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Evaluation Report

Beauveria bassiana strain HF23

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Overview

Registration Decision for *Beauveria bassiana* strain HF23

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 *Beauveria bassiana* HF 23 Technical Product and Balence ES containing the technical grade active ingredient *Beauveria bassiana* strain HF23 to control houseflies in poultry production houses.

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 risk 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 *Beauveria bassiana* strain HF23 and Balence ES.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks[1] to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable 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.

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 present when predicting the impact of pesticides. For more

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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."

information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the PMRA's website at www.pmra-arla.gc.ca.

What Is *Beauveria bassiana* strain HF23?

Beauveria bassiana strain HF23 is a fungus used as a microbial pest control agent to control houseflies in poultry production houses. This fungus causes a fatal disease in insects known as white muscardine disease. The HF23 strain of *B. bassiana* was originally isolated from a housefly in the United States and is reported to be quite specific to houseflies and related flies associated with livestock facilities.

The end-use product, Balence ES, is a commercial class insecticide product that contains *B. bassiana* strain HF23 as the active ingredient. While application in poultry production houses may be considered an indoor use, the chicken manure exposed to the application of Balence ES inside the poultry production house is commonly rendered into fertilizer and used outdoors on agricultural crops. Therefore, this outdoor use was also considered in the human health and the environmental risk assessment.

Health Considerations

Can Approved Uses of *Beauveria bassiana* strain HF23 Affect Human Health?

Beauveria bassiana strain HF23 is unlikely to affect your health when Balence ES is used according to label directions

Exposure to *B. bassiana* strain HF23 may occur during handling of Balence ES. 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 in the hope of identifying any potential to cause disease or toxicity. No significant toxicity and no signs of causing diseases were observed when *B. bassiana* strain HF23 was tested on laboratory animals.

Residues in Water and Food

Dietary risks from food and water are not of concern

The *Food and Drugs Act* prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for the *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act.* 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.

Although Balence ES is intended for use in an indoor environment (i.e. in poultry production houses), chicken manure exposed to the application of Balence ES in the poultry production house may be rendered into fertilizer and used outdoors on agricultural crops. This agricultural use pattern is the only means for residues of the active ingredient to be present on food and feed items. Based on the natural occurrence of *B. bassiana* in cultivated soils and on the limited and indirect agricultural use of the chicken manure, it is not expected that the use of Balence ES will significantly increase the natural environmental background levels of this microorganism.

As there are no direct applications to food and as no significant adverse effects were reported in the Tier I acute toxicity/pathogenicity studies, the establishment of an MRL is not required for *B. bassiana* strain HF23 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. In addition, the likelihood of residues of *B. bassiana* strain HF23 contaminating drinking water supplies is negligible. Consequently, dietary exposure and risk are minimal to non-existent.

Occupational Risks From Handling Balence ES

Occupational risks are not of concern when Balence ES is used according to label directions, which include protective measures

Users of Balence ES can come into direct contact with *B. bassiana* strain HF23 on the skin, in the eyes, or by inhalation. As a standard requirement intended to minimize exposure to high concentrations of a microorganism, the label specifies that users exposed to Balence ES must wear waterproof gloves, a long-sleeved shirt, long pants, a NIOSH approved respirator (with any –95, P-95, R-95 or HE filter), and shoes with socks. Although eye irritation studies submitted by the applicant indicated minimal eye irritation potential, the applicant has also recommended that users of Balence ES wear protective eye wear (e.g. goggles, a face shield or safety glasses).

For bystanders, exposure is expected to be much less than that of workers involved in mixing/loading and application activities and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When *Beauveria bassiana* strain HF23 Is Introduced Into the Environment?

Environmental risks are not of concern

Although Balence ES is intended for use in an indoor environment (e.g. in poultry production houses), chicken manure exposed to the application of Balence ES in the poultry production house may be rendered into fertilizer and used outdoors on agricultural crops. Studies designed to examine the environmental fate of *B. bassiana*

strain HF23 were assessed. *B. bassiana* strain HF23 does not remain viable in chicken manure and chicken litter for long periods of time and is unable to grow at temperatures above 35°C. Application to manure and subsequent composting of the manure is expected to significantly reduce the level of viable cells or spores of *B. bassiana* strain HF23 released into the environment.

The effects of *B. bassiana* strain HF23 on birds was examined. No significant adverse effects were observed in birds when *B. bassiana* strain HF23 was administered orally. Rationales were reviewed to waive avian pulmonary/inhalation/injection, wild mammal, freshwater fish, terrestrial arthropod (including honeybee), aquatic arthropod, non-arthropod invertebrate, and plant testing. The rationales were considered acceptable and significant adverse effects to these non-target organisms are not expected.

Value Considerations

What Is the Value of Balence ES?

***Beauveria bassiana* strain HF23, formulated as the end-use product Balence ES, has value in controlling populations of houseflies in poultry production houses.**

Applying Balence ES, containing spores of *B. bassiana* strain HF23, on various surfaces in poultry production houses can control populations of houseflies by infecting and killing the adult flies. *Beauveria bassiana* strain HF23 provides an alternative to conventional chemical insecticides and is well-suited for incorporation into integrated pest management programs.

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 key risk-reduction measures that must appear on the label of Balence ES to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

- **Human Health**

As a result of concerns with dermal irritation and with users developing allergic reactions through repeated high exposures to *B. bassiana* strain HF23, anyone handling Balence ES must wear waterproof gloves, a long-sleeved shirt, long pants, a NIOSH approved respirator (with any –95, P-95, R-95 or HE filter), and shoes with socks. Eye goggles are not required as the eye

irritation studies submitted indicated minimal eye irritation potential. Although eye irritation studies submitted by the applicant also indicated minimal eye irritation potential, the applicant has recommended that users of Balence ES wear protective eye wear (e.g. goggles, a face shield, or safety glasses).

- **Environment**

As a general precaution, the label prohibits the direct application of the product to aquatic habitats (such as lakes, rivers, sloughs, ponds, coulees, prairie potholes, creeks, marshes, streams, reservoirs, ditches and wetlands), estuaries or marine habitats. The label also directs handlers to not contaminate surface water by improper disposal of equipment wash waters.

As some strains of *B. bassiana* have been shown to be toxic to honeybees, users are directed to avoid applying the product to areas where honeybees are actively foraging or around hives.

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 section of this report or in the Section 12 Notice associated with these conditional registrations. The applicant must submit the following information within the time frames indicated.

Human Health

Product Characterization and Analysis

- A method to distinguish strain HF23 from other naturally occurring strains of *B. bassiana* is required.

- To ensure that in the manufacturing process Balence ES does not contain unacceptable levels of human and animal disease-causing microorganisms, the registrant will be required to include microbe-specific screening methods for coliforms, fecal coliforms, fecal streptococci/enterococci as well as *Salmonella* and *Staphylococcus*. Confirmatory data from five representative production batches will be required.

The applicant must submit this information no later than 1 December 2008.

Other Information

As these conditional registrations relate to a decision on which the public must be consulted[3], 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 (e.g. 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).

[3] As per subsection 28(1) of the *Pest Control Products Act.*

Science Evaluation

***Beauveria bassiana* strain HF23**

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Beauveria bassiana* strain HF23
Function	Control houseflies in poultry production houses
Binomial name	*Beauveria bassiana* strain HF23
Taxonomic designation	
Kingdom	Eumycota
Phylum	Dikarymycota
Subphylum	Ascomycotina
Class	Pezizomycotina
SubClass	Sordariomycete
Order	Hypocreales
Family	Clavicipitaceae
Genus	*Beauveria*
Species	*bassiana*
Strain	HF23
Patent Status information	No Canadian patent status information was provided.
Minimum purity of active	4.75×10^{11} colony forming units (CFU)/g

Identity of relevant impurities of toxicological, environmental and/or significance	The technical grade active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. Beauvericin, a secondary metabolite of *B. bassiana* strain HF23 has been identified in the technical product. Each lot of technical product will be monitored to ensure that beauvericin is at acceptable levels. Although it does not appear on the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* (Canada Gazette, part II, Volume 139, Number 24, pages 2641–2643), a component of one of the formulants in Balence ES is considered to be toxic as defined in section 64 of the *Canadian Environmental Protection Act, 1999*. The PMRA has conducted a risk assessment of this formulant component and has found that the associated risk is acceptable for the proposed use.

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

Technical Product—*Beauveria bassiana* HF23 Technical

Property	Result
Colour	White to light beige
Odour	Slight musty smell
Physical state	Powder
Guarantee	4.75×10^{11} CFU/g
Density	0.181 g/cm^3
Storage stability	N/A
Flammability	N/A
Explodability	Non-explosive

N/A= not applicable

End Use Product—Balence ES

Property	Result
Colour	Dark amber
Odour	Citrus

Property	Result
Physical state	Liquid
Formulation type	Emulsifiable suspension
Guarantee	1.18% *B. bassiana* strain HF23 minimum of 5.6×10^9 CFU/g
Specific gravity	0.92 g/cm^3
Storage stability	14 months when stored at 22–26°C
Corrosion characteristics	Non-corrosive
Flammability	N/A
Explodability	N/A

N/A= not applicable

1.3 Directions for Use

Application rate of the end-use product Balence ES is 9.5 to 16 mL per 100 m^2. Apply the spray to walls, floors, posts and manure concentrating areas where the greatest numbers of pests are located. This is a contact infection material with greatest efficacy against adult flies. Re-treat at intervals of two to seven days as long as pest pressure persists as pest eggs hatch and mature. There is no restriction on the total maximum amount of product that may be applied in a year.

1.4 Mode of Action

Beauveria bassiana is a generalist entomopathogenic fungus that causes white muscardine disease, which is lethal to insects. When spores of the fungus come into contact with an insect host, the spores germinate and hyphae grow into the body of the insect, eventually killing it. After death of the host, a white mycelium grows over the body of the host and produces new fungal spores. The HF23 strain of *B. bassiana* originally was isolated from a housefly in the United States and is reported to be quite specific to houseflies and related flies associated with livestock facilities.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Beauveria bassiana strain HF23 is a Hyphomycete fungus, producing conidia on exposed conidiophores. The genus *Beauveria* is closely related to the genera *Tritirachium* and *Acrodontium*. *Beauveria bassiana* strain HF23 can be identified to the species level by microscopic examination of morphological features, such as conidial production and ramification, by the method of DeHoog (1972).

There was no method submitted for strain-specific identification. The registrant will be required to develop a method using the best available technology to distinguish strain HF23 from other naturally occurring isolates of *B. bassiana.*

2.2 Methods for Establishment of Purity of Seed Stock

Beauveria bassiana strain HF 23 is stored and maintained in the United States Department of Agriculture, Agricultural Research Service Entomopathogenic Fungi Collection (ARSEF Collection, Ithaca, New York) as ascension number 7940.

Practices for ensuring the purity of the seed stock were adequately described in the summary of the method of manufacture 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

The potency (CFU/g) of the technical grade active ingredient and the end-use product is evaluated by counting the total conidia using a hemacytometer. Viability of the technical grade active ingredient and end-use product lots is also assessed by staining cells with fluorescent dyes to differentiate viable cells from non-viable cells which are then counted using fluorescent microscopy.

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

Beauveria bassiana is a ubiquitous microorganism in nature and has been isolated from a wide variety of environments, including cultivated soil where it is most commonly recovered. No effects have been reported for this microbial pest control agent (MPCA) in the United States where it has been conditionally registered since December 2006. Furthermore, in an acute oral toxicity study, no signs of adverse effects were observed in Sprague Dawley rats following oral gavage with 3.20×10^8 CFU/animal of *B. bassiana* strain HF23 suspended in sterile purified water.

Beauvericin, a secondary metabolite produced by *B. bassiana* strain HF23 was isolated and identified in the technical grade active ingredient using acceptable methods. The results of human health and toxicity studies, however, did not indicate any significant adverse effects. Each lot of technical grade active ingredient will be monitored to ensure that beauvericin is at acceptable levels.

Based on the above information, the establishment of a maximum residue limit (MRL) is not required for *B. bassiana* strain HF23 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 control procedures used to limit contaminating microorganisms during manufacture of Balence ES are acceptable. To detect any unusual colonies and to verify colony morphology, the technical product and end-use product are checked for purity by microscopic examination as well as plate counts on standard growth media following standardized methods. In the event that the microbial contaminants in a batch exceed the acceptable limits, the contaminated lot is removed from the production run. These methods, however, are deemed inadequate for detecting human and animal pathogens of concern (see Section 2.6).

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted in Section 2.5, microscopic examination and non-selective plating methods are inadequate for detecting and enumerating microbial contaminants of concern. No methods were submitted to screen specifically for human and mammalian pathogens during manufacture. To ensure that Balence ES does not contain unacceptable levels of human and animal disease-causing microorganisms, microbe-specific screening methods for coliforms, fecal coliforms, fecal streptococci/enterococci as well as *Salmonella* and *Staphylococcus* must be included in the manufacturer's quality assurance program.

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

Results from storage stability testing of five lots of Balence ES showed that after 12–14 months of storage at room temperature (20–27.5°C) Balence ES is stable for a period of up to 14 months under these conditions.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

A survey of published literature has revealed several cases of infection from *B. bassiana*. The cases include reports of deep tissue infection, pulmonary mycosis, empyema, and corneal keratitis in immunocompromised individuals. Given the ubiquitous nature of *B. bassiana* as a common soil microorganism, cases of systemic infection are considered rare. *Beauveria bassiana* does not generally infect healthy individuals and available antifungal therapies offer an effective treatment. Cases of corneal keratitis caused by *B. bassiana* have been reported following traumatic eye injury or eye surgery but, in all cases, have shown excellent prognosis for complete cure. Studies with *B. bassiana* indicate that, like most fungal species, it has some allergic potential.

The PMRA conducted a detailed review of the toxicological database for *B. bassiana* strain HF23. The database for *B. bassiana* HF23 Technical Product is complete (see Appendix I), consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, intraperitoneal infectivity, acute dermal toxicity/irritation, and eye irritation studies) currently required for health hazard assessment purposes. These studies were carried out in accordance with currently accepted international testing protocols and good

laboratory practices. In addition to the studies on the technical grade active ingredient, acute dermal toxicity and eye irritation studies were also submitted for Balence ES. The scientific quality of the data is high, and the database is considered sufficient to characterize the toxicity and infectivity of this microbial pest control agent and end-use product.

In an acute oral toxicity study, no signs of adverse effects were observed in Sprague Dawley rats following oral gavage with 3.20×10^8 CFU/animal of *B. bassiana* strain HF23 suspended in sterile purified water. The test substance was isolated in the feces of dosed rats approximately 10 hours postdosing, but was not detected in the feces, blood and tested tissues by day three. The oral LD_{50} was determined to be $> 3.20 \times 10^8$ CFU/animal and *B. bassiana* strain HF23 is considered to be non-toxic and non-pathogenic to rats via the oral route.

In the acute pulmonary infectivity and toxicity study, Sprague Dawley rats were exposed by the intratracheal route to *B. bassiana* strain HF23 in Butterfield's buffer at a dose of 1.06×10^7 CFU/animal in a volume of 0.1 mL. Animals were observed for up to seven days. There were no signs of adverse clinical effects and all animals gained weight. Two animals dosed with the test substance died shortly after initiation of the study; these deaths were attributed to the anaesthesia used. The test substance was isolated from the lungs of dosed rats approximately one hour postdosing, but was not detected in the feces, blood and tested tissues by day three. The pulmonary LD_{50} was determined to be $> 1.06 \times 10^7$ CFU/animal and *B. bassiana* strain HF23 is considered to be non-toxic and non-pathogenic when administered via the pulmonary route.

In an acute intraperitoneal infectivity study, seven week old Sprague Dawley rats were injected with a 1 mL suspension of *B. bassiana* strain HF23 in sterile purified water at a dose of 2.80×10^7 CFU/animal in 1 mL of sterile purified water. Animals were then observed for up to 21 days. There were no mortalities. All animals gained weight and no significant clinical signs of adverse effects were observed. Two of the animals in the treatment group developed small lumps under the skin in the ventral abdomen. *Beauveria bassiana* strain HF23 was not detected in the lumps and surrounding tissues. Upon necropsy, one male in the treatment group had mottled kidneys. Necropsy findings among females in the treatment group included red ovaries (three animals), enlarged ovaries (one animal), red uterus (two animals), enlarged uterus (one animal), and red lungs (one animal). No lesions or other signs of infectivity were observed. Based on these results, *B. bassiana* strain HF23 was not considered to be pathogenic to rats via the intraperitoneal route.

In a combined acute dermal toxicity and acute dermal irritation study, the backs of New Zealand white rabbits were dermally exposed to 2 g/kg bw of *B. bassiana* strain HF23 for 24 hours. Following exposure, the animals were observed for a period of 14 days. There were no mortalities and no signs of adverse effects. The maximum individual irritation, maximum irritation score (MIS) and maximum average scores (MAS) were all zero. There were no signs of irritation at any point of the study. Based on the results of this study, *B. bassiana* strain HF23 is of low dermal toxicity and is not a dermal irritant. This study was classified as acceptable but supplementary as the test substance was not moistened after application. A replacement study, however, will not be required as dermal toxicity, and dermal irritation studies are not required for technical grade active ingredients.

In a primary eye irritation study, 0.1 mL of *B. bassiana* strain HF23 was instilled into the conjunctival sacs of New Zealand white rabbits. Animals were observed for seven days. Eye irritation was scored at 1, 24, 48, 72 hours, and 7 days after test substance administration. Conjunctival irritation consisting of redness, chemosis and/or discharge were observed in all animals up to 72 hours after dosing. Iritis was observed in one animal after 24 hours. All signs of ocular irritation were absent by day seven. The maximum individual irritation score was determined to be 19 or mildly irritating and the MIS was 15.7 or mildly irritating at the 24 hour time point. The MAS (24, 48, and 72 hours) was 10.6 or non-irritating to minimally/slightly irritating. Based on the results of this study, *B. bassiana* strain HF23 was found to be mildly irritating to the eye.

A dermal toxicity study was conducted with the end-use product, Balence ES. The backs of New Zealand White rabbits were dermally exposed to 2 g/kg bw (4.05×10^9 CFU/mL) for 24 hours. There were no mortalities and no significant signs of adverse effects following application of the test substance. The dermal lethal dose to 50% (LD_{50}) of Balence ES is > 2 g/kg bw in rabbits. Based on the results of this study, Balence ES was found to be of low toxicity when administered via the dermal route.

A dermal irritation study using Balence ES was not submitted. The protocol used to assess dermal toxicity was sufficient for assessing dermal irritation and no signs of adverse effects were noted in the dermal toxicity study. The formulants in Balence ES, however, are potentially irritating to the skin. A study is not required provided that the appropriate personal protective equipment (PPE) to minimize exposure is worn as mandated on the Balence ES label.

In a primary eye irritation study, 1.0 mL of Balence ES (3.45×10^9 CFU/mL) was instilled into the conjunctival sacs of New Zealand White rabbits. Animals were observed for up to 72 hours. Mild conjunctival redness was observed in all 3 animals at 1 and 24 hours after dosing. Redness persisted in 1 animal up to 48 hours. Moderate to heavy discharge was noted in 2 animals after 1 hour, but cleared by 24 hours. No other signs of ocular irritation were observed. At the one hour time point, the maximum individual irritation score was 8. The MIS was 2.7 at the 1 hour time point, and the MAS over the 24, 48 and 72 hour time points was 0.56. These scores indicate that Balence ES was non-irritating to minimally/slightly irritating to the eye.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

The potential for dermal, eye and inhalation exposure for pesticide handlers exists, with the major source of exposure to workers generally being dermal. As 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.

Beauveria bassiana strain HF23 is not known to be a human pathogen and there is no indication that it could penetrate the skin of healthy individuals. Based on the demonstrated lack of adverse effects in the intraperitoneal study, it is the PMRA's opinion that entry of absorbed *B. bassiana* strain HF23 into the body, even through cut skin, should not pose a risk to health.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions. Label statements (e.g. Potential Sensitizer) and risk mitigation measures are required to protect populations that are most likely to be exposed to the products. Such exposure to handlers can be minimized if they wear waterproof gloves, long-sleeved shirts, long pants, NIOSH approved respirators (with any –95, P-95, R-95 or HE filter), shoes and socks.

While submitted studies on *B. bassiana* strain HF23 did not indicate a pulmonary risk, inhalation exposure can be minimized if applicators wear NIOSH approved respirators (with any –95, P-95, R-95 or HE filter)

Although eye irritation studies submitted by the applicant indicated minimal eye irritation potential, the applicant has also recommended that users of Balence ES wear protective eye wear (e.g. goggles, a face shield, or safety glasses).

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 *B. bassiana* strain HF23. The label does not allow applications on turf in residential or recreational areas; therefore, non-occupational exposure and risks to adults, infants and children are low. As the use of Balence ES is confined to the interior of poultry production houses, exposure to infants and children in schools or 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

No direct applications of Balence ES to food and feed crops are proposed. Chicken manure exposed to the application of Balence ES may be composted for use as a fertilizer on agricultural food and feed crops. Composting of the manure, prior to spreading as a fertilizer, results in temperatures of approximately 49 to 60°C which are maintained for several weeks. Spores of *B. bassiana* are not expected to remain viable after composting of the chicken manure as *B. bassiana* strain HF23 did not produce any growth when subjected to temperatures of 35–37°C, (see Section 4.1, Environmental Fate and Behaviour in the Environment). Based on the relatively limited use and the inability of the active ingredient to survive at typical composting temperatures, the exposure to viable *B. bassiana* strain HF23, via use of exposed chicken manure as fertilizer, is expected to be minimal.

Furthermore, the acute oral toxicology data submitted by the applicant indicates that any inadvertent exposure poses minimal risks. *Beauveria bassiana* strain HF23 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study. Higher tier subchronic dietary exposure studies were not required because of the low toxicity of the MPCA and the absence of any indication of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral, pulmonary and intraperitoneal toxicity/infectivity studies. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

Beauveria bassiana strain HF23 produces beauvericin as a secondary metabolite. To ensure beauvericin is at acceptable levels in Balence ES, batches of the technical product are regularly screened for beauvericin using acceptable methods. Dietary exposure to secondary metabolites produced by *B. bassiana* strain HF23 is also not expected given the use pattern of Balence ES.

3.3.2 Drinking Water

Although the use pattern for Balence ES is limited to an indoor use (poultry production houses), the chicken manure exposed to Balence ES will be composted into fertilizer for use on agricultural crops which could lead to *B. bassiana* strain HF23 entering neighbouring aquatic environments via surface-water runoff. *Beauveria bassiana* strain HF23 can potentially survive in water; however, no risks are expected from exposure to this microorganism via drinking water because exposure will be minimal (see rationale in Section 3.3.1, Food), and it showed no harmful effects on animals that were exposed orally in the Tier I acute oral toxicity and infectivity testing. Drinking water is accordingly not being screened for *B. bassiana* as a potential indicator of microbial contamination or as a direct pathogenic contaminant. Furthermore, both percolation through soil and municipal treatment of drinking water will likely reduce the transfer of residues to drinking water. Therefore, the potential of significant transfer to drinking water is minimal to non-existent, and the risk from consuming drinking water containing *B. bassiana* strain HF23 is minimal as there is no evidence of adverse effects from oral exposure to this agent.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculation of acute reference doses and acceptable daily intakes (ADIs) is 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 (e.g. 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 PMRA concludes that *B. bassiana* strain HF23 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 intraspecies 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 *B. bassiana* strain HF23 to human health.

3.4 Maximum Residue Limits

As there are no direct applications to food and as no significant adverse effects were reported in the Tier I acute toxicity/pathogenicity studies, the establishment of an maximum residue limit (MRL) is not required for *B. bassiana* strain HF23 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. The *Food and Drugs Act* prohibits the sale of adulterated food, that is, food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. 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.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty no harm will result from aggregate exposure of residues of *B. bassiana* strain HF23 to the general Canadian population, including infants and children, when the microbial pest control product Balence ES is used as labeled. 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.Given the Balence ES is to be used in poultry production houses and is not allowed for use on turf, residential or recreational areas, dermal and inhalation exposure to the general public will be very low. Furthermore, few adverse effects from exposure to natural populations of *B. bassiana* in the environment have been reported. Even if there were an increase in exposure to this microorganism from the use of Balence ES, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has 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. Besides naturally occurring strains of *B. bassiana* in the environment, the PMRA is not aware of any other microorganisms or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *B. bassiana* strain HF23 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

The temperature range for growth of *B. bassiana* strain HF23 was determined. Dilutions of *B. bassiana* strain HF23 were prepared corresponding to concentrations of 10^4, 10^3, and 10^2 CFU/mL and subcultured onto barley start slurry agar plates. The plates were incubated at either 30°C or at 35–37°C for 14 days. The plates were observed daily for growth of fungi which were identified by colony morphology. Plates incubated at 30°C yielded colonies characteristic of *B. bassiana*. No growth was observed on the plates incubated at the higher temperatures.

Jabb H23 ES, an end-use formulation equivalent to Balence ES, was applied to poultry manure and broiler litter at a rate of 1.08×10^{14} conidia/ha. The viability of the active ingredient was assessed over a period of 55 days. In general, the viability of the applied end-use product gradually declined between days 1 to 14 in manure and between days 1 to 21 in litter. More dramatic declines in viability were observed thereafter. This study was considered to be acceptable but supplementary as critical information with regards to the conditions of the study (e.g. temperature, exposure to light etc.) were not reported.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

A complete ecotoxicology package was reviewed to address the risks of *B. bassiana* strain HF23 to terrestrial organisms.

The acute oral toxicity of *B. bassiana* strain HF23 conidia to 30 WW36 chickens was assessed over a period of 30 days. *Beauveria bassiana* strain HF23 conidia were administered to the birds by gavage at a dose rate of 0.91 g/kg bw (equivalent to 3.9×10^{10} CFU/kg bw) daily for 5 days. Concurrent negative control (dosed with corn oil), filtrate control (dosed with autoclaved *B. bassiana* strain HF23 filtrate) and sterile control (dosed with autoclaved *B. bassiana* strain HF23 conidia) groups (5/sex/group) were also maintained. There were no mortalities and no behavioural abnormalities among the birds in the control and treatment groups. No significant differences in male and female body weights or in feed consumption were observed between the treatment and control groups at any interval. Gross necropsies conducted at the end of the study did not indicate any abnormalities. The lungs, kidneys and livers of selected animals were subjected to a histopathology examination. The histopathology results did not indicate any clear effects attributable to administration of the test substance. Given the absence of any histopathological abnormalities and the lack of health effects during the study, isolation of *B. bassiana* strain HF23 from the organs and tissues was not attempted. This rationale was considered acceptable and infectivity testing was waived. The 30-day acute oral LD_{50} in birds was found to be > 0.91 g/kg bw via the oral route.

A data waiver for avian pulmonary/inhalation/injection toxicity and infectivity testing was submitted. The proposed use for Balence ES is for controlling houseflies in poultry production houses. No direct outdoor uses are proposed, thus minimizing direct exposure to birds. Chicken

manure exposed to Balence ES, however, may be composted and used as a fertilizer leading to potential indirect exposure to non-target organisms. *Beauveria bassiana* strain HF23 has been shown to have limited viability in chicken manure and in chicken litter (see Section 4.1, Fate and Behaviour in the Environment). Composting of the manure, prior to spreading as a fertilizer, results in temperatures of approximately 49 to 60°C, which are maintained for several weeks. No spores are expected to remain viable after composting as *B. bassiana* strain HF23 was found to be unable to grow at temperatures above 35°C (see Section 4.1). Based on the relatively limited use and the inability of the active ingredient to survive at typical composting temperatures, the expected exposure of wild birds to viable *B. bassiana* strain HF23 is expected to be minimal. In the event of exposure, no adverse effects are expected. Chickens exposed to a maximum hazard dose of *B. bassiana* strain HF23 via the oral route did not exhibit any signs of adverse effects. Furthermore, the inability of *B. bassiana* strain HF23 to grow at temperatures above 35°C indicates it will not proliferate at avian body temperatures, which are normally 40°C.

A data waiver for wild mammal toxicity/infectivity testing was submitted. The waiver is based on the rationale that the active ingredient is a naturally occurring soil colonizer, and its level in the environment will not significantly increase with the use of Balence ES, and that an extensive literature search yielded no reports of adverse effects on wild mammals. In addition, human health data performed on laboratory animals showed no detrimental effects.

A data waiver for terrestrial arthropod testing was submitted. *Beauveria bassiana* is ubiquitous in the environment and the proposed use of Balence ES will not significantly augment the naturally occurring levels. No direct outdoor uses are proposed, thus minimizing direct exposure to non-target arthropods. However, chicken manure exposed to Balence ES in the poultry production house may be composted and used as a fertilizer, leading to potential indirect exposure to non-target arthropods. Based on the limited use and the inability of the active ingredient to survive at typical composting temperatures, the expected exposure of non-target arthropods to viable *B. bassiana* strain HF23 is expected to be minimal. Significant adverse effects are also not expected. A summary of a study in which *B. bassiana* was applied to fields of mixed grasses to control grasshoppers was submitted (Brinkman et al., 1996-1999). Carbaryl was used as a control. Neither *B. bassiana* nor carbaryl applications resulted in any noticeable declines in the abundance of aerial insect. Ant and spider abundance declined in all plots following treatment, but rebounded in the following week. The initial decline was attributed to heavy rainfall and resulting decreased activity (as the insects were collected in pitfall traps) rather than to treatment with *B. bassiana* or carbaryl. Adverse effects were not noted in non-target insects including Diptera, Hymenoptera, Lepidoptera, Neruoptera, and Coleoptera.

In a study conducted by Kaufman et al. (2005), applications of Balence ES were compared with pyrethrin treatments for the control of adult houseflies in high-rise, caged-layer poultry facilities. Although adult housefly populations were significantly lower in *B. bassiana*-treated facilities during the spray and postspray periods, no significant differences were observed postspray among populations of *Carcinops pumilio* (adults and larvae), a predator of houseflies, and darkling beetles (adults and larvae), considered a pest in poultry facilities. Based on the results of this study, the use of Balence ES is not expected to pose any more risk than conventional fly control products to non-target terrestrial arthropods. Some strains of *B. bassiana* are known to be toxic to honeybees. The use pattern of Balence ES, however, is not expected to significantly

increase the potential of honeybees coming into contact with the active ingredient in a viable form. The label directs users to avoid applying the product where honeybees are actively foraging or around hives.

A waiver for non-arthropod invertebrate testing was submitted. The active ingredient, *B. bassiana* strain HF23, is a naturally occurring soil colonizer, and its level in the environment will not significantly increase with the use of the Balence ES. Furthermore, the use pattern indicates that any active ingredient that is released into the environment will have limited viability. Therefore, the risk posed by the use of Balence ES to non-arthropod invertebrates is minimal, and further testing is not required.

Studies were not required to address the risks of *B. bassiana* strain HF23 to soil microorganisms as *B. bassiana* is a normal component of the soil. *Beauveria bassiana* strain HF23 produces a secondary metabolite, beauvericin, that has been found to possess antibiotic properties. Each lot of technical product will be monitored to ensure that beauvericin is at acceptable levels.

A waiver for terrestrial plant testing was submitted. The waiver was based on the rationale that *B. bassiana* strain HF23 does not remain viable in chicken manure or litter for long periods of time and that the active ingredient is not a known pathogen of plants. Furthermore, given the use pattern, increased exposure to the active ingredient, due to the use of Balence ES, is not expected to increase significantly.

4.2.2 Effects on Aquatic Organisms

Waivers were submitted to address requirements for freshwater fish and aquatic arthropod testing. *Beauveria bassiana* is ubiquitous in the environment and the use of Balence ES would not significantly augment the natural populations. Furthermore, the use of Balence ES is for controlling houseflies in poultry production houses. There are no direct outdoor uses for Balence ES, thus minimizing direct environmental exposure. Although there are no outdoor applications, chicken manure exposed to Balence ES may be used as a fertilizer leading to potential exposure to aquatic organisms as a result of runoff. *Beauveria bassiana* strain HF23 has been shown to have limited viability in chicken manure and in chicken litter (see Section 4.1, Fate and Behaviour in the Environment). Composting of the manure, prior to spreading as a fertilizer, results in temperatures of approximately 49 to 60°C, which are maintained for several weeks. No spores are expected to remain viable after composting, as *B. bassiana* HF23 was found to be unable to grow at temperatures above 35°C (see Section 4.1, Fate and Behaviour in the Environment). Based on the limited use of Balence ES and the inability of the active ingredient to survive at typical composting temperatures, the expected exposure of aquatic organisms to viable *B. bassiana* strain HF23 is expected to be minimal.

Although a literature search yielded two studies in which *B. bassiana* caused adverse effects to the embryos of inland silverside fish (*Menidia beryllina*), both studies noted that inactivated spores (either heat-killed or detergent-treated) failed to cause significant adverse effects (Middaugh and Genthner 1994, Genthner and Middaugh 1992 (PMRA #1432358)). Given the use of Balence ES, *B. bassiana* strain HF23 is expected to be inactivated in any compost that will be applied to crops and is, therefore, not expected to cause significant adverse effects in fish.

5.0 Value

5.1 Effectiveness Against Pests

Four efficacy studies, all conducted in poultry production houses in the United States, were submitted in support of registration of *B. bassiana* strain HF23 and the associated end-use product Balence ES. The efficacy data demonstrated that Balence ES will control houseflies in poultry production houses at application rates of 9.5–16 mL/100 m^2 (equivalent to 5.4–9.0 × 10^8 conidia/m^2). Considering the mode of action of this product (an entomopathogenic fungus) and the efficacy data, a reapplication interval of every 2 to 7 days, as required, was supported. It is expected that the level of control given by *B. bassiana* products will depend on the specific conditions of the application area (temperature, humidity, poultry litter composition and moisture, etc.).

5.1.1 Acceptable Efficacy Claims

For controlling houseflies in poultry production houses, the application rate is 9.5 to 16 mL per 100 m^2 is acceptable. Apply the spray to walls, floors, posts and manure concentrating areas where the greatest numbers of pests are located. This is a contact infection material with greatest efficacy against adult flies. Re-treat at intervals of two to seven days as long as pest pressure persists as pest eggs hatch and mature. There is no restriction on the total maximum amount of product that may be applied in a year.

5.2 Economics

No economic analysis was conducted for this product evaluation.

5.3 Sustainability

5.3.1 Survey of Alternatives

Alternative active ingredients registered in Canada for controlling houseflies in poultry production houses include chlorpyrifos, dichlorvos, dimethoate, malathion, naled, tetrachlorvinphos, cyfluthrin, lambda-cyhalothrin, permethrin, pyrethrins + piperonyl butoxide, D-trans allethrin + piperonyl butoxide, and silicon dioxide (diatomaceous earth). In addition, the attractive pheromone Z-9-tricosene is registered for housefly control as a trap bait and in attracticide products containing the active ingredient methomyl, azamethiphos, or thiamethoxam.

5.3.2 Compatibility with Current Management Practices Including Integrated Pest Management

The use of Balence ES is fully compatible with current management practices, including integrated pest management. In fact, some of the efficacy trials submitted in support of registration of this product were conducted in poultry production facilities with active integrated pest management programs, including the use of both hymenopteran pupal parasitoids of houseflies and housefly baits containing methomyl.

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

The development of resistance to entomopathogenic fungi has not been documented and, due to the relatively complex nature of the mode of action, is not considered likely. Use of *B. bassiana* strain HF23 within an integrated pest management program would minimize the potential for any incipient resistance to become prevalent in pest populations.

5.3.4 Contribution to Risk-reduction and Sustainability

Beauveria bassiana strain HF23 provides a new alternative to conventional chemical insecticides and an additional management tool for controlling housefly populations in poultry production houses.

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, 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 bioaccumulative. These substances are referred to in the policy as Track 1 substances.

While reviewing *B. bassiana* strain HF23, the PMRA took into account the federal Toxic Substances Management Policy 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, *Beauveria bassiana* HF23 Technical Product, and formulants in the end-use product, Balence ES. The PMRA has reached the following conclusions.

- *Beauveria bassiana* strain HF23 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 no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track 1 criteria.

Therefore, the use of *Beauveria bassiana* HF23 Technical Product 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

- *Beauveria bassiana* strain HF23 does not contain any contaminants of health or environmental concern identified in 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, Balence ES, contains the formulant soybean oil, which is identified in Canada Gazette, part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants of Health of Environmental Concern* as an allergen known to cause anaphylactic-type reactions. Therefore the label for Balence ES will include the precautionary statement: "Warning: this product contains the allergen soybean oil (88.71% weight/weight)" on the principal display panel.

Although it does not appear on the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* (Canada Gazette, part II, Volume 139, Number 24, pages 2641–2643), a component of one of the formulants in Balence ES is considered to be toxic as defined in section 64 of the *Canadian Environmental Protection Act, 1999*. The PMRA has conducted a risk assessment of this formulant component and has found that the associated risk is acceptable for the proposed use.

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for *Beauveria bassiana* HF23 Technical Product and Balence ES are adequate to assess their safety to human health. The technical material was fully characterized, and the specifications were supported by the analysis of a sufficient number of batches.

Although methods and procedures are in place to monitor microbial contamination in *Beauveria bassiana* HF23 Technical Product and Balence ES, no methods were submitted to screen specifically for human and mammalian pathogens during manufacture. A microbe-specific screening method for coliforms, fecal coliforms, fecal streptococci/enterococci, as well as *Salmonella*, and *Staphylococcus* is required, along with confirmatory data demonstrating that contaminants are at acceptable levels.

Storage stability data were sufficient to support an expiration date of 14 months for Balence ES when the product is stored at room temperature (20–27.5°C).

7.2 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *B. bassiana* strain HF23 were determined to be sufficiently complete to permit a decision on registration. *B. bassiana* strain HF23 was of low toxicity in the rat when administered via the oral, pulmonary, intraperitoneal and dermal routes and was not pathogenic or infective via the oral, pulmonary and

intraperitoneal routes. In the oral and pulmonary studies, clearance was established by day three. The technical product was not found to be a dermal irritant and the end-use product was not found to be toxic via the dermal route.

Non-minimal/slight ocular irritation was observed with both the technical product and the end-use product.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions. Exposure to allergens, including *B. bassiana* strain HF23 may cause allergies following repeated exposures. As a result, the signal words "POTENTIAL SENSITIZER" are required on the principal display panels of all technical and end-use products and risk mitigation measured are required to protect populations that are likely to be primarily exposed to the products. Such exposure to handlers and early-entry workers can be minimized if they wear gloves, long-sleeved shirts, long pants, NIOSH approved respirators (with any –95, P-95, R-95 or HE filter), shoes and socks.

When handled according to the label instructions, the pulmonary, dermal and ocular routes are potential routes of exposure to mixer/loaders, handlers and early-entry workers. To minimize risk to workers, use of appropriate PPE will be stipulated on product labels. The labels do not allow application to turf in residential or recreational areas. As the use sites are agricultural, exposure to infants and children in schools or 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.

As there are no direct applications to food and as no significant adverse effects were reported in the Tier I acute toxicity/pathogenicity studies, the establishment of an MRL is not required for *B. bassiana* strain HF23 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.

7.3 Environmental Risk

The environmental fate studies, non-target studies and waivers submitted in support of *B. bassiana* strain HF23 were determined to be sufficiently complete to permit a decision on registration.

The environmental fate studies submitted indicated that *B. bassiana* strain HF23 does not persist in chicken manure or chicken litter and has limited viability at temperatures above 35–37°C.

An acute oral toxicity in chickens concluded that *B. bassiana* strain HF23, with an LD_{50} of > 0.91 g/kg bw, poses a minimal risk to birds when administered via the oral route. The remaining groups of non-target organisms, including birds (pulmonary/inhalation/injection), wild mammals, terrestrial arthropods (including honeybees), non-arthropod invertebrates, terrestrial plants, freshwater fish and aquatic arthropods, were addressed through waivers. The waivers were based on the ubiquitous nature of the active ingredient, the proposed indoor uses, the limited viability of the active ingredient in light of the use and, in some cases, literature reports or study results which indicate a lack of adverse effects.

The waivers were found to be acceptable and testing of non-target organisms was not required. As a general precaution, the label prohibits the direct application of the product to aquatic habitats (such as lakes, rivers, sloughs, ponds, coulees, prairie potholes, creeks, marshes, streams, reservoirs, ditches and wetlands), estuaries or marine habitats. The label also directs handlers to not contaminate surface water by improper disposal of equipment wash waters. Furthermore, as some strains of *B. bassiana* have been shown to be toxic to honeybees, users are directed to avoid applying the product to areas where honeybees are actively foraging or around hives.

7.4 Value

Beauveria bassiana strain HF23, formulated as the end-use product Balence ES, has value in controlling populations of houseflies in poultry production houses.

7.5 Unsupported Uses

All use claims were fully supported as proposed by the applicant.

8.0 Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and in accordance with the Pest Control Products Regulations, has granted conditional registration for the sale and use of the technical grade active ingredient *Beauveria bassiana* HF23 Technical Product and the end-use product Balence ES to control houseflies in poultry production houses.

An evaluation of the current scientific data from the applicant and scientific reports indicated that, under the approved conditions of use, the products have value and do not present an unacceptable risk to human health or the environment.

Although the risks and value have been determined to be acceptable when all risk-reduction measures are followed, as a condition of these registrations, additional scientific information is being requested from the applicant as a result of this evaluation to ensure that the MPCA can be distinguished from naturally occurring strains and the absence of pathogens in the end-use product (see below). (For more details, refer to the Section 12 Notice associated with these conditional registrations.) The applicant will be required to submit this information within the time frames indicated below.

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.

Product Characterization and Analysis

- An identification method to distinguish strain HF23 from other naturally occurring strains of *B. bassiana* is required.

- To ensure that Balence ES does not contain unacceptable levels of human and animal disease-causing microorganisms, the registrant will be required to include microbe-specific screening methods for coliforms, fecal coliforms, fecal streptococci/enterococci as well as *Salmonella* and *Staphylococcus* in the manufacturing process. Confirmatory data from five representative production batches will be required.

The applicant must submit this information no later than 1 December 2008.

List of Abbreviations

°C	degree(s) Celsius
ADI	acceptable daily intake
ARSEF	Agriculture Research Service Entomopathogenic Fungi
bw	body weight
cm^3	cubic centimetre
CFU	colony forming units
FDA	*Food and Drugs Act*
g	gram
kg	kilogram
LD_{50}	lethal dose 50%
m^2	square metre
MAS	maximum average score
MIS	maximum irritation score
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
N/A	not applicable
NIOSH	National Institute for Occupational Safety and Health
NP	nonylphenol
NPE	nonylphenol ethyloxylates
PCPA	*Pest Control Products Act*
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	parts per million
TSMP	Toxic Substances Management Policy

Appendix I Tables

Table 1 Toxicity and Infectivity of *Beauveria bassiana* strain HF23 and Balence ES

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Beauveria bassiana* strain HF23				
Acute Oral Toxicity and Infectivity	Rat—Sprague Dawley - treatment group: 15/sex treated with *B. bassiana* strain HF23, 3.20×10^8 CFU/animal in a 1 mL volume of sterile purified water by oral gavage - shelf control group: 2/sex - room control group: 2/sex - interim sacrifices from the treatment group (3/sex) and room control group (1/sex) on day 3; from the treatment group (3/sex), the shelf control group (1/sex), and the room control group (1/sex) on day 7	$LD_{50} > 3.20 \times 10^8$ CFU/animal	- No mortalities, no signs of adverse effects and no abnormalities upon necropsy. - All animals gained weight. - MPCA recovered from the feces of treatment group rats approximately 10 hours postdosing but was not detected in the feces, blood and tested tissues by day 3. Clearance was established by day 3. **NON-TOXIC, NOT INFECTIVE** **ACCEPTABLE**	PMRA 1178967

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Pulmonary Toxicity and Infectivity	Rat—Sprague Dawley - treatment group: 22/sex treated with *B. bassiana* strain HF23, 1.06×10^7 CFU/animal in a 0.1 mL of Butterfield's buffer via the intratracheal route - shelf control group: 2/sex - room control group: 9/sex - interim sacrifices from the treatment group (3/sex) and room control group (2/sex) approximately 1 hour postdosing, day 3 and day 7; from the shelf control group (1/sex) on day 7	$LD_{50} > 1.06 \times 10^7$ CFU/animal	- Two animals (1/sex) in the treatment group died shortly after initiation of the study; these deaths attributed to anaesthesia. No other mortalities. - No signs of adverse clinical effects. - All animals gained weight. - Test substance isolated from the lungs of dosed rats ~ 1 hour postdosing, but was not detected in the feces, blood or tested tissues by day 3. Clearance established by day 3. **NON-TOXIC, NOT INFECTIVE** **ACCEPTABLE**	PMRA 1178969

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Intraperitoneal Infectivity	Rat—Sprague Dawley - treatment group: 6/sex treated with *B. bassiana* strain HF23, 2.80×10^7 CFU/animal in 1 mL of sterile purified water - room control group: 2/sex - animals were sacrificed at the end of the study (day 21)		- No mortalities. No significant clinical signs of adverse effects. - All animals gained weight. - Two animals (1/sex) in the treatment group developed small lumps under the skin in the ventral abdomen. *Beauveria bassiana* strain HF23 was not detected in the lumps and surrounding tissues. - Upon necropsy, one male in the treatment group had mottled kidneys. Necropsy findings among females in the treatment group included red ovaries (3 animals), enlarged ovaries (1 animal), red uterus (2 animals, enlarged uterus (1 animal) and red lungs (1 animal). One female in the room control group had red ovaries and an enlarged uterus. **NOT INFECTIVE** **ACCEPTABLE**	PMRA 1178970

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Dermal Toxicity/ Irritation	Rabbit—New Zealand white - 5/sex treated with *Beauveria bassiana* strain HF23, 2 g/kg bw, 24 hour exposure	LD_{50} > 2 g/kg bw	- No mortalities and no signs of adverse effects. - No erythema or edema at any time point. - Maximum individual irritation score, MIS and MAS over 24, 48 and 72 hours all 0. **LOW TOXICITY NON- TO MINIMALLY/SLIGHTLY IRRITATING** **ACCEPTABLE BUT SUPPLEMENTARY** as the test substance was not moistened after application. A replacement study, however, will not be required as dermal toxicity and dermal irritation studies are not required for technical grade active ingredients.	PMRA 1178968

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Eye Irritation	Rabbit–New Zealand white - 3 females treated with *B. bassiana* strain HF23, 0.1 mL/eye/animal		- Redness (score = 2) observed in all three animals up to 72 hours. - Chemosis (score = 2) in all three animals at 1and 24 hours subsiding by 48 hours in two of the animals (score = 1) and was absent in these animals thereafter. Chemosis (score = 2) continued to be observed in the third animal up to 72 hours.. - Discharge (score = 3) noted in all three animals at 1 and 24 hours. In two of the animals, discharge was reduced at 48 hours (score = 1) and was absent upon subsequent observations. In the third animal, discharge subsided (score = 2) but persisted up to the 72 hours. - Iritis (score = 1) was observed in one animal at 24 hours. - All signs of ocular irritation absent by day 7. - Maximum individual irritation score = 19 at 24 hours - MIS = 15.7 at 24 hours. - MAS (24, 48, and 72) hours = 10.6 **NON- TO MINIMALLY/SLIGHTLY IRRITATING** **ACCEPTABLE BUT SUPPLEMENTARY**	PMRA 1178971

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Toxicity/Irritation Balence ES				
Acute Dermal Toxicity/ Irritation	Rabbit–New Zealand white - 5/sex treated Balence ES, 2 g/kg bw, 24 hour exposure	LD_{50} > 2 g/kg bw	- No mortalities. - Soft feces observed among one male and three females at 1 and 2.5 hours. This condition was absent in two of the animals by 4 hours but persisted in two animals up to 4 hours and day 3. In the animal with soft feces up to day 3, anogenital staining was also observed from days 1 to 5 and on day 7. -One female animal exhibited a slight weight decrease between days 1 and 7. At the completion of the study on day 14, however, all animals had gained weight. - Irritation not assessed. Dermal irritation not noted in dermal toxicity study. Appropriate PPE to minimize exposure required. Study not required. **LOW TOXICITY** **IRRITATION WAIVED** **ACCEPTABLE**	PMRA 1179032

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Eye Irritation	Rabbit–New Zealand white - 3 females treated with Balence ES, 1.0 mL/eye/animal		- Redness (score = 1) observed in all three animals at 1 and 24 hours. Redness persisted in one animal up to 48 hours. - Discharge (score = 2 and 3) noted in two animals at 1 hour but cleared by 24 hours. - Maximum individual irritation score = 8 at 1 hour - MIS = 2.7 at 1 hour - MAS (24, 48, and 72 hours) = 0.56 **NON- TO MINIMALLY/SLIGHTLY IRRITATING** **ACCEPTABLE**	PMRA 1179033

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Test Substance(s)	Endpoint Value	Significant Effects, Comments	Reference
Terrestrial Organisms					
Vertebrates					
Birds (WW36 chickens)	Oral	i. *Beauveria bassiana* strain HF23 conidia ii. corn oil iii. filtrate control (autoclaved *B. bassiana* filtrate) iv. sterile control (autoclaved *B. bassiana* strain HF23 conidia)	LD50 > 0.91 g/kg bw/day for five days	- No mortalities. - No behavioural abnormalities. - No abnormalities upon gross necropsy. - No significant differences in male and female body weights or in feed consumption. - No treatment-related histopathological abnormalities. **LOW TOXICITY; NOT INFECTIVE**	PMRA 1178960 1486267 1486268

Organism	Exposure	Test Substance(s)	Endpoint Value	Significant Effects, Comments	Reference
Birds	Pulmonary/ Inhalation/ Injection	Waiver submitted. The waiver request is based on the rationale that the end-use product is intended for use in poultry production houses such that the level of the active ingredient, *B. bassiana* strainHF23, in the environment will not significantly increase with the use of Balence ES and that significant adverse effects in birds are not expected. **WAIVER ACCEPTED**			PMRA 1178962 1178963 1432355 1178978 1432360
Wild Mammals	Waiver submitted. The active ingredient is a naturally occurring soil colonizer, whose level in the environment will not significantly increase with the use of Balence ES. An extensive literature search yielded no reports of adverse effects on wild mammals. In addition, acute toxicity and infectivity studies (oral, pulmonary, intraperitoneal and dermal) performed on laboratory animals showed no detrimental effects. **WAIVER ACCEPTED**				PMRA 1178962 1178963 1432355 1432358
Invertebrates					
Terrestrial Arthropods	Waiver submitted. The active ingredient is a naturally-occurring soil colonizer, whose level in the environment will not significantly increase due to the use of Balence ES. Literature indicates that *B. bassiana* strain HF23 is no more harmful to non-target insects than the industry standard, pyrethrin. Some strains of *B. bassiana* are known to be toxic to honeybees. The use pattern, however, is not expected to significantly increase the potential of honeybees coming into contact with the active ingredient in a viable form. Label directs users to avoid application where honeybees are actively foraging or around hives. **WAIVER ACCEPTED**				PMRA 1178962 1178963 1432355 1432358
Non-Arthropod Invertebrates	No study or waiver submitted. The active ingredient is a naturally occurring soil colonizer, whose level in the environment will not significantly increase with the proposed use of Balence ES. Furthermore, the use pattern indicates that any active ingredient that is released into the environment will have limited viability. **WAIVER ACCEPTED**				PMRA 1178962 1178963 1432355
Vascular Plants					
Vascular Plants	Waiver submitted. The active ingredient does not remain viable in chicken manure or litter for long periods of time and the active ingredient is not a known pathogen of plants. Furthermore, given the use patterns, increased exposure to the active ingredient, due to use of Balence ES, is not expected to increase significantly. **WAIVER ACCEPTED**				PMRA 1178962 1178963 1432355 1178978 1432360

Organism	Exposure	Test Substance(s)	Endpoint Value	Significant Effects, Comments	Reference
Aquatic Organisms					
Vertebrates					
Freshwater fish	Waiver submitted. Increased environmental exposure to the active ingredient is expected to be minimal. Given the use for Balence ES, the viability of the active ingredient is expected to be limited. **WAIVER ACCEPTED**				PMRA 1178962 1178963 1178978 1432360
Invertebrates					
Aquatic Arthropods	Waiver submitted. The active ingredient has limited viability in the environment. Furthermore, the use pattern for Balence ES is not expected to significantly increase the level of *B. bassiana* in the environment. **WAIVER ACCEPTED**				PMRA 1178962 1178963 1178978 1432360

References

A. LIST OF STUDIES/INFORMATION SUBMITTED BY REGISTRANT

1.0 The Active Ingredient, Its Properties and Uses

2.0 Methods of Analysis

PMRA 1179028 Evaluation of the viability degradability over time of *Beauveria bassiana* conidia end use product under room temperature storage conditions. 2001. DACO M2.11

PMRA 1179029 Balence 5 lot analysis. 2005. DACO M2.9.1, M2.9.2, M2.10.1, M2.10.2, M2.10.3

PMRA 1179030 Balence Product Chemistry. 2005. DACO M2.7.1, M2.7.2, M2.8, M2.9.1, M2.9.2, M2.9.3, M2.10

PMRA 1178964 *Beauveria bassiana* HF23. Product Chemistry. 2005. MRID 46449601. DACO M1.2, M2.0, M 2.10.2, M2.10.3, M2.12, M2.2, M2.3, M2.5, M2.5, M2.7.1

PMRA 1178965 *Beauveria bassiana* HF23 5 Lot Analysis. 2005. MRID: 46580501. DACO: M2.14,M2.9.2

PMRA 1456315 Email response with attached information regarding the ARSEF strain number. DACO: 2.8.

PMRA 1432352 Balence Product Chemistry. 2005. DACO: M2.10,M2.7,M2.7.1,M2.7.2,M2.8,M2.9,M2.9.1,M2.9.2,M2.9.3

PMRA 1432353 Balence 5 lot analysis. 2005. DACO: 2.14

3.0 Impact on Human and Animal Health

PMRA 1178961 2004, Effect of Milling on and Sensitivity of Detection of *Beauveria bassiana* HF23 in Fortified Rat Tissues, MRID: 46526009, DACO: M4.2.2,M4.2.3,M4.3.3

PMRA 1178963 2003, Growth of *Beauveria bassiana* in Barley Slurry Agar at Two Tempteratures, MRID: 46526011, DACO: M4.9,M8.5

PMRA 1178966 2005, Request of Exemption from the Requirement of Tolerance, MRID: 46526002, DACO: M2.14

PMRA 1178967 2004, Acute Oral toxicity/Pathogenicity Study in Rats with *Beauveria bassiana* HF23, MRID: 46526003, DACO: M4.2.2

PMRA 1178968 2004, Acute Dermal Toxicity/Pathogenicity Study in New Zealand White Rabbits with *Beauveria bassiana* HF23, MRID: 46526004, DACO: M4.4

PMRA 1178969 2004, Acute Pulmonary Toxicity/Pathogenicity Study in Rats with *Beauveria bassiana* HF23, MRID: 46526005, DACO: M4.2.3

PMRA 1178970 2004, Acute Injection Toxicity/Pathogenicity Study in Rats with Beauveria bassian HF23, MRID: 46526006, DACO: M4.3.3

PMRA 1178971 2004, Acute Eye Irritation Study in New Zealand White Rabbits with *Beauveria bassiana* HF23, MRID: 46526007, DACO: M4.9

PMRA 1179031 Waiver Request, DACO: M4.2.2,M4.2.3,M4.3

PMRA 1179032 2004, Acute dermal Toxicity/Pathology Study in new Zealand White Rabbits with *Beauveria bassiana* End-Use Product, DACO: M4.4

PMRA 1179033 2004, Acute Eye Irritation Study in New Zealand White Rabbits with *Beauveria bassiana* HF23 End Use Product, MRID: 46526013, DACO: M4.9

PMRA 1432358 2006, Response to US EPA Letter Dated March 21, 2006, DACO: M2.10,M2.12,M2.7.1,M2.8,M4.9

4.0 Impact on the Environment

PMRA 1178960 2004, *Beauveria bassiana* HF23 Conidia: Microbial Oral Toxicity and Pathogenicity Trest (LD50 or ID50) with Chickens Using a Maximum Hazard Dose, MRID: 46526008, DACO: M9.2.1

PMRA 1178962 2000, Evaluation of Viability Degradation Over Time of *Beauveria bassiana* Conidia Applied to Poultry Manure & Litter, 02100, MRID: 46526010, DACO: M8.5

PMRA 1178963 2003, Growth of *Beauveria bassiana* in Barley Slurry Agar at Two Tempteratures, MRID: 46526011, DACO: M4.9,M8.5

PMRA 1178978 Waiver Request, DACO: M9.2.1,M9.2.2,M9.4,M9.4.1,M9.4.2

PMRA 1432360 2005, Waiver Requests, DACO: M4.9,M9.0

PMRA 1486267 2007, Tissue Collection Sample Assignment, 138174.4100, DACO: M9.2

PMRA 1486268 2007, *Beauveria bassiana* HF23 Conidia: Microbial Oral Toxicity and Pathogenicity Test (LD50 or ID50) with Chickens Using a Maximum Hazard Dose, 13817.4100, DACO: M9.2

5.0 Value

PMRA 1179026 Efficacy Studies for balance-ES. DACO: M10.2.2

PMRA 1179027 Kaufman, P.E., Reasor, C., Rutz, D.A., Ketzis, J.K., & Arends, J.J. 2005. Evaluation of *Beauveria bassiana* applications against adult housefly, *Musca domestica*, in commercial caged-layer poultry facilities in New York state. Biological Control 33: 360-367. DACO: M10.2.2

B. ADDITIONAL INFORMATION CONSIDERED

i) Published Information

1.0 The Active Ingredient, Its Properties and Uses

2.0 Methods of Analysis

PMRA 1443979 Bidochka, M. J., J. E. Kasperski, and G. A. M. Wild. 1998. Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. Can. J. Bot. 76: 1198–1204.

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PMRA 1450834 Domsch, K. H., W. Gams, A. Traute-Heidi. 1993. *Beauveria* Vuill. 1912. *In*: Compendium of soil fungi. Lubrecht & Cramer Ltd. pp. 136-140.

3.0 Impact on Human and Animal Health

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PMRA 1444207 Beaumont, F. H. F. Kauffman, J. G. R. De Monchy, H. J. Sluiter and K. De Vries. 1985. Volumetric aerobiological survey of conidial fungi in the Nroth-East Netherlands. II. Comparison of aerobiological data and skin tests with mould extracts in an asthmatic population. Aller. 40: 181-186.

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PMRA 1443967 Fromtling, R.A., Kosanke, S.D., Jensen, J.M., Bulmer, G.S. 1979b. Fatal *Beauveria bassiana* infection in a captive American alligator. J. Am. Vet. Med. Assoc. 1979. 175(9):934–6. DACO: 2.7.2.

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4.0 Impact on the Environment

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PMRA 1484505 Chang, B.V., Chiang, B.W., Yuan, S.Y., 2005, Biodegradation of nonylphenol in soil. Available online at www.sciencedirect.com. Chemosphere 66 (2007) 1857-1862, DACO: M8.0,M9.0

PMRA 1484528 2001, Canadian Environmental Protection Act, 1999 PRIORITY SUBSTANCES LIST ASSESSMENT REPORT Nonylphenol and its Ethoxylates. Health Canada and Environment Canada, DACO: M8.0,M9.0

PMRA 1484539 European Union Risk Assessment Report 4-NONYLPHENOL (BRANCHED) AND NONYLPHENOL RISK ASSESSMENT 2002 United Kingdom. Institute for Health and Consumer Protection, European Chemicals Bureau, Existing Substances., DACO: M8.0,M9.0

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