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Legislation on the Introduction of Exotic Entomopathogenic Nematodes into Australia and New Zealand

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There are stringent requirements for the importation of all exotic organisms into Australia and New Zealand but since both countries have already permitted the importation and release of some species of both Heterorhabditis *and* Steinernema*, the difficulties of the importation of entomopathogenic nematodes are reduced. In both countries, a series of authorities must be consulted before importation is permitted but only in New Zealand must entomopathogenic nematodes be registered before commercial trials and sales are allowed. Registration not only entails a thorough evaluation of the nematode species and its formulation for a wide range of possible harmful effects to humans, crops and the environment, but efficacy must be demonstrated for each species of nematode in each type of formulation against each pest.*

Keywords: *entomopathogenic nematodes, exotic,* Heterorhabditis*, importation, legislation, regulation,* Steinernema

INTRODUCTION

The fact that Australia and New Zealand are islands, far removed from other major land masses, greatly affects regulatory attitudes, to the introduction of exotic organisms. Firstly, almost every plant food-crop species and nearly all domesticated animals have been introduced in relatively recent times from overseas and a wide range of pests and diseases of these useful species is not yet present in these countries. Secondly, a large number of biological control agents have been introduced, some of which are highly successful because of the absence of conflicting organisms. Thirdly, both countries have a diverse, and often unique, flora and fauna that could be affected adversely by exotic imports. Fourthly, various diseases of humans are not, as yet, established in Australia or New Zealand. Some of the more obvious serious pests and diseases that are not yet present in, but threaten, these countries are various species of fire ant, citrus canker, khapra beetle, screw-worm fly, sunflower downy mildew, varroa mite, European corn borer, giant African snail, foot and mouth disease, Newcastle disease, rabies, plum pox virus and Egyptian alfalfa weevil (Australian Quarantine and Inspection Service (AQIS), 1993).

Examples of major mistakes from the past include the deliberate introduction of rabbits, foxes and cane toads into Australia and of possums into New Zealand, quite apart from the hundreds of accidental introductions of noxious pests and diseases. An example of what might have been

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a serious threat to one of Australia's most successful biological control programmes was a recent importation of an undescribed species of *Cactoblastis* moth, brought into quarantine facilities to examine its potential for the control of tiger pear; following its release for a small-scale field trial it was found, after performing less well than expected, to harbour a *Nosema* species (protozoa) that would have been quite capable of infecting and debilitating the highly successful controlling agent of prickly pear, *Cactoblastis cactorum*, had the population not been promptly eradicated (R. Milner, personal communication, 1995).

With regard to entomopathogenic agents, of the greatest concern would be potential danger firstly to beneficial insects including bees, or to any of the wide range of insects used in the biological control of other insects, weeds or dung and secondly to unique or rare insect fauna.

Australia and New Zealand have more to gain from having strong legislation and its careful policing than some other countries. However, they have adopted rather different means of achieving this and are therefore considered separately in this paper.

AUSTRALIA

Legislation in Australia on the introduction of biological control agents is summarized in Figure 1.

In Australia, the importation into and release from quarantine of all biological control agents is regulated by the Quarantine Act 1908 and the Wildlife Protection (Regulation of Exports and Imports) Act 1982. AQIS of the Department of Primary Industries and Energy administers the Quarantine Act and the Australian Nature Conservation Agency (ANCA) administers the Wildlife Protection (Regulation of Exports and Imports) Act. In general, AQIS is concerned with protecting Australia from pests and diseases of plants and animals whereas ANCA is specifically concerned with any impact (including competition) an introduced organism may have on the indigenous flora and fauna.

A set of procedures, developed and agreed upon by each of the state/territory agricultural and conservation authorities (there are six states and two territories in Australia), together with the Commonwealth Scientific and Industrial Research Organization (CSIRO), was adopted in November 1987. Under these procedures a research organisation wishing to import a biological control agent makes a single application to AQIS. In turn, AQIS registers the application, advises ANCA and circulates the application to the respective nominated officer in all state agricultural and conservation authorities and to CSIRO. The states and CSIRO provide individual responses to AQIS, following internal consultation, by a date specified by AQIS. These responses are copied and conveyed to ANCA for information. Issues raised in responses are taken up and resolved between the applicant and respondent, usually by means of an interim report to the applicants by AQIS. AQIS, on resolution of outstanding issues with respondents and in consultation with ANCA, draws up a permit to import the potential biological control agent into quarantine.

For most organisms, detailed testing is carried out while the organism is kept in quarantine to verify the host specificity of the agent and to ensure that the agent is free from hyper-parasites and disease. However, in the case of entomopathogenic nematodes, most of which show a very broad host range in the laboratory, somewhat different criteria apply. It has already been accepted that *Steinernema* and *Heterorhabditis* species should, like *Bacillus thuringiensis* (*Bt*), be considered as biopesticides which without human assistance usually have little effect on insect populations. That species of both genera are endemic to Australia but are rarely found parasitizing insects under natural conditions is also appreciated. Nevertheless, AQIS, ANCA, CSIRO and the state authorities must be provided with information with which to assess the likelihood of particular nematodes being a problem if released and, as with other organisms, balance that against the benefits of release. This is required not only for exotic species of *Heterorhabditis* and *Steinernema* but also for exotic strains of species endemic to Australia. In the latter case, it would need to be established that an exotic strain was significantly superior to an endemic strain to make the even small risk of release worthwhile.

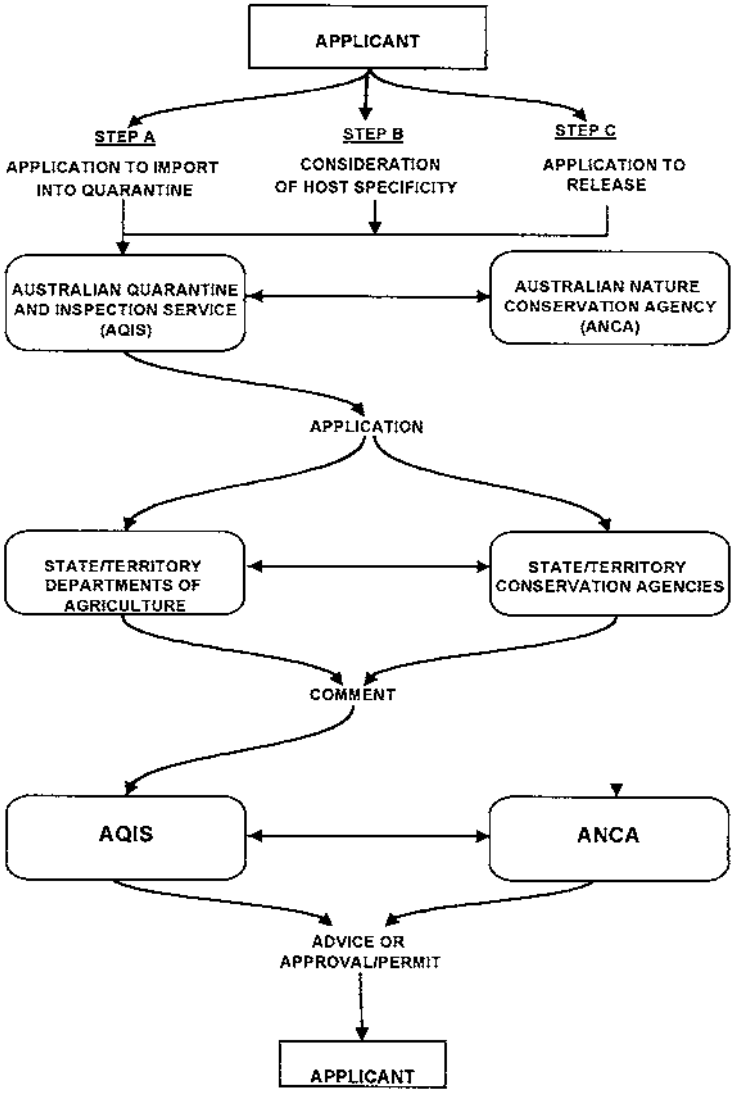


FIGURE 1. Summary of procedures for the introduction of biological control agents into Australia.

On completion of testing, a further application is submitted to AQIS and ANCA. This application, for release of the agent from quarantine, is also registered and similarly referred to cooperating authorities for response, resolution of issues and consideration in consultation prior to release from quarantine.

With most biological control organisms, release from quarantine is followed by multiplication of the organism in Australia and dispersal in appropriate areas, after which the organism looks after itself. However, entomopathogenic nematodes, which are used as bioinsecticides, require continual artificial propagation and repeated application, and therefore may not require the same rigorous laboratory testing for host specificity.

Importation of Nematodes into Approved Quarantine Facilities

All imports of nematodes that have not yet been approved for release require a permit from AQIS and another from ANCA.

As permits for the import of "rhabditid nematodes parasitic in insects" into approved quarantine facilities have been issued over the last 20 years, renewal of these permits by AQIS on a 2-yearly basis is relatively straightforward for prior applicants. New applicants would need to have appropriate quarantine facilities and have had these facilities approved by AQIS. To obtain an import permit from AQIS, an application form PI 983-12/87 (Application to Import Biological Material) must be completed, and from ANCA, application form AP4 (Application for a Permit to Export or Import Wildlife Specimens (whether alive or dead) for the Purpose of Prescribed Scientific Research) is needed. At this stage, the information required by AQIS for its 2-year permit is limited to details of the importer and supplier, country of origin, methods used to ensure freedom from contamination, species and strain name, quantity of material, proposed use while in quarantine and the expected date of completion of work. ANCA, from whom a permit is required for each consignment, also requires specific details of the shipment including ports of loading and unloading with dates, the airline/shipping company, city of dispatch and receipt, sender's approved institution code and recipient's approved institution code.

A most important aspect of these permits falls under the Wildlife Protection (Regulation of Exports and Imports) Act 1982 section 50 (3) "... a person shall, for the purposes of that subsection, be taken to have released an animal from captivity if that animal has escaped from captivity and that person allowed the animal so to escape or failed to take all reasonable measures to prevent the animal from so escaping", and section 50 (4) "The holder of a permit or an authority who contravenes, or fails to comply with, a condition to which the permit or authority is subject by virtue of this section is guilty of an offence punishable, on conviction, by a fine not exceeding $100 000".

Importation of Nematodes Already Approved for Release

Species/strains already found in Australia and species/strains already approved for release, also require permits from AQIS and ANCA for their importation. Similar permits to those above may be issued but the conditions listed obviously do not include maintenance in quarantine facilities. However, AQIS is still concerned that hyper-parasites and diseases are not imported with the approved organism so that each consignment must be accompanied by a sanitary certificate from the appropriate governmental authority (e.g. from USDA if from the US, or from Ministry of Agriculture and Fisheries if from New Zealand). The AQIS permit is similar to that above, but the conditions are different. For example, on a recent permit (1995) to import Bio Flea Halt, a formulation of *S. carpocapsae* from the US, the standard conditions specified were:

- "This permit is not valid unless accompanied by an import permit from the Australian Nature Conservation Agency";
- "Cultures to be imported on sterilised media";
- "This material and any products containing this material must be registered by the National Registration Authority for Agricultural and Veterinary Chemicals prior to commercial sale or distribution for agricultural or veterinary purposes";
- "The culture is to be certified pure";
- "Each consignment must be accompanied by USDA certification stating that the product has been tested and found free of hyperparasites and diseases".

In fact, since this permit was issued, the requirement for registration no longer applies (see below).

Importation and Release of Entomopathogenic Nematodes

Whereas importing nematodes into quarantine is relatively easy, gaining approval for release is considerably more difficult and the following information is required.

Targets

(1) Scientific name (order, family, genus, specie, and author); common name (if any).
(2) Native range and, if determinable, probable centre of origin.
(3) Distribution in Australia, including a map if available, and in any other country where it is a pest or a normal part of the fauna or flora.
(4) Relatives native to Australia (state family names of close relatives if number is large).
(5) Pest status: (a) host organism(s) attacked by it (as appropriate); (b) nature of damage caused; (c) extent of losses caused (average and extremes); (d) estimated value of production loss.
(6) Other control methods available (if any): (a) type of control (chemical, physical, management); (b) effectiveness; (c) costs; (d) any undesirable side-effects.

Agent

(1) Name (order, family, genus, species and author).
(2) Biology of the nematode (in brief).
(3) Native range and, if determinable, probable centre of origin.
(4) Related species and a summary of their host range.
(5) Proposed source(s) of nematode.
(6) Mode of action against target organism and extent of action.
(7) Potential for control of target.
(8) Non-target organisms at risk from agent (include those closely related biologically and those ecologically similar).
(9) Possible interactions with existing biological control programmes (or same or related targets and other targets).
(10) Progress of testing programme and results of testing programme and conclusions. With entomopathogenic nematodes, AQIS and ANCA would be particularly concerned that new species and strains were indeed superior to those already released to make importation worth the risks involved.

The following information may be useful

(11) When and where initial releases are proposed.
(12) Methods to be used for evaluating establishment, dispersal and effect on target and for what period of time.
(13) Methods to be used for evaluating establishment, dispersal and effect on other species in the vicinity of the target and for what period of time.
(14) Collaborative research with other departments.
(15) Assistance to be sought from other departments, e.g. in making releases, mass rearing, secondary distribution, monitoring of spread and effectiveness.
(16) Assistance to be given to other departments, e.g. in making releases in their areas, provision of bulk stocks for release, provision of starter cultures.
(17) Where literature references are cited, a copy of each reference should accompany the application to assist evaluation.

Registration

While permits from AQIS and ANCA allow the importation and release of exotic organisms into Australia, both exotic and indigenous organisms may also require registration before they can be used commercially. Importantly, registration not only requires proof of safety, it also requires proof of efficacy against all pests on which a product will be used.

Registration legislation in Australia has recently been in a state of flux. In 1989, the Australian Agricultural and Veterinary Chemicals Council was established as the national body responsible for the clearance of agricultural and veterinary chemical products prior to state/territory registration. The council published a document *Requirements for Clearance of Agricultural and*

Veterinary Chemical Products in the same year. However, in 1993, the council was disbanded and its role was taken over by the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) which published *Interim Requirements for the Registration of Agricultural and Veterinary Chemical Products* in 1993. These publications and the NRA itself deal not only with chemical products but also with biological, agricultural and veterinary products. The latter are separated into biologically derived chemicals, immunobiologicals, other living organisms and microbial agents. Nematodes are classed as "other living organisms" along with insects, macroscopic parasites and predators, animals and plants. Bacteria, fungi, protozoa, algae and viruses are treated separately as "microbial agents".

The NRA has drafted *Guidelines for Registration and Commercial Trials Clearance of Biological Products* (the Guidelines). Section 1 states that "These guidelines are based on a number of international guidelines for biological pest control agents, but take the Australian situation into account". Section 1 is somewhat comforting to prospective biological control users in that "The NRA recognises that some Biological Products, due to the inherently lower risk posed by them, may be more desirable than synthetic pesticide chemicals and therefore, specific guidelines have been developed which simplify the registration process for these products. Lessening any requirements does not however eliminate the obligation to prove safety".

According to Schedule 3 of the Agricultural and Veterinary Chemicals Code, 1994 which came into effect in March 1995, "any predatory insect, predatory mite or macroscopic parasite" is exempt from registration. Although the Guidelines in section 3.3.3. specifically separate nematodes from macroscopic parasites, the Code overrides the Guidelines. The NRA considers that entomopathogenic nematodes are, for the purposes of the Code, macroscopic parasites and need not be registered (P. Prammer, personal communication, 1995).

At any time in the future, the NRA could require nematodes to be registered before sale. This could occur if, for instance, they were found to be harmful to health during application (e.g. through allergic reactions). Indeed, the recent purported finding of *Providencia rettgeri* (known to be responsible for urinary tract infections in humans) in *Heterorhabditis* species (Jackson *et al*., 1995) does raise the issue of possible health problems.

In the meantime, Australia is fortunate indeed that registration is not required since it would be expensive and require extensive testing and proven efficacy against each pest species. As the market in Australia is relatively small, it is doubtful whether commercial companies would be prepared to go to the trouble and expense involved in registration.

Biological Control Act 1984

The purpose of the Biological Control Act 1984 is to provide immunity from legal action intended to prevent the release of biological control agents for control of target organisms and to prevent, where necessary, small interest groups blocking programmes by recourse to common law.

This act was promulgated after CSIRO developed a biological control strategy for *Echium* spp. (known as 'Paterson's curse' or 'Salvation Jane' depending upon viewpoint). A temporary, and then permanent, injunction was granted by the South Australian Supreme Court to stop control after an application from a group of graziers and apiarists, but there was then no legal mechanism for resolving the conflict. After the legislation, a similar conflict arose between landowners who wanted to control blackberry and apiarists who utilized its pollen; an inquiry established control to be in the national interest and blackberry control was declared under the legislation.

It is unlikely that this Act would be of great importance in regard to import or release of entomopathogenic nematodes, but the Act remains as a safeguard.

NEW ZEALAND

The situation in New Zealand is rather different from that in Australia. While quarantine approval must be sought from the Ministry of Agriculture and Fisheries (MAF) to import new species, the

main control of release and use of exotic nematodes comes from the Pesticides Board, which is responsible for registration.

MAF administers the Biosecurity Act 1993 while the Pesticides Board administers the Pesticides Act 1979.

When application is made to MAF to bring in new species of entomopathogenic nematodes or other organisms, copies of the application are sent to a series of government agencies, including the Department of Conservation, Ministry of Forestry, Department of Health, Ministry of Maori Development and the Parliamentary Commissioner for the Environment. These agencies each then provide their preliminary indication as to whether they rate the risks posed by the importation as (a) negligible, (b) present, (c) significant, (d) serious or (e) unacceptable.

Application forms then need to be sent to Import Management and Quarantine, MAF Quality Management in Wellington for approval.

At the next stage, the nematodes must begin the process of registration, the procedure for which is the same as for registering a chemical. Under the *Guidelines for the Registration of Biological Pesticides*, revised in 1986 and 1989, section 1 states "The Pesticides Act 1979 requires that any pesticide must either be registered or have an experimental use permit (EUP) before it can be sold. Although biological agents when used as pesticides do not come under the Pesticides Act definition of a pesticide, the Governor-General has, by Order in Council, declared them to be pesticides, thus requiring them to be registered. The biological agents defined in this Order are: microbial pathogens, including bacteria, protozoa, rickettsia, fungi, nematodes and viruses or the mutants of any of these agents, intended for sale to control invertebrate pests, weeds, or microbial pathogens of crops."

Thus, unlike the situation in Australia, nematodes are placed firmly among the microbial pathogens and accordingly require registration before they may be sold.

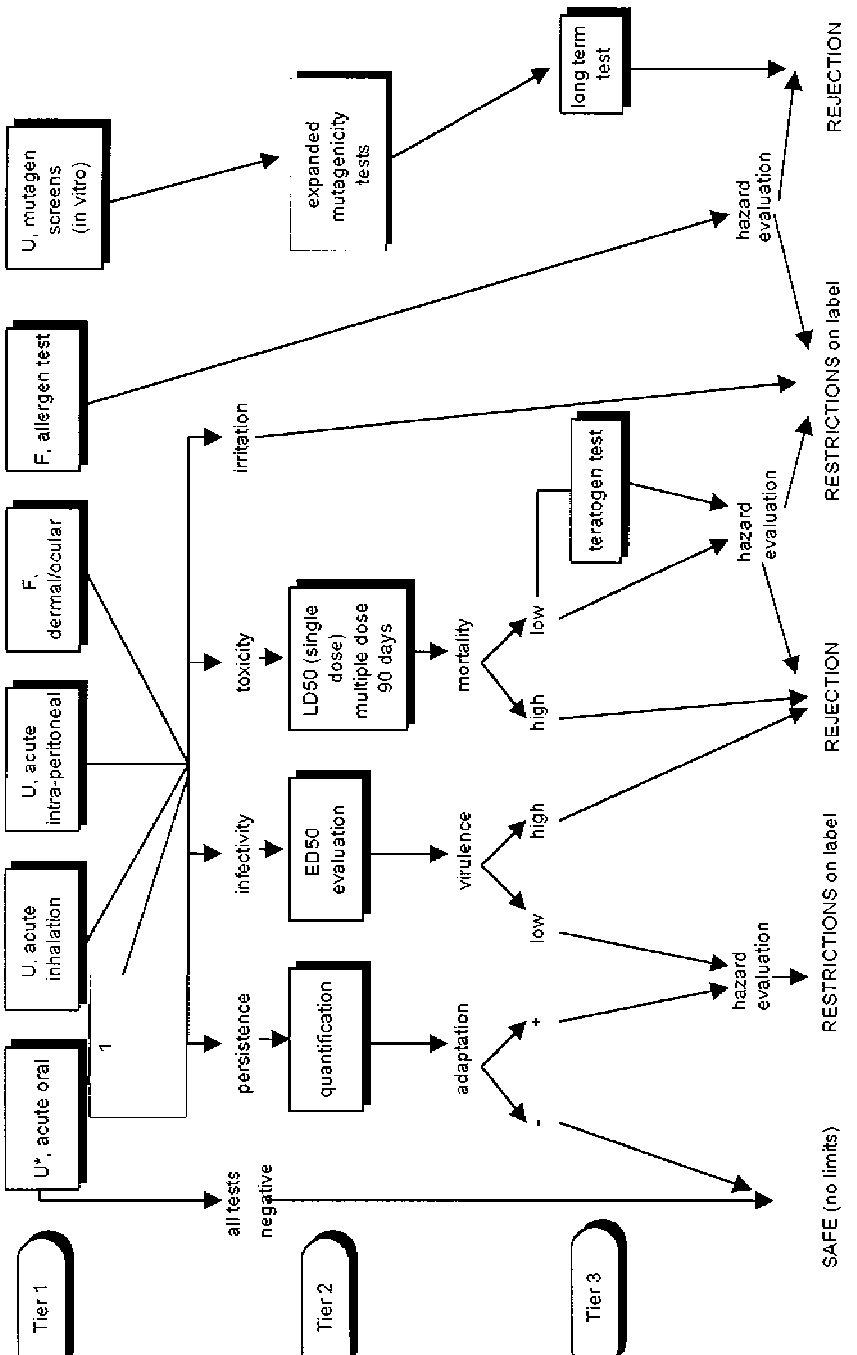
Importation of Nematodes

As with Australia, species of both *Steinernema* and *Heterorhabditis* are present in New Zealand and permits to import entomopathogenic nematodes have been issued previously; this makes it simpler to import further new species. The information required on 'Ministry of Agriculture and Fisheries New Zealand—Te Manatu Ahuwhenua Ahumoana Aotearoa' application to import a new species into New Zealand forms comprises the name and details of importer, species name, zoological classification, life stage to be imported, and the following.

(1) Give a brief synopsis of: (i) any research you have undertaken to date to indicate that this species is a potential candidate for importation into New Zealand (e.g. its suitability to the New Zealand environment and conditions); (ii) any research you did to eliminate other potential species (including any indigenous species) in favour of the proposed species; (iii) whether any research indicated any biological limitations to its distribution throughout New Zealand; (iv) whether you have checked any other restriction (e.g. local body by-laws) which could prevent importation.
(2) What time of year do you propose importation? Please provide reasons.
(3) Which country/countries do you intend to import from?
(4) Have you identified a potential source already? From where?
(5) Where in New Zealand do you intend to establish/hold the proposed species?

If the application is successful, a Plant Import Health Permit is issued by MAF, and this is valid for 1 year. The import permit specifies "Material to be grown/held/processed at" and "Minimum period of quarantine", as well as any general conditions that may be considered necessary. MAF may also give permission to release the organism for experimental purposes or as classical biological control agents, but not for sale.

Once a Plant Import Health Permit has been issued for a nematode, each consignment requires a Permit to Land document to be issued which has one of the following categories left undeleted: 'Authorized unconditionally, prohibited, or authorized subject to the following conditions or



*U = unformulated product, F = formulated product, ED50 = estimated dose exerting effect on 50% of tested individual mice, rats or rabbits

FIGURE 2. Safety testing procedures for bacteria, fungi, protozoa, rickettsia and nematodes required for registration in New Zealand.

treatments'. Any of a number of conditions may apply, including maintenance of the imported nematodes under quarantine conditions.

Release and Registration

There are three steps to full registration with the Pesticides Board.

(1) Experimental Use Permit (Not For Sale)—EPU (NS);
(2) Experimental Use Permit (Limited Sales)—EPU (LS);
(3) Registration.

For each step, progressively more information is required. The information has been extracted in many cases verbatim from *Guidelines for the Registration of Biological Pesticides*, 1989, in which case the text is italicized.

Experimental Use Permit (Not For Sale)

Identification:

- *Systematic name and strain of nematode and the taxonomic description of the nematode, serotype, strain or mutant.*
- *Composition of the unformulated material, microbiological purity, nature and identity of any culture media, impurities and content of extraneous organisms.*

Toxicological data (see Figure 2):

- Acute oral, acute inhalation, acute intra-peritoneal, dermal and eye exposure tests (from tier 1, Figure 2).
- Awareness of the importance of type of formulation, rate of application and equipment used, environmental conditions, protective clothing, personal hygiene and worker attitude.

Environmental and wildlife data:

- *A review on existing general knowledge describing expected effects on environment and its biota. This should include comment on non-target species and on persistence in soil and water and also possible fate in food chains. Any likely undesirable pollution and any likely species at risk should be mentioned. This will provide background information on which to decide what further data may be necessary.*
- *Effects on relevant invertebrates including infectivity.*
- *Other target organisms believed to be at risk. (These should include fish, birds, bees, earthworms, other parasites or predators of the target species.)*
- *Effect on plants, livestock, animals including immunological response.*

Much of this information can be provided from previously published data.

Experimental Use Permit (Limited Sale). As for EPU (NS) together with the following.

Identification:

- The appropriate tests, procedures and criteria used for identification of the biological agent, such as morphology, biochemistry and/or serology.

For the formulated proprietary product:

- *Pro-proprietary name of product.*
- *Type of formulation.*
- *Composition of formulated product, effects of temperature change, method of packaging and storage, retention of biological activity in storage.*
- *Microbiological purity, nature and identity of any culture media, impurities and content of extraneous organisms.*

Toxicological data:

- Acute inhalation, eye and allergen tests.

Efficacy data:

- *Basic information obtained from* replicated *laboratory and field trials will normally be sufficient to determine whether the biological agent will provide reliable control of the pest and to determine limitations of the product.*

Biological properties. *It is important to know which species are attacked and the degree of specificity for the target pest(s), where the agent is naturally occurring and in what circumstances and where appropriate, geographical distribution. Information on the likely biological effects arising from use is required in order to assess possible long term changes in the ecology of the crop and in the environment in general.*

- *Target host species of pest. Pathogenicity or antagonism to pest, infective dose, transmissibility and information on mode of action; history of agent and its use.*
- *Natural occurrence and geographical distribution.*
- *Host specificity range and effects on species other than the target pest (including species most closely related to the target species) to obtain the taxonomic boundary of susceptibility. Studies should include infectivity, pathogenicity and transmissibility.*
- *Infectivity and physical stability in use by specific application method. Effects of temperature, exposure to air, radiation etc. Persistence under the likely environmental conditions of use.*

Registration. As for EPU (LS) but at a more stringent level together with the following.
Toxicological data:

- All tests in tier 1 (Figure 2) should have been completed together with such tests in tiers 2 and 3 (Figure 2) as may have been required for full hazard evaluation.

Residue data:

- Will not usually be appropriate for entomopathogenic nematodes unless the formulation has possible contamination problems for grazing animals, crops, soil or water.

DISCUSSION

While the legislation concerning introduction of exotic nematodes into both Australia and New Zealand appears, at first sight, to be formidable, in practice the situation is not quite as difficult as it seems. This is particularly so because exotic entomopathogenic nematodes of both *Heterorhabditis* and *Steinernema* species have already been imported into and released into both countries over the last 2 decades without any problems arising. When applying to import further exotic species of nematodes, much of the same information and arguments can be utilized, thus saving considerable laborious testing. Further, there can be little doubt that legislative authorities in both countries view the use of biological control agents favourably as opposed to chemical insecticides, and are not inclined to erect unnecessary obstacles to their utilization.

The major difference between the requirements of the two countries is that in Australia entomopathogenic nematodes currently do not have to be registered prior to sale whereas in New Zealand both exotic and indigenous species do. One of the main effects of this is that in New Zealand each particular nematode species in each different formulation must be shown to be effective on each particular pest against which they will be used. Should there be a requirement in the future to register in Australia the same conditions would apply there.

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