

Chapter 6

**POTENTIAL IMPACT OF MICROBIAL INSECTICIDES ON THE
FRESHWATER ENVIRONMENT, WITH SPECIAL REFERENCE
TO THE WHO/UNDP/WORLD BANK, ONCHOCERCIASIS
CONTROL PROGRAMME**

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I. INTRODUCTION

Inland waters, both flowing (lotic) and still (lenitic), are potential sites of collection, and at times concentration, of the multifarious byproducts of human activity. An excessive use of fertilizer on lakes or ponds, followed by runoff, leads to deleterious eutrophication. Equally familiar is the contamination of rivers by industrial wastes. More insidious, though very common, is the slow accumulation in continental waters of products, or compounds resulting from them, used tens or even hundreds of kilometers away and then windborne before being dropped to the earth's surface by rain (acid rains, drift of insecticidal dusts following aerial application, etc.).

Again, the control of disease vectors having aquatic developmental stages may necessitate introducing insecticides directly into waters, where their selectivity for the species under attack is never absolute. In all such cases it is essential to minimize the level of contamination so induced in aquatic ecosystems.

For many years the practical employment of microbial insecticides was restricted to agriculture and forestry, and little attention was paid to the effect of their toxicity to freshwater fauna. Only a few organisms, such as *Daphnia* spp., were tested in the laboratory, and records of the LD₅₀ established for these crustaceans were simply added to other data required for the documentary support for the registration of relevant microbial control products. With growing appreciation of the hazards of transportation of pesticide molecules toward the aquatic biota, and of the direct addition of compounds with antivectorial properties into larval habitats, need has been highlighted for a better understanding of insecticide impact on aquatic organisms and the hydrosphere at large.

The nature of the particular toxicological tests to which microbial insecticides must be submitted in assessing their safety, depends on the manner in which these agents are to be employed in practice. It is obvious, for example, that the registration criteria for the use of *Bacillus moritai* Aizawa and Fujiyoshi (essentially applied against the housefly, *Musca domestica*, in domestic environments) will be far different from those required for *B. thuringiensis* spp. *israelensis* de Barjac if it must be directly introduced into lenitic or lotic waters for control of Culicidae or Simuliidae, respectively.

The consequences are that toxicity studies are often restricted to some laboratory tests carried out on one or two organisms (in general *Daphnia magna* and *Gambusia affinis*). In the case of insecticides introduced directly into water, the research has to concern different biological or ecological levels, right up to the observation of toxin impact on entire ecosystems.

II. WAYS OF EVALUATING THE IMPACT OF MICROBIAL INSECTICIDES ON NONTARGET AQUATIC ORGANISMS (NTOs)

The concept of laboratory evaluation of the potential toxicity of new insecticides to aquatic NTOs is not new. It was even urged as an absolute necessity by some of the experts participating in the meetings of WHO scientific working groups. For example, one such meeting recommended that, ". . . all products of natural origin, able to replace chemical insecticides, living or dead, have to be tested in depth in order to discard any risk of toxicity either to man or to any organisms other than the target species."

More recently the same idea has been reiterated and enlarged upon in another WHO report. "An important consideration for the realization of vector control products is the study of the innocuity spectrum of the candidate compounds to the beneficial and non-target fauna which coexist with the vectors and exercise regulatory pressure upon them. This is true for chemical compounds as well as for biological ones. The ecological innocuity of biological compounds will be measured not only by the immediate response of isolated individuals or groups of organisms, but also by the research of effects on the population of

variate organisms (predators as well as detritivores) which are living in the same biotopes as the target species.^{11,12}

If these principles are valid, the toxicity tests necessary for the registration of a microbial insecticide will differ from one product to another, taking into account the future field of utilization. However, it has to be borne in mind that a product initially conceived for a specific type of use may later, as circumstances demand, be employed for a purpose other than that originally intended, following due modification of its original presentation or formulation.

Nevertheless, considerations of time and money often lead to use of compounds which are not intended to be introduced directly into aquatic biota, being tested only against certain "standard" laboratory organisms. Products destined for use in aquatic vector control must, however, undergo a variety of specific and graduated tests. It is also clear that only those products whose effectiveness against a specific target group has been clearly demonstrated will be submitted to a systematic and complete toxicity screening against aquatic fauna and flora.

III. ECOLOGICAL IMPACT OF THE MAIN MICROBIAL INSECTICIDES ON THE FRESHWATER BIOTA

A. *BACILLUS THURINGIENSIS* BERLINER

It was not until the 1950s, some 4 decades after its isolation (from the Mediterranean flour moth, *Ephesia kuehniella*) and description, that *B. thuringiensis* entered into use as a biological insecticide. Today, it is the sporulating bacterium most commonly used for crop protection and (via its subspecies *israelensis*^{3,4}) vector control. First employed in its primary powder form and subsequently as formulations, *B. thuringiensis* has become generally accepted as an environmentally safe control agent.

Active against larvae of both Culicidae and Simuliidae, *B. thuringiensis* ssp. *israelensis* is directly introduced into water bodies, whether lenitic or lotic. This circumstance has occasioned many toxicity studies, first in the laboratory and later in the field, of its possible impact on freshwater ecosystems. The following pages present a general survey of relevant trials at different levels.

1. Laboratory Tests

a. Influence of Abiotic Factors

It is important to understand that some environmental factors capable of influencing the degree of efficacy of this agent against target vectors can also play an important role with respect to certain NTOs.

For example, the influence of temperature on *B. thuringiensis* ssp. *israelensis* has been extensively studied.⁵⁻⁹ All concerned have concluded that this agent's activity declines rapidly when water temperature decreases. This important finding is potentially negative for the environment, insofar as tropical regions are those chiefly exposed to heavy applications for mosquito or blackfly control. Tropical aquatic NTOs may thus be more vulnerable than those of cold or temperate regions. It has been claimed, also, that storage of the more commonly used formulations (Vectobac[®], Bactimos[®], Teknar[®]) under tropical field conditions does not alter their efficacy. Variations in hydrogen-ion activity, at least between pH 6 and pH 10, have no influence on the activity of *B. thuringiensis*.⁵ No information is available on the possible adverse effects of high electrical conductivity or dissolved salts, although the efficacy of *B. thuringiensis* ssp. *israelensis* against salt marsh mosquito larvae¹⁰ indicates that sea-salt content is not specifically deleterious. However, a high ferrous content has been shown to negate the latter subspecies' larvicidal effect against *Aedes detritus*.¹¹ Similar consequences have been noted in the case of chlorine, which appears to destroy the delta-endotoxin.^{12,13}

A high content of suspended matter in natural waters is held to contraindicate larval mosquito control,¹⁴ leading as it does to a chelation of *B. thuringiensis* ssp. *israelensis* spores with organic particles; these agglomerations rapidly sedimenting, and thus ceasing to be available to a diversity of mosquito larvae.¹⁵ Such a phenomenon must also be considered as negative for benthic detritivorous NTOs, for these would clearly have far greater access to the microbial agent's spores than were the latter to remain in suspension.^{9,16}

Heavy exposure to sunlight/UV radiation leads to a rapid decrease in viable spore content, but does not reduce the larvicidal efficacy of *B. thuringiensis* ssp. *israelensis*.^{17,18} Thus, UV action cannot be considered a basic factor of toxicity reduction for filter-feeding NTOs.

b. Toxicity in Bioassays

Other references to laboratory studies in the present context, indicating no adverse effects of *B. thuringiensis* ssp. *israelensis* to NTOs, include those of Colbo and Undeen,¹⁹ Gallagher,¹¹ Dunn,²⁰ Garcia and colleagues,²¹⁻²⁵ Mastri,²⁶ Larget and de Barjac,^{27,28} Miura et al.,²⁹ Pantuwatana,³⁰ Prasertphon,³¹ and Rajagopalan.³² Garcia et al.,³³ who noted that Dixidae were as susceptible to *B. thuringiensis* as were Culicidae, detected no adverse effects to chironomid larvae below 10⁷ cells per ml. Working with Teknar at 25 and 100 ppm, Dejoux et al.³⁴ reported adverse effects to *Hydra* sp. only, while Schnetter et al.,³⁵ using a locally produced culture of ssp. *israelensis*, recorded 100% mortality in two species of Chironomidae, two of Chaoboridae, and Ceratopogonidae, at dosages ranging from 1.6 to 160 mg/l, but only minimal adverse effect on larval Tipulidae.

Globally, over the past 20 years, numerous trials have been conducted on *B. thuringiensis* toxicity. Many of these were not scientifically comparable with one another. In some cases, too, experimental conditions were imprecise. Nevertheless, the overall results clearly suggest that the introduction of *B. thuringiensis* ssp. *israelensis* into water affects only a handful of taxa. A broad spectrum of invertebrates has been investigated in the present connection. Garcia and colleagues^{21-25,33} furnish preliminary evidence of vulnerability of species beyond the Nematocera to this overwhelmingly mosquito/simuliid entomopathogen. Burges³⁶ and WHO³⁷ summarize other initial field trials as well as laboratory ones. These findings permit us to correlate the absence of toxicity with defined testing conditions, particular formulations or specific concentrations. They suggest, too, that the aquatic stages of Diptera, primarily Culicidae and Simuliidae and their near relatives (Dixidae, Chaoboridae), and (some) Chironomidae are the target families and NTOs most vulnerable to *B. thuringiensis* ssp. *israelensis*.

2. Field Trials

a. Short-Term Studies

Investigations of the rather ephemeral effects of *B. thuringiensis* ssp. *israelensis* on NTOs, have been more numerous than those conducted on a longer-term basis. They are covered in detail by WHO³⁷ and in Chapter 12 herein, and clearly indicate a less-than-drastic adverse impact upon freshwater ecosystems; despite the results of these studies often having been achieved via methodologies not always well adapted to the purpose. Of course, in the case of a catastrophic effect, such immediate consequences as the mortality of aquatic organisms are self-evident.

When the impact is less drastic, only quantitative and comparative studies before and after treatment are able to demonstrate any adverse effect. In this case the methods generally used consist of collecting series of Surber samples and/or core samples in suitable places immediately before the insecticide reaches the aquatic biota, and obtaining other series from the same location at regular intervals after treatment. The use of artificial substrates in the same way⁶ can also be considered a useful method in the case of short-term trials, as well as the study of 24-h *in situ* drift variations, covering an equal period (24 h, 48 h, etc.) before and after insecticide application in the case of treatment of running waters. This method provides useful indication of the immediate response of aquatic ecosystems to insecticides,

microbiological as well as chemical. This method has been in regular use since the earlier days of the World Health Organization/United Nations Development Programme/World Bank/Onchocerciasis Control Programme (WHO/OCP) in the Volta River Basin,^{34,38-40} these field results from the largest long-sustained practical usage of *B. thuringiensis* ssp. *israelensis* to date, revealing only the slightest of hazards to any of the NTOs tested. The two- or threefold increase in the diurnal or nocturnal drift intensity that was sometimes registered, represents only a minimal adverse environmental impact by comparison with the 20- to 40-fold such increase of day-drift regularly occurring in WHO/OCP streams after the application of different chemical compounds employed under identical ecological conditions.

The one drawback to this method is the impossibility of ascertaining precisely what part of the ecosystem participates in the drift as a result of the insecticidal application (whether chemical or microbiological). To explore this question we designed a gutter system able to reveal the drift pattern before and after the application. This allowed in-depth analysis of the participation of each NTO taxon or group of taxa in the increased drift, permitting precise assessment, at the end of each experiment, of the effects of the control agent on the various faunal elements assayed.^{34,41,42} These and related assessments from elsewhere have been summarized by WHO.³⁷ Key papers with respect to WHO/OCP include those of Lacey et al.,⁶ Dejoux et al.,³⁴ Gibon et al.,³⁸ Dejoux,³⁹ Yameogo,⁴⁰ and Elouard and Fairhurst.⁴³ The general outcome may be summed up in the customarily very strong mortality of Simuliidae accompanied by no significant increase of drift-rate for NTOs except for Chironomidae/Orthocladiinae,³⁹ and associated with a lack of adverse effect to the insect fauna (notably predators of simuliids such as Hydropsychidae located on stones in the full current previously thickly inhabited by immature *Simulium damnosum* s.l.).³⁴ The only noninsects detected as being particularly sensitive to *B. thuringiensis* ssp. *israelensis* were molluscs of the family Ancyliidae.³⁹

Relevant observations from places other than West Africa that have reported no adverse effects of *B. thuringiensis* ssp. *israelensis* on Chironomidae and a wide range of other NTOs include some from small streams, e.g., in Newfoundland, Canada, treated with a locally produced culture¹⁹ and in New York, treated with Roger Bellon powder R-153-78 and Sandoz SAN 402/WDC;⁸ and others from ponds, e.g., in Camargue, France, treated with Vectobac.⁴⁴ Reporting on trials with Teknar in a stream in Quebec, Back et al.⁴⁵ found no significant elevation of drift rate for Chironomidae, Ephemeroptera, Plecoptera, and Trichoptera, but reductions of 26 and 39% for larval chironomids of the genera *Eukiefferella* and *Polypedilum*, respectively, on artificial substrates. They also noted a high level of drift for Diptera/Blepharoceridae over the 2 d following application of the microbial preparation. However, in trials with Sandoz SAN 402/WDC in 0.02-ha experimental plots in California, Miura et al.²⁹ noted that while a wide range of other NTOs were unaffected, chironomid larvae collected immediately after treatment showed 100% mortality after 2 d. Severe adverse effects to Chironomidae resulting from the use of Abbott powder ABG-6108 in Florida, were recorded by Ali,⁴⁶ his mortality figures ranging from 23 to 61% (2 kg/ha) to 53 to 88% (10 kg/ha) in 4 × 6-m × 45 to 50-cm deep experimental ponds over a 4-week period; while in the same period there was 27 to 65% control of larval chironomids subjected to 3 kg/ha in a 1-ha golf course pond.

b. Medium-Term Studies

Investigations under this subheading have been fewer than those concerning short-term impacts of microbials on aquatic ecosystems, perhaps because of their costlier nature and the need for participation of qualified personnel over a relatively long period. Also, if we consider several months of regular observation to constitute a medium-term study, a further difficulty becomes apparent; namely that of distinguishing between the eventual impact of the tested compound and the natural evolution of the populations observed.

TABLE 1
Comparison of the Density of Invertebrates Living on Stones in
the Current Between the Section Treated with *Bacillus*
***thuringiensis* ssp. *israelensis*^a and the Untreated Section**

Taxa	Control zone		Treated zone		Difference of average density %
	N/m ²	%	N/m ²	%	
Chironomidae	4402	45.05	4205.5	84.0	-4.5
Simuliidae	94	0.96	39.5	0.79	-58.0
Ceratopogonidae	23.5	0.24	70.0	1.40	+66.4
Tipulidae	284.5	2.92	127.5	2.55	-55.2
Hydropsychidae	5028	51.46	562	11.23	-88.8
Baetidae	38.5	0.39	2	0.04	-94.8
Total	9770.5		5006.5		-48.8%

^a Weekly applications at a dosage of 1.6 mg/l/10' for 9 weeks

It is necessary to know how these populations evolve naturally in order to correlate, for example, an eventual decrease in number of certain taxa to the insecticidal impact, without allowing the data to be distorted by seasonal variations which belong to a natural pattern.

A typical example is provided by the study carried out by Yameogo⁴⁰ on a small stream in central Ivory Coast. Weekly applications of *B. thuringiensis* ssp. *israelensis* (Sandoz 402 I.W. DC formulation) were carried out over 2 months. The evolution of the treated populations of invertebrates during this period was studied and compared to that of a similar population in an untreated section of the same stream.

Traditional methods such as core and Surber samples, utilization of artificial substrates, and *in situ* measurements of drift intensity were used.

Yameogo's conclusions were as follows: "The Chironomidae (Orthocladiinae) demonstrated an immediate response to the first treatment (significant drift increase), but did not appear to be greatly affected by later applications, and their density on artificial substrates and on the stones in the current increased regularly. The Trichoptera/Hydroptilidae demonstrated the same pattern of evolution. However, the Trichoptera/Hydropsychidae and Philopotamidae populations reduced significantly in number during the two months of observation."⁴⁰ We have studied Yameogo's data in more detail in order to take his results still further.³⁴

If we examine the invertebrate fauna living on the stones in the current, it appears that the general pattern of increasing density of invertebrates during the observation period occurs in both the treated and the untreated section of the stream, but with a lower intensity in the treated section, a finding which is not statistically significant for $p = 0.01$.

The Chironomidae were completely unaffected by the treatment and remained numerous in both sections. The Ceratopogonidae were found to be more numerous in the treated section but there was a marked decrease in the density of Hydropsychidae and Tipulidae in the treated area (see Table 1). Similar results were found for the artificial substrates (Table 2), but in the case of sandy bottoms the results differed slightly (Table 3). There, a reduction in the numbers of Chironomini and Tanytarsini was apparent. This could be due to higher sedimentation of the microbial product on the sandy bottom than on the stones which were scoured regularly by the current.

In conclusion, it would appear that a modification of the populations occurs only after 2 months of regular treatments. This conclusion is based mainly on the decrease in density of groups which had never shown a particular sensitivity in short-term studies. Conversely, the Chironomidae which had appeared sensitive in short-term trials were apparently unaf-

TABLE 2
Comparison of the Average Density of Invertebrates Collected
on Artificial Substrates Over a 7-Week Period, in the Treated
and Control Areas

Taxa	% in control zone (7 samples)	% in treated zone (7 samples)	Difference
Chironomini	18.4	18.3	- 0.1
Tanytarsani	8.2	12.3	+ 4.1
Tanypodinae	1.7	2.5	+ 0.8
Orthoclaadiinae	8.4	35.3	+ 26.9
<i>Simulium adersi</i>	4.5	1.5	- 2.5
<i>Simulium hargreavesi</i>	0.2	0.5	+ 0.3
<i>Simulium ruficorne</i>	1.2	0	- 1.2
Baetidae	3.2	8.4	+ 5.2
Caenidae	0.3	1.7	+ 1.4
Hydropsychidae	51.8	14.7	- 37.1
Hydroptilidae	1.0	1.1	+ 0.1
Philopotamidae	0.4	0.9	+ 0.5
Libellulidae	0.1	0	- 0.1
Zygoptera	0.03	0.2	+ 0.17
<i>Potadoma</i> sp.	0.3	1.5	+ 1.2
Oligochaeta	0.27	1.0	+ 0.73

TABLE 3
Comparison of the Density of Invertebrates Living on Sandy
Bottoms in the Current Between the Section Treated with *B.*
thuringiensis ssp. *israelensis*^a and the Untreated Section

Taxa	Control zone		Treated zone		Difference of average density %
	N/dm ³	%	N/dm ³	%	
Chironomini	51	46.4	32.9	39.0	
Tanytarsini	47.8	43.5	8.8	10.4	- 12.1 ^b
Tanypodinae	2.2	2.0	8.3	9.8	
Orthoclaadiinae	1.7	1.5	19.3	22.9	
Ceratopogonidae	0.5	0.5	2.2	2.6	+ 77.3
Caenidae	1.4	1.3	0	0	—
<i>Potadoma</i> sp.	5.4	4.9	9.4	11.1	+ 42.6
Hydropsychidae	0	0	3.5	4.1	—
Total	110		84.4		- 23.3%

^a Weekly applications at a dosage of 1.6 mg/l/10⁷ for 9 weeks.

^b Figure represents difference between treated and control population for all chironomids.

ected by medium-term treatments. In this specific case there is a strong possibility that the decrease in numbers of predacious Hydropsychidae was beneficial to the chironomid population and compensated for any possible direct impact of *B. thuringiensis* ssp. *israelensis* on these midges.

c. Long-Term Studies

At the start of WHO/OCP in 1974, control of the onchocerciasis vector, *Simulium damnosum* s.l., was achieved via weekly applications of Abate[®] (temephos, an organophosphate compound) to production sites throughout the Volta River Basin. Eventual widespread resistance to temephos and cross-resistance to the first alternative chemical pesticide, chlorphoxim, in key cytospecies of the *S. damnosum* complex, necessitated the search for

and use of other suitable control agents, including *B. thuringiensis* ssp. *israelensis*.⁴⁷⁻⁵¹ Long-term environmental impact studies in WHO/OCP have mainly concerned insecticide impact. There has been a strong component for the microbial agent under consideration during the 1980s, and Teknar has lately seen increasingly widespread use at a concentration of 1.2 mg/l/10' via regular weekly applications by helicopter or fixed-wing aircraft. Small rivers are being so treated throughout the year, the larger ones being alternately treated with Teknar during the dry season (low-discharge period) and organic chemical compounds (notably temephos and chlorphoxim) during the season of high water and maximum flow. Unfortunately, alternation of the microbial insecticide with chemical ones (which are less selective) makes it impossible to ascribe adverse effects on NTOs to one or the other control agent.

The dry-season use of *B. thuringiensis* ssp. *israelensis* in streams where the rate of discharge is less than 50 to 75 m³/s,^{49,51} reflects the physical bulk of Teknar by comparison with that of temephos. While the fact that the invertebrate communities exposed to insecticides change somewhat between the wet and dry seasons does not permit determination of the microbial agent's seasonal impact on specific NTOs as well as the aquatic community structure, interesting results have been obtained by one of us (J.-M. E.) from comparing the fauna of rivers following treatment with Teknar for several years, with the fauna known to have existed prior to control.

From mathematical analysis of data from the Maraoué River, which was treated with *B. thuringiensis* ssp. *israelensis* for over a year in 1983 to 1984, the structure of the invertebrate community encountered during the treatment phase can be well characterized. The results indicate that it differs in some respects from that found during both untreated periods and periods of application of temephos or chlorphoxim (Figures 1 and 2). Differences related to diurnal and nocturnal drift are evident, as well as others concerning the invertebrate fauna of the vegetation growing on stones in the current. However, the chief results are similar. For example, it is clear that Trichoptera of the family Ecnomidae (which some regard as a subfamily of the Psychomyiidae) actually benefit from Teknar treatment. Moreover, hydropsychid predators on simuliids do not demonstrate any particular sensitivity, a finding that contradicts those obtained earlier from the medium-term study undertaken on the Kan River by Yameogo.⁴⁰ Two possible explanations for this inconsistency are some difference between the formulations used in the two cases, and a specific resistance on the part of Hydropsychidae having been induced by the alternation of the microbial and chemical insecticides. Overall, though, such alternation as practiced in WHO/OCP on the lower Maraoué River (where resistance to temephos first appeared), does not appear to disrupt NTO populations any more than does each insecticide individually,⁵² while there is no evidence to date of any long-term deleterious effect of *B. thuringiensis* ssp. *israelensis* on the ecosystems of streams receiving weekly applications throughout the dry season.⁴³ The graphs of Figures 3a through 3d exhibit the change of population density of selected groups of invertebrates on stones in rapids after treatments with *B. thuringiensis* ssp. *israelensis* following temephos and chlorphoxim applications. Among the Chironomidae the Tanytarsini seem to be the most sensitive NTOs, a finding in agreement with various short-term studies. It can also be stated that the results of environmental monitoring in WHO/OCP have not revealed any significant direct or indirect effects of operational dosages of Teknar on lotic fish populations.⁴⁹

To conclude, *B. thuringiensis* ssp. *israelensis* is certainly both the most valuable microbial control agent yet commercialized for use against major nematoceran pests and disease vectors, and a conspicuously "safe" insecticide insofar as freshwater NTOs and whole ecosystems are concerned.

Nevertheless, it is urged that international standardization of field as well as laboratory testing, based on a short list of widely distributed freshwater test organisms, is necessary at this time.

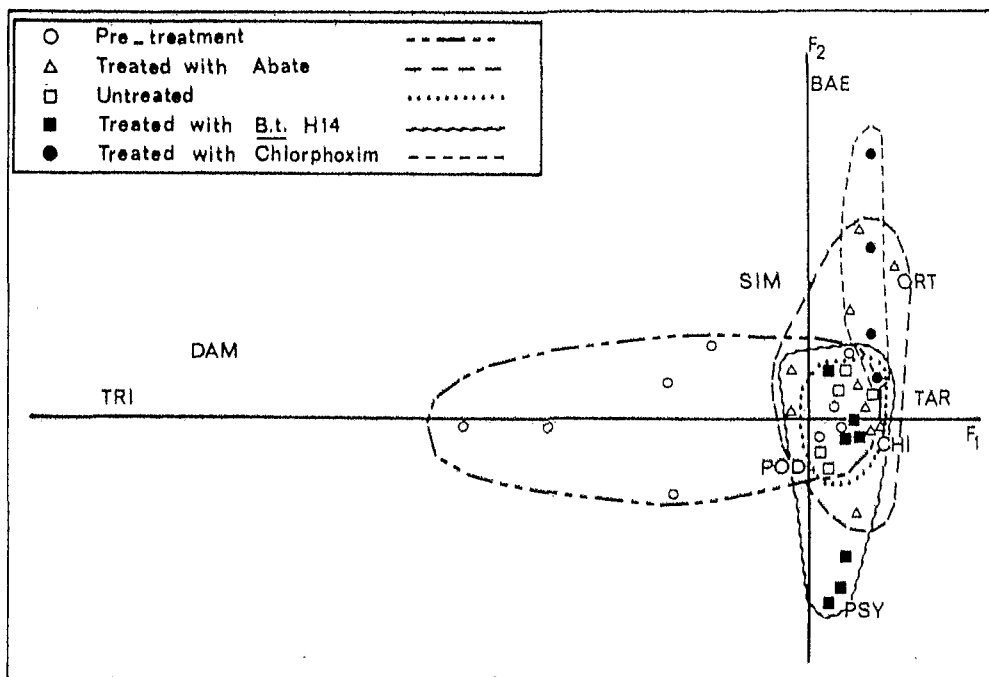


FIGURE 1. Factorial analyses of correspondence were applied to data concerning the densities of benthic fauna living on stones in the current in the Maroué River during the dry season. This river was treated successively with temephos, chlorphoxim, and *Bacillus thuringiensis* ssp. *israelensis*. The axis F_1 draws a clear separation between pretreatment samples associated with a dominance of *Simulium damnosum* and Trichorythidae and all the other samples collected under insecticide treatment conditions. The axis F_2 draws a separation between the period with chlorphoxim treatment associated here with an abundance of Baetidae and the period when *Bacillus thuringiensis* ssp. *israelensis* was used associated with an abundance of Hydropsychidae. BAE = Baetidae; CHL = Chironominae; DAM = *Simulium damnosum*; ORT = Orthocladiinae; PSY = Hydropsychidae; TAR = Tanytarsini; TRI = Trichorythidae; POD = Tanypodinae.

B. *BACILLUS SPHAERICUS* NEIDE 1904

When considering aquatic NTOs, the ability of *B. sphaericus* 1593 (see Chapter 12) to survive and recycle in polluted waters must be taken into account. Survival can be up to 9 months under certain conditions, without decrease of toxicity of the toxin.⁵³ On the other hand, *B. sphaericus* is somewhat susceptible to UV radiation; and it has also been found that the presence of large amounts of suspended material in the water (particularly organic matter) induces a reduction of larvicidal activity against mosquitoes. Such a factor may be considered positive for NTOs if the sedimented organic particles, after chelation with *B. sphaericus*, are not eaten by benthic fauna.

Finally, it has been demonstrated that the toxicity of *B. sphaericus* does not increase significantly when the temperature increases, unlike that of *B. thuringiensis* ssp. *israelensis*.⁹ The same authors have also shown that too much agitation of *B. sphaericus*, during transportation for example, can induce destruction of the spore cells. This leads to a reduction of efficacy. In the same way, this phenomenon can also reduce any toxic effect on NTOs. It is also important to consider that the difficulty remains of standardizing fermentation of the spores and producing batches with equal efficiency towards target fauna. By the same token, the toxicity of each batch produced is subject to variation.

Toxicity studies for *B. sphaericus* are not so numerous as those dealing with *B. thuringiensis* ssp. *israelensis*; but the general results obtained from laboratory and field experiments lead to a similar conclusion, indicating the high innocuity of the different strains commonly used in pest control.

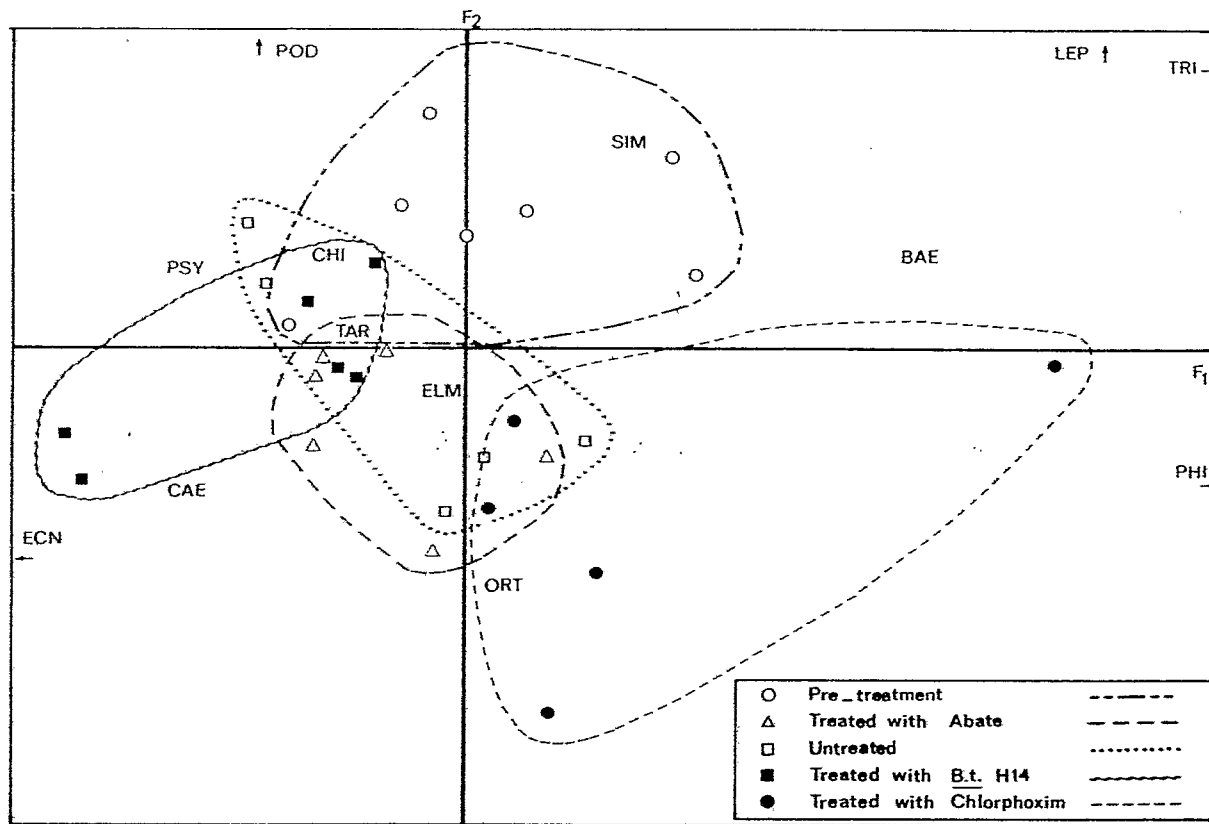


FIGURE 2. The method used in Figure 1 was applied to day drift data and a clear opposition appears again between the period under chlorphoxim treatments and the period under *B. thuringiensis* ssp. *israelensis* treatments here characterized by high densities of Ecnomidae. BAE = Baetidae, LEP = Leptophlebiidae, PHI = Philopotamidae, ECN = Ecnomidae, CAE = Caenidae, ORT = Orthoclaadiinae, PSY = Hydropsychidae, ELM = Elmidae, SIM = other Simuliidae, TAR = Tanytarsini, CHI = Chironomini, POD = Tanypodinae, TRI = Trichorythidae.

Emphasis has been placed mainly on NTOs living in mosquito larval habitats. Going up in the taxonomic classification, studies made on planktonic crustaceans have shown that neither copepods nor cladocerans are affected by concentrations of 10^5 cells per ml. This is the case, e.g., for natural populations of *Acanthocyclops vernalis*, *Moina* spp., *Cypris* spp. and *Ceriodaphnia* spp., tested in California ponds, as well as for *Moina rectirostris*, *Cyprinotus* sp. or *Cypridopsis* sp.⁵⁴ In these experiments, strains 1593 and 2362 were used. Turning to Malacostraca/Decapoda, the North American crayfish, *Orconectes rusticus*, proved similarly unaffected.⁵⁵

Similarly, no adverse effects were detected on Odonata, Ephemeroptera (*Callibaetis* spp.), Heteroptera (Corixidae, Notonectidae), or Coleoptera (Dytiscidae, Hydrophilidae).⁵⁶

Among the Diptera no effects have been noted on *Chironomus stigmatervis* (we have also seen that many chironomid species are not sensitive to *B. thuringiensis* spp. *israelensis*) and it seems that larvae of the genus *Culicoides* (Ceratopogonidae) are affected only at *B. sphaericus* dosages much higher than those needed to kill mosquitoes.²

In a more recent study, Sinègre et al.⁴⁴ tried to control chironomid populations in some shallow brackish-water ponds of the Camargue (France) where mass outbreaks were considered a nuisance. Using BSP 2, a liquid containing the strain 2362 of *B. sphaericus*, at a dosage between 1 to 9 l/ha (i.e., about 3 ppm), the treatment had no discernible effect on the following species: *Chironomus salinarius*, *C. halophilus*, *C. plumosus*, nor on some unidentified Tanytarsini and Tanytopodinae. Such a result suggests that *B. sphaericus* could be less toxic to chironomids than is *B. thuringiensis* spp. *israelensis*, although a similar result was obtained by the same authors, using the latter under the same conditions.

Toxicity for fish appears to be nonexistent at normal dosages. No deleterious effect on fry of *Gambusia affinis* was noted, for example, in a trial using the Stauffer wettable powder formulation of *B. sphaericus* strain 1593, after the fish had been in contact with a solution containing 10^4 and 5×10^4 spores per ml for 96 h.⁵⁴ Neither were *Epilatyx bifasciatus* and *Aphyosemion gardneri* damaged by exposure to comparable dosages.⁵⁷

Similar results have been reported by Chapman⁵⁸ from research carried out in the laboratory. The organisms concerned were again *Gambusia affinis*, crayfish, tadpoles, and different aquatic insects. However, the formulations used in these experiments were "... poor and shelf life tenuous, so little faith can be put in the results" as the author himself points out.

More interesting are the following results quoted by WHO:² "Although field treatments with *B. sphaericus* (1593 and 2362) caused no noticeable effects, a laboratory trial was carried out, in which *B. sphaericus* infected mosquito larvae were offered as the only source of food to several predacious organisms. *Culex* larvae (L4) exposed to 1000 mg/l (100 times the larvicidal rate) were offered for several days to predators, such as dragon-flies, damselfly naiads and the notonectid *Notonecta unifasciata*: the full daily food requirement of these predators was provided by larvae whose guts were filled with a lethal dose of *B. sphaericus* (2362, BSP-1). Predation on treated larvae did not induce any acute adverse effects nor alter the developmental rates of the predacious organisms."²

Such results are of primary importance when we know that the main, if not the unique mode of entry of *B. sphaericus* to an aquatic animal is by ingestion. The lack of effect of such high concentrations on all tested organisms demonstrates the highly selective toxicity of this bacterial agent. Nevertheless, if no effects of the different strains of *B. sphaericus* such as 1593, 1593 M, SSII-1, 2362, or 2297 have been noted at normal dosages required for effective larviciding, the fact remains that pending the production of normalized formulations more studies of toxicity are still required.

C. INFECTIVITY OF BACULOVIRUSES

Mainly effective against defoliant insects, the nuclear polyhedrosis viruses (NPVs) of the genus *Baculovirus* (Baculoviridae) are becoming more and more widely employed since

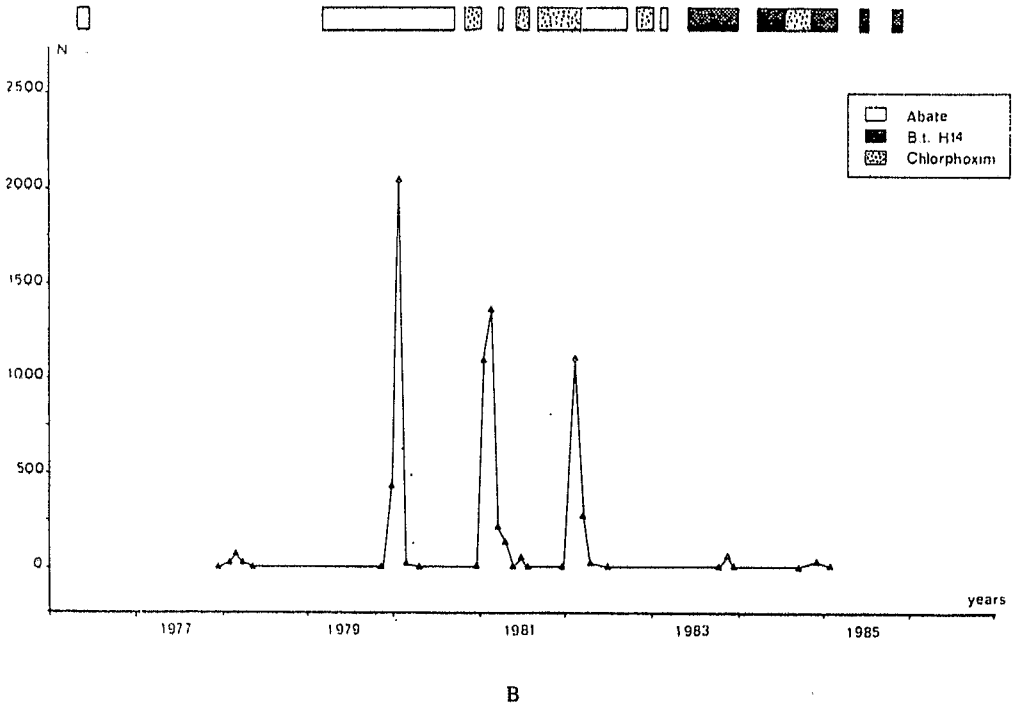
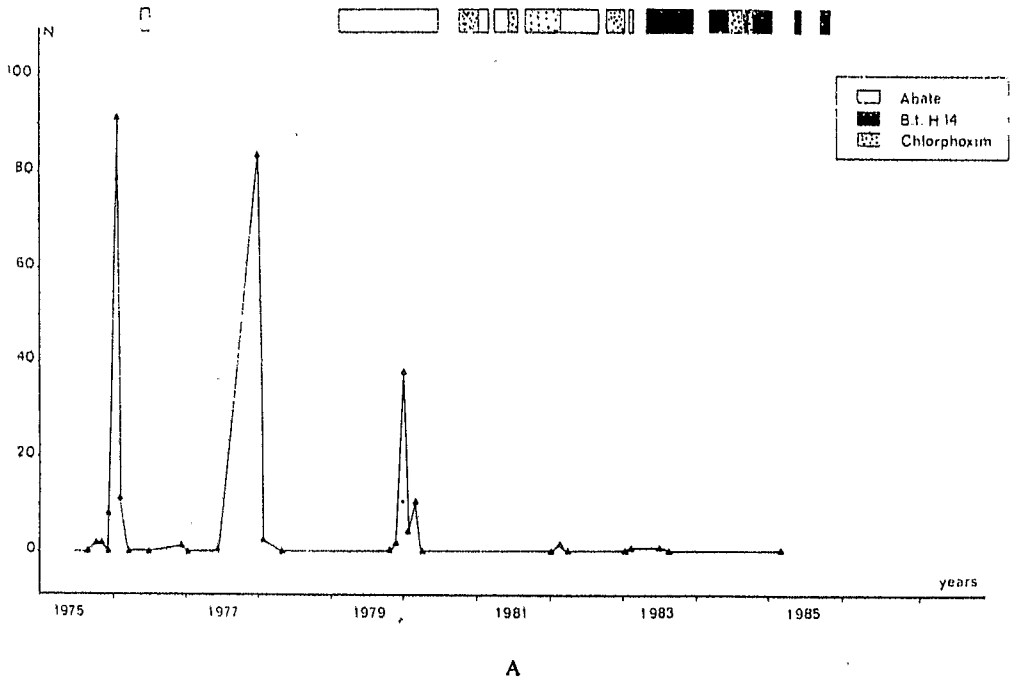


FIGURE 3. Evolution of the average densities of selected benthic organisms on stones in the current (collected by Surber Sampling), in the case of *B. thuringiensis* var. *israelensis* treatments following temephos and chlorphoxim applications. (A) Trichorythidae; (B) Tanytarsini; (C) Hydropsychidae; and (D) Ecnomidae.

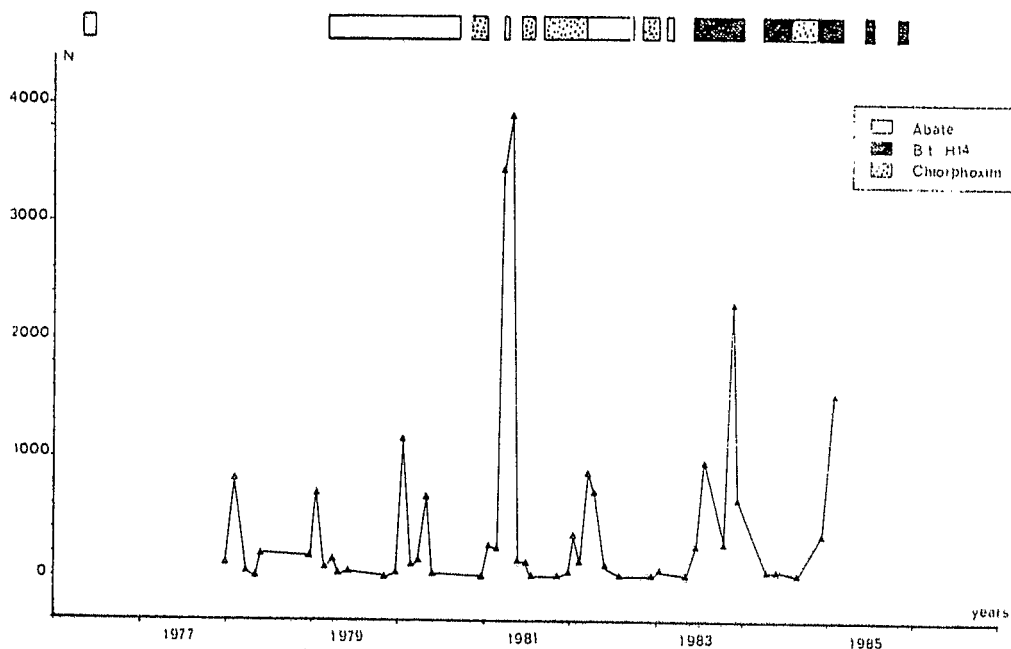


FIGURE 3C

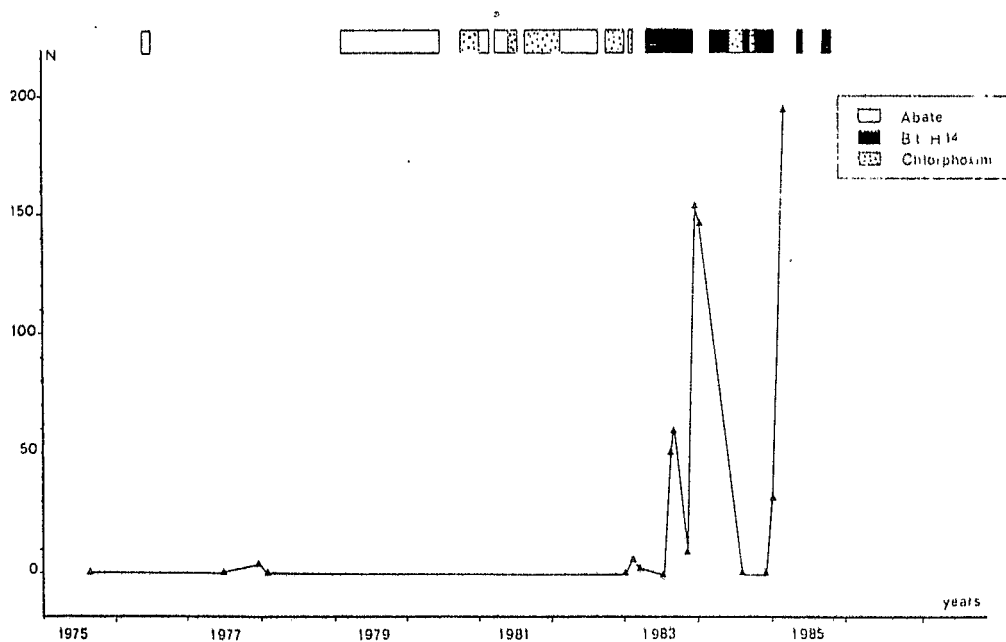


FIGURE 3D

their relatively recent registration, although their pathogenic action has been known for more than 30 years.

Aerial applications inevitably lead to baculoviral preparations reaching rivers or lakes within treated forest areas, and for this reason their pathogenicity among some aquatic organisms has been studied. Also, despite their relative specificity, they prove fatal to many insects (mainly Lepidoptera) which may fall into water and be eaten by fish. Finally, some target lepidopteran species (such as various Pyralidae) represent, during their larval stage,

a significant component of the benthic fauna in certain aquatic habitats. An important reduction of their biomass can then induce local disturbances of the food chain, thus indirectly affecting the fish population.

Before we consider the eventual pathogenicity of baculoviruses to aquatic freshwater fauna, it has to be mentioned — as pointed out by Gröner et al.⁵⁹ — that high concentrations (up to 10/20 units/ha) can frequently be present in the environment without detectable prejudice to the natural populations (vertebrates as well as invertebrates) commonly present in such habitats.

Where the toxicity of aquatic organisms is concerned, it is apparent that once again more research has been carried out on adverse effects to fish than to aquatic invertebrates.

As regards the latter, the most relevant investigations have been carried out by Streams.⁶⁰ His results were presented in an unpublished report cited by Doane and McManus.⁶¹

Frequently abundant species of aquatic invertebrates such as *Daphnia magna*, *Notonecta undulata*, and other waterboatmen (Hemiptera) and *Chironomus thummi* (Diptera) were tested. These organisms were exposed to high but vaguely specified concentrations of gypsy moth NPV, (i.e., "approximately the concentration which would occur when a very shallow pond is sprayed at the rate of $3.75 \times 10/13$ PIB's per hectare").⁶¹ No direct mortality of any of these organisms was observed by the authors. In addition, they state that no harmful effects were observed throughout the development stages of the tested species, a particularly important result. It is also important to note that *Daphnia magna* as well as *Notonecta undulata* and the waterboatmen did not accumulate gypsy moth NPV, as shown by bioassays conducted on tested specimens.

Similar results were obtained by Geraci and Hicks⁶² working on *Daphnia pulex* held in water contaminated by the red-headed pine sawfly (*Neodiprion lecontei*) NPV and kept under observation for 14 d. Their conclusions were later confirmed by Hicks et al.⁶³ using concentrations of 2.4×10 PIB of the same NPV per ml. As well as the absence of direct mortalities, no adverse effects were observed as far as fecundity, lesions or body abnormalities were concerned. Some other invertebrates have also been tested, such as young instars of the penaeid shrimps, *Penaeus setiferus* and *Penaeus aztecus*. In this case, the NPV of the alfalfa looper (*Autographa californica*) was tested, introduced via injections or by feeding. In the case of muscular injection the NPV concentration used about 4.7×10 virus rods per shrimp. After a 30-d observation, shrimp mortality attributable to viral infection did not occur, neither from the injected nor the orally introduced virus. A careful examination of tissues contaminated by NPV injections did not lead to the discovery of any nuclear polyhedra.⁶⁴

Fish toxicity has been the subject of more extensive research, and special studies have been carried out on the physiological effects of a direct contamination by Baculoviridae (incubation, injection, ingestion, etc.). All results indicate an absence of histopathological toxicity for these organisms.⁶⁵⁻⁶⁷ Some 12 species of fish, mainly Salmonidae, have been tested in this way and similar studies carried out on amphibians produced an identical result.

Fish cell cultures exposed to *Baculovirus* (Douglas fir tussock moth NPV) for 24 h showed no pathological changes and no alteration occurred in their growth rate.⁶⁵ In these experiments, cells from chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Salmo gairdneri*) were used. Fingerlings of coho salmon (*Oncorhynchus kisutch*), chinook salmon, and rainbow trout exposed to the same *Baculovirus* by three different routes (i.e., i.p. injection, waterborne exposure, and by feeding) were not adversely affected by any of these contamination methods. No histopathological troubles were detected one month after exposure. This result confirms Wolf's⁶⁸ conclusions, which also established that *Dendroctonus pseudotsugae* NPV had no deleterious effect on amphibian and fish cell lines.

All these initial results have been confirmed by subsequent research; e.g., Moore⁶⁹ established a total absence of harmful effects after the 96-h exposure of 240 juvenile bluegills

(*Lepomis macrochirus*) and 240 brown trout (*Salmo trutta*) to gypsy moth NPV concentrations 100 times higher than those normally effective against that insect.

Similarly, rainbow trout and white suckers (*Catostomus commersoni*) inoculated with spruce budworm NPV were not significantly affected by the virus.⁷⁰ Rainbow trout reactions to red-headed pine sawfly (*Neodiprion lecontei*) NPV incubations or topical applications were also found to be nonexistent by Hicks et al.⁶³

These results all lead to the general conclusion that viruses of the Baculoviridae are nontoxic to aquatic invertebrates as well as fish, although possible long-term effects have not been studied. It is also regrettable that we have no observation relating to global ecosystem modifications which might occur in the case of regular applications of Baculoviridae over several years in the same forest area.

D. INFECTIVITY OF MICROSPORIDIANS

To our knowledge, very little work has been carried out on protozoan pathogenicity to aquatic NTOs, as far as their insecticidal properties are concerned. Some microsporidians which are effective against mosquito larvae or Simuliidae are known to survive in dead insects or in viable infected eggs for more than 10 months.⁷¹ *Nosema stegomyiae* and *Nosema algerae* can be found in their dormant stage in the eggs of *Aedes* for over a year, and are then able to contaminate eventual predators. Spores of *N. algerae* remain viable for 1 to 2 months at 20°C. Weiser also states that "Under conditions of man-guided distribution of spores, *Nosema algerae* or *Vavraia culicis* are able to impact different hosts including mollusks and crayfish."⁷¹

In a study undertaken by Van Essen and Anthony,⁷² nine nontarget aquatic predators were fed with mosquito larvae previously heavily infected with *N. algerae*. Of these NTOs, 50% of the *Notonecta undulata* (Heteroptera) tested developed infections. The other predators (dragonfly, hydrophilid, nepid, megalopteran, decapod, and the larvivorous fish, *Gambusia affinis*) were in no way affected. If we consider that feeding organisms directly with a high dosage of pathogen is much more dangerous for them than a single contact in contaminated water, one can only conclude that *Nosema algerae* will be safe for many other aquatic organisms.

On the other hand, the low mortality rate of mosquito larvae challenged with *N. algerae*, as well as with *V. culicis* (except in the case of anopheline mosquitoes), does not favor the greatly extended use of these Microspora.⁷³

IV. CONCLUSION ON THE HAZARD OF MICROBIAL INSECTICIDES TO FRESHWATER FAUNA

At this point it is perhaps worth quoting various conclusions drawn by other researchers concerning aquatic biotas.

"There is no danger of ill-effect of microbial insecticides on nontarget organisms . . .",⁷¹

"I believe that a pathogen should be registered as safe when there is reasonable evidence that it is so and in the absence of concrete evidence that it is not. A "no risk" situation does not exist, certainly not with chemical pesticides, and even with biological agents one cannot absolutely prove a negative."³⁶

It is now clear that control of disease vectors as well as of agricultural and forest pests cannot be based purely on the use of chemicals because of their generally low specificity, the risk of environmental contamination and also the increasing resistance of insects following intensive applications. Microbial pathogens have great potential, but this necessitates, at least as far as freshwater ecosystems are concerned, more attention to their possible impact.

Despite the relatively large amount of work related to *B. thuringiensis* ssp. *israelensis*, it appears that a lot of questions have only received partial answers. These questions were addressed by Forsberg et al.⁷⁴ in their review of *B. thuringiensis* toxicity, and can be summarized as follows. They remain relevant today, although we have changed the original wording slightly.

1. What are the toxic effects of the components of commercial formulations of microbial insecticides, in the medium and the long-term?
2. What quantities of formulation enter the environment and how persistent are the toxic components in each of their different modes of application?
3. What effects on ecosystems are related to long-term and large-scale field applications of microbial insecticides?
4. Can intensive use of entomopathogens lead to their mutation? Are the microbial agents susceptible to modification by genetic transfer into other forms? Are these new forms capable of producing toxic components, or components with modified toxicity or host specificity?

These questions leave open a rich field of investigation, both at a fundamental and a more practical level, linked to application campaigns. We can conclude that whereas further *B. thuringiensis* ssp. *israelensis* laboratory tests may be superfluous, there is an overall lack of standardized laboratory and field studies covering all microbial insecticides. The setting-up of a well-defined and systematic screening process will enable comparable data to be obtained, and will lead to a better evaluation of toxicity for different conditions of application. In this respect, the safety-testing procedures for bacterial and fungal agents proposed by WHO⁷⁵ could be enlarged upon in order to include more specifications related to aquatic environmental safety.

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