Evaluation Report ERC2010-06

Nosema (Paranosema) locustae Canning

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Overview

Registration Decision for *Nosema (Paranosema) locustae* Canning

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, has granted conditional registration for the sale and use of Nolo BB Concentrate and Nolo Bait Biological Insecticide, containing the technical grade active ingredient *Nosema (Paranosema) locustae* Canning, which may suppress grasshoppers and Mormon crickets in crops and rangelands.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

Although the risks and value have been found acceptable when all risk reduction measures are followed, the applicant must submit additional scientific information as a condition of registration.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of Nolo BB Concentrate and Nolo Bait Biological Insecticide.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act.*

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact."

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (e.g. children) as well as organisms in the environment (e.g. those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the PMRA's website at www.healthcanada.gc.ca/pmra

What Is Nosema (Paranosema) locustae Canning?

Nosema (Paranosema) locustae Canning is a microbial pest control agent which may suppress grasshoppers and Mormon crickets. The spores of this microorganism are ingested by the target pest and then develop primarily in the insect's fat cells. In doing so, the microorganism competes with the host for the energy reserves and as a result the host becomes weak and eventually dies.

The end-use product, Nolo Bait Biological Insecticide, is a commercial class insecticide product that contains *Nosema locustae* as the active ingredient. The end-use product exists as a bait formulation. The product will be applied on crop and rangeland.

Health Considerations

Can Approved Uses of *Nosema (Paranosema) locustae* Canning Affect Human Health?

***Nosema (Paranosema) locustae* Canning is unlikely to affect human health when Nolo Bait Biological Insecticide is used according to label directions**

Exposure to *Nosema (Paranosema) locustae* Canning may occur during handling of Nolo Bait Biological Insecticide. When assessing health risks, several key factors are considered: the microorganism's biological properties (e.g. production of toxic byproducts); reports of any adverse incidents; its potential to cause disease or toxicity as determined in toxicological studies; and the likely levels to which people may be exposed relative to exposures already encountered in nature to other strains of the microorganism. Toxicology studies in laboratory animals describe potential health effects from large doses for the purpose of identifying any potential to cause disease or toxicity. No significant toxicity and no signs of causing diseases were observed when *Nosema locustae* was tested on laboratory animals, but it tested positive in a sensitization study. Besides the microbial pest control agent (MPCA), wheat present in the end-use product is known to be an allergen and must be labelled as such (i.e., Wheat Allergen). Recommended Personal Protective Equipment (PPE), exposure mitigating, and hygiene statements present in the product label are adequate to protect human health when label directions are followed.

Residues in Water and Food

Dietary risks from food and water are not of concern

The *Food and Drugs Act* (FDA) prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for the FDA purposes through the evaluation of scientific data under the *Pest Control Products Act* (PCPA). Each MRL value determines the maximum concentration in parts per million (ppm) of a pesticide allowed in or on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

Nosema (Paranosema) locustae Canning spores occur naturally in soils as they enter the environment from infected grasshoppers. The use of Nolo Bait Biological Insecticide which may suppress grasshoppers and Mormon crickets in croplands and rangelands is not expected to significantly increase natural environmental background levels of this microorganism because Nosema locustae is sensitive to sunlight and heat and is decomposed by other microorganisms, and therefore has low environmental persistence. The spores are not toxic or infective to plants; do not persist on vegetation, and this species is not known to produce any secondary metabolites of toxicological concern. Furthermore, no signs of toxicity or disease were observed when Nosema locustae was administered orally to rats. The establishment of an MRL is therefore not required for Nosema locustae as the likelihood of residues contaminating food and drinking water supplies is negligible to non-existent. As such, dietary exposure and risks are minimal to non-existent.

Occupational Risks From Handling Nolo Bait Biological Insecticide

Occupational risks are not of concern when Nolo Bait Biological Insecticide is used according to label directions, which include protective measures

Users of Nolo Bait Biological Insecticide can come into direct contact with *Nosema (Paranosema) locustae* Canning on the skin, in the eyes, or by inhalation. Pulmonary toxicity, dermal irritation, eye irritation and sensitization studies using *Nosema locustae* in animals have shown low toxicity, no irritation, and a potential for sensitization. Repeated exposure of occupational workers to high concentrations of *Nosema locustae*, as with any other microorganism, can potentially lead to the development of allergic reactions. The signal words “POTENTIAL SENSITIZER” and precautionary statement “May cause sensitization” are required on the product label to warn workers of this potential hazard. Besides the MPCA, wheat present in the end-use product is known to be an allergen and must be labelled as such (i.e., Wheat Allergen). To minimize occupational risk, the label will specify that users exposed to Nolo Bait Biological Insecticide must wear gloves, long-sleeved shirts, long pants, shoes plus socks, eye-wear and a NIOSH approved respirator/mask (with any N, P, R or HE filter).

For bystanders, exposure is considered negligible since the application sites are croplands and rangelands and the product is applied as a bait. On the basis of the low toxicity/pathogenicity profile for *Nosema locustae,* Nolo Bait Biological Insecticide is unlikely to pose an undue risk when bystanders are exposed. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When *Nosema (Paranosema) locustae* Canning Is Introduced Into The Environment?

Environmental risks are not of concern

Nosema (Paranosema) locustae Canning spores occur naturally in soils as they enter the environment from infected grasshoppers. The use of Nolo Bait Biological Insecticide which may suppress grasshoppers and Mormon crickets in croplands and rangelands is not expected to significantly increase natural environmental background levels of this microorganism because *Nosema locustae* is sensitive to sunlight and heat and is decomposed by other microorganisms, and therefore has low environmental persistence. Therefore, it is unlikely that the application of Nolo Bait Biological Insecticide will significantly increase the levels of infective, viable persistent *Nosema locustae* that would adversely affect the dynamics of an ecosystem. There have been no reports of adverse ecological effects in the United States from the application of this biopesticide which was first registered there in 1980.

From the available data and information on the effects of *Nosema locustae* to terrestrial/aquatic organisms, there is reasonable certainty that no harm will be caused to birds, fish, wild mammals, terrestrial and aquatic arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the use of Nolo Bait Biological Insecticide. It is unlikely that *Nosema locustae* will adversely affect non-target organisms because it is an obligate parasite of grasshoppers and crickets. The use of Nolo Bait Biological Insecticide will not pose significant environmental risk when used according to label instructions.

Value Considerations

What Is the Value of Nolo Bait Biological Insecticide

Nolo Bait Biological Insecticide has value in that it may suppress grasshoppers and Mormon crickets in crop and rangeland when applied at a minimum rate of 1.12 kg per hectare. One advantage of Nolo Bait Biological Insecticide is that *Nosema locustae* has little effect on beneficial and other non-target organisms. Therefore, in addition to use in organic production, Nolo Bait Biological Insecticide may be useful in environmentally sensitive areas were conventional insecticides cannot be used and reliable, immediate control is not critical. Nolo Bait Biological Insecticide is compatible with current management practices and conventional crop production systems.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The PMRA is proposing key risk-reduction measures on the labels of Nolo™ BB Concentrate and Nolo Bait Biological Insecticide to address the potential risks identified in this assessment.

Key Risk-Reduction Measures

Human Health
Because of concerns with users developing allergic reactions through repeated high exposure to *Nosema (Paranosema) locustae* Canning, anyone handling or applying Nolo Bait Biological Insecticide must wear waterproof gloves, a long-sleeved shirt, long pants, and shoes plus socks. In addition, mixers/loaders and applicators must wear a NIOSH approved respirator/mask (with any N, P, R or HE filter), and eye-wear.

Environment
As a general precaution, handlers are directed to not contaminate irrigation or drinking water or aquatic habitats by cleaning of equipment or by disposing of wastes. In addition, aerial application is permissible only when meteorological conditions at the treatment site allow for complete and even crop coverage.

What Additional Scientific Information Is Being Requested?

Although the risks and value have been found acceptable when all risk-reduction measures are followed, the applicant must submit additional scientific information as a condition of registration. More details are presented in the Science Evaluation of this Evaluation Report or in the Section 12 Notice associated with these conditional registrations. The applicant must submit the following information within the time frames indicated.

Manufacturing Process of the TGAI:
Minor deficiencies have been identified and the applicant will be required to fill the data gaps.

Manufacturing Process of the End-use Product:
Minor deficiencies have been identified and the applicant will be required to fill the data gaps.

Product Characterization and Analysis
To ensure that the manufacturing process of Nolo BB Concentrate does not result in unacceptable levels of microbial contaminants, the applicant is required to provide the following:

- five certificates of quantitative analysis on microbial contaminants, i.e., bacterial and fungal contaminants using the most recently manufactured batches of the TGAI.
- acceptance limits for each of the microbial contaminants.
- details of the methods used for the bacterial and fungal contaminant analysis.
- description of the steps or measures taken if the batches contain microbial contaminants beyond their acceptable limits.

Storage Stability Testing
The applicant is required to provide a confirmatory storage stability study using the end-use product.

Other Information

As these conditional registrations relate to a decision on which the public must be consulted, the PMRA will publish a consultation document when there is a proposed decision on applications to convert the conditional registrations to full registrations or on applications to renew the conditional registrations, whichever occurs first.

The test data cited in this Evaluation Report (i.e. the test data relevant in supporting the registration decision) will be made available for public inspection when the decision is made to convert the conditional registrations to full registrations or to renew the conditional registrations (following public consultation). If more information is required, please contact the PMRA's Pest Management Information Service by phone (1-800-267-6315) or by e-mail (pmra.infoserv@hc-sc.gc.ca).

Science Evaluation

Nosema (Paranosema) locustae Canning

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Nosema* (*Paranosema*) *locustae* Canning
Function	May suppress grasshoppers and Mormon crickets in crop and rangeland.
Binomial name	*Nosema* (*Paranosema*) *locustae* Canning
Taxonomic designation[1]	
Kingdom	Fungi
Phylum	Microsporidia
Sub-order	Apansporoblastina
Family	Nosematidae
Genus	*Nosema* (*Paranosema*)
Species	*Nosema* (*Paranosema*) *locustae*
Strain	Canning

[1]http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=235221

Patent Status information	No patent
Minimum purity of active	1.0×10^{10} spores/mL

Identity of relevant impurities of toxicological, environmental and/or significance.	The technical grade active ingredient does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards, and no mammalian toxins are known to be produced by *Nosema (Paranosema) locustae* Canning.

1.2 Physical and Chemical Properties of the Active Ingredient and End-Use Product

Technical Product- Nolo BB Concentrate

Property	Result
Guarantee	1.0×10^{10} spores/mL
Physical state	Spore suspension in water
Colour	Beige
Odour	None
Mesh size	8 microns
Specific gravity	1.12

End-Use Product- Nolo Bait Biological Insecticide

Property	Result
Guarantee	2.2×10^{6} spores/g
Physical state	Solid
Formulation type	Solid
Colour	Beige
Odour	Wheat Bran
Bulk density	0.24 – 0.32 g/cm^3
Corrosive characteristics	Non-corrosive

1.3 Directions for Use

Nolo Bait Biological Insecticide may provide suppression of grasshopper and Mormon cricket populations in crop and rangeland.

Use Nolo Bait Biological Insecticide when grasshopper densities reach nine (9) or more grasshoppers per square meter. Grasshoppers are most effectively suppressed when they are young. For best results, apply Nolo Bait Biological Insecticide when most grasshoppers are in the 3rd instar (12 to 19 mm long). Due to the nature of this product (i.e., microsporidial pathogen), efficacy may be affected by such factors as weather (e.g., rain following treatment, temperature), grasshopper population densities, and insect migration.

Apply Nolo Bait Biological Insecticide to crop and rangeland at a minimum rate of 1.12 kg per hectare. This product must be consumed by the target pest in order to be effective. Consumption of a higher number of spores per grasshopper will increase product efficacy and decrease the amount of time required to kill the grasshoppers. Therefore, where greater efficacy or faster population reduction is required, this may be achieved through multiple applications or a higher application rate in order to increase the amount of bait available to each grasshopper. Apply by hand, seed spreader, turbine spreader, or airplane. Concentrate the application in areas of heaviest grasshopper infestation.

1.4 Mode of Action

Nosema locustae is a spore-forming microsporidium protozoan pathogen of orthopteran adipose tissue, and is effective only when ingested. After the bait is consumed, protozoan spores germinate in the insect gut and release sporoplasms, which enter the cells of the fat body. Infection and hypertrophy of the fat body effectively starve the insect host of energy reserves. *Nosema locustae* is not highly virulent and can reach very high numbers of spores in the host before death in 4 to 5 weeks. Transmission is facilitated by necrophagy and contamination of the environment by infected faeces. Grasshoppers eventually die of the infection, but are more likely to be cannibalized by other healthy grasshoppers. This natural behaviour results in the further infection of the population. Infected adult females have decreased egg production, and those eggs may contain *Nosema*, which is ingested by the insects as they chew out of the pod, resulting in death shortly after hatching. The impacts of *Nosema locustae* on grasshoppers include mortality, reduced feeding, reduced reproduction, and reduced migration.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Infections by *Nosema locustae*, as with most microsporidians, are usually diagnosed by the presence of spores. Spores are 3.5 to 5.5 µm long by 1.5 to 3.5 µm in diameter with mean measurements of 2.8 µm to 5.2 µm. Triangulate and elongate megaspores, up to 8 µm long, are common. Spores are generally ellipsoidal, occasionally slightly bent or kidney shaped and refractive to light. Mean lengths of polar filaments extruded by mechanical pressure were 86 µm (maximum 145 µm). The spore consists of a chitinous membrane surrounding the sporeplasm within which the polar filament is coiled. The membrane has two separate layers and the polarplast is about 1.5 µm large at the anterior end of the sporoplasm. The polar filament is coiled within the outer sporoplasm and attaches to the external membrane anteriorly near the polarplast.

A method for strain-specific identification was not submitted. Spores of *Nosema* (*Paranosema*) *locustae* Canning have been identified by microscopic examination.

2.2 Methods for Establishment of Purity of Seed Stock

No mother culture of the active ingredient is maintained by the applicant. *Nosema* (*Paranosema*) *locustae* Canning is constantly replenished through the manufacturing process. Practices for ensuring the purity of the spores were adequately described in the summary of the manufacturing process and quality assurance program.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

There are presently two methods to define the content of *Nosema (Paranosema) locustae* Canning: microscopic examination and via grasshopper infection through a bioassay procedure.

- **Microscopic method:** Spore counting is performed microscopically using a hemacytometer cell counting chamber.

- **Bioassay procedure:** The viability of spores in each batch is tested against different target species at different larval stages using various dose concentrations.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

Nosema (*Paranosema*) *locustae* Canning is a microsporidian pathogen of orthopterans. No adverse effects have been reported for this MPCA in the United States where it has been registered since 1980. No signs of adverse effects were observed when the MPCA was tested orally in rats. Furthermore, *Nosema* l*ocustae* is not known to produce secondary metabolites of toxicological concern.

Based on the above information, the establishment of a maximum residue limit (MRL) is not required for *Nosema locustae* under section 4(d) of the Food and Drugs Act (adulteration of food) as defined under Division 15, section B.15.002 of the Food and Drugs Regulations.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during manufacture of Nolo BB Concentrate are acceptable.

There are no impurities of toxicological concern associated with *Nosema (Paranosema) locustae* Canning and based on the processing and manufacturing methods the possibility of contamination and introduction of unintentional ingredients are minimal.

There are no reports of mammalian toxins produced by this MPCA. There are no reports in the published scientific literature of genotoxic metabolites produced by *Nosema locustae* and there is no evidence to suggest that *Nosema locustae* would produce any genotoxic compound.

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

A study was conducted to determine the possibility of occurrence of any microbial contamination of *Nosema (Paranosema) locustae* Canning spores. Qualitative analyses for bacterial contaminants in five batches of the most recently manufactured TGAI were submitted in support of registration, but were found to be deficient. Consequently, the applicant will be required to provide information on the following:

- five certificates of quantitative analysis on microbial contaminants, i.e., bacterial and fungal contaminants using the most recently manufactured batches of the TGAI.
- acceptable limits for each of the microbial contaminants.
- details of the methods used for the bacterial and fungal contaminant analysis.
- description of the steps or measures taken if the batches contain microbial contaminants beyond their acceptable limits.

2.7 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Although the study submitted to support the 'Storage' statement on the Nolo Bait Biological Insecticide label was acceptable, only one batch of end-use product was tested. A confirmatory study, conducted in a similar manner, is required and must test at least two additional batches of Nolo Bait Biological Insecticide

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

Nosema (Paranosema) locustae Canning is an obligate parasite of orthopterans that requires host infection to grow and multiply. The spores can be found naturally in the soil in inactive forms. Infection of warm-blooded animals is not likely as the microbial pest control agent (MPCA) cannot grow and replicate at body temperatures at or above 37^{o}C.

A detailed review of the toxicological database for Nosema locustae has been completed. The database consists of animal (in vivo) toxicity, irritation and sensitization studies, and incident reporting on immunological effects. Not all studies submitted are currently required by the PMRA for health hazard assessment of MPCAs, but these additional studies were considered in this assessment. All the submitted studies were performed using only the MPCA. Although the dermal toxicity and irritation studies should be conducted with the end-use product as required, given that the end-use product formulation contains no formulants of toxicological concern, testing with a suspension of the MPCA was considered acceptable.

The quality of the test data was acceptable, and the database is sufficient to characterize the toxicity and infectivity of this MPCA and the end-use product. Moreover, a survey of published literature was done to update information on this MPCA and to supplement the hazard assessment.

In an acute oral toxicity/infectivity study there were no mortalities, no significant signs of toxicity, no signs of infectivity, and no treatment related necropsy findings in Sprague Dawley rats (20/sex), when observed for 21 days, following oral gavage with 1 mL *Nosema locustae* spore suspension (2.29×10^8 spores/mL). The MPCA is of low toxicity in the rat when challenged via the oral route. This study is classified as acceptable, and meets the guideline requirement for assessing acute oral toxicity in rats. This study is considered supplemental as an infectivity study because clearance of the MPCA was not assessed, as well, recovery of the MPCA from animal tissue was not attempted and viability or target host infectivity of the spores used were not assessed prior to administration in test animals.

In an acute pulmonary toxicity/infectivity study of 28 days, no mortalities, no signs of toxicity, no changes in body weight gain, no treatment related-necropsy findings and no signs of toxicity were observed in Sprague-Dawley rats (7 ♂, 8 ♀) following intratracheal administration of 40 µL *Nosema locustae* spore suspension (1.89×10^9 spores/mL). The MPCA is of low toxicity and no signs of infectivity were observed in the rat when challenged via the intratracheal route. This acute pulmonary toxicity study is classified as acceptable, and meets the guideline requirement for assessing acute pulmonary toxicity in rats. This study is considered supplementary as an infectivity study because clearance of the MPCA was not assessed, and recovery of the MPCA from animal tissue was not attempted.

In an acute intraperitoneal infectivity study, no mortalities, no significant toxicity, infectivity or pathogenicity were observed in CD1 mice (40/sex) during the observation period of 56 days following intraperitoneal injection of a single dose of 0.25 mL *Nosema locustae* spore suspension (1.4×10^9 spores/mL). The MPCA is of low toxicity and not pathogenic to mice by intraperitoneal injection. This study is classified as acceptable, and meets the guideline requirement for assessing an intraperitoneal infectivity in mice. Although enumeration of the MPCA was not done in tissues collected at necropsy, the clearance pattern of the MPCA was demonstrated in accordance with guideline requirements. The hematology profile, microscopic pathology, and bioassays conducted to study infectivity were found to be adequate to assess the infectivity potential, and determine a pattern of clearance of *Nosema locustae* in mice exposed through the intraperitoneal route.

In a maximum challenge infectivity study of 70 days in rabbits, 32 New Zealand White rabbits (16/sex) were injected with either crude or processed *Nosema locustae* spore suspension (approximately 2×10^8 spores/mL in saline) as follows: one group of 20 animals (10/sex) received intracerebral (0.1 mL processed), intraocular (0.05 mL processed), and intraperitoneal (l.0 mL crude) injections. Three other groups of 4 rabbits each (2/sex) were separately injected, either intracerebrally, intraocularly, or intraperitoneally with test material at similar doses as mentioned above. There were no treatment related mortalities, no clinical signs, and no necropsy findings in this study. No signs of infectivity were observed in rabbits exposed to *Nosema locustae* by intraperitoneal, intracerebral, or intraocular injections. This infectivity study is classified as supplemental with limited use because neither the enumeration of MPCA in the tissues collected at necropsy was conducted nor was the clearance pattern of the MPCA demonstrated. Intracerebral and intraocular injection studies are not required by the PMRA to support the registration of MPCAs, but this multiple injection study is considered and has been reviewed as a maximum challenge safety test.

In a maximum challenge infectivity study of 56 days in mice, 3 groups of 42 white Swiss mice (21/sex) were injected with either crude or processed *Nosema locustae* spore suspension (2×10^8 spores/mL in saline) as follows: a group of 30 animals (15/sex) received intracerebral (0.05 mL processed) and intraperitoneal (l.0 mL crude) injections. Two other groups of 6 mice each (3/sex) were either separately injected intracerebrally or intraperitoneally with test material at similar doses as mentioned above. There were no treatment related mortalities or clinical signs, and no necropsy findings during the study. No signs of infectivity were observed in mice exposed to *Nosema locustae* by intraperitoneal and intracerebral injections. This infectivity study is classified as supplemental with limited use because neither the enumeration of MPCA in the tissues collected at necropsy was conducted nor was the clearance pattern of the MPCA demonstrated.

In a primary eye irritation study, 0.1 mL of *Nosema locustae s*pore suspension in saline (2.29×10^8 spores/mL) was instilled into the conjunctival sac of the left eye of each of the New Zealand White albino rabbits (10/sex) for 24 hours. The right eye of each rabbit, which received a single application of 0.1 mL of 0.8% saline solution, served as the control. Animals were then observed for 14 days, and irritation was scored by the method of Draize. No eye irritation was observed, therefore, the eye irritation score was zero. In this study, *Nosema locustae* is not an eye irritant. Although the PMRA requires irritation testing with the end-use product, given that the end-use product contains no formulants of toxicological concern, testing with a suspension of the MPCA was considered acceptable. This study is classified as acceptable and satisfies the guideline requirement for a primary eye irritation study in rabbits.

In an acute dermal toxicity study, a group of Sprague Dawley rats (7/sex) were dermally exposed to a single dose of 200 µL *Nosema locustae* spore suspension (3.2×10^8 spores/mL) for 24 hours to an area of approximately 10% of body surface area. Following exposure animals were observed for a period of 28 days. The MPCA is of low toxicity as there were no mortalities, no overt signs of toxicity, and no signs of infectivity in the test animals resulting from the exposure. This acute dermal toxicity study is classified as acceptable and satisfies the guideline requirement for a dermal toxicity study in rats. Although the PMRA requires dermal toxicity

testing with the end-use product, given that the end-use product contains no formulants of toxicological concern, testing with a suspension of the MPCA was considered acceptable.

In a primary dermal irritation study six New Zealand White rabbits were dermally exposed to 0.5 mL of *Nosema locustae* spore suspension (2.29×10^8 spores/mL) in 0.8% saline for 24 hours. After exposure animals were observed at 24 and 72 hours, and dermal irritation scored by the method of Draize. No dermal irritation was observed in this study. Application of the test substance caused no effects on body temperature. In this study, *Nosema locustae* was not a dermal irritant based on the primary irritation score of 0. This primary dermal irritation study is classified as acceptable, and satisfies the guideline requirement for a primary dermal irritation study in rabbits. Although the PMRA requires dermal irritation testing on the end-use product, given that the end-use product contains no formulants of toxicological concern, testing with a suspension of the MPCA was considered acceptable.

In a skin sensitization study with *Nosema locustae*, young adult male guinea pigs were tested by intradermal injections. Animals (10 males) were dosed with 0.05 mL of *Nosema locustae* spore suspension (1.7×10^8 spores/mL in distilled water) by intradermal injection on Days 0, 3, 5, 7, 10, 12, 14, 17, 19, and 21 during the induction phase of the study. After 3 weeks of induction exposure (Day 42), test group animals were challenged with 0.05 mL of *Nosema locustae* spore suspension (2.3×10^8 spores/mL). Due to bacterial contamination of test material and in order to assess the magnitude of the contributing component in the challenge suspension to the skin reaction, additional intradermal injections were done. On Day 46, the test group received intradermal injections of 0.05 mL of antibiotic solution at one site and 0.05 mL of an overnight culture of *Staphylococcus saprophyticus* resuspended in sterile 0.85% sodium chloride at a second site. The intensities of erythema and edema at the injection sites were scored and the dimensions of the skin reactions were measured at 24 and 48 hours post-injection by the method of Draize. No mortality was observed during the study and no significant difference in body weight was observed. Animals in the test group showed statistically significant increase in erythema, edema and areas of skin reactions at 24 and 48 hours after the Day 42 challenge, compared with the corresponding Day 0 observations. In this study, technical grade *Nosema locustae* spore suspension tested positive as a dermal sensitizer. This study is classified as acceptable. A dermal sensitization study is not required by the PMRA because the Agency assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing.

A report on hypersensitive incidence presented by the applicant (May 19, 1988) showed no immunological effects to employees of a production facility, where employees had been intimately involved in the extraction, standardization, shipping and handling of *Nosema locustae* spores since the summer of 1982. Also employees were frequently exposed to *Nosema locustae* spores by hands, eyes, nose, clothes, oral cavity, etc. with minimal protection during processing, operational and clean-up activities. In spite of these extensive exposures to the spores with minimal protection, employees had reported no immunological or other adverse effects. Regardless of this finding there is no assurance there would be no immunological effects in the general population resulting from repeated high exposure to this MPCA as there are potentially

sensitive individuals in the population. As indicated previously, all MPCAs are considered potential sensitizers by the PMRA.

Higher tier subchronic and chronic toxicity studies were not required due to the low acute toxicity of the MPCA, and no indications of infectivity or pathogenicity in the test animals treated in the Tier I acute oral toxicity/infectivity, pulmonary toxicity/infectivity, maximum challenge infectivity test, intraperitoneal injection infectivity, and dermal toxicity tests. This MPCA, which is an obligate parasite of insects is unlikely to grow or replicate in the human body at a body temperature of 37°C. However, although the related entomopathogens, *Nosema bombycis* and *Nosema algereae* failed to replicate in mammalian and avian cell cultures at temperatures corresponding to the body temperatures of homeotherms they replicated in the same cell lines at ≤28°C and ≤35°C respectively. *Nosema locustae* is reported in the published literature as not being pathogenic in vertebrates.

There are no reports in the available scientific literature that suggest this MPCA has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodents and rabbits indicate that, following pulmonary and intraperitoneal injection routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated from *Nosema locustae.*

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal and/or inhalation. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. Nosema (Paranosema) locustae Canning has not been identified as a wound pathogen or known to penetrate intact skin of healthy individuals or produce any toxic secondary metabolite that could be dermally absorbed.

Toxicity testing with the MPCA showed no signs of toxicity or infectivity via the oral, dermal, or pulmonary routes of exposure. The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. The submitted sensitization study was positive for this MPCA. Label statements (i.e., Potential Sensitizer) and risk mitigation measures such as personal protective equipment (PPE), including gloves, long-sleeved shirts, long pants, NIOSH approved respirator/mask (with any N-95, P-95, R-95 or HE filter), and shoes plus socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers. In addition to the sensitization potential of the MPCA, wheat present in the end-use product is known to be an allergen and must be labelled as such (i.e., Wheat Allergen) according to PMRA Regulatory Directive, DIR 2006-02: Formulants Policy and Implementation Guidance Document.

The MPCA was shown to not cause dermal or eye irritation. Although the irritation studies submitted tested the MPCA instead of the end-use product, as required by the PMRA, there are no formulants of concern in the end-use product that warrant further testing. However, since the end-use product contains formulants which could be physically irritating to eyes, ocular exposure can be minimized if end-use product applicators wear protective eye-wear.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Nolo Bait Biological Insecticide, and no significant occupational risks are anticipated for this product.

3.2.2 Bystander

Overall, the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for the MPCA and the assumption that precautionary label statements will be followed in the use of Nolo Bait Biological Insecticide.

The label does not allow applications outside of cropland and rangeland; therefore, non-occupational dermal exposure and risk to bystanders is low. Because of this limitation on use sites, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Nosema (Paranosema) locustae Canning is an obligate parasite in orthopterans and is not known to infect plants. In the United States the MPCA is exempt from all tolerance requirements in or on all raw agricultural commodities, where it has been registered for use since 1980. When applied as baits, most of the end-use product will be deposited on the soil surface, rapidly used up by the target pests, and is not expected to persist on the crop before harvest. Moreover, the MPCA is inactivated rapidly by sunlight and heat (temperature over 40°C) or decomposed by soil organisms and poses no known hazards to humans.

The MPCA is not known to produce any secondary metabolite of toxicological concern and there have been no reports of adverse effects to humans from natural populations of *Nosema locustae.* In addition, residues of the microbial pesticide are likely to be removed from treated food by washing, peeling, cooking and processing. Even if residues are not removed, dietary exposure to the microbial agent is unlikely to cause any undue hazard to consumers because no adverse effects were observed at maximum hazard dose levels in the submitted Tier I acute oral toxicity study. Therefore, negligible to no risk is expected for the general population, including infants and children, or animals from residues in or on agricultural commodities.

Although people could be exposed to residues of the MPCA from diet, chronic dietary risks posed by exposure are of no concern. The PMRA did not require subchronic and chronic dietary exposure studies since the Tier I acute oral study demonstrated a low toxicity and no pathogenicity for the MPCA. Because of the low toxicity profile and low exposure potential of the MPCA, there is no concern for chronic risks posed by dietary exposure of sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

The likelihood of *Nosema locustae* entering neighbouring aquatic environments or surface water run-off from field use of Nolo Bait Biological Insecticide is considered low. Application as baits will reduce the soil availability of this MPCA, as the baits are used up within hours by the target hosts or the MPCA is inactivated by sunlight, heat or is decomposed by soil microorganisms. The potential transfer of the MPCA to surface or ground water during run-off can be minimized by following label recommendations.

To avoid contamination of lakes, streams, ponds or other water bodies, aerial application will be restricted by label statements. The label for Nolo Bait Biological Insecticide will instruct users not to contaminate drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes. By following label statements, the possibility of the MPCA entering neighbouring aquatic environments can be minimized. Being an obligate parasite, *Nosema locustae* is not expected to grow or multiply in an aquatic environment.

Municipal treatment of drinking water will also likely remove the transfer of residues to drinking water. Therefore, potential exposure to the MPCA in surface and drinking water is negligible, and consequently, no risks are expected from exposure to this microorganism in drinking water. In the United States, *Nosema locustae* is not listed as the cause of impairment of any water bodies (Docket Number EPA-HQ-OPP-2007-0997) under Section 303 (d) of the Clean Water Act (CWA). Moreover, there were no harmful effects observed in Tier I acute toxicity testing.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses and acceptable daily intakes are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e., no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the Agency concludes that *Nosema locustae* is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects

from prenatal or postnatal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, do not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *Nosema locustae* to human health.

3.4 Maximum Residue Limits

The *Food and Drugs Act* (FDA) prohibits the sale of adulterated food, that is, food containing a pesticide residue that exceeds the established MRL. Pesticide MRLs are established for FDA purposes through the evaluation of scientific data under the PCPA. Each MRL value defines the maximum concentration in parts per million (ppm) of a pesticide allowed in/on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

Since the MPCA is a naturally occurring, host-specific parasite not infective to plants, it is unlikely that the use of Nolo Bait Biological Insecticide as a bait will result in significant residue levels on food and feed. *Nosema locustae* does not persist on vegetation. No adverse effects from dietary exposure have been attributed to natural populations of *Nosema locustae*, and there are no reports of known mammalian toxins of *Nosema locustae* origin. Furthermore, there were no significant signs of toxicity and no signs of pathogenicity observed when *Nosema locustae* spore suspension was administered orally to rats. In the United States, *Nosema locustae* has been exempted from all tolerance requirements in or on all raw agricultural commodities and considered safe, where it has been registered for use since 1980.

Therefore the establishment of an MRL is not required for *Nosema locustae* under Section 4 (d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other published information on the safe use of *Nosema locustae*, there is reasonable certainty no harm will result from aggregate exposure of residues of *Nosema locustae* to the general Canadian population, including infants and children, when the MPCA is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Since the product use is limited to cropland and rangeland only, dermal and inhalation exposure to the general public in residential or recreational areas will be minimal. Furthermore, there have been no reported adverse effects in the human population from exposure to *Nosema locustae* in the environment. Even if there is an increase in exposure to this microorganism from the use of Nolo Bait Biological Insecticide there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. The Agency is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with *Nosema locustae*. No cumulative effects are anticipated if the residues of *Nosema locustae* interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Environmental fate testing is a measure of the ability of an MPCA to survive or replicate in an environment to which it is applied. It provides an indication of non-target organisms which may be exposed to the MPCA, and the magnitude of exposure.

Even though environmental fate data were not required due to the absence of significant toxicological effects in non-target organisms in Tier I testing, the applicant nevertheless submitted three published studies to address the environmental fate of *Nosema (Paranosema) locustae* Canning.

In one study, the impact of *Nosema locustae* on microbial activity and nutrient cycling in soil incubated under laboratory conditions was assessed. Application of *Nosema locustae* at the recommended field rate (1.5×10^{10} spores/ha), had no significant effect on microbial activity and nutrient cycling in loam while application at 10 times the recommended field rate (1.5×10^{11} spores/ha) showed significant effects. *Nosema locustae* caused a reduction in microbial respiration as measured by carbon dioxide evolution, particularly during incubation from Day 14–56, but stimulated nitrogen-mineralization during the first 14 days of incubation which stabilized between Day 14–56 of incubation. *Nosema locustae* reduced the activity of urease during the first and second week by 40% and 70% respectively, but urease activity returned to near normal levels after six weeks. Analysis of field soils six weeks after application of a mixture of *Nosema locustae* and dimethoate showed no adverse effect on microbial activity.

In another study, the persistence of *Nosema locustae* spores in soil, their leaching potential, and their relationship with the indigenous soil microflora were assessed. Three short grass study areas in Saskatchewan were selected. One area was studied for three consecutive years and the other two for two consecutive years. Each of the three sites contained a control and treated plot (100 m × 100 m) separated from each other by at least 0.8 km. The spores were applied to 3 kg of wheat bran as a bait to provide approximately 3.7×10^6 spores per gram of bait. To estimate *Nosema locustae* population in field soils, soil cores (5.5 cm diameter × 3 cm depth) were collected from treated and control plots, and enumeration of spores recovered from soil samples were done by epifluorescence microscopy. To determine the movement of spores in soil, sandy soil was air dried to 2–3% moisture content, sieved and added to fill a plastic cylinder (5.8 cm by

18 cm). *Nosema locustae* spores (3×10^6 spores/mL) in distilled water was added drop by drop to the centre of each column and then 30-mL aliquots of distilled water were added, immediately and 72 h later to simulate the average weekly rainfall. Twenty-four hours after the addition of each aliquot of water, sections of the soil column were sampled, and the movement of spores was determined by epifluorescence microscopy. A control soil column received the same spore aliquot, but no water. Number of spores recovered immediately from two sites after application of bait was about 20% of the number added. From one site 20% of spores were recovered two weeks after the application. Although all the three sites showed low recovery levels of spores, spores were recovered at all sampling times and a low basal level was maintained over 3-4 months. Some of the soil samples contained a larger number of spores/gram indicating presence of infected grasshopper cadavers in the sample. These results showed that spores can persist in soil for several months, and there is entry of *Nosema locustae* spores into the soil ecosystem continuously from infected grasshoppers. Vegetation collected from one site showed detectable spores while spore-like objects which were recovered from vegetation of both control and treated field from the second site were not morphologically similar to *Nosema locustae* spores recovered from infected grasshoppers. Therefore, *Nosema locustae* spores do not persist on vegetation. The spore movement study showed that *Nosema locustae* can move through the soil. This study showed soil actinomycetes preying upon *Nosema locustae* spores, and also several types of soil bacteria that attack the spores, but it was not verified that the spores were actually preyed upon by indigenous soil microorganisms or if they simply serve as nutrients during decomposition.

In another study, persistence and interaction of *Nosema locustae* spores with indigenous soil microflora were assessed. Persistence of *Nosema locustae* spores in model laboratory soil was monitored with fluorescent-membrane filter counts. Approximately 50% of the spores added to the soil were recovered immediately after addition. The number of detectable spores in the soil decreased 1000 fold after 12 weeks of incubation at 27°C, but no decrease in spore recovery resulted in soil incubated at 5°C. Persistence was not related to initial number of spores added to the soil because similar results were obtained when initial level of spores added was 10^4 or 10^6 /g of soil. During the incubation, soil was not allowed to dry out; therefore, desiccation did not cause spore disappearance. The sterile sand control showed that spore persistence was not dependent on temperature. This study indicates that *Nosema locustae* spore persistence in soil depends on predation or antagonism by other soil microorganisms, which is temperature dependent. Soil slide contact techniques showed that *Nosema locustae* spores were attacked by indigenous soil actinomycetes and bacteria at 27°C and not at 5°C. After 5 days of incubation at 27°C, actinomycete hyphae were seen in contact with and sometimes encircling *Nosema locustae* spores, and these hyphae appeared to migrate from one group of spores to another. Small rod-and cocci-shaped bacteria were also observed around *Nosema locustae* spores. After several weeks of incubation at 27°C, few *Nosema locustae* spores were detected on soil slides, but extensive actinomycete hyphae were present. This study indicates that microbial predation affected *Nosema locustae* persistence in the laboratory model soil system in a temperature dependent manner.

4.2 Effects on Non-Target Species

Environmental toxicology studies, including published studies, were submitted to address the risks of *Nosema locustae* to birds, freshwater fish, aquatic arthropod, and honey bees. Data requirements for non-target terrestrial arthropods, and plant toxicity testing were addressed through waiver rationales.

4.2.1 Effects on Terrestrial Organisms:

In an acute oral toxicity/infectivity study of *Nosema locustae* on ring-necked pheasants (*Phasianus colchicus*), 60 12-day-old female chicks were studied for 29 days. Two groups of birds (30/group) were administered, by single oral gavage, either 0.20 mL of sterile distilled water (negative control) or 0.20 mL of technical grade *Nosema locustae* spore suspension (1.89×10^9 spores/mL). The chicks were observed daily for mortalities and clinical signs of disease, weighed weekly and examined thoroughly; hematology variables were measured, histopathological examinations were done and the organ weight to body weight ratio was calculated. There were no mortalities associated with the treatment, no signs of infectivity and no treatment-related toxicity, body weight change, clinical signs or findings at necropsy. Hematological parameters studied were within the normal limits, and there were no microscopic anatomical abnormalities. Microbial clearance was not assessed. This toxicity/infectivity study is classified as supplemental as it does not fully satisfy the guideline requirement for an avian oral toxicity/infectivity study. The guideline maximum challenge concentration (MCC), or maximum hazard dose, was not achieved, and the test concentration was administered only once instead of the required five successive day-administration.

In a maximum hazard intravenous injection study with *Nosema locustae* on ring-necked pheasants (*Phasianus colchicus*), 90 21-day-old (male and female) chicks were studied for 29 days. Three groups of birds (30/group) received separate intravenous injections of 0.05 mL of sterile distilled water (negative control), 0.05 mL of heat inactivated spore suspension (heated at 56°C for 20 minutes; heat-killed control) and 0.05 mL of technical grade *Nosema (Paranosema) locustae* Canning spore suspension (1.89×10^9 spores/mL; test group). The chicks were observed daily for mortalities and clinical signs of disease, weighed weekly and examined thoroughly; hematology variables were measured, histopathological examination was done, and the organ weight to body weight ratio was calculated. In test group birds there were no mortalities associated with the treatment, no signs of infectivity and no treatment-related toxicity, body weight change, clinical signs or findings at necropsy. Hematological parameters studied were within the normal limits, and there were no microscopic anatomical abnormalities. Although the guideline MCC was not achieved in this study and the recovery of the MPCA from tissues collected at necropsy was not attempted, the hematological parameters studied and histopathology methods employed were adequate to address the infectivity potential of the MPCA. As well, the high body temperature of birds (40–42ºC) makes avian infectivity/ pathogenicity unlikely given that *Nosema locustae* is an obligate parasite of insect origin. *Nosema locustae* has no history of pathogenicity to birds and searches in various databases of published scientific literature found no reports of adverse effects. Moreover, no harmful toxins

are produced by *Nosema locustae*. This toxicity/infectivity study is acceptable and satisfies the guideline requirement for avian toxicity/infectivity study in birds.

Nosema locustae has no history of pathogenicity to wild mammals, and searches in various databases of published scientific literature found no reports of adverse effects. Furthermore, the human health and safety studies on the rodents and rabbits submitted in support of registration indicate that there are no pathogenicity nor toxicity concerns from all routes of exposure at the tested doses. This MPCA, which is an obligate parasite of insects, is unlikely to grow or replicate in organisms having a higher body temperature of 37°C. The taxonomically related entomopathogens, *Nosema bombycis* and *Nosema algereae* failed to replicate in mammalian and avian cell cultures at temperatures corresponding to the body temperatures of homeotherms although they showed replication in the same cell lines at ≤ 28°C and ≤ 35°C, respectively. Together, this evidence suggests that the MPCA is unlikely to cause adverse effects on wild mammals.

A published study was submitted to address acute oral infectivity/toxicity in the honey bee (Menapace et al., 1978). In this study, 400 adult honey bees (*Apis mellifera*) were studied in two tests of 200 honey bees each for 26 days. They were individually force fed a dose of 5 µL of 16% sugar solution containing *Nosema locustae* spores. Each bee received one dose of 0, 5×10^1, 5×10^2, 5×10^3, 5×10^4 spores. Thereafter, the caged bees were incubated at 31°C and fed (*ad libitum*) 40% sucrose solution and tap water, and sacrificed after 26 days. The ventriculus and thoracic tissues of dead bees or bees sacrificed at study termination were examined by phase contrast microscopy for the presence of *Nosema locustae* spores. In the first test, 11 of the 200 bees died during the study. Two of the deceased showed *Nosema locustae* (3 spores in one and 1 in the other) in the ventricular contents. One of the 189 surviving bees showed only a single spore in the ventriculus. In the second test, 161 of the 200 bees died during the study due to pre- and post emergence stress from malfunctioning temperature cabinets. No *Nosema locustae* spores were observed in the ventriculi of these dead bees or in sacrificed bees. No spores were found in the thoracic tissues of bees from either test. In this study, *Nosema locustae* spores were not toxic or infective to adult honey bees when exposed by direct ingestion. Although one test was compromised, this study is classified as acceptable.

A waiver was submitted for predatory and parasitic arthropods with the rationale that sarcophagid flies and bee flies are very difficult to maintain in the laboratory. Bee flies and sarcophagid flies are likely to be exposed to *Nosema locustae* spores. Bee flies are widespread predators of grasshopper eggs. They lay eggs on or near grasshopper egg pods, and the developing larvae feed on grasshopper eggs. High levels of predation are not uncommon with bee flies. Sarcophagid flies are another major invertebrate parasite of grasshoppers which may be exposed to *Nosema locustae* as a result of parasitism of infected grasshoppers. Transovarial transmission of *Nosema locustae* is possible in grasshoppers. Spores of *Nosema locustae* have been observed in ovaries and in eggs produced by infected females. It is not known whether bee flies preying on the eggs or sarcophagid flies parasitizing *Nosema locustae* infected grasshoppers will become infected. Such a transmission could be possible, but it need not always be infective in predatory or parasitizing species. A study on the effects of *Nosema locustae* on target and non-target organisms on Cape Verde Islands (West Africa) showed negligible effects on non-

target organisms. No mortality was noted, and transmission was rare and non-pathogenic in non-target species. *Nosema locustae* spores were detected in samples of *Periplaneta americana* (cockroach) and *Butalus occidentalis* (Scorpion) collected from *Nosema locustae* treated fields, but these species showed no pathogenicity in subsequent feeding trials when they were fed with *Nosema locustae* infected mealworms; however, *Nosema locustae* could be detected in the fatty tissue of these test insects.

In the second waiver submitted from further testing of terrestrial arthropod species that may be exposed to the MPCA under operational conditions of use, the applicant argued that exposure of the leafcutter bee (*Megachile rotundata*) was low because bees of this species do not pick up pieces of leaf that have dropped on the ground and therefore, it is unlikely that they would pick up flakes of the *Nosema locustae* bran bait from the ground. Consequently, the bran bait would have to stick to leaves of plants for there to be any potential exposure to the bees. In trials with dimethoate-treated bran bait, leafcutter bees failed to pick up any of the bran. Non-target toxicity and infectivity to non-target terrestrial arthropods by *Nosema locustae* is not expected because it is an obligate parasite of grasshoppers and crickets and primarily a fat body parasite which reproduces slowly in the fat body tissues of host species. It is one of the least virulent pathogens of grasshoppers, and there is no evidence that it will infect any insects other than grasshoppers, some crickets, and a few close relatives. Susceptibility to infection by *Nosema locustae* is generally restricted to Acrididae. Non-target insect exposure to *Nosema locustae* spores is expected to be low because it is formulated in a bait and then applied where targeted pests are present. The applied baits are readily consumed by target pests within a matter of hours. The waiver request from further testing of non-target terrestrial arthropods is accepted based on the current information available which indicates minimal potential toxicity/infectivity to non-target arthropods.

It is unlikely that *Nosema locustae* will affect non-arthropod invertebrates because it is an obligate parasite of grasshoppers and crickets, and there is no evidence of infection in any non-arthropod invertebrate species. This MPCA is present naturally in the soil in low levels as it enters the environment from infected hosts, and it has a low persistence in the soil. Based on the proposed use pattern and the susceptibility of spores to environmental degradation, it is unlikely that the application of Nolo Bait Biological Insecticide will significantly increase the levels of infective, viable *Nosema locustae* spores in soils.

A waiver for terrestrial plant testing was submitted based on the rationale that microsporidia have only been reported to infect animals; they do not cause plant disease, and *Nosema locustae* is a microsporidian that has been registered for use in the United States since 1980 to control locusts and grasshoppers. The waiver request for a plant study is acceptable because *Nosema locustae* is primarily a fat body parasite in grasshopper, its spores do not persist on vegetation, and there is no evidence that they can infect plant species. Plant infectivity and phytotoxic effects have not been reported in the published literature, and *Nosema locustae* has never been associated with plant diseases despite extensive analysis of agricultural diseases by academia, government and industry.

Based on all the available data and information on the effects of *Nosema locustae* to terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the use of Nolo Bait Biological Insecticide. The use of Nolo Bait Biological Insecticide is not expected to pose an unacceptable risk to terrestrial habitats when used according to label instructions.

4.2.2 Effects on Aquatic Organisms

In a 96-hour toxicity study, 100 rainbow trout *(Oncorhynchus mykiss*) and 100 bluegill sunfish *(Lepomis macrochirus*) were exposed (20/concentration) to 5 titrated concentrations of *Nosema locustae* spore suspension under static conditions. The two species were exposed separately to the following concentrations of *Nosema locustae*: 1.5×10^4, 2.2×10^4, 3.3×10^4, 4.9×10^4, and 7.1×10^4 spores/mL. A positive control using a known chemical toxicant was tested on fish from the stock lot. There were no deaths at any concentration tested in either species. The 96-hour LC_{50} was $> 7.1 \times 10^4$ spores/mL for both rainbow trout and bluegill sunfish. This study does not fully satisfy the guideline requirement for a toxicity study in freshwater fish. The viability/infectivity of the MPCA used was not tested, the MCC of 1×10^6 spores/mL was not achieved, and the information provided was not adequate to fully assess the toxicity/infectivity potential of the MPCA. Therefore, this study is classified as supplemental with limited use for assessing toxicity in freshwater fish.

In a 30-day maximum hazard infectivity study in freshwater fish, 30 rainbow trout (*Oncorhynchus mykiss*) fingerlings were studied by injecting 0.05 mL of *Nosema locustae* spore suspension (1.89×10^8 spores/mL) intraperitoneally, feeding 10 grams of pelleted trout ration containing test substance (1.89×10^7 spores/g), and by aquatic exposure to 1.2×10^4 spores/L of test substance for 36 hours. Replacement water flow to the aquaria was stopped during this period. There were no treatment-related mortalities, clinical signs or statistically significant differences in body weight among treatment groups, and no signs of infectivity. Although the intraperitoneal route of exposure studied in this test is environmentally unrealistic, it provides a maximum hazard challenge by bypassing the primary defence mechanisms in fish. The hematological and histopathological examinations performed, adequately established a pattern of clearance of the MPCA from the test fish despite the lack of an attempt to recover viable MPCA from collected tissues. This study is classified as acceptable and satisfies the guideline requirement for a toxicity/infectivity study in freshwater fish.

The applicant provided a published study (Fournie et al., 1990) in support of a waiver request for the non-target aquatic arthropod testing requirement. In this study, three groups (13, 24, and 24) of estuarine grass shrimp (*Palaeomonetes pugio)* were each administered 6–7 µL *Nosema locustae* spore suspension by intrahemocoelic injection under the carapace at the posterior margin of the cephalothorax. The dose was 4.0×10^3 spores per injection for the first two groups (13, 24 shrimp), and 1.5×10^5 spores per injection for the third group (24 shrimp). Injected shrimp were held in static holding tanks and observed for 4 weeks. Water temperature and salinity were maintained at 26ºC and 25%, respectively. Shrimps were sampled every 7 days, and examined either by fresh squash or processed for histological examination. Injections of

Nosema locustae produced no infections in the estuarine grass shrimp *P. pugio.* This study is acceptable and satisfies the guideline requirement for toxicity/infectivity testing in aquatic arthropods.

Nosema locustae is unlikely to infect aquatic non-arthropod invertebrates because it is known only to be an obligate parasite of grasshoppers and crickets with limited insect host range, and is also unlikely to persist in aquatic environments. The likelihood of adverse effects occurring to non-target non-arthropod invertebrates is low because of the proposed use pattern, low aquatic exposure, and susceptibility of the spores to environmental degradation.

Nosema locustae is unlikely to infect aquatic plants. It has only been reported to infect insects and is primarily a fat body parasite in grasshoppers; its spores do not persist on vegetation, and there is no evidence that they can infect plant species. Plant infectivity and phytotoxic effects have not been reported in the published literature. Based on the proposed use pattern, significant aquatic exposure is not expected and spore persistence in aquatic habitats is unlikely. Therefore, no adverse effects are expected should aquatic plants be exposed to this MPCA under operational conditions of use.

Based on all the available data and information on the effects of *Nosema locustae* to aquatic organisms, there is reasonable certainty that no harm will be caused to fish, arthropods, non-arthropod invertebrates, or aquatic plants from the use of Nolo Bait Biological Insecticide. The use of Nolo Bait Biological Insecticide is not expected to pose an unacceptable risk to aquatic habitats when used according to label instructions.

5.0 Value

5.1 Effectiveness Against Pests

A variety of information was evaluated, including published journal articles, grasshopper control handbooks, organic crop surveys, and integrated pest management information. In general, results from the evaluated information indicate that use of Nolo Bait Biological Insecticide against grasshoppers or Mormon crickets may suppress grasshopper populations. Effects will generally not be observed until several weeks following application. Theoretically, high rates of infection with *Nosema locustae* may produce high rates of transmission to subsequent generations of grasshoppers. However, it may be difficult to obtain high rates of infection given the variability in efficacy of *Nosema locustae* bran baits under field conditions. Because *Nosema locustae* requires a relatively long period of time to develop in the host, it requires a long time to debilitate the host and spreads slowly through the population, and therefore does not readily infect other grasshoppers through means other than cannibalism.

One advantage of Nolo Bait Biological Insecticide is that *Nosema locustae* has little effect on beneficial and other non-target organisms. Therefore, in addition to use in organic production, Nolo Bait Biological Insecticide may be useful in environmentally sensitive areas were conventional insecticides cannot be used and reliable, immediate control is not critical.

The minimum application rate of 1.12 kg per hectare (2.5×10^9 spores per hectare) is likely to be too low to provide immediate or consistent suppression or control of populations. Long-term suppression may be possible at this rate: evaluated information indicated that up to 60% control may be possible at the minimum rate, although higher rates are likely required for consistent, reliable suppression or control. From a value perspective, aerial application is supported by the evaluated information, which demonstrated that *Nosema locustae* bran bait can be successfully dispersed via aerial applications.

Based on efficacy or value, there is no reason to require a maximum application rate, as Nolo Bait Biological Insecticide must be consumed by the target pest in order to be effective. Consumption of a higher number of spores per grasshopper will increase product efficacy and decrease the amount of time required to kill the grasshoppers. Therefore, where greater efficacy or faster population reduction is required, this may be achieved by applying multiple applications or a higher application rate in order to increase the amount of bait available to each grasshopper.

While the Mormon cricket can reach high densities in the United States, it does not normally cause problems in Canada. However, *Nosema locustae* will control Mormon crickets as well as grasshoppers. Therefore, inclusion of this potential pest species on the label is supported, even though incidents of pest problems from Mormon crickets are not common.

5.1.1 Acceptable Efficacy Claims

Nolo Bait Biological Insecticide may provide suppression of grasshopper and Mormon cricket populations in crop and rangeland.

Use Nolo Bait Biological Insecticide when grasshopper densities reach nine (9) or more grasshoppers per square meter. Grasshoppers are most effectively suppressed when they are young. For best results, apply Nolo Bait Biological Insecticide when most grasshoppers are in the 3rd instar (12 to 19 mm long). Due to the nature of this product (i.e., microsporidial pathogen), efficacy may be affected by such factors as weather (e.g., rain following treatment, temperature), grasshopper population densities, and insect migration.

Apply Nolo Bait Biological Insecticide to crop and rangeland at a minimum rate of 1.12 kg per hectare. This product must be consumed by the target pest in order to be effective. Consumption of a higher number of spores per grasshopper will increase product efficacy and decrease the amount of time required to kill the grasshoppers. Therefore, where greater efficacy or faster population reduction is required, this may be achieved through multiple applications or a higher application rate in order to increase the amount of bait available to each grasshopper. Apply by hand, seed spreader, turbine spreader, or airplane. Concentrate the application in areas of heaviest grasshopper infestation.

5.2 Phytotoxicity to Host Plants

While the phytotoxicity of Nolo Bait Biological Insecticide to crop or rangeland was not evaluated in this product review, no phytotoxic effects are expected due to the nature of this product (i.e., an orthopteran protozoan pathogen formulated as a bran bait).

5.3 Impact on Succeeding Crops

While the impact on succeeding crops was not evaluated in this product review, no impact is expected from Nolo Bait Biological Insecticide due to the nature of this product (orthopteran protozoan pathogen formulated as a bran bait).

5.4 Economics

No market analysis was assessed for this product review.

5.5 Sustainability

5.5.1 Survey of Alternatives

Registered alternative active ingredients for control of grasshoppers include cypermethrin, malathion, deltamethrin, lambda-cyhalothrin, dimethoate, and carbaryl. Carbaryl is the only other active ingredient registered as a bran bait for use against grasshoppers. There are no products registered for control of Mormon crickets.

5.5.2 Compatibility with Current Management Practices Including Integrated Pest Management

Nolo Bait Biological Insecticide is compatible with current management practices and conventional crop production systems. In addition, due to its host specificity *Nosema locustae* has little effect on beneficial and other non-target organisms; therefore Nolo Bait Biological Insecticide is compatible with non-conventional control practices such as biological control, as well as organic production systems.

5.5.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Due to the nature of this product (i.e., an orthopteran protozoan pathogen), the development of resistance to Nolo Bait Biological Insecticide by grasshoppers and Mormon crickets is not expected.

5.5.4 Contribution to Risk Reduction and Sustainability

Nolo Bait Biological Insecticide has potential to contribute to both risk reduction and sustainability. While the efficacy of this product is limited to claims of suppression, *Nosema locustae* is an orthopteran protozoan pathogen, and has little effect on beneficial and other non-target organisms, and there is very little likelihood of resistance developing to this disease organism. In addition, *Nosema locustae* may remain in the orthopteran population in years following application, and therefore may contribute to long-term suppression of the pest populations.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's Toxic Substances Management Policy (TSMP), which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bio-accumulative. These substances are referred to in the policy as Track 1 substances.

While reviewing *Nosema (Paranosema) locustae* Canning, the PMRA took into account the federal TSMP and followed its Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*. Substances associated with its use were also considered, including microcontaminants in the technical product Nolo™ BB Concentrate, and formulants in the end-use product Nolo Bait Biological Insecticide. The PMRA has reached the following conclusions:

Nosema (Paranosema) locustae Canning, TGAI, does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track-1 criteria. Therefore, the use of Nolo Bait Biological Insecticide is not expected to result in the entry of Track 1 substances into the environment.

6.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, formulants and contaminants in the technical and end-use products are assessed against the formulants and contaminants identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*. This list of formulants and contaminants of health and environmental concern are identified using existing policies and regulations including: the federal Toxic Substances Management Policy; the Ozone-depleting Substance

Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol); and the PMRA Formulants Policy as described in the PMRA Regulatory Directive DIR2006-02, *Formulants Policy and Implementation Guidance Document*. The *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* is maintained and used as described in the PMRA Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act*.

The *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* consists of three parts:

- Part 1:Formulants of Health or Environmental Concern;
- Part 2:Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions; and
- Part 3:Contaminants of Health or Environmental Concern.

The contaminants to which Part 3 applies meet the federal Toxic Substances Management Policy criteria as Track 1 substances, and are considered in section 6.1. The following assessment refers to the formulants and contaminants in Part 1 and Part 2 of the list.

The technical grade active ingredient, Nolo BB Concentrate, does not contain any contaminants of health or environmental concern identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*.

The end-use product, Nolo Bait Biological Insecticide, contains the formulant wheat which is identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions*. Therefore, the label for the end-use product, Nolo BB Biological Insecticide, will include the precautionary statement: “Warning: this product contains the allergen wheat” on the principal display panel.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization and analysis data submitted in support of the TGAI, Nolo BB Concentrate, and the end-use product, Nolo Bait Biological Insecticide, are acceptable. The technical material was fully characterized, and the specifications were supported by the analysis of a sufficient number of batches.

Analysis data for bacterial contaminants in five batches of the most recently manufactured TGAI were found to be deficient. Consequently, the applicant will be required to provide information on the following:

- five certificates of quantitative analysis on microbial contaminants, i.e., bacterial and fungal contaminants using the most recently manufactured batches of the TGAI.
- acceptable limits for each of the microbial contaminants.
- details of the methods used for the bacterial and fungal contaminant analysis.
- description of the steps or measures taken if the batches contain microbial contaminants beyond their acceptable limits.

Although the study submitted to support the 'Storage' statement on the Nolo Bait Biological Insecticide label was acceptable, only one batch of end-use product was tested. A confirmatory study, conducted in a similar manner, is required and must test at least two additional batches of Nolo Bait Biological Insecticide.

7.2 Human Health and Safety

The available information for *Nosema (Paranosema) locustae* Canning is adequate to qualitatively identify the toxicological hazards that may result from human exposure to *Nosema locustae*. Submitted information suggests *Nosema locustae* is of low toxicity and is not pathogenic or infective irrespective of the route of exposure. *Nosema locustae w*as not irritating to the skin and was not an ocular irritant. Skin sensitization study showed *Nosema locustae* as a potential skin sensitizer.

The potential routes of occupational exposure to *Nosema locustae* are pulmonary, dermal and to some extent ocular. Occupational exposure is expected to be minimal if label instructions are followed. Precautionary labelling will alert users of the potential sensitizing hazard of the end-use product. Wearing appropriate PPE stipulated on the end-use product label will mitigate occupational exposure. The label does not allow applications outside of cropland and rangeland; therefore, non-occupational dermal exposure and risk to adults, infants and children are low.

Based on the host specific nature of *Nosema locustae*, its non infectivity to plants, and its formulation as a bait, and historic safe use as an MPCA, it is unlikely that the use of Nolo Bait Biological Insecticide which may suppress grasshoppers and Mormon crickets will result in an increase in residues on treated food/feed stuffs that will be of health concern.

7.3 Environmental Risk

The environmental fate studies, non-target organism studies and waiver requests submitted in support of *Nosema locustae* were determined to be sufficiently complete to permit a decision on registration.

The environmental fate studies submitted indicated that *Nosema locustae* is unlikely to have adverse effects on microbial biomass and nutrient cycling activities in the soil. Spores have the potential to leach depending on the soil types and precipitation events. Persistence of spores on vegetation is unlikely, given that they are readily inactivated by sunlight and high temperatures as well as degradation by other environmental microorganisms.

Based on the proposed use pattern and the susceptibility of spores to environmental degradation, it is unlikely that the application of Nolo Bait Biological Insecticide will significantly increase the levels of infective, viable persistent *Nosema locustae* spores that would adversely affect the dynamics of an ecosystem.

From the available data and information on the effects of *Nosema locustae* to terrestrial and aquatic organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, fish, terrestrial and aquatic arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the use of Nolo Bait Biological Insecticide. It is unlikely that *Nosema locustae* will adversely affect non-target organisms because it is an obligate parasite of grasshoppers and crickets. The use of Nolo Bait Biological Insecticide is therefore not expected to pose unacceptable risks to terrestrial and aquatic habitats when used according to label instructions.

7.4 Value

The information and data reviewed to register Nolo Bait Biological Insecticide are adequate to support a claim of may suppress grasshoppers and Mormon crickets in crop and rangeland, when applied at a rate of a minimum of 1.12 kg per hectare.

7.5 Unsupported Uses

All uses originally proposed in this application are supported.

8.0 Regulatory Decision

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, has granted conditional registration for the sale and use of Nolo BB Concentrate and Nolo Bait Biological Insecticide, containing the technical grade active ingredient *Nosema (Paranosema) locustae* Canning, which may suppress grasshoppers and Mormon crickets in crops and rangelands.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

Although the risks and value have been found acceptable when all risk-reduction measures are followed, as a condition of these registrations, additional scientific information is being requested from the applicant to ensure that the manufacturing process, product characterization and storage stability of *Nosema (Paranosema) locustae* Canning are adequate. For more details, refer to the Section 12 Notice associated with these conditional registrations. The applicant will be required to submit this information by September 30, 2010.

NOTE: The PMRA will publish a consultation document at the time when there is a proposed decision on applications to convert these conditional registrations to full registrations or on applications to renew the conditional registrations, whichever occurs first.

Manufacturing Process of the TGAI:
Minor deficiencies have been identified and the applicant will be required to fill the data gaps.

Manufacturing Process of the End-use Product:
Minor deficiencies have been identified and the applicant will be required to fill the data gaps.

Product Characterization and Analysis
To ensure that the manufacturing process of Nolo BB Concentrate does not result in unacceptable levels of microbial contaminants, the applicant will be required to provide the following:

- five certificates of quantitative analysis on microbial contaminants, i.e., bacterial and fungal contaminants using the most recently manufactured batches of the TGAI.
- acceptance limits for each of the microbial contaminants.
- details of the methods used for the bacterial and fungal contaminant analysis.
- description of the steps or measures taken if the batches contain microbial contaminants beyond their acceptable limits.

Storage Stability Testing
The applicant will be required to provide a confirmatory storage stability study using the end-use product.

List of Abbreviations

^{o}C	degree(s) Celcius
µL	microlitre(s)
µm	micron(s)
cm	centimetre(s)
CWA	United States *Clean Water Act*
FDA	*Food and Drugs Act*
g	gram(s)
h	hour(s)
ha	hectare(s)
kg	kilogram(s)
km	kilometre(s)
L	litre(s)
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
m	metre(s)
mL	millilitre(s)
MAS	maximum average score
MCC	maximum challenge concentration
MPCA	microbial pest control agent
PCPA	*Pest Control Products Act*
PHI	preharvest interval
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	parts per million
RED	Re-registration Eligibility Decision
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Nosema (Paranosema) locustae* Canning and Nolo Bait Biological Insecticide

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of Nosema locustae				
Acute Oral Toxicity/Infectivity 21-day	Sprague Dawley rat Test group: 1 mL *Nosema (Paranosema) locustae* Canning (2.29×10^8 spores/mL.) 20/sex/dose Negative control: 1mL saline solution 20/sex/dose	$LD_{50} > 2.29 \times 10^8$ spores/mL (♂, ♀)	No mortalities, and no significant signs of toxicity or infectivity. No treatment-related changes in body weight or body temperature. Hematological and blood chemistry values were within limits. One male and one female of test group showed dilated pelvis in one kidney each. Enlarged thyroid in 1 test group male. Higher kidney to body weight ratio and higher adrenal to body weight ratio in test group males compared to controls. **LOW TOXICITY, NOT INFECTIVE** Clearance of the MPCA was not assessed. Recovery of the MPCA from animal tissue was not attempted, and viability of spores used was not assessed prior to testing on animals. **ACCEPTABLE** as a toxicity study. **SUPPLEMENTAL** as an infectivity study.	PMRA 1314961

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Pulmonary Toxicity/Infectivity 28-day	Sprague Dawley rat Test group*:* 40 µL *Nosema (Paranosema) locustae* Canning (1.89×10^9spores/mL) (7 ♂, 8 ♀) i. Negative control: 40 µL sterile distilled water (7 ♂, 8 ♀) ii. Heat-killed control: 40 µL heat inactivated (56°C for 20 minutes) spore suspension (8/sex)	$LD_{50} > 1.89 \times 10^9$ spores/mL (♂, ♀)	No mortalities, no signs of toxicity or infectivity, and no change in body weight gain. Intense foreign body reaction producing extensive multifocal granulomatous pneumonia in the heat-killed control and test groups. The pulmonary granulomas showed gram positive elliptical organisms of a size similar to that of *Nosema (Paranosema) locustae* Canning spores. **LOW TOXICITY, NOT INFECTIVE** Clearance of the MPCA was not assessed. Recovery of the MPCA from animal tissue was not attempted. **ACCEPTABLE** as a toxicity study. **SUPPLEMENTAL** as an infectivity study.	PMRA 1313957

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Intraperitoneal toxicity/ Infectivity 56-day	CD1 mice Test group: 0.25 mL *Nosema (Paranosema) locustae* Canning (1.4×10^9spores/mL) (40/sex) i. Negative control: 0.25 mL distilled water (40/sex) ii. Heat-killed treatment 0.25 mL heat inactivated (56°C for 20 minutes) spore suspension (40/sex)	$LD_{50} > 1.4 \times 10^9$ spores/mL (♂, ♀)	No mortalities, significant toxicity, infectivity or pathogenicity. No treatment-related change in body weight or necropsy findings. Blood protein, neutrophil and monocyte concentrations were increased in heat-killed control and test groups; enlargement of spleen noticed in these groups. **LOW TOXICITY NOT PATHOGENIC** Bioassays of tissues and peritoneal lavages from treated animals failed to produce infection when fed to grasshoppers. **ACCEPTABLE**	PMRA 1313958

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Maximum Challenge Infectivity Study Intraperitoneal, intracerebral, and intraocular 70-day	New Zealand White rabbits Injected (16/sex) with *Nosema (Paranosema) locustae* Canning spores (2 × 10^8 spores/mL in saline) as follows: 20 animals (10/sex) received intracerebral (0.1 mL processed), intraocular (0.05 mL processed), and intraperitoneal (1 .0 mL crude) injections. Three other groups of four rabbits each (2/sex) were separately injected either intracerebrally, intraocularly, or intraperitoneally with test material at similar doses as mentioned above. <u>Control</u>: Non-infected grasshopper fat bodies. 1 group of 10 rabbits (5/sex) received intracerebral (0.1 mL processed), intraocular (0.05 mL processed), and intraperitoneal (1 .0 mL crude) injections of control material. Three other control groups of 2 rabbits each (1/sex) were also separately injected either intracerebrally, intraocularly or intraperitoneally with control material at similar doses as mentioned above.	$LD_{50} > 2 \times 10^8$ spores/mL (♂, ♀)	No treatment-related mortalities, significant toxicity, infectivity or pathogenicity. No treatment-related change in body weight or body temperature. Spores were identified in the tissues of two test group rabbits, one in a hepatic lesion and the other in the brain at the site of injection one week after injection. Spores were not detected in urine sediments. Hematology parameters were within limits. **NOT INFECTIVE** Enumeration of MPCA was not done from tissues collected at necropsy. Clearance was not assessed. Accepted as a **SUPPLEMENTAL** study This study is not required by the PMRA.	PMRA 1313956

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Maximum Challenge Infectivity Study Intraperitoneal and intracerebral 56-day	Mice: 3 groups of 42 white Swiss mice (21/sex) were injected with *Nosema (Paranosema) locustae* Canning spores (2×10^8 spores/mL in saline) as follows: One group of 30 animals (15/sex) received intracerebral (0.05 mL processed), and intraperitoneal (l .0 mL crude) injections. Two other groups of 6 mice each (3/sex) were separately injected either intracerebrally or intraperitoneally with test material at similar doses as mentioned above. <u>Control</u>: Control material: non-infected grasshopper fat bodies (32 mice). One control group of 20 mice (10/sex) received intracerebral (0.05 mL processed), and intraperitoneal (l .0 mL crude) injections of control material. Two other control groups of 6 mice each (3/sex) were also separately injected either intracerebrally or intraperitoneally with control material at the same dose as mentioned above.	$LD_{50} > 2 \times 10^8$ spores/mL (♂, ♀)	No treatment related mortalities or necropsy findings or body weight change. Abdominal abscesses were seen in 5 mice receiving test substance. Spore like objects were identified in the abscesses, but no proliferation was noticed. **NOT INFECTIVE** Enumeration of MPCA was not done from tissues collected at necropsy. Clearance was not assessed. Accepted as a **SUPPLEMENTAL** study This study is not required by the PMRA.	PMRA 1313956

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Eye Irritation	New Zealand White albino rabbits 0.1 mL of *Nosema (Paranosema) locustae* Canning spore suspension in saline (2.29×10^8 spores/mL) was instilled into the conjunctival sac of the left eye of each of the rabbits (10/sex) for 24 hours, and left unwashed. Negative control: The right eye of each rabbit, which received a single application of 0.1 mL of 0.8% saline solution. Observations: 24, 48, and 72 hours; 7, and 14 days post exposure. Irritation was scored by the method of Draize.	Eye irritation score was 0	Fluorescein examination showed absence of irritation and corneal damage. No treatment-related changes in body temperature. **NON-IRRITATING** **ACCEPTABLE**	PMRA 1314959
Acute Dermal Toxicity 28-day	Sprague Dawley rat Rats (7/sex) were dermally exposed to 200 µL *Nosema (Paranosema) locustae* Canning spore suspension (3.2×10^8 spores/mL) for 24 hours. i. Negative control: 200 µL distilled water (7/sex) ii. Heat-killed control: 200 µL heat inactivated (56°C for 20 minutes) spore suspension (3.3×10^8 spores/mL), (7/sex).	$LD_{50} > 3.2 \times 10^8$ spores/mL, (♂, ♀)	No mortalities and no treatment-related changes in body weight, body temperature, and necropsy findings. Haematology parameters were within normal limits. No local changes at the application site. **NON-IRRITATING** **LOW TOXICITY** **ACCEPTABLE**	PMRA 1313959

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Dermal Irritation 72 hours	New Zealand White rabbits (6) were dermally exposed to 0.5 mL *Nosema (Paranosema) locustae* Canning spore suspension (2.29×10^8 spores/mL) in saline for 24 hours, and later observed for 72 hours	Dermal irritation score = 0	No signs of irritation during the 72-hour observation period. No treatment-related change in body temperature. **NON-IRRITATING** **ACCEPTABLE**	PMRA 1314960

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Dermal Sensitization 48-day	Induction phase: I. Guinea pigs (10 males) were injected intradermally with 0.05 mL of *Nosema (Paranosema) locustae* Canning spore suspension (1.7×10^8 spores/mL) in antibiotic solution by on days 0, 3, 5, 7, 10, 12, 14, 17, 19, and 21. ii. Control: similarly dosed with (10 males) 0.05 mL killed *Mycobacterium tuberculosis* Challenge phase: (day 42) i. Test group animals were challenged with 0.05 mL of *Nosema (Paranosema) locustae* Canning spore suspension in antibiotic (2. 3×10^8 spores/mL) ii. Control: animals received intradermal challenge of 0.05 mL Tubersol. Due to bacterial contamination of spore suspension and to assess the magnitude of the contributing component in the challenge suspension to the skin reaction, additional intradermal injections were done. Test group received (day 46) intradermal injections of 0.05 mL of antibiotic solution at one site and 0.05 mL of an overnight culture of *Staphylococcus saprophyticus* at a second site while control animals received antibiotic solution at one site (0.05 mL) and *Nosema (Paranosema) locustae* Canning spore suspension at another site (0.05 mL).The intensities of erythema and edema at the injection sites were scored at 24, and 48 hours post-injection by the method of Draize	Showed significant increase in erythema, edema, and area of skin reactions. Tested positive as a skin sensitizer.	No mortalities and no significant change in body weight gain. On Day 10 of induction phase, one test group animal showed elevated, firm, yellow brown mass on the lower left lip, which was found to contain large Gram positive coccoid microorganisms. **DERMAL SENSITIZER** **ACCEPTABLE** Dermal sensitization study is not required for MPCAs.	PMRA 1313960

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Report on hypersensitive incidence (6-year period) of employees in a manufacturing plant where *Nosema (Paranosema) locustae* Canning spores were processed and formulated.	Employees had been intimately involved in the extraction, standardization, shipping and handling of *Nosema (Paranosema) locustae* Canning spores since 1982. Employees were frequently exposed to *Nosema (Paranosema) locustae* Canning spores by hands, eyes, nose, clothes, oral cavity, during processing, operational and clean-up activities, and also during the formulation, shipping, and handling of the end-use product.	No reported immunological effects from exposure to *Nosema (Paranosema) locustae* Canning spore suspensions during a 6-year period.	Dermal Sensitizer according to PMRA 1313960. All microbial products are considered potential sensitizers by the PMRA.	PMRA 1314965

Table 2 Toxicity to Terrestrial Non-Target Species

Organism/Study	Exposure / Doses	Results/Significant Effects/Comments	Reference
Terrestrial Organisms			
Vertebrates			
Birds: Oral Toxicity/Infectivity Ring-necked pheasants (*Phasianus colchicus*) Two groups of birds (30/group): 12-day-old female chicks Study period: 29 days	Single oral gavage. 1) 0.20 mL of sterile distilled water (negative control) or 2) 0.20 mL of technical grade *Nosema (Paranosema) locustae* Canning spore suspension (1.89×10^9 spores/mL). Clearance was not assessed, but hematology and histopathology parameters were studied to assess infectivity.	No treatment-related mortalities or signs of infectivity or abnormal necropsy findings. No significant change in body weight in test group compared to control. Hematological parameters studied were within the normal limits, and there were no microscopic anatomical abnormalities. Results suggest *Nosema (Paranosema) locustae* Canning is non-toxic and non-infective when administered at a single high dose to birds. **SUPPLEMENTAL**	PMRA 1313962
Birds: Maximum hazard intravenous injection Ring-necked pheasants	Intravenous injection: 1) 0.05 mL of sterile distilled water (negative control),	No treatment-related mortalities, signs of infectivity, toxicity or abnormal necropsy findings. No significant change in body weight in the test group compared to controls.	PMRA 1313963

Organism/Study	Exposure / Doses	Results/Significant Effects/Comments	Reference
Three groups of birds ninty, 21-day-old (male and female) chicks (30/group) Study period: 29 days	2) 0.05 mL of heat inactivated (heated at 56°C for 20 minutes, heat-killed control) *Nosema (Paranosema) locustae* Canning spore suspension 3) 0.05 mL of infective technical grade *Nosema (Paranosema) locustae* Canning spore suspension (1.89×10^9 spores/mL, test group). Clearance was not assessed, but hematology and histopathology parameters were studied to assess infectivity.	Hematological parameters studied were within the normal limits, and there were no microscopic anatomical abnormalities; they were adequate to establish a clearance pattern of the MPCA. NOT TOXIC/NOT INFECTIVE **ACCEPTABLE**	
Wild Mammals	No study or waiver was submitted. Data requirements waived based on the results of human health and safety testing data		
Invertebrates **Non-target Arthropods**			
Toxicity/Infectivity study: Honey bee (*Apis mellifera* L.), (Published study) 400 honey bees (2/test of 200 honey bees) Study period: 26 days	Oral exposure 5 µL of 16% sugar solution containing *Nosema (Paranosema) locustae* Canning spores. Each bee received one dose of 0 or 5×10^1 or 5×10^2 or 5×10^3 or 5×10^4 spores. The ventriculus and thoracic tissues were examined by phase contrast microscopy for the presence of Nosema *(Paranosema) locustae* Canning spores. Mortality: 11/200 and 161/200 (not treatment-related). No spores in thoracic tissues. Only 3/400 showed spores in ventriculus (3, 1, and 1 spores each). NOT TOXIC/NOT INFECTIVE **ACCEPTABLE**		PMRA 1313965
Toxicity / Infectivity **Predators and Parasites:** Sarcophagid flies and bee flies	Waiver rationale: very difficult to maintain in the laboratory. No information on infection to bee flies preying on the eggs or sarcophagid flies parasitizing on *Nosema (Paranosema) locustae* Canning infected grasshoppers.		PMRA 1314978

Organism/Study	Exposure / Doses	Results/Significant Effects/Comments	Reference
Waiver	*Nosema (Paranosema) locustae* Canning is an obligate parasite of grasshoppers and crickets. It is one of the least virulent pathogens of grasshoppers and crickets, and there is no evidence that it will infect any insects other than grasshoppers, some crickets, and a few close relatives. **WAIVER ACCEPTED**		
Toxicity / Infectivity Leafcutter Bees *(Megachile rotundafa)* **Waiver**	Waiver rationale: Low exposure to *Nosema (Paranosema) locustae* Canning spores on bran bait because the bees do not pick up pieces of leaf that have dropped on the ground and therefore it is unlikely that they would pick up flakes of the bran bait from the ground. Consequently, the bran bait would have to stick to leaves of plants for there to be any potential of exposure of the bees. In trials with dimethoate treated bran bait, leafcutter bees failed to pick up any of the bran. Low levels of exposure, and no reported adverse effects to leafcutter bees from *Nosema (Paranosema) locustae Canning.* **WAIVER ACCEPTED**		PMRA 1314977
Non-Arthropods			
Non-Arthropod Invertebrates: No waiver submitted.	It is unlikely that *Nosema (Paranosema) locustae* Canning will affect non-arthropod invertebrates because this MPCA is an obligate parasite of grasshoppers and crickets, and there is no evidence of infection in any non-arthropod invertebrate species.		
Plants			
Vascular Plants: **Waiver**	Waiver rationale: Microsporidia have only been reported to infect animals, and no report of diseases in plants. *Nosema (Paranosema) locustae* Canning is an obligatory fat body parasite in grasshoppers and crickets; its spores do not persist on vegetation. There is no evidence that they can infect plant species, and never associated with plant diseases. Plant infectivity and phytotoxic effects have not been reported in the published literature. **WAIVER ACCEPTED**		PMRA 1313967

Table 3 Toxicity to Aquatic Non-Target Species

Organism/Study	Exposure/Dose	Results/Significant Effects/Comments	Reference
Aquatic Organisms			
Vertebrates			
Fish			
Maximum hazard infectivity study. Rainbow trout fingerlings 3 groups(30 fingerlings/group) Study period: 30 days	Exposure: injection, feed, and environmental (36 hours). 1) Negative Control: Injected 0.05 mL of sterile distilled water intraperitoneally. 2) Heat-killed Control: Heat-killed *Nosema (Paranosema) locustae* Canning spore suspension injected intraperitoneally (0.05 mL, 1.89×10^8 spores/mL) and fed 10 grams of contaminated pelleted trout ration (1.89×10^7 spores/g) and exposed to contaminated water (1.2×10^4 spores/L) for 36 hours 3) Test group: *Nosema (Paranosema) locustae* Canning spore suspension injected intraperitoneally (0.05 mL, 1.89×10^8 spores/mL) and fed 10 grams of contaminated pelleted trout ration (1.89×10^7 spores/g) and exposed to contaminated water (1.2×10^4 spores/L) for 36 hours. Clearance was not assessed. No treatment-related mortalities, clinical signs or statistically significant differences in body weight among treatment groups, and no signs of infectivity. Hematological and histopathological examinations performed adequately established a pattern of clearance of the MPCA from the test fish. NOT INFECTIVE **ACCEPTABLE**	PMRA 1313964
96 hour toxicity study Rainbow trout *(Oncorhynchus mykiss,* 100) Bluegill sunfish *(Lepomis macrochirus,* 100)	Environmental exposure The two species were exposed separately to the following concentrations of *Nosema (Paranosema) locustae* Canning spores: 1.5×10^4 , 2.2×10^4, and 3.3×10^4, 4.9×10^4, and 7.1×10^4 spores/mL. (20/con.). Positive control: Known chemical toxicant Viability of the spores was not tested. No mortality at any concentrations tested. The 96-hour LC_{50} was $> 7.1 \times 10^4$ spores/mL for both rainbow trout and bluegill sunfish. Spores of *Nosema (Paranosema) locustae* Canning appear to be non-toxic to freshwater fish. This study is classified as **SUPPLEMENTAL**	PMRA 1378482

Organism/Study	Exposure/Dose	Results/Significant Effects/Comments	Reference
Aquatic Organism			
	Invertebrates		
Aquatic Arthropod Shrimp Infectivity study. Estuarine Shrimp *(Palaeomonetes pugio)* Three groups (13, 24, 24 shrimp each) Control (20 shrimp): Study period: 4 weeks	Intrahemocoelic injection Dosage: 6–7 µL *Nosema locustae* spore suspension. 4.0×10^3 spores per injection for the first 2 groups 1.5×10^5 spores per injection for the third group. Control (20 shrimp): 20 µL saline solution. Spores of *Nosema locustae* were not infective to estuarine shrimp. NOT INFECTIVE **ACCEPTABLE**		PMRA 1313966
Aquatic Non-Arthropod No waiver submitted	*Nosema (Paranosema) locustae* Canning is unlikely to infect aquatic non-arthropod invertebrates because it is known only to be an obligate parasite of grasshoppers and crickets. Likelihood of adverse effects occurring to non-target non-arthropod invertebrates is low.		
	Plants		
Aquatic Plants No waiver submitted	*Nosema (Paranosema) locustae* Canning is an obligatory fat body parasite in grasshoppers and crickets, and they are unlikely to infect aquatic plants. Plant infectivity and phytotoxic effects have not been reported in the published literature.		

Table 4 Alternative Insecticides for Grasshopper Control in Crop and/or Rangeland

Technical Grade Active Ingredient	Insect Claim	Insecticide Classification Group
Carbaryl	Grasshoppers	1A
Cypermethrin	Grasshoppers	3
Deltamethrin	Grasshoppers	3
Dimethoate	Grasshoppers	1B
Lambda-Cyhalothrin	Grasshoppers	3
Malathion	Grasshoppers	1B

Table 5 Label Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed Label Claims	Accepted Label Claims	Unsupported Label Claims and Comments
Grasshoppers in crop and rangeland	Grasshoppers in crop and rangeland	All proposed label claims were supported
Mormon Crickets in crop and rangeland	Mormon Crickets in crop and rangeland	

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