GUIDELINES ON THE DATA REQUIRED FOR REGISTRATION OF BIOLOGICAL/BIOPESTICIDES REMEDIES IN SOUTH AFRICA

Issued by the Registrar: Act No. 36 of 1947, Private Bag X343, Pretoria 0001

Republic of South Africa

Tel. (27 12) 319 7000 / Fax (**27 12) 319 7179**

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1. INTRODUCTION

The purpose of this document is to outline the data and documentation required for the registration of biological remedies regulated under Act 36 of 1947. The requirements differ according to the type of registration that is being sought. Information normally required for the different categories of registration is set out in this document; however, the Act makes provision for the Registrar to call for any further information in order to determine whether a remedy is acceptable in the context of public interest, suitability and biological efficacy. The ultimate goal is to ensure that the data requirements are clear for registration application purposes. It is important that only data with a direct bearing on the registration application are presented.

NOTE: If any of the data or documents called for in the paragraphs that follow are deemed by the applicant to be unnecessary or irrelevant, good scientific argument supporting this view should be presented in an application for a waiver of the requirement for such data or documentation. The application for waiver should be submitted before any tests/trials/studies are conducted and prior to the registration application being submitted to the office of the Registrar.

This document supersedes the biopesticides registration guidelines published in 2010 but it should be read in conjunction with other documents such as residues and data requirements guidelines published by the office of the Registrar etc. For toxicological data requirements, applicants are advised to read guidelines published by the National Department of Health i.e. Requirement: Toxicological assessment of agricultural remedies and stock remedies. Toxicological studies must be conducted following the relevant Organisation for Economic Cooperation and Development (OECD) guidelines (1).

2. DEFINITIONS

"**biological remedy**", **"bioproducts",** "**biological products**" and "**biopesticides**" means any biological remedy, or any mixture or combination of any substance or remedy intended or offered to be used for the destruction, control, repelling, attraction or prevention of any undesired microbe, alga, nematode, fungus, insect, plant, vertebrate, invertebrate, or any product thereof, but excluding any biological remedy in so far as it is controlled under the Medicines and Related Substances Control Act, 1965 (Act 101 of 1965), or the Hazardous Substances Act, 1973 (Act 15 of 1973) (Agricultural remedy as defined in Act 36 of 1947).

"**adjuvant**" means any adjuvant consisting of any chemical substance or biological remedy, or any mixture or combination of such substance or remedy and which, when it is added to an agricultural remedy which is prepared for application in accordance with the approved instructions for use of that agricultural remedy, will enhance the efficiency of such agricultural remedy, to be an agricultural remedy for the purposes of the said Act.

"plant growth regulator" and **"legume inoculant"** means any biological remedy, or any mixture or combination of any substance or remedy intended or offered to be used as plant growth regulator, defoliant, desiccant or legume inoculant, and anything else which the Minister has by notice in the *Gazette* declared an agricultural remedy for the purposes of this Act (Act 36 of 1947) (as defined in Act 36 of 1947).

"**fertilizer**","**biostimulants**", "**biofertilizer**", "**plant growth promoter**" and "**inoculant**", means any substance which is intended or offered to be used for improving or maintaining the growth of plants or the productivity of the soil;
Further defined as: "**Group 3**" which is a fertilizer containing natural or synthetic substance(s) or organism(s) that improve(s) or maintain(s) the physical, chemical or biological condition (fertility) of the soil; and "soil improver" has the same meaning. (Act 36 of 1947)

These products may utilise any of the following "active ingredients";

- **Micro-organisms** (microbial)**:** such as fungi, bacteria and viruses that are antagonistic towards pests and diseases or fungi, bacteria and viruses that have plant characteristic or soil enhancing properties
- **Macro-organisms** (macrobial)**:** such as nematodes and insects
- **Biochemical products and Semio-chemicals:** such as insect sex pheromones, which interfere with mating act as or plant growth regulators.
- **Enzymes and hormones.**
- **Plant extracts:** such as those used to attract insect pests to traps by their scent, as well as those with repelling or killing effect (example garlic, certain essential oils, etc.) or that have plant or soil enhancing properties such as seaweeds etc.
- **Legume inoculants** such as Rhizobia
- **Inoculants:** such as free-living nitrogen-fixers, phosphate-solubilising bacteria, plant growth promoting bacteria.

Products may be registered as 'Agricultural remedies' or 'fertilizer' based on their label claims.

3. REGISTRATION REQUIREMENTS FOR ALL BIOLOGICAL REMEDIES AND BIOLOGICAL FERTILIZERS

The flowing must be submitted

3.1. Proof of payment of the prescribed application fee.

3.2. A covering letter outlining the purpose of the application.

3.3. Three copies of the "Application for the Registration of an Agricultural Remedy" form or "Application for the Registration of a Fertilizer" form obtainable from the DAFF website **www.daff.gov.za** fully completed. For Agricultural Remedies only, this must include all relevant information listed in the Active Ingredient Dossier Index (List I) and the Formulated Product Dossier Index (List II) of which one copy of each must be submitted.

3.4. Identification of the biological active ingredient must include where it was isolated (e.g. soil, from lepidopteran larvae, water, etc.). An accession number assigned to the organism by the manufacturer of the product must be provided. A representative sample/voucher specimen of the active ingredient needs to be deposited and registered in an appropriate collection/culture collection such as Agricultural Research Council (ARC) collections, collections held in South African Museums, University collections or in SANBI collections. An accession/voucher number of this representative sample/voucher must be supplied by the collection authority.
The following must be submitted confirming the identification of the organisms. Any technique used must be scientifically sound and in line with the latest techniques available:

Bacteria = Cultural, microscopic and molecular sequencing technique
Fungi = Morphological, Molecular Techniques (ITS gene region) and DNA bar-cording
Insects = Morphological and DNA bar-cording
Nematodes = Morphological and DNA bar-cording
Viruses = Cultural, microscopic and molecular sequencing technique
Plants extracts/Hormones = Chemical analysis (5 batch or Certificates of Analysis (COAs))

3.5. A mass release permits for microbial and macrobial products issued by the directorate Plant Health/Department of Environmental Affairs.

3.6. If the organism is to be sourced from another country, the country of origin must be declared. All valid permits must be submitted, this could include import documentation including customs and excise documents, import permits for trials from Plant Heath Directorate/Department of Environmental Affairs. A letter from a recognised authority, giving the applicant permission for commercialization of the organism. Applicants should contact the Plant Heath Directorate/Department of Environmental Affairs for more details where necessary.

3.7. If the organism is to be sourced from the local environment, details of its source such as GPS co-ordinates or the name of the district, farm, forest or stand.

3.8. Reports and summary on the pharmacology, toxicology and environmental impact studies of the active ingredient and its metabolites and/or degradation products according to OECD guidelines (1). This requirement also applies to vector control agents/products. Any known physical or physiological injury that may be caused by the organism should be evaluated and reported. These reports can be submitted on a compact disc (CD/DVD). If a remedy containing a new active ingredient is already registered by one or more of the registration authorities of the USA, EU, UK, Japan or Australia, toxicological risk assessment reports from the registration authorities concerned, together with a toxicological risk assessment, by an independent and accredited toxicologist, can be submitted in support of a provisional registration. Provisional registrations are removed after the full toxicology risk

assessment has been done by the Department of Health and the product is recommended for final approval by the Registrar.

3.9. Formulation toxicology:

3.9.1. For a formulation containing a new active ingredient, reports and summary on formulation toxicity according to OECD guidelines (1). These reports can be submitted on a compact disc (CD/DVD).
3.9.2. For a formulation containing an already registered active ingredient(s), formulation toxicity data, generated according to OECD Guidelines should be submitted for hazard classification, or, if test data on formulation toxicity are not available, formulation toxicity can be calculated from the toxicity of the a.i./s and all relevant inert ingredients, using the GHS Acute Toxicity Estimate Procedure for Classification and Labelling of Chemicals. The actual calculation must be submitted. These calculations may be subject to further discussion.
3.9.3. Should there be a change in the isolate or type in the formulation (e.g. due to strains losing pathogenicity in culture over time or introduction of a newer, more effective strain), an application for a formulation change should be made and a new representative sample of the active ingredient needs to be deposited and registered as per 3.4. Formulation toxicity data as per 3.9.2. will be required.

3.10. Reports and summary on the physical properties and storage stability (Shelf life) of the formulated product. These reports must be verified/complied by an independent laboratory complying with acceptable standards such as OECD Good Laboratory Practice (OECD GLP); ISO17025, Good Manufacturing Practice (GMP), accreditation by the Medical Control Council; accreditation by SANAS). Shelf life should be determined in accordance with any available test methodology developed by the applicant, or the relevant FAO Pesticides Specifications, US EPA, and OECD etc. The method used should be described in detail in the laboratory test report. Should an applicant wish to apply for an extended shelf life for a product, this must be accompanied by supporting data.

3.11. Three copies of the proposed label. The label has to conform to the “Regulations relating to Agricultural Remedies” (Gov. Gaz. No 29225, 2006) (3) and the requirements of the "Guidelines for the RSA Classification Code of Agricultural and Stock Remedies and Associated Labelling Practices" (4). Where the remedy will also be marketed in a small pack for the home-garden market the proposed home-garden label should also be submitted. Refer to SANS Code 1268: “Labelling Practices for Agricultural Remedies and Fertilizers registered for Home and Home Garden Sector” issued by SABS, 22 November, 2013 (5).

The label should contain at least the following:

3.11.1. Genus, species and strain name/number of the organism.
3.11.2. Minimum guaranteed active ingredient (spores/ml or spores/g or cfu/ml or cfu/g or organisms/ml or organisms/g) (at expiry date).
3.11.3. Name and address of manufacturer.
3.11.4. Pack size
3.11.5. The range of crops and / or pests on which the product should be used.
3.11.6. Shelf life of the product.
3.11.7. Storage recommendations or restrictions.
3.11.8. Precautionary statements including specificity and host range, potential risks of pathogenicity/infectivity in humans, storage recommendations or restrictions.
3.11.9. Warranty statement.
3.11.10. Compatibility with other registered pest control products/substances if supported by data.
3.11.11. Product batch code and date of manufacturing/expiry date.
3.11.12. Directions for use including application rate, interval, method and frequency of use.

3.12. Experimental data, plus a summary of the data, on the biological efficacy and phytotoxicity (where applicable) on the commodity or commodities concerned. Trials must have been conducted in important production areas of the crop(s) concerned in any SADC country, with at least two thirds of these being in South Africa. The trial localities chosen should represent a range of conditions including different bioclimatic regions, climate/weather, cultivars, agricultural practices and soil characteristics. See Appendix 1 for further details. It should be recognised that microbial plant protection products may in some cases deliver lower levels of control or more variable performance than would be expected for a conventional chemical plant protection product. See Appendix 1 for further details.

3.13. For vector control agents/products efficacy data should be done according to WHOPES guidelines (2).

3.14. If a formulation/mixture has more than one microbe or a product is recommended to be applied with another biological product or other agricultural remedy (mixing partners); compatibility between microbes and/or the other agricultural remedy should be tested and a report must be included. All these combinations must also be supported by efficacy/phytoxicity/residues studies.

3.15. Phytotoxicity arising from the use is usually measured in terms of chlorosis, malformed plant parts, leaf burning, plant wilting, stunting (reduced height), reduced stand, or death. For certain uses, some injury can be tolerated (depending upon the reversibility of effects, or on economic or aesthetic factors), but all injuries should be evaluated and reported. Accordingly, the lack of observable symptoms of phytotoxicity should also be reported.

3.16. Residue data from relevant production areas as per the Guidelines on Residue Study Requirements for Registration of Agricultural Remedies and Setting of Maximum Residue Limits (MRLs) in South Africa (9)

3.17. Data extrapolation for efficacy/residue using Codex crop groups will be considered. Representative commodities within each Codex commodity group and subgroup can be selected and proposed based on consideration of available information. The selection of representative commodities for efficacy data extrapolation purposes must be based on the following:

a) The commodity is likely to be major in terms of production and or consumption.
b) The commodity is most likely similar in morphology, growth habit, pest problems and edible portion to related commodities within a group or subgroup.
c) There is an assumption that all of the commodities in the same group are produced following a similar use pattern or good agricultural practice.
d) All the crops in group/subgroups should be grown or cultivated in South Africa.
e) The applicant has the right to choose which crops should be listed on the label based on the crop grouping concept.
f) The pest(s) occurring in the representative commodity group should be similar to that occurring in the sub groups.

Please refer to Appendix 3 for list of Codex crop groups.

3.18. International or local bee risk studies for all new formulated products as specified in the relevant guidelines. Additional data can be submitted by means of published scientific articles.

3.19. A Safety Data Sheet (SDS) for the formulated product, including the contact details of the company responsible for the product in South Africa.

4. SPECIFIC ADDITIONAL REQUIREMENTS FOR ORGANISMS USED IN BIOLOGICAL REMEDIES AND BIOLOGICAL FERTILIZERS

All the requirements set out in Section 3 apply, these are additional requirements based on the type of organism used.

4.1. Micro-organisms

4.1.1. Shelf life (as per 3.10) and the active ingredient should be viable at an acceptable level for at least six months
4.1.2. If an applicant changes the strain of micro-organism for a registered product, new efficacy data, toxicology must be supplied.
4.1.3. The formulation should not exceed contaminants ≥ 100,000 cfu/g or ml/or not exceed limits set by the Department of Health.
4.1.4. Any known metabolites i.e. primary/secondary must be reported.

4.2. Macro-organisms

(a) **Entomopathogenic nematodes (EPNs)**

Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate parasites of insects. A list of species currently recorded in South Africa can be found in Appendix 4
4.2.1 The full taxonomic description of EPN and its symbiotic bacterium (i.e. genus, species)
4.2.2 The history of the organism and its uses (if applicable)
4.2.3 The life cycle and growth characteristics of the organism
4.2.4 Site of infection, mode of action and of entry into host
4.2.5 The label must include the state and stage of the organism
4.2.6 Efficacy trials are to be conducted on a minimum plot size: 2 x 5 m with 4 replicates. Additional data can be submitted by means of published scientific articles.
4.2.7 Any known metabolites i.e. primary/secondary must be reported.

(b) Insects and other macrobials

4.2.7 Efficacy trials are to be conducted on a minimum plot size: 2 x 5 m with 4 replicates. However, the minimum plot size may need to be adjusted upwards for highly mobile insects such as moths. In this case, plots of 0.5 ha with 2 to 3 replicates for efficacy tests on mobile insects. Additional data can be submitted by means of published scientific publications.

4.2.8 If toxicological studies are not available, any scientific published information to show that the product is not harmful to humans and the environment should be submitted. If there are data gaps identified by the registrar in relation to human and environmental safety, approval may be granted pending submission of additional studies. Any known metabolites i.e. primary/secondary should be reported.

4.3. Biochemical products and semio-chemicals

4.3.1 Reduced residue data requirements may be applicable (refer to residue guidelines of 2015)(9)
4.3.2. No residue data are required where the products/devices (e.g. pheromone traps) do not come into contact with the plant parts i.e. leaves, stems and roots and do not results in chemical residues occurring in plants, or in the situation where the product has been exempted from residues data requirement by the Department of Health. The active ingredients used must not be of toxicological significance in relation to human and environmental health

4.4. Enzymes, hormones and plant extracts

These types of biological products do not have any additional requirements (NB: Data requirements are similar to conventional pesticides. For more information refer to section 3 on the guidance document issued by the Department of Health (General requirements for toxicological assessments of Agricultural Remedies and Stock remedies (19)).

4.5. Legume inoculants and other inoculants

4.5.1. The carrier should have the ability to deliver the right number of viable cells in good physiological condition at the right time and should contain little or no contaminants.
4.5.2 All other microorganisms (contaminants) present in the inoculant must be identified.
4.5.3. Formulations should not contain contaminants **≥** 100,000 cfu/g or ml. Products must be tested for commonly-occurring, and serious, human pathogens such as *E. coli*. These contaminants apply specifically to human pathogens and care needs to be taken not to confuse potential contaminants with mycorrhizae-helper bacteria that are often part of the formulation. Producers will need to explain this in their registration package.
4.5.4. For legume inoculants, any trial work should be carried out in fields that have been free from the legume in question for at least three years over one or two seasons in an area where the crop is commercially grown. Trials must report on development of effective nodules (number (%), size, position on the roots, pink/red colouring internally).
4.5.5. Inoculants should be tested at the recommended rate (1x) and double rate (2x) and compared to a suitable product on the market (if such a comparison product exists). If no suitable commercial products exist, then the untreated control, 1x and 2x rates are used.
4.5.6. Peat carriers should contain at least 5×10^8 cfu/g, perlite (6.5×10^8 cfu/g), and liquid inoculants (2×10^9 cfu/ml).
4.5.7. In seed treatments, there should be a minimum of 100,000 rhizobia per seed.
4.5.8. The inoculant should not contain contaminants ≥ 100,000 cfu/g or ml/or not exceed limits set by the Department of Health.
4.5.9. The bacteria or fungi should be viable at an acceptable level for at least six months.

4.6. Plant growth or plant characteristic promoters, biostimulants and biological fertilizers

4.6.1. In addition to the efficacy trials outlined in section 3, the trials must include sterile pots to illustrate the effect of the microbe without any outside contamination as well as non-sterile pots to show the effect of the microbe when applied to a natural soil containing other microbes.
4.6.2. Formulations should not contain contaminants **≥** 100,000 cfu/g or ml. Products must be tested for commonly-occurring, and serious, human pathogens such as *E. coli*.
4.6.3 Organisms should be viable at an acceptable level for at least six months
4.6.4. In cases where a label claim is made that there is an induced systemic resistance to disease, the formulation will be registered as a biological remedy rather than a biological fertilizer.

REFERENCES AND LIST OF REGULATORY DOCUMENTS

(1) Guidelines published by the Organization of Economic Co-operation and Development (OECD) http://www.oecd.org/env/chemicalsandbiosafety/testingofchemicals/
(2) WHOPES Guidelines : World Health Organisation Pesticide Evaluation Scheme WHOPES http://www.who.int/whopes/en/
(3) Regulations relating to Agricultural Remedies (Gov. Gaz. No. 29225, 22 Sept 2006).
(4) SANS Code 1268: Labelling Practices for Agricultural Remedies and Fertilizers registered for Home and Home Garden Sector Nov 2013 (SABS).
(5) Guideline of the registration process for agricultural remedies 2015 (Registrar, Act No. 36 of 1947).
(6) Manual on Development and use of FAO and WHO Specifications for Pesticides, November 2010. http://whqlibdoc.who.int/publications/2006/9251048576_eng_update3.pdf
(7) FAO Specifications for Agricultural Pesticides http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/lpe/lpe-b/en/
(8) Guidelines for specifying the Shelf Life of Plant Protection Products, CropLife International, 2009 Technical Monogragh No 17.

(9) Guidelines on Residue Study Requirements for Registration of Agricultural Remedies and Setting of Maximum Residue Limits (MRLs) in South Africa (Registrar, Act No. 36 of 1947).
(10) TISA Research Committee Protocol on Flue-Cured Tobacco Smoke Trials.
(11) The WHO recommended classification of pesticides by hazard and guidelines to classification: 2009 (World Health Organisation, 2010).
(12) United Nations Globally Harmonised System of Classification and Labelling of Chemicals (GHS); Part 3: Health Hazards, Chapter 3.1.3 (p. 112) – Classification Criteria for Mixtures; 2011.
(13) Guidelines on Equivalence of Agricultural Remedies (Pesticides) (Registrar, Act No. 36 of 1947, November 2000).
(14) Australian Pesticides and Veterinary medicines Authority standards http://www.apvma.gov.au/products/constituents/standards/index.php.
(15) EU Pesticides Database http://ec.europa.eu/sanco_pesticides/public/index.cfm
(16) Guidelines for the registration of Biopesticides, (DAFF, November 2010)
(17) Guidelines for the registration of allowed inputs for organic agriculture (Not yet issued)
(18) Organic Standard SANS 1369:201x for use in Organic agriculture (not yet finalised)
(19) General requirements for toxicological assessments of agricultural and stock remedies.
(20) Registration Guidelines for Minor Uses (Minor Crops) in South Africa, (DAFF, 2010)
(21) EPPO standard series PP1 (efficacy evaluation of plant protection products) (EPPO: http://pp1.epo.org/).
(22) Department of Health, South Africa, General requirements for toxicological assessments of agricultural and stock remedies.
(23) CropLife South Africa, Protocol for scientific verification of quality and specifications of pesticides that have reached a two year shelf life, 2015.
(24) Agricultural Research Council, wine fermentation/vinification test.
(25) South African Government gazette No. R. 383 25 February 1983, Fertilizers, Farm Feeds, Agricultural remedies and Stock remedies Act, 1947 (Act 36 of 1947); Declaration of certain substances and remedies to be agricultural remedies.
(26) Guidelines for the registration of adjuvants in South Africa, October 2010.
(27) Draft guidelines for the registration of adjuvants in South Africa, Registrar of Act 36 of 1947, 2015.

APPENDIX 1: GENERAL TRIAL REQUIREMENTS FOR BIOLOGICAL REMEDIES / BIOLOGICAL FERTILIZER

1. INTRODUCTION

The purpose of this document is to outline the general requirements for field trials for the registration of Biological remedies for pest control in conventional agricultural crops and forestry and Biological fertilisers. The word “pest” is used in its broad sense, meaning insect or other animal pest species, plant pathogen or weed. The information is relevant to most crop/pest situations

Trial sites must be available for inspection by officials of Act 36 at any time following commencement of such trials. Trials must be conducted under conditions of Good Agricultural Practices (GAP). It should be noted that agricultural practices change from time to time, and researchers should familiarize themselves with the latest trends and changes in application technology and agricultural practices.

Data must be generated using sound scientific principles and experimental design and analysed using appropriate statistical methods. Applicants must be able to demonstrate that when their product is used according to label directions, it is effective for the purpose claimed and that its application to the target plant will not cause any unintended effects. It is the responsibility of the applicant to present adequate data to support any claims made on the product’s proposed label.

Data should demonstrate that:

- The remedy/fertilizer is not adversely harmful to humans and the environment.
- the remedy/fertilizer applied is effective
- its side effects on the treated crop are negligible and manageable
- its effects (in the case of some remedies) on subsequent crops are negligible and manageable
- its effects on non-target organisms such as beneficial insects and pollinators are negligible and manageable.
- It has some target specificity

2.1 TRIAL MATERIAL

The test substance or formulation used in trials should be the same product for which registration is sought. Sample material used in registration trials must be no more than two years old when the trials are conducted.

2.2 NUMBERS AND LOCALITIES OF TRIALS

2.2.1 Efficacy Trials

A minimum of three successful field trials must be done for each end use (crop/pest species) to demonstrate the efficacy of the product. In order to prove consistency of activity over a range of environmental conditions, the trials should be located in a variety of locations, spanning the bioclimatic regions, where relevant (refer to Appendix 2), and taking into account seasonal variation i.e. weather/climate, irrigation practices cultivars and soil characteristics in which the crop/pest combination are found and for which registration is sought.

For new active ingredients, the trials must be conducted over a minimum of two seasons or cropping cycles and under a range of environmental conditions relevant to the crop/pest as mentioned above.

In the case of new formulations of registered active ingredients, or products containing equivalent active ingredients from generic manufacturers, one season’s trial work will suffice provided that the

trials are conducted under a range of environmental conditions relevant to the crop/pest as mentioned above. Alternatively, trials should also be conducted in different growing seasons if the crop occurs in one geographic area or bioclimatic zone. For soil acting products, physical soil analysis reports done by an AgriLasa/GLP/ISO17025 accredited laboratory should be submitted. Such reports should be from soil samples taken not more than five years before or after the trial was initiated.

2.2.2 Phytotoxicity / Selectivity Trials / Yield Trials

In most cases, a minimum of three successful field trials must be done on each crop or group of related crops to demonstrate the crop safety of the product. The trials should be located in the same or a similar range of locations to the efficacy trials. Efficacy trials can also be used for visual phytotoxicity evaluations, provided that the appropriate treatments are included in these trials. The use of different cultivars is highly recommended.
For new active ingredients, the trials must be conducted over a minimum of two seasons or cropping cycles, on different cultivars and, in the case of soil-applied remedies, on soils with different physical and chemical characteristics. For herbicides applied in agronomic (field) crops, yield trials must be conducted on a range of different cultivars and, where relevant, under irrigated or dryland conditions and on different soil types, including soils on which phytotoxicity is more likely to occur.
In the case of new formulations of registered active ingredients, or generic products, a minimum of three trials in one or more season will be required provided that the trials are conducted under a range of environmental conditions relevant to the crop as mentioned above, including, if relevant, soil types on which phytotoxicity is more likely to occur. If the crop occurs in one geographic area or bioclimatic zone then trials should be conducted in more than one growing season.

2.3 EXPERIMENTAL DESIGN

Efficacy and phytotoxicity trials should be designed and laid out in such a way as to permit a statistical evaluation. Usually a randomised block design is adequate.
It is essential to ensure adequate pest pressure, evenly distributed through the trial site for trials to give meaningful efficacy data.

Treatments must be replicated at least four times, however, in order to allow for meaningful statistical analysis, the Error Degrees of Freedom should be 12 or more and thus the replicate number should be adjusted according to the Error Degrees of Freedom. For example, if you have one product at two rates plus an untreated control and a reference product in the trial you need to have 5 replicates in order to have 12 Error Degrees of Freedom.
The design must include an untreated Control treatment. In the case of herbicide efficacy trials, this can be in the form of an untreated control strip alongside each treated plot.

The remedy being tested must be compared to an appropriate reference product. This could be another remedy having the same or similar active ingredient, one with a pest control spectrum similar to that of the remedy being tested, or one that is commonly used in the specific crop or situation concerned.

Experiments with a new remedy (new active ingredient) should include a range of dosages in order to determine the optimum rate(s) for effective pest control without significant crop damage.
Once optimum rates have been determined for different crop/pest combinations, further trials should be done using these rates. The selected optimal rate(s) should provide consistent results on the targeted pest species.

Efficacy trials with new formulations or generic remedies must include the optimum rate(s) of active ingredient as determined above and these rates should reflect the proposed commercial rate(s).

Phytotoxicity trials must contain at least a single (1x) and double (2x) dosage to demonstrate crop safety.

If the remedy is to be applied in mixtures then the trials must be designed so as to demonstrate the properties of these combinations such as synergism, additive effect, antagonism, compatibility, crop safety/phytotoxicity, and residues etc. In cases where an adjuvant/fertilizer/botanical extract is added to the spray mixture efficacy, phytotoxicity and residue data will be needed – refer to the Adjuvant Registration Guidelines and residue guidelines.

If the proposed rate of any product in a tank mixture differs from the recommended rate when the component products are applied alone, the trials must be designed so as to demonstrate the effectiveness and crop safety of the mixture with these rates of the different components. Similarly, if any pest claimed to be controlled by a tank mixture is in addition to pests controlled by the components individually at equivalent rates, then supporting data are needed for the new pest claim.

2.4 APPLICATION METHOD AND WATER VOLUME

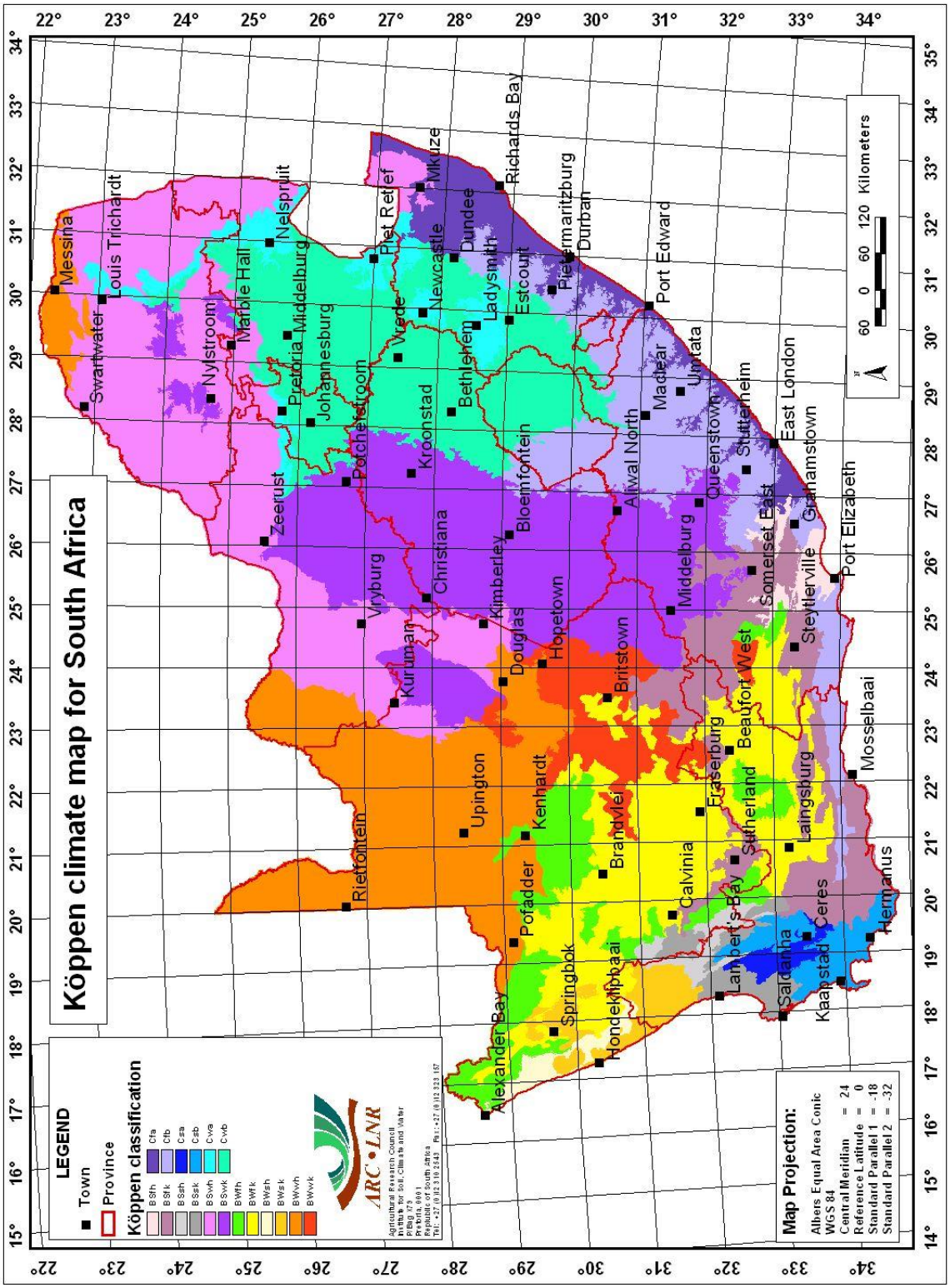
All application equipment used in trials must be properly calibrated.
The application pattern should be similar to that used in commercial practice, both in particle size and distribution and in deposition on the treated surfaces.
The method(s) of application should be similar to the method(s) which will be recommended on the product label and these should reflect the current application technology and good agricultural practices.
Optimum spray volumes must be determined for the product concerned for each application method. A range of spray volumes should be tested if it is expected that different spray volumes will result in different levels of performance of the product.
The spray volume and the carrier or diluents should be similar to those that are, or will be recommended for commercial use. If it is intended to recommend more than one, or a range of application volumes, e.g., ground and aerial application, trial data must be generated to support such recommendations. Efficacy should be demonstrated on one or more of the target species in at least one trial at each of the application volumes. Similarly, at least one selectivity trial should be done at each application volume to prove that the reduced water volume and increased concentration of the pesticide do not cause any phytotoxicity to the target crop. This can be done simultaneously with the efficacy trials mentioned above.

2.5 DATA RECORDING

The following should be recorded and reported in the Trial Report:
Trial Sponsor: Details of Company seeking registration
Trial Objective: The reason for doing the trial
Person Responsible for the Trial: Name & contact details of the person who established and evaluated the trial.
Locality of Trial site: Town/City, Farm name and GPS coordinates.
Trial Co-operator: Name & contact details
Crop details: Common and scientific names of crop; Cultivar name, planting date (or age).
Pest details: Common and scientific names of pest(s). If codes or abbreviations are used these must be clearly explained.
Products evaluated: For each product included in the trial, give Trade name and/or Code name, Active ingredient, a.i. content, Formulation type, Registration number (if registered) and name of supplier or registration holder
Date of Trial Establishment: Normally date of first treatment
Trial Design: Trial layout, number of replicates, statistical design.

Plot size: Length x breadth (area) or number of trees or vines or crop rows
Row width, Plant spacing & Plant population/ha: For soil applied products or seed treatment trials, also give planting depth
Planting Method: Method & type of planting equipment if relevant
Soil Analysis: Trials with soil-applied products & seed treatments, for example, the soil analysis reports should be from soil samples taken not more than five years before or after the trial was initiated. The reports should include: % clay, silt, sand, organic matter, soil classification and soil classification.
Application Equipment: Make and type of equipment, including spray nozzle type if relevant.
Spray Pressure: If relevant, in bar or kPa
Spray volume applied: Volume in ℓ/ha or ℓ/tree at each application
Application dates: Date and time of each application
Crop and pest stages of growth at each application: e.g., Zadock, BBCH: These must agree with crop and pest stages to be claimed on the label.
Environmental conditions at application: Air temperature, humidity, cloud cover, wind strength & direction, soil moisture if relevant.
Topography of Trial Site: degree and direction of slope
Precipitation: Time and amount of first rainfall or irrigation after application and, if possible, weekly or monthly precipitation for the duration of the trial.
Good Agricultural Practices: List standard practices applied in the husbandry of the crop concerned, including fertilisation, standard pesticide treatments, etc.
Timing of evaluations: Date and number of days after treatment (DAT) for each evaluation.
Evaluation Methods: Describe methods used to assess efficacy & phytotoxicity; describe any rating system used.
Crop and pest stages of growth at each evaluation: e.g., Zadoks, BBCH
Assessment sampling: Describe sampling area or sample size used when doing assessments.
Results – Efficacy: Present data in table format; graphs or photographs may also be used to illustrate treatment effects. Data from counts, ratings, etc. should preferably be converted, where possible, into % control. Appropriate statistical analyses should be performed on the data.
Results – Phytotoxicity: Describe any symptoms seen. The number of evaluations would depend on the nature of the product and duration of symptoms being present. Data should be presented in table format. Even if there were no symptoms seen, tables should be presented to indicate that the levels of damage dropped to zero or were at zero since application. **Discussion and Conclusions:** A discussion of the factual evidence from the trial and an accurate interpretation of the results, but do not include recommendations based on the results of one trial only.
Discussion and Conclusions: A discussion of the factual evidence from the trial and an accurate interpretation of the results, but do not include recommendations based on the results of one trial only.

APPENDIX 2: BIOCLIMATIC REGIONS IN SOUTH AFRICA



APPENDIX 3: CODEX COMMODITY CROP GROUPINGS

Table 1: Commodity Crop Groupings and Possible Crop Extrapolations for efficacy data extrapolation (Adopted from Codex and Australian Guidelines). NB: Refer to the latest Codex or minor crops guidelines for latest changes in Crop Groups.

Crops		**Possible Extrapolation**	
		From	**To**
Citrus fruit	**Subgroup 1** Lemons Limes Mandarins **Subgroup 2** Grapefruit Oranges Tangelos	Oranges + Lemons or Oranges + Limes or Oranges + Mandarins	Whole group
Pome fruit	Apple Crab apple Pear Quince	Apples + Pears	Whole group
Stone fruit	**Subgroup 1** Apricot Nectarine Peach **Subgroup 2** Cherries Plums Prunes	Peaches + Nectarines + Cherries or Peaches + Plums + Cherries Peaches	Whole group Nectarines, plums
Berries and other small fruit	**Subgroup 1** Blackberry Boysenberry Cranberry Raspberry **Subgroup 2** Blueberry Currants Gooseberry **Other** Grapes Strawberry	Grapes + strawberry and one other from subgroups 1 or 2 Raspberry Currants	Whole group Subgroup 1 Subgroup 2
Bulb vegetable	**Subgroup 1** Garlic Onions Shallots **Subgroup 2** Chives Spring onions **Subgroup 3** Leeks **Subgroup 4** Fennel bulb	Onions+ Spring onions or Onions + shallots or Onions + Leeks Onions (green) or shallots	Whole group Subgroups 1, 2 and 3

Crops		Possible Extrapolation	
		From	**To**
Brassica vegetables	**Subgroup 1** Cauliflower Broccoli **Subgroup 2** Cabbage **Subgroup 3** Brussels sprouts	Cauliflower + Cabbage + Brussels sprouts or Broccoli + Cabbage + Brussels sprouts	Whole group
Capsicum	Chilli (green) Bell peppers Baby green pepper Piquante Jalepeno Paprika Peppadews	Paprika + peppadew + baby green pepper	Whole group
Fruiting vegetables - cucurbits	**Subgroup 1** Cucumber Bitter melon Zucchini (baby marrow) Patty pans Baby gems **Subgroup 2** Melons Pumpkin Squash **Subgroup 3** Gherkin	Melon + Cucumber + Zucchini Melons	Whole group Subgroup 2
Legume vegetables (succulent seeds and immature pods)	Beans (green) Peas (green)	Beans (green) + Peas (green)	Whole group
Pulses dry	Peas Beans Chickpea Lentils Lupin Soybean	Field peas (dry) + faba beans (dry) + lupins or Beans (dry) + chickpeas + lupins or Beans (dry) + Peas (dry) + Lupins	Whole group

Crops		Possible Extrapolation	
		From	**To**
Root and tuber vegetables	**Subgroup 1** Carrot Parsnip **Subgroup 2** Beetroot Swede Turnip **Subgroup 3** Sweet potato Potato Yam **Subgroup 4** Radish Horseradish **Subgroup 5** Chicory	Potato + carrot + beetroot or Potato + carrot + swede or Potato + carrot + radish	Whole group
Stalk and stem vegetables	Artichoke Asparagus Celery Witloof Rhubarb	Celery + asparagus + artichoke Celery	Whole group Rhubarb
Cereal grains	**Subgroup 1** Wheat Triticale Cereal rye **Subgroup 2** Barley Oats **Subgroup 3** Maize Sorghum Millet **Subgroup 4** Rice	Wheat + barley + oats Maize + sorghum Wheat or barley Wheat Rice	Subgroups 1 and 2 Subgroup 3 Oats, rye, triticale, durum wheat (treatments applied before GS32 only) Whole group except rice for post harvest treatment only Rice
Grasses for sugar or syrup production	Sugarcane	Sugarcane	Sugarcane
Leguminous Pastures	Lupins, Lucerne, Medics *(Medicago* spp), Serradella and Clover,	Lupins + Clover	Whole group
Tree nuts	Almonds Cashew Chestnuts Hazelnuts Macadamia Pecan Pistachios Walnuts	Pecan + Macadamia	Whole group

Crops		Possible Extrapolation	
		From	**To**
Herbs	Many	Parsley, mint (extrapolations to a group on a case by case basis)	Whole group

For crops not indicated on this table- full developmental residue/efficacy data is recommended

APPENDIX 4: NEMATODE STATUS IN SOUTH AFRICA

Two major genera are acknowledged: *Heterorhabditis* and *Steinernema* which are mutualistically associated with bacteria (Family: Enterobacteriaceae) of the genera *Photorhabdus* and *Xenorhabdus*, respectively (Kaya & Gaugler 1993)(6).

The current status of nematodes found in South Africa:

Nematode species	Report of occurrence	Associated bacteria	Reference of bacteria
H. bacteriophora	Malan *et al*., 2006 (7); Hatting *et al*., 2009 (8); Malan *et al*., 2011 (9)	Unknown	-
*H. noenieputensis***	Malan *et al*., 2014 (10)	*P. noenieputensis***	Ferreira *et al*. 2013a (17)
*H. safricana***	Malan *et al*., 2008 (11)	Unknown	-
H. zealandica	Malan *et al*., 2006 (7)	*P. zealandica***	Ferreira *et al*. 2013a (17)
*S. citrae***	Stokwe *et al*., 2011 (12)	Unknown	-
*S. innovationi***	Çimen *et al*., 2014a (13)	Unknown	-
*S. khoisanae***	Nguyen *et al*., 2006 (14) Hatting *et al*., 2009 (8)	*X. khoisanae***	Ferreira *et al*., 2013b (18)
*S. tophus***	Çimen *et al*., 2014b (15)	Unknown	-
*S. sacchari***	Nthenga *et al*., 2014 (16)	Unknown	-
S. yirgalemense	Malan *et al*., 2011 (9)	*X. indica*	Ferreira *et al*., 2014 (19)

(6) Kaya, H.K. & Gaugler, R., 1993. Entomopathogenic nematodes. Annual Review of Entomology 38, 181-206.
(7) Malan, A.P., Nguyen, K.B. & Addison, M.F., 2006. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the southwestern parts of South Africa. African Plant Protection 12, 65-69.
(8) Hatting, J., Stock P.S. & Hazir, S., 2009. Diversity and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in South Africa. Journal of Invertebrate Pathology 102, 120-128.
(9) Malan, A.P., Knoetze, R. & Moore, S.D., 2011. Isolation and identification of entomopathogenic nematodes from citrus orchards and their biocontrol potential against false codling moth. Journal of Invertebrate Pathology 108, 115-125.
(10) Malan, A.P., Knoetze, R. & Tiedt, L.R., 2014. Heterorhabditis noenieputensis n. sp. (Rhabditida: Heterorhabditidae), a new entomopathogenic nematode from South Africa. Journal of Helminthology 88, 138-151.
(11) Malan, A.P., Nguyen, K.B., De Waal, J.Y. & Tiedt, L., 2008. Heterorhabditis safricana n. sp. (Rhabditida: Heterorhabditidae), a new entomopathogenic nematode from South Africa. Nematology 10, 381-396.
(12) Stokwe, N.F., Malan, A.P., Nguyen, K.B., Knoetze, R. & Tiedt, L., 2011. Steinernema citrae n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. Nematology 13, 567-587.
(13) Çimen, H.C., Lee, M.-M., Hatting, J., Hazir, S. & Stock, S.P., 2014a. Steinernema innovationi n. sp. (Panagrolaimomorpha: Steinernematidae), a new entomopathogenic nematode species from South Africa. Journal of Helminthology (In press).
(14) Nguyen, K.B., Malan, A.P. & Gozel, U., 2006. Steinernema khoisanae n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. Nematology 8, 157-175.
(15) Çimen, H.C., Lee, M.-M., Hatting, J., Hazir, S. & Stock, P.S., 2014b. Steinernema tophus sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from South Africa. Zootaxa 3821, 337-253.
(16) Nthenga, I., Knoetze, R., Berry, S., Tiedt, L.R. & Malan, A.P., 2014. Steinernema sacchari n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. Nematology 16, 475-494.
(17) Ferreira, T., Van Reenen, C., Pagès, S., Tailliez, P., Malan, A.P. & Dicks, L., 2013a. Description of Photorhabdus luminescens subsp. noenieputensis subsp. nov., a symbiotic bacterium associated with a new Heterorhabditis species related to Heterorhabditis indica. International Journal of Systematic and Evolutionary Microbiology 63, 1853-1858.
(18) Ferreira, T., Van Reenen, C.A., Endo, A., Spröer, C., Malan, A.P. & Dicks, L.M.T., 2013b. Description of Xenorhabdus khoisanae sp. nov., a symbiont of the entomopathogenic nematode Steinernema khoisanae. International Journal of Systematic and Evolutionary Microbiology 63, 3220-3224.
(19) Ferreira, T., Van Reenen, C.A., Tailliez, P., Pagès, S., Malan, A.P. & Dicks, L.M.T., 2014. First report of the symbiotic bacteria, Xenorhabdus indica, associated with the entomopathogenic nematode Steinernema yirgalemense. Journal of Helminthology (In press).

APPENDIX 5: IMPORT REQUIREMENTS OF UNREGISTERED BIOLOGICAL REMEDIES OR BIOLOGICAL FERTILIZERS

This import permit under ACT 36 of 1947, is issued for the import of unregistered biological remedies or biological fertilizers for use in efficacy/residue trials for registration, laboratory work or export.

The procedure for an import permit for agricultural remedies is as follows:

1. Motivation letter or protocol.

2. The description of the biological remedy or biological fertilizer. This should include the active ingredient identification, content and type of formulation and the intended use of it.

3. Specify the quantity of biological remedy or biological fertilizer imported.

4. Indicate the port of entry and exit.

6. Copy of the material safety data sheet.

7. If the consignment is to be exported, attach documentation e.g. registration status of the biological remedy or biological fertilizer in the destination country.

8. If the biological remedy or biological fertilizer is imported for trial purposes, attach the trial protocol. In the case of laboratory tests, the name and address of the laboratory concerned.

9. A Permit from Directorate Plant Health, DAFF will be required

10. Proof of payment of the application fee.