# **Environmental Assessment of Larvicide Use** D. Calamari, L. Yameogo, J-M./Hougard and C. Lévêque

The objective of the Onchocerciasis Control Programme (OCP) is to eliminate onchocerciasis as a disease of public importance and as an obstacle to socio-economic development. The OCP was initially based solely on the control of the blackfly vector, Simulium damnosum sensu lato, by insecticide spraying of the breeding sites on river systems, where larval stages develop. Results of monitoring the environmental effects and the process of risk assessment for new insecticides are reviewed. The achievements of this strategy are outlined here by Davide Calamari, Laurent Yameogo, Jean-Marc Hougard and Christian Leveque.

Onchocerciasis (river blindness) is caused by a filarial worm Onchocerca volvulus; the microfilariae live in the skin and cause debilitating skin lesions and, ultimately, blindness. West Africa used to be the most affected area in terms of the distribution and severity of clinical manifestations of the disease. Since 1974, successful control has been undertaken through the Onchocerciasis Control Programme (OCP)<sup>1-3</sup>.

The strategy of larviciding is designed to interrupt the transmission of the parasite for longer than the longevity of the adult worms in a human host (estimated to be ~14 years) by destroying larval stages of the vector through aerial application of insecticides at breeding sites. The development of the aquatic stage from egg to pupae is around one week, hence insecticide application is undertaken weekly. At the peak of larviciding activities, during the years 1986-1991, ~50000 km of river were treated in an area of over 1000000 km<sup>2</sup>.

Recently, the introduction of a microfilaricide (ivermectin) for mass chemotherapy suggests that the combined use of ivermectin and larviciding should reduce the required duration of vector control to 12 years<sup>4</sup>. Despite the introduction of ivermectin, and in the absence of a macrofilaricide, vector control remains the method of choice to achieve the goals of the OCP<sup>5</sup>.

Because prolonged and regular use of insecticides presents a potential risk to the aquatic environment, an ecological group was set up, prior to launching the OCP, in order to: (1) organize a long-term monitoring programme of the aquatic fauna; (2) identify criteria for the selection of pesticides for operational use, and determine the conditions by which insecticide use can be optimized in relation to season and environmental factors in different areas of the OCP; and (3) review the nature of the agricultural development process being undertaken and proposed in the areas liberated from onchocerciasis, and identify the environmental and human ecological implications of such development.

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Although methods, procedures and protocols for the monitoring and risk assessment programmes have evolved since 1974, with the OCP responding to continually changing situations (insect re-invasion, resistance, variability in hydrology, etc.), basic concepts remain. The monitoring activities conducted by National Hydrobiological Teams and the ecotoxicological research carried on by the Hydrobiological Unit of the OCP, which also coordinates and assists the field work in the OCP area, are reviewed annually.

The criteria for the evaluation of insecticide impact on aquatic environment include: (1) the vector control activities should not reduce the number of invertebrate species, or cause a marked shift in the relative abundance of species; (2) the pesticides applied should have neither a direct impact on fish, nor an effect on the life cycle of fish species; (3) bioaccumulation and biomagnification through food webs should be avoided; (4) human activities in the control area should not be impaired; and (5) temporary and seasonal variations in non-target invertebrate populations are acceptable.

A network of sampling stations exists throughout the OCP area where selection criteria have been met<sup>6</sup>. Initially, there were 40 sampling sites, but as the programme evolved together with the treatment strategy, the number has been reduced to ten for invertebrates and ten for fish (Fig. 1).

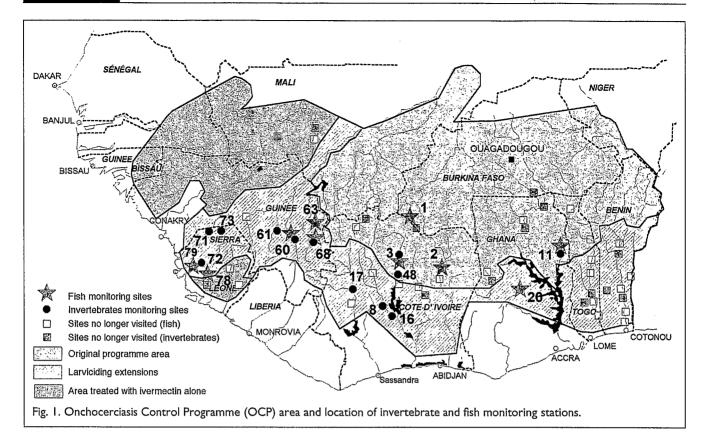
Details of the methods and sampling techniques for OCP monitoring programmes have been described<sup>7-9</sup>. It was realized, however, that information on the biology of the aquatic fauna in West Africa was generally limited, and so specific studies were undertaken to provide ecological baseline information.

#### Vector control

Vector control has faced three major obstacles<sup>5</sup>. (1) It was established that the border of the initial area (654 000 km<sup>2</sup> spread over seven countries) was invaded by migrating infective blackflies originating from outside the designated area. To protect the core area, vector control was extended (between 1986 and 1990) in the west and southeast, with two additional countries joining the OCP. (2) Five years after the beginning of the OCP, resistance of Simulium damnosum to temephos was detected<sup>10</sup>. This organophosphorous compound, the only insecticide used at the beginning of the programme, was selected because of its efficacy, its carry (distance over which it remains effective), its limited impact on non-target fauna and also its acceptable cost. After the development of insect resistance to another organophosphate (chlorphoxim) was detected, the OCP adopted a strategy of rotational use of different classes of insecticides, with different modes of action, to prevent development of further resistance. (3) Seven insecticides  $\frac{1}{2}$  are currently used<sup>5</sup>: six of them formulated as emulsifiable concentrates (temephos, phoxim, pyraclofos, permethrin, etofenprox and carbosulfan), while the seventh ica liquid concentrate of a biological insecticide,

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*Bacillus thuringiensis* H-14 (Bt-14). The rotational use of insecticides has been particularly effective, with only limited resistance to the organophosphates currently in use, while the susceptibility of the *Simulium* populations to other classes of compounds remains unchanged.

The consideration of environmental concerns has resulted in a number of limitations and restrictions in the use of the available insecticides for the rotational vector control.

#### Invertebrate and fish monitoring results

For the monitoring process, invertebrate data have been collected in each river and analysed independently, distinguishing, for each river, the three types of sampling technique adopted. Invertebrates collected directly from the river-bed by means of a Surber net (named invertebrate community, living exactly at the sampling station), day and night drift, where the nets capture animals transported by water (named invertebrate assemblages, which include organisms from upper river locations).

Numerical analysis methods to assess the variations in invertebrate community structure during the periods of treatment and during the periods when larviciding is suspended permit the evaluation of two principal properties of the biological communities: (1) their resistance (capacity of contrasting stress factors); and (2) their resilience (capacity to recover after stress).

The taxonomic levels as well as the functional feeding group (trophic role) of the invertebrates sampled have been determined; the analysis of the biological variation has been focused on these structural and functional attributes. The OCP concern is to prevent the loss of faunal biodiversity, and to maintain the quality of the biomass available for the higher trophic levels in the food webs, while conserving the stability of the energetic flows (quantity). As the invertebrate communities represent lower levels of the aquatic food webs, any change can be a signal of potential detrimental effects on the ecological characteristics of the whole river system.

On the basis of preliminary results, the following analyses were undertaken: (1) invertebrate taxonomic diversity; (2) relative abundance of functional groups; (3) rank abundance models; and (4) multivariate analysis<sup>11</sup>.

The taxonomic diversity indices calculated during pretreatment periods showed a normal structure of invertebrate communities. The result was obtained from relative abundance of functional groups and diversity indices and from rank abundance models.

During separate insecticide treatments, some rivers showed only limited changes in invertebrate community structure; in others, great changes occurred.

Individual larvicides have different impacts on river fauna, depending on the river system to which they are applied. Moreover, each insecticide shows a characteristic distribution of effects on various invertebrate taxa, thereby providing a 'fingerprint', because of selective action on particular groups of insects, according to the mode of action of different types of larvicides<sup>12</sup>.

Rotational use of the larvicides has led to difficulties in interpreting some biological data because of the additional effects related to previous treatment history. This is most frequently seen during a period of suspension, when the invertebrate recovery is related to the type and the severity of the stresses that occurred during past treatments.

A first survey of the impact of the larviciding campaign was made after the initial phase of the programme, when only temephos, chlorphoxim and Bt-14 were applied. This demonstrated an initial deleterious impact on non-target fauna during the first year (limited reduction in biomass and number of taxa). However, notwithstanding the continuation of the treatments, there was a partial recovery within a year. Long-term studies (several years) showed such types of insecticides had little impact<sup>6,7</sup>.

A recent evaluation of 20 years of OCP monitoring data<sup>11,13</sup>, for a period when different groups of chemical substances (eg. carbosulfan and permethrin) were \* used, showed greater effects of the insecticides on the invertebrate fauna, particularly the rarefaction of a few taxa and, for example, in the case of permethrin, a biomass reduction. However, the ability of the aquatic biota to recover was demonstrated, even if, at a slow rate. Therefore, the taxonomic and functional variability in non-target invertebrate fauna seems to be compatible with the range of biological variation that would normally occur in these river systems<sup>11,13</sup>.

In the normal situation, invertebrate communities are rarely constant in taxonomic and trophic composition because of natural stresses (eg. unusual hydrological conditions, such as drought and spate events), which occur frequently. When these factors are taken into consideration, the 'biological variations previously discussed are ecologically acceptable': a conclusion confirmed by analysis on fish monitoring<sup>14,15</sup>.

Fish populations at most monitoring sites show no evidence of a reduction in species richness after almost 20 years of larviciding in the original area of the programme, and seven years of larviciding in Guinea<sup>15</sup>. In an earlier evaluation<sup>6,16</sup>, the same conclusions were reached after ten years of larviciding with temephos, chlorphoxim and Bt H-14. Although the total number of species could mask changes in species composition, results from all rivers showed no loss of species after 20 years of larviciding.

Fish catches per unit of effort (ie. a standard time during which nets remain in the river) with a standardized set of gill nets has demonstrated a seasonal pattern, with high catches at the low water period (January – April) and lower catches in the wet season (August – November). Long-term, yearly fluctuations are well correlated to the hydrological pattern, as has been observed for African rivers<sup>17</sup>.

The coefficient of condition, a ratio between weight and length (corpulence), of the principal species of fish fluctuates around the expected means, which have not changed significantly during the programme. This suggests that larviciding has no effects on food availability, and that the feeding habits of fish have not altered<sup>18</sup>.

There has, therefore, been no evidence of fish mortality due to larviciding at the operational doses in the OCP area. However, fluctuations have been observed in the abundance of biomass and richness of fish species. These variations are correlated to hydrological patterns, and can also be attributed to the pressure exerted by local fisherman, in certain areas.

As a result of the first ten years monitoring the use of organophosphate insecticides and Bt-14, the Ecological Group decided to reduce the number of monitoring stations, and to change monitoring strategies by abandoning drift-sampling<sup>19</sup>.

Moreover, during the annual evaluation of the research on new insecticides (see below), and as derived from monitoring data, several means of reducing the impacts of new chemicals have been suggested. The following proposals have been made: (1) temephos and Bt-14 are considered as harmless for the environment and can be used without restrictions; (2) pyraclofos might

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show some toxicity (for example, in an accidental overdose) against the non-target fauna, particularly fish, and it is recommended that it be used at discharges above  $15 \text{ m}^3 \text{ s}^{-1}$  but without any restriction on the number of cycles; (3) permethrin and carbosulfan should only be used above 70 m<sup>3</sup> s<sup>-1</sup> and (if possible) for not more than six weeks per year on the same stretch of river; and (4) although the toxicity of etofenprox for fish and crustaceans is less than permethrin, its utilization should remain limited to discharges above  $15 \text{ m}^3 \text{ s}^{-1}$ , but without any restriction on the number of annual cycles.

For these reasons, the OCP has established a satellite transmission network for recording water discharge, allowing for in-time management of hydrological data in the treatment of the rivers<sup>20</sup>.

## **Risk assessment for new larvicides**

In addition to the above monitoring activities, the OCP has carried out a number of ecotoxicological studies on candidate larvicides.

Acute toxicological tests have been performed on African fish species according to standard protocols<sup>21</sup> to obtain original data on fish toxicology. Short-term impact on non-target invertebrates was also studied using a system of artificial gutters (semifield equipment). Qualitative and quantitative drift in such systems have been evaluated at different concentrations, including the operational dose, and the larvicides classified according to their general toxicity and the typology of the susceptibility of the principal taxa established<sup>22,23</sup>.

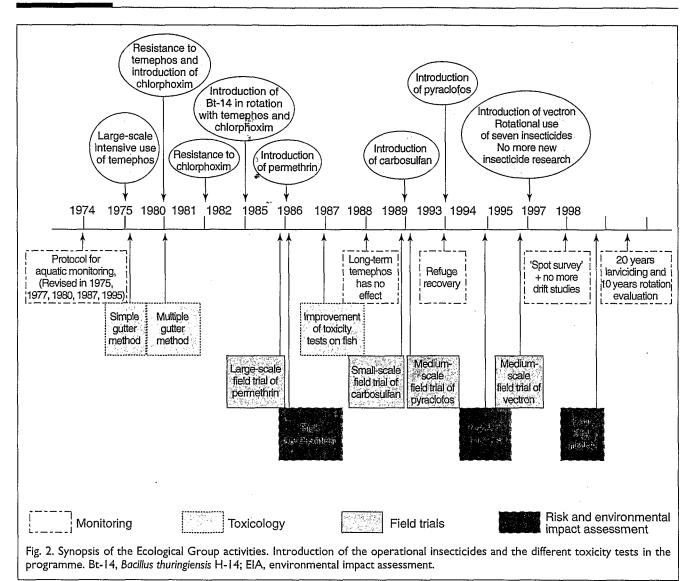
Bt-14 has proved to be the most selective and the least environmentally damaging larvicide of the seven. The other insecticides can be classified as follows, in increasing order of toxicity: temephos, chlorphoxim, pyraclofos, etophenprox, permethrin and carbosulfan.

Among the taxa studied, the Baetidae were the most susceptible to the chemical larvicides. The Hydropsychidae were fairly susceptible, while the Chironomidae were the organisms least susceptible to most of the insecticides. Results suggest that the more toxic the insecticides were in gutter tests, the more the population abundance differed from the reference ones in the field.

A risk assessment in different steps was performed for every new larvicide to be used in the OCP. After the first trials, where efficacy on blackflies was evaluated (as well as physical properties such as dispersion and viscosity), a review of the literature was performed, together with some laboratory tests on fish, and gutter tests on invertebrates. The Ecological Group reviewing the results recommended, in cases of acceptable toxicological results, small-scale pilot studies in the field, with spräying to be carried out a few meters upstream of the evaluation zone.

For the most toxic insecticides that have many advantages to their possible use (such as low cost, wide range of applications in relation to discharges and long carry distance), large-scale studies below the operational dose<sup>5</sup> were recommended. This allows a complete risk assessment scheme to be obtained<sup>24</sup> before the insecticide is used as an operational larvicide. Permethrin, for example, was tested in this way<sup>25</sup>. After 15 weekly applications at its operational dose, benthic fauna density and diversity were severely affected. However, both drift and benthic fauna recovered almost to pretreatment levels a month after the treatment was terminated, but one taxa remains absent. Fish showed

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some evidence of stress, but remained in the treatment zone, allowing catches to be compared with those of the control station. No fish mortality was observed, nor any variation in the condition factors of the fish.

All these studies have allowed the OCP and the Ecological Group to agree on the optimal use of larvicides that limits the environmental impact. A synopsis of the activities of the Ecological Group is shown in Fig. 2.

## Environmental impact and development

The success of the OCP in vector control in West Africa has allowed extensive areas of previously abandoned valleys to be resettled and developed.

Thus, despite the long-term use of larvicides in the rivers of the OCP area, the quality of the aquatic environment has been preserved, largely because of the precautions taken, and because of the absence of human population pressure in the areas most severely affected by the illness. However, the situation is now changing rapidly, and there is an increasing concern for the aquatic and terrestrial environments, because of population movements and land recolonization.

It was recognized, therefore, that there was a need for the formulation of an appropriate methodology for environmental impact assessment. A pilot project in the Upper Leraba Basin was launched in 1993, the primary objectives being: (1) to assess the present environmental situation in the basin, with a view to determining potential sources of impact on the aquatic environment; (2) to quantify chemical loads and assess the modification to the physical environment; and (3) to identify simple study methods applicable to other areas, and investigate ways to minimize impacts.

An evaluation of the changes in the physical environment was made for the period between 1972 and 1993. These studies showed that about 75% of the original savanna woodland had been cleared in the past few years for settlement and agricultural development. There had been no observed significant disturbance of the riverine forest and associated floodplain grasslands of the main rivers system, and organic loads and nutrients were only of relevance along limited stretches of the main Leraba River, where point sources of contamination can be identified (eg. human and cattle wastes).

- The presence in river water of pesticides used for cotton protection was calculated from the quantity of chemicals used locally by means of simulation models, which permitted the concentrations of pesticides in the river to be estimated. Although the predicted concentrations of pesticides in river water were not at levels that would cause damage or give cause for alarm, they could nevertheless be considered as early warning signals<sup>26</sup>.

#### Conclusions

The OCP has repeatedly been able to demonstrate that, despite the weekly application of larviciding over a long period, there has been no significant deterioration of the aquatic environment.

The result of the OCP activity should not only be regarded as a success in curbing and controlling river blindness, but also as an important example of sound environmental management in a programme that, from the beginning, had included ecological considerations in planning vector control activities.

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## Websites of Interest

PCR Resources Much information and many useful links can be found at the Alkami Biosystems homepage at http://www.alkami.com

The Society of Nematologists The homepage, at http://ianrwww.unl.edu/ianr/plntpath/nematode/son/sonhome/htm, has helpful links to University departments that do relevant work and show illustrations on their websites.

Leishmania Genome Network A new section containing a Leishmania Genome Directory, to be updated monthly, has been added to the site at http://www.ebi.ac.uk/parasites/leish.html. The Directory currently contains listings for all 555 Leishmania genes and their orthologs/ paralogs in other parasite genera.

**National Center for Infectious Diseases/CDC** This website at http://www.cdc.gov/ncidod is a useful source of information and has links to electronic journals and so on.

Another malaria website The Royal Perth Hospital, Western Australia has devised an attractive one for medical practitioners and for laboratory scientists at http://www.rph.wa.gov.au/labs/haem/malaria/index.html. It covers history, prophylaxis, diagnosis and treatment and is good for teaching.

Web translator For translations between English and main European languages try http://babelfish.altavista.digital.com/cgi-bin/translate

Integrated Control of Pathogenic Trypanosomes and their Vectors The new EC Concerted Action initiative supported by DGXII of the European Commission under the INCO-DC (International Co-operation with Developing Countries) Programme of the Fourth Framework will be co-ordinated by the University of Glasgow, UK. It has 27 developing country, European and International partners and will run for four years from 1 July 1998. It is complementary to the FAO/IAEA/OUA-IBAR/WHO Programme Against African Trypanosomosis (PAAT), but will not be restricted to Africa. Details from Mark Eisler (m.eisler@vet.gla.ac.uk).

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Novel malaria transmission blocking targets

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