Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Risk Assessment on the Importation of Honey Bee (*Apis mellifera*) Packages from the United States of America

(V13)

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Animal Health Risk Assessment
Animal Health Science Division
Animal Health Science Directorate

Évaluation des risques zoosanitaires
Division de la santé des animaux
Direction des sciences de la santé des animaux

Canada

EXECUTIVE SUMMARY

This assessment is a science-based evaluation to assist risk managers in decision making and risk mitigation. This assessment is not scientific research. Assumptions that have been made and may have influenced the results are listed and detailed in the document.

The Animal Health Risk Assessment (AHRA) unit of the Canadian Food Inspection Agency (CFIA) conducted a risk assessment to provide scientific information and advice in support of the Canadian National Animal Health Program for the development of import policy. The CFIA's Animal Import/Export Division asked the AHRA to update and assess the likelihood of biological hazards spreading and/or becoming established in Canada, and their likely consequences as a result of the importation of honey bee (*Apis mellifera*) packages from the United States of America (U.S.).

There are about 8,000 beekeepers in Canada, fewer than half of whom manage commercial beekeeping operations. The nature of commercial beekeeping operations differs across the country: some beekeepers specialize in honey production, others specialize in delivering pollination services, and many beekeepers combine both activities. Canadian beekeepers may import bee packages (each consisting of a queen bee and two or three pounds of worker bees packaged together) from Australia, New Zealand and Chile. In 1987, in response to the outbreak in the U.S. of two parasitic mites (honey bee tracheal mite, *Acarapis woodi*; and varroa mite, *Varroa destructor*), the Canadian federal Department of Agriculture closed the border to the importation of honey bees from the continental U.S. Importations of honey bee queens were allowed from Hawaii in 1993. Following the CFIA's 2003 risk assessment, the Agency allowed the importation of honey bee queens from the U.S. to help strengthen the genetics of Canada's domestic bee population and meet demands for queen bees. The importation of package bees from the U.S. continues to be prohibited.

This qualitative risk assessment is based on the approach recommended by the World Organisation for Animal Health (OIE) and consists of the characterization of hazards with entry, exposure and consequence assessments. The qualitative assessment includes the likelihood of the introduction of the hazards into Canada with the importation of honey bee packages from the U.S. (entry assessment); the likelihood of potential hazards spreading and/or becoming established within the domestic honey bee population in Canada (exposure assessment); and the expected magnitude of the resulting consequences (consequence assessment).

Africanized honey bees, antibiotic-resistant American foulbrood, small hive beetle and amitraz-resistant varroa mite are identified as hazards associated with the importation of honey bees from the U.S. The cause of the colony collapse disorder (CCD) is still unclear; it is generally considered to be multifactorial. Due to the fact that there is no specific biological agent identified, CCD was not considered a hazard for this risk assessment.

Key risk factors considered in the assessment are the distribution and prevalence of honey bee diseases in the U.S., the extensive migratory beekeeping industry, the overwintering of colonies

in the southern part of the U.S., the lack of interstate movement controls, and the absence of a national honey bee management program.

Summary of the Risk Estimates

Hazard	Entry Probability	Exposure Probability	Consequence Estimate	Risk Estimate
Africanized honey bee	Moderate to High	Small	Moderate	Low to Moderate
American foulbrood - Oxytetracycline resistant	High	Moderate to High	Moderate	Moderate
Small hive beetle	High	Low to Small	Moderate	Low to Moderate
Varroosis (varroa mite) - Amitraz-resistant	High	High	Moderate	Moderate

Please note that the probability range represents the level of uncertainty. Such uncertainties need to be taken into account when making a decision.

Conclusions of the current risk assessment are similar to the previous scientific evaluation conducted in 2003; there is still a high probability of introducing diseases and pests into Canada due to importation of honey bees from the continental United States. The risk assessment does not provide new scientific evidence to remove or decrease the current import control measures in place, thus allowing only the importation of honey bee queens from the United States.

As such, the risk assessment provides scientific support for the import control measures that are currently in place for the importation of honey bees from the U.S. These measures allow honey bee queens to be individually inspected for signs of disease before importation into Canada. Such verification is not possible with honey bee packages.

TRACKING FORM

Status of the Document

Please note that this document may be updated, replaced or made obsolete by other documents at any time. It is inappropriate to cite this document as anything other than a work in progress. Discussion of this document is invited.

Process Initiation and Dates

Request from: Dr. Connie Rajzman, Veterinary Program Specialist – Import/Export Live Animals and Germplasm Section, Animal Import/Export Division, CFIA

Date: received on March 12, 2013

Risk Assessment Versions and Dates

Draft #1: CFIA, Internal peer review. The draft document was released to: Animal Health Risk Assessment, Plant Health Risk Assessment, Plant Health and Biotechnology, and Animal Health Directorate, Animal Import/Export Division (2013-06-18)

Draft #2: The draft risk assessment was released for editing (2013-07-24)

Draft #3: External peer review (2013-08-09)

Final document: Document released to the Terrestrial Animal Health Directorate, Import/Export Division (2013-09-23)

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RISK ASSESSMENT REQUEST

Name of requester: Dr. Connie Rajzman
Date Submitted: March 5, 2013

TITLE: Importation of Honeybee Packages from the United States of America

Prioritization of Request:

Top Priority	High Visibility Issue/ Urgent Immediate Need	☒
Very High Priority	Active Trade Issue/ Could Have a Direct Impact on Trade	☐
High Priority	New Commodity or Origin/ New Emerging Disease/ New Policy	☐
Regular Priority	Policy Revisions, Updates, Disease Status Reviews	☐
Low Priority	Others	☐

Type of service requested: Include mitigating measures ☐

Risk Assessment	
Full Risk Assessment of commodity or activity	☒
Integrated Risk Assessment (with Interdepartmental Collaborations)	☐
Scientific Evaluation (including Literature Review)	☐
Hazard identification	☐
Disease Status Evaluation	☐
Review of National & International Standards	☐
Fact Sheet	☐
Others (please specify):	☐

BACKGROUND INFORMATION:

History, background and rationale of the request
A risk assessment was done on this commodity in 2003, there is a need to have it updated. Requests for import permits continue to be received by the CFIA.

Description of commodity or activity to be assessed

- honeybee packages from the United States of America.
- packages are shipments of worker bees in 2 to 3 pound packages containing 20,000 or more bees.

Volume, quantity and frequency of commodity or activity

Unknown. Importation has been prohibited since 1987. The importation of queen bees was allowed again in 2004 however packaged bees remained prohibited.

Time-frame associated with commodity or activity

Immediate need.

WHAT IS YOUR SPECIFIC QUESTION FOR THIS REQUEST?

To assess the likelihood that imported honeybee packages from the USA, according to Section 160 of the Health of Animals Regulations, would not, or would not likely to, result in the introduction into Canada, or the spread within Canada, of a vector, disease or toxic substance.

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LIST OF ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Service
AFB	American foulbrood
AHB	Africanized honey bee
AHRA	Animal Health Risk Assessment unit
CCD	Colony collapse disorder
CFIA	Canadian Food Inspection Agency
EFB	European foulbrood
EHB	European honey bee
HBTM	Honey bee tracheal mite
mrVAR	Varroa mite resistant to coumaphos,[1] fluvalinate[2] and amitraz[3] (multi-resistant)
OIE	World Organisation for Animal Health
OTC	Oxytetracycline
rAFB	Oxytetracycline-resistant American foulbrood
rVAR	Acaricide-resistant varroa mite
SHB	Small hive beetle
U.S.	United States of America
USDA	United States Department of Agriculture
VAR	Varroa mite

[1] The commercial name of coumaphos is CheckMite+®.
[2] The commercial name of fluvalinate is Apistan®
[3] The commercial name of amitraz is Apivar®.

GLOSSARY

Africanized honey bee	It is a hybrid variety of the European honey bee (EHB) (*A. mellifera*) produced by cross-breeding of two subspecies of the western honey bee in Brazil with the African bee (*A. mellifera scutellata)* that subsequently spread into South, Central and North America.
Attendant	One of several worker bees that attend to the needs of the queen bee. Usually five or six attendants are shipped with each caged queen bee.
Brood	The eggs, larvae and pupae that will develop into adult bees.
Cluster	Tight formation of a group of bees that overwintering bees use to maintain their body temperature. Bees consume stored honey, which is converted by the bees into heat. The temperature inside the cluster is maintained between 20°C and 30°C.
Drifting bee	Foraging bee that inadvertently enters a hive other than its own. Generally, bees loaded with pollen and nectar are allowed to enter a hive unchallenged even if they are not a member of that hive. Drifting is more common when hives are placed closely together in apiaries.
European honey bee	Also named Western honey bee, the honey bee, *Apis mellifera*, found throughout the Western world though it is originally thought to be near Eastern in origin. This bee is carried from Europe to all areas around the world.
Package	Shipment of worker bees consisting of a queen bee and two or three pounds of worker bees packaged together. Each pound represents about 3,500 bees.
Pest	Unwanted organism that may be a parasite, disease pathogen, predator or insect pest. The term *pest* is used in this document as a generic term to refer to any of these living organisms.
Robber bee	Bee engaging in foraging behaviour in which bees from one hive collect the nectar and honey stores from another hive. In general, weak hives are more likely to be robbed, because they are poorly defended.
Swarming	Reproduction at the level of the colony. Swarming occurs when the queen bee leaves a colony with a large group of worker bees (in a swarm) to form a new honey bee colony.

BACKGROUND

CURRENT CONTEXT

Canada

In Canada, the nature of commercial beekeeping operations differs across the country, with smaller operations that often have fewer than 100 hives[4] being more common in Quebec, Ontario, British Columbia and the Maritimes, in contrast with the larger beekeeping operations in Alberta, Manitoba and Saskatchewan, which typically have many thousands of hives per operation. Large commercial honey bee (*Apis mellifera*) operations (>300 colonies) account for a small proportion of honey bee farms (13%) but own around 83% of honey bee colonies (Melhim et al., 2010).

Canadian beekeepers produced 90.9 million pounds of honey in 2012, a 13.8% increase from 2011. The province of Alberta is the top producer of honey in Canada, with 40.5 million pounds (Canadian Honey Council, 2012; Statistics Canada, 2012). Across Canada, some beekeepers specialize in honey production, others specialize in delivering pollination services, and many beekeepers combine both activities. In 2011, the total value of the honey produced in Canada amounted to $151 million, up 4.5% from 2010. The value of pollination services by honey bees accounts for an estimated $1.3 to $1.7 billion annually (Melhim et al., 2010).

In Canada, beekeepers are supported by professional apiarists who belong to the Canadian Association of Professional Apiculturists (http://capabees.org). The members of that association are employed by universities, colleges, or provincial or federal governments in the field of apiculture. Provincial governments have legislative and regulatory authorities and programs in place to manage and control the spread of bee diseases, in close collaboration with the Canadian Food Inspection Agency (CFIA).

Currently, bee packages (each consisting of a queen bee and two or three pounds of worker bees packaged together) can only be imported, under import requirements, from Australia, New Zealand and Chile. Although the use of bee packages is a good approach to start new colonies, packages are also a direct way to transport many pathogens, diseases and parasites. The quality of bee packages is highly dependent on that of the colonies of origin and on the producers' management practices. In 1987, in response to the outbreak in the United States of America (U.S.) of two parasitic mites (the honey bee tracheal mite [HBTM], *Acarapis woodi*; and the varroa mite [VAR], *Varroa destructor*), the Canadian federal Department of Agriculture closed the border to the importation of honey bees from the continental U.S. In 1993, the CFIA allowed honey bee queen imports from Hawaii (CAPA, 1994). Following the CFIA's 2003 risk assessment, the Agency allowed the importation of honey bee queens from the U.S. under specific conditions to help strengthen the genetics of Canada's domestic bee population and meet industry`s needs. The importation of package bees from the U.S. continues to be prohibited.

[4] From now on, the terms *hive* and *colony* will be used interchangeably to refer to honey bee colony.

In 2012, Canadian beekeepers imported $2.5 million worth of package bees ($2.1 million from New Zealand and $400,000 from Australia), $500,000 less than in the previous year. Canadian beekeepers imported 198,000 queens, worth $3.9 million, in 2012, about the same quantity as in the previous year. Of those queens, 170,000 (86%) came from the U.S. (Hawaii and continental U.S.), 18,000 (9%) came from Australia, 8,000 (4%) came from New Zealand, and 2,000 (1%) came from Chile. Demand is driven primarily by the level of colony losses experienced in the preceding year.

Since the winter of 2006–2007[5], Canadian beekeepers have experienced significantly elevated overwinter losses. National average winter losses, which had typically claimed about 15% of all hives, rose to about 35% in 2006–2007, with rates, ranging between 20% to 30%, the following years, before returning to 15.3% in 2011–2012 (CAPA, 2012a). In 2013, overall winter losses were 28.6% ranging from 17%- 46.4% (CAPA, 2013).

United States of America

In the U.S., there were 2.62 million colonies[6] producing honey in 2012, a slight (5%) increase over 2011 (2.49 million), and the number of beekeepers is estimated at 115,000 to 125,000. Of those colonies, over 2 million are reported to belong to commercial migratory beekeepers, who move their colonies from state to state and rent them each year to pollinate agricultural crops (National Honey Board, 2013). Honey production from producers with five or more colonies totalled 147 million pounds, down 1% from 2011. Yield per colony averaged 56.1 pounds, down 6% from 59.6 pounds in 2011 (USDA-NASS, 2013). The package bee[7] industry is based mainly in the southern part of the U.S. and in California.

The emergence in the U.S. and other countries of colony collapse disorder (CCD),[8] defined as a high number of colony losses and die-offs, has attracted a great deal of public attention. The cause is still unclear, but it is generally agreed that colony collapse disorder is not the result of a single culprit (Johnson, 2010).

For the U.S. beekeeping industry, the key issues are the distribution and prevalence of honey bee diseases in the U.S., the extensive migratory beekeeping industry, the overwintering of colonies in the southern part of the U.S., the lack of interstate movement controls, and the absence of a honey bee management program at the national level and in many states.

[5] Some provinces may have experienced high rates of winter kill prior to that period (Currie et al., 2010).
[6] Colonies producing honey in more than one state were counted in each state where the honey was produced (USDA-NASS, 2013).
[7] In the selling bee industry, colonies are split and sold in the early spring as nuclei (4-5 or more frames of bees and a queen per nucleus) or worker bees collected and packaged for sell with a caged queen. The method of assembling packages can vary depending on the producer, but package bees are typically obtained by shaking many colonies into a large mesh cage, creating a mixture of workers from many colonies. Then two or three pounds of bees are transferred into a smaller package with a young mated queen (Strange et al., 2008; MAAREC, 2012).
[8] Colony collapse disorder is characterized by a rapid loss of adult worker bees, few or no dead bees found in the hive, the presence of immature bees, a small cluster of bees with a live queen present, and pollen and honey stores in the hive not touched by scavenger pests (e.g.: wax moth or small hive beetle).

RISK ASSESSMENT

1 INTRODUCTION

1.1 Scope of the Risk Assessment

The Animal Health Risk Assessment (AHRA) unit of the CFIA conducted a risk assessment to provide scientific information and advice in support of the Canadian National Animal Health Program for the development of import policy. The CFIA's Animal Import/Export Division asked the AHRA to update and assess the likelihood of biological hazards spreading and/or becoming established in Canada and their likely consequences as a result of the importation of honey bee (*A. mellifera*) packages from the U.S. (AHRA, 2003).

The following is the taxonomic classification for the commodity of interest; the honey bee, *Apis mellifera* (Bee-info, 2013; ITIS, 2013a; Wikipedia, 2013):

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Hymenoptera
Family:	Apidae
Subfamily:	Apinae
Tribe:	Apini Latreille, 1802
Genus:	*Apis* Linnaeus, 1758
Species:	*mellifera* Linnaeus, 1758 (honey bee)[9]

1.2 Methodology

This qualitative risk assessment is based on the approach recommended by the World Organisation for Animal Health (OIE) and consists of the characterization of hazards with entry, exposure and consequence assessments. The qualitative assessment includes the likelihood of the introduction of the hazards into Canada with the importation of honey bee packages from the U.S. (entry assessment); the likelihood of potential hazards spreading and/or becoming established within the domestic honey bee population in Canada (exposure assessment); and the expected magnitude of the resulting consequences (consequence assessment). The likelihood definitions and risk estimates are provided in Appendix 1.

[9] From now on, the terms *honey bee* and *bee* will be used interchangeably to represent the honey bee species, *Apis mellifera*, unless it is necessary to distinguish *A. mellifera* from other bee species.

1.3 Data Systems Searched and Key Words Used

A literature review was performed using the PubMed, ScienceDirect and Apidologie websites. An Internet search was also conducted using the Google and Google Scholar search engines. The following key words were used, alone or in combination:
Honey bee, Bee, Apis mellifera, Nosema, Diseases, Canada, United States, Small hive beetle, Varroosis/varroa, Paenibacillus larvae, American foulbrood, Resistant, Africanized honey bee, Colony collapse disorder (CCD), Tropilaelaps mites, Apocephalus borealis, Import, Oxytetracycline resistance, Miticide/acaricide resistance, Winter mortality, Viruses

In addition, specific websites of interest were explored, including those of the OIE, ProMED-Mail, and the United States Department of Agriculture (USDA).

1.4 General Approach

A literature review was conducted to determine disease agents and pests that could be considered hazards based on the following inclusion criteria:

- The disease or pest is notifiable to the OIE, and/or reportable and/or notifiable in Canada as per the *Health of Animals Act* and *Regulations* (CFIA, 2012);
- The agent is present in honey bees in the U.S.;
- The disease agent or pest (or strain) is not present in Canada, and/or control and eradication programs exist; and/or
- The disease agent represents a public health concern with economic consequences.

The hazard identification process assessed the biological disease agents and pests that could potentially be introduced with a commodity or activity and for which pathways exist for exposure of the agents or pests to susceptible animals and humans. In Canada, provincial governments have legislative and regulatory authorities and programs in place to manage and control the spread of bee diseases, in close collaboration with the CFIA. Each disease agent or pest was assessed based on the current disease status in Canada and the existence of provincial control programs. Information on provincial legislative controls for honey bee diseases is compiled and summarized in Appendix 2.

In order for a hazard to be introduced into domestic and feral honey bee populations in Canada as a result of the importation of honey bee packages from the U.S., the following main elements of an entry and exposure scenario pathway must occur:

Entry (release) pathway:

- The agent must be present at a high enough prevalence in the bee hives of the exporting country;
- The honey bees must have direct or indirect contact with infected individuals and/or pests and/or vectors;
- The infection must be transmitted to honey bee workers within the bee hives; and
- Infected honey bees (infected packages) must be imported.

Exposure pathway:

- Infected honey bees must come into contact with Canadian domestic and wild honey bees;
- The dose of the agent must be sufficient to establish infection; and
- The infection must be transmitted to honey bees and honey bee hives.

The consequence assessment describes the likelihood of a hazard to be established within the honey bee population as well as the hazard's causal effect on bee colony health, which may in turn lead to economic consequences.

The starting point for this document was the CFIA's Risk Assessment on Honey Bees from the United States (AHRA, 2003).

1.5 Likelihood Definitions

In this qualitative risk assessment, the following terminology will apply (*see Appendix 1* for more details):

Table 1 – Likelihood Definitions

Likelihood Definitions	
Negligible	The event would be virtually unlikely to occur.
Extremely low	The event would be extremely unlikely to occur.
Very low	The event would be very unlikely to occur.
Low	The event would be unlikely to occur.
Small	The event would be minimally likely to occur.
Moderate	The event would be fairly likely to occur.
High	The event would likely occur.

2 UNCERTAINTIES AND RESEARCH GAPS

2.1 Uncertainties

The uncertainties that were identified during the course of this assessment include:

- *Geographical location*: Since there is an extensive migratory beekeeping industry, there is a lack of information on the traceability of origin in the U.S.; the geographical transmission of honey bee diseases across the country is accentuated.
- *Honey bee disease health status*: There is no honey bee program at the national level; there is no detail information on the presence and distribution of bee viruses; there is no information on the health status, presence and distribution of feral honey bee colonies in both countries.
- *Epidemiology of disease vectors*: There is uncertainty regarding the ability of some pests to adapt to climatic conditions specific to Canada (e.g.: cooler conditions).
- *Volume of bee packages imported*: There is uncertainty regarding the number of bee packages that would potentially be imported from the USA.

In this qualitative risk assessment, a probability range was used to represent the level of uncertainty. Such uncertainties need to be taken into account when making a decision.

2.2 Research Gaps

The research gaps that were identified during the course of this assessment include:

- Epidemiological data on the prevalence and distribution of honey bee diseases and pests from Canadian provinces and U.S. states;
- Surveillance data to determine the proportion of hives affected with pesticide- and/or antimicrobial-resistant pests;
- Scientific data and additional information on feral colonies, specifically in areas close to the Canada–U.S. border;
- Studies on the ability of the small hive beetle (SHB) and Africanized honey bee (AHB) to survive during winter in northern areas; and
- Geographical studies on the phorid parasitism of honey bees in North America, and measurements of the effect of various densities of phorid parasitism on hive health.

3 ASSUMPTIONS

The following assumptions were made and have influenced the results:

- Given the extensive migratory beekeeping industry and the lack of interstate movement controls, it was assumed that the hazards identified were widely distributed across the U.S. in domestic and feral honey bee populations; and
- It was assumed that the prevalence of diseases and pests, particularly pesticide- and/or antimicrobial-resistant pests, was higher in migratory hives.

4 HAZARD IDENTIFICATION

Hazard identification is the process of identifying the biological agents that could potentially be introduced with a commodity or activity and for which pathways exist for exposure of the agents to susceptible animals and humans.

Table 2 – Hazards Associated with Honey Bee Packages from the U.S.

Diseases/Pests	Agents	Diseases Listed		Occurrence		Hazard
		OIE[1]	CFIA[2]	Canada	U.S.	
Small hive beetle	***Aethina tumida***	**Y**	**IM**	**Sporadic/ limited distribution**	**Clinical disease**	**Yes**
Varroosis (varroa mite)	***Varroa destructor*** **(acaricide-resistant)**	**Y**	**IM**[3]	**Clinical disease**	**Clinical disease**	**Yes**
Acarapisosis, or tracheal mite infestation	*Acarapis woodi* (tarsonemid mite)	Y	AN	Clinical disease	Clinical disease	No
American foulbrood	***Paenibacillus larvae*** **(oxytetracycline-resistant)**	**Y**	**AN**[4]	**Sporadic/ limited distribution**	**Clinical disease**	**Yes**
European foulbrood	*Melissococcus plutonius*	Y	AN	Clinical disease	Clinical disease	No
Tropilaelaps infestation ****	*Tropilaelaps clareae*, *T. koenigerum*, *T. thaii* and *T. mercedesae*	Y	Not listed	Never reported	Never reported	No
Nosematosis	*Nosema apis* and *N. ceranae*	N	AN	Worldwide	Worldwide	No
Africanized honey bee ****	**Hybrid varieties of *A. mellifera***	**N**	**Not listed**	**Never reported ***	**Limited distribution ******	**Yes**
Parasitic fly	*Apocephalus borealis*[5]	N	Not listed	Never reported	Limited distribution	No
Bee viruses	18 viruses have been identified***	N	Not listed	Present (or unknown)	Present (or unknown)	No

Source: This information is based on the OIE report available for January to June 2012 (OIE, 2012b)

[1] Y = yes, N = no.
[2] IM = immediately notifiable, which are diseases that are primarily foreign to Canada and for which there are no control or eradication programs; laboratories are required to notify the CFIA. AN = annually notifiable, which are diseases present in Canada for which no federal program exists; data is being collected primarily to meet Canada's international obligations for surveillance as well as for public health purposes.
[3] Fluvinate-resistant Varroa mite is immediately notifiable.
[4] The American foulbrood is annually notifiable but resistance to oxytetracycline is not notifiable.
[5] Potentially a new emerging issue (more research needs to be conducted to understand its epidemiology).
* Disease never reported in Canada (Canadian Honey Council, 2013).
** Distribution of AHB (USDA-ARS, 2011) (*see Appendix 3*).
*** Chen & Siede (2007).
**** Listed in several provinces Bee acts and associated regulations

4.1 Diseases, Biological Agents and Pests Not Considered to Be Hazards

- Because they are either absent in the exporting country or present in both the exporting and importing countries, and because they are not under official control in Canada, acarapisosis, European foulbrood (EFB), nosematosis and *Tropilaelaps* infestation are not considered to be hazards associated with importation of honey bee packages from the U.S.

- *Nosema ceranae* has been reported in Canada and the U.S. for many years, and it is now considered to have spread worldwide (Pernal et al., 2007; Chen et al., 2008; Williams et al., 2008; Currie et al., 2010; Runckel et al., 2011).

- *Apocephalus borealis* is a species of North American phorid fly[10] that is known to parasitize bumblebees and paper wasps and has recently been observed attacking honey bees in a limited area[11] of the U.S. (Core et al., 2013). There are uncertainties regarding the geographical distribution, epidemiology and expression of this novel characteristic (host shifting). This phenomenon has not been reported in Canada. It is considered by some experts to be an opportunistic infestation, as it evolved to parasitize other species (*Personal communications with subject matter experts (SME)*, April 2013). It could represent an emerging hazard; however, based on the current limited scientific information, it was not considered a hazard for the purpose of this risk assessment.

- Many viruses have been reported to infect honey bees worldwide (Chen & Siede, 2007). There are uncertainties regarding the presence and distribution of viruses in both countries. Some of them, such as deformed wing virus, black queen cell virus, Israeli acute paralysis virus, sacbrood virus, Kashmir bee virus, chronic bee paralysis virus, and acute bee paralysis virus, are known to be present in Canada and the U.S. (Chen et al., 2005, 2006; Williams et al., 2009; Currie et al., 2010; Runckel et al., 2011). Due to their presence and the absence of official control in Canada, these viruses were not considered to be hazards.

- The colony collapse disorder has been reported in the U.S. and other countries and it is defined as a high number of colony losses and die-offs. The cause of the CCD is still unclear. It is generally agreed that CCD is not the result of a single culprit; many pests, pathogens, environmental or management factors have been suggested to act individually or in combination to impact colony health. Due to the fact that there is no specific biological agent identified, CCD was not considered a hazard for this risk assessment.

[10] Distribution of the species has been reported in northern areas (Brown, 1993).
[11] The geographical distribution of the infection of honey bees by this fly has been mapped (ZomBee Watch, 2012).

4.2 Diseases, Biological Agents and Pests Considered to be Hazards

- Africanized honey bee, antibiotic-resistant American foulbrood (AFB, resistant to oxytetracycline [rAFB]), SHB and amitraz-resistant VAR (acaricide-resistant [rVAR]) are hazards of concern associated with the importation of honey bee packages from the U.S.

- Although there are currently no federal control programs for any of these hazards, in Canada, legislative controls for honey bee diseases and pests do exist at the provincial level. Although AHB is not reported in Canada, it is considered a biological hazard for the bee industry in Canada with economic impacts and represents a public health concern.

  Note: Based on the current scientific evidence, the outcomes of the updated hazard identification did not change from the previous risk assessment in 2003 (AHRA, 2003).

4.2.1 Africanized Honey Bee

(For additional information, see AHRA, 2003)

- Africanized honey bee (AHB) is a hybrid variety of the European honey bee (EHB) (*A. mellifera*) produced by cross-breeding of two subspecies of the western honey bee in Brazil with the African bee (*A. mellifera scutellata)*.

- The hybrid progenies have expanded into most of South, Central America and Mexico, reaching as far as the southern U.S. and subtropical parts of the Americas. The northernmost areas that AHB reaches are below the 34°N latitude in the state of Nevada (1999). The progression of the annual migration of AHB in the U.S. is continually monitored and mapped (USDA-ARS, 2011) (*see Appendix 3*).

- These hybrids have retained most of the unfavourable traits of their African ancestors, such as swarming more frequently, being more likely to abandonment the colony (abscond) as a response to stress, having greater defensiveness in comparison with other honey bees, living more often in ground cavities, deploying in greater numbers for defence, and pursuing perceived threats over much longer distances from the hive.

- Africanized honey bee has never been detected in Canada. It is named under provincial legislation in Alberta, Saskatchewan, Ontario, Quebec, New Brunswick, Nova Scotia and Prince Edward Island (*see Appendix 2*).

- Because it exhibits highly defensive behaviour, AHB presents a threat to public and animal health as well as to the Canadian beekeeping industry because of the significant impact on productivity and potential trade issue with live honey bee material. The introduction of AHB into Canada may necessitate changes to some established management practices.

Africanized Honey Bee

Given that AHB is distributed in most of the southern states, has never been detected in Canada, is named under the provincial legislation in most Canadian provinces, and represents a threat to public and animal health with economic consequences for the honey bee industry, AHB is considered a hazard.

4.2.2 American Foulbrood

(For additional information, see AHRA, 2003; OIE, 2012a, 2013.)

- American foulbrood is a worldwide bacterial disease of the larval and pupal stages of *A. mellifera* and other *Apis* species. Caused by the spore-forming, Gram-positive bacterium *Paenibacillus larvae*, AFB can produce over a billion spores in each infected larva (Lindström et al., 2008). The spores remain infectious for more than 35 years and withstand heat, cold, draught and humidity. The lethal dose (LD_{50}[12]) for day-old larvae is 35 spores (Shimanuki et al., 1992).

- Adult honey bees become contaminated with spores as they perform housecleaning duties to remove dead larvae and scales. The adults may then infect larvae directly through the feeding process or indirectly through the contamination of honey, which is later fed to larvae. The spores spread the disease when wax or queens are transferred or when combs or contaminated honey is exchanged. American foulbrood may also be spread through robber bees that carry honey contaminated with spores and act as potential vectors of the disease, or by beekeepers through the feeding of honey and pollen from infected hives or the use of infected tools or hive equipment (Pernal & Melathopoulos, 2006; Lindström et al., 2008). Swarms originating from clinically diseased colonies may represent an efficient transmission route (Genersch, 2010).

- Combs of infected apiaries may show distinctive clinical signs. Larvae change colour from white to brown as they start to degenerate. Eventually, a small black scale firmly attaches to the brood cell (and contains millions of spores). In infected hives, the colony has a mottled look owing to empty cells, there may be a typical smell, and the brood is slimy. Subclinical infections are common and require laboratory diagnosis.

- Treatment with antibiotics will destroy the vegetative bacteria but will not kill the spores. The use of antibiotics may mask signs of disease in some hives. Burning the hives and the contaminated equipment is often recommended to destroy the spores. Oxytetracycline (OTC) has been the only effective approved antibiotic for many years (Spivak & Reuter, 2001). Tylosin tartrate was approved in 2005 in the U.S. and recently in Canada for the treatment of AFB. Residue in honey is more of a concern with the use of tylosin tartrate than OTC.

- American foulbrood occurs in the continental U.S. and Canada; however, strains resistant to OTC (rAFB) have been widely reported in the U.S. (Miyagi et al., 2000; Feldlaufer M[13] quoted in Bren, 2002; Evans, 2003). In Canada, rAFB has been reported sporadically and in limited areas, with the exception of Alberta, where it is considered widespread.

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[12] The LD_{50} is the dose at which 50% of the larvae will die.
[13] Research Chemist, Bee Research Laboratory, USDA, Beltsville, MD, U.S.

- American foulbrood is on Canada's annually notifiable list of diseases. AFB and/or rAFB is a named disease in all provinces (except Newfoundland), which have control programs in place and/or provide pest management strategies (*see Appendix 2*).

Table 3 – Oxytetracycline-Resistant American Foulbrood in Canadian Provinces

Province	**Current Situation**
British Columbia[1]	1998: first case reported Few incidental findings in early 2000s No other cases reported
Alberta[2]	1999: first case reported 2001: 5% of honey bee operations were positive 2010: 10% of honey bee operations were positive Distribution: widespread
Saskatchewan[3]	2009: first case reported Currently five operations have tested positive Distribution: limited
Manitoba[4]	2003: first case reported 2004: three more operations in the same areas 2005: total of 10 operations in limited areas 2012: two operations tested positive Distribution: limited
Ontario	Never reported
Quebec[5]	2007: first case isolated from colonies without clinical signs Not reported since 2007
New Brunswick[6]	2006: first case reported; one operation tested positive No reports of resistance since 2006
Nova Scotia[7]	2006: first case reported; one operation tested positive Not reported since 2006
Prince Edward Island[8]	2006: first case reported; one operation tested positive Not reported since 2006

Source: Provincial apiarists, May 2013.

[1] The use of OTC in combination with management practices has been found to be very effective against AFB.
[2] No quarantine or movement controls are in place; tylosin is used.
[3] With one exception, all cases were contained in the same area, representing approximately 10% of all colonies in the province. Movement controls are applied on the rAFB cases currently reported. The cases appear to be connected.
[4] Operations that tested positive were placed under quarantine and movement controls. Colonies were treated with tylosin, and equipment was irradiated. In 2004, an epidemiological link was established with previous cases. In 2005, six out of seven operations in the same area as previous years were connected. Restrictions are applied to the positive operations.
[5] Since 2005, each strain of AFB isolated has been tested for OTC resistance.
[6] The positive colony was burned, and other colonies in the apiary were placed in quarantine and treated with tylosin.
[7] The colony was destroyed, and equipment was irradiated. Testing has been done yearly and found no occurrence of rAFB. Additional testing was done and found no other positive cases.
[8] All colonies were quarantined, placed under movement controls and destroyed. Additional testing was done and found no other positive cases.

American Foulbrood

In the U.S. honey bee population, the AFB agent, *P. larvae*, has demonstrated variable levels of resistance to oxytetracycline treatment. Although AFB is present in Canada, rAFB has only been reported sporadically since the late 1990s, in limited areas. Most Canadian provinces have control programs in place.

The prophylactic use of oxytetracycline to manage AFB disease is significantly compromised by the rAFB strains. Therefore, the AFB agent that is resistant to antibiotic (oxytetracycline) is considered a hazard.

4.2.3 Small Hive Beetle

(For additional information, see AHRA, 2003; OIE, 2012a, 2013.)

- In SHB infestation, honey bee (*A. mellifera*) colonies are infested by the beetle *Aethina tumida*. *Bombus* species are also considered to be susceptible. Small hive beetle was introduced into the U.S., Canada and Australia by the commercial movement of bees.

- Small hive beetle has a high reproductive potential. Each female can produce about 1,000 eggs in its four to six months of life. After two to six days, the eggs hatch and the emerging larvae begin to feed voraciously on brood comb, bee eggs, pollen and honey in the hive. At maturity (approximately 10 to 29 days after hatching), the larvae exit the hive and burrow into the soil around the hive entrance. Adult beetles emerge after an average of three to four weeks, although pupation can take between 8 and 60 days depending on temperature and moisture levels.

- The life span of adult SHB depends on environmental conditions, such as temperature and humidity. Beetles reach sexual maturity in about seven days, and adults can survive for up to 190 days if fed on honey and pollen. Temperatures between 15°C and 45°C and humidity levels higher than 34% are required for larval development and pupation (Annand, 2011, and Meikle & Patt, 2011, cited in Cuthbertson et al., 2013). Adult beetles become inactive when temperatures fall below 20°C (Wenning, 2001). Hence, once SHB is established within a localized environment, it is extremely difficult to eradicate (Hood, 2004; Annand, 2008).

- The adult beetle is attracted to bee colonies. Odours from various hive products (honey and pollen) and adult bees are very attractive to flying beetles (Elzen et al., 1999a). To spread, an infestation does not require contact with adult bees. However, the movement of adult bees, honeycomb and other apiculture products and used equipment associated with beekeeping may all cause infestations to spread to previously unaffected colonies. In addition, there is scientific evidence that SHB can act as a potential vector of deformed wing virus in honey bee and *Paenibacillus larvae*, the agent of AFB (Eyer et al., 2009; Schäfer et al., 2010).

- Small hive beetle is not notifiable in the U.S. The pest was first introduced into South Carolina in 1996 and was then detected in Florida in June 1998. It has been found and become established in most of the states on the East Coast, along the Canadian border, on the West Coast (in Oregon), and in the central U.S. (in Iowa, Arkansas, Kansas and Oklahoma), and was also detected in Hawaii (NAPIS, 2013b). It is assumed to be widely spread in the U.S. (Ellis & Ellis, 2010).

- Small hive beetle is an immediately notifiable disease in Canada and is a named disease under provincial legislation in Alberta, Saskatchewan, Ontario, Quebec, New Brunswick, Nova Scotia and Prince Edward Island. It is not named in British Columbia, Manitoba or

Newfoundland and Labrador, but those provinces have the authority to control movements of bees and equipment (*see Appendix 2*).

- Historically, SHB has been reported only a few times in Canada: in 2002 (Manitoba, in unprocessed beeswax) and in 2006 (Manitoba and Alberta). Recently, there have been a few sporadic and controlled outbreaks in Quebec, Ontario[14] and Manitoba in southern areas close to the Canada–U.S. border. Control measures applied by the provincial authorities have been able to prevent the spread of SHB. Infested hives have been either destroyed or kept under quarantine and subjected to movement control measures.

Small Hive Beetle

Small hive beetle is notifiable to the OIE and immediately notifiable in Canada as per the *Health of Animals Act* and *Regulations*. The agent is present in honey bees in the U.S. and in Canada; however, in Canada, SHB has been reported sporadically and in limited areas since 2002. Most Canadian provinces have control programs in place. Therefore, SHB is considered a hazard.

[14] Information about the quarantine area, surveillance and control zones can be found at: http://www.omafra.gov.on.ca/english/food/inspection/bees/12rep.htm#small

4.2.4 Varroa Mite

(For additional information, see AHRA, 2003; University of Georgia College of Agricultural and Environmental Science, 2012; OIE, 2012a; 2013; FERA, 2013)

- Varrosis is caused by a mite (*Varroa destructor*) capable of devastating honey bee colonies. The native host of *V. destructor* is the Asian honey bee (*Apis cerana*). Unlike that species, *A. mellifera* has limited or no natural defences against VAR. *Varroa destructor* has become a serious pest of *A. mellifera* around the globe.

- Varroa mites are external honey bee parasites that attack both the adults and the brood, with a distinct preference for drone brood. These mites suck the hemolymph from both the adults and the developing brood. It is generally agreed that VAR is playing a role in winter colony mortality. The mite can also act as a vector for viruses of honey bees (e.g. deformed wing disease and acute paralysis virus).

- Varroa mites spend most of their life cycle within the brood cells of maturing bee pupae. Once a pupa has developed into an adult bee and emerged from the brood cell, the mites spend short periods of time on adult bees until they infect to maturing bee larvae before cell capping to initiate another reproductive cycle. If left untreated, an infested colony will usually die within two to three years.

- The life span of an individual mite depends on temperature and humidity and can extend up to 150 days or more. In winter, VAR overwinter solely on the bodies of adult bees, until brood rearing commences the following spring. Mites cannot survive more than a few days without bees to feed on (e.g. on combs or equipment). The infestation spreads by direct contact from adult bee to adult bee, drifting bee and by the movement of infested bees and bee broods to other bee colonies.

- There is no chemical treatment with 100% effectiveness. The mites have developed resistance to some synthetic miticide agents, which could be caused by the overuse or misuse of products and mismanagement of the disease. Cross-resistance to multiple synthetic miticides has been reported. Amitraz is currently the only approved synthetic miticide that is effective against fluvalinate- and coumaphos-resistant strains of VAR in Canada.

- Varroa mite occurs in both the U.S. and Canada. The U.S. National Honey Bee Pests and Diseases Survey has been conducted since 2009 and has estimated the prevalence of VAR in the U.S. for 2009, 2010 and 2011 at 87.1%, 92.4% and 91.8%, respectively (at least one mite per 100 bees) (USDA-APHIS, 2013a).

- In the U.S., several reports have indicated that VAR has developed resistance to chemical treatments:
  - Fluvalinate-resistant VAR is widely distributed in the U.S. (Elzen et al., 1999b).

- In 2002, four years after the registration of the use of coumaphos, mites resistant to coumaphos were reported in Florida, Maine and New Jersey (MAAREC, 2002; Pettis, 2004).
- Amitraz has been incorporated into management practices in commercial beekeeping operations (Runckel et al., 2011). Amitraz resistance has also been observed in the honey bee mite population in the U.S. (Elzen et al., 2000b).
- It could be reasonable to assume that some mites are resistant to amitraz (rVAR) but the prevalence is still unknowns (*Personal communications with SME,* September 2013).
- There have been reports that fluvalinate, coumaphos and amitraz resistance is spreading throughout the U.S. (in Arizona, California, Florida, Maine and North Dakota) and that cross-resistance may also occur (Sammataro et al., 2005).

• In Canada, fluvinate-resistant varroa mite is a CFIA immediately notifiable disease. Strains resistant to fluvalinate and coumaphos are known to be present (Currie et al., 2010). However, amitraz-resistant VAR have not yet been reported in Canada (Vandervalk, 2013). Amitraz was made available to beekeepers in 2008, after an emergency use permit was delivered (CAPA, 2008; Canadian Honey Council, 2008).

• Provincial legislative controls for VAR, including resistant strains (rVAR), are in place in all provinces except Quebec (*see Appendix 2*).

Varroa mites (resistant strains)

Varroa mites are widespread in both the continental U.S. and Canada. In Canada, resistance to the miticide amitraz has not been reported, and there are provincial legislative controls in place in most provinces. In the U.S., mites resistant to fluvalinate, coumaphos and amitraz are present. There is an intense migratory beekeeping industry with no interstate movement controls on the honey bees. Therefore, amitraz-resistant VAR are considered a hazard.

5 RISK ASSESSMENT APPROACH

5.1 Factors Affecting the Entry Assessment

Entry assessment is a process that consists of describing the biological pathway(s) necessary for an importation activity to introduce pathogenic agents into a particular environment. Considerations include the disease situation in the exporting region, the health status of the premises and animals, and the pathogenesis of the disease agent.

- Based on the literature review, the risk factors listed in the CFIA's Risk Assessment on Honey Bees from the United States (AHRA, 2003) are still valid, as follows:
  - The highly migratory nature of the U.S. beekeeping industry, coupled with the need to maintain disease at very low levels to ensure strong hives for pollination, has resulted in increased exposure to diseases and increased levels of treatment (higher dose and multiple prolonged period of treatment), leading to increased resistance of parasites and diseases in the USA honey bees.
  - There is no national honey bee management program, along with no interstate movement controls. In the continental U.S., national management programs do not include surveillance or control programs for honey bee diseases and pests of interest to this assessment. Unless local surveillance and control programs indicate otherwise, it must be assumed that most honey bee diseases and pests of interest are widely distributed in the U.S. honey bee population.
    - Note: Since 2009, the USDA Animal and Plant Health Inspection Service (APHIS) has conducted a yearly national survey of honey bee pests and diseases with the objective of verifying the absence of exotic threats (*Tropilaelaps* mites, slow paralysis virus and *A. cerana*) and documenting which honey bee diseases, parasites and pests are present in the U.S. (USDA-APHIS, 2013a). The survey expanded from three states in 2009 to more than half of the states in 2011. Although limited, those surveys provide valuable information.
  - State inspectors, or in some cases municipal or county inspectors, are responsible for implementing state disease control and surveillance programs. The level of inspection and legislated controls vary from state to state (Somerville, 2003). It is unlikely that inspectors are able to obtain good coverage of hives in states where there is a great deal of migratory beekeeping. Interstate movement controls are limited or nonexistent so that hives may be moved quickly once a crop comes into bloom.
  - Given the extent of the migratory industry and the absence of movement controls, zoning of the U.S. for bee diseases would be very difficult.

- o In some instances, U.S. hives are located close enough to the Canadian border that diseases and pests could be spread naturally and/or through swarming and the normal foraging activities of bees. Cases of SHB in Canada in recent years have been associated with natural incursions.

- Package bees are produced in the southern U.S. states, where AHB established, and in California, areas where large numbers of bee colonies congregate for overwintering and pollinate almonds.

- The revised U.S. regulations for the importation of honey bees and other pollinator bees went into effect on November 22, 2004 (Electronic Code of Federal Regulations, 2013).

  - In the continental U.S., APHIS does not regulate the interstate movement of bees. In Hawaii, the interstate movement and importation of bees is prohibited.
  - Currently, the importation of honey bees (queens and packages) into the U.S. is allowed only from Canada and New Zealand (USDA-APHIS, 2013b).

- Number of bee packages that would potentially be imported from USA and their specific origin are unknown.

  - Annually, Canadian beekeepers import currently[15] approximately 40,000 honey bee packages (Australia, Chile and New-Zealand). Due to lower transportation costs and rapidity of obtaining the packages, it would be expected that the U.S. beekeepers will rapidly become an important supplier of bee packages. A higher demand for bee packages from the USA would result in an increase of the prices of packages, which could limit the importation from the USA.
  - Furthermore, considering the growing agricultural industry requiring pollinators, the fact that US beekeepers must replace their colonies due to high winter losses, the number of bee packages actually available may in fact be limited.

[15] Canadian beekeepers used to import up to 80,000 bee packages.

5.2 Factors Affecting the Exposure Assessment

Exposure assessment is a process that consists of describing the probability that humans or animals in Canada could be exposed to hazardous pathogens from the importation of honey bee packages from the U.S. This description is based on the probability of direct and indirect contacts and the probability of infectious contacts.

- Based on the literature review, the risk factors listed in the CFIA's Risk Assessment on Honey Bees from the United States (AHRA, 2003) are still valid, as follows:
  - Interprovincial movement controls and limited migratory beekeeping have been largely effective in slowing the establishment and spread of honey bee diseases and pests in Canada.
    - Interprovincial movements of bee colonies exist in few provinces. Bee inspections and provincial permits are required to move colonies between provinces. Bee colonies are moved because they are contracted for crop pollination, or for overwintering. There is uncertainty about the degree of hive movement in and between other provinces, although it is thought to be minimal: In Western Canada, interprovincial movement involves the transport of colonies from Alberta and Manitoba to southern British Columbia for overwintering. Before they are moved back to their respective provinces, Alberta's bees and to a lesser extent bees from Manitoba are used for contracted crop pollination in British Columbia. In Atlantic Canada, extra colonies are required for berry crops in northern New Brunswick and are contracted from Ontario (from which more than 14,000 colonies were contracted in 2011) (OMAFRA, 2011) and Québec. There is also movement of colonies from Ontario to Québec, and some movement from Ontario and Nova Scotia to Prince Edward Island.
    - According to the consultation conducted by Serecon Management Consulting Inc. (2012), 9% of the respondent beekeepers had rented their colonies at least once, with an average of 392 colonies having been rented (range from 1 to 10,000). Also, 29% of the respondents had moved their colonies at least one time in 2011 (average of 2 times). If colonies had been moved, the average distance reported was 121 km (range from 0.1 to 1,708 km), with commercial beekeepers moving their colonies over greater distances, averaging 231 km versus 45 km for hobby farms. Most of the beekeepers reported using their own truck to move their hives, transporting their hives at night, using entrance screens on hives or netting over the load to minimize bees flying away during transport.

- In Canada, provincial governments have authorities and programs in place to manage and control the spread of bee diseases, in close collaboration with the CFIA.
    - The legislative controls for honey bee diseases for each province are presented in Appendix 2. While the provinces have the authority to implement necessary measures to maintain the health of honeybees, there is variation regarding the rigour with which controls are applied in each province.

- The CFIA, in collaboration with producers, industry associations, academia and provincial governments, has developed the National Bee Farm-Level Biosecurity Standard (CFIA, 2013). The objective of the national standard is to provide a consistent, country-wide approach to the implementation of biosecurity practices for both small- and large-scale operations. The Biosecurity Standard forms the basis of a comprehensive voluntary program designed to provide practical guidance to prevent the introduction and the spread of pests in the three main Canadian bee sectors: honey bees, alfalfa leafcutting bees, and bumblebees (Serecon Management Consulting Inc., 2012).

- Provincial apiculture programs in Canada are focused on disease and pest prevention, pest surveillance, integrated pest management, education and awareness, and movement controls. As reported by CAPA (2012a), workshops were conducted to promote integrated pest management (IPM) practices to beekeepers with particular attention given to surveillance programs to monitor pests and diseases with emphases on *Nosema ssp.*, the small hive beetle and *Varroa*. Attention has also focussed on proper disease identification, monitoring pest population, winter management, rotation of treatments and discouraging misuse and off-label use of medications.

- Demand for bee packages is highest in Alberta, followed by Manitoba, British Columbia, and is very low in other provinces as reported by industry associations and provincial apiculturists (*Personal communications with SME*, May 2013). Alberta, Saskatchewan and Manitoba obtain most of their bee stock from sources outside of Canada, whereas Ontario, Quebec and the Atlantic provinces source their bees from their own regions (Serecon Management Consulting Inc., 2012).

- Health Canada's Pest Management Regulatory Agency is responsible for pesticide regulation in Canada. The import, sale or use of unregistered pesticides in Canada is a violation of the *Pest Control Products Act*, as is using a pesticide in a manner other than directed on the product label.
    - Health Canada is issuing fines to beekeepers for the illegal use of miticides (Health Canada, 2013).
    - Such fines may be incentive to producers to comply with the usage of miticides as recommended on the label and limiting the overuse and misuse of miticides.

5.3 Factors Affecting the Consequence Assessment

Consequence assessment is the process that consists of describing the relationship between specified exposures to a risk agent and the economic consequences of those exposures. It typically includes a specification of the impact on health in the animal and human populations sustained under given exposure scenarios. In other words, consequence assessment is the process of developing a description of the relationship between the specified exposures to a risk agent and health and other consequences for animals and humans that are exposed.

- A comprehensive cost analysis should be conducted to assess the impact of introducing bee diseases and pests into Canada. It should not only consider the various sectors of the bee industry, but also sectors such as: the agricultural industry relying on pollinators, public health, implementation of awareness and emergency plans, and outdoor recreational activities industry.

    - Such analysis would support the decision making process by providing the global impact on Canadian economic and health.

- Historically, Canada has enjoyed climatic conditions that ensure an enviable animal (including bees) health status. Harsh winter conditions prevent the entry and survival of pests and pathogen agents adapted to warmer climates.

    - Climatic conditions are different from conditions in Southern USA and to some extent from many of the states. There is also difference in conditions across the country. Colder temperature have prevented the establishment of some pests in bee populations, limiting it natural migration within Canada.
    - As the result of global warming, favorable ecological niches to some of those pests and pathogens will develop. Consequently to the importation of bee packages and natural migration, bee pests and pathogen will enter and will establish and spread in many areas across Canada.

- Research, development of new treatments and management strategies for controlling and limiting the impacts of the hazards will influence the magnitude of the consequence estimates.

5.4 Africanized Honey Bee

5.4.1 Entry Assessment

Africanized honey bee (AHB) is not an OIE-listed disease, and there are no recommendations for import conditions.

<u>Distribution of Africanized Honey Bees in the United States</u>

- Since its introduction into Texas in 1990, AHB has been monitored by the USDA (USDA-ARS, 2011). This program has followed the evolution of AHB spread using swarm baits. Over the years, AHB has slowly expanded its geographical range (*see Appendix 3*). To date, it has been found in Texas, Arizona, New Mexico, California, Nevada, Utah, Louisiana, Oklahoma, Arkansas, Alabama and Florida. Sporadically, the state of Georgia has been reported and eradicated few Africanised swarms, and is still officially AHB-free (*Personal communications with SME*, September 2013).

- There is uncertainty regarding the prevalence of AHB in American hives. In Florida, more than 70% of the feral colonies captured in swarm traps were Africanized colonies (Hall et al., 2013). In Arizona, the percentage of feral colonies exhibiting African DNA in 1999 was approximately 74% (Harrison et al., 2006). There is uncertainty with respect of number of bee packages that will be imported and their origin.

- The process of Africanization has been due to the interaction of several mechanisms including paternal genes from mating with African drones in managed colonies, and due to loss of maternal European genotypes in feral populations (Guzman et al, 2011). Regardless if colonies are managed or feral, the asymmetric gene flow allowed the Africanized bees to become successful invaders and spread fast.

- There is little information on the proportion of affected hives in areas where AHB has been reported. No information is available on the number of bee hives destroyed because of increased aggressiveness. Predicted range distributions have been modelled, and distribution is considered to be limited to the southern half of the U.S. (Schneider et al., 2004; Harrison et al., 2006; Vital et al., 2012).

- Migratory beekeeping in the U.S. could introduce African genes into previously unaffected areas and out of predicted range of distributions (as reported in some areas in Madera and Stanislaus Counties, California (*Personal communications with SME*, September 2013). However, most hives showing aggressive and/or defensive behaviour typical of AHB would likely be removed prior to transport, as beekeepers would not want to manage such bees. It is not easy to distinguish AHB from EHB. The Africanization of a colony can take place sometime prior to its detection.
    - Compared with the situation in 2003, AHB has expanded to more states. Based on the above, it is assumed that AHB is likely present in apiaries, or in surrounding areas, within feral populations in the southern U.S.

- It is unknown whether the low incidence of AHB reported in northern states is the result of environmental conditions that are not favourable for AHB survival, management practices, the lack of surveillance programs for reporting of cases, limited stays by migratory beekeepers who might have AHB, or some combination thereof. Migration of AHB into northern areas of the U.S. and into Canada is unlikely. Climatic and environmental conditions are likely to stop its progression toward northern areas, as conditions did in southern Argentina (Mistro et al., 2005; Schneider et al., 2004).

Africanized Honey Bees in Contact with European Honey Bees

- Package bees are produced mainly in the southern states and in California, at the end of the overwintering period. Many beekeepers move their colonies to California and southern parts of the U.S. for the winter. Some colonies may already have some level of hybridization.

- Because the overwintering season happens in the same geographical areas as the distribution of AHB, one can assume that there are greater opportunities for EHB to be exposed to AHB genetic material. An Africanized queen could potentially replace (take over from) an EHB queen in an existing colony, newly emerged EHB queens can mate with Africanized drones, and artificially inseminated queens could be fertilized with genetic material from Africanized drones. In these cases, queens will produce Africanized progenies (workers and queens). Africanized drones are produced by Africanized queens.

- Africanized honey bee genes and behaviour can be masked for several months while colonies develop and increase in size (Winston, 1992). This delay provides an opportunity for AHB drones and queens to be raised and to reproduce.
  - ➢ It is likely that some bees in the colonies would contain Africanized genetic material.

Africanized Honey Bees in Colonies and Packages

- Certifying populations free of AHB is an issue. The mitochondrial DNA and the morphometric analysis used to identify Africanization in samples of bees are not 100% reliable; mitochondrial DNA is maternally inherited,[16] and hybrids might not be detected through morphometric analysis because of the different degrees of hybridization (Guzman-Novoa, 2012).
  - ➢ No further developments in identification methods have occurred; testing will not accurately detect the presence of Africanized stock in bee populations or packages.

- If an AHB colony is established, it is likely to be destroyed or re-queened (as aggressiveness is an unwanted trait) and would not be included in the assembly of bee packages. However, AHB could be overwintered in stock not yet showing aggressive behaviour.

[16] A drone is haploid and contains the maternal genetic material.

- Bee packages are composed of a mixture of worker bees and drones from various colonies and include an unrelated young mated queen[17]. Strange et al. (2008) found variability in drone proportions in packages that ranged from 0.004% to 5.1%, with one package containing 20.3% drones (n = 48 packages, from six producers). It was suggested that the packages with a low proportion of drones originated from producers using drone excluders[18] (also named queen excluders). There was also variability in the practices used by producers to assemble packages (Strange et al., 2008).
  - Assembling packages by shaking hives and not using an excluder are likely to result in AHB workers and a proportion of drone bees being included in packages.

- Several of the AHB cases reported previously (in Florida, New York and South Carolina [NAPIS, 2013a]; in Tennessee in 2012 [http://news.tn.gov/node/8656]; and in Virginia in 2000 (http://community.seattletimes.nwsource.com/archive/?date=20000721&slug=4032773) seem to be the result of human-assisted spread through the shipment of queens and packaged bees or other shipments from AHB states.

Based on the foregoing, the likelihood of introducing Africanized drone and worker bees is estimated to be **moderate to high**.

5.4.2 Exposure Assessment

Africanized Honey Bees in Contact with Canadian Honey Bees

- Africanized honey bees are exotic to Canada. They have never been reported in Canada or close to the Canada–U.S. border. Demand is high for packages in Alberta, Manitoba and British Columbia, which are areas where interprovincial movement of bees is most important.
  - If introduced with packages, it is likely that AHB will be observed first in the western provinces, and the movement of bee and potentially feral colonies will contribute to the arrival of AHB in other areas; and
  - Considering that AHB can only be distinguished by molecular or morphometric analysis, movement controls are unlikely to prevent the spread of AHB if bee colonies have not yet demonstrated aggressiveness.

Transmission of Africanized Genes into Canadian Populations of European Honey Bees

- Canadian bees could be exposed to African genes following the importation in the early summer of honey bee packages containing Africanized bees. Africanized drones are likely to fly away when packages are dissembled and placed in bee hives, and would reproduce

[17] Queens included in packages do not represent an issue, as they should meet the same import conditions required for the importation of queen honey bees from the U.S. (CFIA, Automated Import Reference System).
[18] Queen or drone excluder: mesh grid that allows smaller bees (workers) to pass through but excludes drones and queens.

with virgin EHB queens during mating flight, resulting in the production of some Africanized queens (F1). The density of Africanized drones within a given area will increase over time during the season. Africanized queens produce more drones per colony than do EHB queens, so drone populations in an area tend to favour Africanized bees. European honey bees also accept drifting Africanized drones into their colonies (vanEngelsdorp & Caron, 2006). It is likely that at least one Africanized drone from an imported package will mate with an EHB (domestic or feral).

- A proportion of workers within packages could be Africanized. Such workers would be replaced by the queen (EHB) progenies within a few weeks after the new colony is established.
- Soon after packages are opened, the queen occasionally does not survive, and the newly formed colony is left queenless. On some occasions, workers (which could be Africanized) could lay eggs, which would produce only drones (Africanized). Although it is biologically plausible, there is an extremely low likelihood that workers would stay in that new hive and lay eggs, given the absence of bonding with the new hive and no brood to look after.
  - ➢ The transmission of Africanized genes to EHB is likely to happen if Africanized drones were to be introduced.

<u>Spreading and Establishment of Africanized Honey Bees</u>

- European honey bee queens mated to Africanized drones will likely produce Africanized workers and queens, and then a hive may quickly become Africanized during the summer after the introduction of AHB (Schneider et al., 2004; vanEngelsdorp & Caron, 2006). There is a strong natural characteristic favouring the Africanized bees (Schneider et al., 2004). Africanized honey bee colonies grow faster, have a high rate of reproductive swarming and travel long distances (about 170 km) before selecting a new site to establish their nests, and AHB robs other colonies when nectar resources are low or lacking (Winston, 1992; Ellis & Ellis, 2012).
- Africanized honey bees could also become established in feral colonies, with the possible consequence of EHB queens of both feral and domestic populations being fertilized by AHB drones. Africanized bees may take over existing EHB colonies (Ellis & Ellis, 2012).
- Colonies with defensive traits would likely be destroyed or re-queened by beekeepers before winter (as beekeepers would not want to manage such bees). If genes were overwintered in stock not showing aggressive behaviour, they would be eliminated at some point in the future if the behaviour of the colonies changed. This would result in a wide distribution of Africanized genes in many areas by beekeepers or by natural means (swarms).
- The practice of overwintering domestic bee populations would benefit AHB survival: some beekeepers use indoor facilities, some use methods that could reduce heat loss from the hive (e.g. a shelter or tarp), additional wintering feed and others move their colonies to warmer areas.
- It is generally considered that AHB has a limited potential to become established and would probably not survive the cold Canadian winter as feral colonies. Estimates of the eventual limits of AHB in North America are still highly speculative. They have a predicted range of 34°N latitude (Schneider et al., 2004). It has been estimated that the northern distribution of

AHB will be limited by a 120-consecutive-day isoline of temperatures not exceeding 10°C during the winter (Southwick et al., 1990). The higher metabolic rate of AHB (in comparison with EHB) as well as a tendency to nest in poorly protected sites may preclude AHB from persisting through a long winter season like that in Canada, with the potential exception of southern British Columbia (as reported Harrison et al., 2006) and southern Ontario.

- Migration of AHB to the northern U.S. and Canada is unlikely. However, AHB might reach northern states through migratory beekeeping and the importation of queens reared in Africanized areas (Guzman-Novoa, 2012). If it happens, AHB will slowly introgresses its genes into EHB populations.

There has been no significant evidence to show that AHB could become established in the Canadian climate. Although the exposure of EHB to imported AHB is likely to happen, the likelihood that AHB would become established within the Canadian bee population following their introduction with package bees and their subsequent spread is estimated to be **small**.

5.4.3 Consequence Assessment

Consequences for Bees

- Africanized honey bee does not have an impact on bee health in terms of death or disease but will have an impact on the genetic makeup of Canadian honey bees as AHB gradually introgresses its genes into EHB populations. Colonies will slowly begin to display highly defensive behaviour. Management of Africanized hives is difficult.

Consequences for Public (Beekeeper) Health and Well-Being

- There would be a potential negative impact on human health if contamination of the gene pool ultimately results in aggressive behaviour that leads to stinging incidents of beekeepers and of the general public. The most significant negative impact on human health may be increased levels of stress for the Canadian public, farmer and beekeepers, induced by fear and uncertainty. There may be liability issues if honey bee packages imported from areas at risk for AHB were implicated in stinging incidents in Canada. It is likely that more stinging incidents associated with AHB will be reported in comparison with EHB incidents. Below are a few recent examples:
  - As of 2012, U.S. officials had reported a total of 23 people killed as the consequence of AHB stinging incidents since AHB was introduced in 1990 (Guzman-Novoa, 2012).
  - The highly defensive responses of Africanized bees in Mexico resulted in more than 3,000 stinging incidents, including 410 people killed, between 1988 and 2000; that is an average of 31.5 deaths per year, which translates to 0.32 annual deaths per million people. Africanized bees have caused the death of about 1,000 people in 30 years, according to mortality statistics in Latin America (Guzmán-Novoa et al., 2011).
  - In March 2013, park employees in Florida were attacked by AHB after disturbing the bees, resulting in over 100 stings per person.

- In July 2012, a man died in California after being stung by AHB.
- In California, a couple were hospitalized after being attacked by bees.
- In Texas in March 2012, bees attacked three people and killed a horse (http://www.khou.com/news/texas-news/Swarming-bees-kill-a-horse-in-Hood-County-144643625.html).
- In Tennessee in April 2012, a beekeeper was stung many times by AHB.

<u>Economic Consequences, Industries and Authorities</u>

- It is likely that the reaction from the public to the presence of AHB genetics in Canada would be negative. As in the U.S., considerable investment in public education to avoid stinging incidents and to calm public worries will be required. There will be divergence on who should share the costs and responsibilities between provincial and federal authorities for responding to stinging incidents, removing and destroying AHB colonies, and ensuring public health awareness and emergency responding plans.
- Consequences for the beekeeping industry include a disruption in beekeeping activities:
    - ➢ Possible restrictions on the movement of bees from operations where AHB was found.
    - ➢ Losses of potential apiary sites and bees in urban locations, restrictions on beekeeping in certain areas to protect the Canadian public.
    - ➢ Costs for the destruction of aggressive hives; costs for re-queening.
    - ➢ Loss of potential business of selling local Canadian bees based on current provincial regulation.
    - ➢ Cost associated with certification procedures to ensure that queens produced for sale are purely European.
    - ➢ Decrease in honey production, given that Africanized colonies tend to swarm more frequently.
    - ➢ Beekeepers quitting the industry because of the aggressive nature of the bees.
    - ➢ Discourage future generation of beekeepers.
- Media attention could have a considerable negative impact on the beekeeping industry.
- Consequences for Canadian fruit and vegetable producers include the possibility that the pollination of certain crops close to urban areas could be compromised if restrictions on the placement of hives were put into place.
- Livestock and wildlife could also be considered at risk if a massive attack were provoked.
    - In Florida in April 2006, AHB attacked a farm worker, killed a goat and a sheep, and injured several other animals (http://phys.org/news65105069.html#nRlv).
- There could be potential, not inconsequential economic losses due to reluctance to engage in outdoor sports and recreational activities.
- There has been no assessment done on the effect on indigenous species of insects (or cavity-nesting birds) that could compete for the same environment to establish nests and harvest food.

Statements Attenuating the Consequences

- The results of an economic study published in 2010 (Livanis & Moss, 2010) showed that AHB did not significantly affect the production of honey in the U.S., that no hives were lost to AHB, and that the economic impacts were likely due to the cost of management measures taken by the beekeepers to prevent AHB from overtaking domestic hives.
- The defensive characteristic of AHB colonies could be reduced to a level not different from those of European colonies after only two generations of mating between Africanized queens and European drones (Guzman-Novoa & Page, 1993). Considering the fact that AHB could potentially become established in only a few areas in Canada, the impact of AHB could be reduced by introducing and saturating mating areas with European drones.
  - The efficacy and cost of such control methods would need to be determined, and consideration should be given to the ongoing inclusion of Africanized drones through importation.
  - A cost-benefit analysis should be conducted to assess the impact of introducing bee diseases and pests into Canada.

Based on the foregoing, the consequence is estimated to be **moderate**.

5.4.4 Risk Estimate

Based on the estimates for entry (moderate to high), exposure (small) and consequence (moderate), the overall risk estimate is **low to moderate**.

5.5 American Foulbrood

5.5.1 Entry Assessment

American foulbrood is an OIE-listed disease (OIE, 2012a). For the importation of live bees and drones with or without associated brood combs, the OIE recommends that the bees come from a country or zone/compartment (under study) that is officially free from American foulbrood.

Note: Natural transmission of rAFB to Canadian hives could happen through the normal activities of bees. Most incursions will happen along the border and are likely to be detected by beekeepers after treatment for AFB has failed.

Distribution of American Foulbrood in the United States

- American foulbrood is widely reported in the U.S. There is no information on the prevalence of AFB resistant to OTC. In 2002, it was already agreed that rAFB had spread across large parts of the U.S. (Feldlaufer M[19] quoted in Bren, 2002). In 2003, it was stated that rAFB had been confirmed in 27 states (AHRA, 2003).
    - ➢ Given that spores can be easily transmitted between colonies and throughout the beekeeping industry, it is likely that rAFB is present in most U.S. states.
- There is a lack of information on the prevalence of AFB and its resistance to OTC.

American Foulbrood in Contact with Bees and Colonies

- In general, only very young larvae are susceptible to AFB. Just a few spores are sufficient to initiate a fatal infection via contaminated food (Genersch, 2010). The LD_{50}[20] for day-old larva is 35 spores.
- It is estimated that one dead larva may produce 2.5 billion spores, resulting in massive contamination of the hive. Adult bees become contaminated by cleaning the dead larvae and can then contaminate other cells or the food source.
- Bees carry spores of AFB within their mouthparts and digestive tract (Goodwin et al., 1996). Bees have been shown to egest viable spores in their fecal material over a period of more than two months (Wilson, 1972).
    - ➢ These egesta represent a potential source of contamination.
- The rate of carriage is dependent on the location of bees within the hive. Samples of bees collected from the brood nest, honey superchamber and edge frame carried higher loads of spores in comparison with honey samples and bee samples taken by the entrance (Gillard et al., 2008). Bees taken from the brood comb of infected hives carried on average 193 spores, sufficient to initiate infection in young larvae.
    - ➢ Bees from different locations in the hive will be included in packages.

[19] Research Leader, Bee Research Laboratory, USDA, Beltsville, MD, U.S.
[20] The LD_{50} is the dose at which 50% of the larvae will die.

- The prevalence of subclinical and/or transient infection is not known. Hives may not show signs of disease but test positive for *Paenibacillus larvae* (Gillard et al., 2008; Pernal & Melathopoulos, 2006; Hornitzky, 1998).
  - There is uncertainty as to whether bees from subclinically infected hives would contain sufficient spores to initiate an infection.
- Spores may easily be transmitted between colonies (directly by bees and by beekeeping management practices) (Lindström, 2006; Genersch, 2010).
  - As packages are assembled from many hives, there is a potential for carrier bees from infected hives to be included.
- A proportion of inoculated hives are resistant to infection and may clear infectivity from the hive even after symptoms have occurred (Spivak & Reuter, 2001). Bees with hygienic behaviour will quickly remove infected dying larvae from the hive, prior to the formation of spores, thereby removing the source of infection.

<u>American Foulbrood in Packages</u>

- Spores are heavily concentrated in and on contaminated hive equipment and, to a lesser degree, in hive products such as honey and pollen.
  - It is assumed that the quantity of spores potentially carried by packaged bees would constitute only a small fraction of that found in contaminated equipment.
- The prophylactic use of antibiotics for AFB may mask the signs of disease in hives (Genersch, 2010). Spore levels may be high in treated hives, given that antibiotics do not kill spores but only prevent the establishment of infection in larvae, thereby preventing the expression of disease.
- Management practices used by migratory beekeeping operators to ensure that colonies are healthy and can sustain transportation could include routine antibiotic use. As described in Runckel et al. (2011), preventive antimicrobial treatments (OTC in the spring and tylosin tartrate later in the season) are part of the management practices in U.S. large-scale migratory beekeeping operations.
  - Bees in packages are likely to be assembled from various colonies with potentially different levels of AFB infectivity and resistance to OTC.
  - The U.S. Food and Drug Administration approved in 2005 the use of tylosin tartrate and in 2012 the use of lincomycin hydrochloride to control AFB (http://www.fda.gov/AnimalVeterinary/ResourcesforYou/AnimalHealthLiteracy/ucm309134.htm).
- In an experiment by Pankiw and Corner (1966), four of six hives containing packaged bees from known infected hives showed evidence of infection after two brood cycles.
- It has been suggested that spores do not persist indefinitely within the bee's digestive system under natural conditions. Sugar syrup fed to packaged bees for two to three days prior to releasing them allows for the passage of most of the spores through the digestive system, therefore reducing the chances that an infective dose will be fed to a young larva (Spivak, 2000). However, Pankiw and Corner (1966) demonstrated that AFB does develop in colonies produced from packaged bees taken from infected hives, even after the bees had been fed sugar syrup and held for three days prior to introduction to the hive.

- Feeding sugar syrup to packaged bees may not be sufficient to eliminate infectivity in heavily contaminated packages.

Based on the foregoing, the likelihood of introducing OTC-resistant strains of AFB is estimated to be **high**.

5.5.2 Exposure Assessment

Contact with Canadian Honey Bees

- American foulbrood is an annually notifiable disease in Canada and a named disease in the regulations of all provinces except Newfoundland and Labrador. In Nova Scotia, AFB and rAFB are named diseases. Provinces have control programs in place and/or provide pest management strategies.
- In the consultation conducted by Serecon Management Consulting Inc. (2012), 48% of respondents said that they had encountered AFB. This percentage may be underestimated, because more respondents from the groups that represent commercial operations, rent bees for pollination, sell bees and/or have more than 16 years of industry experience or hired staff have encountered AFB as compared with their sub-group counterparts.
- Bee packages potentially infected with AFB and/or rAFB would likely be imported into the major honey-producing areas in Canada.
- Demand is high for packages in Alberta, Manitoba and British Columbia, which are areas where interprovincial movement of bees is most important. It is likely that rAFB infection would be observed in these provinces first.
  - OTC-American foulbrood is considered widespread in Alberta, but few occurrences have been found in Saskatchewan and Manitoba. No epidemiological links have been made between these provinces. Movement controls appear to be effective in preventing spread, as no cases have been reported in British Columbia for years.
- American foulbrood is highly contagious through the spread of spores. The incubation period is considered by the OIE to be 15 days. Only a few spores could initiate an infection, and the LD_{50} for day-old larva is 35 spores.
- Under experimental conditions, large quantities of spores were required to establish AFB infections in a hive. Low-level infections developed in four of five colonies inoculated with 5 million spores (Goodwin et al., 1994).
- Imported infected bees would significantly increase exposure over and above that due to the natural spread of rAFB. New foci of infection would be expected in previously unaffected areas.
- Spores remain viable for a long period, and there is a potential for repeat low-level exposure, resulting in infection.

Transmission of American Foulbrood (Resistant American Foulbrood)

- An AFB infection can spread to other honey bee colonies located within up to an 8-km radius (typically 3.2 km). American foulbrood can spread anywhere infected colonies are located and whenever contaminated equipment is exchanged, no matter what the distance (http://www.omafra.gov.on.ca/english/food/inspection/bees/afb-biology.htm).
- Infection with rAFB through imported bees would likely spread to the other colonies within the apiary and the operation, as prophylactic treatment with OTC would be ineffective.
- American foulbrood (resistant or not) would be expected to spread rapidly throughout hives (by robber bees, drifting bees, the use of contaminated hive tools and equipment in non-infected hives, and the feeding of contaminated honey or pollen) within affected apiaries and, less rapidly, between apiaries not managed by the same beekeeper. The extent of spread is dependent upon the management practices of individual apiaries.
  - ➢ There is the potential for further spread if rAFB is not rapidly detected.
- Establishment of rAFB in feral colonies (where still present) and abandoned bee hives would sustain infection in the bee population.
- In some occasion, a proportion of inoculated hives may be resistant to infection and may clear infectivity from the hive even after symptoms have occurred (Spivak & Reuter, 2001).
- In one study, subclinical infections were common when an infected hive was placed near uninfected hives. Upon the removal of the diseased hives, the prevalence of subclinical infection decreased to zero after four weeks.
  - ➢ There is potential for the bacteria causing AFB to be cleared from a hive.
- Diagnosis of AFB is based on identification of the pathogenic agent and the presence of clinical signs; testing for antibiotic resistance requires a specific test.
- In most provinces, early reports of finding rAFB will result in actions to control it, such as irradiation, burning and replacement of infected equipment, and movement controls.
- Symptoms of AFB are suppressed by therapeutic and prophylactic application of antibiotics, principally OTC. This prevents the bacterium from multiplying in the gut of the larvae. Tylosin tartrate, another antibiotic, has been recently approved to treat AFB in Canada.
  - ➢ There is an issue with residues in honey with the use of tylosin.
  - ➢ The spread of rAFB, if introduced, would be slowed by early detection, control measures and the use of tylosin.

Natural Introduction and Exposure

- The exposure assessment for rAFB as a result of imported honey bees must be considered in relation to the expected spread without imports, through natural spread or the spontaneous development of resistance (as the result of misuse and off-label use of OTC).
  - ➢ Natural transmission of AFB/rAFB disease to Canadian hives is likely to happen through normal honey bee activities. The natural spread of rAFB, if introduced and undetected, would be expected to proceed gradually northward. Spread due to human activity would be limited, given that interprovincial movements require a permit and the limited migratory beekeeping given that hives are not generally moved great distances in Canada. Early detection and control measures applied by provincial

authorities will limit the spread of rAFB from the U.S.

- Spontaneous development of resistance:
  - Resistant AFB has been reported sporadically in most of the provinces and has been kept under control. Management strategies and disease prevention have been emphasized by provincial authorities. There is uncertainty regarding the rigour with which these practices are applied by beekeepers in each province. Between 40% and 45% of respondents in the consultation said that they had used OTC for prevention in the spring or fall (Serecon Management Consulting Inc., 2012).
  - The availability of another antibiotic allowing the rotation of therapeutic agents would reduce the likelihood that an OTC-resistant strain of *P. larvae* will develop.

  Based on current knowledge, it can be concluded that there is a very low to low likelihood that rAFB will expand rapidly throughout the domestic bee population from natural spreading from the U.S. or from spontaneous development.

Based on the foregoing, the exposure of Canadian honey bees to rAFB as the result of imported bee packages from the U.S. is estimated to be **moderate to high**.

5.5.3 Consequence Assessment

Consequences for Bees

- Adult bees are carriers of but are not affected by *P. larvae.*
- Young larvae are susceptible to the disease, die, and produce a great number of spores, resulting in massive contamination of the hive.

Economic Consequences for Beekeepers

- One consequence of the presence and establishment of rAFB in bee colonies would be the additional monetary and labour costs of treating infected colonies with another antibiotic (tylosin) after OTC treatment has failed to control the infection:
  - Losses of infected hives, as the result of delay in controlling the disease with initial OTC treatment, and consequently, losses of honey and other bee products.
  - Destruction or disinfection of contaminated tools and equipment.
  - Cost of replacing infected equipment.
  - Losses due to contamination by tylosin of honey and bee products (honey and pollen).
  - Possible losses of markets (trade issue) due to potential presence of antibiotic residue in honey and other bee products, associated with the use of tylosin.
- Labour and time costs consumed by the management of rAFB may be more complex with the use of tylosin, which cannot be applied during honey production, because of its higher propensity to leave residues in honey.

    - Introduction of hygienic measures to reduce spread to unaffected hives, and the use of other tools and equipment.
    - Time to destroy and disinfect tools and equipment as well as hives (irradiation).
    - Transfer of bees into clean hives.
    - Periodic inspection and removal of infected brood.
    - Time and money required to develop and implement new control measures, apply additional biosecurity practices, purchase extra supplies, and educate the beekeepers and their employees.
- There will be costs associated with the need for increased testing to detect resistance in previously low-risk areas and for modifications in inspection programs (certifications for movement permits) because of resistance problems in multiple, random locations.
- Another potential negative impact would be the public perception that honey could be contaminated with antibiotics, given that people want to consume food that is "wholesome" and "natural."

Based on the foregoing, the consequence assessment is **moderate**.

5.5.4 Risk Estimate

Based on the estimates for entry (high), exposure (moderate to high) and consequence (moderate), the overall risk estimate is **moderate**.

5.6 Small Hive Beetle

5.6.1 Entry Assessment

Small hive beetle is an OIE-listed disease (OIE, 2012a). For the importation of live worker bees, drone bees or bee colonies with or without associated brood combs, the OIE recommends that the bees come from a country or zone that is officially free from *Aethina tumida* infestation; that the bees and accompanying packaging presented for export have been inspected and do not contain *A. tumida* or its eggs, larvae or pupae; and that the consignment of bees is covered with fine mesh through which a live beetle cannot enter.

<u>Distribution of Small Hive Beetle in the United States</u>

- Small hive beetle was first confirmed in Florida in 1998 and had spread to 30 states by 2003. It is reported in Hawaii. It is assumed that SHB has spread to much of the U.S. (Ellis & Ellis, 2010).
- Small hive beetle has been reported and is considered established in some of the states along the Canada–U.S. border, from the East Coast (Maine) to North Dakota (NAPIS, 2013b).
- It is believed that SHB has been dispersed as a result of migratory beekeeping practices and perhaps through packaged bees and used equipment (*Personal communications with SME*, May 2013).
- There is uncertainty regarding the prevalence of SHB in American hives. There is little information on the proportion of affected hives in areas where the beetle has been reported.
- As mentioned by Hood (2011), no effective sampling methodology has been developed to estimate the total number of beetles in a colony without doing a full count. This lack may limit the knowledge of the situation.
    - ➢ Compared with the situation in 2003, SHB has expanded to most states. Based on the above, it is assumed that SHB is likely present in most beekeeping areas in most states.
    - ➢ The absence of movement controls and the lack of inspection of colonies being moved may favour the spread of SHB through migratory beekeeping practices.
- Natural transmissions of SHB to Canadian hives have occurred on a few occasions since 2008 in areas close to the Canada–U.S. border, and have been kept under control. Considering the extensive migratory bee industry in the U.S. and the fact that SHB has spread into most states, more incursions of SHB from the northern states into Canada would have been expected.
    - ➢ The reason for the small number of natural transmissions to Canadian hives, whether low prevalence in northern states, environmental conditions that are not favourable for SHB establishment, sufficient density of bee colonies so that SHB does not have to travel long distances to find new hosts to infest, or a combination thereof, needs to be determined.

Small Hive Beetle in Contact with Bees and Colonies

- Small hive beetle is attracted by bee colonies and can disperse easily among apiaries within an area. Odours from hive products and adult bees were found to be significantly attractive to flying adult beetles (Elzen et al., 1999a), and the beetles are also thought to be attracted to their own pheromone (cited in Hood, 2004a).
- Adult beetles are active flyers and are able to fly a distance of about 13 to 16 km.
  - ➢ Because of its ability to track bees, SHB is likely to be found in many colonies in areas of high-density bee populations, such as the southern part of the U.S. during the overwintering season and/or in other areas during the blooming season.
- The female beetle lays eggs (1,000 during its lifetime) directly on feed sources or in crevices or cavities within the hive away from the bees. Damage to hives occurs mainly during the larval stage. Newly hatched larvae immediately begin feeding on honey, pollen and, preferably, bee brood (Ellis & Ellis, 2010). Infestation could be severe with only a few adult beetles in a hive.
  - ➢ The number of beetles within an area can increase rapidly.
- Adult bees do not carry this pest, although SHB eggs were observed to be attached to worker bees in Florida (Elzen et al., 1999c). This further suggests that the importation of bees could be a potential import route.
- Although the adult SHB is readily visible, its aversion to light makes it rapidly seek cover, running to hide in cracks and crevices, when hive material is opened for inspection.
  - ➢ This natural behaviour makes SHB very difficult to detect when adults exist in low numbers in a hive. Infested colonies could be chosen to be included in the assembly of packages.

Small Hive Beetle in Packages

- Packages are typically assembled by shaking various colonies into a box; the practice of shaking bees into packages is likely to also dislodge SHB adults and larvae from the hive and into the package. In a study on colony infestation levels, Spiewok et al. (2007) screened colonies for SHB by shaking combs to dislodge SHB from hidden places.
- Migrating larvae can survive for up to 48 days after feeding ceases and then still develop into viable adults (Cuthbertson et al., 2013). It is probable that both adults and larvae will be transported with packages and caused an infestation. During the inspection of imported queens from the U.S., SHB larvae have been found (CAPA, 2012b). Mature larvae could become a source of infestation if they were drop onto the ground under environmental conditions (soil type and humidity) that are beneficial to their development.
- In 2006, SHB was reported in Alberta and was traced back to an importation of an infested bee packages from Australia.
- Because larvae pupate within close proximity to hives, it is plausible that emerging SHB would be found on package boxes, pallets, containers and other equipment lying on the ground while the apiarist is assembling the packages. This represents a potential source of package contamination.
- In Michigan and Maine, SHB cases are associated with packaged bees from the southern U.S. (*Personal communications with SME*, May 2013).

  - Shaking hives into packages will dislodge SHB adults and larvae; it is likely that some packages will carry SHB.
  - The fact that imports of queen bees, which are handpicked and individually inspected, have been found with SHB (adults and larvae) provides evidence that SHB larvae and adults would likely be introduced into packages at some point during package assembly and shipment.
- Package bee equipment used to assemble packages could be exposed to the small hive beetle, where eggs and young larvae could be found and transhipped with package bees as found in shipments of queens from Hawaii.
  - Inappropriate storage of equipment for package bees may result in its infestation before use.
- Based on a study on the control of SHB in bee packages, there is evidence that SHB will leave the package if it can. In one trial, the authors tested the effect of using coumaphos strips in honey bee transport packages, to reduce the spread of SHB. More than half the beetles (smaller adults) escaped the packages through the mesh ventilation panel, were not recovered and were not killed by the strips (Baxter et al., 1999b).
- Coumaphos has been demonstrated to be highly effective in controlling the beetles, causing 99.9% mortality after 72 hours (also effective to a certain degree against larvae) (Baxter et al., 1999a; Elzen et al., 1999a).
  - Insecticide could be used in hives or packages to control SHB. However, attention must be given to toxicity of coumaphos to bees if exposed in closed environment (such as a package) to more than 6 hours (*Personal communications with SME*, September 2013).

Based on the foregoing, the likelihood of introducing small hive beetle is estimated to be **high**.

5.6.2 Exposure Assessment

<u>Small Hive Beetle in Contact with Canadian Honey Bees</u>

- Small hive beetle is a notifiable disease in Canada and a named disease in most of the provinces. Provinces have legislative authorities to set measures to prevent further spread, and SHB-specific surveillance programs are in place in most provinces.
- Packaged bees potentially infested with SHB would likely be imported into the major honey-producing areas in Canada.
- Demand for packages is high in Alberta, Manitoba and British Columbia, which are areas where interprovincial movement of bees is more important. It is likely that SHB infestation would be observed in these provinces first from introduced packages. However, surveillance programs and other control measures (movement controls and quarantine) that are in place in these provinces will reduce the likelihood of SHB establishment and spread.
  - Because of its aversion to light, SHB could be difficult to detect during post-entry inspections if it exists in low numbers in the package.

- Because bee packages are disassembled by shaking bees into a hive, hidden beetles would likely be dislodged and fall into the assembled hives, and larvae could fall onto the ground and continue their development cycle.
- It is reasonable to assume that if adults are left in the packaging material, they may fly to attractive honey bee colonies in the apiary.

<u>Small Hive Beetle Infestation in a Colony</u>

- Weakened and compromised honey bee colonies (e.g. newly formed colonies that originated from packages and could be weakened from stress and low numbers) are at greatest risk of SHB infestation, with only a few adults required to establish a severe infestation. In contrast, strong and healthy colonies have been observed with many adult SHB and little resulting damage. However, in heavily infested areas, even strong honey bee colonies have succumbed to the effects of SHB and died (Hood, 2004a).
- One active SHB female can produce about 1,000 eggs in its four to six months of life. Adult beetles are mature one week after emerging from the soil.
- Low numbers of beetles have been observed in most of the cases reported in Canada in recent years. Limited damage has been observed in smaller colonies (SHB Working Group, Key Case Management Information, 2013).
    - ➢ These observations support the fact that few beetles are needed to establish an infestation.

<u>Transmission between Colonies, Spreading and Establishment</u>

- If SHB is introduced into an apiary through the importation of bee packages, other colonies in the apiary and subsequently within a short distance from it would likely be exposed and become infested with SHB.
    - ➢ The spread of infestation does not require contact between adult bees. Dispersal of the infestations can follow or accompany swarms.
    - ➢ Adult SHB can fly long distances and could potentially infest other hives in the surrounding area (newly emerged adults are more likely to fly, whereas mature adult beetles are less active and tend to stay in dark areas).
    - ➢ Feral colonies could become infested and spread SHB to other areas. Switching to alternate hosts (such as bumblebees) and feral colonies could present a viable survival strategy for SHB when honey bee hives are less abundant or temporarily unavailable (Hoffmann et al., 2008, cited in Cuthbertson et al., 2013).
- It is generally agreed that migratory beekeepers bring colonies that are infested with SHB.
    - ➢ This is supported by state apiarists, who report increased numbers of SHB with the return of migratory bees (*Personal communications with SME*, May 2013).
    - ➢ Although the interprovincial movement of colonies is limited and requires a permit, such movement would likely contribute to the spread of SHB to other areas if it’s presence is not detected early enough to be prevented (low numbers of beetle could go unnoticed for a period of time).
- Sales of bees and equipment, in which SHB could be hidden, are limited in Canada, as reported in the Honey Bee Benchmark Consultation Report (Serecon Management

Consulting Inc., 2012).

- In dead honey bee colonies or exposed beekeeping equipment, SHB infestations can increase dramatically, as there is a large source of larval food and no protection by guard bees.
- Adult beetles can also survive and reproduce independently in other natural environments, and could survive for six months or longer.
- Small hive beetle can establish populations in temperate regions because of its ability to overwinter; beetles exploit the cluster behaviour of the bees to survive (Neumann & Elzen, 2004). Adult SHB has been demonstrated to overwinter in honey bee clusters during the harsh winters in Minnesota (Wenning, 2001).
  - ➢ Small hive beetle has survived in overwintered hives in Canada and has successfully restarted its reproductive cycle post-overwintering. The survival rate could vary according to geographical location in Canada. In Ontario, some hives infested with SHB were still infested the following spring. In Quebec, research results have shown that SHB may be able to survive through the winter and reproduce the following spring (*Personal communications with SME*, May 2013); while previous conclusion suggested that SHB may not survive overwintering (Giovenazzo & Boucher, 2010). In Manitoba in the spring of 2013, no live beetles were found during the inspection of hives that had been infested in 2012 (*Personal communications with SME*, May 2013).
- Even though SHB is reported in northern states, there is no evidence to suggest that this pest is well established and expanding in affected areas (*Personal communications with SME*, May 2013).
- There is uncertainty regarding the ability of the beetle to eventually adapt to cooler conditions.
  - ➢ Whether SHB could survive in Canada and in what areas still need to be determined.
- Indoor overwintering facilities, moving bee to warmer climate and winter wrapped bee hives in Canada as well as honey houses and equipment storage facilities may be conducive to the survival of adult SHB.
- Because of low temperatures during the winter, it is highly unlikely that egg laying and larval development would occur. Therefore, populations would be expected to decline over the overwintering period through attrition.
- Good management practices will help control infestation, including maintaining strong, healthy and populous colonies (good coverage of bees on the comb seems to prevent SHB from gaining a foothold) and locating apiary sites on dense clay-based soils to help break the life cycle by inhibiting the formation of pupae.
- Coumaphos is the only registered product to treat SHB in Canada. Coumaphos-treated corrugated cardboard placed on the bottom board of the hive has been shown to be moderately effective against adult SHB but is not effective against larvae that are located in the brood, as they do not receive sufficient exposure to the pesticide. Soil treatment with permethrin may be used to interrupt the life cycle of SHB but does not prevent the damage associated with the larval stage in the hive.
  - ➢ The application of these recommendations to hives from which packages originate would likely reduce the likelihood of exposure of Canadian bee populations.

Natural Introduction and Exposure

- Occurrences of SHB have been reported in areas close to the border in three provinces, and SHB has survived the winter in Ontario. Cases were reported in 2008, 2009, 2010, 2011 and 2013 in the south-western area of the province of Quebec; in 2010, 2011 and 2012 in Ontario[21]; and in 2012 few beetles were found in 2 beekeeping operations in the southern part of the provinces of Manitoba. Quarantine was established in all cases, and colonies were destroyed or kept under control measures in zoned areas to prevent the spreading. Infested beekeeping operations were either located within close range of the US border, or were epidemiologically link to or close to a quarantine area[22].
- In the consultation, 60% of the respondents said that they monitored the presence of SHB in their hives, and 42% said that they monitored their extraction facility for SHB (Serecon Management Inc., 2012).

  Based on current knowledge and experience, it can be concluded that there is a very low to small likelihood that SHB will spread rapidly across the domestic populations of bees from natural spread from the U.S.

Based on the foregoing, the exposure is estimated to be **low to small**.

5.6.3 Consequence Assessment

Consequences for Bees, Colonies

- Small hive beetle is particularly a problem in weakened, newly established or split (nuclei) hives. In these cases, worker bees do not have full coverage of the brood, allowing SHB to reproduce undisturbed. Some colonies seem to tolerate numerous adults without any significant damage (Sanford, 1999, cited in AHRA, 2003).
- Damage is more severe in newly drawn-out comb, whereas established brood comb is sturdier and less susceptible to damage (Delaplane, 2000). However, even strong honey bee colonies have succumbed to the effects of SHB and died in heavily infested areas (Hood, 2004a).
- The adults and larvae feed on honey, pollen and bee brood, resulting in spoilage of stored honey. The fermented honey left behind in dead colonies is repellent to honey bees and cannot be marketed by the beekeeper.
- Bees may abandon the hive once the fermented rancid honey starts to drip from the comb.
- SHB can act as a potential vector of other pathogens.

[21] Information about the quarantine area, surveillance and control zones can be found at: http://www.omafra.gov.on.ca/english/food/inspection/bees/12rep.htm#small
[22] Reports submitted to the OIE can be seen at: http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review/viewsummary

Economic Consequences for Beekeepers, Industries and Authorities

- Although SHB is considered to be mostly a nuisance pest in its native area of sub-Saharan Africa, it has become a major problem for many beekeeping operations in the U.S. (Elzen et al, 1999a; Hood, 2004a). This is typical for the introduction of a pest in a previously uninfested area, where natural controls may not be present.
- Within its first few years in Florida, SHB destroyed thousands of colonies and caused an estimated US$3 million in damage to the beekeeping industry (Ellis, 2003).
- In the U.S., the most severe infestations have been found in and around honey houses (Sanford, 1999, cited in AHRA, 2003).
- The establishment of SHB will impact beekeepers as a result of:
    - Colony losses; the costs of replacing hives and damaged equipment (combs) would be significant for beekeepers.
    - Losses of honey production and other bee products, because of spoiled products.
    - Cost associated with the construction of beetle-proof honey houses, specific storage facilities for equipment, and the modification of honey house to reduce moisture.
    - More intense labour and higher costs associated with management measures, such as treatment, control of beetles, surveillance, and more frequent honey extraction.
    - At first, movement restrictions from a known infested area to a non-infested area (until beetles are widespread across all honey bee production areas).
    - Small hive beetle larvae may cause severe damage in honey supers that are left unattended. In areas where this pest is located, honey must be extracted and stored without delay to avoid damage.
    - Losses associated with trade issues.
- In honey houses and extracting facilities, SHB can spoil honey when honeycomb is exposed for long periods of time. It is recommended that beekeepers promptly extract any exposed honeycomb and clean the facilities immediately after extraction.
- Wax cappings should also be stored in beetle-proof containers and rendered as soon as possible.
- There could be an impact on the export of bees and some bee products, as other countries will impose restrictions because of the risk that beetles will be shipped with these commodities.
- There will be costs to provincial governments to control and attempt to eradicate SHB, and to issue certifications for export and movement permits.
- *Bombus* species populations are susceptible to infestation by SHB (Hoffmann et al., 2008). Small hive beetle infestation in Canada will also negatively affect the associated industries using *Bombus* species as pollinators as well as the wild *Bombus* population.
- There could be an impact on crops relying on pollinators, if a large number of colonies are destroyed by SHB and also if bumblebee numbers decline because of SHB infestation.
- Adult SHB is a scavenger and may infest fruit crops such as cantaloupe, strawberry, blueberry and grape.

Based on the foregoing, the consequence assessment is **moderate**.

5.6.4 Risk Estimate

Based on the estimates for entry (high), exposure (low to small) and consequence (moderate), the overall risk estimate is **low to moderate**.

5.7 Varroa Mite (Amitraz-Resistant and Multi-Resistant)

5.7.1 Entry Assessment

Varroosis is an OIE-listed disease (OIE, 2012a). For the importation of live honey bee queens, worker bees and drones with or without associated brood combs, the OIE recommends that bees come from a country or zone/compartment (under study) that is officially free from varroosis.

Distribution of Varroa Mite in the United States

- A high prevalence of VAR has been reported (USDA-APHIS, 2013a).
- Amitraz-resistant mites (rVAR) and multi-resistant mites (mrVAR; resistant to the synthetic miticides fluvalinate, coumaphos and amitraz) are present in the U.S.

Varroa Mite in Colonies

- Varroa mite spends most of its life cycle within the brood cells of maturing bee larvae, but when there is no brood to reproduce, adult VAR can remain on adult bees for more than 150 days.
- Mites can spread through swarming and by drifting, foraging and robber bees as well as infested packages or queens. Mites can spread rapidly within an apiary in a short time.
- Given the highly migratory nature of the U.S. beekeeping industry, there is the potential for rVAR to be spread to any state through the movement of infested hives. Bee breeding colonies are also susceptible unless controls are put in place to prevent contact with bees from neighbouring hives that are potentially infested.
  - Because VAR is easily transmissible, many colonies and apiaries in a beekeeping area could be affected.
- Pesticide treatments do not eliminate VAR. The purpose of treatment is to reduce the mites to a manageable level while minimizing pesticide damage to the bees.
  - Proper use of miticides (as recommended by the manufacturer) and rotation of the miticides used will limit the development of resistance. There have been anecdotes and reports of miticide misuse (MAAREC, 2002; *Personal communications with SME*, April 2013).
  - Resistant mites could lose their resistance to a product if they are left untreated for a certain period (Milani & Della Vedova, 2002; Elzen & Westervelt, 2004).

Varroa Mite in Packages

- Given the prevalence of VAR mites in colonies and the fact that shaking bees into packages may also dislodge additional mites from the frames and hive, it is reasonable to expect that many bees will carry VAR, including VAR resistant to miticides. The level of infestation will be variable depending on date of last treatment before making the packages, what control agent used, time of the year (spring, summer vs. fall) and sampling method.

- Since packages typically contain approximately 6,500 to 10,000 bees (two-three pounds), the bees are not individually inspected. It is likely that numerous amitraz-resistant VAR and mrVAR will be contained in each package.
  - This is highlighted by the fact that mites have been detected on queen bee imports[23], even if they are handpicked and individually inspected.

Based on the foregoing, the likelihood of importing bee packages with resistant VAR is estimated to be **high**.

5.7.2 Exposure Assessment

Contact with Canadian Honey Bees

- Fluvalinate-resistant varroosis is a notifiable disease in Canada, and VAR is a named disease in most provincial regulations. Provinces have legislative authorities to set measures to prevent further spread of VAR, particularly if miticide resistance is detected.
- Imported infested bee packages would likely be distributed throughout most major honey-producing areas in Canada. If amitraz-resistant strains were introduced into apiaries, other colonies within the apiaries would likely be exposed and become infested with that resistant strain, given that VAR management with amitraz would be ineffective.
- The importation of packaged bees infested with rVAR would significantly increase exposure over and above exposure due to natural spread. Not only would the quantity of introduced resistant mites be increased, but infested bees with mrVAR and amitraz-resistant VAR would be dispersed over a larger geographical area in a relatively short period, resulting in multiple foci of infestation and further spread of mrVAR. In contrast, natural cross-border spread would likely be confined mostly to border areas, for at least some years, in provinces where there is little migratory movement of hives.
  - The fact that mites have been found on occasion on queen imports from the U.S., which are handpicked and inspected, provides evidences that several VAR, including amitraz-resistant strains and mrVAR, would likely to be in contact with bee colonies if packages are imported from the U.S.

Transmission of Varroa Mite

- Because demand for packages is high and because interprovincial movements occur between Alberta, Manitoba and British Columbia, it is likely that imported amitraz-resistant VAR would become widespread first within these provinces (as the treatment would be ineffective). Synthetic miticides are used more by beekeepers in Alberta, Saskatchewan and Manitoba, and Alberta beekeepers less often use multiple synthetic products (Serecon Management Consulting Inc., 2012).

[23] Import conditions state that queens should come from operations with1% VAR or less. Varroa mites found in queen cages during the post-entry inspection are removed (Alberta provincial apiarist, May 2013).

- Interprovincial movement requires a permit. Conditions regarding VAR have been established, and colonies must be tested and found to have 1% VAR or less.
- If left untreated or unnoticed (below the threshold to cause significant losses), amitraz-resistant VAR would continue to reproduce and spread naturally or through management practices to other areas and other apiaries.
- Infestations with rVAR would be detected through passive surveillance, as beekeepers experiencing difficulties with VAR control will seek the advice of provincial apiarists. However, the spread of rVAR could have already happened by then.
- Varroa mites are known to spread rapidly from hive to hive. The rate of spread to other apiaries would depend on the density of hives (both managed and feral) in the area. Mites spread naturally via swarms and foraging and robber bees. Swarms can move several miles, creating new foci of infestation in previously unaffected areas. Mites may also be spread through various management practices, such as splitting and relocation of hives. It is likely that rVAR would be spread to other colonies and areas from natural transmission and/or management practices, if miticide resistance is not detected rapidly.
  - ➢ It is likely that VAR would spread to many areas.
- The spread of resistance is proven to be rapid. The VAR mites resistant to fluvalinate was first reported in Canada in 2001, and by 2002 it was already spread in most of the province (CAPA, 2003; Canadian Honey Council, 2010). Similarly when coumaphos resistance was first discovered in 2003[24], by 2006 it was reported in many provinces (CAPA, 2004; CAPA, 2007; Canadian Honey Council, 2010).
- Reversible resistance (or reversion) to miticides has been reported. If fluvalinate is unused for some years, VAR appears to regain sensitivity to it (Milani & Della Vedova, 2002; Elzen & Westervelt, 2004).
  - ➢ Amitraz is now a widely used synthetic product by beekeepers in Canada (CAPA, 2012b). It may be expected that VAR mites would regain sensitivity to fluvalinate and perhaps to coumaphos in time, offering optional effective treatment. However, the efficacy of the miticide after reversion may only last few treatments before the resistance is back high enough that it is ineffective (CAPA, 2008).
  - ➢ It is reported in CAPA (2012c), that the efficacy of fluvalinate was measured at approximately 90% and coumaphos was only 17%. Reversion to coumaphos may be, according to this report, not as probable as the reversion to fluvalinate.

<u>Natural Introduction and Exposure</u>

- The exposure assessment for amitraz-resistant VAR and mrVAR as a result of imported honey bees must be considered in relation to the expected spread without imports, through natural spread or spontaneous development of resistance as the result increased levels of treatment (misuse) (Watkins, 1996).
  - ➢ The natural spread of mites across the Canada–U.S. border is the only significant source of amitraz-resistant mites if imports of honey bees from the U.S. are not allowed. For the most part, the spread of resistant mites would be expected to proceed

[24] VAR has rapidly developed resistance to coumaphos, between 1 to 4 years after its emergency registration in 2002.

gradually northward. Spread due to human activity would be limited, given that interprovincial movements require a permit and the limited migratory beekeeping.

- Resistance to amitraz would be first detected in beekeeping operations along the border.

➢ Spontaneous development of resistance (and spread):

- Because of the development of resistance to other synthetic products, Canadian beekeepers use amitraz as the first-choice synthetic miticide (CAPA, 2012b). Organic acid is also widely used by beekeepers in Canada, with a comparable proportion of beekeepers reporting that they use synthetic products and organic acid (Serecon Management Consulting Inc., 2012). The use of organic acid and integrated pest management strategies may result in the other synthetic miticides (more likely fluvalinate) regaining their efficacy against VAR as the result of not being used.
- Provincial government apiarists have been monitoring Amitraz resistance and responding to any reports of Amitraz failure to control mites to investigate resistance. They also educate producers on the risk of misused or overused miticides and emphasize integrated pest management (rotation of treatments and use of organic acid) and disease prevention to control VAR. There is uncertainty regarding the rigour with which these practices are applied by beekeepers in each province. A gap in the practice of using multiple products and rotating their use was identified by Serecon Management Consulting Inc. (2012), which reported that just 40% to 50% of respondents mentioned alternating and rotating miticides.
- Enforcement by the Pest Management Regulatory Agency of the *Pest Control Products Act*, which regulates the proper use of miticides, would help prevent the off-label use of amitraz.

Based on the foregoing, it can be concluded that there is an extremely low to small likelihood that amitraz resistance would develop spontaneously in Canada if import is not considered.

In conclusion, introduced mrVAR and amitraz-resistant VAR would likely spread to other hives in Canada. The exposure to Canadian bees is estimated to be **high**. Overall, the time taken for amitraz-resistant VAR to become widespread, considering that it could develop spontaneously or migrate with swarm bees, would be substantially shortened.

5.7.3 Consequence Assessment

Consequences for Bees, Colonies

- Varroa mites weaken bees by feeding on hemolymph, and the bees are more susceptible to pathogens.
- Uncontrolled VAR infestations cause most hives to collapse within two years; as shown in 2007 and 2008 winter (Canadian Honey Council, 2008; CAPA, 2013). Uncontrolled VAR is associated with high winter colony loss (Guzmán-Novoa et al., 2010; Currie et al., 2010).

- Varroa mites can also act as vectors for viruses of the honey bee (e.g. deformed wing disease and acute paralysis virus).

Economic Consequences for Beekeepers and Industries

- Varroa mites were rated the most serious pest or disease by the respondents to the Honey Bee Benchmark Consultation (Serecon Management Consulting Inc., 2012).
- If amitraz-resistant or multi-resistant strains of VAR become established in Canada, apiarists will experience economic hardship as a result of:
    - ➢ The loss of colonies and the cost associated to replace the hives.
    - ➢ The costs of replacing hives.
    - ➢ Losses of honey production as the result of delay in controlling the mites with the initial treatment.
    - ➢ More intense labour and higher costs to adopt control measures, with might have variable results and require proper window for treatments such as use of formic acid, oxalic acid, and thymovar; and increased exposure to harmful chemicals.
    - ➢ Potential movement restrictions from a known infested area to a non-infested area (until resistance to amitraz is widespread across all honey bee production areas).
    - ➢ Possible losses of markets (trade issue).
- A reduction in the availability of healthy populous colonies for crop pollination will affect the viability of many horticultural and agricultural industries and will have an impact on the national economy.
- Varroa is widespread in Canada. Its management is based on a limited number of approved acaricides available in Canada. The introduction of amitraz-resistant strains of VAR would jeopardize the management of this parasite. Amitraz is regularly used by beekeepers as a standard treatment and is currently the only effective synthetic acaricide approved to treat VAR in Canada (Vandervalk, 2013).
    - ➢ Over time, the presence of resistant strains of VAR has obliged beekeepers to reconsider the use of standard treatments. In Canada, the use of fluvalinate, coumaphos, formic acid, oxalic acid, thymovar and now amitraz is approved to treat VAR. Although still part of management options, the synthetic reliable acaricides (i.e. fluvalinate and coumaphos) are no longer being used as standard treatments because of the development of resistance.
    - ➢ If amitraz resistance developed, there is no reliable acaricide that can be used at any time of the year as needed and does not require special conditions to work as in other organic chemicals. Currently research has been carried out to screen effective acaricides and develop new alternative acaricides to replace amitraz if needed (Vandervalk, 2013).

The consequence assessment for the spread and establishment of amitraz-resistant VAR and mrVAR via the importation of bee packages throughout the honey-producing areas is considered to be **moderate**.

5.7.4 Risk Estimate

Based on the estimates for entry (high), exposure (high) and consequence (moderate), the overall risk estimate is **moderate**.

CONCLUSION

In this qualitative risk assessment, Africanized honey bee, antibiotic-resistant American foulbrood, small hive beetle and amitraz-resistant varroa mite were identified as hazards associated with the importation of honey bees packages from the continental U.S.

In the entry assessment, the key risk factors assessed in the continental U.S. were the distribution and prevalence of honey bee diseases in the U.S., the extensive migratory beekeeping industry, the overwintering of colonies in southern part of the U.S., the lack of interstate movement controls, and the absence of a national honey bee management program.

In addition, the following scientific considerations were taken into account in the exposure assessment:

- Disease control management practices and regulations, the control of interprovincial movements, and the limited migratory beekeeping industry in Canada have helped to limit the spread of diseases.
- Africanized honey bee has not been reported in Canada.
- Oxytetracycline-resistant strains of AFB have been reported in Canada; however, provinces are actively implementing control programs if rAFB is detected.
- Small hive beetle has been reported in limited areas of three provinces, close to the Canada–U.S. border, and has been kept under control with implementation of control program currently in place.
- Strains of VAR resistant to fluvalinate and coumaphos are reported across the country. The use of amitraz has been approved to control the disease. It is currently the only effective and reliable synthetic miticide used by beekeepers in Canadian their integrated pest management strategy, and no resistance has been reported.

Table 4 – Summary of the Risk Estimates

Hazard	Entry Probability	Exposure Probability	Consequence Estimate	Risk Estimate
Africanized honey bee	Moderate to High*	Small	Moderate	Low to Moderate
American foulbrood - Oxytetracycline-resistant	High	Moderate to High	Moderate	Moderate
Small hive beetle	High	Low to Small	Moderate	Low to Moderate
Varroosis (varroa mite) - Amitraz-resistant	High	High	Moderate	Moderate

**The probability range represents the level of uncertainty. Such uncertainties need to be taken into account when making a decision.*

Conclusions of the current risk assessment are similar to the previous scientific evaluation conducted in 2003; there is still a high probability of introducing diseases and pests into Canada due to importation of honey bees from the continental United States. The risk assessment does not provide new scientific evidence to remove or decrease the current import control measures in place, thus allowing only the importation of honey bee queens from the United States.

As such, the risk assessment provides scientific support for the import control measures that are currently in place for the importation of honey bees from the U.S. These measures allow honey bee queens to be individually inspected for signs of disease before importation into Canada. Such verification is not possible with honey bee packages.

APPENDIX 1

DEFINITION OF RISK ANALYSIS TERMS AND RISK ESTIMATE TABLES

Entry assessment

Entry assessment consists of describing the biological pathway(s) necessary for an importation activity to introduce pathogenic agents into a particular environment. Entry assessments typically include (a) a description of the types, amounts, timing and probabilities of the release of risk agents, and (b) a description of how these attributes might change as a result of various actions or events. Entry assessment is the process of developing a description of the relevant characteristics of the risk source that establishes its potential for creating harm by releasing or otherwise introducing risk agents into portions of the environment that are accessible to animals and humans.

Exposure assessment

Exposure assessment consists of describing the relevant conditions and characteristics of animal and human exposures to risk agents produced or released by a given risk source. Exposure assessments typically include (a) a description of the intensity, timing, frequency and duration of exposure, (b) routes of exposure (e.g. ingestion, inhalation or insect bite), and (c) the number, species and characteristics of populations that might be exposed. Exposure assessment is the process of developing a description of the relevant conditions and characteristics of animal and human exposures to risk agents produced or released by a specified source of risk.

Consequence assessment

Consequence assessment consists of describing the relationship between specified exposures to a risk agent and the economic consequences of those exposures. Consequence assessments typically include a specification of the impact on health in the animal and human populations sustained under given exposure scenarios. In other words, consequence assessment is the process of developing a description of the relationship between the specified exposures to a risk agent and health and other consequences for animals and humans that are exposed.

Risk estimation

Risk estimation consists of integrating the results from entry assessment, exposure assessment and consequence assessment to produce quantitative measurements of health and environmental risks. These measurements typically include (a) estimated numbers of people who would experience health impacts of various severities over time, (b) measurements indicating the nature and magnitude of adverse consequences for the natural environment, and (c) probability distributions, confidence intervals and other means for expressing the uncertainties in these estimates.

LIKELIHOOD DEFINITIONS			
Likelihood	**Descriptive Definition**	**Probability Range**	
		Minimum	**Maximum**
Negligible	The event would be virtually unlikely to occur.	10^{-7}	10^{-6}
Extremely low	The event would be extremely unlikely to occur.	10^{-6}	10^{-5}
Very low	The event would be very unlikely to occur.	10^{-5}	10^{-4}
Low	The event would be unlikely to occur.	10^{-4}	10^{-3}
Small	The event would be minimally likely to occur.	10^{-3}	10^{-2}
Moderate	The event would be fairly likely to occur.	10^{-2}	10^{-1}
High	The event would likely occur.	10^{-1}	1

CONSEQUENCE DEFINITIONS	
Consequences	**Description**
Negligible	The probability and the costs and losses associated with the economic factors are insignificant.
Very low	The probability and the costs and losses associated with the economic factors are minor.
Low	The probability and the costs and losses associated with the economic factors are low.
Moderate	The probability and the costs and losses associated with the economic factors are intermediate
High	The probability and the costs and losses associated with the economic factors are severe.
Extreme	The probability and the costs and losses associated with the economic factors are catastrophic.

Entry and Exposure Probability Matrix

ENTRY PROBABILITY							
High	Negligible	Extremely low	Very low	Low	Small	Moderate	High
Moderate	Negligible	Negligible	Extremely low	Very low	Low	Small	Moderate
Small	Negligible	Negligible	Negligible	Extremely low	Very low	Low	Small
Low	Negligible	Negligible	Negligible	Negligible	Extremely low	Very low	Low
Very low	Negligible	Negligible	Negligible	Negligible	Negligible	Extremely low	Very low
Extremely low	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Extremely low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	Negligible	**Extremely low**	**Very low**	**Low**	**Small**	**Moderate**	**High**

EXPOSURE PROBABILITY

The matrix represents the product of the maximum values of the entry and exposure probabilities, which have been estimated qualitatively.

REFERENCE TABLE FOR THE RISK ESTIMATION MATRIX

This table represents integration of the likelihood assessment (probabilities of entry and exposure assessments) with the consequence assessment.

Risk Estimate Matrix

PROBABILITY						
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Small	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
Very low	Negligible	Negligible	Negligible	Very low	Low	Moderate
Extremely low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low
	Negligible	**Very low**	**Low**	**Moderate**	**High**	**Extreme**

CONSEOUENCES

The probability on the ordinate axis is the mean estimated probability obtained from the probability matrix for a qualitative risk assessment.

The consequences on the abscissa represent the expected consequences.

APPENDIX 2

PROVINCIAL LEGISLATIVE CONTROLS FOR HONEY BEE DISEASES

(As of May 2013)

Province	# of Beekeepers Registered	Named Diseases	Movement Controls		Authority to Inspect, Quarantine, Treat and Destroy Bees and Equipment?	Other Comments
			Interprovincial	Intraprovincial		
BC	2,000	AFB, EFB, chalkbrood, HBTM, sacbrood, nosema, VAR, and any other bee disease listed by the Regulations.	No bee, hive or equipment may be brought into the province without an import permit. Permit issuance is based on inspection results prior to import and approved apiary location.	Movement controls of bees and equipment between 14 bee districts are in place. Beekeepers must notify the province when selling bees in the province.	Yes	
AB	800 (number of registered bee colonies: 282,000)	Africanized bee (in the Act) The Regulation includes: AFB, VAR, HBTM, EFB, chalkbrood, nosemosis (*Nosema apis*), sacbrood, *Tropilaelaps* and SHB *In the process of being added in 2013: Nosema ceranae,* Asian giant hornet (*Vespa mandarinia*), Asian honey bee (*Apis cerana*), Cape honey bee (*Apis mellifera capensis*)	Permit is required to import bees, in line with international trade conditions regarding pests and diseases (1% VAR or less, no AFB and no SHB), and equipment must be free of AFB. Bees must be treated with a pesticide (does not apply to overwintered bees in BC or to bees located within 25 km of the AB border).	Beekeepers must report purchases of bees and equipment and get a provincial permit before moving bees.	Yes	Apivar is registered for VAR. No resistance to amitraz is reported. Apivar is the only effective synthetic miticide being used by beekeepers.

Province	# of Beekeepers Registered	Named Diseases	Movement Controls		Authority to Inspect, Quarantine, Treat and Destroy Bees and Equipment?	Other Comments
			Interprovincial	Intraprovincial		
SK	750	AFB, VAR, tracheal mite, AHB, SHB, *Tropilaelaps clareae*	Permit is required to import bees and equipment. Importation is allowed using risk mitigation protocols based on CFIA importation protocols. rAFB, SHB are included in protocols.	Beekeepers must report purchases of bees and equipment and must obtain a permit to sell bees and equipment, which includes a health inspection for VAR, SHB, AFB.	Yes	Movement controls are applied on the rAFB cases currently reported.
MB	520	AFB, EFB, HBTM, *Nosema* spp., chalkbrood spp., *Varroa* spp., *Tropilaelaps* spp., other diseases as named	Certificate, satisfactory to the inspector, must be issued by the place of origin for bees and equipment to be brought into the province.	Where disease is confirmed, bees and equipment may not be sold or moved without a permit. Sales of bees and equipment must be reported.	Yes	No resistance in VAR population to amitraz has been confirmed. Resistance to coumaphos and fluvalinate have been confirmed and deemed to be widespread.
ON	3,200	AFB, EFB, VAR, HBTM, AHB, SHB, *Tropilaelaps*, *Euvarroa*, *Apis cerana*, *Nosema* spp., giant Asian hornet (*Vespa velutina*, *V. mandarinia*), large hive beetle, Cape honey bee, giant honey bee complex, dwarf honey bee complex, resistant strains of *Nosema* spp. July 2013: updates to the *Bees Act* regulations	Permit is required from the provincial apiarist to import bees and equipment. Provincial apiarist must be notified when bees are received from outside ON. Receiving or transporting pests is prohibited.	Permit is required to move bees to, from or within a quarantine area. Permit is required to sell or remove bees.	Yes	

Province	# of Beekeepers Registered	Named Diseases	Movement Controls		Authority to Inspect, Quarantine, Treat and Destroy Bees and Equipment?	Other Comments
			Interprovincial	Intraprovincial		
QC	500	AFB, SHB, *Tropilaelaps* spp., Africanized bee	Health certificate is required, and authorization for introduction must be given.	No specific controls can be applied for named diseases in the Regulation.	Yes	Each report of AFB is sampled and tested for OTC resistance.

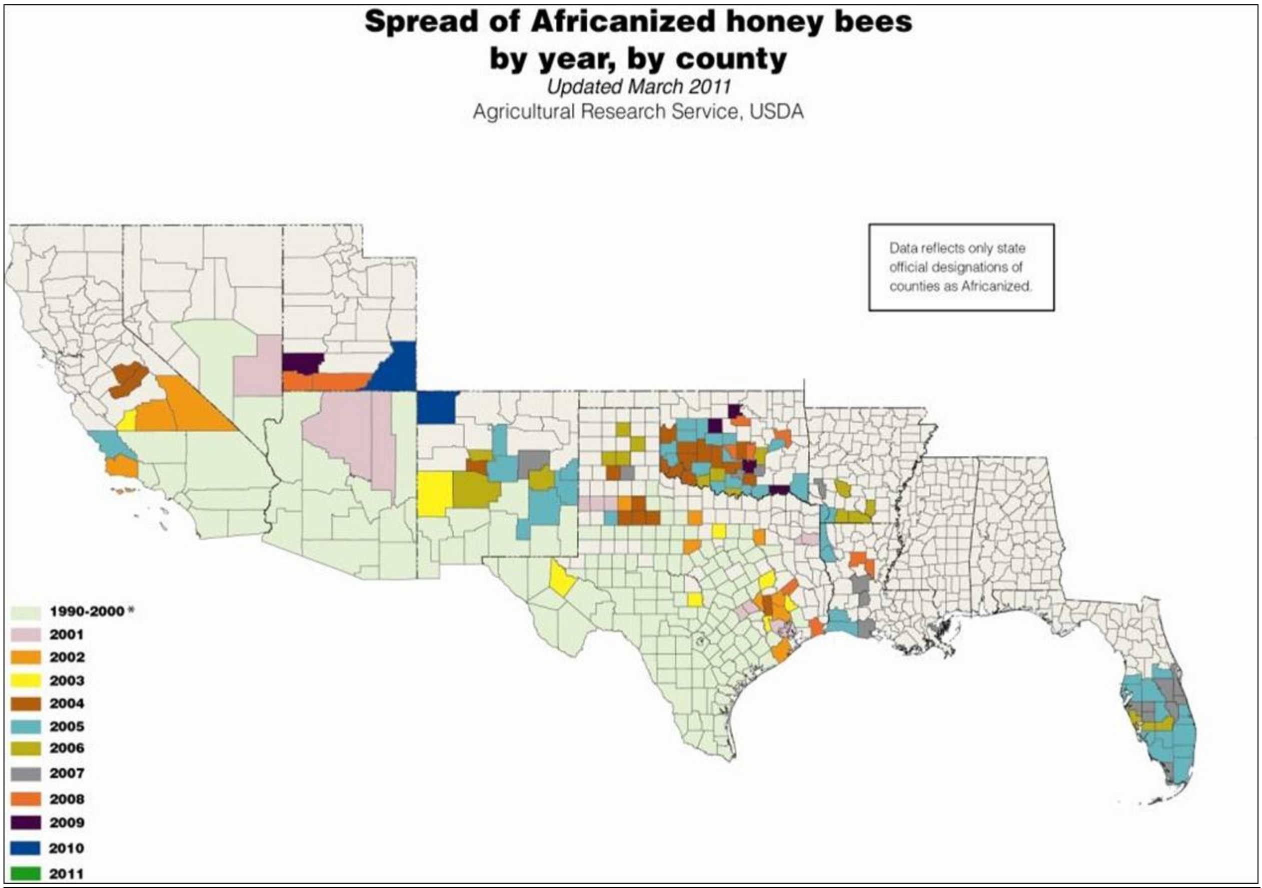
Province	# of Beekeepers Registered	Named Diseases	Movement Controls		Authority to Inspect, Quarantine, Treat and Destroy Bees and Equipment?	Other Comments
			Interprovincial	Intraprovincial		
NB	244 (22 commercial: 50 or more colonies)	Regulated diseases: the Act does not make mention of diseases regarding importation. Diseases regulated in the Regulation: AFB, VAR, tracheal mite. NOTE: In the Regulation, NB enforces AFB but no longer enforces VAR or tracheal mite. The Act states that no person shall keep bees with AFB, EFB, sacbrood, Nosema, Honey Bee Mite (Tracheal mite) , VAR mite, Chalkbrood or any other contagious or infectious disease	*Apiary Inspection Act*: No controls mentioned regarding interprovincial movement. Although this is not mentioned in the Act or Regulation, NB does not permit any colonies with AFB. The following condition in quotes is enforced by NB even though it is not listed in the Act or Regulation: "If an apiary has more than 2% of colonies with AFB, then no colonies are permitted to enter NB. No colonies are permitted from an apiary if that apiary has had rAFB within the previous two years. NB does not permit any colonies from an apiary where the SHB has been found upon inspection for interprovincial movement." *Regulation:* American Foulbrood, Varroa mite, Tracheal mite. NOTE: In the Regulation, NB enforces American foulbrood but no longer enforces varroa mite or tracheal mite.	For all contagious or infectious diseases: None shall be moved to cause the spread of disease, once the disease is known. Colonies with the following diseases may be quarantined: AFB, EFB, sacbrood, nosema, honey bee mite (tracheal mite), VAR, chalkbrood or any other contagious or infectious disease. In practical terms, colonies with SHB, rAFB or AHB would be quarantined.	Yes, for "any other contagious or infectious disease"; this would include but not be limited to SHB, rVAR, rAFB, AHB.	NB does not enforce the interprovincial movement control for varroa mite or tracheal mite since these diseases are endemic in NB.

Province	# of Beekeepers Registered	Named Diseases	Movement Controls		Authority to Inspect, Quarantine, Treat and Destroy Bees and Equipment?	Other Comments
			Interprovincial	Intraprovincial		
NS	315 (30 commercial)	AFB, rAFB, EFB, chalkbrood, nosematosis, sacbrood, VAR, fluvalinate-resistant VAR, coumaphos-resistant VAR, HBTM, *Tropilaelaps*, SHB, AHB, Cape honey bee	Permit is required to import bees and equipment. Permit is issued after a certificate of inspection from the exporting province is received. NS Bee Health Protocol is in place for inspection requirements and movement.	Beekeepers must be registered. A certificate of inspection to sell bees and equipment is required.	Yes	
PEI	Not mandatory. Approx. 6 commercial and 40 hobbyists	Class A (exotic to province): HBTM, SHB Class B (present, subject to control): VAR, AFB Restricted bees: AHB, Asian honey bee, Asian hornet, Cape honey bee	Permit is required to import bees and equipment. Permit is issued after a certificate of inspection from the exporting province is received. PEI *Bee Health Regulations* are in place for inspection requirements and movement.	None	Yes	No control measures are taken for AFB-positive hives.

Province	# of Beekeepers Registered	Named Diseases	Movement Controls		Authority to Inspect, Quarantine, Treat and Destroy Bees and Equipment?	Other Comments
			Interprovincial	Intraprovincial		
NL	Registration not required in NL. Approx. 35	VAR, HBTM	Border is closed to imports of bees, except from those places proven to be free of VAR, HBTM, greater wax moth and SHB. Permit is required to import bees. Imported bees and equipment are to be held in quarantine for 12 months, after which the Director of Animal Health issues a certificate declaring them free of disease.	Permit is required to move bees through a quarantined area.	Inspect, quarantine and treat: Yes. Destroy: No; bees and/or equipment will be seized and returned to place of origin.	

APPENDIX 3

DISTRIBUTION OF AFRICANIZED HONEY BEE IN THE UNITED STATES OF AMERICA



Source: USDA-ARS (2011).

REFERENCES

[AHRA] Animal Health Risk Assessment (2003). *Risk Assessment on Honey Bees from the United States – K6*. Canadian Food Inspection Agency.

Annand N, 2008. Small hive beetle management options. Primefacts NSW Department of Primary Industries, Australia. http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0010/220240/small-hive-beetle-management-options.pdf on 2013-06.

Annand N, 2011. *Small Hive Beetle Biology: Providing Control Options*. Report for the Australian Government Rural Industries Research and Development Corporation.

Baxter J, Elzen PJ & Wilson WT (1999a). Control of the small hive beetle (*Aethina tumida*). *American Bee Journal* 139 (4): 308.

Baxter JR, Elzen PJ, Westervelt D, Causey D, Randall C, Eischen FA & Wilson WT (1999b). Control of the small hive beetle, *Aethina tumida* in package bees. *American Bee Journal* 139 (10): 792-793.

Bee-info (2013). Biology of bee – Taxonomy [Internet]. Retrieved from http://www.bee-info.com/biology-bee/taxonomy.html on 2013-03-14.

Bren L (2002). Treating minor species: A major animal health concern [Internet]. Retrieved from http://www.fda.gov/AnimalVeterinary/NewsEvents/FDAVeterinarianNewsletter/ucm110709.htm on 2013-04-30.

Brown BV (1993). Taxonomy and preliminary phylogeny of the parasitic genus *Apocephalus*, subgenus *Mesophora* (Diptera: Phoridae). *Systematic Entomology* 18 (3): 191-230.

Canadian Honey Council (2008). Request for Emergency Registration - Apivar Pest Control StripTM for Control of Apistan – CheckMite –Resistant Varroa Mites (*Varroa destructor*) on Honey Bees (*Apis mellifera*) in CANADA - 2008

Canadian Honey Council (2010). Description of Emergency – Apivar® for Control of Varroa Mites (*Varroa destructor*) on Honey Bees (*Apis mellifera*) in CANADA – 2010.

Canadian Honey Council (2012). Production and value of honey 2012 [Internet]. Retrieved from http://www.honeycouncil.ca/news.php?nType=Canadian on 2013-03-22.

Canadian Honey Council (2013). Africanized honey bees [Internet]. Retrieved from http://www.honeycouncil.ca/africanized_honeybees.php on 2013-03-22.

[CAPA] Canadian Association of Professional Apiculturists (1994). Annual Meeting, January 9-11, 2014, New Brunswick [Internet]. Retrieved from http://capabees.org/content/uploads/2013/02/CAPAProceedings1994.pdf on 2013-09-10.

[CAPA] Canadian Association of Professional Apiculturists (2003). Canada Association of Professional Apiculturists Proceedings 2003. Niagara Falls, Ontario, Canada, December 2002.

[CAPA] Canadian Association of Professional Apiculturists (2004). Canada Association of Professional Apiculturists Proceedings 2004. Winnipeg, Manitoba, Canada, January 2004.

[CAPA] Canadian Association of Professional Apiculturists (2007). Canada Association of Professional Apiculturists Proceedings 2007. Langley, BC, Canada, January 2007.

[CAPA] Canadian Association of Professional Apiculturists (2008). Canada Association of Professional Apiculturists Proceedings 2008/9. Niagara Falls, Ontario, Canada, December 2008.

[CAPA] Canadian Association of Professional Apiculturists (2012a). CAPA Statement on Honey Bee Wintering Losses in Canada (2012) [Internet]. Retrieved from http://capabees.org/content/uploads/2012/10/2012capawintloss1.pdf on 2013-06.

[CAPA] Canadian Association of Professional Apiculturists (2012b). Canada Association of Professional Apiculturists Proceedings 2011. Winnipeg, MB, Canada, 26 January 2012.

[CAPA] Canadian Association of Professional Apiculturists (2012c). 2012/2013 Business Meeting, Draft Meeting Minutes. Québec city, QC, November15, 2012.

[CAPA] Canadian Association of Professional Apiculturists (2013). CAPA Statement on Honey Bee Wintering Losses in Canada (2013) [Internet]. Retrieved from http://capabees.org/content/uploads/2013/06/2013-CAPA-Statement-on-Colony-Losses-final.pdf on 2013-09-09.

[CFIA] Canadian Food Inspection Agency (2012). Terrestrial animal diseases [Internet]. Retrieved from http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/eng/1300388388234/1300388449143 on 2013-03-20.

[CFIA] Canadian Food Inspection Agency (2013). National bee farm-level biosecurity standard [Internet]. Retrieved from http://www.inspection.gc.ca/animals/terrestrial-animals/biosecurity/standards-and-principles/national-bee-farm-level-bisosecurity-standard/eng/1365794112591/1365794221593?chap=3#s8c3 on 2013-06.

Chen Y, Pettis JS & Feldlaufer MF (2005). Detection of multiple viruses in queens of the honey bee *Apis mellifera* L. *Journal of Invertebrate Pathology* 90 (2): 118-121.

Chen Y, Evans J & Feldlaufer M (2006). Horizontal and vertical transmission of viruses in the honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology* 92 (3): 152-159.

Chen Y & Siede R (2007). Honey bee viruses. *Advances in Virus Research* 70: 33-80.

Chen Y, Evans JD, Smith IB & Pettis JS (2008). *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. *Journal of Invertebrate Pathology* 97 (2): 186-188.

Core A, Runckel C, Ivers J, Quock C, Siapno T, DeNault S, Brown B, DeRisi J, Smith CD & Hafernik J (2013). A new threat to honey bees, the parasitic phorid fly *Apocephalus borealis*. *PloS ONE* 7 (1): e29639.

Currie RW, Pernal SF & Guzmán-Novoa E (2010). Honey bee colony losses in Canada. *Journal of Apicultural Research* 49 (1): 104-106.

Cuthbertson AGS, Wakefield ME, Powell ME, Marris G, Anderson H, Budge GE, Mathers JJ, Blackburn LF & Brown MA (2013). The small hive beetle *Aethina tumida*: A review of its biology and control measures. *Current Zoology*. In press. Retrieved from http://www.actazool.org/site_media/onlinefirst/downloadable_file/2013/02/05/SHB_Review___FINAL.pdf on 2013-05-23.

Delaplane KS (2000). The small hive beetle, *Aethina tumida*: A new beekeeping pest [Internet]. Bugwood Entomology and Forest Resources Library. Retrieved from http://www.bugwood.org/factsheets/small_hive_beetle.html on 2013-06.

Electronic Code of Federal Regulations (2013). Title 7: Agriculture, Part 322—Bees, Beekeeping Byproducts, and Beekeeping Equipment [Internet]. Retrieved from http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&sid=6c2bf4aa764f4e0c50862e17c87ecde8&rgn=div5&view=text&node=7:5.1.1.1.7&idno=7#7:5.1.1.1.7.1 on 2013-04-12.

Ellis JD (2003). The ecology and control of small hive beetles (*Aethina tumida Murray*). Volume I. PhD dissertation, Rhodes University, Grahamstown, South Africa, October 2003.

Ellis JD, Hepburn R, Luckman B & Elzen P (2004). Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera: Nitidulidae). *Environmental Entomology* 33 (4): 794-798.

Ellis JD and Ellis A (2010). Small Hive Beetle, *Aethina tumida* Murray (Insecta: Coleoptera: Nitidulidae) [Internet]. Fact Sheet EENY-474. Retrieved from http://edis.ifas.ufl.edu/pdffiles/IN/IN85400.pdf on 2013-02-15.

Ellis JD and Ellis A (2012). African Honey Bee, Africanized Honey Bee, Killer Bee, *Apis mellifera scutellata* Lepeletier (Insecta: Hymenoptera: Apidae) [Internet]. Fact Sheet EENY-429. Retrieved from http://edis.ifas.ufl.edu/pdffiles/IN/IN79000.pdf on 2013-05-01.

Elzen PJ, Baxter JR, Westervelt D, Randall C, Delaplane KS, Cutts L & Wilson WT (1999a). Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae), attacking European honey bees in the Western Hemisphere. *Apidologie* 30 (5): 361-366.

Elzen PJ, Eischen FA, Baxter JF, Elzen GW & Wilson WT (1999b). Detection of resistance in US *Varroa jacobsoni* Oud. (Mesostigmata: Varroidae) to the acaricide fluvalinate. *Apidologie* 30 (1): 13-17.

Elzen PJ, Baxter JR, Westervelt D, Randall C, Cutts L, Wilson WT, Ridvhrn GS & Delaplane KS (1999c). Status of the small hive beetle in the US. *Bee Culture* 127 (January): 27-28.

Elzen PJ, Baxter JR, Westervelt D, Randall C & Wilson WT (2000a). A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae), in Florida, USA. *Apidologie* 31 (5): 593-594.

Elzen PJ, Baxter JR, Spivak M & Wilson WT (2000b). Control of *Varroa jacobsoni* Oud. resistant to fluvalinate and amitraz using coumaphos. *Apidologie* 31 (3): 437-441.

Elzen P & Westervelt D (2004). A scientific note on reversion of fluvalinate resistance to a degree of susceptibility in *Varroa destructor*. *Apidologie* 35 (5): 519–520.

Evans JD (2003). Diverse origins of tetracycline resistance in the honey bee bacterial pathogen *Paenibacillus larvae*. *Journal of Invertebrate Pathology* 83 (1): 46-50.

Eyer M, Chen YP, Schäfer MO, Pettis J & Neumann P (2009). Small hive beetle, *Aethina tumida*, as a potential biological vector of honeybee viruses. *Apidologie* 40 (4): 419-428.

[FERA] Food and Environment Research Agency (2013). BeeBase – Varroa [Internet]. Retrieved from https://secure.fera.defra.gov.uk/beebase/index.cfm?pageid=93 on 2013-04-15.

Genersch E (2010). American foulbrood in honeybees and its causative agent, *Paenibacillus larvae*. *Journal of Invertebrate Pathology* 103 (Supplement): S10-S19.

Gillard M, Charriere JD & Belloy L (2008). Distribution of *Paenibacillus larvae* spores inside honey bee colonies and its relevance for diagnosis. *Journal of Invertebrate Pathology* 99 (1): 92-95.

Giovenazzo P & Boucher C (2010). A scientific note on the occurrence of the small hive beetle (*Aethina tumida* Murray) in Southern Quebec. *American Bee Journal* 150 (3): 275-276.

Goodwin RM, Perry JH & Ten Houten A (1994). The effect of drifting honey bees on the spread of American foulbrood infections. *Journal of Apicultural Research* 33 (4): 209-212.

Goodwin RM, Perry JH & Haine HM (1996). A study on the presence of *Bacillus larvae* spores carried by adult honey bees to identify colonies with clinical symptoms of American foulbrood disease. *Journal of Apicultural Research* 35 (3-4): 118-120.

Guzmán-Novoa E & Page RE (1993). Backcrossing Africanized honey bee queens to European drones reduces colony defensive behavior. *Annals of the Entomological Society of America* 86 (3): 352-355.

Guzmán-Novoa E, Eccles L, Calvete Y, Mcgowan J, Kelly PG & Correa-Benítez A (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie* 41 (4), 443-450.

Guzmán-Novoa E, Benítez AC, Espinosa-Montaño LG & Guzmán-Novoa G (2011). Colonization, impact and control of Africanized honey bees in Mexico. *Veterinaria México* 42 (2): 149-178.

Guzmán-Novoa E (2012). Report on Africanized honey bees. In: *Canadian Association of Professional Apiculturists Proceedings 2011*, Winnipeg, MB, Canada, 26 January 2012, 32-33.

Hall HG, Zettel-Nalen C & Ellis JD (2013). African Honey Bee: What You Need to Know [Internet]. Fact Sheet ENY-114. Retrieved from http://edis.ifas.ufl.edu/pdffiles/MG/MG11300.pdf on 2013-05-01.

Harrison JF, Fewell JH, Anderson KE & Loper GM (2006). Environmental physiology of the invasion of the Americas by Africanized honeybees. *Integrative and Comparative Biology* 46 (6): 1110-1122.

Health Canada (2013). Enforcement Bulletin, Apr. 17, 2013, Edmonton, AB, Canada.

Hoffmann D, Pettis JS & Neumann P (2008). Potential host shift of the small hive beetle (*Aethina tumida*) to bumblebee colonies (*Bombus impatiens*). *Insectes Sociaux* 55 (2): 153-162.

Hood WM (2004). Overview of the small hive beetle, *Aethina tumida*, in North America. *Bee World* 81 (3): 129-137.

Hood WM (2004). The small hive beetle, *Aethina tumida*: A review. *Bee World* 85 (3): 51-59.

Hood WM (2011). *Handbook of Small Hive Beetle IPM*. Extension Bulletin 160, Clemson University Cooperative Extension Program, October 2011.

Hornitzky MAZ (1998). The spread of *Paenibacillus larvae* subsp *larvae* infections in an apiary. *Journal of Apicultural Research* 37 (4): 261-265.

[ITIS] Integrated Taxonomic Information System (2013a). *Apis mellifera* Linnaeus, 1758 [Internet]. Retrieved from http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=154396 on 2013-03-14.

Johnson R (2010). Honey Bee Colony Collapse Disorder [Internet]. Publication 7-5700; RL33938. Retrieved from http://www.fas.org/sgp/crs/misc/RL33938.pdf on 2013-03-28.

Lindström A. (2006). Distribution and Transmission of American Foulbrood in Honey Bees. PhD dissertation, Faculty of Natural Resources and Agricultural Sciences, Department of Entomology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Lindström A, Korpela S & Fries I (2008). The distribution of *Paenibacillus larvae* spores in adult bees and honey and larval mortality, following the addition of American foulbrood diseased brood or spore-contaminated honey in honey bee (*Apis mellifera*) colonies. *Journal of Invertebrate Pathology* 99 (1): 82-86.

Livanis G & Moss CB (2010). The effect of Africanized honey bees on honey production in the United States: An informational approach. *Ecological Economics* 69 (4): 895-904.

Meikle WG & Patt JM (2011). The effects of temperature, diet, and other factors on development, survivorship, and oviposition of *Aethina tumida* (Coleoptera: Nitidulidae). *Journal of Economic Entomology* 104 (3): 753-763.

Melhim A, Weersink A, Daly Z & Bennett B (2010). Beekeeping in Canada: Honey and Pollination Outlook [Internet]. CANPOLIN No. 6. Retrieved from http://www.uoguelph.ca/canpolin/Publications/Outlook-Beekeeping-CANPOLIN06.pdf on 2013-03-28.

[MAAREC] Mid-Atlantic Apiculture Research and Extension Consortium (2002). BeeAware – Use It Wisely or Risk Losing It [Internet]. Retrieved from https://agdev.anr.udel.edu/maarec/wp-content/uploads/2010/03/BeeAware402.pdf on 2013-04-16.

[MAAREC] Mid-Atlantic Apiculture Research and Extension Consortium (2012). Package bees [Internet]. Retrieved from https://agdev.anr.udel.edu/maarec/beginning-beekeeping-2/package-bees/ on 2013-03-22.

Milani R & Della Vedova G (2002). Decline in the proportion of mites resistant to fluvalinate in a population of *Varroa destructor* not treated with pyrethroids. *Apidologie* 33 (4), 417-422.

Mistro DC, Rodrigues LAD & Ferreira Jr. WC (2005). The Africanized honey bee dispersal: A mathematical zoom. *Bulletin of Mathematical Biology* 67 (2): 281-312.

Miyagi T, Peng CYS, Chuang RY, Mussen EC, Spivak MS & Doi RH (2000). Verification of oxytetracycline-resistant American foulbrood pathogen *Paenibacillus larvae* in the United States. *Journal of Invertebrate Pathology* 75 (1): 95-96.

[NAPIS] National Agricultural Pest Information System (2013a). Survey status of Africanized honey bee – *Apis mellifera scutellata* (2005) [Internet]. Retrieved from http://pest.ceris.purdue.edu/map.php?code=ISAEAEA# on 2013-05-01.

[NAPIS] National Agricultural Pest Information System (2013b). Survey status of small hive beetle – *Aethina tumida* (All years) [Internet]. Retrieved from http://pest.ceris.purdue.edu/map.php?code=INBJQEA&year=alltime on 2013-04-19.

National Honey Board (2013). Honey industry facts [Internet]. Retrieved from http://www.honey.com/tools-tips-and-resources/honey-industry-facts on 2013-04-15.

Neumann P & Elzen PJ (2004). The biology of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae): Gaps in our knowledge of an invasive species. *Apidologie* 35 (3): 229-247.

[OIE] World Organisation for Animal Health (2011). Guidelines for Assessing the risk of non-native animals becoming invasive [Internet]. Retrieved from http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/OIEGuidelines_NonNativeAnimals_2012.pdf on 2013-09-12.

[OIE] World Organisation for Animal Health (2012a). Terrestrial Animal Health Code [Internet]. Retrieved from http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/ on 2013-04-10.

[OIE] World Organisation for Animal Health (OIE) (2012b). World Animal Health Information Database Interface: Animal health situation [Internet]. Retrieved from http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation on 2013-03-19.

[OIE] World Organisation for Animal Health (2013). Diseases of Bees [Internet]. Retrieved from http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/BEES-EN.pdf on 2013-04-10.

[OMAFRA] Ontario Ministry of Agriculture, Food and Rural Affairs (2011). 2011 Ontario provincial apiarist annual report [Internet]. Retrieved from http://www.omafra.gov.on.ca/english/food/inspection/bees/11rep.htm on 2013-05.

Pankiw P & Corner J (1966). Transmission of American foulbrood by package bees. *Journal of Apicultural Research* 5: 99-101.

Pernal SF & Melathopoulos AP (2006). Monitoring for American foulbrood spores from honey and bee samples in Canada. *Apiacta* 41: 99-109.

Pernal S, Melathopoulas A & Van Haga A. (2007) Nosema Disease – Diagnosis and Control [Internet]. Canadian Association of Professional Apiculturists. Retrieved from http://capabees.org/content/uploads/2013/02/nosema.pdf on 2013-03-03 .

Pettis JS (2004). A scientific note on *Varroa destructor* resistance to coumaphos in the United States. *Apidologie* 35 (1): 91-92.

Runckel C, Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R & DeRisi JL (2011). Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, *Nosema*, and *Crithidia*. *PloS ONE* 6 (6): e20656.

Sammataro D, Untalan P, Guerrero F & Finley J (2005). The resistance of varroa mites (Acari: Varroidae) to acaricides and the presence of esterase. *International Journal of Acarology* 31 (1): 67-74.

Sanford MT (1999). Small Hive Beetle [Internet]. Fact Sheet ENY-133. Retrieved from http://ufdcimages.uflib.ufl.edu/IR/00/00/14/88/00001/AA25700.pdf on 2013-06.

Schäfer M, Ritter W, Pettis J & Neumann P (2010). Small hive beetles, *Aethina tumida*, are vectors of *Paenibacillus larvae*. *Apidologie* 41 (1): 14-20.

Schäfer MO, Ritter W, Pettis JS & Neumann P (2011). Concurrent Parasitism Alters Thermoregulation in Honey Bee (Hymenoptera: Apidae) Winter Clusters. *Ann. Entomol. Soc. Am.* 104 (3): 476-482.

Schneider SS, DeGrandi-Hoffman G & Smith DR (2004). The African honey bee: Factors contributing to a successful biological invasion. *Annual Review of Entomology* 49: 351-376.

Serecon Management Consulting Inc. (2012). Honey Bee Benchmark Consultation Report, National Bee Farm-Level Biosecurity Standard. Final Draft Submission. Prepared for the Office of Animal Biosecurity, Canadian Food Inspection Agency, Ottawa, ON, Canada.

Small Hive Beetle Working Group, Key Case Management Information (2013). Report received from Dr. Amanda Amaratunga, Canadian Food Inspection Agency, February 2013.

Shimanuki H, Knox DA, Furgala B, Caron DM & Williams JL (1992). Diseases and pests of honey bees. In: Graham JM, ed. *The Hive and the Honey Bee*. Hamilton, IL, U.S.: Datant & Sons.

Somerville D (2003). Study of the Small Hive Beetle in the USA: A Report for the Rural Industries Research and Development Corporation. RIRDC Publication No 03/050, NSW Agriculture Department, Australia.

Southwick EE, Roubik DW & Williams JM (1990). Comparative energy balance in groups of Africanized and European honey bees: Ecological implications. *Comparative Biochemistry and Physiology A* 97 (1), 1-7.

Spiewok S, Pettis JS, Duncan M, Spooner-Hart R, Westervelt D & Neumann P (2007). Small hive beetle, *Aethina tumida*, populations I: Infestation levels of honey bee colonies, apiaries and regions. *Apidologie* 38 (6): 595-605.

Spiewok S, Duncan M, Spooner-Hart R, Pettis JS & Neumann P (2008). Small hive beetle, *Aethina tumida*, populations II: Dispersal of small hive beetles. *Apidologie* 39 (6): 683-693.

Spivak M (2000). Preventative antibiotic treatments for honey bee colonies. *American Bee Journal* 140 (11): 867-868.

Spivak M & Reuter GS (2001). Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behaviour. *Apidologie* 32 (6): 555-565.

Spleen AM, Lengerich EJ, Rennich K, Caron D, Rose R, Pettis JS, Henson M, Wilkes JT, Wilson M, Stitzinger J, Lee K, Andree M, Snyder R & vanEngelsdorp D (2013). A national survey of managed honey bee 2011-12 winter colony losses in the United States: Results from the Bee Informed Partnership. *Journal of Apicultural Research* 52 (2): 44-53.

Statistics Canada (2012), Production and value of honey and maple products, 2012 [Internet]. Retrieved from http://www.statcan.gc.ca/daily-quotidien/121214/dq121214c-eng.pdf on 2013-09-10.

Strange JP, Cicciarelli RP & Calderone NW (2008). What's in that package? An evaluation of quality of package honey bee (Hymenoptera: Apidae) shipments in the United States. *Journal of Economic Entomology* 101 (3): 668-673.

University of Georgia College of Agricultural and Environmental Science (2012). Honey bee disorders: Honey bee parasites [Internet]. Retrieved from http://www.ent.uga.edu/bees/disorders/honey-bee-parasites.html on 2013-04-15.

[USDA-APHIS] United States Department of Agriculture Animal and Plant Health Inspection Service (2013a). 2011–2012 National Honey Bee Pests and Diseases Survey Report [Internet]. Retrieved from http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/2011_National_Survey_Report.pdf on 2013-04-22.

[USDA-APHIS] United States Department of Agriculture Animal and Plant Health Inspection Service (2013b). Permits: Regulated organism and soil permits [Internet]. Retrieved from http://www.aphis.usda.gov/plant_health/permits/organism/index.shtml on 2013-04-24.

[USDA-ARS] United States Department of Agriculture Agricultural Research Service (2011). Spread of Africanized honey bees by year, by county [Internet]. Retrieved from http://www.ars.usda.gov/Research/docs.htm?docid=11059&page=6 on 2013-03-19.

[USDA-NASS] United States Department of Agriculture National Agricultural Statistics Service (2013). Honey [Internet]. Retrieved from http://www.abfnet.org/associations/10537/files/Hone-03-18-2013.pdf on 2013-03-22.

Vandervalk LP (2013). *New options for Integrated Pest Management of Varroa destructor (Acari: Varroidae) in colonies of Apis mellifera (Hymenoptera: Apidae) under Canadian Prairies conditions (Thesis)*. Master of Science, Department of Agricultural, Food, and Nutritional Science, University of Alberta. 130 p.

vanEngelsdorp D & Caron DM (2006). Final African Honey Bee Action Plan [Internet]. Mid-Atlantic Research and Extension Consortium African Honey Bee Working Group. Retrieved from https://agdev.anr.udel.edu/maarec/wp-content/uploads/2010/03/AHBactionPlan06.pdf on 2013-05-30.

Villa JD, Rinderer TE & Stelzer JA (2002). Answers to the puzzling distribution of Africanized bees in the United States or "Why are those bees not moving east to Texas?" *American Bee Journal* 142 (7), 480-483.

Vital MVC, Hepburn R, Radloff S & Fuchs S (2012). Geographic distribution of Africanized honey bees (*Apis mellifera*) reflects niche characteristics of ancestral African subspecies. *Natureza & Conservação* 10 (2):184-190.

Watkins M (1996). Resistance and its relevance to beekeeping. *Bee World* , 77(4), 15-22.

Wenning CJ (2001). Spread and threat of the small hive beetle. *American Bee Journal* 141 (9): 640-643.

Wikipedia (2013). Honey bee [Internet]. Retrieved from http://en.wikipedia.org/wiki/Honey_bee on 2013-03-18.

Williams GR, Shafer ABA, Rogers REL, Shutler D & Stewart DT (2008). First detection of *Nosema ceranae*, a microsporidian parasite of European honey bees (*Apis mellifera*), in Canada and central USA. *Journal of Invertebrate Pathology* 97 (2): 189-192.

Williams GR, Rogers REL, Kalkstein AL, Taylor BA, Shutler D & Ostiguy N (2009). Deformed wing virus in western honey bees (*Apis mellifera*) from Atlantic Canada and the first description of an overtly-infected emerging queen. *Journal of Invertebrate Pathology* 101 (1): 77-79.

Wilson WT (1972). Resistance to American foulbrood in honey bees: XII. Persistence of viable *Bacillus larvae* spores in the feces of adults permitted flight. *Journal of Invertebrate Pathology* 20 (2): 165-169.

Winston ML (1992). The biology and management of Africanized honey bees. *Annual Review of Entomology* 37: 173-193.

ZomBee Watch (2012). ZomBee Watch [Internet]. Retrieved from https://www.zombeewatch.org/?navigation on 2013-04-03.