *S. damnosum* species complex (see Figure 1) transmit *O. volvulus* in West Africa.

The adult *O. volvulus* in humans lives in fibrous nodules, which are either, subcutaneous and palpable or lie deep in the connective and muscular tissues. The adult female measures about 30-80cm long and the males about 3-5cm (Crosskey, 1990). The female worm is viviparous and for greater part of its life-span estimated around 12 years, produces millions of microfilariae, which migrate from the nodules to invade the skin, eyes and some other organs. The microfilaria which measures 250-300 micrometres in length is the pathogenic stage of the parasite and causes most of the disease manifestations of onchocerciasis. The microfilariae can live in the connective tissues for up to two years and are ingested from the skin by blood feeding *Simulium* vectors. The ingested microfilariae then penetrate the stomach walls into the abdominal cavity of the vector but only those that migrate to the thorax develop successfully (Omar and Garms, 1977). In the thorax muscles, they shorten and fatten to become the first stage larvae (L_1). The L_1 which measures 190-350 microns, moults to become the second larval stage (L_2), which is longer (350-550 microns) and after the subsequent moult becomes the infective third

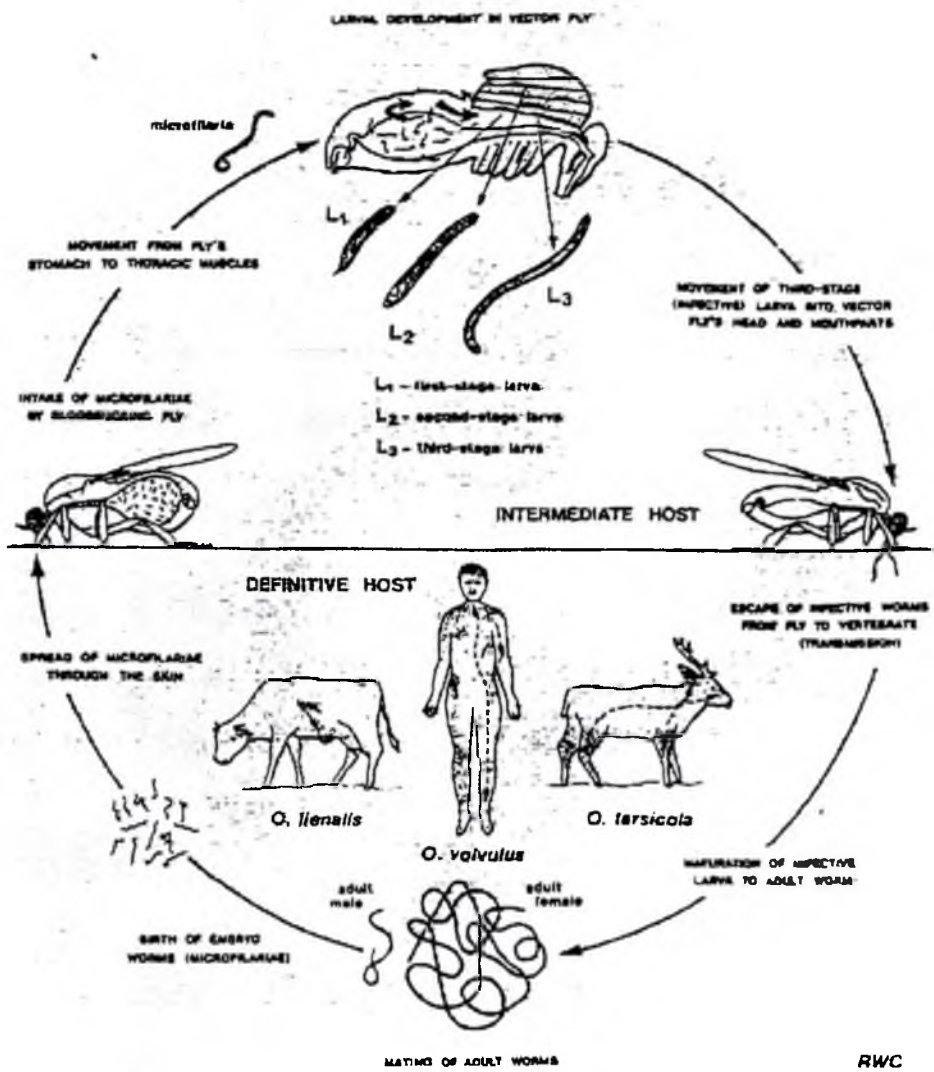


Fig. 1 Schematic illustration of the life cycle and transmission of *Onchocerca volvulus* (Leuckart, 1893) (Crosskey, 1990).

larval stage (L_3). The L_3 is motile and migrates to the head of the fly where it remains infective till it is released with the next blood meal. The infective larvae escape via the proboscis, into the bite wound and then migrate into the human host. The duration of the development from ingested microfilariae to the infective stage in the vector is usually between 6-12 days.

In the human host, they moult twice without multiplying to reach the adult stage. Their presence in humans will cause a fibrotic reaction of the dermal layers of the skin generating the characteristic nodules. In about nine to twelve months after infection, the female parasite matures, mates and starts to shed a new generation of microfilariae. The first microfilariae produced by adult females may appear in the skin some 10-15 months after infestation (Crosskey, 1990). Although the route of the immature worm inside the human host is unknown, they appear to be attracted to existing nodules and may settle on their surface to form satellite or composite nodules (WHO, 1995).

A person is capable of becoming infected after being bitten by an infectious blackfly carrying the *Onchocerca volvulus* parasites. However, the disease does not appear to take a stronghold until after the victim has received many infective bites over a period of time (Dudley & Feltmate1992).

1.2.5 The Life Cycle of *Simulium damnosum* Species Complex

The *Simulium damnosum* species complex in West Africa comprises: *S. damnosum* s. str., *S. sirbanum, S. sudanense, S. dieguerense, S. squamosum, S. yahense, S. sanctipauli, S. leonense* and *S. soubrense*.

The life cycle of *S. damnosum* s.l. involves an aquatic and terrestrial stages (Figure 2). All the pre-adult stages of eggs, larvae and pupae are aquatic whilst the adult fly is terrestrial. The female fly bites man, needing blood to mature each batch of eggs laid. Before taking its first blood meal, the female mates and stores the sperm in a spermatheca, which it uses to fertilise successive batches of eggs. The eggs are laid attached to solid immersed surfaces and substrates or to vegetation in the fastest flowing parts of a river or stream. In warm tropical waters, the eggs hatch in about one or two days and the larvae remain attached to the substrate by spinning silk pads on which they cling onto with their posterior hook circlets. They will also move to find optimum conditions of food and oxygen supply in the current. The larvae are particulate-feeders and use the cephalic fans on the head to filter particles from the flowing water. They go through seven instars after six to nine days depending on temperature and species, and when fully grown, spin silken cocoons to pupate inside. The pupal stage lasts from three to six days which also depends on temperature and the species. When mature, the cocoon splits and the adult fly accompanied by a bubble of air rises to the water surface. It takes to flight, mates, and the females blood-feed to continue the life cycle. The adult female fly lives at most for about three or four weeks, but the male lives for a few days.

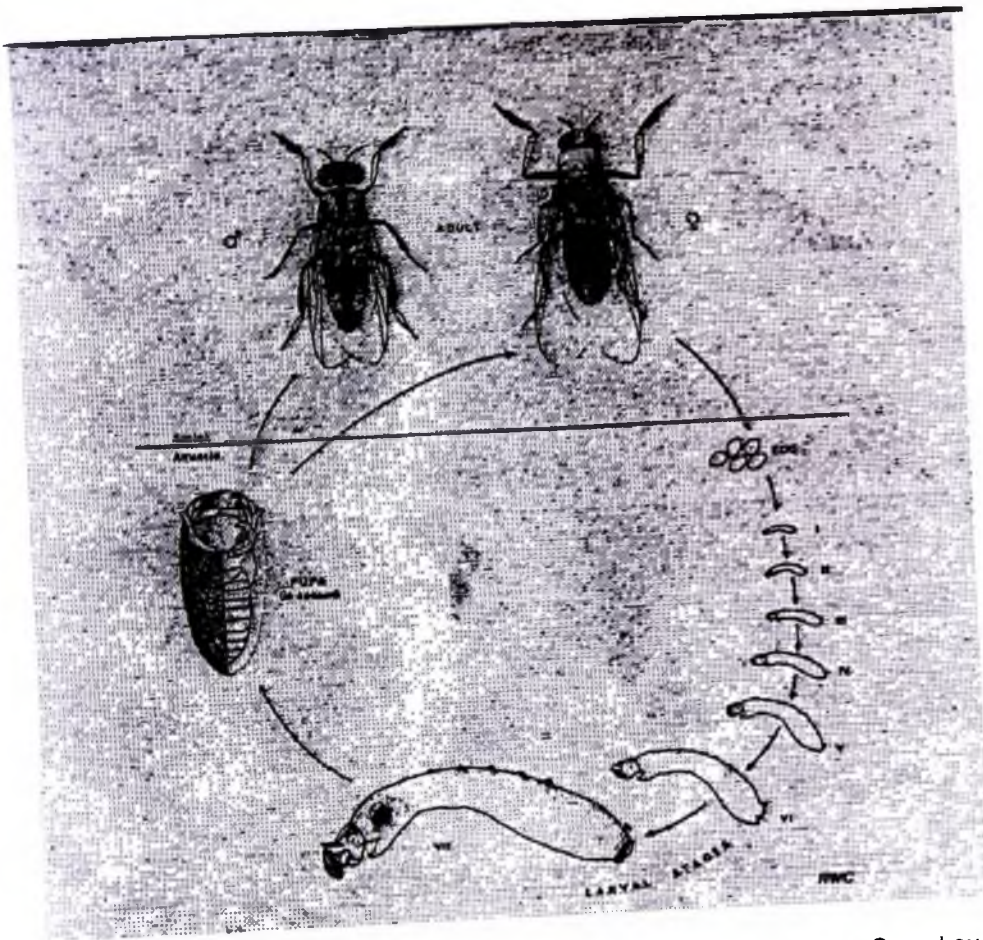


Fig. 2 The life cycle of *Simulium damnosum* (Theobald, 1903) s. l. (Crosskey, 1990)

The requirement for fast flowing waters restricts the distribution of the species. This usually coincides with the areas where the precambrian basement rock forms the land surface and is exposed in the river beds in the shape of resistant rapids that break the flow and provide the classic breeding site (Crosskey, 1981). In the more northerly regions of the savanna, the breeding sites are localised to perennial rivers with rapids during the dry season. During the periodic wet season, breeding extends to other rivers that cease in the dry season. The negative areas correspond closely to sedimentary areas, which are characterised by sandy riverbeds (Crosskey, 1981; Paris, 1989). In the south, lagoons, coastal plains and the sea limit the distribution. There are no east-west limitations on the distribution of the breeding sites of the vectors.

1.2.6 Ecological Determinants of Survival and Distribution of *S. damnosum* Larvae.

All fresh waters contain dissolved and particulate inorganic and organic compounds and gases which vary greatly depending on the geology, topography, climate, season, time of day, depth and biota (Grunewald, 1981). All compounds are vitally important to the existence of aquatic organisms and there is an intensive and continuous exchange of ions and gases between the latter and the surrounding water. It is therefore likely that, the immature stages of Simuliidae, which colonise various kinds of waters all over the world, are influenced in their survival and distribution by their physico-chemical environment.However, the correlation between physico-chemical parameters of the aquatic environment and its blackfly fauna is rarely fully documented although some laboratory studies have been conducted on temperature (Mokry, 1976; Ross and Merritt, 1978) on pH, conductivity and nitrogen compounds (Grunewald, 1978; Grunewald and Grunewald, 1978).

Carlsson (1968; 1970) and Disney (1975) have published information on the water chemistry of rivers harbouring *S. damnosum* s.l populations in West, Central and East Africa. However, none of the authors differentiated between breeding sites of the various species of the *S. damnosum* -complex. As members of this species complex differ in their distribution, biology, ecology and behaviour, it would not be surprising if the larval stages differed in their habitat requirements with respect to hydro-chemical and physical conditions.

Some studies, however, have shown that the survival and longevity of *S. damnosum* larvae depend on physical parameters such as temperature, turbidity, hydrogen ion concentration (pH), conductivity, water current, parent rock among others. It is very easy to imagine because some physical or chemical state is present in waters of a particular habitat that the larvae are either present or absent. For example, larvae could be absent in a particular location because of the high incidence of aquatic weeds or algae.

According to Crosskey (1990), the four physico-chemical parameters affecting the distribution of immature stages of *S. damnosum* are mostly water temperature, water velocity, hydrogen ion content and conductivity (concentration of dissolved salts).

1.2.6.1 Water temperature

In the rivers and streams of the savanna zone of West Africa, the immature stages of *S. damnosum* s. str. and *S. sirbanum,* develop at water temperatures ranging from 24°C to 33°C, with mean value of 27°C. Similar values have been obtained from large rivers of the tropical rain forest zone where *S. sanctipauli* and *S. soubrense* breed (Grunewald, 1976a). Also in the rain forest zone, the water temperature of the small, heavily shaded streams harbouring larvae and pupae of *S. yahense* have been found to be lower with a mean value of 25°C (23°C-30°C). Similar values (mean, 25°C; range 22-29°C) have been recorded for *S. squamosum* (Grunewald 1976a,b; Quillevere *et al* 1976, 1977).

Quillevere *et al* (1976, 1977) found that, water temperature was much lower during the rainy season than in the dry season at the larval habitats of *S. damnosum, S. sirbanum, S. sanctipauli,* and *S. soubrense* in Cote d' Ivoire. However, for the larval sites of *S. yahense* and *S. squamosum* the authors found that the difference in temperature between the dry and rainy season was much less pronounced.

1.2.6.2 Water velocity

Blackfly larvae depend upon a particular current velocity, not only for their feeding activities but also for preventing the accumulation of depleted water around them which is a direct consequence of their metabolism (Ruttner, 1926).

Since there is considerable variation in velocity and turbulence over very short distances (Ambuhl, 1962), most of the relevant measurements made from rivers and streams are of little significance for any particular individual of a blackfly species. However, studies have shown that for *S. damnosum* species complex in West Africa, the water current velocity ranged from 0.4m/s (Carlsson, 1968) to 2.40m/s (Elsen and Hebrard, 1977).

In East and Central Africa, Grunewald (1976a) has reported velocities as high as 2.18m/s for the Kibwezi form of *S. damnosum* and also found larvae of Kisiwani form developing in currents as low as 0.68m/s. Under laboratory conditions, Grunewald (1976a) reared the Kisiwani form from egg to adult at only 0.43m/s.

1. 2. 6. 3 Hydrogen ion concentration and conductivity

The hydrogen-ion concentration and conductivity are as important as water temperature and water velocity in the distribution of the immature stages of the *S. damnosum* complex.

On the basis of differences in pH and conductivity of larval habitat, Grunewald (1976a,b) classified the species of the *S. damnosum* complex into three main groups. The larval habitat of the first group, *S. yahense*, and *S. sanctipauli*, are characterised by acidity, hydrogen ion content ranging from pH 5.7-6.2, and conductivity always below 50µS. The larval habitats of the second group of *S. damnosum s.s. S. sirbanum, S. squamosum,* and *S. soubrense*, are characterised by either weak acidity or weak alkalinity and conductivity values of 150µS. In the last group, of the Kibwezi and Kisiwani forms, the larval habitats are characterised by high alkalinity with pH 7.7-10, with conductivity values from 400-950µS, which were the result of very high levels of dissolved salts.

1. 2. 6. 4 Water chemistry of *S. damnosum* habitats

In unpolluted oxygenated rivers, only trace quantities of nitrite were found. In water harbouring larval stages of the *S. damnosum* complex, the concentration of nitrite seldom exceeded 0.009mg/litre; although the two East African forms, Kibwezi and Kisiwani, were found in water with maximum concentration of up to 0.039mg/litre and 0.125mg/litre respectively (Grunewald, 1976a, b).

As with nitrite, the content of nitrate was also found to be very low in the water of all the sites that were investigated. In West Africa, nitrate concentration of

around 1.0mg/litre can occur but the highest nitrate concentration recorded was 3.164mg/litre at a *S. squamosum* breeding site in the rain forest zone of Cameroon. In East Africa, the quantity of nitrate was even lower than in West Africa, with the highest being recorded in a larval habitat of the Kisiwani form which contained 1.578mg/litre (Grunewald, 1981).

In "closed" rearing systems in the laboratory, however, an increase in mortality of Paleartic blackfly larvae was observed when the concentration of nitrogen accumulated to 10.0mg/litre as a result of decaying food particles and larval metabolic waste-products (Grunewald, 1973).

In West Africa, the water of rivers and streams investigated by Grunewald (1976 a; b) and Quillevere *et al* (1976; 1977) were generally very poor in phosphate. The concentrations of phosphate in the form of ionic orthophosphate did not exceed 0.5mg/litre. The highest values of dissolved phosphate of about 0.27mg/l were found in waters breeding the savanna species of *S. damnosum* and *S. sirbanum* and in the rivers of the rain forest zone harbouring *S. sanctipauli* and *S. soubrense* (Grunewald, 1976a, b; Quillevere *et al.*, 1976, 1977). In the small streams of the forest zone where immature stages of *S. yahense* and *S. squamosum* occur, the phosphate content hardly exceeds 0.2 mg/litre. Quillevere *et al* (1977) found the phosphate concentration in the rain forest zone to be lower in the rainy season than in the dry one, while this did not vary with seasons in the savanna zone.

In natural, unpolluted watercourses, phosphate and the nitrogen compounds did not directly affect the immature stages of the *S. damnosum* complex. But being

essential nutrient salts for the growth of plants and algae. Phosphate, ammonium and nitrate may affect larval populations indirectly.

Analyses of iron, silicate and aluminium in blackfly larval habitats did not indicate any relation between these parameters and the distribution of immature stages of the *S. damnosum* complex (Grunewald, 1976a, b; Quillevere *et al* 1976, 1977). Neither did iron and silicate influence the survival rate of the larvae of *B. erythrocephala* in the laboratory (Grunewald, 1973).

The amount of organic matter in rivers and streams varies in West Africa. Quillevere *et al.*, (1976, 1977) found higher values of organic matter at breeding sites of *S. yahense* and *S. squamosum* than those of *S. sanctipauli* and *S. soubrense* during dry seasons. In the rainy season, however, there was an increase of organic matter at the sites of the two latter species, with the concentration reaching a similar level to that recorded for *S. yahense* and *S. squamosum* sites. In the savanna zone, Quillevere *et al* (1976, 1977) found higher levels of organic substances at breeding sites of *S. damnosum* and *S. sirbanum* in the dry than in the rainy season. The organic matter in the blackfly larval habitats, as determined by the consumption of potassium permanganate, gives no real indication of the amount of nutrients available to the larvae. This is because dissolved organic matter, like humic acids is included and not all forms of organic matter are oxidised by permanganate. However, organic matter as organic acids may affect *S. damnosum* complex larvae by reducing the hydrogen ion concentration (Shapiro, 1957) and by intensive ion exchange processes (Gessner, 1959).

1.2.7 The Control of Onchocerciasis.

In principle, there are two approaches to the control of onchocerciasis, either through the reduction in the numbers of the vector thereby reducing transmission or by killing the parasite in the human host. However, onchocerciasis control activities, until recently, have used insecticides against larval and adult stages of the vectors. Two periods can be recognised in the use of insecticides for control and these are pre- and the OCP eras.

The pre-OCP period can be divided fundamentally into two different eras. A period of about 15 years before DDT became available and 25 years during which DDT was available and before it fell into disrepute owing to its persistence and the undoubted environmental damage caused by its gross overuse in public health programmes.

The first attempt to control a *Simulium* vector of *O. volvulus* took place in Chiapas State, Mexico in 1932 (Walsh, 1990). Streams in the coffee estates and associated villages were weeded on a monthly basis. However, despite considerable efforts there was little effect on *Simulium* densities. Tests were then carried out using Calcium chloride, Sodium chloride, Paris green and creoline which was found to be effective at one part per thousand but a planned campaign scheduled for 1937 had to be abandoned owing to opposition by local residents (Davies, 1983).

Another notable control scheme of the pre-DDT era, which also employed the clearance of vegetation, concerned the very small focus of Riana in the Nyanza Province of Kenya, which covered about 42km^2. The vector in question was the *S*

neavei which was known to occur where there was dense undergrowth and tree cover and was practically confined to this well-defined foci because of its restricted flight range. The clearing operations were carried out in two stages along the two rivers involved in 1943 and 1944. By 1948, *S. neavei* had been eliminated from the area (Buckley, 1951). Success was achieved because the biting habits and flight range of the fly were correctly understood then (WHO, 1987).

The DDT era, strictly speaking, was an operational research into the efficacy of DDT as a larvicide, rather than a control scheme designed to reduce transmission of *O. volvulus*. Captain Fairchild of the US Army Sanitary Corps, had access to DDT in 1943 and 1944 when it was almost considered a secret war weapon and the very limited supplies were available only to the military. Fairchild teamed up with Barreda, a civilian, and in the Yepocapa focus of Guatemala tested a DDT xylene emulsion at 0.1mg/l for one hour in small streams which achieved highly satisfactory results against *S. ochraceum* (Jamnback, 1981).

Control through aerial application against the adult stages, though not practical due to the dispersal habits of the fly, has also been attempted with some success in Uganda (Barnley, 1958). However, it is believed that this result was probably due to the larviciding effect of the DDT, which had accidentally entered water. The initial adulticiding operations was suspended and eradication thereafter was achieved through larviciding (McCrae, 1978).

The OCP era began with the establishment of the programme in West Africa in 1974. Larviciding was the sole means of control adopted by the OCP until recently when it was complemented with the drug ivermectin. Prior to this, the only

drugs available for the treatment of onchocerciasis were suramin and diethylcarbamazine (WHO, 1987). Although suramin is known to be an effective macrofilaricide (Ashburn *et al.*, 1949) and has been used successfully in limited mass treatment (Dawood, 1978; Rougemont *et al.*, 1980, 1984), the difficulties associated with its mode of administration and its toxicity limited its usefulness (Awadzi *et al.*, 1995). Diethylcarbamazine on the other hand has microfilaricidal action, which has to be given over several days but it also produces severe, adverse (Mazzotti) reactions (Awadzi & Gilles, 1980). These drawbacks therefore excluded the use of both drugs for routine treatment of onchocerciasis.

1. 2. 7. 1 Control of onchocerciasis using ivermectin

The advent of an effective microfilaricide called (ivemectin, MSD) Mectizan® and its registration in October 1987 in France for the treatment of human onchocerciasis were breakthroughs in the control of onchocerciasis.

The introduction of ivermectin therefore added a new tool to onchocerciasis control strategies. Ivermectin is a highly effective microfilaricide, which if given at the required dosage of 150μg/kg of body weight, at an interval of one year, prevents the development of symptoms. It also appears to have some cumulative effect on the adult worm and its reproductive capacity (Abiose, 1998). Ivermectin, which is a macrocyclic lactone, was originally developed for the treatment of the ectoparasites and endoparasites of domestic animals and livestock but was found to be highly effective against *O. cervicalis* in horses and *O. gibsoni* in cattle.

In 1982, Mectizan was tested in human patients with onchocerciasis and found to reduce the microfilarial counts in skin snips of infected individuals significantly for a period of six months to one year (Aziz *et al.*, 1982). In subsequent clinical trials, it was found not only to be an effective, and long-acting microfilaricide, but also had no serious adverse effects and therefore was suitable for mass treatment (Coulaud *et al.*, 1983, 1984; Greene *et al.*, 1985; Lariviere *et al.*, 1985; Awadzi *et al.*, 1986, 1989; Diallo *et al.*, 1986; White *et al.*, 1987). The results of some other clinical trials also revealed that ocular microfilarial loads reached their lowest levels six months after treatment and remained low until 12 months post-treatment (Taylor *et al.*, 1986; Newland *et al.*, 1988; Dadzie *et al.*, 1989). After several large-scale field studies the drug's safety, its acceptability and effectiveness against onchocerciasis was confirmed (De Sole *et al.*, 1989; Remme *et al.*, 1989a; Abiose *et al.*, 1993) and it is now the drug of choice for the treatment and control of the disease. The rationale for the strategy of ivermectin usage is to reduce the transmission of *O. volvulus* to levels low enough to prevent the development of severe eye lesions, blindness and skin lesions by reducing microfilariae load. The strategy also implies that the treatment has to be sustained long enough to enable the adult parasites to die out in the population.

Currently, the task of eradicating human onchocerciasis in Africa is under the auspices of the African Programme for Onchocerciasis Control (APOC). The APOC was created to tackle the problem in endemic African countries which were not within the OCP (Remme, 1995). The goal of the APOC is to eliminate onchocerciasis as a public health problem, by implementing community-directed

Mectizan ® (Ivermectin, MSD) treatment and maintaining it for 12 years in about 19 African countries where the disease is endemic.

1.2.7.2 Control of onchocerciasis using chemical insecticides

The OCP provides the only example of sustained use of chemical insecticides for onchocerciasis control. Six insecticides are now used in the OCP area, namely, Temephos, Pyraclofos, Phoxim, Permethrin, Carbosulfan and *Bacillus thuringiensis* serotype H-14. (*Bt* H-14) which is a biological insecticide (Hougard *et al*, 1993).

Temephos, an organophosphorus, is the insecticide of choice, because it is cheap, requires low operational doses and it has a carry of as much as several tens of kilometres when water levels are high. Phoxim is an organophosphorus and it does not endanger the environment, but is less effective and has a limited range. Permethrin is a pyrethroid, which has a more limited range than temephos and Pyraclofos, though its operational dose is very low, so that its use is not limited by high rates of flow. However, because it is somewhat toxic to non-target fauna, it is never used at less than $70m^3/s$ and when possible, it is applied for not more than 6 weeks per year to the same stretch of water (WHO, 1994). Carbosulfan is a carbamate with a carry and toxicity similar to that of Permethrin, but both its price and operational dose are much higher therefore its use is therefore restricted to rates of flow between $70m^3/s$ (the toxicity threshold) and $150m^3/s$ (the cost threshold).

1.2.7.2.1 Criteria for selecting chemical insecticides for onchocerciasis control

The insecticide formulations that are used for large-scale campaigns have to satisfy a wide range of requirements. They must be highly effective against the vectors, but must be safe for the rest of the environment. Each formulation must be specially devised for blackfly control, the supply of the insecticide must be guaranteed over a long period of time and the cost kept as low as possible. Biodegradable constituents are required but there must also be maximum carry downstream from the point of application. In addition, since the vectors are under constant insecticide pressure in very extended control zones, alternative larvicides must be available, preferably belonging to different chemical classes, so that any resistance to one or more compounds (cross resistance) can be avoided or dealt with promptly.

Generally, there is no known chemical insecticide, which is ideal because it has all the desirable characteristics. An insecticide, however, becomes widely used when it possesses many of the desired properties. It is also impossible to rate the various properties in order of importance because different situations demand different conditions.

According to Kurtak *et al.* (1987), in selecting a candidate insecticide for testing in the OCP, the following criteria should be considered:

1. It must be active against all larval instars of *S. damnosum* s.l. Ideally, the concentration required to kill all of the larvae with a 10-minute exposure

should not exceed 0.1 mg/litre but concentrations up to 0.3 mg/litre might be acceptable.

2. The distance that the larvicide remains nearly 100% effective downstream (carry) should approach 20km in large rivers.
3. Since the larvicide is added to rivers, which also serve as source of water to humans, formulations with a rat oral toxicity of LD_{50} greater than 250mg/kg body weight are ruled out. In practice, this implies that the dilute formulations of moderately toxic materials can be tested and or used.
4. The ideal formulation should have no acute or long-term toxic effect on fish even at considerable overdoses. Toxicity to non-target invertebrates should be minimum but it is recognised that some reduction will occur. In principle, no important group of invertebrates should be eliminated, but shifts in proportions are considered to be tolerable.
5. The preferred formulation should be an emulsifiable concentrate, which has a lower specific gravity than water and forms a stable emulsion without agitation. Wettable powders and flowable formulations, especially those, which must be diluted with water, are more difficult and dangerous to mix, load and apply, and should generally be avoided. The formulation should not degrade rapidly when stored as a concentrate in unprotected containers in a hot climate where the air temperature often reaches 40°C.

6. The applied larvicides should not be so stable as to lead to its accumulation in the food web.
7. Formulations with solutions that corrode the spraying equipment and the aircraft itself should be avoided.

However, for microbial insecticides, the successful field application depends on the nature of the agent, their entomopathological properties and the ability for cost-effective mass production in suitable formulation (Merdan, 1982).

1.2.7.3 Control of onchocerciasis using the biological insecticide *Bacillus thuringiensis* **serotype H-14.**

Currently for onchocerciasis vector control, microbial larvicides are regarded as alternatives to chemical insecticides, which are now being gradually phased out and its use discouraged for a variety of reasons. Some of the reasons are the development of resistance among the vectors to an ever-widening range of chemicals, the high cost and the unavailability of chemicals, particularly in the developing countries where the disease is endemic.

Progressively, the adverse environmental impact of many chemicals is assuming importance in the decision-making. Most people regard microbial larvicides as desirable because of the minimal environmental impact, which leaves the beneficial fauna unharmed, and thus, making it possible to conserve natural control agents. The use of microbial larvicides avoids a rebound of the vectors in enhanced intensity such as experienced after the effect of chemical insecticide has declined (Burges, 1982).

In the OCP, *Bacillus thuringiensis* is applied aerially in a similar manner as that of chemical insecticides. *Bacillus thuringiensis* serotype H-14 formulations are usually used by OCP to clear the species that have developed resistance to the organophosphates, pyrethroids and carbamates. Once this is achieved, the programme reverts to the chemical insecticides. This is because, although *B. thuringiensis* H-14 is practically neutral, with few side effects except in the larvae of some groups of Diptera (Dejoux *et al.*, 1985) it is expensive compared to chemical insecticides.

1.2.8 Biological Characteristics of *Bacillus thuringiensis* Serotype H-14

Bacillus thuringiensis Berliner 1915 is a spore-forming bacterium that produces a crystal of toxic protein (delta-endotoxin) with each spore. The species *B. thuringiensis* has been divided on the basis of the flagellar antigens of the vegetative cells (H-antigens) or the antigenic composition of the crystal into 14 serotypes, in some of which there are two subtypes. The isolation of *B. thuringiensis* serotype H-14 was first achieved by analysing both moist and dry soil samples from ponds or stream banks which were known mosquito breeding sites in the Negev, Israel (Goldberg *et al.*, 1977, Goldberg and Margalit , 1977a and 1977b).

The *Bacillus thuringiensis* group of bacteria is composed of aerobic, gram-positive endospore-forming rods. Taxonomically, *B. thuringiensis* is closely related to *B. cereus* except that the species *B. thuringiensis* is characterised by the production of a crystalline protein body (*B. thuringiensis* delta-endotoxin or crystalline parasporal body) in the cell during the phase of spore formation. This protein body stains like other cell material and it is formed outside the exosporium and separates readily from the liberated spores.

Together with a few biochemical characteristics, sub-specific division of *B. thuringiensis* is based on the flagellar antigens of the vegetative cell (H-antigens), the antigenic composition of the delta-endotoxin, esterase types and production of "toxins" other than the parasporal crystal.

In *B. thuringiensis* strains, as many as seven different toxic components produced during the growth cycle can be identified, one of which, the beta-exotoxin

is heat-stable, acts as an Adenosine tri-phosphate (ATP) analog and has some toxic effects on birds and mammals as well as on insects (WHO, 1979).

Until recently, 13 serotypes of *B. thuringiensis* had been identified, all showing a high pathogenicity for larvae of Lepidoptera. Only two strains, belonging respectively to the serotype 1 (*thuringiensis*) and the serotype 3a, 3b(*kurstaki*), exhibited some effectiveness against young mosquito larvae of most susceptible species *Aedes aegypti, A. triseriatus* and *Culex tarsalis*. Preparations from other strains had pathogenicity for mosquitoes but at concentrations of about 100mg/l, which is too high to be operationally useful (Hall *et al*, 1977; 1979 & Panbangred *et al* , 1979).

In contrast to this pattern, *Bacillus thuringiensis* serotype H-14 does not demonstrate useful activity against the larval stage of Lepidoptera but is highly pathogenic to mosquito larvae (de Barjac, 1978 a, b, c). The serotype H-14 has all the biochemical and morphological characteristics common to all strains of *Bacillus thuringiensis;* biotests on the Lepidoptera *Anagasta kuhniella* have shown that it does not produce the heat stable beta-exotoxin. *Bacillus thuringiensis* serotype H-14 produces unusual protein crystals of all shapes and sizes whilst other strains of the bacterium typically produce diamond-shaped protein crystals (de Barjac, 1978a). The larvicidal activity of *Bacillus thuringiensis* serotype H-14 is associated with the proteic delta-endotoxin in these crystals from which it can be extracted using dilute alkali solution. This is also the case of the delta-endotoxins produced by the other strains of *Bacillus thuringiensis*. The amino acid content of the delta-

endotoxin produced by serotype H-14 is similar to those of the toxins produced by other strains of the bacterium.

The active agent can be fully extracted using chloroform and partially using petroleum ether with minute amounts being extracted into acetone and methanol. The partial heat stability indicates that the substance may have a low molecular weight. Some strains also produce a water-soluble adenine nucleotide termed *thuringiensis* or beta-exotoxin, which has been fully characterised and synthesised (WHO, 1979). This beta-exotoxin has some toxic effects on birds and mammals, as well as, on a broad-spectrum effect on invertebrates when ingested. Therefore, in North America and Western Europe, commercial formulations of *B. thuringiensis* insecticides are exclusively based on strains, which do not produce this beta-exotoxin (WHO, 1979).

Bacillus thuringiensis H-14, being a biological control agent which is environmentally friendly, biodegradable, highly selective for Diptera, and thus, very safe for non-target fauna and above all for the fact that no resistance has yet been observed is the reason that it has attracted a lot of attention. Conservative estimate suggests that over 500 tons have been used without any visible environmental harm. No harmful effects have been recorded in safety tests with bees, vertebrates, mammals and man. Most beneficial insects are unharmed even at enormous doses (WHO, 1979).

1.2.8.1 Mode of Action of *Bacillus thuringiensis* endotoxins

The bacterium is rarely able to spread among pest populations but persists on foliage for periods varying from three days to six weeks depending on conditions. The crystals are insoluble in water and so the spores and crystals constitute a particulate insecticide, formulated usually as a wettable powder or emulsion. This microbial insecticide is comparable to a stomach poison and has no contact action.

During sporulation, the bacterium synthesises parasporal inclusions containing one or more delta-endotoxins, classified as Cry proteins (Hoefte and Whiteley, 1989). In these inclusions, the toxins exist as inactive protoxins. When the inclusions are ingested, by insect larvae, they dissolve in the mid-gut and the protoxin is converted to the active form by 'trimming' with gut proteases. These toxins diffuse through the peritrophic membrane and bind to specific receptors on the brush-border membrane of mid-gut columnar cells. These create leakage pores that cause cell swelling, cell lysis, disruption of gut integrity and eventually death of insect (Ellar, 1998). There is an irreversible step that follows binding of toxin to the brush-border membrane and it is thought to be associated with the insertion of all or part of the toxin into the membrane. This insertion of toxin molecules into the membrane is believed to be necessary for the formation of a pore that mediates the potentially lethal increase in membrane permeability (Knowles and Dow, 1993).

Usually, the insect dies from within a few hours to about three weeks, depending on the dose ingested and the type of pathogenic action induced (WHO, 1979). The concentration and affinity of toxin binding sites on the mid-gut brush-

border membrane often, but not always, correlate positively with the susceptibility of a larva to a toxin (Wolfersberger, 1990).

Due to their high specificity for these unique receptors on the membrane of the gut epithelial cells, the delta-endotoxins are harmless to non-target insects. The fact that they are proteins means that they can be biodegraded readily.

The most conspicuous feature of the alimentary canal in the blackfly is its very long, tubular and capacious mid-gut where all the digestion takes place. Ingested material is packed into the gut more or less continuously and can often be seen through the semi-translucent body wall as an almost solid column filling the length of the gut from which waste is regularly voided through the anus.

The mid-gut which is functionally like that of other aquatic dipterous detrivores has a digestive enzyme system and high alkalinity that enable the larvae to exploit algal food better than vascular plant derivatives (Martin *et al*, 1985). In the fore- and hindguts, the hydrogen ion concentration is at about pH 10.0-11.4 (Lacey & Federici, 1979; Undeen, 1979). This very high alkalinity has practical value since it is the major reason why *B. thuringiensis* crystalline toxin can be used for blackfly control since the insecticide is activated by the high gut alkalinity when ingested by the filtering larvae.

1.2.8.2 Commercial production of *Bacillus thuringiensis* (H-14) formulations

Bacillus thunngiensis serotype H-14 is produced by submerged liquid fermentation. It can be grown on nutrient agar media that is usually used for the commercial production of other strains of the bacterium (Goldberg *et al*., 1977,

Goldberg and Margalit, 1977a and 1977b). It can also be cultivated on a sporulation medium containing yeast autosylate and also on medium, which has casaminoacids and sodium citrate (Vankova *et al*, 1978). *Bacillus thuringiensis* serotype H-14 used for the preparation of the standard reference formulation " IPS-78" is cultivated on a medium which contains wheat flour (15g), glucose (10g), peptone (5g), yeast extract (5g), $KH_2PO_4.7H_2O$ (0.1g), $MgSO_4.7H_2O$ (0.5g), NaCl (3g), $FeSO_4$ (0.1g) and water at 30^0C for 35 to 40 hours. The spore-crystal is then precipitated with acetone and collected by centrifugation (de Barjac, 1978). *Bacillus thuringiensis* formulations are produced commercially as either wettable dispersable powders, liquid concentrates or as granules.

The institutions or companies that have sent primary powder samples to WHO to date are: Abbott Laboratories, USA; All-Union Scientific Research Institute of Agricultural Microbiology, Leningrad-Pushkin , USSR; All-Union Scientific Research Institute on Bacterial Preparations, Moscow D-242, USSR; Institut Pasteur, Paris, France; Roger Bellon Laboratories, Monts, France and Sandoz Laboratories, Homestead, USA.

CHAPTER TWO

MATERIALS AND METHODS

2.1 The Study Area

The field station where the assays were carried out is situated on the west bank of the River Pra at Daboase in the Western Region, Ghana.

Daboase town (50° 4"N, 1° 39"W) lies in the tropical rainforest belt of South-western Ghana. There are two main seasons of wet and dry. The rainy season is usually from April to October and the dry season from November to March; the area receives about 1000 – 1500mm of rain annually. The mean ambient temperature varies between 25^{0}C and 30^{0}C with extremes of 21^{0}C and 38^{0}C.

Thick canopies of trees at some places fringe the course of the river, but at some points, the riverbanks are denuded of vegetation and are exposed totally to the direct rays of the sun. The river is perennial, but the volume and flow rates vary and tend to be seasonal, being higher during rains.

2.2 Biological Material

2.2.1 Formulations of *Bacillus thuringiensis* (serotype H-14)

The *B. thuringiensis* H-14 insecticides used for the study were manufactured and supplied by Abbott Laboratories, USA as VectoBac®. They were usually delivered in 5-litre containers in batches. However, since the potency of the formulations in each batch varies, the data of only one formulation, which was designated as a standard/reference by the manufacturers was subjected to analyses for this study.

2.2.2. Field collection of *Simulium damnosum* sensu lato.

Simulium damnosum s. l., larvae were collected from the complex breeding sites on the River Pra at Bosomase, located 5km upstream of Daboase (Figure 3). They were then transported under cool conditions in iceboxes to the field station, taking care that the humidity inside the box was high and that the larvae did not suffer from excessive heat stress and dehydration. Back at the field station, the larvae were carefully detached from their natural supports and placed in the gutters of the assay system (see section 2.3.1) where they re-attach and were left undisturbed for at least 12 hours (usually overnight) before the assay.

Fig. 3 Field collection of *Simulium damnosum* species complex larvae

2.3 Field Bioassays of *Bacillus thuringiensis* (H-14) Formulations

The assays of the Bt H-14 formulations were carried out using the mini-gutter system.

2.3.1 The mini-gutter system

The mini-gutter is an open circuit system used in the OCP for assaying both synthetic insecticides and formulations of *B. thuringiensis* H-14 (Figure 4). It was developed by Guillet and Escaffre in 1979 mainly for testing suspension concentrates such as *Bacillus thuringiensis* and some water-based flowables.

It consists of series of eight metal gutters (or troughs) each measuring 1.5m long by 7cm wide and 3cm deep and painted white. The troughs are mounted in parallel on a metal frame that is provided with metal legs each about one metre high. The assembly of troughs is slightly inclined towards the lower (open) ends of the gutters so that water flows downstream. A rectangular collector tank of about 30 litres in capacity is mounted on metal legs at the upper end of the assembly of gutters. The tank has at the base of its broadside eight individual taps and eight nozzles, which open into the gutters such that each gutter receives water from a tap and a nozzle. The taps are used so that the rate of flow of water in each gutter could be regulated. Water from the river is pumped into the collector tank through flexible PVC pipes using a motorised pump and an in-built float, which acts as a constant pressure head that prevents overflow.

Fig. 4 Mini-gutter system for testing *Bacillus thuringiensis* H-14 formulations

A 1.5 litre plastic bottle, cut off at its base and fitted at its mouth with a rubber tubing 8mm in diameter, is supported upside down by mounted frames attached to the collector tank. The tip of the rubber tubing is fitted with a nozzle and this served as the apparatus for consistent delivery of the insecticide solution into each gutter. A series of baffles built near the upper end of the gutters ensures that the solutions of insecticides flowing in the gutter are thoroughly mixed during treatment.

2.3.2 Method of testing *Bacillus thuringiensis* (H-14) formulations

Before each treatment, test solutions were freshly prepared by pipetting 10ml of the concentrate sample and making it up to one litre with river water (solution A). Then 100ml of this solution A was pipetted into fresh plastic bottle containing 900ml of river water (solution B). It is from this solution B that exact volumes, which gave the desired concentrations, were introduced into each gutter. The volume of solution B needed to arrive at the desired concentration was determined using the following equation:

$$V = \frac{Q \times C \times 100}{10 \times A}$$

where V = Volume of solution B required;

Q = Discharge rate in litre per 10 sec;

C = Concentration desired in mg/l/10sec.

A = Concentration of solution B in mg/l;

The concentration of solution B, indicated as A in the equation is deduced from the basis that the stock is 1000mg/l thus solution B is invariably always 1 0mg/l (0.001%). An initial trial run with water was always carried out to arrive at a complete discharge in exactly 10 minutes (dispensing rate) by adjusting the nozzles of the insecticide receptacles. From this, the volume of test solution needed in each receptacle was then determined.

For each assay, the following insecticide dosage rates (mg/l/10sec); of 480, 360, 240, 120, 60, 30, and 15 were tested and each assay included a standard and

a control, which had no insecticide. After the application of the insecticides, the larvae detaching from the gutter (dead or moribund) were caught in nets attached to the lower end of the gutters. Six hours after dosing, dead and living larvae were collected separately into tubes containing 70% alcohol. The assay for each VectoBac® formulation was replicated 3-4 times.

The numbers obtained were first counted in the field and recorded; then back at base in the laboratory, they were sorted out again this time to exclude very young larvae (first to fourth instars). The corrected numbers were then used to calculate the lethal concentration using probit analysis (Finney, 1971).

For the present study, however, only the bioassay results using the standard formulation of VectoBac®, which was always run alongside several other batches, were analysed. The standard was chosen for analysis because the results would give an insight into its performance under varying or fluctuating environmental and physico-chemical conditions that occurred during the study period.

The median lethal concentration value or the dosage that kills 50% of insects exposed to it (LC50) determined the performance. The LC_{50} is often used as a single index to summarise susceptibility, because in theory, it can be estimated with greater precision than the lethal concentrations of either end of the range (Finney, 1971).

2.3.3 Analysis of insecticide test data

The principle and procedure leading to the estimation of the lethal concentrations involve exposing batches of insects for a specified time to come into contact with different concentrations of the insecticide or place the larvae for a specified time, in water to which are added different concentrations of the insecticide.

The tests are usually carried out using four insecticide concentrations, one of, which should give a complete kill in the preliminary test, and at least one that should result in less than 50% mortality. Each test is repeated four times at each selected concentration.

The observed mortality rate for each concentration in an assay is then corrected for the natural mortality rate, that is, the control batch of unexposed insects. If the mortality rates in the control are higher than 5%, the assay may be disregarded, but above 20% certainly invalidates the test. The corrected mortality rate is determined using Abbott's formula (WHO, 1963), which is given as follows:

$$\text{Corrected \%mortality} = \frac{\text{Observed \%mortality} - \text{Control \%mortality}}{100 - \text{Control \%mortality}} \times 100$$

Thus, applying the formula to an observed mortality rate of 13.6% at the concentration of 0.5% DDT and a control mortality rate of 9.3%, the corrected mortality rate then is:

$$\frac{13.6 - 9.3}{100 - 9.3} \times 100 = 4.7\%$$

The present study used a macro program written in Lotus statistical software for desktop computers that was developed in the OCP The principles for estimating the LC's are the same, but the program also computes chi-square and produces a plot, which shows the variability of the results obtained. The data of the measured physico-chemical parameters can also be fed into the programme therefore a typical output includes also all the information on the water conditions of each test.

2.4 Studies on River Water Conditions Influencing the performance of VectoBac®

2.4.1 Measurement of physico-chemical parameters of river water

The river water temperature, pH, conductivity and turbidity were measured each time an assay was carried out. In addition to these, water samples were collected for chemical analysis. However, due to logistic constraints, the analyses could not be carried out for each assay. For this, representative water samples collected during the wet and dry seasons were studied.

The hydrogen ion concentration or pH was measured in the field using a GALLENKAMP 640-model pH-meter and the conductivity was measured using a JENWAY 4020 Conductivity meter. The turbidity was measured using turbidimeter (DRT model 100B).

For the chemical analysis, the water samples were collected into 1.5 litre plastic containers, transported, kept in the dark in the laboratory until ready to use. For this analysis, the following parameters were measured; alkalinity, hardness of water, cation and anion concentrations, heavy metals and dissolved solids.

50ml of the water sample was titrated with 0.02M sulphuric acid (H_2SO_4), using Methyl orange as indicator when the pH was 8.3 or above and phenolphthalein when the pH was 4.5 and below and then the formula below was applied to determine alkalinity:

$$\text{Alkalinity} = \frac{\text{Average Titre Value}}{\text{Sample Volume}} \times 1000 \text{ mg/litre}$$

Where, average titre value = Average volume of acid

The total hardness was determined by titrating 50ml of the water sample with 0.01M EDTA solution, using Murexide indicator and applying the formula;

$$\text{Total Hardness} = \frac{\text{Average Titre value}}{\text{Sample Volume}} \times 1000 \text{mg/litre}$$

The hardness due to Calcium was also determined by titrating 50ml of the water with 0.5M EDTA using Eriochrome black T indicator and then applying the formula;

$$Ca^{2+} = \frac{400.8 \times \text{Average titre value}}{\text{Sample volume}} \times 1000 \text{mg/litre}$$

Hardness due to Magnesium was, however, determined by deducting the value of hardness due to calcium from that of total hardness.

In determining the chloride content of the samples, 50ml of the water was titrated against Silver nitrate solution using Potassium chromate as indicator and then applying the formula;

$$Cl^- = \frac{\text{Average Titre Value} - \text{Blank titre} \times 1000}{\text{Sample Volume}}$$

The concentrations of Ca^{2+}, HCO_3, Mg^{2+} and Non CO_3 Hardness were all determined as follows:

Ca^{2+} Hardness = Ca^{2+} /0.4 mg/litre

HCO_3 Hardness = Alkalinity in mg/litre x 1.22

Mg^{2+} Hardness = Total Hardness – Calcium Hardness

Non CO_3 Hardness = Total Hardness – CO_3 Hardness

To determine the amount of total solids present in each sample, 100ml of the water sample was pipetted into a pre-weighed dish and mixed thoroughly using a magnetic stirrer. It was then evaporated to dryness on a steam water bath. The dry evaporated sample was then placed in an oven at 103-105°C for one hour. It was subsequently allowed to cool in a desiccator and then weighed. The values were then used into the formula:

$$\text{Total Solids} = \frac{\text{Residue (dried)} \times 1000}{\text{Sample Volume}}$$

For total dissolved solids, 100ml of the water sample was pipetted and stirred using a magnetic stirrer and poured onto a glass-fibre filter with applied vacuum. There were three successive washings using 10ml of reagent grade water while allowing complete drainage between washings and the suction continued for three minutes after filtration was complete.

The filtrate (with several washings) was transferred to a pre-weighed evaporating dish and dried on a steam bath. The dried sample was then placed in the oven for one hour and then cooled in a desiccator and weighed. The values were then used in the formula;

$$\text{Total dissolved Solids} = \frac{1000 \times \text{Residue} \times 1000}{\text{Sample Volume}}$$ (APHA, 1998).

Sodium, potassium, manganese, nitrate, silicate and iron contents of the water samples were determined using the UV SPECTROPHOTOMETER (PU. 8625/UV/VIS).

The trace elements were determined using the AA SPECTROMETER Model UNICAM 969 and the dry weights (mass) of dissolved solids were determined using a balance (METTLER AE 200).

2.4.2 Statistical Analytical Methods

2.4.2.1 Multiple regression analysis

Multiple regression analysis was used to identify and select the parameters that affected the potency of the standard formulation of VectoBac ®.

Multiple regression analysis is a very powerful tool for investigating relationships. The principle involves testing the significance of particular regression coefficients and then applying logical approach to selecting the best set of independent variables, where best is to be interpreted as including all variables which really affect the dependent variable and only those remain the same (Kirkwood, 1988). Thus, for three variables, x_1, x_2 and x_3 the significance of the effect of x_1 is tested by comparing the residual sum of squares for the regression on all three variables and for the regression on x_2 and x_3 only. The difference between the residual sums of squares is compared with the residual mean square for the full model and the F-test is used.

In order to arrive at a good model, three methods are usually employed for this analysis. These are; forward entry, stepwise selection and backward elimination. The forward entry method selects the parameter with the highest level of significance first before any other. The stepwise selection method enters the parameters in their order of significance with the parameter having the highest level of significance being entered first before any other. The backward elimination method removes the parameter with the least level of significance completely out of the model and does not consider it at all in any computations.

Thus, for this study, multiple regression was used as an exploratory tool because one of the end points of the analysis is the identification of 'good' predictor variables. The dependent variable in each case was the LC_{50} values and the independent variables were pH, conductivity, turbidity and river temperature. The criteria for selection was set at probability of F- to – enter value, as either equal to or less than 0.05 and for removal at either equal to or greater than 0.1.

2.4.2.2 Linear discriminant function analysis

The main purpose of linear discriminant function analysis (LDF), is to predict group membership from a set of variables and in many cases it is carried to the point of actually putting cases into groups (classification). Basically, a discriminant function score is predicted from the sum of the series of the variables, each weighted by a coefficient. There is one set of discriminant function coefficients for the first discriminant function, a second set of coefficients for the second discriminant function and so forth. Cases get separate discriminant function scores for each discriminant function when their scores on variables are inserted into the equation;

$$D_i = d_{i1}z_1 + d_{i2}z_2 + \ldots\ldots\ldots d_{ip}z_p.$$

Where D is the standardised score on the ith discriminant function, z is the standardised score on each variable and d_{i}, is the discriminant coefficient.

Just as D_i can be calculated for each specimen, a mean value of D_i can be calculated for each group. Thus, members of each group considered together have a mean score on a discriminant function that is the distance of the group in

standard deviation units from the zero mean of the discriminant function. Cases are assigned into groups using the basic classification equation;

$$C_j = c_{jo} + c_{j1}X_1 + c_{j2}X_2 + \ldots\ldots\ldots\ldots c_{jp}X_p$$

Where C_j, is the score on the classification function for the group j, X_1 is the raw score of the variable and c, the associated classification function of the variable, and c_{jo}, a constant. This method then can be used to predict to which subgroup a new individual is likely to belong. Discriminant function analysis can also be performed in a stepwise manner to select the characters most useful in predicting group membership. Statistical criteria are used in the analysis to determine the order of entry of the characters and it enables the researcher to produce a reduced set.

Discriminant function analysis was used to assign into two groups of strong and weak performance of the standard *B. thuringiensis* formulation using the LC_{50} values that were obtained. For the analysis using the LC_{50} values, 50mg/l was chosen as the cut-off point separating the two groups. This value was chosen because 50mg/l was approximately the midpoint of the range obtained which was 107.07mg/l. Linear discriminant function analysis was then applied to the dataset to reveal the variables that best separates the two groups and also for this study, that is, to reveal the variables that would give 100% separation.

2.5 Laboratory Studies of the Effect of Conductivity on Potency of VectoBac®

2.5.1 Laboratory-based assay system for testing VectoBac®.

A simple system capable of rearing *Simulium damnosum* s. l. from the egg stage to normal sized adults (Boakye *et al.*, 2000) was adopted to test the effect of conductivity on the potency of VectoBac®. in the laboratory. The system consists of 1-litre glass beaker containing water, a hollow chamber constructed from two 50ml polypropylene centrifuge tubes fitted together in pairs, through which a rubber tube that is connected at one end to an air stone and the other end to an air pump. When switched on, the air pump generates strong currents and turbulent conditions in the chamber sufficient enough to support *S. damnosum* larvae (Figures 5a and 5b).

2.5.2 Study Design

Before each experiment, the rearing unit was switched on initially to aerate the river water in the beaker for about one hour, a condition, which was found very necessary for the vitality of the larvae. Thereafter, about 40-50 living larvae were introduced into each unit and left for about an hour for them to establish. Those that died or were weak (those which did not respond to vibrations caused in the water) were then removed. The water was drained off and replaced immediately with an equal

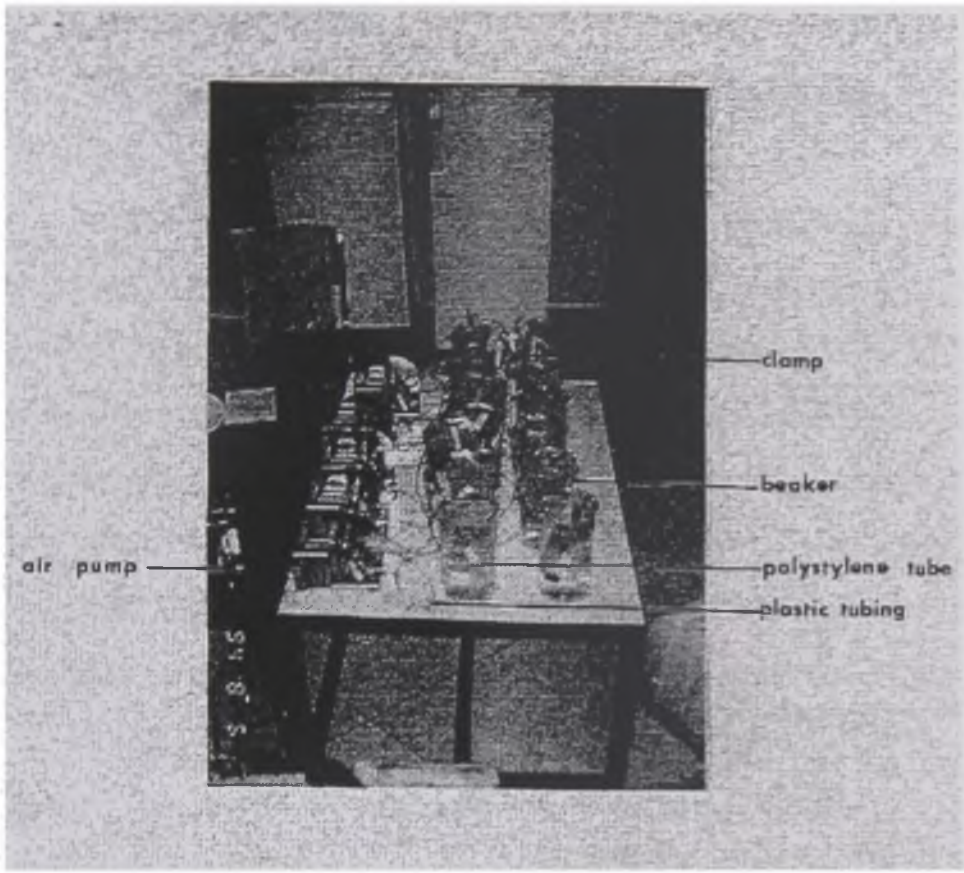


Fig. 5a. A typical setup of rearing units for assaying VectoBac in th laboratory.

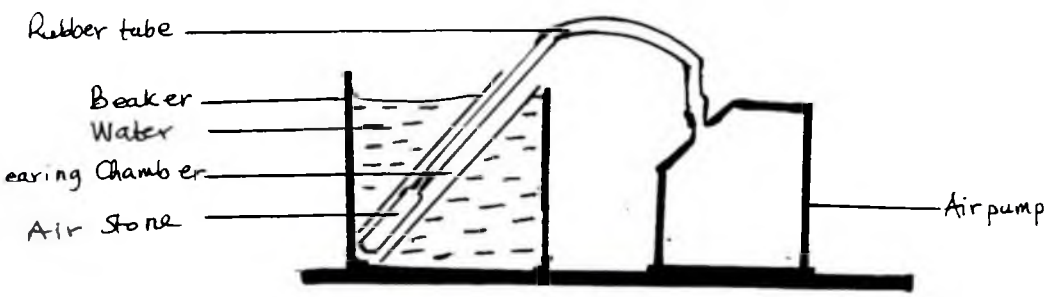


Fig. 5b. Schematic diagram of a rearing unit used for testing VectoBac in the laboratory (after Boakye et al., 2000).

volume of fresh aerated water. The larvae, which remained attached, were left undisturbed for another two hours before the larvicide was introduced.

To test the effect of conductivity of the Bt H-14 formulation, two approaches were taken. One was to directly increase the conductivity of the insecticide solution only before treatment and the other was to increase the conductivity of water medium in the system. The four levels of conductivity used for the experiments in both cases were natural (range 125.1-138.5; mean 133.3μS), 1500μS, 2000μS, and 3000μS. The final concentrations corresponding to 0.05mg/l, 0.1mg/l, 0.2mg/l, 0.4mg/l and 0.8mg/l of VectoBac® were obtained in the chamber by adding 50μl, 100μl, 200μl, 400μl and 800μl, respectively, of freshly prepared 10% solution.

For each bioassay three sets of experiments were conducted. The first experiment tested the bio-insecticide under normal conditions, that is, the conductivities of water and the insecticide solution were not altered. This experiment also served as the control for the rest of the two experiments. In addition, there was always a control that had no insecticide.

The second experiment tested the effect of increased conductivity of the test water only with that of the insecticide remaining unaltered. This was achieved by adding 5M Sodium chloride solution (NaCl) into each beaker until the desired conductivity was reached. The experiment in addition had two in-built controls with no insecticides, one unit, which had the natural conductivity of the water and the other with, the water conductivity raised to the desired test level. Thus, for each of these experiments, seven test units were used, which corresponded with the two

controls mentioned above and the five different final concentrations of the insecticide.

For the third set of experiment, the natural conductivity of the river water was maintained, whilst that of the 10% VectoBac® solution was raised to the desired level by adding 5M NaCl solution. After altering the conductivity, the insecticide was left standing with frequent mixing at intervals for ten minutes before it was introduced into the test chamber. A control was set up for these experiments, which had no insecticide. Thus, for each reaction condition, at least six test units were set up; the control, 0.05mg/l, 0.1mg/l, 0.2mg/l, 0.4mg/l and 0.8mg/l of the insecticide.

Ten minutes after applying the insecticide, the water in the chamber was discarded and immediately replaced with fresh, aerated water and the system stopped after six hours. The water in each test unit was drained and the larvae transferred into separate white trays, which contained aerated water. From these trays, the larvae were sorted out as either dead (usually detached), alive or were too small in size. The data was then used to calculate the lethal concentration values.

All three experiments for each of the different conductivity levels were carried out at the same time and each which in turn was replicated five times.

CHAPTER THREE

RESULTS

3.1 The Performance of the Standard VectoBac® Formulation

The standard VectoBac® formulation (#12AS/26-398-N9) was tested alongside 45 others that were evaluated in the field during the study period which lasted 15 months (February 1997- April 1998). Over 1,000 bioassays were conducted and of these a total of 130 were those of the standard that was chosen for the present study. The concentrations used in the bioassays were 480mg/l, 360mg/l, 240mg/l, 120mg/l, 60mg/l, 30mg/l and 15mg/l of the insecticide. In some cases, which depended on the availability of *S. damnosum* larvae, not all the concentrations listed above were tested.

The detailed results of lethal concentration values (LC_{50} LC_{95} and LC_{99}) of the standard formulation obtained during the study period are shown in Appendix I, and a typical output of the computer program used for calculating LC values is shown in Figure 6.

The results obtained showed that the performance of the formulations varied during the study period (Table 1). The mean LC_{50} obtained was 69.04mg/l (range = 107.07mg/l; median = 71.92mg/l). The mean LC_{95} was 284.97mg/l (range = 521.85mg/l; median = 266mg/l) and the mean LC_{99} was 517.05mg/l (range = 107.07mg/l; median =454.4mg/l) respectively. Except for the LC_{95} ($P > 0.05$), the observed variations in performance were significant when tested using non-parametric statistical methods.

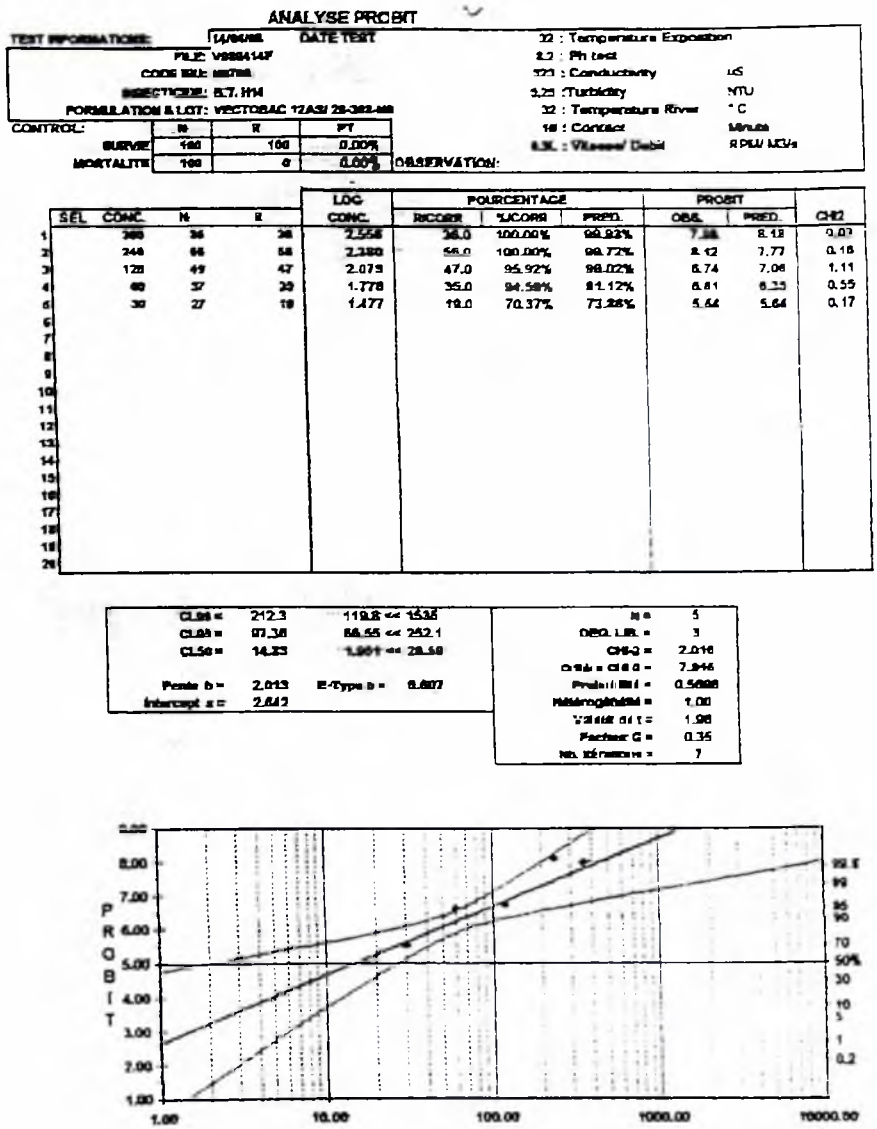


Fig. 6. A typical output of the lotus software program used for the computation of LC values of VectoBac® from bioassay data.

TABLE: 1
Summary of LC values of the standard VectoBac obtained during the study period. The Kolmogorov-Smirnov Z-test was used to test the significance in the variation observed.

Lethal Concentration	Mean	Median	Standard Error	Min - Max (Range)	Significance of variation
LC_{50}	69.04	71.92	2.96	14.83-121.9 (107.07)	P = 0.032 Z = 1.44
LC_{95}	284.97	266.0	12.76	66.55-588.4 (521.85)	P = 0.238 Z = 1.03
LC_{99}	517.05	454.40	24.12	101.3-1130 (1028.7)	P = 0.029 Z = 1.46

TABLE: 2
Summary of river water condition data obtained during the study period. The Kolmogorov-Smirnov Z-test was used to test the significance in the variation observed.

Water condition	Mean	Median	Standard Deviation	Min - Max (Range)	Significance of variation
River Temperature (°C)	29	29	1.77	25-33 (8)	P = 0.000 Z = 2.79
PH	8.2	8.2	23.21	8.1-8.6 (0.50)	P = 0.000 Z = 3.75
Conductivity (µS)	396.8	156	565.13	114-1999 (1885)	P = 0.000 Z = 4.54
Turbidity (NTU)	23.9	15	0.14	4.18-73 (68.82)	P = 0.000 Z = 3.80

The best performances were recorded on two occasions. the first in February 1997 and the other in April 1998. The LC_{50} values for the tests carried out on these occasions were 24.16mg/l and 14.83mg/l respectively. The estimated LC_{90} values were 66.55mg/l and 97.36mg/l and those for LC_{99} were 101.3mg/l and 212.3mg/l respectively. The worst performance was recorded in April 1997 and the estimated LC_{50}, LC_{95} and LC_{99} values were 121.9mg/l, 588.4mg/l and 1130mg/l respectively.

3.2 The River Conditions of Temperature, Conductivity, pH and Turbidity

The results obtained for the temperature, conductivity, pH and turbidity are summarized in Table 2.

3.2.1 River temperature

The temperature of the river water recorded was found to vary over the study period and it ranged from 25°C recorded in August 1996 to 33°C recorded in April 1998, with a mean value of 29°C (range = 8°C; median = 29°C). The observed variation within this temperature range was found to be significant (Kolmogorov-Smirnov Z Test, P = 0.000).

3.2.2 Conductivity

The conductivity of the river water ranged from a low of 114μS, which was recorded in May 1997 to a high of 1999μS, during February 1998 The mean water conductivity was 396.8μS (range = 1885μS; median =156μS) and the observed variation was also highly significant (Kolmogorov-Smirnov Z Test, P = 0 000).

TABLE 3: Dry and wet season data of mean values of physico-chemical parameters of the River Pra at Bosomase (1997-1998)

Water Condition	Parameter	Dry season	Wet season
Alkalinity	Alkalinity	61.79 mg/l	62.40 mg/l
Hardness	Ca^{2+} hardness	38.76 mg/l	40.71 mg/l
	Mg^{2+} hardness	19.26 mg/l	18.17 mg/l
	Total hardness	56.45 mg/l	57.03 mg/l
Solids	Suspended solids	27.30 mg/l	24.36 mg/l
	Dissolved solids	104.73 mg/l	164.36 mg/l
	Total solids	132.03 mg/l	188.73 mg/l
Cations	Ca^{2+}	18.60 mg/l	16.07 mg/l
	Na^{+}	275.78 mg/l	8.65 mg/l
	K^{+}	18.40 mg/l	6.18 mg/l
	Mg^{2+}	2.60 mg/l	4.12 mg/l
	Mn^{2+}	0.03 mg/l	0.03 mg/l
	Fe^{2+}	2.98 mg/l	2.92 mg/l
	NH_4^{+}	0.19 mg/l	0.44 mg/l
Anions	SiO_2	1.77 mg/l	1.32 mg/l
	Cl^{-}	9.36 mg/l	6.9 mg/l
	SO4-	1.33 mg/l	1.19 mg/l
	HCO_3^{-}	63.16 mg/l	74.30 mg/l
	F^{-}	4.23 mg/l	4.46 mg/l
	NO_3^{-}	0.32 mg/l	0.24 mg/l
	NO_2^{-}	0.07 mg/l	0.04 mg/l
	PO_4^{-}	0.22 mg/l	0.15 mg/l
Heavy metals	Cadmium	<0.03 mg/l	<0.03 mg/l
	Lead	<0.03 mg/l	<0.03 mg/l
	Zinc	<0.03 mg/l	<0.03 mg/l
	Nickel	<0.03 mg/l	<0.03 mg/l
	Chromium	<0.03 mg/l	<0.03 mg/l

3.2.3 pH (Hydrogen-ion concentration)

The pH of the river water ranged from 8.1 to 8.6 over the study period. The mean pH was 8.2 (median = pH 8.2) and the variation, which existed within the range, was also highly significant (Kolmogorov-Smirnov Z Test, P = 0.000).

3.2.4 Turbidity

The turbidity of the river water ranged from 4.18NTU recorded in April 1998 to 73NTU during September 1997. The mean turbidity value was 23.9NTU (median = 15NTU) and the variation, which existed within the range, was also significant (Kolmogorov-Smirnov Z Test, P = 0.000).

3.3 Chemical Analysis of River Water Samples

A total of six water samples collected in the wet and dry seasons were analysed and the detailed results are shown in APPENDIX II. Table 3 lists the mean values of the parameters that were measured and compares the dry and wet season data.

It was observed that, generally, with the exception of total solids and cations, all the other parameters, namely, the alkalinity, water hardness, heavy metals and anion contents did not differ appreciably between the wet season and dry season samples.

The mean alkalinity values as determined by $CaCO_3$ concentrations for the dry and wet seasons were 61.8 mg/l and 62.4mg/l respectively.

The total water hardness which is basically due to both Calcium and Magnesium ions, for the dry and wet season samples were 56.5mg/l and 57.0mg/l, respectively.

The heavy metals, Cadmium, Lead, Zinc, Nickel and Chromium were all present in traces (i.e. less than 0.03mg/l) in all the samples that were analysed. Mercury, as a heavy metal, was not studied because the requisite reagents were not available.

Among the anions that were studied, the mean Chloride (Cl^-) concentration was 9.4mg/l for the dry season samples which was relatively higher than the 6.9mg/l obtained for those of the wet season. Bicarbonate (HCO_3^-) levels were relatively higher in the wet season samples with a mean value of 74.3mg/l than in

those of the dry season, which was 63.2mg/l. The rest did not differ appreciably. The mean silicate (SO_4^-) concentrations recorded in the wet and dry season samples were 1.3mg/l and 1.2mg/l respectively. The mean concentration of nitrate (NO_3^-) in the dry season samples was 0.32mg/l and 0.24 mg/l for those of the wet season. The nitrite (NO_2^-) levels for the dry and wet season samples were 0.07mg/l and 0.04mg/l respectively. The average phosphate ($P0_4^-$) levels for the dry and wet season samples were 0.22mg/l and 0.15mg/l respectively. The average levels of fluoride (F^-) for the dry and wet season samples were 4.23mg/l and 4.46mg/l respectively.

Dissolved solids were found to be largely responsible for the significantly different total solids concentrations observed between the dry and wet season samples. The average dissolved solids for the dry and wet season samples were 104.73mg/l and 164.36mg/l respectively whilst those of suspended solids were 27.3 and 24.36mg/l respectively. Thus, the average of total solids contents for the dry and wet season samples were 132.03mg/l and 188.72mg/l respectively.

Among the cations that were studied, Sodium (Na^+), Calcium (Ca^{2+}) and Potassium (K^+) were those found to be present in appreciable quantities in all the samples. The largest difference between the dry and wet season samples was observed in Na^+ levels, which were 275.8mg/l in the dry and 8.65mg/l in the wet season samples. The average K^+ level was 18.4mg/l in the dry season samples, which was about three times higher than the 6.18mg/l recorded in those collected in the wet season. The mean Ca^{2+} level was 18.6mg/l for the dry season and 16.07mg/l for the wet season samples. The average Magnesium ion (Mg^{2+})

concentration was 2.6mg/l for the dry season and 4.12mg/l for the wet season samples. The average Iron (Fe^{2+}) concentration was about the same. it was 2.98mg/l for the dry season and 2.92mg/l for the wet season samples. The average Ammonium ion (NH_4^+) concentration was 0.19mg/l for the dry season and 0.44mg/l for the wet season samples. Manganese (Mn^{2+}) was present in traces (less than 0.03mg/l) in all the samples that were analysed.

To summarise, large significant differences were found between four parameters when the data of the dry and wet season samples were analysed. These were the total solids content, which was high in the wet season (188.7mg/l), compared to 132mg/l for the dry season. Sodium, Potassium and Chloride ions were higher (27.6mg/l, 18.4mg/l and 9.4mg/l) in the dry season, than in the wet season (8.7mg/l, 6.18mg/l and 6.9mg/l).

3.4 The Identification of River Water Conditions Influencing the Performance of VectoBac®

3.4.1 Univariate statistical method

An exploratory analysis of the relationship and trends existing between each water parameter and performance (LC_{50}) of the VectoBac® was carried out using graphical methods and the results are shown in Figures 7-10 and Table 5.

The analysis revealed that high potency, that is, low LC_{50} values were observed when river water temperatures were from 30°C and above (Figure 7). Temperatures of 29°C and below were observed to coincide with the point when LC_{50s} were above 42.92mg/l. Similarly, high conductivity values were associated with good performance of the standard VectoBac® (Figure 8). Here also, LC_{50} values below 42.92mg/l were associated with conductivity levels above 133µS whilst higher LC_{50} values were consistently below this threshold. Low turbidity was also found to be associated with good performances of the standard VectoBac® (Figure 9), also revealing that the lowest turbidity levels (below 4.18NTU) were consistently associated with good performance of the LC_{50} being below 42.92mg/l. No relationship, however, could be discerned when the pH and the performance data were similarly analysed (Figure10).

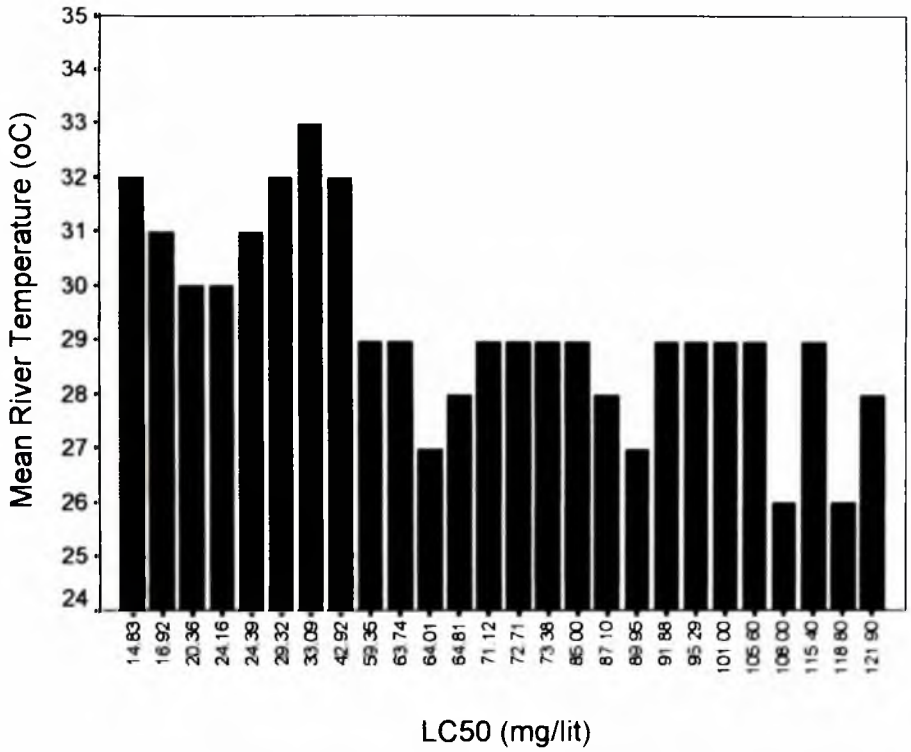


Fig. 7

Graph showing the relationship between mean river temperature and LC50 values obtained during the study period

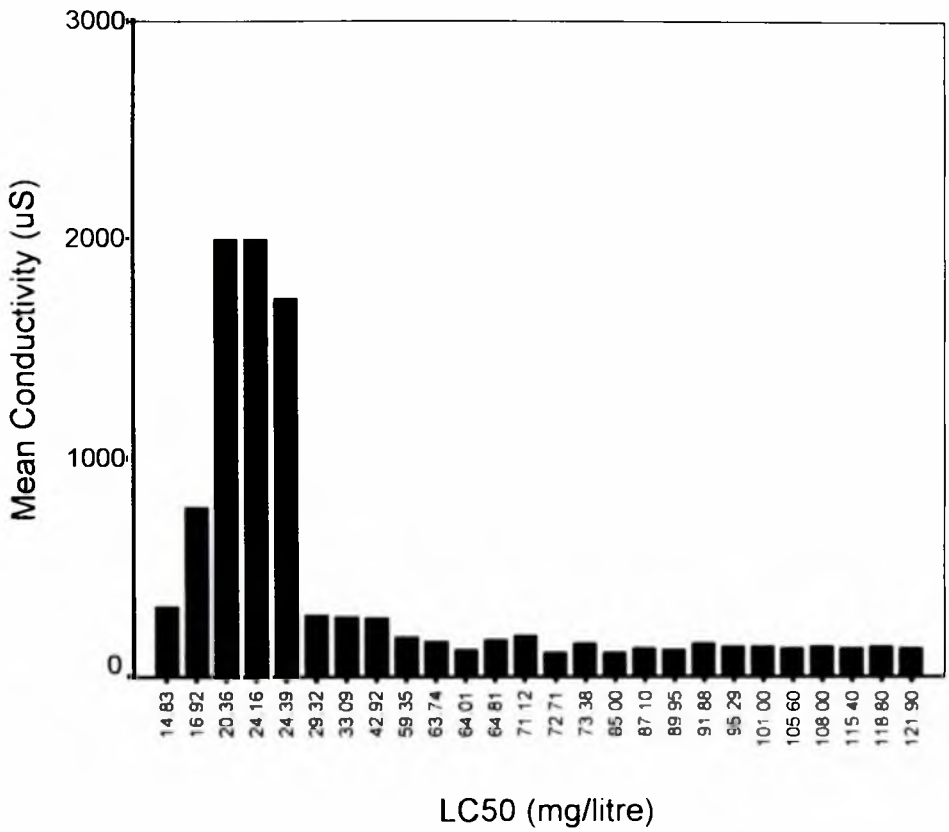


Fig. 8

Graph showing the relationship between mean conductivity and LC50 values obtained during the study period.

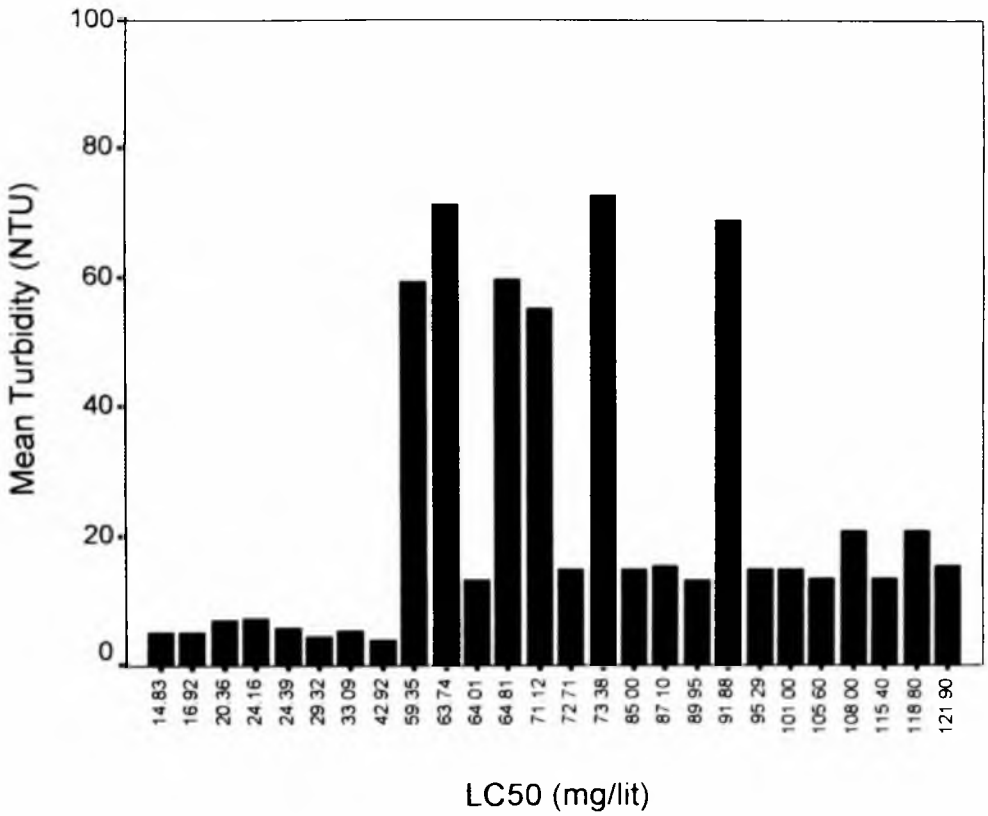


Fig. 9

Graph showing the relationship between mean turbidity and LC50 values obtained during the period of study

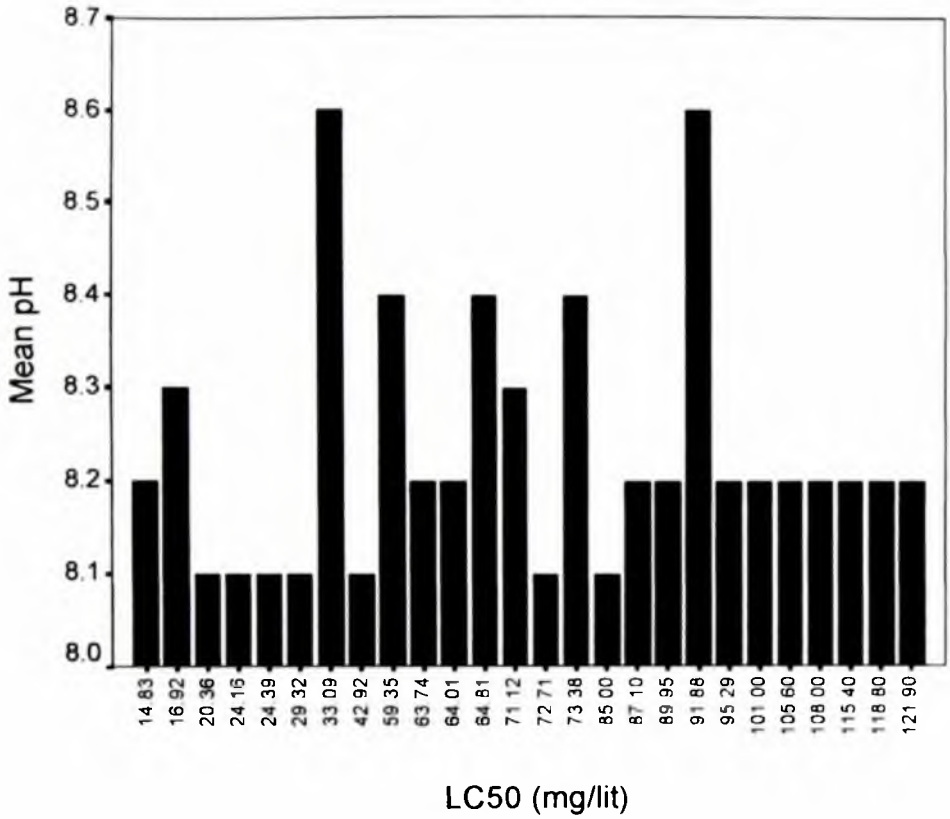


Fig.10

Graph showing the relationship between mean pH and LC50 values the study period.

3.4.2 Multivariate Statistical Methods

3.4.2.1 Multiple regression analysis

The details of results obtained when the dataset was subjected to multiple regression analysis are shown in Table 6. All the three models of multiple regression that were applied first selected river temperature then conductivity and turbidity in that order. In all cases, the pH was excluded in the analysis.

When correlation analyses were carried out, it revealed that LC values were negatively correlated to river temperature (Pearson correlation R = -0.752; significant P= 0.000) and to conductivity (Pearson correlation R = -0.617; significant P= 0.000), but positively correlated to turbidity (Pearson correlation R = 0.213; significant P= 0.008). Although the correlation with pH was positive this was not significant (Pearson correlation R = 0.052; significant P= 0.279)

3.4.2.2 Linear discriminant function analysis (LDF)

Using 50mg/l as the cut-off LC_{50} value, 90 cases out of 130 fell above this limit and were assigned to the weak performance group and the rest to the strong group. A 100% correct classification of cases was achieved using river temperature, conductivity and turbidity with the stepwise LDF selection criteria set at F to Enter = 3.84 and F to remove = 2.71 (Table 4). The derived classification functions obtained that can be used to classify new products as either weak or strong are listed in Table 5

TABLE: 4
Summary result of stepwise discriminant function analysis performed to select parameters that best separates strong and weak performance of the standard VectoBac.

Step	Variable entered	F- statistic	Significance
1	River temperature	.341	.000
2	Conductivity	.165	.000
3	Turbidity	.123	.000

TABLE: 5
Derived classification functions by stepwise discriminant analysis for the separation of strong and weak performance using river temperature, conductivity and turbidity.

Water condition	Classification function coefficient (Strong)	(Weak)
River temperature	43.914	37.850
Conductivity	5.834E-02	4.629E-02
Turbidity	-0.664	-0.498
Constant	-715.691	-531.282

3.5 Laboratory Studies on the Effect of Conductivity on Potency of VectoBac®

The results of the experiments carried out to investigate the effect of conductivity on the performance of the standard *Bt* formulation are shown in Tables 7 and 8 and Figures 11-13. The experiments were carried out using a total of 3,672 larvae and Tables 7.0-7.6 show this and the respective percent mortalities obtained for each experiment. Table 8 on the other hand lists the respective LC values and the relative median potency values. The relative median potency (RMP) was computed using SPSS software package and it tests the significance of observed differences between LC values of experiments. The criteria for establishing significance was if RMP value obtained between any two levels is equal to or greater than 1.

The temperatures and conductivities of the water used for the experiments ranged from 23.6°C – 31.2°C and 125.μS - 138.54μS, respectively.

The LC_{50}, LC_{95} and LC_{99} values obtained for the control experiments (i.e. under natural condition) were 0.13579mg/l, 1.11438mg/l and 2.66561mg/l, respectively (Table 7.0).

For the experiments that increased the conductivity of the water medium only to 1,500μS, the LC_{50}, LC_{95} and LC_{99} values obtained were 0.11521mg/l, 0.94553mg/l and 2.26172mg/l respectively (Table 7 1). The respective values for that of the insecticide only were 0.12526mg/l, 1.02803mg/l and 2.45906mg/l (Table 8). The RMP value

TABLE: 6
Model Summary of multiple regression analysis of field data using LC_{50} values as the dependent variable.

Model	Correlation R	Adjusted R square	Standard error	Statistic F	Significance P
1[a]	.752	.562	22.3120	166.392	.000
2[b]	.846	.712	18.0931	160.345	.000
3[c]	.853	.721	17.7993	112.198	.000

a. river temperature
b. river temperature and conductivity
c. river temperature, conductivity and turbidity.

TABLE: 7.0
Results of Experiment to study the effect of Conductivity on the Potency of VectoBac. (Normal water; Conductivity = 134.7µS)

Insecticide Concentration (mg/l)	Number of larvae	Number of dead larvae	% Mortality	Adjusted % Mortality
0.05	102	27	26.4	20.6
0.1	99	42	42.4	39.4
0.2	108	66	61.1	61.1
0.4	93	72	77.4	77.4
0.8	111	99	89.2	89.2

TABLE: 7.1 Results for Water Conductivity raised to 1500µS

Insecticide Concentration (mg/l)	Number of larvae	Number of dead larvae	% Mortality	Adjusted % Mortality
0.05	99	27	27.3	25.8
0.1	102	45	44.1	44.1
0.2	99	60	60.6	60.6
0.4	102	93	91.2	82.4
0.8	105	96	91.4	91.4

TABLE: 7.2 Results for *Bt* Conductivity raised to1500µS

Insecticide Concentration (mg/l)	Number of larvae	Number of dead larvae	% Mortality	Adjusted % Mortality
0.05	105	27	25.7	23.7
0.1	105	45	42.9	42.9
0.2	93	60	64.5	64.2
0.4	114	96	84.2	81.8
0.8	105	93	88.6	88.6

TABLE: 7.3 Results for Water Conductivity raised to 2000µS

Insecticide Concentration (mg/l)	Number of larvae	Number of dead larvae	% Mortality	Adjusted % Mortality
0.05	99	36	36.4	36.1
0.1	93	48	51.6	51.6
0.2	96	69	71.9	71.9
0.4	102	99	97.1	89.7
0.8	105	102	97.1	96.6

TABLE: 7.4 Results for *Bt* Conductivity raised to 2000µS

Insecticide Concentration (mg/l)	Number of larvae	Number of dead larvae	% Mortality	Adjusted % Mortality
0.05	99	27	27.3	27.3
0.1	111	57	51.4	51.4
0.2	96	72	75	72.2
0.4	114	99	86.8	86.8
0.8	99	96	96.9	95.2

TABLE: 7.5. Results for Water Conductivity raised to 3000µS

Insecticide Concentration (mg/l)	Number of larvae	Number of dead larvae	% Mortality	Adjusted % Mortality
0.05	117	33	28.2	28.2
0.1	102	51	50	50
0.2	105	72	68.6	68.6
0.4	102	90	88.2	86.2
0.8	96	93	96.9	95

TABLE: 7.6 Results for *Bt* Conductivity raised to 3000µS

Insecticide Concentration (mg/l)	Number of larvae	Number of dead larvae	% Mortality	Adjusted % Mortality
0.05	105	36	34.3	33.7
0.1	99	54	54.5	54.5
0.2	93	72	77.4	74.5
0.4	99	87	87.9	87.9
0.8	102	96	94.1	94.1

TABLE: 8
Results of LC values obtained at the different conductivities of water and VectoBac and the relative median potency indicating significant differences in the LC values between water and the insecticide at that conductivity level.

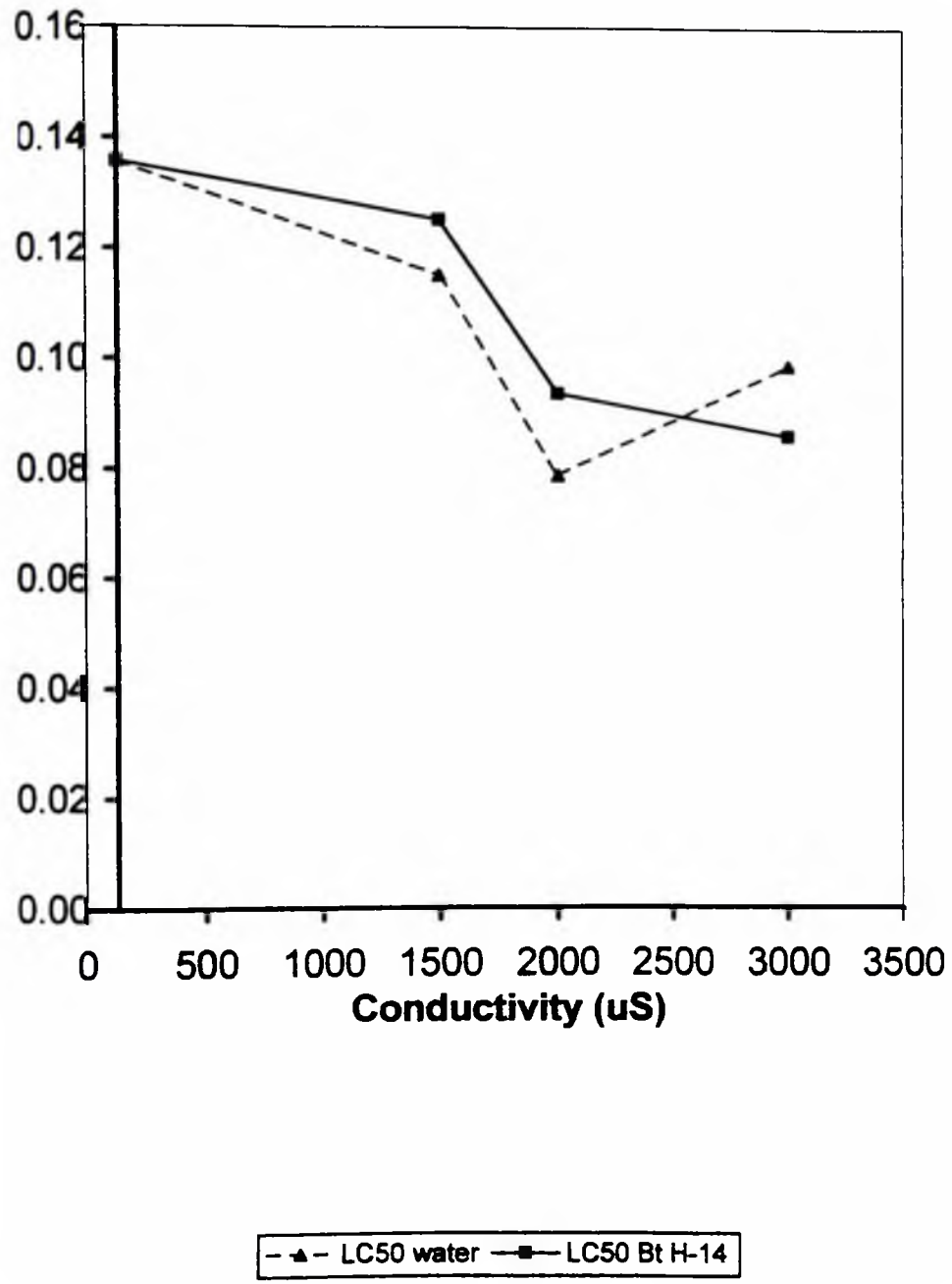
Conductivity (μS)	Concentration of Insecticide (mg/l)						Relative Median Potency
	Water			Bt			
	LC_{50}	LC_{95}	LC_{99}	LC_{50}	LC_{95}	LC_{99}	
133	0.13579	1.11438	2.66569	0.13579	1.11438	2.66561	
1500	0.11521	0.94553	2.26172	0.12526	1.02803	2.45906	0.9198
2000	0.07884	0.64706	1.54776	0.09388	0.77043	1.84287	0.8399
3000	0.09872	0.81016	1.93791	0.08591	0.07508	1.68655	1.1490

obtained for the observed differences between the two experiments was 0.9198 and this was considered not significant.

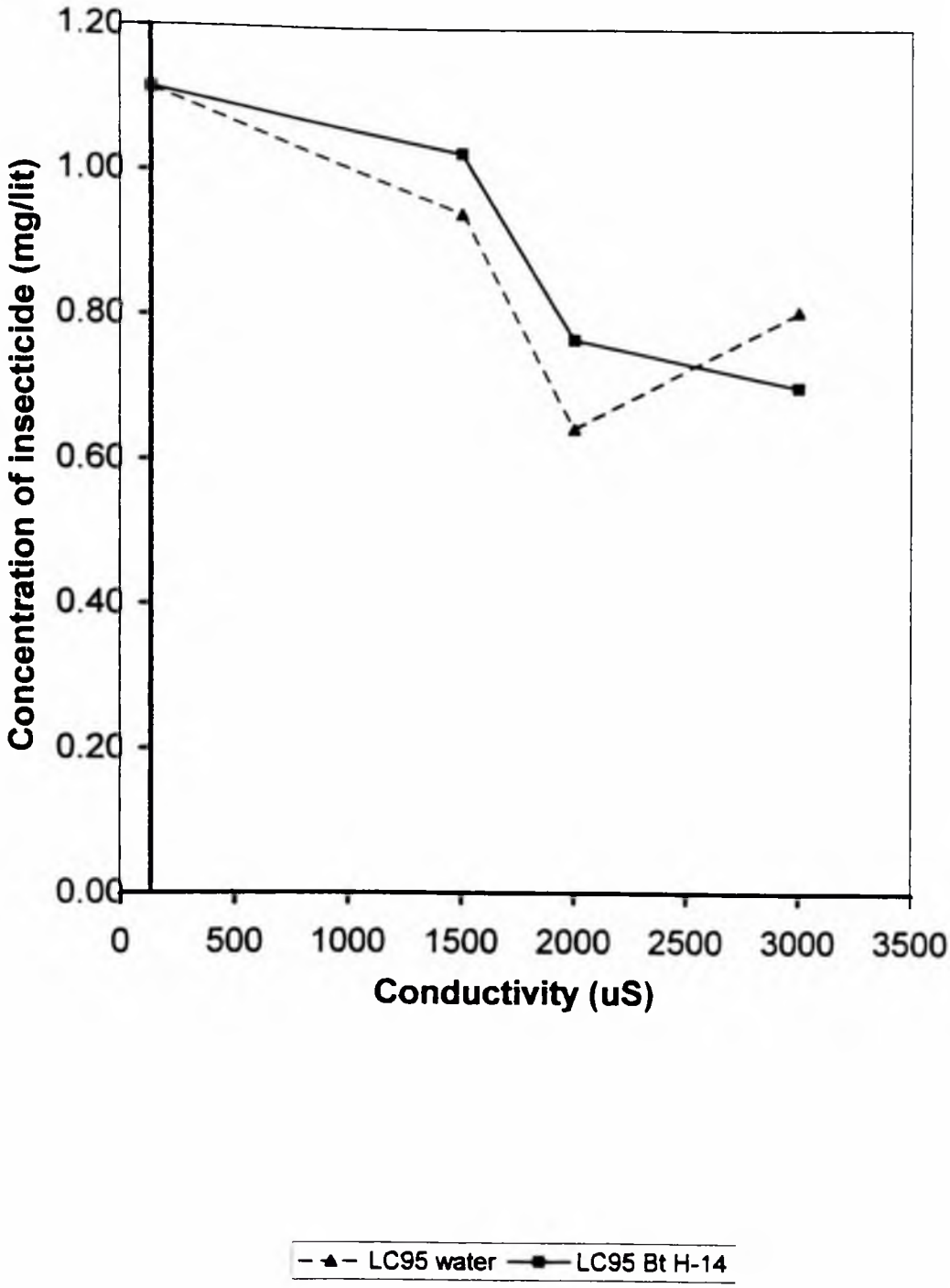
For the experiments that increased the conductivity of water only to 2000μS, the LC_{50}, LC_{95} and LC_{99} values obtained were 0.07884mg/l, 0.64706mg/l and 1.54776mg/l (Table 8). The respective values for increased conductivity of the insecticide solution only were 0.09388mg/l, 0.77043mg/l and 1.84287mg/l. The RMP value obtained for the observed differences between the two was 0.8399 and this was also considered not significant.

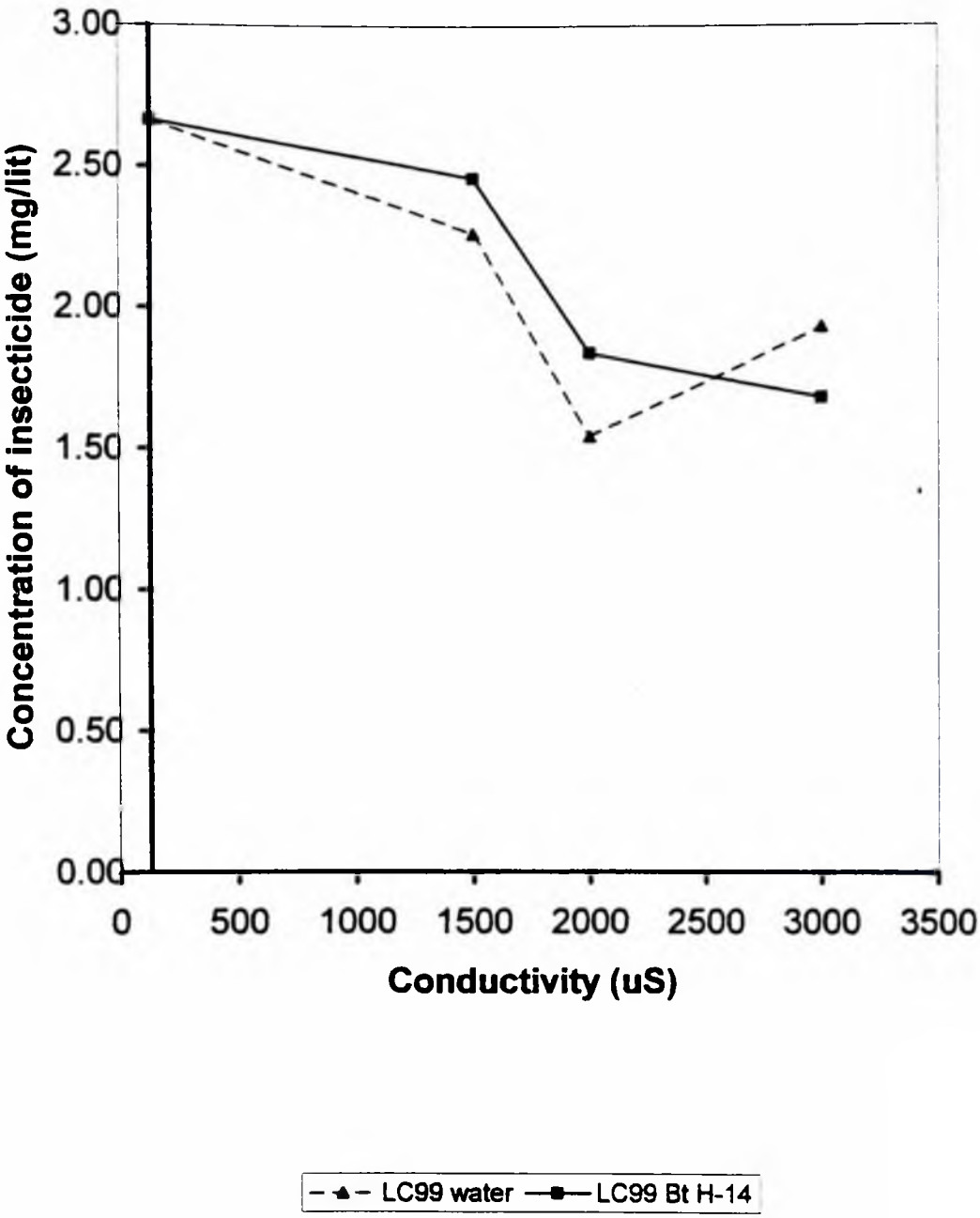
For the experiments that increased the conductivity of water to 3000μS, the LC_{50}, LC_{95} and LC_{99} values obtained were 0.09872mg/l, 0.81016mg/l and 1.9379mg/l, respectively (Table 8). The respective values for increased conductivity of the insecticide solution were 0.08591mg/l, 0.70508mg/l and 1.68655mg/l (Table 8). The RMP value obtained for the observed differences between the two experiments, however, was 1.149 and this was significant.

With regards to trends, of LC_{50} values, it was observed that increasing water conductivity only to 1,500μS, 2,000μS and 3,000μS enhanced potency by 15.2% (from 0.13579mg/l to 0.11521mg/l), 41.9% (from 0.13579mg/l to 0.07884mg/l) and 27.3% (0.13579 to 0.0.09872 and 0.11521mg/l), respectively. Whilst increased conductivity of the insecticide at similar levels enhanced potency by 7.8% (from 0.13579mg/l to 0.12526mg/l), 30.9% (from 0.13579mg/l to 0.09388mg/l) and 36.7% from (0.13579 to 0.0.09872 and 0.11521mg/l), respectively (Figure 11).



The same trends of improved performances were observed with the LC_{95} and LC_{99} values (Figures 12 and 13).





CHAPTER 4

DISCUSSION AND CONCLUSION

The study has clearly shown that temperature, conductivity and turbidity of river water influence the performance of VectoBac® formulations against *S. damnosum* s.l. larvae in the field. Why water temperature was the most significant of the three parameters is unknown. But it is likely that its effect might be indirect through increased metabolism and thereby increased ingestion rates by the insect. The finding that at temperatures below 29°C performances were poor corroborates this suggesting that there is a temperature range with an optimum, for toxin uptake. This also presupposes an upper limit and this should be easy to determine in the laboratory.

The temperatures of rivers and streams which support the breeding of *S. damnosum* s.l. in West Africa vary depending on the ecological zone and the season, and these in turn determine the distribution of the members of the species complex (Ocran *et al.*, 1982). In the savanna areas where *S. sirbanum* and *S. damnosum* s.str. occur, the water temperatures can range from 24°C to 33°C with a mean of around 27°C for the year (Grunewald, 1976). Similar temperature ranges were found in the large rivers of the rainforest zone, where *S. sanctipauli* s.l. and *S. soubrense* occur, but in heavily shaded rivers and streams, which support *S. yahense* and *S. squamosum*, temperatures can range from 22°C to 30°C with an annual mean of about 25°C (Grunewald, 1976a, b; 1978; Quillevere *et al*, 1977, 1976). In West Africa, water temperatures are usually lower in the rainy season

than in the dry season and the differences can be more pronounced in the savanna zone and the large forest rivers (Quillevere *et al*, 1976; 1977,). This implies that for effective use of VectoBac® in the sub-region for blackfly control, river temperature should be taken into consideration, perhaps by selecting only those rivers at 30°C and above for treatment.

Why and how water conductivity influences the potency of VectoBac® is also not clear. However, it is believed that after ingestion, delta-endotoxin inclusion is solubilised and activated in the mid-gut, ultimately producing a toxin-induced lysis of susceptible mid-gut epithelial cells (Knowles, 1994). It is also believed that cytolysis proceeds via the initial interaction of an activated toxin with specific membrane receptors, followed by the formation of a 1-2nm diameter pore leading to cell death by colloid-osmotic lysis (Knowles and Ellar, 1987). This belief is supported by experiments using brush border membrane vesicles (BBMV) prepared from insect mid-guts which have demonstrated the presence of high affinity receptors for delta endotoxins in susceptible insects (Hofmann, *et al.*, 1988; Van Rie *et al.,* 1989). The characteristics of the toxin lesion formed in the membrane after the initial interaction are still under investigation (Knowles and Dow, 1993). Using *Manduca sexta* midgut BBMV, the delta-endotoxin was observed to alter the membrane permeability for monovalent cations, anions and neutral solutes (Carrol and Ellar, 1993). Recent evidence suggests that delta-endotoxins can form a 2nm diameter pore in *Manduca sexta* midgut BBMV (Carrol and Ellar, 1997) or in a planar lipid bilayer to which these BBMV were fused (Martin and Wolfersberger, 1995).

Delta-endotoxins have been shown in *in vivo* experiments to form channels in lipid bilayers in the absence of putative receptor molecules (Grochulski *et al.*, 1995; Schwartz *et al.*, 1993; Slatin *et al.*, 1990). However, fusing receptor-bearing BBMV to these lipid bilayers decreases greatly the toxin concentration needed to produce membrane conductance changes (Lorence *et al.*, 1995; Martin and Wolfersberger, 1995).

If this phenomenon should explain the results obtained by the present study, then it stands to reason that the river water conductivity influences the environment of the insect's gut. This is not hard to imagine because blackflies larvae as particulate feeders, continuously ingest substrates and with them the dissolved ions responsible for the water conductivity levels. From the results of the chemical analysis, it seems that the high conductivity levels were due to a large extent on Na^+ in the form of NaCl and to a lesser extent K^+ These ions are abundant in seawater and I believe that especially during the dry season, the river at the study site is influenced by the tides of the Atlantic Ocean into which the river flows about 20km downstream. Conductivity above 1,000µS as found during the present study is unusual in rivers of West Africa that support blackflies. Grunewald (1976a;1976b) for example, reported conductivity levels less than 50µS for larval habitats of *S. yahense* and *S. sanctipauli* and up to 150µS for the rest of the species complex. Thus for operational purposes, the information that high conductivity leads to increased performance might not be extremely important when using VectoBac® for blackfly control. The present study moreover, does not provide any basis to draw a firm conclusion that there should be a minimum conductivity level threshold

for the use of VectoBac®. Thus, when the best performance (LC_{50} = 14.83mg/l) was recorded the conductivity was 323µS, which is far below the highest of 1999µS, but then the temperature was 32°C, which was high. Also the difference in conductivity between this performance LC_{50} threshold of 42.92mg/l and the next of 59.35mg/l, was only 89.4µS which makes it difficult to envisage that this made the difference.

The results of the laboratory experiments, however, suggest that the effect of water conductivity might involve something more than just changing the environment of the gut of *S. damnosum* s.l. The experiments revealed that, changing the conductivity of the insecticide alone could enhance the potency of VectoBac® substantially by 36.7% when it was raised to 3000µS. Whilst that of water at similar conductivity level improved by 27.9%. It must be noted, however, that 41.9% was achieved at conductivity of 2000µS. It is unlikely that the few drops of NaCl added to the insecticide could drastically alter the conductivity of 1 litre of water in the rearing chamber. (This was later verified in separate experiments). Thus, it seems only likely that, NaCl, does potentiate either through increased uptake of toxins, or it induces conformational change in the toxin, making it more effective in binding to receptors on the cells lining the gut of the insect. Whichever way, there should be an optimum concentration above, which this effect should either decline. This is what was most probably observed when the performance declined after water conductivity was raised from 2000µS to 3000µS.

Turbidity was the least significant of the three influencing parameters. However, unlike in the case of conductivity, an optimum range can be discerned

from the results obtained. In all cases, good performance of VectoBac® (i.e. with LC_{50} below 42.92mg/l), the water turbidity was below 7.30NTU. This limit of water turbidity is almost two times lower than the least recorded value associated with the weak performance group, which was 13.3 NTU. Most river waters in West Africa can become very turbid during the rains due mainly to mud and organic matter washed from upstream, and the R. Pra is no exception. That, total solids were found to be higher in the wet season samples than those of the dry season can be taken as supporting evidence, but reasons why turbidity influences the performance of VectoBac® can only be speculative. It is possible that ingested fine mud particles present themselves between the receptors and the toxins thereby preventing them from binding to the surface of the cells lining the gut. Alternatively, the toxins could be adsorbed onto the surfaces of the fine particles thereby making them unavailable for binding. Whatever the reason, the implications of this finding are that, for an effective operational use of VectoBac®, turbid waters should be avoided.

That the observed pH range did not influence the performance of VectoBac® is to be expected because it is lower than that found in the alimentary canal of *S. damnosum* s.l. The mid-gut of *S. damnosum* s.l. which is functionally like that of any other aquatic dipterous detrivore has a digestive enzyme system and a very high alkaline environment that enables the larvae to exploit algal food better than vascular plant derivatives (Martin *et al.*, 1985). The pH within the fore and hind gut is also very high and it ranges from about pH 10-11.4 (Lacey and Federici, 1979; Undeen, 1979). It is this high alkalinity that activates the toxins of *B*

thuringensis. Although highly alkaline habitats of Kibwezi and Kiswani forms in East Africa with pH values of 7.7-10 are known (Grunewald, 1976a,b), in West Africa, rivers that support *S. damnosum* have pH ranging from 5 8. Therefore, even though pH values higher than that found in the gut may enhance performance because they are positively correlated, it is unlikely that this can be exploited operationally in West Africa.

In view of the above, it is little wonder that the performance of VectoBac® has been variable in the OCP However, the present study has established the basis upon which the product can be evaluated under any river water conditions of temperature, conductivity and turbidity. Products can be assigned to the weak or strong performance group, using the basic classification formula:

$C = C_o + C_{tem}X_{tem} + C_{con}X_{con} + C_{turb}X_{turb}$

where C, is the score on the classification function for the group, C_o the group constant and X_{tem}, X_{con} and X_{turb} are the raw scores of temperature, conductivity and turbidity. Thereafter the assayed product is then assigned to the group to which it has the highest value.

From this study therefore, scores for weak and strong performances can be computed using the classification functions;

$$C_{strong} = -715.691 + 43.914X_{temp.} + 5.834^{E-02}X_{cond.} + -0.664X_{turb.}$$

And

$$C_{weak} = -531.282 + 37.85X_{temp.} + 4.629^{E-02}X_{cond.} + -0.498X_{turb.}$$

The study also provides a basis for evaluating the real potency of any batch of VectoBac® assayed in the field using the values of the constants from the MR analyses. For this, the LC_{50} values can be predicted from the MR equation;

$$y = a + b_{temp}.X_{temp}. + C_{cond}.X_{con}d. + d_{turb}.X_{turb}.$$

where y is the predicted LC_{50} value, a is a constant, b, c and d are the respective coefficients and X the raw score.

Using this equation, the real LC_{50} value for VectoBac® can be predicted from the following equation;

$$LC_{50} = 439.757 + -12.19X_{temp}. + -2.6243^{E-02}X_{cond}. + -0.166X_{turb}.$$

The laboratory-based system developed for assaying the product, however, overcomes the vagaries associated with field conditions. It also has other advantages over the mini-gutter system, which has to be sited in the field near rivers and requires huge logistic support. Understandably, it does not simulate closely the field situations of blackfly habitats and of the product in rivers, which the mini gutter system does. However, the cited advantages, make this system more attractive for routine assays of any insecticide for blackfly control and therefore warrants further evaluation.

For onchocerciasis control, *B. thuringensis* H-14 formulations should be improved to be ultimately comparable to the choice chemical insecticides being used by the OCP, in terms of efficiency, dosage rates and costs. If this is achieved, then together with its advantages of selectivity and non hazardous effect on the environment, *B. thuringensis* H-14 has the potential of being the ideal and recommended larvicide for blackfly control in developing countries. Thus, if

increase in potency, can be achieved by increasing conductivity of the insecticide solution then this study has provided the basis for more research into the development of better formulations

In conclusion, the present study has shown that river temperature, conductivity and turbidity influence the performance of VectoBac® against *S. damnosum* larvae. River temperature and conductivity were positively correlated with its performance whilst turbidity was negatively correlated. Thirty degrees centigrade appear to be the minimum threshold below which, the performance of VectoBac® falls significantly. It was also observed that low turbidity improves the performance of the insecticide with a threshold value of 4.18NTU above, which the performance of the insecticide falls significantly. The laboratory studies showed that increased conductivities of either the water medium or the insecticide solution improved considerably the performance of VectoBac® and this creates an avenue for developing better products. The study has also shown that the laboratory-based system can be used for testing VectoBac®, but this will need further evaluation before it can be adopted for routine use.

REFERENCES

ABIOSE, A. (1998). Onchocercal eye disease and the impact of Mectizan treatment. *Annals of Tropical Medicine and Parasitology* **92,** supplement No. 1, S11-S22.

ABIOSE, A., JONES, B. R., CONSEUS, S. N., MURDOCH, I., CASSELS-BROWN, A., BABALOLA, O. E., ALEXANDER, N. D. E., NUHU, I., EVANS, J., IBRAHIM, U. F. & MAHMOOD, A. O. (1993). Reduction in incidence of optic nerve disease with annual ivermectin to control onchocerciasis. *Lancet*, **i,** 130-134.

ANDERSON, J. & FUGLSANG, H., (1977). Ocular Onchocerciasis. *Tropical Diseases Bulletin*, **74,** 257-272.

AMBUHL, H. (1962). Die Besonder heiten der Wasserstromung in physikalischer, chemischer und biologischer Hinsicht. *Scweiz. Z. Hydrol.* **24,** 367-382.

AMERICAN PUBLIC HEALTH ASSOCIATION (1998). *Standard methods for the examination of Water and Waste Water*. (20th Edition). (Ed. CLESCERI, L. S., GREENBERG, A. E., & TRUSSELL, R. R.), United Book Press, Baltimore.

ASHBURN, L. L., BURCH, T. A. & BRADY, F. J. (1949). Pathologic effects of suramin, hetrazan and arsenamide on adult *Onchocerca volvulus*. *Boletin de la Oficina Sanitaria Panamericana*, **28**, 1107-1117.

AWADZI, K. & GILLES, H. M. (1980). The chemotherapy of onchocerciasis. III. A comparative study of DEC and Metrifonate. *Annals of Tropical Medicine and Parasitology*, **74,** 199-210.

AWADZI, K., DADZIE, K. Y., SCHULZ-KEY, H., GILLES, H. M., FULFORD, A. J., & AZIZ, M. A. (1986). The chemotherapy of onchocerciasis. XI. A double-blind comparative study of ivermectin, diethylcarbamazine and placebo in human onchocerciasis in Northern Ghana. *Annals of Tropical Medicine and Parasitology*, **80**, 433-442.

BUCKLEY, J. J. C. (1951). Studies on human onchocerciasis and *Simulium* in Nyanza Province, Kenya. II. The disappearance of *S. neavei* from a bush cleared focus. *Journal of Helminthology*, **25,** 213-222.

BURGES, H. D. (1982). The role of pathogens in insect control. In: Basic biology of microbial larvicides of vectors of human Diseases. *Proceedings of a consultation convened by the Scientific Working Groups on Biomedical Sciences and Biological Control, pp*5-9. *WHO, Geneva.*

CARLSSON, G., (1968). Benthonic fauna in African water courses with special reference to blackfly populations. *Scandinavian Institute of African Studies Research Report,* **31-13.**

CARLSSON, G., (1970). Biology of immature stages of blackflies in parts of East Africa with special reference to *Simulium damnosum*. WHO *Mimeographed Document WHO/ONCHO 70. 81. WHO/VBC/70 232.*

CARROL, J. & ELLAR, D. J. (1993). An analysis of *Bacillus thuringiensis* delta-endotoxin action on insect-midgut-membrane permeability using a light scattering assay. *European Journal of Biochemistry,* **214**, 771-778.

CARROL, J. & ELLAR, D. J. (1997). Analysis of the large aqueous pores produced by a *Bacillus thuringiensis* protein insecticide in *Manduca sexta* midgut-brush-border-membrane vesicles. *European Journal of Biochemistry,* **245,** 797-804.

CARROL, J., WOLFERSBERGER, M. G., ELLAR, D. J. (1997). The *Bacillus thuringiensis* Cry 1Ac toxin-induced permeability change in *Manduca sexta* midgut brush border vesicles proceeds by more than one mechanism. *Journal of Cell Science*, **110**, 3099-3104.

COLLESS, D. & Mc ALPINE, D., (1970). Diptera. Simuliidae. In: *The insects of Australia.* Melbourne University Press, Carlton, Victoria. Australia.

COULAUD, J. P., LARIVIERE, M., GERVAIS, M.C., GAXOTE, P., AZIZ, A., DELOUL, A. M. & CERAC, J. (1983). Traitement de l'onchocercose humaine par l'ivermectine. *Bulletin de societe de Pathologie Exotique*, **76**, 681-688.

COULAUD, J. P., LARIVIERE, M., AZIZ, M. A., GERVAIS, M.C., GAXOTE, P., DELOUL, A. M. & CERAC, J. (1984). Ivermectin in onchocerciasis. *Lancet,* **ii,** 526-527

CROSSKEY, R. W., (1969). A reclassification of the Simuliidae (Diptera) of Africa and its Islands. *Bulletin of the British Museum (Natural History), Entomological Supplement,* **14,** 195pp.

CROSSKEY, R. W., (1973a). Family Simuliidae. In: *A Catalogue of the Oriental Region,* **I**, 424-430, University Press of Hawaii, Honolulu,

CROSSKEY, R. W., (1981). A review of Simulium damnosum s.l., and human onchocerciasis in Nigeria, with special reference to geographical distribution and the development of a Nigerian National Control campaign. *Tropenmedizin und Parasitologie,* **32,** 2-16.

CROSSKEY, R. W., (1990). The Natural History of Blackflies. *John Wiley and Sons, Chichester.*

DADZIE, K. Y., REMME, J., ROLLAND, A. & THYLEFORS, B. (1989). Ocular onchocerciasis and intensity of infection in the community. II. West African rainforest foci of the vector *Simulium yahense*. *Tropical Medicine and Parasitology*, **40**, 348-354.

DAVIES, L., DOWNE, A. E. R., WEITZ, B. & WILLIAMS, C. B. (1962). Studies on black flies (Diptera: Simuliidae) taken in a light trap in Scotland. II. Blood meal identification by precipitin tests. *Transactions of the Royal Entomological Society, London* **114**, 21-27.

DAVIES, J. B. (1983). Onchocerciasis vector control – 50 years ago. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, **77**, 139 – 140.

DAWOOD, M. S. (1978). Field treatment of onchocerciasis in the Sudan with suramin preparations and Banocide. WHO, Alexandra.

de BARJAC, H., (1978a). Une nouvelle variete de *Bacillus thuringiensis* tres toxique pour les moustiques. *B. thuringiensis* var. *israelensis* serotype 14. *Comptes Rendus de l'Academie des Sciences Serie D, Sciences de la vie. (Paris)* **286D,** 797-800.

de BARJAC, H.(1978b). Toxicite de *Bacillus thuringiensis* var. *israelensis* pour les larves *d'Aedes aegypti* et *d'Anopheles stephensi*. *Comptes Rendus Academie des Sciences Serie D. Sciences de la vie* (Paris), **286D,** 1175 – 1178.

de BARJAC, H. (1978c). Un nouveau candidat a la lutte biologie contre les moustiques: *Bacillus thuringiensis* var. *israelensis*. *Entomophaga*, **23**: 309 – 319.

DEJOUX, C., GIBON, F & YAMEOGO, L. (1985). Toxicite pour la faune non-cible de quelques insecticides nouveaux utilises en milieu aquatique tropical. *Revue de Hydrobiologie Tropicale,* **18,** (1), 31-49.

DE SOLE, G., AWADZI, K., REMME, J., DADZIE, K. Y., BA, O., GIESE, J., KARAM, M., KEITA, F. M. & OPOKU, N. O. (1989). A community trial of ivermectin in the onchocerciasis focus of Asubende, Ghana. II. Adverse reactions. *Tropical Medicine and Parasitology*, **40,** 375-382.

DIALLO, S., AZIZ, M. A., LARIVIERE, M., DIALLO, J. S., DIOP, I., N' DIR, O., BANDIANE, S., PY, D., SCHULZ-KEY, H., GAXOTE, P. & VICTOIUS, A. (1986). A double-blind comparison of the efficacy and safety of ivermectin and diethylcarbamazine in a placebo controlled study of Senegalese patients with onchocerciasis. *Transactions of the Royal society of tropical Medicine and hygiene*, **80**, 927-934.

DISNEY, R. H. L. (1975). A survey of blackfly populations (Diptera. Simuliidae) in West Cameroon. *Entomologist's Monthly Magazine*, **111,** 211-227.

DUDLEY, D. W & FELTMATE, B. W. (1992). Aquatic Insects. *Redwood Press Ltd, Melksham.*

DUKE, B. O. L. (1990). Human onchocerciasis - an overview of the disease. *Acta Leidensia,* **59,** 9-24.

DUKE, B. O. L. & ANDERSON, J. (1972). A comparison of the lesions produced in the cornea of the rabbit's eye by microfilariae of the forest and Sudan savanna strains of *Onchocerca volvulus* from Cameroon. I. The clinical picture. *Tropenmedizin und Parasitologie*, **23,** 354-368.

DUKE, B .O. L., LEWIS, D. J. & MOORE, P. G. (1966). *Onchocerca-Simulium* complexes. I Transmission of forest and Sudan savanna strains of *Onchocerca volvulus*, from Cameroon by *Simulium damnosum* from various West African bio-climatic zones. *Annals of Tropical Medicine and Parasitology*, **60,** 318-336.

ELLAR, D. J. (1998). Bacterial toxins in biological control: the structure and function of *Bacillus thuringiensis* delta-endotoxins. *Journal of Applied Microbiology,* **84**, 149S-151S.

ELSEN, P. & HEBRARD, G. (1977). Methodes d'echantillonage des populations pre-imaginales de *Simulium damnosum* Theobald, 1903 (Diptera: Simuliidae) en Afrique de l'Ouest. II. Observations sur le choix de couleurs, l'evolution du peuplement et la reparation horizontale au moyen de rubans en plastique. *Tropenmedizin und Parasitologie,* **28**, 471 - 477

FINNEY, D. J. (1971). Probit Analysis. 3rd Edition. *Cambridge University Press, Cambridge.*

GESSNER, F. (1959). Hydrobotanik. Die physiologischen grundlagen der pflazenverbreitung im Wasser. **II**, *VEB Deutscher Verlag der Wissenschaften, Stoffhaushalt. Berlin*

GOLDBERG, L. J., GOLDBERG, E. M. & MARGALIT, J. (1977). Potential application of a bacterial spore, ONR 60A to mosquito larval control/demonstrated rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univittatus, Aedes aegypti* and *Culex pipiens* (complex). Mimeographed document *WHO/VBC/77.662*, 8pp. Geneva.

GOLDBERG, L. J. & MARGALIT, J. (1977a). ONR 60A: A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univittatus, Aedes aegypti* and *Culex pipiens* (complex). *Naval biosciences Laboratory, Technical Progress Report*, **53**, Part1: 80-88.

GOLDBERG, L. J. & MARGALIT, J. (1977b). Bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univittatus, Aedes aegypti* and *Culex pipiens*. *Mosquito News*, **37**: 355-358.

GREENE, B. M., TAYLOR, H. R., CUPP, E. W., MURPHY, R. P., WHITE, A. T., AZIZ, M. A., SCHULZ-KEY, H., D' ANNA, S. A. & WILLIAMS, P. N. (1985). Comparison of ivermectin and diethylcarbamazine in the treatment of onchocerciasis. *New England Journal of Medicine*, **313,** 133-138.

GROCHULSKI, P., MASSON, L., BORISOVA, S., PUSZTAI-CAREY, M., SCHWARTZ, J. L., BROUSSEAU, R. & CYGLER, M. (1995). *Bacillus thuringiensis* Cry 1Ac (a) insecticidal toxin crystal structure and channel formation. *Journal of Molecular Biology*, **254**, 447-464.

GRUNEWALD, J., (1973). Die hydrochemischen Lebensbedingungen der praimaginalen stadien von *Boophthora erythrocephala* De Geer (Diptera: Simuliidae). 2. Die Entwicklung einer zucht unter experimentellen Bedingungen. *Z. Tropenmed. Parasit.* **24,** 232-249.

GRUNEWALD, J., (1976a). The hydrochemical and physical conditions of the environment of the immature stages of some species of the *Simulium* (*Edwardsellum*) *damnosum* complex (Diptera). *Tropenmedizin und Parasitologie*, **27,** 438-454.

GRUNEWALD, J. (1976b). The hydrochemical and physical conditions of the environment of the aquatic stages of some West African cytotypes of the *Simulium damnosum* complex, and studies on the water quality of some potential locations for a rearing laboratory. *World Health Organisation Report, 1-27.*

GRUNEWALD, J. (1978). Die Bedeutung der Stickstoff-Exkretion und Ammoniak- Empfindlichkeit von Simuliiden-Larven (Diptera) fur den Aufbau von Laboratorium skulturen. *Zeitschaft angewandt Entomologie* **85,** 52 - 60.

GRUNEWALD, J. (1981). Hydrochemical and physical characteristics of the larval sites of the species of the *Simulium damnosum* complex. In Blackflies. *The future for biological methods in Integrated Control.* [Ed. Laird, M.] pp 227-235. Academic Press, London and New York .

GRUNEWALD, J. & GRUNEWALD, E. B., (1978). Der Einfluss derwasserstoffionen-und Gesamtionenkonzentration sowie derlonenkomposition auf die aquatischen stadien zweier zyto artendes *Simulium damnosum* Komplexes (Diptera: Simuliidae) Ostafrikas. *Arch Hydrobiol.* **82,** 419-431.

GRUNEWALD, J., GRUNEWALD, E. B., RAYBOULD, J. N. & MHIDDIN, H. K. (1979). The Hydrochemical and Physical characteristics of the breeding sites of the *Simulium neavei* Roubaud group and their associated crabs in the Eastern Usambara Mountains in Tanzania. *Int. Revue, ges. Hydrobiol.*, **64,** 71-88.

GUILLET, P., ESCAFFRE, M., OUEDRAOGO, M. & QUILLEVERE, D. (1980). Mise en evidence d'une resistance au temephos dans le complexe *Simulium damnosum* [*S. sanctipauli et S soubrense*] en Cote d'Ivoire. (Zone du programme de lutte contre l'onchocercose dans la region du bassin de la Volta). *Cahiers ORSTOM Entomologie et Medicale Parasitologie*, **18,** 291-299.

HALL, I. M., ARAKAWA, K. Y., DULMAGE, H. T. & CORREA, J. A. (1977). The pathogenicity of strains of *Bacillus thuringiensis* to larvae of *Aedes* and *Culex* mosqiutoes. *Mosquito News*, **37**: 246 – 251.

HALL, I. & ARAKAWA, K. (1979). Bioassays against *Aedes, Culex* and *Anopheles* mosquitoes. **In**: Monograph on *Bacillus thuringiensis*, Dulmage, H. T. (Ed) in press

HOEFTE, H. & WHITELEY, H. R. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Review*, **53**, 242-255.

HOFMANN, C., VANDERBRUGGEN, H., HOFTE, H., VAN RIE, J., JANSENS, S., & VAN MELLAERT, H. (1988). Specificity of *Bacillus thuringiensis* delta-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect mid-guts. *Proceedings of the National Academy of Sciences, USA*, **85**, 7844-7848.

HOUGARD, J. M., POUDIOUGO, P., GUILLET, P. BACK, C., AKPOBOUA, K. B. & QUILLEVERE, D. (1993). Criteria for the selection of larvicides by the Onchocerciasis Control Programme in West Africa. *Annals of Tropical Medicine and Parasitology*, **87**, 435 – 442.

JAMNBACK, H. (1981). The origins of blackfly control programmes. Pp71 – 73 in [Laird M. Ed.] Blackflies: the future for biological methods in integrated control. New York and London, *Academic Press*,

KETTLE, D. S. (1992). Medical and Veterinary Entomology. *C.A.B. International, Wallingford* UK.

KIRKWOOD, B R. (1988). Essentials of Medical Statistics. *Blackwell Scientific Publications, Oxford* UK.

KNOWLES, B. H. (1994). Mechanism of action of *Bacillus thuringiensis* delta-endotoxins. *Advanced Insect Physiology*, **24,** 275-308.

KNOWLES, B. H. & DOW, J. A. T. (1993). The crystal delta-endotoxins of *Bacillus thuringiensis*: models for their mechanism of action on the insect gut. *BioEssays* **15**, 469-476.

KNOWLES, B. H. & ELLAR, D. H. (1987). Colloid-osmotic lysis is a general feature of the mechanism of action of *Bacillus thuringiensis* delta-endotoxins with different insect specificity. *Biochemistry and Biophysics Acta* **924**, 509-518.

KURTAK, D. C., OUEDRAOGO, M., BARRO, T., & GUILLET, P., (1982). Preliminary note on the appearance in Ivory Coast of resistance to chlorphoxim in *Simulium soubrense/ sanctipauli* larvae already resistant to temephos (Abate®). *WHO unpublished document* WHO/VBC/82. 850.

KURTAK, D C , JAMNBACK, H., MEYER, R , OCRAN, M & RENAUD, P (1987). Evaluation of larvicides for the control of *Simulium damnosum* s. l. (Diptera: Simuliidae) in West Africa. *Journal of the American Mosquito Control Association,* Vol. **3, No. 2,** 201-210.

LACEY, L. A. & FEDERICI, B. A. (1979). Pathogenesis and midgut histopathology of *Bacillus thuringiensis* in *Simulium vittatum* (Diptera: Simuliidae). *Journal of Invertebrate Pathology*, **33,** 171-182.

LACEY, L.A., ESCAFFRE, H., PHILIPPON, B., SEKETELI, A., & GUILLET, P (1982). A large river treatment with *Bacillus thuringiensis* H-14 for the control of *S. damnosum* s.l., In: Onchocerciasis Control Programme. *Tropenmedizin und Parasitologie,* **33**. 97-101.

LARIVIERE, M., VINGTAIN, P., AZIZ, M. A., BEAUVAIS, B., WEIMANN, D., DEROUIN, F., GINOUX, J., SCHULZ-KEY, H., GAXOTE, P., BASSET, D. & SARFATI, C. (1985). Double-blind study of ivermectin and diethylcarbamazine in African onchocerciasis patients with ocular involvement. *Lancet*, **ii,** 172-177.

LORENCE, A., DARSZON, A., DIAZ, C., LIEVANO, A., QUINTERO, R. & BRAVO, A. (1995). Delta-endotoxins induce cation channels in *Spodoptera frugiperda* brush border membranes in suspension and in planar bilayers. *FEBS Lett.* **360**, 217-222.

MANSON-BAHR, P. E. C. & APTED, F. I. C. (1982). *Manson's Tropical Diseases*. (18th Edition). Bailliere, Tindall, London.

MARTIN, F. G. & WOLFERSBERGER, M. G. (1995). *Bacillus thuringiensis* delta-endotoxin and larval *Manduca sexta* midgut brush-border membrane vesicles act synergistically to cause very large increases in the conductance of planar lipid bilayers. *Journal of Experimental Biology,* **198,** 91-96.

MARTIN, M. M., KUKOR, J. J., MARTIN, J. S. & MERRITT, R. W. (1985). The digestive enzymes of larvae of the blackfly, *Prosimulium fuscum* (Diptera; Simuliidae). *Comparative Biochemistry and Physiology*, **82B,** 37-39.

MAZZOTTI, L. (1959). Presencia de microfilarias de *Onchocerca volvulus* en el liquido cefalorraquideo de enfermos tratados con hetrazan. *Revista Instituto de salubridad y Enfermedades Tropicales*, **19,** 1-5

MERDAN, A. I. (1982). Field use of Microbial Insecticides. *Proceedings of a consultation convened by the Scientific Working Group on Biomedical Sciences and Biological control. WHO, Geneva*. pp11 - 17

McCRAE, A. W. R. (1978). Intermittent eradication of *Simulium damnosum* Theobald on the Nile from Jinja, Uganda 1951-1977. *Proceedings of the Medical Entomology Centenary Symposium*, pp133-134

MOKRY, J. E. (1976). Laboratory studies on the larval biology of *Simulium venustum* Say (Diptera: Simuliidae). *Canadian Journal of Zoology* **54,** 1657-1663.

MULLER, R. (1975). Worms and Disease. *A manual of Medical Helminthology*. Heinemann, London:

NELSON, G. (1970). Onchocerciasis. *Advances in Parasitology*, **8,** 173-224

NEWLAND, H. S., WHYTE, A. T., GREENE, B. M., D' ANNA, S. A., KEYVAN-LARIJANI, E., AZIZ, M. A., WILLIAMS, P. N. & TAYLOR, H. R. (1988). Effect of single-dose ivermectin on human *Onchocerca volvulus* infection with onchocercal ocular involvement. *British Journal of Ophthalmology*, **75,** 163-169.

OCRAN, M. H., DAVIES, J. B., AGOUA, H., GBOHO, C. & OUEDRAOGO, J. (1982). Water temperatures in *Simulium damnosum* breeding rivers of the Onchocerciasis Control Programme area. *WHO/VBC/82.* 848. 9pp.

OMAR, M. S. & GARMS, R. (1977). Lethal damage to *Simulium metallicum* following high intakes of *Onchocerca volvulus* microfilariae in Guatemala. *Tropenmedizin und Parasitologie*, **28,** 109 -119.

PARIS, F. (1989). Geography on onchocerciasis blindness in Northern Cameroon. In: *Parasitic Diseases; Treatment and control,* (Ed. Miller, M. J. & Love, E. J.), pp 221-229.CRC Press, Boca Raton

PHILIPPON, B. (1977). Etude de la transmission *d'Onchocerca volvulus* (Leukart, 1893) (Nematoda, Onchocercidae) par *Simulium damnosum* Theobald, 1903 (Diptera: Simuliidae). *Travaux et Documents de L' O.R.S.T.O.M.,* **63,** 1-308.

PROST, A. & VAUGELADE, J. (1981). La surmortalite des aveugles en zone de savane ouest-africaine. *Bulletin of the World Health Organisation,* **59,** 773-776.

QUILLEVERE, D., GOUZY, M., SECHAN, Y. & PENDRIEZ, B. (1976). Etude du complexe *Simulium damnosum* en Afrique de l'Ouest. IV. Analyse de l'eau des gites larvaires en saison seche. *Cahiers ORSTOM Serie Entomologie Medicale et Parasitologie,* 1**4,** 315-330.

QUILLEVERE, D., GOUZY, M., SECHAN, Y. & PENDRIEZ, B. (1977). Etude du complexe *Simulium damnosum* en Afrique de l'Ouest. VI. Analyse de l'eau des gites larvaires en saison des pluies; comparaison avev la saison seche. *Cahiers ORSTOM Serie Entomologie Medicale et Parasitologie,* **15,** 195-207

RAPER, A. B. & LADKIN, R. G. (1950). Endemic dwarfism in Uganda. *East African Medical Journal*, **29**, 339.

REMME, J., BAKER, R. H. A., DE SOLE, G., DADZIE, K. Y., ADAMS, M. A., ALLEY, E. S., AVISSEY, H. S. K. & WALSH, J. F. (1989). A community trial of ivermectin in the onchocerciasis focus of Asubende, Ghana. I. Effect on the microfilarial reservoir and the transmission of *Onchocerca volvulus*. *Tropical Medicine and Parasitology*, **40,** 367-374.

RODGER, F. C. (1960). The pathogenesis and pathology of ocular onchocerciasis. *American Journal of Ophthalmology*, **49,** 104-594.

ROSS, D. H. & MERRITT, R. W. (1978). The larval instars and population dynamics of five species of blackflies (Diptera: Simuliidae) and their responses to selected environmental factors. *Canadian Journal of Zoology,* **56,** 1633-1642.

ROUGEMONT, A., THYLEFORS, B., DUNCAN, M., PROST, A. & RANQUE, P H. (1980). Traitement de l'onchocercose par la suramine a faibles doses progressives dans le collectivites hyperendemiques d'Afrique occidentale. I. Resultats parasitologiques et surveillance ophthalmologique en zone de transmission non interrompue *Bulletin of the World Health Organisation*, **58,** 919-922

ROUGEMONT, A., HIEN, M., THYLEFORS, B , PROST, A & ROLLAND, A (1984). Traitement de l'onchocercose par la suramine a faibles doses progressive dans les collectivites hyperende uniques d'Afrique occidentale. 2. Resultats cliniques parasitologique et ophthalmologique en zone de transmission controlee. *Bulletin of the World Health Organisation*, **62,** 261-269.

RUBTZOV, I. A. (1969). Man and the blood sucking flies On the characteristics of their interrelationships in historical times *Memorie Soc Ent. Ital.* **48,** 263-268

RUTTNER, F (1926). Bemerkungen uber den Sauerstoffgehalt der Gewasser und dessen respiratorischen Wert. *Naturwissenschaften,* **14,** 1237-1239

SCHWARTZ, J. L., GARNEAU, L., SAVARIA, D., MASSON, L., BROUSSEAU, R. & ROUSSEAU, E. (1993). Lepidopteran-specific crystal toxins from *Bacillus thuringiensis* from cation- and anion-selectivein planar lipid bilayers. *Journal of Membrane Biology*, **132,** 53-62.

SHAPIRO, J. (1957). Chemical and biological studies on the yellow organic acids of lake water. *Limnology and Oceanography*, **2,** 161-179.

SLATIN, S. L., ABRAMS, C. K. & ENGLISH, L. (1990). Delta-endotoxins form cation-selective channels in planar lipid bilayers. *Biochemistry and Biophysics Research Commentary*, **169**, 765-772.

STONE, A. (1965). Family Simuliidae. In: A catalogue of the Diptera of America North of Mexico. *Agricultural Handbook*, **276,** 181-189.

TAYLOR, H. R., MURPHY, R. P., NEWLAND, H. S., WHITE, A. T., D'ANNA, S. A., KEYVAN-LARIJANI, E., AZIZ, M. A., CUPP, E. W. & GREENE, B. M. (1986). Treatment of onchocerciasis. The ocular effects of ivermectin and diethylcarbamazine. *Archives of Ophthalmology*, **104,** 863-870.

UNDEEN, A. H. (1979). Simuliid larval midgut pH and its implications for control. *Mosquito News*, **39,** 391-392.

VAN RIE, J., JANSENS, S., HOFTE, H., DEGHEELE, D. & VAN MELLAERT, H. (1989). Specificity of *Bacillus thuringiensis* delta-endotoxins *European Journal of Biochemistry*, **186,** 239-247.

VANKOVA, J., WEISER, J., RYTIR, V. & HOFMAN, J. (1978). The pathogenicity of a strain of *Bacillus thuringiensis* var. *israelensis* to mosquito larvae. *Proceedings of the International Colloquim on Invertebrate Pathology,*Prague, 1978, pp219-222.

VULCANO, M. A. (1967). Family Simuliidae. In: A catalogue of the Diptera of the Americas south of the United States, *Departamento de Zoologia, Secretaria de Agricultura*, Sao Paolo, **16,** 44pp.

WALSH, J. F. (1990). Review of vector control prior to the OCP. *Acta Leidensia*, **59,** 61-78.

WHITE, A. T., NEWLAND, H. S., TAYLOR, H. R., ERTMANN, C. D., KEYVAN-LARIJANI, E., NARA, A., AZIZ, M. A., D'ANNA, S. A., WILLIAMS, P. N. & GREENE, B. M. (1987). Controlled trial and dose-finding study of ivermectin for treatment of onchocerciasis. *Journal of Infectious Diseases*, **156,** 463-470.

WOLFERSBERGER, M. G. (1990). The toxicity of two *Bacillus thuringiensis* delta-endotoxins to gypsy moth larvae is inversely related to the affinity of binding sites on midgut brush border membranes for the toxins. *Experientia,* **46,** 475-477.

WORLD HEALTH ORGANISATION (1963). Insecticide resistance and vector control. Thirteenth Report of the WHO Expert Committee on Insecticides. *World Health Organisation Technical Report Series*, **265,** 51-61.

WORLD HEALTH ORGANISATION (1979). Report of a WHO meeting on Standardisation and Industrial Development of Microbial Control Agents, New Orleans, USA, 29 January to 2 February 1979. *WHO Mimeographed Document, TDR/BCV/79.01*, 23pp.

WORLD HEALTH ORGANISATION (1987) WHO Expert Committee on onchocerciasis. Third report. *World Health Organisation Technical Report Series*, **752,** 1-167

WORLD HEALTH ORGANISATION (1994) Onchocerciasis Control Programme. Expert Advisory Committee. Report of the 18th session *Document, OCP/EAC15.3,*. Ouagadougou.

WORLD HEALTH ORGANISATION (1995) WHO Expert Committee on onchocerciasis Control. *World Health Organisation Technical Report Series*, **852,** 1-105.

APPENDICES

Appendix 1

Results of field data showing the lethal concentrations (LC_{95} & LC_{99}).

	seqno	lotno	testdate	file_id	conc	log_conc
1	1	12AS/26-398-N9	27.02.97	V970227E	240	2 380
2	2	12AS/26-398-N9	27.02.97	V970227E	120	2.079
3	3	12AS/26-398-N9	27.02.97	V970227E	60	1 778
4	4	12AS/26-398-N9	27.02 97	V970227E	30	1 477
5	5	12AS/26-398-N9	27.02.97	V970227E	15	1 176
6	1	12AS/26-398-N9	26 02 97	V970226E	240	2 380
7	2	12AS/26-398-N9	26.02.97	V970226E	120	2 079
8	3	12AS/26-398-N9	26.02.97	V970226E	60	1 778
9	4	12AS/26-398-N9	26.02 97	V970226E	30	1 477
10	5	12AS/26-398-N9	26.02.97	V970226E	15	1.176
11	1	12AS/26-398-N9	28 02 97	V970228E	240	2 380
12	2	12AS/26-398-N9	28.02.97	V970228E	120	2 079
13	3	12AS/26-398-N9	28.02.97	V970228E	60	1 778
14	4	12AS/26-398-N9	28.02.97	V970228E	30	1.477
15	5	12AS/26-398-N9	28.02.97	V970228E	15	1 176
16	1	12AS/26-398-N9	25.02.97	V970225E	480	2 681
17	2	12AS/26-398-N9	25.02.97	V970225E	240	2 380
18	3	12AS/26-398-N9	25.02.97	V970225E	120	2 079
19	4	12AS/26-398-N9	25.02 97	V970225E	60	1.778
20	5	12AS/26-398-N9	25 02 97	V970225E	30	1 477
21	1	12AS/26-398-N9	23 09 97	V970923A	360	2 556
22	2	12AS/26-398-N9	23.09.97	V970923A	240	2.380
23	3	12AS/26-398-N9	23.09 97	V970923A	120	2 079
24	4	12AS/26-398-N9	23.09.97	V970923A	60	1.778
25	5	12AS/26-398-N9	23.09.97	V970923A	30	1 477
26	1	12AS/26-398-N9	24.09.97	V970924A	360	2.556
27	2	12AS/26-398-N9	24 09 97	V970924A	240	2.380
28	3	12AS/26-398-N9	24.09 97	V970924A	120	2 079
29	4	12AS/26-398-N9	24.09 97	V970924A	60	1.778
30	5	12AS/26-398-N9	24.09.97	V970924A	30	1 477
31	1	12AS/26-398-N9	25.09.97	V970925A	360	2 556
32	2	12AS/26-398-N9	25 09 97	V970925A	240	2 380

	notested	nokilled	chisqu	temp	ph	condu_ty	turbidty
1	154	154	04	29-30	8 10	1999 0	7 33
2	160	158	.44	29-30	8.10	1999 0	7 33
3	146	139	2.40	29-30	8.10	1999.0	7.33
4	136	92	92	29-30	8 10	1999.0	7 33
5	195	38	91	29-30	8 10	1999 0	7.33
6	149	147	1 24	30-31	8 10	1732 0	6 03
7	130	127	93	30-31	8 10	1732 0	6 03
8	131	116	1.69	30-31	8 10	1732 0	6 03
9	114	71	.13	30-31	8 10	1732.0	6.03
10	161	14	2.99	30-31	8 10	1732.0	6 03
11	91	90	.50	29-29	8 10	1999 0	7 21
12	144	139	.06	29-29	8 10	1999 0	7 21
13	97	93	5 50	29-29	8 10	1999 0	7 21
14	228	162	32	29-29	8 10	1999 0	7 21
15	208	66	10 95	29-29	8 10	1999 0	7 21
16	133	133	.74	29.5-31	8.30	778.0	5 28
17	126	126	.32	29.5-31	8 30	778.0	5.28
18	132	132	.73	29.5-31	8 30	778 0	5.28
19	128	128	90	29 5-31	8 30	778 0	5 28
20	153	153	6 44	29.5-31	8 30	778 0	5 28
21	60	60	21	29.5	8 40	155 4	73 00
22	61	59	43	29.5	8 40	155 4	73 00
23	55	39	4 21	29.5	8 40	155 4	73 00
24	27	8	1.37	29.5	8 40	155 4	73 00
25	30	5	2.55	29.5	8 40	155 4	73 00
26	45	45	.23	28.5	8 20	166 8	71 50
27	40	38	1.15	28.5	8 20	166 8	71.50
28	38	27	4.22	28.5	8 20	166 8	71 50
29	51	26	.11	28.5	8.20	166 8	71.50
30	35	7	.70	28.5	8 20	166 8	71 50
31	35	35	30	29.5	8 60	156 7	69.20
32	51	46	4 22	29 5	8 60	156 7	69 20

	riv_temp	flowrate	killrate	lc50	lc95	lc99
1	30.0	.30	100.00	24.16	66.55	101.30
2	30 0	.30	98.75	24.16	66.55	101.30
3	30.0	.30	95.21	24.16	66.55	101.30
4	30 0	.30	67.64	24.16	66.55	101.30
5	30.0	.30	19.48	24.16	66 55	101 30
6	31.0	.30	98.66	24.39	105.70	194.00
7	31.0	.30	97.69	24.39	105.70	194.00
8	31.0	.30	88.55	24.39	105.70	194.00
9	31.0	.30	62.27	24.39	105.70	194.00
10	31.0	.30	25.45	24.39	105.70	194.00
11	30.0	.30	98 90	20.36	88.29	162.20
12	30.0	.30	96.53	20.36	88 29	162 20
13	30.0	.30	95.88	20 36	88 29	162.20
14	30.0	.30	71.05	20 36	88.29	162 20
15	30.0	.30	31.72	20.36	88.29	162.20
16	31.0	.30	99.25	16.92	118 80	266.30
17	31.0	.30	99.21	16.92	118.80	266.30
18	31.0	.30	96.97	16.92	118.80	266.30
19	31.0	.30	90.62	16.92	118 80	266 30
20	31.0	.30	65.36	16.92	118.80	266 30
21	29.0	.30	100.00	73 38	266 60	455 00
22	29.0	.30	96.72	73.38	266 60	455.00
23	29.0	.30	70.91	73.38	266 60	455 00
24	29.0	.30	29.63	73.38	266 60	455.00
25	29.0	.30	16.67	73.38	266 60	455 00
26	29.0	.30	100.00	63.74	295 80	558 80
27	29.0	.30	95.00	63 74	295 80	558 80
28	29.0	30	71.05	63.74	295 80	558 80
29	29.0	.30	50.98	63.74	295.80	558.80
30	29 0	30	20.00	63.74	295.80	558.80
31	29.0	30	100.00	91.88	374 60	670 50
32	29 0	30	90.09	91.88	374.60	670 50

	seqno	lotno	testdate	file_id	conc	log_conc
33	3	12AS/26-398-N9	25 09 97	V970925A	120	2.079
34	4	12AS/26-398-N9	25.09 97	V970925A	60	1.778
35	5	12AS/26-398-N9	25 09 97	V970925A	30	1.477
36	1	12AS/26-398-N9	21.05 97	V970521D	360	2.556
37	2	12AS/26-398-N9	21.05.97	V970521D	240	2.380
38	3	12AS/26-398-N9	21 05 97	V970521D	120	2.079
39	4	12AS/26-398-N9	21.05 97	V970521D	60	1 778
40	5	12AS/26-398-N9	21 05 97	V970521D	30	1.447
41	1	12AS/26-398-N9	20 05 97	V970520A	360	2.556
42	2	12AS/26-398-N9	20 05 97	V970520A	240	2.380
43	3	12AS/26-398-N9	20.05 97	V970520A	120	2.079
44	4	12AS/26-398-N9	20.05 97	V970520A	60	1.778
45	5	12AS/26-398-N9	20 05 97	V970520A	30	1.477
46	1	12AS/26-398-N9	20.05 97	V970520D	360	2 556
47	2	12AS/26-398-N9	20 05 97	V970520D	240	2 380
48	3	12AS/26-398-N9	20.05 97	V970520D	120	2.079
49	4	12AS/26-398-N9	20 05 97	V970520D	60	1 778
50	5	12AS/26-398-N9	20.05.97	V970520D	30	1.477
51	1	12AS/26-398-N9	21.05.97	V970521A	360	2.556
52	2	12AS/26-398-N9	21 05 97	V970521A	240	2.380
53	3	12AS/26-398-N9	21 05 97	V970521A	120	2.079
54	4	12AS/26-398-N9	21 05 97	V970521A	60	1 778
55	5	12AS/26-398-N9	21 05 97	V970521A	30	1.477
56	1	12AS/26-398-N9	22.05 97	V970522A	360	2.556
57	2	12AS/26-398-N9	22.05.97	V970522A	240	2 380
58	3	12AS/26-398-N9	22.05.97	V970522A	120	2.079
59	4	12AS/26-398-N9	22 05 97	V970522A	60	1.778
60	5	12AS/26-398-N9	22.05 97	V970522A	30	1 477
61	1	12AS/26-398-N9	22 05 97	V970522D	360	2.556
62	2	12AS/26-398-N9	22 05 97	V970522D	240	2.380
63	3	12AS/26-398-N9	22.05.97	V970522D	120	2 079
64	4	12AS/26-398-N9	22.05.97	V970522D	60	1.778

	notested	nokilled	chisqu	temp	ph	condu_ty	turbidty
33	45	29	1.75	29.5	8.60	156.7	69.20
34	23	5	95	29.5	8.60	156.7	69.20
35	26	3	1.68	29.5	8.60	156.7	69.20
36	219	189	4.34	26.5-27	8.20	141.6	21.10
37	185	161	5.94	26.5-27	8.20	141.6	21.10
38	166	87	.09	26.5-27	8.20	141.6	21.10
39	151	26	1.96	26.5-27	8.20	141.6	21.10
40	139	9	.15	26.5-27	8.20	141.6	21.10
41	139	134	2.43	26-28	8.20	136.6	13.60
42	133	105	2.53	26-28	8.20	136.6	13.60
43	131	69	.84	26-28	8.20	136.6	13.60
44	114	17	6.67	26-28	8.20	136.6	13.60
45	157	20	8.58	26-28	8.20	136.6	13.60
46	134	123	.19	26-28	8.20	136.6	13.60
47	149	133	4.29	26-28	8.20	136.6	13.60
48	151	69	3.36	26-28	8.20	136.6	13.60
49	123	18	3.38	26-28	8.20	136.6	13.60
50	133	10	2.09	26-28	8.20	136.6	13.60
51	178	166	.01	26.5-27	8.20	141.6	21.10
52	195	163	.00	26.5-27	8.20	141.6	21.10
53	151	81	.14	26.5-27	8.20	141.6	21.10
54	166	41	14	26.5-27	8.20	141.6	21.10
55	142	8	.00	26.5-27	8.20	141.6	21.10
56	121	119	84	27-27.5	8.20	146.7	15.00
57	144	126	1.36	27-27.5	8.20	146.7	15.00
58	180	98	5.96	27-27.5	8.20	146.7	15.00
59	194	49	.16	27-27.5	8.20	146.7	15.00
60	133	10	95	27-27.5	8.20	146.7	15.00
61	173	170	1.38	27-27.5	8.20	146.7	15.00
62	78	68	84	27-27.5	8.20	146.7	15.00
63	121	71	1.91	27-27.5	8.20	146.7	15.00
64	131	30	2.66	27-27.5	8.20	146.7	15.00

	riv_temp	flowrate	killrate	lc50	lc95	lc99
33	29.0	.30	64.07	91.88	374.60	670.50
34	29.0	.30	20.92	91.88	374.60	670.50
35	29.0	.30	10.61	91.88	374.60	670.50
36	26.0	.30	86.30	118.80	503.60	916.10
37	26.0	.30	87.02	118.80	503.60	916.10
38	26.0	.30	52.40	118.80	503.60	916.10
39	26.0	.30	17.20	118.80	503.60	916.10
40	26.0	.30	6.45	118.80	503.60	916.10
41	29.0	.30	96.40	105.60	513.40	988.60
42	29.0	.30	78.94	105.60	513.40	988.60
43	29.0	.30	52.66	105.60	513.40	988.60
44	29.0	.30	14.90	105.60	513.40	988.60
45	29.0	.30	12.73	105.60	513.40	988.60
46	29.0	.30	91.79	115.40	428.40	737.60
47	29.0	.30	89.26	115.40	428.40	737.60
48	29.0	.30	45.69	115.40	428.40	737.60
49	29.0	.30	14.62	115.40	428.40	737.60
50	29.0	.30	7.51	115.40	428.40	737.60
51	26.0	.30	93.26	108.00	414.30	723.10
52	26.0	.30	83.59	108.00	414.30	723.10
53	26.0	.30	53.63	108.00	414.30	723.10
54	26.0	.30	24.68	108.00	414.30	723.10
55	26.0	.30	5.61	108.00	414.30	723.10
56	29.0	.30	98.35	101.00	363.80	618.70
57	29.0	.30	87.50	101.00	363.80	618.70
58	29.0	.30	54.43	101.00	363.80	618.70
59	29.0	.30	25.24	101.00	363.80	618.70
60	29.0	.30	7.49	101.00	363.80	618.70
61	29.0	.30	98.27	95.29	347.60	594.10
62	29.0	.30	87.18	95.29	347.60	594.10
63	29.0	.30	58.67	95.29	347.60	594.10
64	29.0	.30	22.89	95.29	347.60	594.10

	seqno	lotno	testdate	file_id	conc	log_conc
65	5	12AS/26-398-N9	22.05.97	V970522D	30	1.477
66	1	12AS/26-398-N9	23.05.97	V970523A	360	2.556
67	2	12AS/26-398-N9	23.05.97	V970523A	240	2.380
68	3	12AS/26-398-N9	23.05.97	V970523A	120	2.079
69	4	12AS/26-398-N9	23.05.97	V970523A	60	1.778
70	5	12AS/26-398-N9	23.05.97	V970523A	30	1.477
71	1	12AS/26-398-N9	23.05.97	V970523D	360	2.556
72	2	12AS/26-398-N9	23.05.97	V970523D	240	2.380
73	3	12AS/26-398-N9	23.05.97	V970523D	120	2.079
74	4	12AS/26-398-N9	23.05.97	V970523D	60	1.778
75	5	12AS/26-398-N9	23.05.97	V970523D	30	1.477
76	1	12AS/26-398-N9	24.05.97	V970524A	360	2.556
77	2	12AS/26-398-N9	24.05.97	V970524A	240	2.380
78	3	12AS/26-398-N9	24.05.97	V970524A	120	2.079
79	4	12AS/26-398-N9	24.05.97	V970524A	60	1.778
80	5	12AS/26-398-N9	24.05.97	V970524A	30	1.477
81	1	12AS/26-398-N9	24.05.97	V970524D	480	2.681
82	2	12AS/26-398-N9	24.05.97	V970524D	240	2.380
83	3	12AS/26-398-N9	24.05.97	V970524D	120	2.079
84	4	12AS/26-398-N9	24.05.97	V970524D	60	1.778
85	5	12AS/26-398-N9	24.05.97	V970524D	30	1.477
86	1	12AS/26-398-N9	25.05.97	V970525A	360	2.556
87	2	12AS/26-398-N9	25.05.97	V970525A	240	2.380
88	3	12AS/26-398-N9	25.05.97	V970525A	120	2.079
89	4	12AS/26-398-N9	25.05.97	V970525A	60	1.778
90	5	12AS/26-398-N9	25.05.97	V970525A	30	1.477
91	1	12AS/26-398-N9	25.05.97	V970525D	360	2.556
92	2	12AS/26-398-N9	25.05.97	V970525D	240	2.380
93	3	12AS/26-398-N9	25.05.97	V970525D	120	2.079
94	4	12AS/26-398-N9	25.05.97	V970525D	60	1.778
95	5	12AS/26-398-N9	25.05.97	V970525D	30	1.477
96	1	12AS/26-398-N9	20.01.98	V980120A	360	2.556

	notested	nokilled	chisqu	temp	ph	condu_ty	turbidty
65	90	10	2.06	27-27 5	8 20	146 7	15 00
66	84	81	46	27	8 20	138 1	15 50
67	100	97	2 96	27	8 20	138 1	15.50
68	114	66	7 75	27	8 20	138 1	15.50
69	136	43	.54	27	8 20	138.1	15.50
70	135	16	.88	27	8 20	138 1	15.50
71	138	115	1.83	27	8 20	138 1	15 50
72	151	123	2 43	27	8 20	138 1	15 50
73	237	118	04	27	8 20	138 1	15 50
74	167	38	.00	27	8 20	138 1	15 50
75	157	10	.07	27	8 20	138 1	15.50
76	111	110	.07	27	8 10	114 0	15 10
77	139	134	.20	27	8 10	114 0	15 10
78	123	83	7 01	27	8 10	114 0	15 10
79	106	44	.01	27	8 10	114 0	15.10
80	99	15	.71	27	8 10	114 0	15 10
81	103	102	.07	27	8 10	114 0	15 10
82	76	70	.05	27	8 10	114.0	15.10
83	104	64	2.67	27	8 10	114 0	15.10
84	147	45	1.41	27	8 10	114 0	15 10
85	125	18	1.92	27	8 10	114 0	15 10
86	101	101	.22	26-26 5	8 20	132 8	13 30
87	153	148	3.83	26-26 5	8 20	132 8	13 30
88	154	124	3 59	26-26.5	8 20	132 8	13 30
89	156	69	.91	26-26.5	8.20	132.8	13.30
90	152	26	3.62	26-26.5	8.20	132 8	13.30
91	161	145	.38	26-26.5	8 20	132.8	13.30
92	119	104	1.32	26-26.5	8 20	132 8	13 30
93	142	80	1.77	26-26.5	8 20	132 8	13 30
94	109	41	24	26-26.5	8 20	132 8	13 30
95	131	19	.00	26-26.5	8 20	132 8	13.30
96	49	48	.24	29.5	8.40	169 1	60.10

	riv_temp	flowrate	killrate	lc50	lc95	lc99
65	29 0	30	11 10	95 29	347 60	594 10
66	28 0	30	96 43	87 10	336 20	588 30
67	28 0	30	97 00	87 10	336 20	588 30
68	28 0	.30	57 89	87 10	336 20	588 30
69	28 0	.30	31 61	87 10	336 20	588 30
70	28 0	.30	11 84	87 10	336 20	588.30
71	28 0	.30	83.33	121 90	588 40	1130 00
72	28 0	30	81 45	121 90	588 40	1130 00
73	28.0	.30	49.78	121 90	588 40	1130 00
74	28 0	.30	22 74	121.90	588 40	1130 00
75	28 0	.30	6.36	121 90	588 40	1130 00
76	29 0	.30	99.10	72 71	265.40	453 80
77	29.0	.30	96.40	72 71	265 40	453 80
78	29.0	.30	67 47	72.71	265 40	453 80
79	29.0	.30	41 50	72 71	265 40	453 80
80	29.0	.30	15.14	72 71	265 40	453 80
81	29.0	.30	99.03	85.00	350 60	630.70
82	29.0	.30	92.10	85.00	350 60	630.70
83	29.0	.30	61.53	85.00	350.60	630.70
84	29.0	.30	30 60	85.00	350.60	630.70
85	29.0	30	14 39	85.00	350.60	630.70
86	27.0	.30	100 00	64.01	231 20	393 60
87	27 0	.30	96 73	64 01	231 20	393 60
88	27.0	.30	80.52	64.01	231 20	393.60
89	27.0	30	44.22	64.01	231 20	393.60
90	27.0	.30	17.09	64.01	231 20	393.60
91	27 0	30	90.06	89.95	499 20	1015.00
92	27 0	30	87.39	89.95	499 20	1015.00
93	27 0	30	56 33	89.95	499 20	1015.00
94	27 0	30	37 61	89 95	499 20	1015 00
95	27 0	30	14.50	89 95	499.20	1015 00
96	28 0	30	97 96	64 81	230 00	388 80

	seqno	lotno	testdate	file_id	conc	log_conc
97	2	12AS/26-398-N9	20 01 98	V980120A	240	2 380
98	3	12AS/26-398-N9	20 01 98	V980120A	120	2 079
99	4	12AS/26-398-N9	20 01 98	V980120A	60	1 778
100	5	12AS/26-398-N9	20 01 98	V980120A	30	1 477
101	1	12AS/26-398-N9	21 01 98	V980121A	360	2 556
102	2	12AS/26-398-N9	21 01 98	V980121A	240	2 380
103	3	12AS/26-398-N9	21 01 98	V980121A	120	2 079
104	4	12AS/26-398-N9	21 01 98	V980121A	60	1 778
105	5	12AS/26-398-N9	21 01 98	V980121A	30	1 477
106	1	12AS/26-398-N9	22 01 98	V980122A	360	2 556
107	2	12AS/26-398-N9	22 01 98	V980122A	240	2 380
108	3	12AS/26-398-N9	22 01 98	V980122A	120	2 079
109	4	12AS/26-398-N9	22 01 98	V980122A	60	1 778
110	5	12AS/26-398-N9	22 01 98	V980122A	30	1 477
111	1	12AS/26-398-N9	17 04 98	V980417F	240	2 380
112	2	12AS/26-398-N9	17 04 98	V980417F	120	2 079
113	3	12AS/26-398-N9	17 04 98	V980417F	60	1 778
114	4	12AS/26-398-N9	17 04 98	V980417F	30	1 477
115	5	12AS/26-398-N9	17 04 98	V980417F	15	1 176
116	1	12AS/26-398-N9	16 04 98	V980416F	360	2 556
117	2	12AS/26-398-N9	16 04 98	V980416F	240	2 380
118	3	12AS/26-398-N9	16 04 98	V980416F	120	2 079
119	4	12AS/26-398-N9	16 04 98	V980416F	60	1 778
120	5	12AS/26-398-N9	16 04 98	V980416F	30	1 477
121	1	12AS/26-398-N9	15 04 98	V980415F	360	2 556
122	2	12AS/26-398-N9	15 04 98	V980415F	240	2 380
123	3	12AS/26-398-N9	15 04 98	V980415F	120	2 079
124	4	12AS/26-398-N9	15 04 98	V980415F	60	1 778
125	5	12AS/26-398-N9	15 04 98	V980415F	30	1 477
126	1	12AS/26-398-N9	14 04 98	V980414F	360	2 556
127	2	12AS/26-398-N9	14 04 98	V980414F	240	2 380
128	3	12AS/26-398-N9	14 04 98	V980414F	120	2 079

	notested	nokilled	chisqu	temp	ph	condu_ty	turbidty
97	90	88	89	29.5	8.40	169.1	60.10
98	78	64	17	29.5	8.40	169.1	60.10
99	94	35	5.13	29.5	8.40	169.1	60.10
100	54	12	.55	29.5	8.40	169.1	60.10
101	52	52	.08	28	8.40	184.6	59.60
102	69	68	.08	28	8.40	184.6	59.60
103	40	35	.01	28	8.40	184.6	59.60
104	59	27	.93	28	8.40	184.6	59.60
105	51	9	.49	28	8.40	184.6	59.60
106	64	62	1.11	24	8.30	193.0	55.60
107	53	51	.26	24	8.30	193.0	55.60
108	38	30	.36	24	8.30	193.0	55.60
109	43	22	2.72	24	8.30	193.0	55.60
110	65	4	1.54	24	8.30	193.0	55.60
111	143	142	.01	32	8.60	281.0	5.39
112	151	147	1.64	32	8.60	281.0	5.39
113	180	126	8.99	32	8.60	281.0	5.39
114	155	69	1.03	32	8.60	281.0	5.39
115	99	23	1.19	32	8.60	281.0	5.39
116	64	64	.10	32	8.10	285.0	4.54
117	67	66	.64	32	8.10	285.0	4.54
118	59	57	.37	32	8.10	285.0	4.54
119	60	42	2.73	32	8.10	285.0	4.54
120	62	34	.98	32	8.10	285.0	4.54
121	71	70	.00	32	8.10	274.0	4.18
122	68	65	.09	32	8.10	274.0	4.18
123	88	77	.21	32	8.10	274.0	4.18
124	59	40	.39	32	8.10	274.0	4.18
125	71	23	.40	32	8.10	274.0	4.18
126	36	36	.03	32	8.20	323.0	5.25
127	55	55	.16	32	8.20	323.0	5.25
128	49	47	1.11	32	8.20	323.0	5.25

	riv_temp	flowrate	killrate	lc50	lc95	lc99
97	28.0	.30	97 78	64 81	230 00	388.80
98	28 0	30	82 05	64 81	230 00	388 80
99	28.0	30	37 22	64 81	230 00	388 80
100	28 0	.30	22 21	64.81	230 00	388 80
101	29.0	.30	100.00	59.35	176 50	277 20
102	29.0	.30	98.55	59 35	176 50	277 20
103	29.0	.30	87.50	59 35	176 50	277 20
104	29.0	.30	45.76	59.35	176 50	277 20
105	29.0	.30	17.64	59 35	176 50	277 20
106	29.0	.30	96.88	71.12	250 70	422 60
107	29.0	.30	96.23	71.12	250 70	422 60
108	29.0	.30	78.95	71.12	250 70	422.60
109	29.0	.30	51.16	71 12	250 70	422 60
110	29.0	.30	6.15	71 12	250 70	422 60
111	33.0	.30	99.30	33 09	143 90	264 50
112	33.0	.30	97.35	33 09	143 90	264 50
113	33.0	.30	70.00	33 09	143 90	264 50
114	33.0	.30	44.51	33.09	143 90	264 50
115	33.0	.30	23.22	33 09	143 90	264 50
116	32.0	.30	100.00	29 32	151 10	297 90
117	32.0	.30	98.51	29.32	151 10	297 90
118	32.0	.30	96.61	29 32	151 10	297 90
119	32.0	.30	70.00	29 32	151 10	297 90
120	32.0	.30	54.84	29.32	151 10	297 90
121	32.0	30	98.59	42.92	201.40	382.20
122	32.0	30	95.59	42.92	201.40	382.20
123	32.0	.30	87.50	42.92	201 40	382.20
124	32.0	.30	67.80	42.92	201 40	382 20
125	32.0	30	32.39	42.92	201 40	382 20
126	32 0	30	100.00	14 83	97 36	212 30
127	32 0	30	100.00	14.83	97.36	212 30
128	32.0	30	95.92	14 83	97 36	212.30

	seqno	lotno	testdate	file_id	conc	log_conc
129	4	12AS/26-398-N9	14 04 98	V980414F	60	1.778
130	5	12AS/26-398-N9	14 04 98	V980414F	30	1 477
131						
132						
133						
134						
135						
136						
137						
138						
139						
140						
141						
142						
143						
144						
145						
146						
147						
148						
149						
150						
151						
152						
153						
154						
155						
156						
157						
158						
159						
160						

	notested	nokilled	chisqu	temp	ph	condu_ty	turbidty
129	37	35	55	32	8.20	323.0	5.25
130	27	19	17	32	8.20	323.0	5.25
131							
132							
133							
134							
135							
136							
137							
138							
139							
140							
141							
142							
143							
144							
145							
146							
147							
148							
149							
150							
151							
152							
153							
154							
155							
156							
157							
158							
159							
160							

Appendix II

RESULTS SHEET OF WATER QUALITY ANALYSES

Sample source: Bosomase, R. Pra **Date of Analysis:** March, 1997

Parameter	Formula	Units	Value	Notes WHO guideline
Alkalinity		Mg/l	71.15	-
Total Hardness	As $CaCO_3$	Mg/l	65.15	500
Total Dissolved solids		Mg/l	95	1000
Suspended solids		Mg/l	15.7	-
Total solids		Mg/l	110.7	-
Calcium	Ca	Mg/l	24.91	200
Sodium	Na	Mg/l	804	200
Potassium	K	Mg/l	42.36	30
Magnesium	Mg	Mg/l	4.2	150
Manganese	Mn	Mg/l	<0.03	0.1
Iron	Fe	Mg/l	2.6	0.3
Chloride	Cl	Mg/l	20.1	250
Sulphate	SO_4	Mg/l	1.1	400
Bi-carbonate	HCO_3	Mg/l	63.5	
Flouride	F	Mg/l	4.11	1.5
Silica	SiO_2	Mg/l	3.2	
Ammonium	NH_4-N	Mg/l	0.51	
Nitrate	NO_3-N	Mg/l	0.41	10
Nitrite	NO_2-N	Mg/l	0.10	-
Phosphate	PO_4-P	Mg/l	0.36	-
Cadmium	Cd	Mg/l	<0.03	-
Lead	Pb	Mg/l	<0.03	0.05
Zinc	Zn	Mg/l	<0.03	5.0
Nickel	Ni	Mg/l	<0.03	
Chromium	Cr	Mg/l	<0.03	
Ca^{2+} Hardness	As $CaCO_3$	Mg/l	39.51	
Mg^{2+} Hardness	As $CaCO_3$	Mg/l	29.10	

RESULTS SHEET OF WATER QUALITY ANALYSES

Sample source: Bosomase, R. Pra **Date of Analysis:** April, 1997

Parameter	Formula	Units	Value	Notes WHO guideline
Alkalinity		Mg/l	60.21	-
Total Hardness	As $CaCO_3$	Mg/l	60.20	500
Total Dissolved solids		Mg/l	101.2	1000
Suspended solids		Mg/l	18.2	-
Total solids		Mg/l	119.4	
Calcium	Ca	Mg/l	16.21	200
Sodium	Na	Mg/l	16.17	200
Potassium	K	Mg/l	10.56	30
Magnesium	Mg	Mg/l	1.83	150
Manganese	Mn	Mg/l	<0.03	0.1
Iron	Fe	Mg/l	2.8	0.3
Chloride	Cl	Mg/l	3.2	250
Sulphate	SO_4	Mg/l	1.4	400
Bi-carbonate	HCO_3	Mg/l	60 1	-
Flouride	F	Mg/l	4 01	1.5
Silica	SiO_2	Mg/l	1.10	-
Ammonium	NH_4-N	Mg/l	<0 03	
Nitrate	NO_3-N	Mg/l	0.35	10
Nitrite	NO_2-N	Mg/l	0.09	
Phosphate	PO_4-P	Mg/l	0.27	-
Cadmium	Cd	Mg/l	<0 03	-
Lead	Pb	Mg/l	<0.03	0.05
Zinc	Zn	Mg/l	<0 03	5.0
Nickel	Ni	Mg/l	<0 03	-
Chromium	Cr	Mg/l	<0 03	-
Ca^{2+} Hardness		Mg/l	40 11	-
Mg^{2+} Hardness		Mg/l	21 36	

RESULTS SHEET OF WATER QUALITY ANALYSES

Sample source: Bosomase, R. Pra **Date of Analysis**: May, 1997

Parameter	Formula	Units	Value	Notes WHO guideline
Alkalinity		Mg/l	51.20	
Total Hardness	As $CaCO_3$	Mg/l	41.50	500
Total Dissolved solids		Mg/l	119.1	1000
Suspended solids		Mg/l	32.1	
Total solids		Mg/l	151.2	
Calcium	Ca	Mg/l	15.03	200
Sodium	Na	Mg/l	10.10	200
Potassium	K	Mg/l	6.91	30
Magnesium	Mg	Mg/l	1.02	150
Manganese	Mn	Mg/l	<0.03	0.1
Iron	Fe	Mg/l	3.2	0.3
Chloride	Cl	Mg/l	2.1	250
Sulphate	SO_4	Mg/l	0.95	400
Bi-carbonate	HCO_3	Mg/l	57.0	-
Flouride	F	Mg/l	3.97	1.5
Silica	SiO_2	Mg/l	0.86	
Ammonium	NH_4-N	Mg/l	0.36	-
Nitrate	NO_3-N	Mg/l	0.24	10
Nitrite	NO_2-N	Mg/l	0.05	
Phosphate	PO_4-P	Mg/l	0.11	
Cadmium	Cd	Mg/l	<0.03	-
Lead	Pb	Mg/l	<0.03	0.05
Zinc	Zn	Mg/l	<0.03	5.0
Nickel	Ni	Mg/l	<0.03	
Chromium	Cr	Mg/l	<0.03	-
Ca^{2+} Hardness		Mg/l	39.15	
Mg^{2+} Hardness		Mg/l	11.51	-

RESULTS SHEET OF WATER QUALITY ANALYSES

Sample source: Bosomase, R. Pra **Date of Analysis:** April , 1998

Parameter	Formula	Units	Value	Notes WHO guideline
Alkalinity		Mg/l	54.0	
Total Hardness	As $CaCO_3$	Mg/l	44.0	500
Total Dissolved solids		Mg/l	118.0	1000
Suspended solids		Mg/l	48.0	
Total solids		Mg/l	166.0	
Calcium	Ca	Mg/l	14.67	200
Sodium	Na	Mg/l	7.18	200
Potassium	K	Mg/l	2.30	30
Magnesium	Mg	Mg/l	1.78	150
Manganese	Mn	Mg/l	<0.03	0.1
Iron	Fe	Mg/l	3.56	0.3
Chloride	Cl	Mg/l	4.8	250
Sulphate	SO_4	Mg/l	1.5	400
Bi-carbonate	HCO_3	Mg/l	65.9	
Flouride	F	Mg/l	4.57	1.5
Silica	SiO_2	Mg/l	1.03	
Ammonium	NH_4-N	Mg/l	<0.03	-
Nitrate	NO_3-N	Mg/l	0.19	10
Nitrite	NO_2-N	Mg/l	0.01	-
Phosphate	PO_4-P	Mg/l	<0.03	
Cadmium	Cd	Mg/l	<0.03	
Lead	Pb	Mg/l	<0.03	0.05
Zinc	Zn	Mg/l	<0.03	5.0
Nickel	Ni	Mg/l	<0.03	
Chromium	Cr	Mg/l	<0.03	
Ca^{2+} Hardness		Mg/l	36.67	
Mg^{2+} Hardness		Mg/l	7.33	-

RESULTS SHEET OF WATER QUALITY ANALYSES

Sample source: Bosomase, R. Pra **Date of Analysis:** May, 1998

Parameter	Formula	Units	Value	Notes WHO guideline
Alkalinity		Mg/l	56.0	
Total Hardness	As $CaCO_3$	Mg/l	54 4	500
Total Dissolved solids		Mg/l	208.0	1000
Suspended solids		Mg/l	19 0	-
Total solids		Mg/l	227.0	-
Calcium	Ca	Mg/l	14 03	200
Sodium	Na	Mg/l	8 14	200
Potassium	K	Mg/l	5 98	30
Magnesium	Mg	Mg/l	4.7	150
Manganese	Mn	Mg/l	<0.03	0.1
Iron	Fe	Mg/l	3 25	0.3
Chloride	Cl	Mg/l	9 6	250
Sulphate	SO_4	Mg/l	1 81	400
Bi-carbonate	HCO_3	Mg/l	68 3	-
Flouride	F	Mg/l	4 69	1 5
Silica	SiO_2	Mg/l	0 74	
Ammonium	NH_4-N	Mg/l	0 46	-
Nitrate	NO_3-N	Mg/l	0 23	10
Nitrite	NO_2-N	Mg/l	0 04	
Phosphate	PO_4-P	Mg/l	0 09	-
Cadmium	Cd	Mg/l	<0 03	-
Lead	Pb	Mg/l	<0 03	0 05
Zinc	Zn	Mg/l	<0.03	5.0
Nickel	Ni	Mg/l	<0.03	-
Chromium	Cr	Mg/l	<0 03	-
Ca^{2+} Hardness		Mg/l	35 08	-
Mg^{2+} Hardness		Mg/l	19 32	-

RESULTS SHEET OF WATER QUALITY ANALYSES

Sample source: Bosomase, R Pra **Date of Analysis:** July, 1998

Parameter	Formula	Units	Value	Notes WHO guideline
Alkalinity		Mg/l	80 0	
Total Hardness	As $CaCO_3$	Mg/l	75 20	500
Total Dissolved solids		Mg/l	166.0	1000
Suspended solids		Mg/l	22 0	-
Total solids		Mg/l	188.0	-
Calcium	Ca	Mg/l	19 16	200
Sodium	Na	Mg/l	7 72	200
Potassium	K	Mg/l	5 65	30
Magnesium	Mg	Mg/l	6 63	150
Manganese	Mn	Mg/l	<0 03	0 1
Iron	Fe	Mg/l	2 33	0 3
Chloride	Cl	Mg/l	9 0	250
Sulphate	SO_4	Mg/l	0 8	400
Bi-carbonate	HCO_3	Mg/l	97 6	-
Flouride	F	Mg/l	4.71	1 5
Silica	SiO_2	Mg/l	2.35	-
Ammonium	NH_4-N	Mg/l	0 49	-
Nitrate	NO_3-N	Mg/l	0 25	10
Nitrite	NO_2-N	Mg/l	0 04	-
Phosphate	PO_4-P	Mg/l	0 25	-
Cadmium	Cd	Mg/l	<0 03	-
Lead	Pb	Mg/l	<0 03	0 05
Zinc	Zn	Mg/l	<0 03	5 0
Nickel	Ni	Mg/l	<0 03	-
Chromium	Cr	Mg/l	<0 03	-
Ca^{2+} Hardness		Mg/l	47 90	-
Mg^{2+} Hardness		Mg/l	23 70	-