IMPORT RISK ANALYSIS:

HONEY BEE (*Apis mellifera*) GENETIC MATERIAL

Biosecurity Authority
Ministry of Agriculture and Forestry
Wellington
New Zealand

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Approved for release

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1. EXECUTIVE SUMMARY

In association with several external consultants, MAF has completed an analysis of the risks associated with the importation of honey bee (*Apis mellifera*) genetic material - queens, queen cells, eggs and semen. The major reason for carrying out this analysis is to find acceptable conditions under which genes for varroa tolerance can be introduced into New Zealand. Although MAF did develop an IHS for bee semen in 1998, practical difficulties with its implementation have meant that it has not been used.

This risk analysis has closely followed the development of a broader analysis of the risks of importing honey bee hive products. While many parts of these analyses are similar, the nature of the commodities considered presents different questions and challenges for each analysis.

The risk analysis on bee genetic material concluded that post-arrival quarantine would be required for a number of organisms of potential concern, and these are summarised in Chapter 43 of this document.

Due to the uncertainty surrounding deformed wing virus and its association with varroa and colony collapse as well as the well-recognised risk posed by European foul brood, the only form of genetic material that can practically comply with recommended conditions is semen.

2. INTRODUCTION

This document is an analysis of the biosecurity risks posed by the importation of honey bee (*Apis mellifera*) genetic material[1].

Under the Biosecurity Act 1993, 'risk goods' are defined as:

> *"any organism, organic material, or other thing, or substance, that (by reason of its nature, origin, or other relevant factors) it is reasonable to suspect constitutes, harbours, or contains an organism that may cause unwanted harm to natural and physical resources or human health in New Zealand, or interfere with the diagnosis, management, or treatment, in New Zealand, of pests or unwanted organisms."*

The Ministry of Agriculture and Forestry (MAF) is responsible for issuing import health standards under the Biosecurity Act that specify the requirements to be met before 'risk goods' may be imported.

Under the Hazardous Substances and New Organisms Act (1996), the importation of new organisms is the responsibility of the Environmental Risk Management Authority. However, because honey bees of the species *A. mellifera* are already present in New Zealand, importation of genetic material from this species is not considered to be a new organism.

Because of the likelihood that honey bee (*A. mellifera*) genetic material may harbour organisms that may cause unwanted harm, it is considered by the Director of Animal Biosecurity (a Chief Technical Officer under the Biosecurity Act) to be a 'risk good' under the Biosecurity Act.

2.1 Commodity Definition

The following forms of *Apis mellifera* genetic material are considered in this risk analysis:

- Queens - 3 different shipping methods
- Queen cells - capped or uncapped
- Eggs
- Semen (fresh)

2.1.1 Queens

The honey bee queen is the main form of transfer of *Apis mellifera* genetic material world-wide, and a substantial trade exists in this commodity, both within and between major beekeeping countries. Mated queen bees may be shipped in the following forms:

- a small screened cage (called a 'queen cage') accompanied by 5-10 worker bees and a food source (sugar or sugar and honey fondant),

- a larger screened cage (called a 'package') accompanied by an artificial swarm of worker bees and drones and a sugar syrup food source, or

[1] *Apis* species other than *A. mellifera* are included as hazardous organisms in this risk analysis, since it is possible that these species could enter New Zealand if (for whatever reason) the import was derived from the wrong species of bee. The subject of this risk analysis is, however, only purposeful importation of *A. mellifera* genetic material.

- a box (called a ‘nucleus or nuc’) accompanied by a large number of worker bees and drones with beeswax combs containing honey, pollen and brood (eggs, larvae and pupae).

To reduce the risk of spreading honey bee pests and diseases, queen honey bees are generally shipped in new cages or containers not previously in contact with bees, and the food source accompanying the queens must either not be honey or be treated to kill disease-causing organisms such as American foul brood (Hansen, 1984), European foul brood (Hornitzky and Smith, 1998) and many others .

Therefore, in this risk analysis, the commodity ‘queens' will refer to both queens and attendant worker bees in any of the above three forms, that are:

- shipped in new cages or containers not previously in contact with bees, and
- supplied with a food source that does not contain honey, or contains honey that has been sterilised by gamma irradiation using a cobalt-60 source at a dose rate of 14kGy.

2.1.2 Queen cells

A queen cell is a beeswax cell that contains a honey bee queen at any development stage prior to emergence. Queen cells are generally transferred from a ‘queen rearing’ colony to ‘queenless’ colonies prior to the queen emerging as an adult. The queen cells are generally capped (i.e., sealed prior to pupation), although unsealed queen cells can also be transferred.

There is a small trade in this commodity, generally within countries. Queen cells are normally transported in specially designed portable incubators to maintain a temperature of about 34^{o}C, since pupae will die if exposed to lower temperatures for prolonged periods.

2.1.3 Eggs

Honey bee eggs are a minor and insignificant form of transfer of *Apis mellifera* genetic material. There is no routine trade in honey bee eggs, and they do not appear to be used by bee breeding units to transfer genetic material over long distances.

Direct transfer of eggs from worker cells into queen cells using instruments such as forceps is impractical because honey bee eggs are very fragile (Weiss, 1983a). Commercial devices have been developed to encourage the queen to lay eggs in artificial cells that can then be transferred individually for queen rearing purposes.

There is no record of ova (the mature reproductive cell while still within the queen bee’s ovaries) being used for transfer of *A. mellifera* genetic material.

Honey bee eggs can remain viable for up to three days provided the egg at time of removal from the colony is at least 1.5 days old (Weiss, 1983b). Eggs can withstand temperatures as low as 15˚C, and have been successfully mailed long distances.

2.1.4 Semen

Semen collected from honey bee drones is a minor, although important, form of transfer of *Apis mellifera* genetic material. No routine trade exists in this commodity, although honey bee semen is used in honey bee breeding programmes, and is sometimes sent between breeding units within or between countries.

The semen is collected by everting and ejaculating mature honey bee drones and then taking up the semen that has been deposited on the exposed genitalia using a specially constructed syringe (Laidlaw, 1977). The semen is held directly in the syringe or transferred into a capillary tube (Harbo, 1985). In most cases, the semen is held in a buffered saline or 'Kiev' citrate solution, with an antibiotic such as sulphanilamide to protect the semen from bacterial contamination (Ruttner, 1976; Laidlaw, 1977; Harbo, 1985). Semen is generally collected from a number of drones, and is sometimes homogeneously mixed (Moritz, 1983).

Research has been carried out in an attempt to prolong the storage period of honey bee semen, including the use of freezing techniques (Harbo, 1983; Cobey, 1983). However, rapid freezing and thawing of honey bee semen causes significant decreases in cell viability compared to fresh semen (Peng et al, 1992). Homogeneous mixing of semen also significantly decreases viability (Collins, 2000a) whereas unmixed semen retains its viability remarkably over time, with 79.5% viability after six months (Collins, 2000b). Honey bee semen is therefore generally stored at room temperature in an unmixed form. No significant loss of viable spermatozoa was found over a period of six weeks with honey bee semen held at either 12^{o}C or 25^{o}C (Collins, 2000c).

2.2 Methodology

The methodology used in this risk analysis follows the guidelines in Section 1.3 of the *International Animal Health Code of the Office International Des Epizooties* (OIE, 2002). In New Zealand, the OIE risk analysis framework is applied as described in *Import Risk Analysis Animals and Animal Products* (Murray, 2002). The process is shown in Figure 1.

The Hazard Identification process begins with the collation of a list of organisms associated with honey bees. The OIE list of bee diseases was used as a starting point, and other organisms were included for various reasons. In particular, as the OIE list does not include any bee viruses, a number of which are of concern to New Zealand. In addition a range of other bee disease-causing organisms (e.g., tropilaelaps), and undesirable genetic material (e.g., Africanised honey bees) were added.

Next, for each organism listed, the epidemiology is discussed, including a consideration of the following questions:

1) whether the various forms of *Apis mellifera* genetic material that could be imported could potentially act as a vehicle for the introduction of the organism,

2) whether it is exotic to New Zealand but likely to be present in exporting countries,

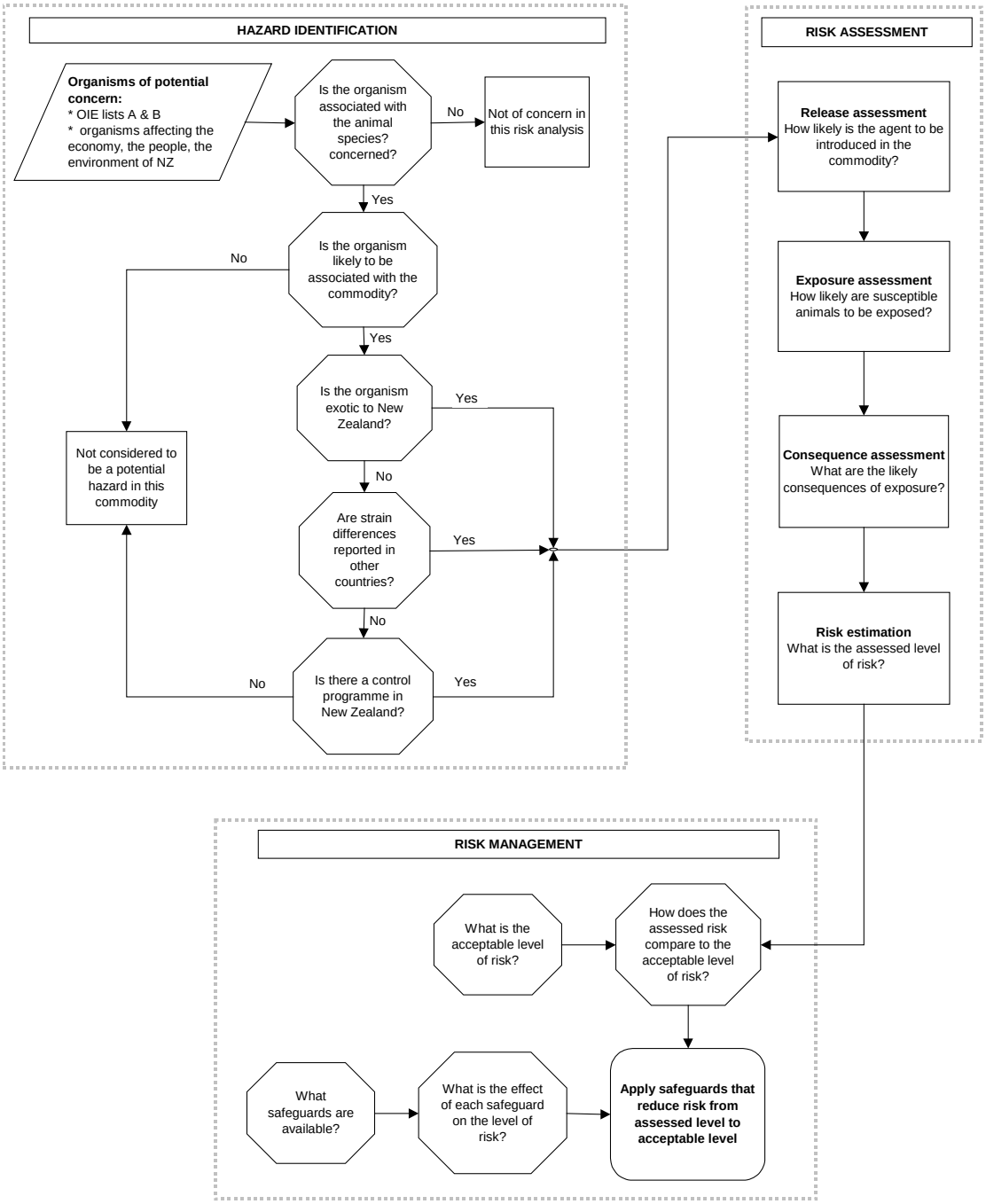
3) if it is present in New Zealand,

a) whether it is "under official control", which could be by government departments, by national or regional pest management strategies or by a small-scale programme, or

b) whether more virulent strains are known to exist in other countries.

For any organism, if the answers to questions one and either two or three are 'yes', it is classified as a potential hazard.

Figure 1. The risk analysis process.



For each potential hazard, the following analysis is carried out:

1) Risk Assessment

   a) Release assessment - the likelihood of the organism being imported in the commodity.

   b) Exposure assessment - the likelihood of animals or humans in New Zealand being exposed to the potential hazard.

   c) Consequence assessment - the consequences of entry, establishment or spread of the organism.

   d) Risk estimation - a conclusion on the risk posed by the organism based on the release, exposure and consequence assessments. If the risk estimate is non-negligible, then the organism is classified as a hazard.

2) Risk management

   a) Risk evaluation - a determination is made as to whether sanitary measures are necessary.

   b) Option evaluation - identify the options available for managing the risk, and consider risk reduction effects.

   c) Recommended measures - the recommendation of the appropriate option or combination of options that achieve a negligible likelihood of entry, spread or establishment, while minimising negative trade effects.

Table 1 lists the organisms that are considered in this risk analysis, together with some of the key information pertaining to each organism. Further details, including the results of the hazards identification, the risk assessment and the recommended risk management measures, can be found in the chapters on the individual agents.

Table 1: Organisms Considered in this risk analysis

Common Name/ Disease	**Chapter**	**Scientific Name**	**Exotic?**	**OIE List?**	**Under Official Control or Unwanted Organism?**	**More Virulent Strains Overseas?**
Acute paralysis virus	3	Acute paralysis virus	No	No	No	No
Africanised honey bee	39	*Apis mellifera scutellata* and its hybrids	Yes (3)	No	Unwanted	n/a
American foulbrood	20	*Paenibacillus larvae larvae*	No	Yes	Official control	No (4)
Amoeba disease	36	*Malpighamoeba mellificae*	No	No	No	No
Apis iridescent virus	4	*Apis* iridescent virus	Yes (2)	No	No	n/a
Arkansas bee virus	5	Arkansas bee virus	Yes (2)	No	No	n/a
Bee louse	28	*Braula coeca*	Yes (2)	No	Unwanted	n/a
Bee paralysis	6	Chronic paralysis virus	No	No	No	No
Bee virus X	7	Bee virus X	No	No	No	No
Bee virus Y	8	Bee virus Y	No	No	No	No

Berkeley bee virus	9	Berkeley bee virus	Yes (2)	No	No	n/a
Black queen cell	10	Black queen cell virus	No	No	No	No
Cape honey bee	40	*Apis mellifera capensis*	Yes (2)	No	Unwanted	n/a
Chalkbrood	26	*Ascosphaera apis*	No	No	No	Yes (5)
Chronic paralysis associate virus	11	Chronic paralysis associate virus	No	No	No	No
Cloudy wing virus	12	Cloudy wing virus	No	No	No	No
Deformed wing virus	12	Deformed wing virus	Yes (2)	No	No	n/a
Egypt bee virus	12	Egypt bee virus	Yes (2)	No	No	n/a
European foulbrood	21	*Melissococcus plutonius*	Yes (3)	Yes	Unwanted	n/a
External acarine mites	29	*Acarapis dorsalis, A. externus*	No	No	No	No
Filamentous virus	15	Filamentous virus	No	No	No	No
Gregarine disease	37	*Gregarinidae*	Yes (2)	No	No	n/a
Honey bee races (1)	41	*Apis mellifera carnica, A. m. caucasica*	Yes (2)	No	No	n/a
Honey bees	42	*Apis spp. other than A. mellifera*	Yes (2)	No	Unwanted	n/a
Kashmir bee virus	16	Kashmir bee virus	No	No	No	No
Nosema	38	*Nosema apis*	No	Yes	No	No
Powdery scale disease	23	*Paenibacillus larvae pulvifaciens*	Yes (2)	No	No	n/a
Sacbrood	17	Sacbrood virus	No	No	No	No
Septicaemia	24	*Pseudomonas aeruginosa*	No	No	No	No
Slow paralysis virus	18	Slow paralysis virus	Yes (2)	No	No	n/a
Small hive beetle	30	*Aethina tumida*	Yes (2)	No	Unwanted	n/a
Spiroplasmas	25	*Spiroplasma melliferum, S. apis*	Yes (2)	No	No	n/a
Stonebrood	27	*Aspergillus* spp.	No	No	No	No
Thai sacbrood	19	*Thai sacbrood virus*	Yes (2)	No	No	n/a
Tracheal mite	31	Acarapis woodi	Yes (3)	Yes	Unwanted	n/a
Tropilaelaps	32	*Tropilaelaps clareae, T. koenigerum*	Yes (3)	No	Unwanted	n/a
Varroa	33	*Varroa destructor*	No	Yes	Official control	No (6)
Varroa	34	*Varroa jacobsoni, V. underwoodi, V. rindereri, Euvarroa sinhai, E. wongsirii*	Yes (3)	No	Unwanted	n/a
Wax moth (greater & lesser)	35	*Galleria mellonella; Achroia grisella*	No	No	No	No
Paenibacillus alvei	22	*Paenibacillus alvei*	Yes (3)	No	No	n/a

n/a = for exotic organisms, the question of more virulent strains overseas does not arise
Note 1 – other than Africanised honey bees and the Cape honey bee
Note 2 – not reported
Note 3 – not found during surveys
Note 4 – strains resistant to oxytetracycline are present overseas
Note 5 – limited evidence of strain variation in virulent of *Ascosphaera apis*, but no evidence of direct link to severity of chalkbrood
Note 6 – strains resistant to various miticides are present overseas

2.3 Uncertainty

For many honey bee pathogens, and in particular for honey bee viruses, the risk analysis poses particular problems as a result of the generally limited information that is available in the scientific literature.

In the Hazard Identification process outlined above, Question 2 may be difficult to answer objectively for honey bee viruses, since records of particular viruses often represent the location of individual research workers rather than the actual distribution of the organism (Allen and Ball, 1996).

For most of the honey bee viruses, further difficulties are encountered in the release assessment step. Indeed, a detailed search of agricultural research databases failed to find any reports of honey bee viruses being contained in honey bee semen or eggs. However, almost all bee viruses are non-enveloped and non-occluded single-stranded RNA viruses that do not remain infective for long outside the body of their host (Ball, 1999), and a rapid loss of infectivity has been shown in experiments using dead larvae killed by sacbrood (Bailey, 1976), and also in work carried out on Kashmir bee virus (Anderson and Gibbs, 1988). White showed in 1913 that sacbrood virus is killed by prolonged exposure at 30-35^{o}C (Bailey, 1976), and Kashmir bee virus is very susceptible to proteolytic modification (Bailey et al, 1979). Thus, bee viruses are generally considered to be very vulnerable outside the host, and unless there is information to the contrary, this risk analysis assumes that any viruses that may be present in semen at the time of collection will be quickly inactivated.

However, elsewhere when such uncertainty is encountered, a precautionary approach is adopted, in consideration of the available scientific evidence. Where assumptions are necessary in order to reach conclusions, these are made explicitly.

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3. ACUTE PARALYSIS VIRUS

3.1 Hazard Identification

3.1.1 Aetiologic Agent: Acute paralysis virus.

3.1.2 OIE List: None.

3.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

3.1.4 Epidemiology

Acute paralysis virus is a virus found in *Apis mellifera*. The virus is generally present as an inapparent infection in adult honey bees (Bailey et al, 1963). However, it has been shown to kill both adult bees and brood in colonies infested with *Varroa destructor* (Ball and Allen, 1988). It appears that the mite induces replication of the virus when the mite feeds on virus-infected bees. It is not known what activates the latent infection of acute paralysis virus when in association with *V. destructor* (Ball, 1994). Mites can also act as a vector in the spread of the virus from bee to bee (Ball, 1989). Acute paralysis has been suggested as one of the causes of parasitic mite syndrome, although not all colonies showing the syndrome have been found to have the virus (Hung et al, 1996).

Honey bee larvae can also become infected with the virus by ingesting food contaminated with viral particles secreted by infected nurse bees (Ball and Allen, 1988).

Acute paralysis virus has been found in many parts of the world, including New Zealand (Allen and Ball, 1996; Anderson, 1988).

3.1.5 Conclusion

Acute paralysis virus is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore acute paralysis virus is not classified as a potential hazard for the purposes of this analysis.

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4. APIS IRIDESCENT VIRUS

4.1 Hazard Identification

4.1.1 Aetiologic Agent: *Apis* iridescent virus.

4.1.2 OIE List: None.

4.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

4.1.4 Epidemiology

Apis iridescent virus causes clustering disease in *Apis cerana* colonies (Bailey and Ball, 1978). However, although the virus readily multiplies in *A. mellifera* in the laboratory (Bailey et al, 1976), neither the disease nor the virus have been reported in *A. mellifera* in nature.

Apis iridescent virus has been found only in *A. cerana* and has been reported only from Kashmir and Northern India (Ball and Bailey, 1997).

4.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, *Apis* iridescent virus must be classified as a potential hazard for the purposes of this analysis.

4.2 Risk Assessment

4.2.1 Release Assessment

To become infected or contaminated, the commodity would have to be in contact with a colony that is already infected with *Apis* iridescent virus. Since the virus has not been found in *Apis mellifera* in nature, the commodity would have to come into contact with *A. cerana*. Since the commodities are all forms of *A. mellifera* genetic material, and since *A. cerana* is unable to mate *with A. mellifera* or co-exist in *A. mellifera* colonies (Koeniger and Koeniger, 2000), the only chance of contamination would be through *A. cerana* worker bees coming into contact with the commodity during robbing of the donor colony, or through momentary contact between *A. cerana* and *A. mellifera* workers during foraging. The likelihood of contamination or infection of the commodity via these routes is negligible.

Apis iridescent virus has been reported only in India. Freedom from the organism is probable for consignments originating from outside Asia where *A. cerana* is not present.

4.2.2 Exposure Assessment

Since there is no record of natural infection of the virus in *A. mellifera*, the likelihood of the virus becoming established in *A. mellifera* if it were introduced is negligible.

4.2.3 Consequence Assessment

Since the disease caused by *Apis* iridescent virus has been reported only in *A. cerana*, the consequences of the virus being introduced into New Zealand are negligible, since *A. cerana* is not present. There are no other likely consequences of the virus entering New Zealand, including no likely effects on native insects, since honey bee viruses cannot be cultivated in other insects or in insect cell tissue culture (Ball, 1999).

The consequence assessment is therefore negligible.

4.2.4 Risk Estimation

The likelihood of contamination or infection of commodities is negligible. The probability of establishment of the organism in New Zealand is also negligible, as is the likelihood of any significant consequences resulting from that establishment. The risk estimation is therefore negligible.

4.3 Risk Management

4.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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5. ARKANSAS BEE VIRUS

5.1 Hazard Identification

5.1.1 Aetiologic Agent: Arkansas bee virus.

5.1.2 OIE List: None.

5.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

5.1.4 Epidemiology

Arkansas bee virus is a little-known virus found in *Apis mellifera* which has not been reported outside the United States. The virus was originally reported in Arkansas when apparently healthy bees were injected with extracts of pollen loads taken from foraging bees (Bailey and Woods, 1974). The virus has also been found in bees in California. Adult bees injected with the virus die in about 14 days, but show no other outward signs of disease (Bailey and Woods, 1974). Arkansas bee virus has been isolated from honey bee pupae infected with Berkeley bee virus (Lommel et al, 1985).

5.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, Arkansas bee virus must be classified as a potential hazard for the purposes of this analysis.

5.2 Risk Assessment

5.2.1 Release Assessment

To become infected or contaminated, the commodity would have to be in contact with a colony that is already infected with Arkansas bee virus, or be visited by a foraging bee infected with the virus.

The likelihood of contamination is unknown, but it is assumed that if the donor colony has the virus, then the commodities are also likely to have the virus.

Although no work has been done on degradation and loss of infectivity of Arkansas bee virus *per se*, because of the close similarity in physical characteristics between almost all honey bee viruses (Ball, 1999), it is unlikely that honey bee semen stored away from honey bees would carry infective levels of the virus for any significant length of time[1]. It is unclear, however, whether this is also the case for eggs.

Since the virus has been found in adult bees and pupae, it is assumed that adult queen bees and adult queen bees emerging from queen cells could carry the virus.

[1] See section 2.3 for a discussion of the uncertainty surrounding this issue.

Arkansas bee virus has so far been reported only in the United States, so if a consignment of queen bees, queen cells or eggs comes from that country then the likelihood of release is non-negligible.

5.2.2 Exposure Assessment

There is no information on the natural means of transmission of Arkansas bee virus from bee to bee, although experimental infection is through inoculation of adult bees. However, because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

5.2.3 Consequence Assessment

Arkansas bee virus has not been associated with any production losses or other significant adverse effects in honey bee colonies, irrespective of whether the colonies have infestations of haemolymph-feeding parasites such as varroa or tracheal mite. . It is therefore unlikely that the virus would have any such effects if introduced into New Zealand. The virus is also unlikely to result in justified restrictions on bee exports from New Zealand since there are no official control programmes for honey bee viruses anywhere in the world.

Since there are no surveillance programmes for honey bee viruses in New Zealand, it is unlikely that the virus would be detected until it became well established.

Arkansas bee virus is unlikely to have any effects on New Zealand native insects since honey bee viruses cannot be cultivated in other insects or in insect cell tissue culture (Ball, 1999).

The consequence assessment is therefore negligible.

5.2.4 Risk Estimation

The likelihood of contamination or infection of commodities coming from a colony containing the virus is assumed to be high. The likelihood of establishment of the organism in New Zealand via these commodity is non-negligible. However, the likelihood of any significant consequences resulting from that establishment is negligible. The risk is therefore considered to be negligible.

5.3 Risk Management

5.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

References

Bailey L, Woods RD. Three previously undescribed viruses from the honey bee. *Journal of General Virology* 25, 175-186, 1974

Ball BV. An introduction to viruses and techniques for their identification and characterisation. In: Colin ME, Ball BV, Kilani M (eds*). Bee Disease Diagnosis* Pp 69-80. Centre International de Hautes Etudes Agronomiques Mediterraneennes, Zaragoza, 1999

Lommel SA, Morris TJ, Pinnock DE. Characterisation of nucleic acids associated with Arkansas bee virus. *Intervirology* 23, 199-207, 1985

6. BEE PARALYSIS

6.1 Hazard Identification

6.1.1 Aetiologic Agent: Chronic paralysis virus.

6.1.2 OIE List: None.

6.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

6.1.4 Epidemiology

Bee paralysis is a disease of adult *Apis mellifera* caused by the chronic paralysis virus. Although symptoms of bee paralysis have been described for over 100 years, the cause of the disease was not identified until 1963 (Bailey et al, 1963).

The disease has two distinct sets of symptoms (Bailey, 1975). In the first, bees are observed with abnormal trembling of both the wings and body. The bees also often have bloated abdomens and wings unhooked at the hammuli. Heavily infected colonies can suddenly collapse, with large numbers of dead bees found at the entrance (Bailey, 1969).

The second set of symptoms is known as "hairless black" disease, because the thorax and abdomen of affected bees are denuded of hair, giving the bees both a shiny and blacker appearance. The hair removal is the result of other bees pulling at the affected bees when they enter the colony. Affected bees die within a few days (Drum and Rothenbuhler, 1983).

Chronic paralysis virus has a world-wide distribution (Allen and Ball, 1996), and is present in New Zealand (Anderson, 1988). Susceptibility to bee paralysis has been shown to be linked to hereditary factors (Kulincevic and Rothenbuhler, 1975). The incidence of bee paralysis is typically quite low (Bailey and Ball, 1991).

6.1.5 Conclusion

Chronic paralysis virus is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore chronic paralysis virus is not classified as a potential hazard for the purposes of this analysis.

References

Allen M, Ball B. The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162, 1996

Anderson DL. Pathologist report. *The New Zealand Beekeeper* 199, 12-15, 1988

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Drum NH, Rothenbuhler WC. Non-stinging aggressive responses of worker honey bees to hive mates, intruder bees and bees affected with chronic bee paralysis. *Journal of Apicultural Research* 22, 256-260, 1983

Kulincevic JM, Rothenbuhler WC. Selection for resistance and susceptibility to hairless black syndrome in the honey bee. *Journal of Invertebrate Pathology* 25, 289-295, 1975

7. BEE VIRUS X

7.1 Hazard Identification

7.1.1 Aetiologic Agent: Bee virus X.

7.1.2 OIE List: None.

7.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

7.1.4 Epidemiology

Bee virus X is a virus found in *Apis mellifera*. The virus has experimentally been shown to multiply in the alimentary canal of adult bees when they have consumed viral particles, but not when injected into bees' haemolymph. It may therefore be restricted to the bee's alimentary canal (Ball and Bailey, 1997). Bee virus X has been found in dead bees in association with the protozoan *Malphighamoeba mellificae*, but multiplies equally as well in the absence of the organism (Bailey et al, 1983). Bee virus X shortens the life of adult bees at a rate similar to *M. mellificae*, and during winter the virus accelerates the death of bees infected with the protozoan (Ball and Bailey, 1997).

Bee virus X has been reported in Europe, Australasia, Argentina, Canada and Iran (Allen and Ball, 1996). Bee virus X has been found in New Zealand (Anderson, 1988).

7.1.5 Conclusion

Bee virus X is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore bee virus X is not classified as a potential hazard for the purposes of this analysis.

References

Allen, M, Ball B. The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162, 1996

Anderson DL. Pathologist report. *The New Zealand Beekeeper* 199, 12-15, 1988

Bailey L, Ball BV, Perry JN. Association of viruses with two protozoan pathogens of the honey bee. *Annals of Applied Biology* 103, 13-20, 1983

Ball BV, Bailey L. Viruses. In: Morse R, Flottum K (eds). *Honey Bee Pests, Predators, and Diseases* Third Edition. Pp 11-33. AI Root, Ohio, 1997

8. BEE VIRUS Y

8.1 Hazard Identification

8.1.1 Aetiologic Agent: Bee virus Y.

8.1.2 OIE List: None.

8.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

8.1.4 Epidemiology

Bee virus Y is a virus found in *Apis mellifera*. The virus multiplies when viral particles are eaten by adult bees, but not when injected into their haemolymph, so it may be restricted to the bee's alimentary canal. The virus only multiplies in the alimentary canal of adult bees when *Nosema apis* is present. However, there are no known symptoms of viral infection (Ball and Bailey, 1997). Over-wintering colonies show significantly greater bee losses when infected with the virus and *N. apis* than with *N. apis* alone (Bailey et al, 1983).

Bee virus Y has been reported in Europe, North America and Australasia (Allen and Ball, 1996), including New Zealand (Anderson, 1988).

8.1.5 Conclusion

Bee virus Y is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore Bee virus Y is not classified as a potential hazard for the purposes of this analysis.

References

Allen M, Ball B. The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162, 1996

Anderson DL. Pathologist report. *The New Zealand Beekeeper* 199, 12-15, 1988

Bailey L, Ball BV, Perry JN. Association of viruses with two protozoan pathogens of the honey bee. *Annals of Applied Biology* 103, 13-20, 1983

Ball BV, Bailey L. Viruses. In: Morse R, Flottum K (eds.) *Honey Bee Pests, Predators, and Diseases* Third Edition. Third Edition. Pp 11-32. AI Root, Ohio, 1997

9. BERKELEY BEE VIRUS

9.1 Hazard Identification

9.1.1 Aetiologic Agent: Berkeley bee virus.

9.1.2 OIE List: None.

9.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

9.1.4 Epidemiology

Berkeley bee virus is a virus found in *Apis mellifera*. It was identified in the original isolate of Arkansas bee virus and in Californian bees, but is not related to any other known bee virus (Lommel et al, 1985). Nothing is known about its effects on bees or whether it can multiply without being associated with Arkansas bee virus (Ball and Bailey, 1997).

Berkeley bee virus has not been reported outside the United States.

9.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, Berkeley bee virus must be classified as a potential hazard for the purposes of this analysis.

9.2 Risk Assessment

9.2.1 Release Assessment

To become infected or contaminated, the commodity would have to be in contact with a colony that is already infected with Berkeley bee virus or be visited by a foraging bee infected with the virus.

The likelihood of contamination is unknown, but it is assumed that if the donor colony has the virus, then the commodities are also likely to have the virus.

Although no work has been done on degradation and loss of infectivity of Berkeley bee virus *per se*, because of the close similarity in physical characteristics between almost all honey bee viruses (Ball, 1999), it is unlikely that honey bee semen stored away from honey bees would carry infective levels of the virus for any significant length of time[1]. It is unclear, however, whether this is also the case for eggs.

Since the virus has been found in adult bees and pupae, it is assumed that adult queen bees and adult queen bees emerging from queen cells could carry the virus.

Berkeley bee virus has so far been reported only in the United States, so if the consignment of queen bees, queen cells or eggs comes from that country then the likelihood of release is non-negligible.

[1] See section 2.3 for a discussion of the uncertainty surrounding this issue.

9.2.2 Exposure Assessment

There is no information on the natural means of transmission of Berkeley bee virus from bee to bee, although experimental infection is through inoculation of adult bees. However, because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

9.2.3 Consequence Assessment

Berkeley bee virus has not been associated with any production losses or other significant adverse effects in honey bee colonies, irrespective of whether the colonies have infestations of haemolymph-feeding parasites such as varroa or tracheal mite. . It is therefore unlikely that the virus would have any such effects if introduced into New Zealand. The virus is also unlikely to result in justified restrictions on bee exports from New Zealand since there are no official control programmes for honey bee viruses anywhere in the world.

Since there are no surveillance programmes for honey bee viruses in New Zealand, it is unlikely that the virus would be detected until it became well established.

Berkeley bee virus is unlikely to have any effects on New Zealand native insects since honey bee viruses cannot be cultivated in other insects or in insect cell tissue culture (Ball, 1999).

The consequence assessment is therefore negligible.

9.2.4 Risk Estimation

The likelihood of contamination or infection of commodities coming from a colony containing the virus is assumed to be high. The likelihood of establishment of the organism in New Zealand via these commodity is non-negligible. However, the likelihood of any significant consequences resulting from that establishment is negligible. The risk is therefore considered to be negligible.

9.3 Risk Management

9.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

References

Ball BV, Bailey L. Viruses. In: Morse R, Flottum K (eds.) *Honey Bee Pests, Predators, and Diseases* Third Edition. Pp 11-32. AI Root, Ohio, 1997

Lommel SA, Morris TJ, Pinnock DE. Characterisation of nucleic acids associated with Arkansas bee virus. *Intervirology* 23, 199-207, 1985

10. BLACK QUEEN CELL

10.1 Hazard Identification

10.1.1 Aetiologic Agent: Black queen cell virus.

10.1.2 OIE List: None.

10.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

10.1.4 Epidemiology

Black queen cell is a disease of *Apis mellifera* queen brood caused by the black queen cell virus. The queen dies in the prepupal or pupal stage, and the dead brood changes the cell wall of the queen cell to dark brown or black. The dead brood contains many particles of the virus. The disease is most noticeable when large numbers of queen cells are produced for queen rearing purposes (Bailey and Ball, 1991).

Black queen cell virus contrasts with sacbrood in that black queen cell virus does not multiply easily when fed to worker larvae, adult worker bees or drones, or when injected into adult worker bees or drones (Bailey and Woods, 1977). Black queen cell virus is, however, a common infection of field bees (Bailey and Ball, 1991).

Black queen cell virus appears to multiply only in worker bees that are also infected with *Nosema apis*. Over-wintering colonies show significantly greater bee losses when infected with the virus and *N. apis* than with *N. apis* alone (Bailey et al, 1983). The virus has been reported in Europe, North America and Australasia (Allen and Ball, 1996). Black queen cell virus has been found in New Zealand (Anderson, 1988).

10.1.5 Conclusion

Black queen cell virus is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore black queen cell virus is not classified as a potential hazard for the purposes of this analysis.

References

Allen M, Ball B. The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162, 1996

Anderson DL. Pathologist report. *The New Zealand Beekeeper* 199, 12-15, 1988

Bailey L, Ball BV. *Honey Bee Pathology*. Academic Press, London, 1991

Bailey L, Ball BV, Perry JN. Association of viruses with two protozoan pathogens of the honey bee. *Annals of Applied Biology* 103, 13-20, 1983

Bailey L, Woods RD. Two more small RNA viruses from honey bees and further observations on sacbrood and acute bee paralysis viruses. *Journal of General Virology* 37, 175-182, 1977

11. CHRONIC PARALYSIS ASSOCIATE VIRUS

11.1 Hazard Identification

11.1.1 Aetiologic Agent: Chronic paralysis associate virus.

11.1.2 OIE List: None.

11.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

11.1.4 Epidemiology

Chronic paralysis associate virus is a virus found in *Apis mellifera*. The virus is always associated with chronic paralysis virus but is serologically distinct. It does not multiply when injected alone into bees, and is probably therefore a satellite of chronic paralysis virus, inhibiting multiplication of that virus (Ball et al, 1985). Chronic paralysis associate virus may be of significance in the defence mechanisms of honey bees against chronic paralysis virus (Bailey and Ball, 1991). It is more evident in queens than in worker bees (Bailey et al, 1980).

No information could be obtained regarding the distribution of chronic paralysis associate virus. Recent investigation suggests that the virus is present in New Zealand (Todd and Ball, 2003).

11.1.5 Conclusion

Chronic paralysis associate virus is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore chronic paralysis associate virus is not classified as a potential hazard for the purposes of this analysis.

References

Bailey L, Ball BV. *Honey Bee Pathology*. Academic Press, London, 1991

Bailey L, Ball BV, Carpenter JM, Woods RD. Small virus-like particles in honey bees associated with chronic paralysis virus and with a previously undescribed disease. *Journal of General Virology* 46, 149-155, 1980

Ball BV, Overton HA, Buck KW. Relationships between the multiplication of chronic bee paralysis virus and its associate particle. *Journal of General Virology* 66, 1423-1429, 1985

Todd J, Ball BV. Viruses in New Zealand bees. *Bee Craft* 85, 12-13, 2003

12. CLOUDY WING VIRUS

12.1 Hazard Identification

12.1.1 Aetiologic Agent: Cloudy wing virus.

12.1.2 OIE List: None.

12.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

12.1.4 Epidemiology

Cloudy wing virus is a virus found in *Apis mellifera*. The virus produces an opaqueness in the wings of adult bees when the bees are heavily infected. The opaqueness is caused by crystalline structures of viral particles between muscle fibres. Heavy infection results in bee mortality (Bailey and Ball, 1991).

Honey bees could not be infected experimentally with cloudy wing virus by feeding adult bees with the virus or injecting it into their haemolymph (Ball and Bailey, 1997). Natural infection may occur between bees over short distances in the air (Bailey et al, 1980). Heavy infection can cause colony death (Bailey and Ball, 1991). There is no seasonal incidence of infection (Bailey et al, 1983).

Cloudy wing virus has been reported in Europe, North America and Australasia (Allen and Ball, 1996), including New Zealand (Anderson, 1988).

12.1.5 Conclusion

Cloudy wing virus is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore cloudy wing virus is not classified as a potential hazard for the purposes of this analysis.

References

Allen M, Ball B. The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162, 1996

Anderson DL. Pathologist report. *The New Zealand Beekeeper* 199, 12-15, 1988

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Ball BV, Bailey L. Viruses. In: Morse R, Flottum K (eds). *Honey Bee Pests, Predators, and Diseases* Third Edition. Pp 11-33. AI Root, Ohio, 1997

13. DEFORMED WING VIRUS

13.1 Hazard Identification

13.1.1 Aetiologic Agent: Deformed wing virus.

13.1.2 OIE List: None.

13.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

13.1.4 Epidemiology

Deformed wing virus is a virus found in *Apis mellifera.* Pupae infected with deformed wing virus at the white-eye stage of development survive to emergence but have poorly developed wings and soon die (Bailey and Ball, 1991). However, the virus multiplies slowly, and brood infected at an earlier stage emerge normally, although their productivity and lifespan are reduced (Ball, 1993).

Although it has been reported to kill honey bees in the absence of varroa in Britain and South Africa, the virus is usually found in *A. mellifera* colonies infested with the mite, where it is associated with mortality of both adult bees and brood (Bailey and Ball, 1991).

Little information is available on the incidence of the virus in the absence of varroa. The virus was, however, detected serologically in 69% of dead bee samples collected from varroa-infested colonies in midsummer in Poland (Topolska et al, 1995), and in over 90% of varroa-infested colonies in England (Ball, 2001). Varroa has also been implicated in the spread of deformed wing virus. The virus has been detected in *V. destructor* (*jacobsoni)* and the ability of varroa to transmit the virus has been demonstrated experimentally (Bowen-Walker et al, 1999).

Deformed wing virus has been recorded in *A. mellifera* from many European, Middle Eastern, North African and Asian countries, and in South Africa. It has not been reported from North or South America, the South Pacific, Australia or New Zealand (Allen and Ball, 1996). A preliminary investigation of bee and varroa samples from 32 New Zealand honey bee colonies did not detect deformed wing virus (Todd and Ball, 2002).

13.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, deformed wing virus must be classified as a potential hazard for the purposes of this analysis.

13.2 Risk Assessment

13.2.1 Release Assessment

To become infected or contaminated, the commodity would have to be in contact with a colony that is already infected with deformed wing virus, or be visited by a foraging bee infected with the virus, or become infested with a varroa mite carrying the virus.

The likelihood of contamination is unknown, but it is assumed that if the donor colony has the virus, then the commodities are also likely to have the virus.

Although no work has been done on degradation and loss of infectivity of deformed wing virus *per se*, because of the close similarity in physical characteristics between almost all honey bee viruses (Ball, 1999), it is unlikely that honey bee semen stored away from honey bees would carry infective levels of the virus for any significant length of time[1]. It is unclear, however, whether this is also the case for eggs.

Since the virus has been found in adult bees and pupae, it is assumed that adult queen bees and adult queen bees emerging from queen cells could carry the virus.

Deformed wing virus has so far been reported in the Europe, Asia, the Middle East and Africa, so if a consignment of adult queen bees, queen cells or eggs comes from any of those areas then the likelihood of release is non-negligible.

13.2.2 Exposure Assessment

The only information on the natural means of transmission of deformed wing virus from bee to bee is via varroa, although it is presumed to also be able to spread between bees in the absence of varroa since infections causing mortality have been reported in areas where the mite was not present. Because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

13.2.3 Consequence Assessment

Deformed wing virus has been associated with bee mortality, particularly in the presence of varroa. Reports from several countries indicate that there is a significant link between deformed wing virus, varroa, and honey bee colony collapse (Martin et al, 1998; Nordstrom et al, 1999). While there are currently no published studies verifying a causal relationship between the virus and colony death, in the United Kingdom, where almost all varroa samples contain the deformed wing virus (Ball, 2001), it has been concluded that long term research carried out in that country has revealed that the virus is the cause of the majority of honey bee colony deaths ascribed to varroa (Martin et al, 2003). A computer simulation model has suggested that deformed wing virus provides an explanation for the lack of correlation observed between varroa mite numbers in a colony and colony collapse (Martin, 2001).

Colony collapse associated with varroa has a significant negative impact on both beekeeper incomes and the profitability of providing proper strength colonies for paid pollination services (Tew, 1999).

Deformed wing virus is unlikely to result in justified restrictions on bee exports from New Zealand since it has a widespread distribution and there are no official control programmes in place for it anywhere in the world.

[1] See section 2.3 for a discussion of the uncertainty surrounding this issue.

Since there are currently no surveillance programmes for honey bee viruses in New Zealand, it is unlikely that deformed wing virus would be detected until it became well established.

Deformed wing virus is unlikely to have any effects on New Zealand native insects since honey bee viruses cannot be cultivated in other insects or in insect cell tissue culture (Ball, 1999).

13.2.4 *Risk Estimation*

The likelihood of contamination or infection of queen bees, queen cells or eggs coming from a colony containing the deformed wing virus is assumed to be high. The likelihood of establishment of the organism in New Zealand via those commodities is non-negligible. Since there are significant consequences associated with the virus in overseas countries, it cannot be ruled out that similar consequences would also result in New Zealand if the virus became established. The risk for queen bees, queen cells and eggs is therefore considered to be non-negligible. However, the risk for semen is considered to be negligible.

13.3 Risk Management

13.3.1 Risk Evaluation

Since the risk estimate for deformed wing virus is considered to be non-negligible, sanitary measures would need to be employed to reduce the risks to a negligible level.

13.3.2 Option Evaluation

13.3.2.1 Risk management objective

The objective is to effectively manage the risk of deformed wing virus by ensuring the imported *Apis mellifera* genetic material does not carry the organism when given a biosecurity clearance in New Zealand.

13.3.2.2 Options available

The OIE Code does not include recommendations regarding any honey bee viruses.

No routine testing techniques appear to have been developed that give a high probability of determining whether live queen bees, queen cells or eggs contain infective amounts of deformed wing virus without destroying the commodity in the process. Therefore it is unclear how the use of a post-arrival quarantine facility (White and Rhodes, 1988) would effectively mitigate against release of the organism, since in all cases either the commodity itself (queen cells and eggs), or eggs produced from a nuclei colony containing the commodity (queen bees or queen cells), would finally be moved to a release area without verification of freedom from the organism.

The only remaining option is for the commodity (in the case of queen bees, queen cells or eggs) to come from a country or territory officially free of the virus. However, since there are no official control programmes for the organism anywhere in the world, and since the distribution of the organism is not well documented, this option is also not suitable.

13.3.2.3 Recommended Sanitary Measures

For honey bee queens, queen cells and eggs

Since there are no suitable measures for these commodities, their importation will not be permitted.

For semen

No sanitary measures required.

References

Allen M, Ball B. The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162, 1996

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Ball B. The damaging effects of *Varroa jacobsoni* infestation. In: Matheson E. (ed). *Living with Varroa* Pp 9-16. IBRA, Cardiff, 1993

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White B, Rhodes JW. Importation of queen bees - quarantine facility and procedures [in Australia]. In: White B, Rhodes JW (eds*). Bee Keeping in the Year 2000. Proceedings of the Second Australian and International Beekeeping Congress, Surfers Paradise, Gold Coast, Queensland, Australia, July 21-26* Pp 65-67. Federal Council of Australian Apiarists' Assn., Brisbane, 1988

14. EGYPT BEE VIRUS

14.1 Hazard Identification

14.1.1 Aetiologic Agent: Egypt bee virus.

14.1.2 OIE List: None.

14.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

14.1.4 Epidemiology

Egypt bee virus is a virus found in *Apis mellifera* that is distantly related serologically to deformed wing virus. Nothing is known of its natural history (Ball and Bailey, 1997). Young pupae injected with the virus die in about 7 or 8 days, but researchers have been unable to propagate the virus in adult bees (Bailey and Ball, 1991).

Egypt bee virus has been isolated from dead bees from Egypt (Bailey et al, 1979) and France (Chastel et al, 1990).

14.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, Egypt bee virus must be classified as a potential hazard for the purposes of this analysis.

14.2 Risk Assessment

14.2.1 Release Assessment

To become infected or contaminated, the commodity would have to be in contact with a colony that is already infected with Egypt bee virus, or be visited by a foraging bee infected with the virus.

The likelihood of contamination is unknown, but it is assumed that if the donor colony has the virus, then the commodities are also likely to have the virus.

Although no work has been done on degradation and loss of infectivity of Egypt bee virus *per se*, because of the close similarity in physical characteristics between almost all honey bee viruses (Ball, 1999), it is unlikely that honey bee semen stored away from honey bees would carry infective levels of the virus for any significant length of time[1]. It is unclear, however, whether this is also the case for eggs.

Since the virus has been found in adult bees and pupae, it is assumed that adult queen bees and adult queen bees emerging from queen cells could carry the virus.

[1] See section 2.3 for a discussion of the uncertainty surrounding this issue.

Egypt bee virus has been reported only in Egypt and France, so if a consignment of adult queen bees, queen cells or eggs comes from either of those countries then the likelihood of release is non-negligible.

14.2.2 Exposure Assessment

There is no information on the natural means of transmission of Egypt bee virus from bee to bee, although experimental infection is through inoculation of pupae. However, because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

14.2.3 Consequence Assessment

Egypt bee virus has a very limited distribution and has not been associated with any production losses or other significant adverse effects in honey bee colonies, irrespective of whether the colonies have infestations of haemolymph-feeding parasites such as varroa or tracheal mite. It is therefore unlikely that the virus would have any such effects if introduced into New Zealand. The virus is also unlikely to result in justified restrictions on bee exports from New Zealand since there are no official control programmes for honey bee viruses anywhere in the world.

Since there are no surveillance programmes for honey bee viruses in New Zealand, it is unlikely that the virus would be detected until it became well established.

Egypt bee virus is unlikely to have any effects on New Zealand native insects since honey bee viruses cannot be cultivated in other insects or in insect cell tissue culture (Ball, 1999).

The consequence assessment is therefore negligible.

14.2.4 Risk Estimation

The likelihood of contamination or infection of commodities coming from a colony containing the virus is assumed to be high. The likelihood of establishment of the organism in New Zealand via these commodity is non-negligible. However, the likelihood of any significant consequences resulting from that establishment is negligible. The risk is therefore considered to be negligible.

14.3 Risk Management

14.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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15. FILAMENTOUS VIRUS

15.1 Hazard Identification

15.1.1 Aetiologic Agent: Filamentous virus.

15.1.2 OIE List: None.

15.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

15.1.4 Epidemiology

Filamentous virus is a virus found in *Apis mellifera*. The virus replicates in the fat bodies and ovarian tissues of adult workers and queens. The infection results in the haemolymph of heavily infected bees taking on a milky white appearance, caused by large numbers of particles of the virus. No other symptoms have been identified (Ball and Bailey, 1997). The virus shows an annual multiplication cycle, with a peak in mid-spring and a trough in late summer (Bailey and Ball, 1991).

Like black queen cell virus and bee virus Y, filamentous virus multiplies in adult bees only when they are also infected with *Nosema apis*. Also similarly, bees infected with the virus and *N. apis* die in greater numbers in winter than those infected with *N. apis* alone, although the trend is not as significant as with black queen cell virus and bee virus Y (Bailey et al, 1983).

Filamentous virus was first identified in the United States (Clark, 1978). Filamentous virus has been found in North America, Australia, Europe, Russia and Japan (Ball and Bailey, 1997). The virus is present in New Zealand (Bailey et al, 1981).

15.1.5 Conclusion

Filamentous virus is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore filamentous virus is not classified as a potential hazard for the purposes of this analysis.

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16. KASHMIR BEE VIRUS

16.1 Hazard Identification

16.1.1 Aetiologic Agent: Kashmir bee virus.

16.1.2 OIE List: None.

16.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

16.1.4 Epidemiology

Kashmir bee virus was first detected from *Apis cerana* in India (Bailey and Woods, 1977). However, the virus is generally found as an inapparent infection in adult *A. mellifera* (Anderson and Gibbs, 1988). There is some evidence suggesting that bee mortality is caused by Kashmir bee virus in colonies infested with *Varroa destructor (jacobsoni)* (Hung et al, 1996). Virus multiplication is rapid when even small numbers of viral particles are injected into adult bees or pupae, resulting in death to the bee within three days (Ball and Bailey, 1997). Suggestions of Kashmir bee virus causing bee mortality without the presence of varroa have been made (Bailey and Ball, 1991; Sammataro, 1997), but no evidence has been presented to support this contention.

Closely related but serologically distinct strains of Kashmir bee virus have been detected in the United States (Bruce et al, 1995) and in Australia (Bailey et al, 1979). Strains of Kashmir bee virus found in Canada and Spain more closely resemble acute paralysis virus than previously identified strains of Kashmir bee virus (Allen and Ball, 1995).

Kashmir bee virus has been found in North America, Europe and Australasia, including New Zealand (Allen and Ball, 1996; Anderson, 1985).

16.1.5 Conclusion

Kashmir bee virus is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore Kashmir bee virus is not classified as a potential hazard for the purposes of this analysis.

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17. SACBROOD

17.1 Hazard Identification

17.1.1 Aetiologic Agent: Sacbrood virus.

17.1.2 OIE List: None.

17.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

17.1.4 Epidemiology

Sacbrood is a disease of *Apis mellifera* larvae caused by the sacbrood virus. Sacbrood virus may be present in young adult bees without causing obvious disease (Bailey, 1969). Infected bees pass the virus in food to young larvae, which then become infected and die in the prepupal stage (Bailey, 1969). Fluid accumulates in the larva, resulting in a distinct watery sac, while the body colour changes to grey-white and then yellow (Ball and Bailey, 1997). The larva finally dries out to a scale and turns dark brown to black.

Larvae fed the virus when more than two days old survive the infection and carry the virus as adults (Anderson and Gibbs, 1988). Adult bees infected with the virus show a change in behaviour, including a loss of appetite for pollen (Bailey, 1969). Adult worker lifespan and metabolic rate is also reduced by the infection (Bailey and Ball, 1991).

Sacbrood has a seasonal occurrence, with outbreaks often in the spring. The disease normally disappears spontaneously during summer (Ball, 1999). Sacbrood is the most common viral disease of bees reported (probably because of its easily identified symptoms), and occasionally results in substantial losses of brood in colonies (Dall, 1985).

Sacbrood has been reported in every continent where honey bees are kept and is present in New Zealand (Matheson, 1997).

17.1.5 Conclusion

Sacbrood virus is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore sacbrood virus is not classified as a potential hazard for the purposes of this analysis.

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18. SLOW PARALYSIS VIRUS

18.1 Hazard Identification

18.1.1 Aetiologic Agent: Slow paralysis virus.

18.1.2 OIE List: None.

18.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

18.1.4 Epidemiology

Slow paralysis virus is a virus found in *Apis mellifera*. The virus causes mortality after 12 days in adult bees when injected into haemolymph (Bailey, 1976). The virus has not been identified with a disease itself, but has been associated with adult bee mortality in colonies infested with *Varroa destructor* (Ball and Bailey, 1997). Nothing further is known of the virus's natural history (Ball and Bailey, 1997).

Slow paralysis virus has been recorded in Britain, Fiji and Western Samoa (Allen and Ball, 1996).

18.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, slow paralysis virus must be classified as a potential hazard for the purposes of this analysis.

18.2 Risk Assessment

18.2.1 Release Assessment

To become infected or contaminated, the commodity would have to be in contact with a colony that is already infected with slow paralysis virus, or be visited by a foraging bee infected with the virus, or become infested with a varroa mite carrying the virus.

The likelihood of contamination is unknown, but it is assumed that if the donor colony has the virus, then the commodities are also likely to have the virus.

Although no work has been done on degradation and loss of infectivity of slow paralysis virus *per se*, because of the close similarity in physical characteristics between almost all honey bee viruses (Ball, 1999), it is unlikely that honey bee semen stored away from honey bees would carry infective levels of the virus for any significant length of time[1]. It is unclear, however, whether this is also the case for eggs.

Since the virus has been found in adult bees, it is assumed that adult queen bees and adult queen bees emerging from queen cells could carry the virus.

[1] See section 2.3 for a discussion of the uncertainty surrounding this issue.

Slow paralysis virus has so far been reported in Britain, Fiji and Western Samoa, so if the consignment of adult queen bees, queen cells or eggs comes from any of those areas then the likelihood of release is non-negligible.

18.2.2 Exposure Assessment

There is no information on the natural transmission of slow paralysis virus. Infection in adult bees has been proven experimentally. However, because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

18.2.3 Consequence Assessment

While slow paralysis virus has been associated with bee mortality in the presence of varroa, no production losses or other significant adverse effects in honey bee colonies have been reported. It is likely that effects of the virus have not been isolated from effects of varroa and its associated parasitic mite syndrome (Hung et al, 1996). The probability is that if slow paralysis virus became established in New Zealand, effects from the virus would not be noticed by beekeepers as being significantly greater than the effects already being experienced due to varroa infestation. The virus is also unlikely to result in justified restrictions on bee exports from New Zealand since there are no official control programmes for honey bee viruses anywhere in the world.

Since there are no surveillance programmes for honey bee viruses in New Zealand, it is unlikely that the virus would be detected until it became well established.

Slow paralysis virus is unlikely to have any effects on New Zealand native insects since honey bee viruses cannot be cultivated in other insects or in insect cell tissue culture (Ball, 1999).

The consequence assessment is therefore negligible.

18.2.4 Risk Estimation

The likelihood of contamination or infection of commodities coming from a colony containing the virus is assumed to be high. The likelihood of establishment of the organism in New Zealand via these commodity is non-negligible. However, the likelihood of any significant consequences resulting from that establishment is negligible. The risk is therefore considered to be negligible.

18.3 Risk Management

18.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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19. THAI SACBROOD VIRUS

19.1 Hazard Identification

19.1.1 Aetiologic Agent: Thai sacbrood virus.

19.1.2 OIE List: None.

19.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

19.1.4 Epidemiology

Thai sacbrood virus is a virus found in *Apis cerana*. The virus is believed to be closely related to sacbrood virus, but has distinctive properties (Ball and Bailey, 1997). Thai sacbrood has been reported to cause severe brood mortality in *A. cerana* (Verma et al, 1990).

Although Thai sacbrood has been found to multiply in *A. mellifera* in the laboratory, it has not been reported to cause disease signs in *A. mellifera* in localities where the bee was in close proximity to *A. cerana* (Allen, 1995).

Thai sacbrood virus is widely distributed on *A. cerana* throughout Southeast Asia (Ball and Bailey, 1997).

19.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, Thai sacbrood virus must be classified as a potential hazard for the purposes of this analysis.

19.2 Risk Assessment

19.2.1 Release Assessment

To become infected or contaminated, the commodity would have to be in contact with a colony that is already infected with Thai sacbrood virus, or be visited by a foraging bee infected with the virus. However, since the virus has not been found in *Apis mellifera*, it is likely that the commodity would have to come into contact with *A. cerana*. Since the commodities are all forms of *A. mellifera* genetic material, and since *A. cerana* is unable to mate with *A. mellifera* or co-exist in *A. mellifera* colonies (Koeniger and Koeniger, 2000), the only chance of contamination would be through *A. cerana* worker bees coming into contact with the commodity during robbing of the donor colony, or through momentary contact between *A. cerana* and *A. mellifera* workers during foraging. The likelihood of contamination or infection of the commodity via these routes is negligible.

Although no work has been done on degradation and loss of infectivity of Thai sacbrood virus *per se*, because of the close similarity in physical characteristics between almost all honey bee viruses (Ball, 1999), it is unlikely that honey bee semen stored away from honey bees would

carry infective levels of the virus for any significant length of time[1]. It is unclear, however, whether this is also the case for eggs.

Since the virus has been found in larvae, it is assumed that queen cells could carry the virus. It is unknown whether adults carry the virus, although adult *A. mellifera* do carry sacbrood virus (Ball and Bailey, 1997).

Thai sacbrood virus is widely distributed throughout Asia on *A. cerana*. Freedom from the organism is likely for consignments originating from outside Asia where *A. cerana* is not present.

19.2.2 Exposure Assessment

Since there is no record of natural infection of the virus in *A. mellifera*, and since the likelihood of the commodities carrying the virus is negligible, the likelihood of the virus becoming established in *A. mellifera* in New Zealand is also negligible.

19.2.3 Consequence Assessment

Since the sacbrood disease caused by the virus has been reported only in *A. cerana*, there is little likelihood of this disease becoming established in New Zealand (since *A. cerana* is not present). Therefore, the consequences of its introduction as far as honey bees in New Zealand is concerned is negligible.

Thai sacbrood virus is unlikely to have any effects on New Zealand native insects since honey bee viruses cannot be cultivated in other insects or in insect cell tissue culture (Ball, 1999).

There are no other likely consequences of the virus entering New Zealand. Therefore the likelihood of any significant consequences resulting from its introduction is negligible.

19.2.4 Risk Estimation

The likelihood of contamination or infection of commodities is negligible. The likelihood of establishment of the organism in New Zealand is also negligible, as is the likelihood of any significant consequences resulting from that establishment. The risk is therefore considered to be negligible.

19.3 Risk Management

19.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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[1] See section 2.3 for a discussion of the uncertainty surrounding this issue.

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20. AMERICAN FOULBROOD

20.1 Hazard Identification

20.1.1 Aetiologic Agent: Family *Bacillaceae, Paenibacillus larvae* subsp. *larvae* (White) Heyndrickx et al.

20.1.2 OIE List: B.

20.1.3 New Zealand's Status: Present in New Zealand. Under official control.

20.1.4 Epidemiology

American foulbrood is a disease of *Apis mellifera* larvae and pupae caused by the spore-forming bacterium *Paenibacillus larvae* subsp. *larvae* (Bailey and Ball, 1991). Larvae become infected by ingesting spores contaminating their food (Woodrow, 1943). The number of spores required to infect a larva increases with larval age. As few as ten spores can infect 24 hour old larvae, whereas larger numbers are needed to infect larvae over two days old (Woodrow, 1943; Brodsgaard, 1998). The spores germinate soon after they enter the larval gut and penetrate the body cavity through the gut wall (Bailey and Ball, 1991). The infected larvae then quickly die and about 2500 million spores are formed (Sturtevant, 1932). Additional larvae are infected by bees performing house-cleaning duties (Bailey and Ball, 1991). *P. l. larvae* spores can remain viable for over 35 years (Haseman, 1961).

The progression of American foulbrood disease in honey bee colonies has been shown to follow three different scenarios, which occur in about equal proportions. Infection develops quickly resulting in colony death, disappears and does not reoccur, or disappears and then reappears in about three weeks with resulting colony death (Goodwin and Van Eaton, 1999). American foulbrood is one of the most significant bee diseases world-wide, and causes annual losses in the United States of over US$5 million (Shimanuki, 1997).

Control of American foulbrood disease is generally either through the prophylactic feeding of oxytetracycline to honey bee colonies, or the destruction by burning of colonies found with clinical symptoms, or the destruction of individual combs found with clinical symptoms (Matheson and Reid, 1992).

There are no reports of strains of *P. l. larvae* with differing pathogenicity. However, strains have been reported with varying resistance to oxytetracycline (Alippi, 1999; Miyagi et al, 2000). There is no evidence suggesting that any organisms other than *A. mellifera* are hosts of *P. l. larvae*. Apart from larvae and pupae of *A. mellifera*, very few media have been found that will induce germination or sporulation of the bacterium. These include unheated egg yolk, egg yolk with carrot extract and peptone, and glucose-peptone with thiamine and trace elements. However, in all cases an innoculum of many millions of spores is needed to start growth on these media (Bailey and Ball, 1991).

American foulbrood has been found on all continents and in most beekeeping countries, including New Zealand. It has not been found in parts of South America, most of Africa or on the Indian subcontinent (Matheson, 1997).

20.1.5 Conclusion

Although *P. l. larvae* is present in New Zealand, it is under an official control programme in the form of a National Pest Management Strategy under the Biosecurity Act 1993. Therefore *P. l. larvae* is classified as a potential hazard for the purposes of this analysis.

20.2 Risk Assessment

20.2.1 Release Assessment

To become infected with the organism or contaminated with spores, the commodity would have to come from a colony that is already infected with American foulbrood. Visitation of the commodity by foraging bees is unlikely to be a suitable pathway since spore transfer by this means would be insufficient to initiate infection.

The likelihood of infection or contamination occurring depends on whether the colony contains a sufficient population of *Paenibacillus larvae larvae.* Colonies with either clinical or sub-clinical infections would have sufficient spores for contamination to occur (Goodwin and Van Eaton, 1999).

Infected queen cells or mated queen bees are the only commodities likely to carry significant spores of the organism. It is unlikely that sufficient spores of *P. l. larvae* would be deposited on queen cells or eggs to initiate an infection in a colony receiving either of those commodities.

Honey bee semen is unlikely to contain spores of *P. l. larvae*, since semen is obtained from within the drone. The only possibility for contamination to occur would be by the insemination syringe momentarily touching the exoskeleton of a drone during eversion.

American foulbrood is present in many countries, so if a consignment of queen bees or queen cells comes from one of those countries then the likelihood of release is non-negligible.

20.2.2 Exposure Assessment

Queen insemination is not an exposure pathway, since spores of the organism must be fed to young larvae to create an infection. Therefore the likelihood of exposure via semen is negligible.

Spores of *P. l. larvae* have low infectivity. The lowest concentration of spores that have been fed to colonies and reported to become infected is 50 million spores/L of syrup (Goodwin et al, 1994; Sturtevant, 1932). The lowest number to create an infection is 5 million spores, fed in 100 mls of sugar solution (Goodwin et al, 1994).

Although queens have tested positive for *P. l. larvae* spores, and while it is theoretically possible for queen honey bees to transmit American foulbrood from one colony to another, it is unlikely that queens would carry enough spores to initiate an infection (Goodwin and Van Eaton, 1999). Where spore-carrying queens from infected colonies were intentionally placed in uninfected hives, they did not infect the new colonies (Wilson and Alzubaidy, 1975). However, honey used to make queen cage candy can contain infective doses of *P. l. larvae* spores (see Section 2.1.1 for further discussion on this issue).

Infected larvae and pupae contain large numbers of *P. l. larvae* spores (Goodwin and Van Eaton, 1999). An infected queen cell would have a high probability of transmission if placed in another honey bee colony.

Because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible. However, given the other information presented on exposure, the likelihood of exposure is assessed as low for queen bees, but high for queen cells.

20.2.3 Consequence Assessment

Although there are no reports of strains of *P. l. larvae* with differing pathogenicity, there are strains that are reported to have varying resistance to oxytetracycline. New Zealand has for the last 50 years had a policy of not feeding antibiotics for American foulbrood control and it is currently not permitted by the Biosecurity (National American Foulbrood Pest Management Strategy) Order 1998. It is unlikely that oxytetracycline will be fed in New Zealand for American foulbrood control. As long as New Zealand does not feed oxytetracycline, the importation of these strains should have no consequences.

The major direct consequence of importing *P. l. larvae* into New Zealand would be to any New Zealand colonies that might be infected by a contaminated consignment. The American Foulbrood National Pest Management Strategy requires colonies infected with the disease to be destroyed. Costs to beekeepers from the disease have been estimated at $2.9 million per annum (NBA, 1997). Direct costs associated with a hive lost to American foulbrood have been estimated at $325 (cost of hive, destruction costs, loss of production). There is also the risk that if contaminated consignments resulted in widespread distribution of *P. l. larvae*, then some infections might not be detected before the organism was spread through robbing to surrounding colonies.

Therefore the consequences of introduction are considered to be non-negligible.

20.2.4 Risk Estimation

The risk estimate for semen is negligible.

The likelihood of contamination of the commodities considered is limited to queen cells and queens. The likelihood of exposure and establishment of the organism in New Zealand is non-negligible. The likelihood of significant consequences resulting from that establishment is also non-negligible. As a result, the risk for American foulbrood is considered to be non-negligible and the organism is classified as a hazard.

20.3 Risk Management

20.3.1 Risk Evaluation

Since the risk estimate for American foul brood associated with queens or queen cells is non-negligible, and since *P. l. larvae* is under official control in New Zealand, risk management measures are warranted.

20.3.2 Option Evaluation

20.3.2.1 Risk management objective

Although New Zealand is justified in imposing risk management measures against American foul brood as a result of there being an official control program in place in this country, under the principle of non-discrimination covered in article 2.3 of the WTO Sanitary and Phytosanitary agreement, the measures imposed must not be greater than those achieved under the rules of the official control program. The relevant rule under the National Pest Management Strategy is rule 31(1) which prohibits the movement or sale of bee products from hives known or suspected to be clinically affected by American foul brood. Therefore, it is appropriate to impose measures on imported bee products to provide the same level of protection that would be achieved by the application of that rule.

20.3.2.2 Options available

The OIE *Code* includes recommendations for live bees regarding American foulbrood, which may be used as a basis for developing appropriate measures.

Testing techniques have been developed that give a high probability of determining whether a honey bee colony contains *P. l. larvae* (Goodwin and Van Eaton, 1999).

Although sterilisation by gamma radiation has been shown to kill *P. l. larvae* (Hornitzky, 1994), it is inappropriate in this case since all life stages of *A. mellifera* are killed by gamma radiation. The chemical disinfectants Vircon (90% for 10 minutes) and sodium hypochlorite (1% for 30 minutes) have been shown to deactivate *P. l. larvae* spores, although the activity is only contact in nature (Goodwin and Haine, 1998). Both disinfectants are toxic to bees.

20.3.2.3 Recommended sanitary measures

<u>For honey bee queens and queen cells</u>

Each consignment must be either:

1) from hives from a country or part of the territory of a country free from American foulbrood (see Appendix I), or

2) from hives sampled and found free of American foulbrood (culture of adult bees) within seven days of shipment.

<u>For semen and eggs</u>

No sanitary measures required.

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21. EUROPEAN FOULBROOD

21.1 Hazard Identification

21.1.1 Aetiologic Agent: Family *Enterococcaceae, Melissococcus plutonius* (White) Bailey and Collins.

21.1.2 OIE List: B.

21.1.3 New Zealand's Status: Exotic to New Zealand. Listed on the unwanted organisms register as a notifiable organism.

21.1.4 Epidemiology

European foulbrood is a disease of *Apis mellifera* larvae caused by the bacterium *Melissococcus plutonius* (Bailey, 1957). An infection is established when a larva ingests contaminated food and the bacteria begin to grow vigorously within the gut. The multiplying bacteria compete with the larva for food, creating a higher than normal demand for food provided by nurse bees (Shimanuki, 1997). The incubation period for the disease is four days (Bailey and Gibbs, 1962).

Larvae usually die when they are 4-5 days old, with the bacteria destroying the peritrophic membrane and then invading the intestinal epithelium (Tarr, 1938). Some infected larvae survive and the bacteria are discharged with the faeces on the wall of the brood cells (Bailey, 1959). The bacteria are removed by house-cleaning worker bees, which then act as a vector to contaminate larval food.

Clinical symptoms of European foulbrood are more likely when either the ratio of nurse bees to diseased larvae decreases for some reason, or nurse bees are recruited away from larval feeding by the demands of a nectar flow. When this imbalance occurs, infected larvae that have a higher than normal demand for food are not removed and visual signs of the disease in the form of diseased larvae in combs begin to appear (Alippi, 1999). Once sufficient nurse bees are again able to clean out dead infected larvae, the disease usually subsides (Bailey and Ball, 1991).

There are a number of secondary invader bacteria associated with *M. plutonius*, including *Lactobacillus eurydice, Paenibacillus alvei, P. apiarius, Brevibacillus laterosporus* and *Enterococcus faecalis*. These bacteria do not cause the disease but have an effect on the odour and appearance of dead brood associated with the disease (Alippi, 1999).

Honey bee colonies are usually more seriously affected in the spring and early summer (Tarr, 1938; White, 1920). Control of European foulbrood is generally through the feeding of oxytetracycline in either sugar syrup, or powdered sugar dusted on combs, once symptoms become apparent (Shimanuki, 1997).

Strains of *M. plutonius* appear to be closely related (Bailey and Gibbs, 1962), although one strain from Brazil was less closely related (Bailey, 1984). The effect of the strain difference is not known.

European foulbrood is found on all continents, including Australia (Matheson, 1997), although it has not been reported from Western Australia. European foulbrood has not been reported from New Zealand.

21.1.5 Conclusion

The organism is exotic to New Zealand and is listed on the unwanted organisms register as a notifiable organism. Therefore *M. plutonius* is classified as a potential hazard for the purposes of this analysis.

21.2 Risk Management

21.2.1 Release Assessment

To become infected or contaminated, the commodity would have to the commodity to come from a colony that is already infected with *Melissococcus plutonius,* or be visited by a foraging bee infected with the organism

Honey bee semen is unlikely to contain *M. plutonius,* since semen is obtained from within the drone. The only possibility for contamination to occur would be by the insemination syringe momentarily touching the exoskeleton of a drone during eversion.

It is unclear whether sufficient amounts of *M. plutonius* would be deposited on queen bees, queen cells or eggs to initiate an infection in a colony receiving one of those commodities. However, it is assumed that contamination is possible. It is considered that infected queen cells are likely to have sufficient *M. plutonius* to initiate an infection.

European foulbrood is present in most countries, so if a consignment of queen bees, queen cells or eggs comes from one of those countries then the likelihood of release is non-negligible.

21.2.2 Exposure Assessment

Queen insemination is not an exposure pathway, since spores of the organism must be fed to young larvae to create an infection. Therefore the likelihood of exposure via semen is negligible.

For other commodities, because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

21.2.3 Consequence Assessment

Honey bee colonies can be destroyed or seriously crippled by European foulbrood (Bailey and Ball, 1991). Nevertheless, in areas with uninterrupted nectar flows, the infection usually remains slight and colonies can cope with the infection without assistance (Shimanuki, 1997; Alippi, 1999). However, since European foulbrood can be a major problem for hives used for pollination (Shimanuki, 1997), it would likely have implications for the more than 70,000 colonies in New Zealand used for kiwifruit pollination. Beekeepers in Australia and

elsewhere find it necessary to feed antibiotics to control European foulbrood, and this would probably also be necessary if the disease were introduced to New Zealand. The feeding of antibiotics to honey bees has implications for the American Foulbrood National Pest Management Strategy, which relies on beekeepers being able to diagnose clinical signs of American foulbrood. Feeding antibiotics has been reported to suppress American foulbrood disease signs, thus making it more difficult to detect and control (Oldroyd et al, 1989).

Although the presence of European foulbrood would probably not result in restrictions being placed on the export of bees and bee products from New Zealand, the feeding of antibiotics to honey bees would have a negative effect on honey exports, as it is likely that some importing countries would require New Zealand honey to be tested to ensure it does not contain antibiotic residues.

European foulbrood is unlikely to have any effects on New Zealand native insects since it is restricted to honey bees.

Therefore, although there is uncertainty surrounding effects on honey bee colonies, there are likely to be significant effects on hives used for commercial pollination, increased costs to beekeepers through the need to feed antibiotics to their honey bee colonies, and increased costs to honey exporters. The consequences of introduction would be severe.

21.2.4 Risk Estimation

The risk estimate for semen is negligible. For the other commodities under consideration, the likelihood of *M. plutonius* being present in the imported commodities is non-negligible. The likelihood of exposure and establishment of the organism in New Zealand is also non-negligible. If European foulbrood became established in New Zealand the consequences would be severe. Therefore, the risk posed by *M. plutonius* is non-negligible and the organism is classified as a hazard.

21.3 Risk Management

21.3.1 Risk Evaluation

Since the risk estimate for European foulbrood in queens, queen cells and eggs is non-negligible, sanitary measures would need to be employed to effectively manage the risks to reduce them to a negligible level.

21.3.2 Option Evaluation

21.3.2.1 Risk management objective

The objective is to effectively manage the risk of European foulbrood by ensuring the imported *Apis mellifera* queens, queen cells and eggs do not carry *Melissococcus plutonius* when given a biosecurity clearance in New Zealand.

21.3.2.2 Options available

The OIE Code includes recommendations regarding European foulbrood, which can be used as a basis for developing appropriate measures.

Testing techniques have been developed in order to determine whether honey, brood, and bee products contain *M. plutonius* (Hornitzky and Smith, 1998; Govan et al, 1998). However, it is unclear whether these test methods would be suitable for the various forms of *A. mellifera* genetic material. If queen bees, queen cells and eggs are capable of carrying infective doses of *M. plutonius*, it is also unclear how the use of a post-arrival quarantine facility (White and Rhodes, 1988) would effectively mitigate against release of the organism, since in all cases either the commodity itself (queen cells and eggs) or eggs produced from a nuclei colony containing the commodity (queen bees or queen cells) would finally be moved to a release area without verification of freedom from the organism.

Although sterilisation by gamma radiation has been shown to kill *M. plutonius* (Hornitzky, 1994), it is inappropriate in this case since all life stages of *A. mellifera* are killed by gamma radiation.

Therefore, the only option that is suitable in the case of queen bees, queen cells or eggs is that they come from a country or territory officially free of European foulbrood. Post-arrival quarantine may be used to provide an incubation-based withholding period for consignment testing.

21.3.2.3 Recommended sanitary measures

<u>For honey bee queens, queen cells and eggs</u>

Each consignment should meet the following criteria:

1) Be from hives from a country or part of the territory of a country free from European foulbrood (see Appendix I); or

2) Be from hives sampled and found visually free of European foulbrood within seven days of shipment; and
3) Be placed in post-arrival quarantine (see Appendix II); where
   a) Accompanying worker bees should be killed and examined for *M. plutonius* by bacterial culture and PCR; and
   b) All recipient nuclei colonies should consist of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with oxytetracycline; and
   d) All recipient nuclei colonies should be sampled and found visually free of European foulbrood at a date beyond the incubation period of the disease (4 days).

If the original consignment is found to contain *M. plutonius* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the nuclei in the second confined area (nuclei receiving eggs or recently hatched larvae) have been examined and found to be free of *M. plutonius* (bacterial culture and PCR). All original imported material should be destroyed.

<u>For semen</u>

No sanitary measures required.

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22. PAENIBACILLUS ALVEI

22.1 Hazard Identification

22.1.1 Aetiologic Agent: Family *Bacillaceae, Paenibacillus alvei* (Cheshire and Cheyne) Ash et al.

22.1.2 OIE List: None.

22.1.3 New Zealand's Status: Uncertain - has been isolated in New Zealand on one occasion.

22.1.4 Epidemiology

Paenibacillus alvei is an aerobic, spore-forming, opportunistic saprophyte that is often recovered from diseased larvae of honey bees (*Apis mellifera*) colonies infected with Melissococcus plutonius (the causative agent of European foulbrood) (Djordjevic et al, 2000). However, unlike the primary bacterial honey bee pathogens *M. plutonius* (the causative agent of European foulbrood)and *Paenibacillus larvae larvae* (the causative agent of American foulbrood), *P. alvei* has also been isolated from a variety of sources in diverse geographic sites: from wax moth cultures in Arizona (Gilliam, 1984), from humans (Reboli et al, 1989), from mosquito larvae in India (Balaraman et al, 1979), from milk in India (Munkukndan et al, 1979), from soil in Egypt (Hafez and El-Mohandes, 1999) and from ewe's milk in Spain (Roman-Blanco et al, 1999).

Paenibacillus alvei is a secondary invader of *Apis mellifera* larvae that have been killed by other pathogens (Gochnauer, 1981). Bailey et al (1973) found that 10^5 cells of *P. alvei* fed to individual honey bee larvae caused no mortality. When the same concentration of bacterial cells was fed with sacbrood virus, less than half of the larvae failing to pupate contained *P. alvei*. Thus even when introduced with a primary pathogen, multiplication of *P. alvei* does not always occur. Two Russian studies (Skrypnik, 1984 and Kardokov et al, 1975) concluded that under certain circumstances *P. alvei* may be pathogenic to honey bee larvae under laboratory conditions. However, there is no indication that *P. alvei* is a primary pathogen under field conditions.

There is no comprehensive information on the world distribution of *P. alvei*, although since it is frequently associated with *Melissococcus plutonius,* (Bailey and Ball, 1991), its distribution is likely to be similar. As such, the presence of *P. alvei* spores is used in many countries as an indicator of European foulbrood. *P. alvei* has also been found in larvae purportedly killed by American foulbrood (Alippi, 1991; Alippi, 1997), although it is possible that in such cases European foulbrood may have been previously present in the colonies.

P. alvei has been found in Australia in 46% of 120 samples of adult bees (Hornitzky and Karlovskis, 1989) and 16% of 505 honey samples (Hornitzky and Clark, 1991). *P. alvei* complicates microbiological tests for American foulbrood in Australia, necessitating the addition of naladixic acid to culture media (Hornitzky and Nicholls, 1993). *P. alvei* was also found in 56% of honey samples in Argentina (Alippi, 1995; Alippi, 1997).

P. alvei has not been detected in Western Australia where *M. plutonius* is also not found (Hawkins C, 2001).

The status of *P. alvei* in New Zealand is uncertain. While there has been one report of *P. alvei* being isolated from a New Zealand source in 1980 (pers. comm. B. Ball IACR-Rothamsted 2002), no further isolations have been reported. In New Zealand, surveillance for European foulbrood in honey bee larvae is based on microscopy, culture and PCR. Although many suspect samples have been processed by official diagnostic laboratories, none have been found to contain *P. alvei*. Confirmation of suspect larvae for American foulbrood is done by microscopy and culture. New Zealand laboratories have not experienced problems with *P. alvei* overgrowing plates used to culture *P. l. larvae*, as is experienced in Australia (Hornitzky and Nicholls, 1993). Although no dedicated surveys for *P. alvei* have been carried out in New Zealand, the lack of any routine detection of the organism during laboratory testing for either European foulbrood or American foulbrood suggests that if *P. alvei* is present in New Zealand, it is at a low prevalence in beehives. However, it is also likely that without *M. plutonius* the presence of *P. alvei* may remain undetected unless active surveillance and laboratory testing is undertaken. No information could be obtained on the presence of the organism in other (non-honey bee) niches in the New Zealand environment.

Larve killed by other pathogens can exhibit post mortem changes related to *P. alvei* that are similar to clinical symptoms of *P. larvae larvae* (Djordjevic et al, 2000).

22.1.5 Conclusion

P. alvei is a saprophyte, not a primary pathogen of *Apis mellifera* under field conditions. There is no evidence that it can cause complications in the diagnosis of American foulbrood in the absence of *M. plutonius*. Therefore, *P. alvei* is not classified as a potential hazard for the purposes of this analysis.

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23. POWDERY SCALE DISEASE

23.1 Hazard Identification

23.1.1 Aetiologic Agent: Family *Bacillaceae, Paenibacillus larvae* subsp. *pulvifaciens* (Katznelson) Heyndrickx et al.

23.1.2 OIE List: None.

23.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

23.1.4 Epidemiology

Powdery scale disease is a disease of *Apis mellifera* larvae caused by the spore-forming bacterium *Paenibacillus larvae* subsp. *pulvifaciens*. The organism can be detected by culture or by PCR technology (Alippi et al, 2002).

The disease produces scales that are the remains of dead larvae. The scales are dry and powdery with a light brown to yellow coloration. The scales crumble when handled. The symptoms are somewhat similar to stonebrood (Shimanuki, 1997).

Little is known about the biology of the organism (Alippi, 1999), but powdery scale disease is rare and is not considered of economic importance. It is thought that the bacterium is commonly found on honey bees but only becomes pathogenic under stress conditions (Shimanuki, 1997). Bailey and Ball (1991) suggest that *P. l. pulvifaciens* is a saprophyte that is a fortuitous and ill-adapted pathogen of bees.

There is little information available on the distribution of *P. l. pulvifaciens*. Powdery scale disease has been reported in the United States (Gilliam and Dunham, 1978) and spores of *P. l. pulvifaciens* have been found in honey produced in Mexico (Alippi, 1999). Neither the disease nor the causative organism have been reported in New Zealand.

23.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, harbour *P. l. pulvifaciens* must be classified as a potential hazard for the purposes of this analysis.

23.2 Risk Assessment

23.2.1 Release Assessment

To become infected or contaminated, the commodity would have to come from a colony that is already infected with powdery scale disease, or be visited by a foraging bee that is carrying the organism. The commodity could be contaminated with spores of the organism, since infection can only take place in honey bee larvae and pupae.

There is no information available regarding the mode of transmission of the disease, or the level of spores in a colony needed to bring about contamination. However, since the disease occurs only rarely, spore transfer and germination are unlikely to be highly efficient. Transmission is assumed to be no greater than for *Paenibacillus larvae larvae*, a member of the same genus that causes a disease of significant economic importance (American foulbrood).

Honey bee semen is unlikely to contain *M. plutonius*, since semen is obtained from within the drone. The only possibility for contamination to occur would be by the insemination syringe momentarily touching the exoskeleton of a drone during eversion.

Unless spores of the organism are highly infective, it is unlikely that sufficient spores of *P. l. pulvifaciens* would be deposited on honey bee eggs to initiate an infection in a colony receiving that commodity. If the epidemiology of the organism is similar to *P. l. larvae*, mated queen bees and queen cells are the only forms of biological pathway that are likely to carry significant spores of the organism to initiate an infection.

Powdery scale disease has been reported in the United States, and *P. l. pulvifaciens* has been found in honey from Mexico, so the likelihood of release is non-negligible for consignments from at least those two countries.

23.2.2 Exposure Assessment

Queen insemination is not an exposure pathway, since spores of the organism must be fed to young larvae to create an infection. Therefore the likelihood of exposure via semen is negligible.

For queens and queen cells, because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

23.2.3 Consequence Assessment

Since the incidence of powdery scale disease is rare and the disease is not considered of economic importance, it is unlikely that *P. l. pulvifaciens* would have significant effects if introduced or established in New Zealand. The disease is also unlikely to result in justified restrictions on bee and bee product exports from New Zealand since there are no official control programmes for powdery scale disease anywhere in the world.

Powdery scale disease is unlikely to have any effects on New Zealand native insects since it appears to be restricted to honey bees.

Therefore the consequences of introduction are likely to be negligible.

23.2.4 Risk Estimation

The likelihood of contamination or infection of queen bees or queen cells coming from a colony containing the organism is assumed to be high. The likelihood of establishment of the organism in New Zealand via that commodity is assumed to be non-negligible. However, the

likelihood of any significant consequences resulting from that establishment is negligible. Therefore the risk estimation is considered to be negligible.

23.3 Risk Management

23.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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24. SEPTICAEMIA

24.1 Hazard Identification

24.1.1 Aetiologic Agent: Family *Pseudomonadaceae, Pseudomonas aeruginosa* (Schroeter) Migula.

25.1.2 OIE List: None.

24.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

24.1.4 Epidemiology

Septicaemia is a disease of adult *Apis mellifera* caused by the bacterium *Pseudomonas aeruginosa*. The organism is found commonly in soil and water and is not specific to honey bees. There has been some dispute over the proper classification of the organism (Alippi, 1999).

Septicaemia occurs in adult honey bees when stress in the colony increases. Symptoms include a colour change of bee haemolymph and degeneration of muscle tissue. Connective tissues of the thorax, legs, wings and antennae are destroyed, so that the dead bee falls apart when handled. Death is within 24 hours of infection (Alippi, 1999). Infection is thought to be through the bee's spiracles (Shimanuki, 1997). Septicaemia may also be a complication resulting from the instrumental insemination of queen honey bees (Mackensen, 1969), and is one reason antibiotics are added to honey bee semen solutions used for this purpose (Ruttner, 1976).

Streptomycin has been used to control infections of septicaemia in honey bee colonies, but development of resistant strains of the bacteria has limited the compound's usefulness (Shimanuki, 1997).

No information is available on the distribution of septicaemia in honey bees, although it is believed to occur world-wide (Shimanuki, 1997), since the causative organism is ubiquitous.

24.1.5 Conclusion

P. aeruginosa is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore *P. aeruginosa* is not classified as a potential hazard for the purposes of this analysis.

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25. SPIROPLASMAS

25.1 Hazard Identification

25.1.1 Aetiologic Agent: Family *Spiroplasmataceae*, *Spiroplasma melliferum* Clark, *Spiroplasma apis* Crozier and Crozier.

25.1.2 OIE List: None.

25.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

25.1.4 Epidemiology

Spiroplasmas are bacteria belonging to the class Mollicutes (Clark, 1978). Although 12 different spiroplasmas have been isolated from bees (Clark et al, 1985), only a few have been associated with bee mortality (Alippi, 1999).

S. melliferum was found to severely infect workers and drones (Clark et al, 1985). After it is ingested, the spiroplasma multiplies in the haemolymph until it reaches a level where the bee dies (Clark, 1977). Infected bees become sluggish and die within a week (Clark, 1982; Shimanuki and Knox, 2000). The bee-infecting spiroplasma in the United States is reported to be capable of destroying as many as 40% of foraging bees during the nectar flow (Clark, 1978).

S. apis was found to cause a lethal infection called May disease in France (Mouches et al, 1982; Mouches et al, 1984). Infected bees were flightless and had swollen abdomens and shaky movements (Mouches et al, 1984). Colonies recovered spontaneously in mid-summer (Bailey and Ball, 1991).

It has been suggested (Clark, 1977) that the spiroplasmas infecting honey bees are actually plant-derived. The spiroplasma found in tulip poplar (*Liriodendron tulipifera*) nectar has been demonstrated to kill honey bees and was used to explain the spiroplasmosis discovered in bees in the United States (Clark, 1978). The mechanism of spread of spiroplasmas between bees and between colonies is unknown, although it has been speculated that it may be through faeces of infected bees and other insects deposited on plant surfaces (Clark et al, 1985). Spiroplasmas may be enzootic in honey bee colonies, or they may cause enzootic infections in other insects prevalent on flowering plants and then make their way into honey bees (Bailey and Ball, 1991).

Spiroplasmas of bees have not been widely studied so there is limited information available on their distribution. Spiroplasma-infected bees have been reported from France (Mouches et al, 1982), North America and Hawaii (Clark, 1978) and Peru (Shimanuki, 1997).

25.1.5 Conclusion

Since spiroplasmas have not been reported in New Zealand, under the criteria presented in Section 2.2, they must be classified as a potential hazard for the purposes of this analysis.

25.2 Risk Assessment

25.2.1 Release Assessment

To become infected or contaminated, the commodity would have to come from a colony that is already infected with spiroplasmas, or be visited by a foraging bee that is carrying the organism. The commodity could be either directly infected or contaminated via honey bee faeces.

It is possible that honey bee semen might contain spiroplasmas. Contamination could occur during eversion through the momentary touching of the insemination syringe to the exoskeleton of a drone, the rupturing of the drone's internal tissues, or the semen being contaminated by drone faeces

Unless they are highly infective, it is unlikely that sufficient spiroplasmas would be deposited on honey bee eggs to initiate an infection in a colony receiving that commodity.

Mated queen bees, queen cells and semen are therefore the biological pathways that are likely to carry significant amounts of the organism(s) to initiate an infection.

Since spiroplasmas have been reported from North America, Hawaii, South America and Europe, the likelihood of release is non-negligible for consignments coming from any of these areas.

25.2.2 Exposure Assessment

Queen insemination is a potential exposure pathway, since infection occurs through the bee's haemolymph, and during insemination the insemination syringe is capable of rupturing the internal tissues of the queen. However, such rupturing would result in the death of the queen, either during or following insemination.

For other commodities, because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, exposure is likely.

With no other information to the contrary the likelihood of exposure from queen bees and queen cells is assessed as high, and exposure through insemination is moderate, especially if the sperm solution has been routinely treated with antibiotic.

25.2.3 Consequence Assessment

Spiroplasmas are reported to cause disease symptoms each year in France, with resulting large numbers of dead or moribund adult bees appearing in the front of hives (Mouches et al, 1984). In extreme cases, spiroplasmas have also been reported to be capable of destroying as many as 40% of foraging bees during the nectar flow (Clark, 1978), although such reports are very rare. Colonies recover spontaneously in mid-summer (Bailey and Ball, 1991).

Although it is not possible to accurately predict how spiroplasmas would manifest themselves if the organism(s) became established in New Zealand, there are few reports in the literature of colony mortality associated with spiroplasmas. The effects appear to be transitory, and

with a pronounced seasonal peak similar to nosema (Bailey and Ball, 1991). Leading beekeeping texts all regard spiroplasmas as a disease of only minor importance.

The presence of spiroplasmas are unlikely to result in justified restrictions on bee and bee product exports from New Zealand since there are no official control programmes for spiroplasmas anywhere in the world.

S. melliferum and *S. apis* are the major examples of significant spiroplasma pathogenesis for insects in nature (Clark et al, 1985). Spiroplasmas also cause elimination of male progeny (male-killing) in certain species of *Drosophila*. In plants, spiroplasmas are associated with several plant diseases, including citrus stubborn disease and corn stunt (Clark, 1977).

A literature search of spiroplasma pathogenicity in plants and animals for the period 1970-2002 revealed that only three plant pathogenic spiroplasmas are currently known: *Spiroplasma citri,* the agent of citrus stubborn, *S. kunkelii* the causal agent of corn stunt, and. *S. phoeniceum*, responsible for periwinkle yellows. Spiroplasma pathogenicity in animals has been shown for *S. melliferum* and *S. apis* in honey bees, in the form of male-killing in *S. ixodetis* and other unidentified spiroplasmas in ladybird beetles (*Harmonia axyridis* and *Adalia bipunctata*), and in the form of male- killing in *S. poulsonii* in neo-tropical *Drosophila* species. As well, pathogenicity of *S. citri* (the causative agent of citrus stubborn) and *S. floricola* (isolated from tulip tree flowers) have been experimentally induced in the greater wax moth (*Galleria mellonella*). Finally, *S. taiwanense*, originally isolated from mosquitoes, has been shown in the laboratory to cause pathogenicity in female *Aedes aegypti* mosquitoes.

There are no reports of *S. apis* or *S. melliferum* causing pathogenicity in insects other than *Apis mellifera,* although Clark (1982) has shown that spiroplasmas from five species of Hemiptera were unable to infect honey bees. *S. melliferum* appears to cause an intestinal infection that is not particularly harmful to other insects, while *S. apis* may be confined to honey bees (Bailey and Ball, 1991).

Although the literature on spiroplasmas and insects is not extensive, the evidence suggests that neither *S. melliferum* nor *S. apis* would cause detrimental effects to either New Zealand native insects or plants.

Therefore the consequences of introduction are considered to be negligible.

25.2.4 Risk Estimation

The likelihood of contamination or infection of queen bees, queen cells or semen coming from a colony containing the organism is non-negligible. The probability of establishment of the organism in New Zealand via that commodity is also assumed to be non-negligible. However, the likelihood of any significant consequences resulting from that establishment is negligible. The risk estimation is therefore also negligible.

25.3 Risk Management

25.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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26. CHALKBROOD

26.1 Hazard Identification

26.1.1 Aetiologic Agent: Family *Ascosphaeraceae*, *Ascosphaera apis* Maassen ex Claussen Olive and Spiltoir).

26.1.2 OIE List: None.

26.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

26.1.4 Epidemiology

Chalkbrood is a disease of *Apis mellifera* brood caused by the fungus *Ascosphaera apis*. Infection of honey bee larvae appears to be by ingestion of spores (Heath and Gaze, 1987), although it has also been suggested to occur by surface inoculation of the larval cuticle (Gilliam et al, 1978). Diseased larvae die and desiccate, taking on either a white or greyish 'mummified' appearance.

The effects of chalkbrood have been reported to range from "transient and not considered serious" to "persistent and damaging" (Bailey, 1963). Decreases in honey production of 5% (Heath, 1982) to 37% (Yacobson et al, 1991) have been recorded. Although chalkbrood has been reported to sometimes kill colonies overseas (Anderson, 1938) this apparently does not occur in New Zealand (Reid, 1993). Colonies with chalkbrood are sometimes unable to produce a surplus honey crop or sufficient food for winter (De Jong, 1976).

However, determining cause and effect is difficult with chalkbrood as it is not a simple infectious disease. *Ascos. apis* is often present in hives that have never shown symptoms of chalkbrood, and there appears to be a strong genetic component to chalkbrood susceptibility (Gilliam and Vandenberg, 1997). Races of bees with an excessive tendency to swarm are considered to be the most susceptible, and some colonies are more adept than others at containing and limiting spread of the fungus by various hygienic behaviours (Spivak and Gilliam, 1991). Breeding programmes in several countries have shown that strains of bees with good hygienic behaviour (uncapping and removal) have less clinical symptoms of the disease (Gilliam and Vandenberg, 1997). However, in addition to bee genetics, a range of environmental and management factors have been reported to contribute to the development of the disease in bee colonies (Gilliam and Vandenberg, 1997).

Chalkbrood is present in North and South America, Europe, northern Africa and most of Asia (Matheson, 1997). It is also present in New Zealand (Palmer-Jones, 1964; Reid, 1988), where it was first detected in 1984 in Northland, and as in other countries, it spread rapidly following its initial discovery (Gilliam and Vandenberg, 1997; Reid, 1993).

Chalkbrood does not appear to cause the problems in New Zealand that have been reported in Israel (Yacobson et al, 1991). The disease had a very low incidence in Israel between 1984-1990. However, in the following year, chalkbrood was found in almost every apiary. Colonies with clinical chalkbrood signs in one apiary produced 37% less honey than hives with no clinical signs.

There are reports of varying virulence between strains of *Ascos. apis* under laboratory conditions (Glinski and Chmielewski, 1989; Sawathum and Ritter, 1995), and one such report claims differences in virulence between chalkbrood strains of up to eighteen-fold (Glinski and Chmielewski, 1982). However, given the multifactorial nature of chalkbrood disease, in particular effect of management, genetic and environmental factors (Gilliam and Vandenberg, 1997), it is difficult to assess whether there is a causal relationship between losses of brood and production and natural chalkbrood infections.

26.1.5 Conclusion

Chalkbrood is present in New Zealand, but is not under official control. Although *Ascos. apis* is not listed on the unwanted organisms register, the possibility of more virulent strains existing abroad cannot be ruled out. Therefore *Ascos. apis* must be classified as a potential hazard for the purposes of this analysis.

26.2 Risk Assessment

26.2.1 Release Assessment

Since infection can only take place in honey bee larvae fed or in contact with spores of the organism, the likelihood of release is a function of the likelihood of the commodities being contaminated with spores of the organism. To become contaminated with spores, the commodity would have to come from a colony that is already infected with chalkbrood, or be visited by a foraging bee that carries the organism.

Semen is unlikely to contain spores of *Ascos. apis*, since it is obtained from within the drone. The only possibility for contamination to occur would be by the insemination syringe momentarily touching the exoskeleton of a drone during eversion.

Mated queen bees, queen cells and possibly eggs are the likely forms of biological pathway that might carry spores of the organism.

26.2.2 Exposure Assessment

Although the infective dose of *Ascos. apis* is not known, the ability of chalkbrood infection to spread is believed to be low (Bailey, 1963). It is also difficult to induce chalkbrood infection experimentally by inoculation of honey bee colonies (Puerta et al, 1999). Nevertheless, it is apparent from the first appearance of chalkbrood in countries such as Australia, New Zealand, etc., that establishment is possible, and its introduction is presumably through a commodity or commodities capable of harbouring spores of *Ascos. apis*. Moreover, it is known that honey can carry infective spores and mycelial elements of the this organism (Anderson et al, 1997).

Queen insemination is not an exposure pathway, since spores of the organism must be fed to young larvae to create an infection. Therefore the likelihood of exposure via semen is negligible.

For other commodities, because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-

negligible. However, given the difficulty of inoculation the likelihood of establishment is considered to be low.

26.2.3 Consequence Assessment

Since the virulence of *Ascos. apis* in New Zealand has not been compared to the virulence of this organism in other countries, it is not possible to determine the consequences of introducing *Ascos. apis* from abroad. As both the resistance of bee stocks (Milne, 1983) and environmental conditions (Bamford, 1989) affect the severity of chalkbrood infections, differences in reported severity of chalkbrood disease in various countries should not be linked to *Ascos. apis* strain differences without supporting experimental evidence.

If a more severe form of chalkbrood were to become established in New Zealand, it could adversely affect production and the pollinating efficiency of some colonies, at least in the short term. There are unlikely to be additional control costs, however, since chalkbrood is not usually controlled by beekeepers except for the requeening of colonies with more resistant stock (Heath, 1982). Chalkbrood is also unlikely to result in justified restrictions on the export of bees and bee products from New Zealand since there are no official control programmes for chalkbrood anywhere in the world.

Although *Ascos. apis* has been isolated from species of solitary bees overseas (Gilliam and Vandenberg, 1997), the form of chalkbrood that is present in New Zealand does not appear to affect New Zealand native insects, and it is considered unlikely that other strains of the organism would have any adverse effects on such native insects.

Although it is possible that strains of *Ascos. apis* more virulent than those already in New Zealand are present overseas, there is no supporting experimental evidence that these strains are linked directly to differences in severity of chalkbrood. Any adverse affects from increases in severity of chalkbrood are likely to be transitory, since honey bees show a marked variability in susceptibility to chalkbrood infection (Gilliam and Vandenburg, 1997), and beekeepers are likely to requeen seriously affected colonies with more resistant stock. The consequence assessment is therefore negligible.

26.2.4 Risk Estimation

The likelihood of contamination of queens, queen cells and eggs is considered to be non-negligible. The likelihood of establishment of the organism in New Zealand via those commodities is low. However, the likelihood of any long-term, significant consequences resulting from that establishment is negligible. The risk is therefore considered to be negligible.

26.3 Risk Management

26.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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27. STONEBROOD

27.1 Hazard Identification

27.1.1 Aetiologic Agent: Family *Trichocomaceae, Aspergillus flavus* Link, and other species.

27.1.2 OIE List: None.

27.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

27.1.4 Epidemiology

Stonebrood is a disease of *Apis mellifera* brood caused by the fungus *Aspergillus flavus*, or less frequently other *Aspergillus* species such as *A. fumigatus* (Gilliam and Vanderburg, 1997). These fungi are ubiquitous and are commonly found in soil. They infect and kill other insects and sometimes cause respiratory diseases in animals, particularly humans and birds (Bailey and Ball, 1991). Infection in bees is usually via the gut (Burnside, 1930) by honey bee larvae ingesting conidiophores. The internal tissues are quickly overgrown with mycelium, which break through the body wall and grow into the brood comb cell wall. Infected larvae and pupae are transformed into hard, stone-like mummies after death. Adult honey bees are attacked when fungal spores are ingested (Burnside, 1930). After the spores germinate within the alimentary canal, the resulting mycelia attack the softer tissues.

Stonebrood has been reported from North America, Europe, Venezuela and Australia (Hornitzky et al, 1989) but not from New Zealand. *A. flavus* has, however, been isolated from dead *Vespula vulgaris* larvae in New Zealand (Glare et al, 1996), and *A. fumigatus* has been isolated from animals in New Zealand (Baxter et al, 1980; Thompson et al, 1978). Although stonebrood has not been reported in New Zealand, the presence of both pathogens suggests that the disease could occasionally occur in beehives in this country, but infections are probably minor and escape notice. Stonebrood is rare and considered of minor importance by beekeepers (Gilliam and Vanderburg, 1997).

27.1.5 Conclusion

Stonebrood has not been identified in New Zealand, although the causative organisms are present. *Aspergillus* species are not listed on the unwanted organisms register, and there is no evidence to suggest that more virulent strains exist abroad. Therefore *Aspergillus* species are not classified as a potential hazard for the purposes of this analysis.

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28. BEE LOUSE

28.1 Hazard Identification

28.1.1 Aetiologic Agent: Family *Braulidae, Braula coeca* Nitzsch.

28.1.2 OIE List: None.

28.1.3 New Zealand's Status: Exotic to New Zealand. Listed on the unwanted organisms register as a notifiable organism.

28.1.4 Epidemiology

The bee louse (*Braula coeca*) is a pest of honey bee combs. It is a wingless fly that lives as a commensal in the honey bee colony and is transported by bees. The adult fly is carried around on the thorax or abdomen of worker, drone and queen honey bee adults and feeds from the host's mouthparts (Imms, 1942). Adult females lay eggs in honeycomb just before the cells are capped. Upon hatching, the larvae construct tunnels of wax that act as a shelter for the pupal stage. The life cycle takes about 3 weeks (Caron, 1981).

It has been suggested that the bee louse can overwinter as eggs or pupae (Manley, 1948), but no data have been produced to support this suggestion. Adults die within six hours of hatching if they do not attach themselves to an adult bee (Herrod-Hempsall, 1931). The bee louse is thought to spread from one colony to another via robber bees, drifting bees and in swarms distributed by beekeepers (Caron, 1981).

The larval tunnelling of the bee louse detracts from the value of comb honey being produced (Caron, 1981). Heavy louse infestations on queen bees have also been suggested as a cause of supersedure (Eckert and Shaw, 1960; Caron, 1981). The actual loss to beekeepers of either of these two events does not appear to have been quantified.

The bee louse has been found on all continents (Caron, 1981) and in Tasmania. Although Smith and Caron (1985) have incorrectly interpreted the world-wide distribution maps of Nixon (1982) and reported it as being present in New Zealand, in fact the bee louse has not been recorded in New Zealand (Matheson, 1997).

28.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, the bee louse must be classified as a potential hazard for the purposes of this analysis.

28.2 Risk Assessment

28.2.1 Release Assessment

For the commodity to become contaminated it would have to come from a colony that is already infested with bee louse, or be visited by a foraging bee that is carrying the organism, or for a stray bee louse to fly on to the commodity prior to shipment (although the likelihood of this is negligible).

Queen bees could be infested with *Braula coeca* adults, and queen cells could be contaminated with eggs or small larvae. The likelihood of honey bee semen or eggs containing the organism is negligible.

The bee louse is present in many countries, so if a consignment of queen bees or queen cells comes from one of those countries the likelihood of release is non-negligible.

28.2.2 Exposure Assessment

Because imported *Apis mellifera* queens or queen cells would most likely be used to establish foundation stock for a breeding programme, or as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

28.2.3 Consequence Assessment

The bee louse has the potential to cause problems for comb honey production. The tunnelling of *B. coeca* larvae can cause vein-like markings on the face of cappings that detract from the appearance of the finished product (Couston, 1977). It has been suggested that severe infections may decrease the efficiency of queens (Bailey, 1963; Bailey and Ball, 1991), cause paralysis and impaired egg laying (Kessler, 1987), cause the queen to supersede (Caron, 1981), and cause the death of developing bees (Marcangeli et al, 1993). There do not, however, appear to be any published data to support these suggestions.

As the bee louse is found in most countries, introduction of the organism in New Zealand would be unlikely to produce justified restriction on the export of bees from New Zealand.

The bee louse is unlikely to have any effects on New Zealand native insects since it is restricted to honey bees.

While the consequences of establishment of the bee louse in New Zealand are likely to be of low significance, they are nevertheless considered to be non-negligible.

28.2.4 Risk Estimation

the likelihood of contamination or infection of commodities coming from a colony containing the virus is non-negligible. The likelihood of exposure is considered to be non-negligible. The likely consequences of introduction are non-negligible The risk is therefore considered to be non-negligible, and the bee louse is classified as a hazard.

28.3 Risk Management

28.3.1 Risk Evaluation

Since the risk estimate for *Braula coeca* is non-negligible, sanitary measures would need to be employed to effectively manage the risks to reduce them to a negligible level.

28.3.2 Option Evaluation

28.3.2.1 Risk management objective

The objective is to effectively manage the risk of *B. coeca* by ensuring that the imported *Apis mellifera* queens and queen cells do not carry the organism when given a biosecurity clearance in New Zealand.

28.3.2.2 Options available

One option would be to allow imports of *A. mellifera* genetic material only from countries free of the *B. coeca*. However, since suitable insecticides are available to control *B. coeca* (Kessler, 1987), and since post-arrival quarantine facilities can be used to effectively mitigate against release of the organism (White and Rhodes, 1988), sanitary measures can be designed that include the use of these insecticides, as well as post-arrival quarantine, to provide assurance that progeny released into New Zealand are not infested with the organism. Post-arrival quarantine is required to mitigate against the possibility of incorrect administration of the insecticide, or development of resistance to the insecticide by the organism.

28.3.2.3 Recommended sanitary measures

<u>For queen bees</u>

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *B. coeca* (see Appendix I), or

2) Be from hives treated with an insecticide effective against *B. coeca* (e.g., Perizin) beginning three weeks prior to shipment and through to the time of shipment; and
3) Be from hives sampled and found free of *B. coeca* within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All accompanying worker bees should be killed and visually examined for *B. coeca;* and
   b) Each queen should be placed into a nucleus hive consisting of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with an insecticide effective against *B. coeca* (e.g., Perizin); and
   d) Separate confined areas should be used for holding the importing material and rearing the progeny, with transfer of genetic material to the second confined area via eggs or recently hatched larvae.

If the original consignment is found to contain *A. woodi* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the recipient nuclei have been examined and found to be free of *B. coeca*. All remaining imported material should be destroyed.

For queen cells

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *B. coeca* (see Appendix I), or

2) Be from hives treated with an insecticide effective against *B. coeca* (e.g., Perizin) beginning three weeks prior to shipment and through to the time of shipment; and
3) Be from hives sampled and found free of *B. coeca* within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All queen cells should be drenched with an insecticide effective against *B. coeca* (e.g., Perizin) prior to placement in recipient nuclei colonies; and
   b) Each queen cell should be placed into a nucleus hive consisting of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with an insecticide effective against *B. coeca* (e.g., Perizin).
   d) Separate confined areas do not need to be used.

If the original consignment is found to contain A. woodi during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the recipient nuclei have been examined and found to be free of *B. coeca*. All remaining imported material should be destroyed.

For semen or eggs

No sanitary measures required.

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29. EXTERNAL ACARINE MITES

29.1 Hazard Identification

29.1.1 Aetiologic Agent: Family *Tarsonemidae, Acarapis externus* Morgenthaler and *A. dorsalis* Morgenthaler.

29.1.2 OIE List: none.

29.1.3 New Zealand's Status: Both external acarapis mites are present in New Zealand. Not under official control.

29.1.4 Epidemiology

Acarapis externus and *A. dorsalis* are external mites parasitic on adult *Apis mellifera*. The mites, which are microscopic in size, have modified mouthparts used to pierce the cuticle of adult bees and suck blood. Both mites are restricted to honey bees as hosts and feed externally on bees (Delfinado-Baker and Baker, 1982).

A. externus is found generally on the neck of the bee and on pits on the back of the bee's head, while *A. dorsalis* occupies a groove across the top of the bee's thorax. Neither species appear to affect bees adversely (Eickwort, 1997), although the pest status of the two species has never been thoroughly investigated (De Guzman et al, 2001).

Both mites have a worldwide distribution (Bailey and Ball, 1991). *A. externus* and *A. dorsalis* are present in New Zealand (Clinch, 1976).

29.1.5 Conclusion

Both species of external acarine mite are present in New Zealand. Neither is under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore *A. externus* and *A. dorsalis* are not classified as a potential hazard for the purposes of this analysis.

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30. SMALL HIVE BEETLE

30.1 Hazard Identification

30.1.1 Aetiologic Agent: Family *Nitidulidae, Aethina tumida* Murray.

30.1.2 OIE List: None.

30.1.3 New Zealand's Status: Exotic to New Zealand. Listed on the unwanted organisms register as a notifiable organism.

30.1.4 Epidemiology

The small hive beetle (*Aethina tumida*) is a pest of honey bee combs. The adult beetle invades honey bee colonies, where female beetles lay eggs that hatch within six days. The beetle larvae eat honey bee eggs, larvae, pupae, honey and pollen (Lundie, 1940). The larvae also tunnel in sealed honey and can render it unfit for human consumption (Elzen et al, 1999a).

The larval stage lasts between 10 and 20 days (Taber, 1999). The beetle larvae leave the hive when mature and burrow into the soil in front of the hive to pupate (Fore, 1999). The pupal stage takes between two weeks and two months. Adult beetles can survive for up to six months (Taber, 1999). They can survive for five days without food or water (Pettis and Shimanuki, 1999).

Control of the small hive beetle is generally by applying 40% permethrin as a soil drench in front of the beehive, and the use of 10% coumaphos strips (Check-Mite+) within the colony (Elzen et al, 1999b).

The small hive beetle has been reported from Africa, the United States (Mostafa and Williams, 2002) and Australia (CSIRO, 2002). The beetle was first reported in the United States in South Carolina in 1996. Infestations have since been found in Georgia, North and South Carolina, Florida (Fore, 1999; Hood, 2000), and a number of other states.

Although in Africa the damage caused by the small hive beetle is similar to that of the greater wax moth (*Galleria mellonella*), the beetle has resulted in much more significant losses in the south-eastern United States, where one large operation alone estimated a loss of nearly 10,000 colonies (Eischen et al, 1999).

30.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, Small hive beetle must be classified as a potential hazard for the purposes of this analysis.

30.2 Risk Assessment

30.2.1 Release Assessment

To become contaminated, the commodity would have to come from a colony that is infested with small hive beetle. The commodity could either be infested with *Aethina tumida* adults or

contaminated with eggs or small larvae. The likelihood of contamination would for queens or queen cells is non-negligible. There likelihood of honey bee semen or eggs containing the organism is negligible.

The small hive beetle is present in the United States, Africa and Australia, so if a consignment of queen cells, nucleus hives, package bees or queens comes from one of those areas then the likelihood of release is non-negligible.

30.2.2 Exposure Assessment

The small hive beetle is able to fly and is attracted to the combination of hive products plus bees. If any larvae of the beetle successfully pupate, or if adults are brought into New Zealand, there is a significant likelihood that the organism could find its way to a hive. , Because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

30.2.3 Consequence Assessment

There is not enough information available on the distribution of *A. tumida* in temperate climates to estimate its likely impact should it be introduced into New Zealand. The small hive beetle does not appear to be a major problem in southern Africa (Elzen et al, 1999), possibly because the African bee (*Apis mellifera scutellata*) is more defensive against beetle infestations. New Zealand honey bees are likely to show a similar vulnerability to *A. tumida* as bees in the United States and Australia, since New Zealand bees are more closely related to strains present in these countries than to African honey bees. Significant colony losses in New Zealand are therefore possible, and beekeepers would need to use pesticides to control the beetles. Because of the limited distribution of the small hive beetle throughout the world, their presence in New Zealand would also likely result in restrictions being imposed on exports of queens and package bees.

The small hive beetle is unlikely to have any effects on New Zealand native insects. However, the beetle has been shown to be able to complete its life cycle in colonies of *Bombus impatiens*, a bumble bee species (Ambrose et al, 2000). Bumble bees are not native to New Zealand, but are significant feral pollinators. Colonies of bumble bees are also used to pollinate glasshouse tomatoes.

The consequences of establishment of the small hive beetle in New Zealand are likely to be high.

30.2.4 Risk Estimation

There is a non-negligible likelihood of contamination for all commodities except semen and eggs. The likelihood of establishment of the organism in New Zealand via queens or queen cells is considered to be non-negligible. The likelihood of significant consequences resulting from that establishment is high. As a result, the risk for the small hive beetle is considered to be non-negligible and it is classified as a hazard.

30.3 Risk Management

30.3.1 Risk Evaluation

Since the risk estimate for *Aethina tumida* is non-negligible, sanitary measures would need to be employed to effectively manage the risks to reduce them to a negligible level.

30.3.2 Option Evaluation

30.3.2.1 Risk management objective

The objective is to effectively manage the risk of *A. tumida* by ensuring the imported *Apis mellifera* genetic material does not carry the organism when given a biosecurity clearance in New Zealand.

30.3.2.2 Options available

One option would be to allow imports of *Apis mellifera* genetic material only from countries free of the *A. tumida*. However, since suitable insecticides are available to control of the pest (Elzen et al, 1999), and since post-arrival quarantine facilities can be used to effectively mitigate against release of the organism (White and Rhodes, 1988), sanitary measures can be designed that include the use of these insecticides, as well as post-arrival quarantine, to provide assurance that progeny released into New Zealand are not infested with the organism. Detection of infestation is possible using Check-Mite+ and sticky boards. Post-arrival quarantine is required to mitigate against the possibility of incorrect administration of the insecticide, or development of resistance to the insecticide by the organism.

30.3.2.3 Recommended sanitary measures

<u>For queen cells, queen bees</u>

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *A. tumida* (see Appendix I), or

2) Be from hives treated with an insecticide effective against *A. tumida* (Check-Mite+) beginning 26 days prior to shipment and continuing up to the time of shipment; and
3) Be from hives sampled (Check-Mite+ and sticky boards) and found free of *A. tumida* within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All queen cells should be drenched with an insecticide effective against *A. tumida* (Check-Mite+) prior to placement in recipient nuclei colonies; and
   b) All recipient nuclei colonies should consist of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with an insecticide effective against *A. tumida* (Check-Mite+).
   d) Separate confined areas do not need to be used.

If the original consignment is found to contain *A. tumida* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the recipient nuclei have been examined and found to be free of A. tumida (Check-Mite+ and sticky boards). All original imported material should be destroyed.

For semen or eggs

No sanitary measures required.

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31. TRACHEAL MITE

31.1 Hazard Identification

31.1.1 Aetiologic Agent: Family *Tarsonemidae, Acarapis woodi* Rennie.

31.1.2 OIE List: B.

31.1.3 New Zealand's Status: Exotic to New Zealand. Listed on the unwanted organisms register as a notifiable organism.

31.1.4 Epidemiology

Acarapis woodi is a parasitic mite of *Apis mellifera.* The mite causes acarapisosis, a disease of the respiratory system of adult honey bees (Bailey and Ball, 1991). Female mites enter the first thoracic spiracle of an adult bee that is usually less than three days old. Once inside the tracheae, the mite lays between five and seven eggs, which hatch over three or four days (Morgenthaler, 1931). The mite goes through a six-legged larval stage followed by a pharate nymphal stage, developing into an adult male in 12 days, or a female in 15 days (Delfinado-Baker and Baker, 1982).

All stages (eggs, larvae, nymphs and adults) of *A. woodi* live exclusively in the tracheae, except mated females, which leave to enter the tracheae of another adult bee. Since the mated female can live outside the bee for only a few hours, spread of the mite is only through direct contact between bees (Sammataro and Needham, 1996).

Honey bees with high infestations of the mite have a shortened life-span (Giordani, 1965). Honey bee colonies with high infestations of the mite show increased losses of bees, especially in spring (Otis and Scott-Dupree, 1992). High infestation has been shown to lead to very high overwintering mortality rates of colonies in temperate climates (Phibbs, 1996). Since the mite was first reported in the United States, beekeepers have lost tens of thousands of colonies and millions of dollars to the disease (Wilson and Pettis, 1997).

Control of tracheal mite is generally through the use of either menthol or formic acid as a fumigant within the beehive (Wilson and Pettis, 1997).

A. woodi has been reported from most areas of the world. The only significant beekeeping countries where it has not been reported are Australia and New Zealand (Matheson, 1997).

31.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, *A. woodi* must be classified as a potential hazard for the purposes of this analysis.

31.2 Risk Assessment

31.2.1 Release Assessment

To become contaminated the commodity would have to come from a colony that is already infested with tracheal mite, or be visited by a foraging bee that is carrying the mite. The

likelihood of queen cells, honey bee semen or eggs containing the organism is negligible, since the only time the mite is external is during the adult phoretic stage.

The tracheal mite is present in most countries, so if a consignment of queen bees comes from one of those countries then the likelihood of release is non-negligible.

31.2.2 Exposure Assessment

Tracheal mites can exist in adult bees for long periods of time, and can migrate from bee to bee. Because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

31.2.3 Consequence Assessment

It is likely that honey bees in this country would be as susceptible to tracheal mites as honey bees in north-eastern United States, where, following their introduction in 1984, tracheal mites caused the death of over 30% of colonies in the winter of 1995-1996 (Finley et al, 1996; Tew, 1996). Therefore, severe consequences could be expected for the New Zealand beekeeping and pollination industries if tracheal mite were introduced. In addition to hive losses, the need to use chemicals to control the mite would pose additional production costs both in terms of treatment and the labour involved in administering it.

Tracheal mite is unlikely to have any effects on New Zealand native insects since it is restricted to honey bees.

Therefore the consequences of introduction are considered to be high.

31.2.4 Risk Estimation

The likelihood of contamination is negligible for semen, queen cells and eggs. Therefore the risk for those commodities is considered negligible.

For queens, the likelihood of contamination is non-negligible. The likelihood of exposure and establishment for tracheal mites associated with queens is high, and the likelihood of significant consequences resulting from that establishment is high. Therefore the risk for *Acarapis woodi* is considered to be non-negligible and it is classified as a hazard.

31.3 Risk Management

31.3.1 Risk Evaluation

Since the risk estimate for *Acarapis woodi* in queens is non-negligible, sanitary measures would need to be employed to effectively manage the risks to reduce them to a negligible level.

31.3.2 Option Evaluation

31.3.2.1 Risk management objective

The objective is to effectively manage the risk of *A. woodi* by ensuring the imported *Apis mellifera* genetic material (in the form of queen bees) does not carry the organism when given a biosecurity clearance in New Zealand.

31.3.2.2 Options available

The OIE Code includes recommendations regarding tracheal mite that can be used as a basis for developing appropriate measures.

One option would be to allow imports of *Apis mellifera* genetic material only from countries free of *A. woodi*. However, since suitable insecticides are available to control the mite (Wilson and Pettis, 1997), and since post-arrival quarantine facilities can be used to effectively mitigate against release of the organism (White and Rhodes, 1988), sanitary measures can be designed that include the use of these insecticides, as well as post-arrival quarantine, to provide assurance that progeny released into New Zealand are not infested with the organism.

31.3.2.3 Recommended sanitary measures

<u>For queen bees</u>

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *A. woodi*, or

2) Be from hives treated with an miticide effective against *A. woodi* (menthol or formic acid) beginning 19 days prior to shipment and continuing up to the time of shipment; and
3) Be from hives sampled and found free of *A. woodi* (honey bee tracheae dissection or ELISA) within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All accompanying worker bees should be killed and examined for *A. woodi* (honey bee tracheae dissection or ELISA); and
   b) Each queen bee should be placed in a nucleus hive composed of bees from New Zealand.
   c) All recipient nuclei colonies should be treated with a miticide effective against *A. woodi* (menthol or formic acid); and
   d) Separate confined areas should be used for holding the importing material and rearing the progeny, with transfer of genetic material to the second confined area via eggs or recently hatched larvae.

If the original consignment is found to contain *A. woodi* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the nuclei in the second confined area (nuclei receiving eggs or recently hatched larvae) have been examined and

found to be free of A. woodi (honey bee tracheae dissection or ELISA). All original imported material should be destroyed.

For queen cells, semen or eggs

No sanitary measures required.

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32. TROPILAELAPS

32.1 Hazard Identification

32.1.1 Aetiologic Agent: Family *Laelapidae, Tropilaelaps clareae* Delfinado and Baker, *Tropilaelaps koenigerum* Delfinado-Baker and Baker.

32.1.2 OIE List: None.

32.1.3 New Zealand's Status: Exotic to New Zealand. Listed on the unwanted organisms register as unwanted organisms.

32.1.4 Epidemiology

Tropilaelaps clareae is a mite, originally parasitic on *Apis dorsata,* which is now also a parasite of *A. mellifera* (Aggarwal, 1988). *T. koenigerum* is smaller than *T. clareae* and has been collected from *A. dorsata* (Delfinado-Baker and Baker, 1982), *A. mellifera* and *A. cerana* (Abrol and Putatunda, 1995).

Adult female and immature stages of tropilaelaps feed on the haemolymph of honey bee larvae. The mite is phoretic on adult bees, but is unable to feed on them (Rinderer et al, 1994). Mite reproduction takes place in both drone and worker brood, although drone brood is preferred. Parasitism can reach 90% of brood cells (Burgett et al, 1983). Mated female mites enter a brood cell before it is capped and then lay eggs. The eggs hatch and development follows through a larval stage, protonymph and deutonymph. Males and females are produced in equal proportions and mating takes place inside the cell. Adult females leave the cell when the bee emerges and stay on adult bees for about 1.4 days before entering another cell to begin reproduction (Kitprasert, 1984). The mites are reported to be able to survive without bee brood for only two days (Woyke, 1984; Woyke, 1985; Koeniger and Muzaffar, 1988) or three days (Rinderer et al, 1994).

Damage to *A. mellifera* colonies from infestation by *T. clareae* can be severe (Burgett and Akrantanakul, 1985). If left unchecked, the mite population can rapidly cause the death of the colony (Rinderer et al, 1994). Although *T. koenigerum* has been reported on *A. mellifera*, no information has been presented on its effects on that species of bee.

Control of tropilaelaps is either through pyrethroids that are also used to control varroa (De Jong, 1997), or by caging the queen to eliminate brood in the colony (Woyke, 1985), since tropilaelaps are not able to survive for more than three days without brood.

T. clareae has been found in southeast Asia, Afghanistan, China and Kenya (De Jong, 1997). *T. koenigerum* has been found in Sri Lanka (Delfinado-Baker and Baker, 1982), Nepal (Delfinado-Baker et al, 1985) and India (Abrol and Putatunda, 1995).

32.1.5 Conclusion

Since *T. clareae* and *T. koenigerum* have not been reported in New Zealand, under the criteria presented in Section 2.2, these mites must be classified as potential hazards for the purposes of this analysis.

32.2 Risk Assessment

32.2.1 Release Assessment

For a commodity to become contaminated it would have to come from a colony that is already infested with tropilaelaps, or be visited by a foraging bee that is carrying the mite. The likelihood of queens being contaminated with tropilaelaps adults is considered to be non-negligible, as is the likelihood of queen cells being contaminated with adults, eggs or nymphal stages. However, the likelihood of contamination of semen or eggs is negligible.

Tropilaelaps is present in south and southeast Asia, and Africa, so if a consignment of queens bees or queen cells comes from one of those areas then the likelihood of release is non-negligible.

32.2.2 Exposure Assessment

Although tropilaelaps cannot survive away from brood for more than three days, imports of *Apis mellifera* queens are likely to be via air freight, the duration and so could possibly fall within this infectivity period. Because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be high.

32.2.3 Consequence Assessment

The establishment of *Tropilaelaps clareae* would likely cause severe consequences for the New Zealand beekeeping and horticultural industries. T. *clareae* is considered to be a more serious pest than varroa in southeast Asian countries where both mites exist (Woyke, 1989). The presence of *T. clareae* could have a major effect on the export of queens and package bees from New Zealand, although the inability of the mite to survive on adult bees should reduce the possibility of live bee exports transporting *T. clareae*.

Although *T. koenigerum* has been found on *A. mellifera*, its effects have not been reported. For the purposes of this analysis, the consequences of the establishment of *T. koenigerum* in New Zealand are assumed to be similar to those for *T. clareae*.

Tropilaelaps is unlikely to have any effects on New Zealand native insects since it is restricted to honey bees.

Therefore the consequences of introduction are considered to be high.

32.2.4 Risk Estimation

The likelihood of contamination of commodities is limited to queens and queen cells. The likelihood of introduction and establishment of the organism in New Zealand via those commodities is high. The likelihood of significant consequences resulting from that establishment is also high. Therefore the risk for tropilaelaps is considered to be non-negligible and it is classified as a hazard.

32.3 Risk Management

32.3.1 Risk Evaluation

Since the risk estimate for tropilaelaps is non-negligible, sanitary measures would need to be employed to effectively manage the risks to reduce them to a negligible level.

32.3.2 Option Evaluation

32.3.2.1 Risk management objective

The objective is to effectively manage the risk of tropilaelaps by ensuring the imported *Apis mellifera* genetic material in the form of queen bees and queen cells does not carry the organisms when given a biosecurity clearance in New Zealand.

32.3.2.2 Options available

The OIE Code does not include recommendations regarding tropilaelaps.

One option would be to allow imports of *A. mellifera* genetic material only from countries free of tropilaelaps. However, since tropilaelaps mites cannot survive away from brood, a withholding period in a quarantine facility away from other bees could effectively mitigate against the release of the organism via queen bees. For queen cells, suitable pesticides are available to control tropilaelaps (Woyke, 1984), and post-arrival quarantine facilities can be used to effectively mitigate against release of the mites (White and Rhodes, 1988). As a result, sanitary measures can be designed to provide assurance that progeny released into New Zealand are not infested with the tropilaelaps.

32.3.2.3 Recommended sanitary measures

<u>For queen bees</u>

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from tropilaelaps (see Appendix I) or

2) Be from hives treated with a miticide effective against tropilaelaps (e.g., Apistan) beginning three weeks before shipment and through to the time of shipment; and
3) Be from hives sampled and found free of tropilaelaps (Apistan and sticky boards) within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) Queen bees and accompanying workers should be held away from other adult bees and brood for a period twice the recognised longest period for survival of the mite away from brood (i.e., six days).
   b) No recipient nuclei or separation of confined areas is required.

If the original consignment is found to contain tropilaelaps during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of

progeny from post-arrival quarantine should only be granted at the end of the withholding period. All remaining imported material should be destroyed.

For queen cells

Each consignment meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from tropilaelaps (see Appendix I), or

2) Be from hives treated with a miticide effective against tropilaelaps (e.g., Apistan) beginning three weeks before shipment and through to the time of shipment; and
3) Be from hives sampled and found free of tropilaelaps (Apistan and sticky boards) within seven days of shipment; and
4) Be placed in post-arrival quarantine (see Appendix II); where
   a) All queen cells should be emerged into nuclei colonies containing no brood, and remaining broodless for 6 days; and
   b) All recipient nuclei colonies should consist of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with a miticide effective against tropilaelaps (e.g., Apistan).
   d) Separate confined areas do not need to be used.

If the original consignment is found to contain tropilaelaps during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted at the end of the withholding period. All remaining imported material should be destroyed.

For semen or eggs

No sanitary measures required.

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33. VARROA

33.1 Hazard Identification

33.1.1 Aetiologic Agent: Family *Varroidae, Varroa destructor* Anderson and Trueman.

33.1.2 OIE List: B.

33.1.3 New Zealand's Status: Present in New Zealand. Under official control.

33.1.4 Epidemiology

Varroa destructor, known until recently as *V. jacobsoni* (Anderson and Trueman, 2000), is a mite originally parasitic on *Apis cerana* that is now also a parasite of *A. mellifera.* The mite is believed to have successfully first parasitised *A. mellifera* last century in Japan and eastern Siberia (Ritter, 1981). The mite causes varoosis, a disease of honey bee brood and adults.

Adult female varroa mites leave adult bees and invade either worker or drone brood cells before they are capped. The mites prefer to invade cells containing drone larvae (Fuchs, 1990). Eggs begin to be laid about 60-70 hours after the cell is sealed (Ifantidis, 1983). Five to six eggs are laid, the first being male and the remainder female (Rhem and Ritter, 1989). Following egg hatch, the mite goes through two juvenile stages (protonymph and deutonymph) before taking on adult body shape. The mother mite establishes a feeding site on the pupa, which her offspring then use to feed on the haemolymph as they grow. The new generation of mites mate in the cell before the host bee emerges. Only mature female mites survive to leave the cell when the bee emerges (Ifantidis, 1983). The mature female mites stay on adult bees usually for about seven days, piercing the body wall of the bee between the abdominal segments and feeding on the haemolymph (Bailey and Ball, 1991). Varroa can remain on adult bees for far longer periods, as evidenced by its ability to persist in colonies in cold climates with broodless periods of 120 days and longer (Korpela et al, 1992).

Varroa has been blamed for the destruction of hundreds of thousands of honey bee colonies in areas where it has come into contact with *A. mellifera* (Chun, 1965; Ritter, 1981; De Jong, 1997; Tew, 1999). Varroa has a range of damaging effects on individual honey bees (Ball, 1993), as well as on the honey bee colony, including colony death (De Jong, 1997).

Control of varroa is generally through the use of pyrethroids and other chemicals applied directly to the colony in the form of contact strips or fumigants. Populations of varroa mites in some overseas countries have developed resistance to various control products (Milani, 1999).

V. destructor is found in all significant beekeeping countries with the exception of Australia (Matheson, 1997). The entire South Island of New Zealand is reported to be free of varroa.

33.1.5 Conclusion

Although *V. destructor* is present in New Zealand, it is under official control. Therefore under the criteria presented in Section 2.2, it must be classified as a potential hazard for the purposes of this analysis.

33.2 Risk Assessment

33.2.1 Release Assessment

For the commodity to become contaminated, it would have to come from a colony that is already infested with *Varroa destructor*, or be visited by a foraging bee that is carrying the mite. The likelihood of queens being contaminated with adult varroa mites is considered to be non-negligible, as is the likelihood of queen cells being contaminated with adults, eggs or nymphal stages. However, the likelihood of contamination of semen or eggs is negligible.

V. destructor is present in most countries, so if a consignment of queen bees or queen cells comes from one of those countries then the likelihood of release is non-negligible.

33.2.2 Exposure Assessment

Varroa can exist on adult bees for long periods of time, and can also be transmitted in brood. Because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be high.

33.2.3 Consequence Assessment

The spread of *V. destructor* through North America has been claimed to be the biggest catastrophe to befall apiculture there since honey bees were introduced (De Jong, 1997; Tew, 1999). Usually all colonies that do not receive chemical treatment die within two to four years (De Jong, 1997).

If *V. destructor* were to spread to the South Island of New Zealand, experience in other countries suggests that it would also destroy all feral colonies, or at least reduce their life expectancy (Loper, 1995). While varroa might have a positive effect on American foulbrood incidence, through destruction of feral and unmanaged colonies, any benefits would be outweighed by the need for the South Island beekeeping industry to use chemical control measures against varroa, and the loss of pollination provided by feral colonies. The need to use chemicals would pose additional production costs both in terms of treatment and the labour involved in administering it. Some treatments, such as Apistan strips, have been demonstrated to produce undesirable residues in wax (Wallner, 1999).

Varroa has developed resistance to a number of varroa control products overseas, including fluvalinate, flumethrin, acrinathrin (Milani, 1999). The introduction of these resistant strains could have a negative effect on control of *V. destructor* in New Zealand, since fluvalinate-resistant strains have negatively affected control of the mite in northern Italy. Trials conducted when *V. destructor* was identified in New Zealand demonstrated that the strain introduced was not resistant to fluvalinate or flumethrin (Goodwin and McBrydie, 2000; Taylor and Goodwin, 2001). Whether it is resistant to other varroa control products is not known.

The introduction of fluvalinate or flumethrin-resistant mites to the North Island would have a negative effect on varroa control since these are the most commonly used varroa control products. Increased colony deaths could have a serious effect on the supply of hives for commercial pollination.

Varroa is unlikely to have any effects on New Zealand native insects since it is highly to live and reproduce only on *Apis* spp.

Therefore the consequences of introduction are considered to be high.

33.2.4 Risk Estimation

The likelihood of contamination is negligible for semen and eggs. Therefore the risk for those commodities is considered negligible.

For queens and queen cells, the likelihood of contamination is high. The likelihood of exposure and establishment for varroa mites associated with queens and queen cells is high, and the likelihood of significant consequences resulting from that establishment is high. Therefore the risk for *Varroa destructor* is considered to be high and it is classified as a hazard.

33.3 Risk Management

33.3.1 Risk Evaluation

Since the risk estimate for *Varroa destructor* is non-negligible, sanitary measures would need to be employed to effectively manage the risks to reduce them to a negligible level.

33.3.2 Option Evaluation

33.3.2.1 Risk management objective

The objective is to effectively manage the risk of *V. destructor* by ensuring the imported *Apis mellifera* genetic material in the form of queen bees and queen cells does not carry the organism when given a biosecurity clearance in New Zealand.

33.3.2.2 Options available

The OIE Code includes recommendations regarding varroa that can be used as a basis for developing appropriate measures.

One option would be to allow imports of *A. mellifera* genetic material only from countries free of the miticide-resistant *V. destructor*, and then only into the infected areas of New Zealand. However, it is difficult to know if mites in a country have developed resistance to a particular chemical. A testing regime for resistance on an individual import basis would be costly and impractical. Miticides could be used to treat commodities, but again it is not possible to know if any mites contaminating an import are resistant to the chemical chosen. The only suitable option is post-arrival quarantine facilities, which can be used to effectively mitigate against release of the organism (White and Rhodes, 1988).

33.3.2.3 Recommended sanitary measures

<u>For queen bees and queen cells</u>

Each consignment should meet the following criteria:

1) Be from hives from a country or part of the territory of a country free from *V. destructor* (see Appendix I), or

2) Be from hives sampled and found free of *V. destructor* (alcohol wash) within seven days of shipment; and
3) Be in new cages or containers not previously in contact with bees; and
4) Be placed in post-arrival quarantine (see Appendix II); where
   a) All accompanying worker bees should be killed and examined (alcohol wash) for *V. destructor*; and
   b) Each queen bee/cell should be placed into a nucleus hive consisting of varroa-free bees from New Zealand; and
   c) Separate confined areas should be used for holding the importing material and rearing the progeny, with transfer of genetic material to the second confined area via eggs.

If the original consignment is found to contain *V. destructor* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the nuclei in the second confined area (nuclei receiving eggs) have been examined and found to be free of *V. destructor* (alcohol wash). All remaining imported material should be destroyed.

For semen or eggs

No sanitary measures required.

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34. VARROA (OTHER THAN *VARROA DESTRUCTOR*)

34.1 Hazard Identification

34.1.1 Aetiologic Agent: Family *Varroidae, Varroa jacobsoni* Oudemans, *V. underwoodi* Delfinado-Baker and Aggarwal, *V. rindereri* Guzman and Delfinado-Baker, *Euvarroa sinhai* Delfinado and Baker, *E. wongsirii* Lekprayoon and Tangkanasing.

34.1.2 OIE List: None.

34.1.3 New Zealand's Status: Exotic to New Zealand. *Varroa underwoodi* is listed on the unwanted organisms register as an unwanted organism.

34.1.4 Epidemiology

Varroa jacobsoni is a mite parasite of *Apis cerana*. The species was originally thought to be the mite responsible for parasitism of *A. mellifera* worldwide. However, recent genetic research has shown that the mite parasitising *A. mellifera* is a new species, *V. destructor* (Anderson and Trueman, 2000). *V. jacobsoni* was the first species identified in the genus in Java in 1906, but little further morphological analysis on the genus was carried out until recently. *V. jacobsoni* has now been found to be incapable of reproducing on *A. mellifera* (Anderson and Sukarsih, 1996).

V. underwoodi is a mite parasite of *A. cerana* (Delfinado-Baker and Aggarwal, 1987). The mite closely resembles *V. destructor* in appearance and is found in low levels in drone cells of *A. cerana* (De Jong, 1997). Its lifecycle has so far not been studied, but it is likely to be similar to *V. destructor*. Parasitism of *A. mellifera* has so far not been reported.

V. rindereri is a mite parasite of *A. koschevnikovi*. The mite resembles *V. jacobsoni*, but is longer and wider, and has more long hairs (setae) along each side of the body. Its biology and distribution is not known, and there are no reports of it being able to parasitise *A. mellifera* (Otis and Kralj, 2001).

Euvarroa sinhai is a mite parasite of *A. florea* (Delfinado and Baker, 1974). The mite is smaller than *V. destructor*. *E. sinhai* has a similar lifecycle to *V. destructor*, except that mites only enter drone brood cells to reproduce. Adult mites are phoretic on adult worker bees and drones (Akrantanakul and Burgett, 1976). Experimental infestations of *E. sinhai* on *A. mellifera* and *A. cerana* have been demonstrated (Mossadegh, 1990; Koeniger et al, 1993), suggesting that they may be potential candidates for parasitism by the mite (De Jong, 1997).

E. wongsirii is a mite parasite of *A. andreniformis*. It has a biology similar to *E. sinhai*. *E. wongsirii* is slightly shorter and wider than *E. sinhai*, with a strongly triangular shape. *E. sinhai* is pear-shaped. *E. wongsirii* has not been found in association with *A. mellifera* (Otis and Kralj, 2001).

V. jacobsoni is present in Indonesia, Malaysia and New Guinea (Anderson and Trueman, 2000). *V. underwoodi* has been found in Nepal (Delfinado-Baker and Aggarwal, 1987) and South Korea (De Jong, 1997). *V. rindereri* appears to be localised to the island of Borneo (Otis and Kralj, 2001). *E. sinhai* has been found in Thailand (Akrantanakul, 1975), India and Sri Lanka (Koeniger et al, 1993), and Iran (Mossadegh, 1990). *E. wongsirii* has been found in

peninsular Malaysia and Thailand, and probably also occurs in eastern India, Indochina and western Indonesia (Otis and Kralj, 2001).

34.1.5 Conclusion

Imports of *A. mellifera* genetic material could harbour species of varroa other than *V. destructor.* These species are exotic to New Zealand and are listed on the unwanted organisms register as notifiable organisms. As a result *V. jacobsoni, V. underwoodi, V. rindereri* and *E. sinhai* and *E. wongsirii* are classified as a potential hazards for the purposes of this analysis.

34.2 Risk Analysis

34.2.1 Release Assessment

For the commodity to become contaminated it would have to come from a colony that is already infested with the varroa species, or be visited by a foraging bee that is carrying the mite. The likelihood of queens being contaminated with adult varroa mites is considered to be non-negligible, as is the likelihood of queen cells being contaminated with adults, eggs or nymphal stages. However, the likelihood of contamination of semen or eggs is negligible.

Varroa jacobsoni, V. underwoodi, V. rindereri and *Euvarroa sinhai* and *E. wongsirii* are present in several Asian countries, so if a consignment of queen bees or queen cells comes from one of those countries then the likelihood of release is non-negligible.

34.2.2 Exposure Assessment

It is unclear whether *V. underwoodi* and *V. rindereri* can exist on adult bees or brood of *Apis mellifera*, since the mite has so far not been reported on this honey bee species. However, since the two mites are closely related to *V. destructor*, including parasitism of *A. cerana*, and since *V. destructor* was able to transfer from *A. cerana* to *A. mellifera*, there is a non-negligible likelihood that either *V. underwoodi* or *V. rindereri* would be capable of parasitising *A. mellifera* if they were to come into contact with that honey bee species.

E. sinhai has been shown to be capable of infesting *A. mellifera* experimentally, so there is also a non-negligible likelihood of parasitism of that honey bee species, probably by either *E. sinhai or E. wongsirii*.

V. jacobsoni has been found in association with *A. mellifera* adults and brood (Anderson, 1994), but is unable to reproduce in *A. mellifera* colonies. Establishment is not possible for *V. jacobsoni* away from *A. cerana*.

Varroa can exist on adult bees for long periods of time, and can also be transmitted in brood. Because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be high.

The likelihood of exposure is therefore high for *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*, but negligible for *V. jacobsoni*.

34.2.3 Consequence Assessment

Since it is unclear whether *V. underwoodi, V. rindereri, E. sinhai* or *E. wongsirii* are capable of parasitism of *A. mellifera*, it is also unclear what would be the consequences if any of these species became established in New Zealand.

However, if *A. mellifera* showed as little resistance to parasitism by one of these species as it has shown to *V. destructor*, the consequences of establishment would be severe.

Since so little is known about any of these species, and in particular their possible parasite relationships with *A. mellifera*, and since it was many years before it became evident that *V. destructor* was a problem for *A. mellifera* (Crane, 1978), this analysis takes a precautionary approach regarding the possible impact of the mites in the New Zealand environment.

None of these species of varroa are likely to have any effects on New Zealand native insects since they are highly to live and reproduce only on *Apis* spp.

Because of the possible high level of damage, the closeness in genetic relationship between *V. destructor* and these mites species, and the lack of any information to the contrary, the likelihood of adverse consequences for *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* are non-negligible.

34.2.4 Risk Estimation

The likelihood of contamination is negligible for semen and eggs. Therefore the risk for those commodities is considered negligible.

For queens and queen cells, the likelihood of contamination by *Varroa jacobsoni, V. underwoodi, V. rindereri* and *Euvarroa sinhai* and *E. wongsirii* is high.

Because of reports of possible cross-species parasitism, the likelihood of exposure is high for *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*.

The consequences of introduction are assumed to be non-negligible for *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*.

Therefore, although the risk for *Varroa jacobsoni* is considered to be negligible, the risk for *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* is considered to be non-negligible and they are classified as hazards.

34.3 Risk Management

34.3.1 Risk Evaluation

Since the risk estimate for *Varroa underwoodi, V. rindereri, Euvarroa sinhai* and *E. wongsirii* is non-negligible, sanitary measures would need to be employed to effectively manage the risks to reduce them to a negligible level.

34.3.2 Option Evaluation

34.3.2.1 Risk management objective
The objective is to effectively manage the risk of *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* by ensuring the imported *Apis mellifera* genetic material in the form of queen bees and queen cells does not carry either organism when given a biosecurity clearance in New Zealand.

34.3.2.2 Options available

The OIE Code includes recommendations regarding varroa that can be used as a basis for developing appropriate measures.

One option would be to allow imports of *A. mellifera* genetic material only from countries free of *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*. However, since post-arrival quarantine facilities can be used to effectively mitigate against release of the organisms (White and Rhodes, 1988), sanitary measures can be designed to provide assurance that progeny released into New Zealand are not infested with either organism.

34.3.2.3 Recommended sanitary measures

<u>For queen bees and queen cells</u>

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*, or

2) Be from hives treated with a miticide effective against varroa (e.g., Apistan) beginning 24 days before shipment and through to the time of shipment; and
3) Be from hives sampled and found free of *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* (Apistan/sticky boards and alcohol wash) within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All accompanying worker bees should be killed and examined (alcohol wash) for *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*; and
   b) Each queen bee/cell should be placed into a nucleus hive consisting of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with a miticide effective against varroa (e.g., Apistan); and
   d) Separate confined areas should be used for holding the importing material and rearing the progeny, with transfer of genetic material to the second confined area via eggs.

If the original consignment is found to contain *V. underwoodi, V. rindereri, E. sinhai* or *E. wongsirii* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the nuclei in the second confined area (nuclei receiving eggs) have been examined (Apistan/sticky boards and alcohol wash) and found to be free of *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*. All remaining imported material should be destroyed.

For semen or eggs

No sanitary measures required.

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35. WAX MOTHS

35.1 Hazard Identification

35.1.1 Aetiologic Agent: Family *Pryalidae*, *Galleria mellonella* L., *Achroia grisella* Toumanoff.

35.1.2 OIE List: None.

35.1.3 New Zealand's Status: Both wax moths are present in New Zealand. Not under official control.

35.1.4 Epidemiology

The greater wax moth (*Galleria mellonella*) is a pest of honey bee combs. The larval stage of the moth feeds on honey, nectar, pollen and beeswax. Bee brood may also be attacked when the larvae are short of food. The development cycle (egg, larva, pupa) of the moth varies from four weeks to six months, depending on food availability. Adult females live from three days to one month (Ben Hamida, 1999).

G. mellonella can cause considerable damage to honey bee colonies, destroying weak colonies and causing desertion. The moth is considered a serious pest of honey bees, especially in tropical conditions (FAO, 1986).

The greater wax moth is present in most parts of the world, although it is limited in its distribution by its inability to withstand very low temperatures (Williams, 1997). The moth is found in the warmer parts of New Zealand (Reid, 1988).

G. mellonella spreads between hives via the flight of adult females, or the human-assisted movement of beeswax combs containing either eggs or larvae from one hive to another. Eggs generally hatch in eight to 10 days, but hatching may be prolonged for up to 30 days at low temperatures.

The lesser wax moth (*Achroia grisella*) has a similar biology to *G. mellonella*, but is less widely distributed worldwide. The moth is generally of minor importance, but can destroy neglected combs (Williams, 1997). The moth is found throughout New Zealand (Reid, 1988).

Control of both species of wax moth is generally through the fumigation of stored combs with chemicals such as paradichlorobenzene, or by spraying stored combs with formulations of *Bacillus thuringiensis*. There are no reports of strain variation among greater and lesser waxmoths abroad. (Williams, 1997).

35.1.5 Conclusion

The greater and lesser waxmoths are present in New Zealand, they are not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore *G. mellonella* and *A. grisella* are not classified as potential hazards for the purposes of this analysis.

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36. AMOEBA DISEASE

36.1 Hazard Identification

36.1.1 Aetiologic Agent: Family *Entamoebidae, Malpighamoeba mellificae* Prell.

36.1.2 OIE List: None.

36.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

36.1.4 Epidemiology

Amoeba disease is a disease of adult *Apis mellifera* caused by the parasitic protozoan *Malpighamoeba mellificae*. Cysts of *M. mellificae* germinate in the rectum of adult honey bees and then travel through the alimentary canal to lodge in the Malpighian tubules, which function in bees in a similar fashion to the liver (Crane, 1990). The amoeba encysts in the Malpighian tubules, and the cysts are then deposited in faeces. The cysts transmit the parasite to new hosts when the cysts come into contact with an adult bee's mouthparts during routine comb cleaning (Bailey, 1955).

Amoeba disease presents no clear symptoms and there is no experimental evidence that infections of *M. mellificae* shorten adult honey bee lifespans or cause dysentery in infected colonies. Strain variations in virulence have not been reported (Fries, 1997).

Amoeba disease is ubiquitous, and has been identified in all continents where *A. mellifera* is kept (Matheson, 1997). *M. mellificae* has been found in honey bees in New Zealand (Anderson, 1987).

36.1.5 Conclusion

M. mellificae is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore *M. mellificae* is not classified as a potential hazard for the purposes of this analysis.

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37. GREGARINE DISEASE

37.1 Hazard Identification

37.1.1 Aetiologic Agent: Family *Gregarinidae, Monoica apis, Apigregarina stammeri, Acuta rousseaui* and *Leidyana apis*.

37.1.2 OIE List: None.

37.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms .register

37.1.4 Epidemiology

Gregarine disease is a disease of adult *Apis mellifera* caused by four species of protozoan parasites in the Family *Gregarinidae* (Shimanuki et al, 1992). The organism attaches itself to the gut epithelium of honey bees where it encysts (Stejskal, 1955), destroying epithelial cells.

Although it is known that the organism produces spores which are passed through the bee in faeces (Hitchcock, 1948), the mechanism of spread of gregarine disease between bees and between colonies is not known. Suggested routes include package bees (Stejskal, 1965), contaminated water (Stejskal, 1965), bumble bees (Hitchcock, 1948), cockroaches (Stejskal, 1955), and contaminated comb (Hitchcock, 1948).

The damage gregarine disease causes to honey bees is unclear. Reported infection rates have varied between 12 and 300 per bee in the United States (Hitchcock, 1948) and up to 3000 per bee in Venezuela (Stejskal, 1955). Although gregarines do cause pathological changes in the cells where they attach (Stejskal, 1965), there is little evidence that they cause measurable damage to infected bees (Bailey and Ball, 1991; Oertel, 1965). The economic importance of gregarine disease has yet to be determined (Oertel, 1965), but it is thought that bees infested by gregarines may not be able to work efficiently and may die prematurely (Stejskal, 1965). On the other hand, it has also been suggested that there is little reason to control gregarine infections in temperate climates (Fries, 1997) as bees in such areas are less likely to be infected than those in tropical regions (Hitchcock, 1948). Warm climates are probably more favourable to gregarine disease, since the organism is killed by freezing (Stejskal, 1955).

Honey bees parasitised by gregarines have been reported from Venezuela, North Africa, North America, France, Italy and Switzerland (Hitchcock, 1948; Stejskal, 1955). There are no reports of gregarines in honey bees in New Zealand.

37.1.5 Conclusion

Since gregarines have not been reported in New Zealand, under the criteria presented in Section 2.2, these organisms must be classified as a potential hazard for the purposes of this analysis.

37.2 Risk Assessment

37.2.1 Release Assessment

For the commodity to become infected or contaminated it would have to come from a colony that is already infected with gregarines, or be visited by a foraging bee that is carrying the organism(s).

There is little information regarding the mode of transmission of the disease, and no information regarding the level of gregarine spores in a colony needed to bring about contamination. However, since the disease occurs only rarely, spore transfer and germination are unlikely to be highly efficient.

Honey bee semen is unlikely to contain spores of gregarines, since semen is obtained from within the drone. The only possibility for contamination to occur would be by the insemination syringe momentarily touching the exoskeleton of a drone during eversion.

Unless spores of gregarines are highly infective, it is unlikely that sufficient spores would be deposited on honey bee eggs to initiate an infection in a colony receiving that commodity.

Queens and queen cells are the only forms of biological pathway that are likely to carry significant spores of gregarines to initiate an infection, and the risk of release associated with these two commodities is considered to be non-negligible.

37.2.2 Exposure Assessment

Queen insemination is not an exposure pathway, since spores of the organism must be fed to young larvae to create an infection. Therefore the likelihood of exposure via semen is negligible.

Because imported *Apis mellifera* queens and queen cells would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, the likelihood of exposure is considered to be non-negligible.

37.2.3 Consequence Assessment

Gregarines appear to be of little consequence to honey bee colonies in temperate regions, but could possibly cause some insignificant problems for bees in the more sub-tropical areas of the North Island of New Zealand. The disease is unlikely to result in justified restrictions on bee and bee product exports from New Zealand since there are no official control programmes for gregarines anywhere in the world.

Although bumblebees and cockroaches have been suggested as mechanisms of spread of gregarine disease between bees, there is no information to suggest the disease would cause any effects to New Zealand native insects.

Therefore the consequences of introduction are considered to be negligible.

37.2.4 Risk Estimation

Since the likelihood of release for semen or eggs is negligible, the risk associated with those commodities is considered to be negligible.

The likelihood of contamination or infection of queen bees or queen cells coming from a colony containing gregarines is assumed to be high. The probability of establishment of gregarines in New Zealand via those commodities is assumed to be non-negligible. However, the likelihood of any significant consequences resulting from that establishment is negligible. The risk is therefore also considered to be negligible for queens and queen cells.

37.3 Risk Management

37.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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38. NOSEMA DISEASE

38.1 Hazard Identification

38.1.1 Aetiologic Agent: Family *Nosematidae*, *Nosema apis* Zander.

38.1.2 OIE List: B.

38.1.3 New Zealand's Status: Present in New Zealand. Not under official control.

38.1.4 Epidemiology

Nosema disease (nosemosis) is a disease of adult *Apis mellifera* caused by the parasitic protozoan *Nosema apis*. Spores of *N. apis* are ingested by an adult bee and germinate in the ventriculus within 10 minutes (Bailey, 1955a). A polar filament in the spore penetrates an epithelial cell in the bee's ventriculus and inoculates the host cell with sporoplasm. Multiplication of the parasite occurs, followed by the production of more spores of two distinct types (Fries et al, 1992). Spores are excreted from the bee, where they are picked up by other bees during comb cleaning. Spores can remain viable in faeces for more than a year (Bailey and Ball, 1991).

Spore levels of infected bees can range from 30 to 200 million spores per bee (Bailey and Ball, 1991). Heavy infection can cause inflammation of the digestive tract, dysentery, reduced nutrient uptake, increased physiological ageing and reduced longevity, reduced ability to secrete larval food, and metabolic disorders in the queen (Fries, 1997). Infection levels follow a seasonal progression, with lowest levels in the summer and highest in the late winter and early spring (Bailey, 1955b).

Despite not having overt clinical symptoms, the effects of nosemosis on honey bees can be dramatic (Shimanuki et al, 1992), including reduced honey production (Kauffeld et al, 1972) increased winter colony losses (Fries, 1988), and queen loss and supersedure (Jay and Dixon, 1982).

Control of nosema disease is generally through the prophylactic feeding of fumagillin in syrup, generally in the early spring and sometimes in the autumn (Fries, 1997).

N. apis has a cosmopolitan distribution, and is probably present wherever honey bees are kept (Matheson, 1997). *N. apis* is present in New Zealand (Anderson, 1988).

38.1.5 Conclusion

N. apis is present in New Zealand, it is not under official control, and there is no evidence to suggest that more virulent strains exist abroad. Therefore *N. apis* is not classified as a potential hazard for the purposes of this analysis.

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39. AFRICANISED BEE

39.1 Hazard Identification

39.1.1 Scientific Name: Family *Apidae, Apis mellifera scutellata* Lepeletier and its hybrids.

39.1.2 OIE List: None.

39.1.3 New Zealand's Status: Exotic to New Zealand. Listed on the unwanted organisms register as a notifiable organism.

39.1.4 Description

Apis mellifera scutellata is a subspecies of honey bee naturally occurring in an extensive range of eastern and southern Africa from Ethiopia to the Cape (Ruttner, 1986). The subspecies was introduced into Brazil from Africa in 1956 in an attempt to breed a strain of bees that would be more suitable to tropical conditions (Winston, 1992).

Since its introduction, the subspecies has spread into much of South America, all of Central America, Mexico, and into some areas of the south-western United States (Winston, 1992; Thoenes, 1999). The bee is regarded to a greater or lesser extent as a hybrid with local populations of bees, and is thus referred to more correctly as an 'Africanised' bee. Crane has reviewed research that explains why the progeny of hybridisation with *A. m. scutellata* forms a population that achieves dominance over European sub-species (Crane, 1990).

Africanised bees have a number of behavioural traits that make them difficult to manage, the most important being their exceptionally high level of defensive behaviour (Collins et al, 1982), and their lower honey production (Rinderer, 1988). It is believed that they have the potential to be the single most severe insect pest in the United States (Dietz, 1992).

39.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.2, *A. m. scutellata* must be classified as a potential hazard for the purposes of this analysis.

39.2 Risk Assessment

39.2.1 Release Assessment

The imported commodities could be 'contaminated' by Africanised genes if :

- an imported queen had been mated with one or more drones with Africanised genes, or the queen had been derived from an egg produced by a queen with Africanised genes, or both; or
- an imported queen had been artificially inseminated with semen obtained from one or more drones with Africanised genes; or
- imported semen had been collected from one or more drones with Africanised genes; or
- imported eggs had been derived from a queen with Africanised genes; or
- the larvae used to develop an imported queen cell had been derived from an egg produced by a queen with Africanised genes.

For any exports from a country that has Africanised bees, the likelihood of release is non-negligible.

39.2.2 Exposure Assessment

The initial introduction of Africanised bees into Brazil was through the importation of pure-bred *Apis mellifera scutellata* queens into that country, with 26 of those queens being released accidentally as swarms, and also the propagation and distribution of hybrid queens to beekeepers in Southern Brazil (Winston, 1992). Whether the genetic material from a single introduction (especially if that introduction does not contain all *A. m. scutellata* genes) would be swamped by European bees in New Zealand is unknown. However, the apparent dominance of Africanised over European characteristics (Fierro et al, 1988) suggests that there is a non-negligible likelihood that a single introduction could eventually become dominant in this country.

Should Africanised bee genetic material be introduced and the genes not swamped by the local gene pool, they would probably spread over most of New Zealand, if predictions made by some scientists in the United States (Dietz and Vergara, 1995) are correct. However, it has also been suggested that Africanised bees will not successfully colonise further north or south than the 33rd parallel (Eischen, 1994) or overwinter in areas with mean monthly temperatures less than 15.5^{o}C (Taylor, 1985). If this is correct, then Africanised bees would be unlikely to establish in New Zealand in feral colonies except in Northland, since Auckland has six months with mean monthly temperatures less than 15^{o}C.

Because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, there is a likelihood of spread by humans throughout New Zealand. Due to the uncontrolled nature of honey bee mating behaviour, the likelihood of New Zealand strains being exposed to Africanised bee genetic material is also high.

Therefore, the likelihood of exposure is high.

39.2.3 Consequence Assessment

Should Africanised bees become established in New Zealand, the consequences on beekeeping are likely to be severe. It is likely that the export of queens and package bees would stop, or at least be seriously affected.

The behaviour of Africanised bees would also affect beekeeping practices. Many Latin American countries now require bees to be kept 200–300m from roads, agricultural fields and dwellings (Winston, 1992). A similar requirement in New Zealand would mean that much of the country would become unavailable to beekeepers. Major difficulties would also occur if a high percentage of the 75,000 colonies used for kiwifruit pollination were to become Africanised. Restrictions could prohibit the use of Africanised honey bees for pollination in such situations. It is highly likely that the keeping of bees in built-up areas would be prohibited.

European strains of honey bee existing as feral colonies in New Zealand would be displaced by Africanised colonies as a result of preferential mating behaviour, a shorter development time for Africanised queen bees (DeGrande-Hoffman et al, 1998), and the increased

production of Africanised swarms. Nesting sites not suitable for European colonisation would be utilised by Africanised swarms (Crane, 1990).

The behaviour of Africanised bees would also pose a significant potential public health problem, with increased stinging incidents and increased public resources devoted to swarm and feral colony destruction (Dietz, 1992).

While there does not appear to be any published evidence suggesting that Africanised honey bees would have any adverse effects on New Zealand native insects or plants, it is possible that the behaviours of these bees (e.g., aggressiveness, high density of feral colonies, competition for food resources) could have some adverse effect on some native species. However, a review of ecological impacts of introduced honey bees world-wide found no studies showing detrimental impacts of honey bees on population abundance of any native animals or plants (Butz Huryn, 1997).

Therefore the consequences of introduction are considered to be high.

39.2.4 Risk Estimation

The likelihood of release if an exporting country has Africanised bees is non-negligible. While there is uncertainty surrounding the likelihood of Africanised bees being able to establish in New Zealand's climate, the likelihood of exposure is considered to be non-negligible. The serious impact on beekeeping, horticulture and agriculture, and public health, if Africanised bees were to become established means the consequences of introduction and establishment would be high. Therefore the risk is considered to be high and the organism must therefore be classified as a hazard.

39.3 Risk Management

39.3.1 Risk Evaluation

Since the risk estimate for Africanised honey bees is non-negligible, sanitary measures would need to be employed to effectively manage the risks to reduce them to a negligible level.

39.3.2 Option Evaluation

39.3.2.1 Risk management objective

The objective is to effectively manage the risk of Africanised bees by ensuring the imported *Apis mellifera* genetic material does not carry genes of Africanised bees when given a biosecurity clearance in New Zealand.

39.3.2.2 Options available

The OIE Code does not include recommendations regarding Africanised bees.

One option would be to allow imports of *A. mellifera* genetic material only from countries free of the Africanised honey bees. However, since suitable testing techniques are available that give a high probability of determining whether individual bees are Africanised (Sheppard and Smith, 2000; Collins et al, 2000), and since post-arrival quarantine facilities can be used

to effectively mitigate against release of the organism (White and Rhodes, 1988), sanitary measures can be designed that include these techniques to provide assurance that progeny released into New Zealand do not contain Africanised genes.

39.3.2.3 Recommended sanitary measures

For honey bee queens

Consignments must:

1) Be from a country or part of the territory of a country free from Africanised honey bees (see Appendix I), or

2) Be from hives sampled and found free of Africanised honey bees (morphometric analysis or PCR using nuclear DNA) within seven days of shipment; and
3) Be placed in post-arrival quarantine (see Appendix II) where:
   a) Each queen bee should be placed into a nucleus hive consisting of bees from New Zealand; and
   b) All accompanying worker bees should be killed and examined for Africanisation (morphometric analysis or PCR using nuclear DNA); and
   c) Adult bee progeny should be examined for Africanisation (morphometric analysis or PCR using nuclear DNA).
   d) Because the genetic material of Africanised bees is transferable in eggs, there is no need to separate the confined area holding the imported material and the confined area where progeny are reared (see Appendix II).

If the original consignment is found to contain africanised genes during testing in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once testing shows those progeny to have an acceptably low probability of being Africanised. The original imported material should be destroyed.

For honey bee queen cells and eggs

Consignments must:

1) Be from a country or part of the territory of a country free from Africanised honey bees (see Appendix I), or

2) Be from hives sampled and found free of Africanised honey bees (morphometric analysis or PCR using nuclear DNA) within seven days of shipment; and
3) Be placed in post-arrival quarantine (see Appendix II), where:
   a) Each queen cell or egg should be placed into a nucleus hive consisting of bees from New Zealand; and
   b) Adult bee progeny should be examined for Africanisation (morphometric analysis or PCR using nuclear DNA); and
   c) Because the genetic material of Africanised bees is transferable in eggs, there is no need to separate the confined area holding the imported material and the confined area where progeny are reared (see Appendix II).

If the original consignment is found to contain africanised genes during testing in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once testing shows those progeny to have an acceptably low probability of being Africanised. The original imported material should be destroyed.

For honey bee semen

Consignments must:

1) Be from a country or part of the territory of a country free from Africanised honey bees (see Appendix I), or

2) Be from hives sampled and found free of Africanised honey bees (morphometric analysis or PCR using nuclear DNA) within seven days of shipment; and
3) Be placed in post-arrival quarantine (see Appendix II), where:
   a) the semen is examined for Africanisation (PCR using nuclear DNA)
   b) the adult progeny reared from the inseminated queen should be examined for Africanisation (morphometric analysis or PCR using nuclear DNA).
   c) Because the genetic material of Africanised bees is transferable in semen, there is no need to separate the confined area holding the imported material and the confined area where progeny are reared (see Appendix II).

If the original consignment is found to contain africanised genes during testing in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once testing shows those progeny to have an acceptably low probability of being Africanised. The original imported material should be destroyed.

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40. CAPE HONEY BEE

40.1 Hazard Identification

40.1.1 Scientific Name: Family *Apidae, Apis mellifera capensis* Escholtz.

40.1.2 OIE List: None.

40.1.3 New Zealand's Status: Exotic to New Zealand. Listed on the unwanted organisms register.

40.1.4 Description

The Cape honey bee (*Apis mellifera capensis*) is a subspecies of *A. mellifera* found in the Cape region of southern Africa. The bee is notable for its high level of thelytoky, which is the ability to produce diploid (female) adults from unfertilised eggs generated from laying workers (Verma and Ruttner, 1983). Thelytoky does exist in other subspecies of *A. mellifera*, but is very rare. These other subspecies almost invariably produce haploid adults from laying workers, and the drone is the only honey bee caste that is haploid. As a result, in subspecies other than *A. m. capensis*, colonies without a mated queen, or a fertilised egg that can be used to rear a new queen, are generally unable to replenish their population of worker bees. Such colonies eventually perish (Winston, 1987).

When colonies of other subspecies of honey bee are kept within flight range *of A. m. capensis,* laying workers of the Cape bee are likely to enter the colonies (Johannsmeier, 1983). The laying workers mimic a series of queen pheromones and are able to successfully escape reproductive suppression from the resident queen and adult bees. Social parasitism occurs, with the laying workers producing diploid eggs. The pheromone mimicry causes a breakdown in reproductive regulation, resulting in reproductive anarchy in the colony (Wossler, 2002).

In southern Africa, *A. m. scutellata* colonies are successfully usurped by *A. m. capensis* workers, and the result is colony death, since once the *A. m. scutellata* queen disappears no new adult queens of either race are observed in the usurped colonies (Martin et al, 2002). A population model has been constructed to evaluate the impact of parasitism of *A. m. capensis* laying workers on populations of *A. m. scutellata*, both in apiaries and in the wild. The model shows that *A. m. capensis* infestations are likely to be fatal for kept hives of *A. m. scutellata* irrespective of beekeeping activities to compensate for colony losses, although population dynamics achieve equilibrium for wild populations (Moritz, 2002).

The Cape honey bee is currently limited in distribution to its natural range, although the area may be larger than originally thought, with a line of hybridisation with *A. m. scutellata* (Crewe et al, 1994).

40.1.5 Conclusion

Since *A. m. capensis* has not been reported in New Zealand, under the criteria presented in Section 2.2, this subspecies must be classified as a potential hazard for the purposes of this analysis.

40.2 Risk Assessment

40.2.1 Release Assessment

The imported commodities could be 'contaminated' with *Apis mellifera capensis* genes if :

- an imported queen had been mated with one or more drones with *Apis mellifera capensis* genes, or a queen had been derived from an egg produced by a queen or laying worker with *A. m. capensis* genes, or both; or
- an imported queen had been artificially inseminated with semen obtained from one or more drones with *A. m. capensis* genes; or
- an imported queen had been accompanied by one or more worker bees with *A. m. capensis* genes; or
- imported semen had been collected from one or more drones with *A. m. capensis* genes; or
- imported eggs had been derived from a queen or laying worker with *A. m. capensis* genes; or
- the larvae used to develop an imported queen cell had been derived from an egg produced by a queen or laying worker with *A. m. capensis* genes.

The likelihood of exposure occurring depends on whether the exporting country has the Cape honey bee. Since the cape honey bee appears to be limited to southern Africa, the likelihood of release is non-negligible for consignments from that area.

40.2.2 Exposure Assessment

It is unclear what the gene frequency of *A. m. capensis* genetic material needs to be in either a mixed mating, or mixed semen used for artificial insemination, to impart thelytoky in progeny. It is also unclear what would eventuate if a single *A. m. capensis* worker were released into a colony of *A. mellifera* in New Zealand. It is possible that genetic material from a single introduction (especially if that introduction does not contain all *A. m. capensis* genes) would be swamped by European bees in New Zealand. However, the apparent dominance of *A. m. capensis* over *A. m. scutellata* suggests that there is a non-negligible likelihood that a single introduction could eventually become dominant in this country.

Because imported *Apis mellifera* genetic material would most likely be used to establish foundation stock for a breeding programme, or less likely as replacement queens to head production colonies, it is considered that there is a non-negligible likelihood of exposure and establishment of the organism in New Zealand.

40.2.3 Consequence Assessment

The consequence of introducing *A. m. capensis* genetic material into New Zealand in any of the commodity forms is uncertain. However, if *A. m. capensis* genetic material were introduced and the genes were not swamped by the local gene pool, it is possible that they could spread over at least the warmer parts of New Zealand, since the sub-species is noted for its overwintering ability in climatic conditions of the Cape Town region (Sheppard, 1997).

The social parasitism and usurpation displayed by *A. m. capensis* suggests that even at low frequencies in the wild honey bee population, the sub-species could cause an on-going threat to beekeeping activities with other sub-species of honey bee (Moritz, 2002). Beekeepers

could lose substantial numbers of colonies to the parasitism and activities such as splitting and requeening to make up losses might not be able to overcome those losses. Losses could also impact on the price and availability of hives used for commercial pollination activities. Establishment of the sub-species would also likely stop (or at least seriously affect) the export of queens and package bees from New Zealand.

There does not appear to be any published evidence suggesting that the Cape honey bee would have any adverse effects on New Zealand native insects or plants. Apart from social parasitism and usurpation of other *Apis mellifera*, there are no other detrimental effects associated with this sub-species. A review of ecological impacts of introduced honey bees world-wide found no studies showing detrimental impacts of honey bees on population abundance of any native animals or plants (Butz Huryn, 1997).

In view of the potential impact on beekeeping and commercial pollination if *A. m. capensis* were to become established in New Zealand, the consequences of introduction are considered to be non-negligible.

40.2.4 Risk Estimation

The likelihood of release if an exporting country has the Cape honey bee is non-negligible. The likelihood of exposure and establishment of the organism in New Zealand is assumed to be non-negligible, as are the possible consequences. The organism is therefore be classified as a hazard.

40.3 Risk Management

40.3.1 Risk Evaluation

Since the risk estimate for the Cape honey bee is non-negligible, sanitary measures would need to be employed to effectively manage the risks to reduce them to a negligible level.

40.3.2 Option Evaluation

40.3.2.1 Risk management objective

The objective is to effectively manage the risk of Cape honey bees by ensuring the imported *Apis mellifera* genetic material does not carry genes of *A. m. capensis* when given a biosecurity clearance in New Zealand.

40.3.2.2 Options available

The OIE Code does not include recommendations regarding Cape honey bees.

One option would be to allow imports of *A. mellifera* genetic material from countries where Cape honey bees are present providing testing techniques are used to determine whether the material contains *A. m. capensis* genes. However, it is unclear whether suitable testing techniques are available that give a high probability of determining whether individual bees contain such genes, and if they are available, they do not appear to be used on a routine basis, as is the case with Africanised bee identification. The only suitable option is therefore not to allow imports of *A. mellifera* genetic material from such countries.

40.3.2.3 Recommended sanitary measures

Each consignment must be from hives from a country or part of the territory of a country free from Cape honey bees (see Appendix I).

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41. HONEY BEE RACES OTHER THAN *A. M. SCUTELLATA* AND *A. M. CAPENSIS*

41.1 Hazard Identification

41.1.1 Scientific Name: Family *Apidae, Apis mellifera carnica* Pollmann, *A. m. caucasica* Gorbatschev, and others.

41.1.2 OIE List: None.

41.1.3 New Zealand's Status: Exotic to New Zealand. Not listed on the unwanted organisms register.

41.1.4 Description

During the evolution of the *Apis mellifera* species, local populations in Europe and Africa are thought to have become separated from each other by geographic barriers. As a result, the populations became differentiated into distinct regional types, often with identifiable differences in morphology. The types are referred to as races (Crane, 1990).

Ruttner (1986) has analysed geographic differences in *A. mellifera*, and has identified 23 distinct races, including *A. m. scutellata* (see Chapter 39) and *A. m. capensis* (see Chapter 40), belonging to three distinct branches (southern/eastern Europe, northern/western Europe and Africa). New Zealand is known to have two of these races: *A. m. ligustica* (known as the 'Italian bee'); and *A. m. mellifera* (known as the 'European black bee' or the 'German black bee'). *A. m. ligustica* is the predominate race in New Zealand, with *A. m. mellifera* also used (Matheson, 1997). Husbanded colonies in New Zealand are often hybrids of the two races. This is because *A. m. mellifera* characteristics appear to predominate in feral colonies (thus providing a genetic reservoir for cross-mating), and also because many beekeepers, either unintentionally or purposely, maintain hybrids in their honey bee stocks.

The main honey bee races used worldwide in commercial beekeeping are *A. m. ligustica*, *A. m. carnica* (the 'Carniolan bee'), and to a lessor extent *A. m. caucasica* (the 'Caucasian bee') (Dietz, 1992). *A. m. scutellata* and its hybrids are now distributed throughout the Americas, as a result of importations of the race into Brazil from Africa in 1956 (see Chapter 39). *A. m. mellifera* is found as feral stock in both the United States and the Pacific, as a result of the importation of the race by immigrants in the 1800's. It fell out of favour in commercial beekeeping with the introduction of *A. m. ligustica* in the mid-1800's (Sheppard, 1997). The remaining races of *A. mellifera* tend to be confined to their areas of origin, and have not achieved any wide acceptance as economically important strains elsewhere in the world. This Chapter will therefore concentrate on the Carniolan bee and the Caucasian bee, since they are the two races most likely to be imported as genetic material into New Zealand.

The Carniolan bee is dark in colour, with greyish brown hairs. Behaviourally it is characterised by gentleness during manipulation, rapid population build-up, adjustment of brood production to available resources, good overwintering in cold climates, and excellent honey production (Ruttner, 1986). It's natural range is confined to central Europe, and there are natural areas of hybridisation with *A. m. ligustica*. The two races are closely related (Dietz, 1992). It is a commercially important bee in North America (Sheppard, 1997), and is the most popular bee kept in Germany (Dietz, 1992). The bee has also been imported into Australia under quarantine (White and Rhodes, 1988).

The Caucasian bee is also dark in colour, with grey hairs and a long proboscis. It displays extreme gentleness on the combs, slow spring build-up, and a low inclination to swarm. It is known to deposit greater amounts of propolis on hive parts than many other races (Ruttner, 1986). It also appears to be prone to nosema infection, but is nevertheless regarded as a good honey producer (Dietz, 1992). The natural range of the race is confined to a small area of the Caucasus Mountains, but it has been used commercially in a number of countries, including the United States (Sheppard, 1997). The bee has also been imported into Australia under quarantine (White and Rhodes, 1988).

Both races of bee have been imported into New Zealand at various times during the development of beekeeping in this country. However, no legal imports of genetic material other than *A. m. ligustica* have been made for at least the last 45 years (Matheson, 1997), and there do not appear to be any morphometric or DNA analyses proving that either Carniolan or Caucasian bees currently exist in a distinguishable form in New Zealand.

41.1.5 Conclusion

Imports of honey bee queens, eggs, semen or queen cells could contain *A. m. carnica* or *A. m. caucasica* genetic material. The races also appear to be exotic to New Zealand. Therefore, under the criteria presented in Section 2.2, *A. m. carnica* and *A. m. caucasica* must be classified as a potential hazard for the purposes of this analysis.

41.2 .Risk Assessment

41.2.1 Release Assessment

The imported commodities could be 'contaminated' with *Apis mellifera carnica* or *A. m. caucasica* genes if :

- an imported queen had been mated with one or more drones with *Apis mellifera carnica* or *A. m. caucasica* genes, or a queen had been derived from an egg produced by a queen with *A. m. carnica* or *A. m. caucasica* genes, or both; or
- an imported queen had been artificially inseminated with semen obtained from one or more drones with *A. m. carnica* or *A. m. caucasica* genes; or
- imported semen had been collected from one or more drones with *A. m. carnica* or *A. m. caucasica* genes; or
- imported eggs had been derived from a queen with *A. m. carnica* or *A. m. caucasica* genes; or
- the larvae used to develop an imported queen cell had been derived from an egg produced by a queen with *A. m. carnica* or *A. m. caucasica* genes.

The likelihood of exposure occurring depends on whether the exporting country has recognised lines of either Carniolan or Caucasian bees. The races are kept in a number of important beekeeping countries, so if a consignment of queen bees, queen cells, semen or eggs comes from one of those countries then the likelihood of release is non-negligible.

41.2.2 Exposure Assessment

Unlike *A. m. mellifera* or *A. m. scutellata,* there does not appear to be any record of either Carniolan or Caucasian bees predominating naturally in areas where they have been

introduced outside their range. In order to develop a pure-bred line of either Carniolan or Caucasian bees it would be necessary to import considerable amounts of genetic material in one or more forms, and then either undertake sophisticated line-breeding and back-crossing, or closed population breeding, to maintain the purity and brood variability of the lines over time (Laidlaw and Page, 1997).

Therefore Carniolan or Caucasian bees are only likely be sustained as a result of on-going management by beekeepers. Unless the New Zealand environment is significantly different to other environments outside their natural range where the bees have been introduced, it is highly unlikely that either race would become established as self-sustaining feral populations in New Zealand for any length of time. Such self-sustaining populations have not eventuated as a result of both races being introduced into this country in the past. The probability of establishment resulting from an exposure is therefore assessed as negligible.

41.2.3 Consequence Assessment

There does not appear to be any published evidence suggesting that either Carniolan or Caucasian bees would have any adverse effects on New Zealand native insects or plants. A review of ecological impacts of introduced honey bees world-wide found no studies showing detrimental impacts of honey bees on population abundance of any native animals or plants (Butz Huryn, 1997).

Neither race of bee would cause any significant adverse consequences for human health, apart from stinging. Based on experience in countries where either one or both of the races are already present, stinging would be similar in level to that already experienced by other stinging insects (wasps, bumblebees, etc.). There are no scientific studies to suggest that levels of aggressiveness (including stinging) in hybrids between these races and races currently in New Zealand would be any greater than the levels of aggressiveness in hybrids already occurring between *A. m. ligustica* and *A. m. mellifera*. Many New Zealand beekeepers currently cope with these levels of aggressiveness in their colonies, and are prepared to do so in exchange for perceived positive characteristics (such as reduced food consumption) that also result from such hybridisation. Unless it drastically impairs/prohibits beekeeping practices or causes significant public health risk (as in the case of *A. m. scutellata* and its hybrids), honey bee aggressiveness is a matter of individual preference, not adverse economic consequence.

Because both Carniolan and Caucasian bees are regarded world-wide as races of economic importance with desirable commercial beekeeping characteristics, and because no significant adverse consequences are likely to result even if they are introduced and maintained by beekeepers in New Zealand, the consequence of their introduction are likely to be negligible.

41.2.4 Risk Estimation

The likelihood of release occurring if an exporting country has either Carniolan or Caucasian bees is non-negligible. However, the likelihood of self-sustaining feral establishment of the races in New Zealand is negligible, as is the probability of the bees producing significant negative consequences even if they are maintained by beekeepers as pure-bred lines or hybrids. The risk is therefore considered to be negligible.

41.3 Risk Management

41.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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42. HONEY BEES OTHER THAN *APIS MELLIFERA*

42.1 Hazard Identification

42.1.1 Scientific Name: Family *Apidae*, *Apis andreniformis* Smith, *A. cerana* F., *A. florea* F., *A. dorsata* F., *A. laboriosa* Sakagami, *A. koschevnikovi* Buttel-Reepen, *A. nuluensis* Tingek, Koeniger & Koeniger, *A. nigrocincta* Smith.

42.1.2 OIE List: None.

42.1.3 New Zealand's Status: Exotic to New Zealand. Listed on the unwanted organisms register.

42.1.4 Description

There are at least four species of bees in the *Apis* genus. *A. mellifera* is already in New Zealand and worldwide is the species most commonly managed by humans. The other three species are *A. cerana*, *A. florea* (the dwarf honey bee) and *A. dorsata* (the giant honey bee), all of which have not been introduced into New Zealand. More recently it has been suggested that other species of *Apis* exist. These include *A. andreniformis* (the small dwarf honey bee), *A. laboriosa* (a large, specialised mountain bee) and *A. koschevnikovi* (Dietz, 1992), as well as *A. nigrocincta* and *A. nuluensis* (Takahashi et al, 2002).

A. cerana occurs in Asia and as far south as New Guinea. *A. florea* occurs in Asia and as far west as Iran. It has also been introduced to Africa. *A. dorsata* is restricted to south and southeast Asia (Ruttner, 1986). *A. andreniformis* occurs in southeast Asia and as far north as southern China. *A. laboriosa* occurs at high altitudes in Nepal. *A. koschevnikovi* occurs in northern Borneo (Dietz, 1992). *A. nigrocincta* and *A. nuluensis* are found in Borneo and Sulawesi/Mindanao respectively (Takahashi et al, 2002).

The only detrimental effect that has been identified with different *Apis* species is inter-specific robbing behaviour, particularly between *A. cerana* and *A. mellifera (*Fell, 1997).

42.1.5 Conclusion

Although no hybridisation is possible between *A. mellifera* and other *Apis* species, imports of honey bee queens, eggs, semen or queen cells could contain genetic material of other *Apis* species if (for whatever reason) those imports were derived from the wrong species of bee. *Apis* species other than *A. mellifera* are exotic to New Zealand and are listed on the unwanted organisms register. As a result *Apis* species other than *A. mellifera* are classified as a potential hazard for the purposes of this analysis.

42.2 Risk Assessment

42.2.1 Release Assessment

The commodity could become 'contaminated' with genetic material of *Apis* spp other than *A. mellifera* if:

- an imported queen or queen cell had come from a species of *Apis* other than *A. mellifera*; or
- imported semen had been collected from one or more drones of a species of *Apis* other than *A. mellifera*; or
- imported eggs had been derived from a queen of a species of *Apis* other than *A. mellifera*.

The likelihood of such event s would obviously depend on whether the exporting country has a species of *Apis* other than *A. mellifera*. Distribution is limited to south and southeast Asia, as well as Africa (*A. florea*), so there is a possiblility of such events for consignments from these areas. However, it is highly unlikely that an imported consignment of bees that is purportedly *A. mellifera* would mistakenly be composed of a different species of *Apis*, since the species are all grossly dissimilar to *A. mellifera* in size and other visual characteristics. Therefore, the likelihood of release is considered to be negligible.

42.2.2 Exposure Assessment

Other species of *Apis* are unable to co-exist in colonies of *A. mellifera*, and they are incapable of successfully mating and hybridising with the species (Koeniger and Koeniger, 2000). Imports of species of *Apis* other than *A. mellifera* in the form of queen cells, eggs or semen would therefore be incapable of establishment in New Zealand since their survival is totally dependent on a colony structure of adults of the same species. Imports of queens in the form of single queens accompanied by a small number of attendant bees would also be unlikely to establish, since the number of attendants would be insufficient to develop a sustainable colony structure. Moreover, since only one species other than *A. mellifera* (*A. cerana*) is capable of being kept in man-made hives, the likelihood of exposure considered to be negligible.

42.2.3 Consequence Assessment

It is unclear what consequences would arise from inadvertent introduction into New Zealand of genetic material of an *Apis* species other than *A. mellifera*. While the species are all endemic to Asia, they are found throughout a range of climatic situations and altitudes. Thus for the purposes of this risk analysis it is assumed that they are all able to persist in at least some regions of New Zealand.

The species are all beneficial nectar and pollen feeding insects capable of pollinating flowering plants, and one (*A. cerana*) is capable of being kept in hives. The only likely adverse effect would be inter-specific robbing behaviour, but this is not likely to be of significant consequence to either *A. mellifera* beekeeping or New Zealand native insects.

None of the species would cause any significant adverse consequences for human health, apart from stinging, which based on experience where the species are endemic would be similar in level to that already experienced by other stinging insects (*Apis mellifera*, wasps, bumblebees).

There does not appear to be any published evidence suggesting that species of *Apis* other *than A. mellifera* would have any adverse effects on New Zealand native insects or plants. A review of ecological impacts of introduced honey bees world-wide found no studies showing detrimental impacts of honey bees on population abundances of any native animals or plants (Butz Huryn, 1997).

Therefore the consequences of introduction species of *Apis* other *than A. mellifera* are considered to be negligible.

42.2.4 Risk Estimation

The likelihood of accidental 'contamination' of consignments with genetic material of species of *Apis* other *than A. mellifera* is considered to be negligible. In addition, both the likelihood of establishment and the the likelihood of any significant negative consequences resulting from any chance establishment are negligible. The risk is therefore considered to be negligible.

42.3 Risk Management

42.3.1 Risk Evaluation

Since the risk is considered to be negligible, risk management measures are not required.

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43. SUMMARY OF RECOMMENDED SANITARY MEASURES

Note: the section numbering for recommended sanitary measures is the same as in the body of the risk analysis, so that readers can more easily refer to individual chapters.

DEFORMED WING VIRUS

13.3.2.3 Recommended Sanitary Measures

For honey bee queens, queen cells and eggs

Since there are no suitable measures for these commodities, their importation will not be permitted.

For semen

No sanitary measures required.

AMERICAN FOUL BROOD

20.3.2.3 Recommended sanitary measures

For honey bee queens and queen cells

Each consignment must be either:

1) from hives from a country or part of the territory of a country free from American foulbrood (see Appendix I), or

2) from hives sampled and found free of American foulbrood (culture of adult bees) within seven days of shipment.

For semen and eggs

No sanitary measures required.

EUROPEAN FOUL BROOD

21.3.2.3 Recommended sanitary measures

For honey bee queens, queen cells and eggs

Each consignment should meet the following criteria:

1) Be from hives from a country or part of the territory of a country free from European foulbrood (see Appendix I); or

2) Be from hives sampled and found visually free of European foulbrood within seven days of shipment; and
3) Be placed in post-arrival quarantine (see Appendix II); where
   a) Accompanying worker bees should be killed and examined for for *M. plutonius* by bacterial culture and PCR; and
   b) All recipient nuclei colonies should consist of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with oxytetracycline; and
   d) All recipient nuclei colonies should be sampled and found visually free of European foulbrood at a date beyond the incubation period of the disease (4 days).

If the original consignment is found to contain *M. plutonius* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the nuclei in the second confined area (nuclei receiving eggs or recently hatched larvae) have been examined and found to be free of *M. plutonius* (bacterial culture and PCR). All original imported material should be destroyed.

For semen

No sanitary measures required.

BEE LOUSE

28.3.2.3 Recommended sanitary measures

For queen bees

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *B. coeca* (see Appendix I), or

2) Be from hives treated with an insecticide effective against *B. coeca* (e.g., Perizin) beginning three weeks prior to shipment and through to the time of shipment; and
3) Be from hives sampled and found free of *B. coeca* within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All accompanying worker bees should be killed and visually examined for *B. coeca;* and
   b) Each queen should be placed into a nucleus hive consisting of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with an insecticide effective against *B. coeca* (e.g., Perizin); and
   d) Separate confined areas should be used for holding the importing material and rearing the progeny, with transfer of genetic material to the second confined area via eggs or recently hatched larvae.

If the original consignment is found to contain A. woodi during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of

progeny from post-arrival quarantine should only be granted once the recipient nuclei have been examined and found to be free of *B. coeca*. All remaining imported material should be destroyed.

For queen cells

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *B. coeca* (see Appendix I), or

2) Be from hives treated with an insecticide effective against *B. coeca* (e.g., Perizin) beginning three weeks prior to shipment and through to the time of shipment; and
3) Be from hives sampled and found free of *B. coeca* within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All queen cells should be drenched with an insecticide effective against *B. coeca* (e.g., Perizin) prior to placement in recipient nuclei colonies; and
   b) Each queen cell should be placed into a nucleus hive consisting of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with an insecticide effective against *B. coeca* (e.g., Perizin).
   d) Separate confined areas do not need to be used.

If the original consignment is found to contain A. woodi during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the recipient nuclei have been examined and found to be free of *B. coeca*. All remaining imported material should be destroyed.

For semen or eggs

No sanitary measures required.

SMALL HIVE BEETLE

30.3.2.3 Recommended sanitary measures

For queen cells, queen bees

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *A. tumida* (see Appendix I), or

2) Be from hives treated with an insecticide effective against *A. tumida* (Check-Mite+) beginning 26 days prior to shipment and continuing up to the time of shipment; and
3) Be from hives sampled (Check-Mite+ and sticky boards) and found free of *A. tumida* within seven days of shipment; and

4) Be in new cages or containers not previously in contact with bees ; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All queen cells should be drenched with an insecticide effective against *A. tumida* (Check-Mite+) prior to placement in recipient nuclei colonies; and
   b) All recipient nuclei colonies should consist of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with an insecticide effective against *A. tumida* (Check-Mite+).
   d) Separate confined areas do not need to be used.

If the original consignment is found to contain *A. tumida* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the recipient nuclei have been examined and found to be free of A. tumida (Check-Mite+ and sticky boards). All original imported material should be destroyed.

For semen or eggs

No sanitary measures required.

TRACHEAL MITE

31.3.2.3 Recommended sanitary measures

For queen bees

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *A. woodi*, or

2) Be from hives treated with an miticide effective against *A. woodi* (menthol or formic acid) beginning 19 days prior to shipment and continuing up to the time of shipment; and
3) Be from hives sampled and found free of *A. woodi* (honey bee tracheae dissection or ELISA) within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All accompanying worker bees should be killed and examined for *A. woodi* (honey bee tracheae dissection or ELISA); and
   b) Each queen bee should be placed in a nucleus hive composed of bees from New Zealand.
   c) All recipient nuclei colonies should be treated with a miticide effective against *A. woodi* (menthol or formic acid); and
   d) Separate confined areas should be used for holding the importing material and rearing the progeny, with transfer of genetic material to the second confined area via eggs or recently hatched larvae.

If the original consignment is found to contain *A. woodi* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the nuclei in the second

confined area (nuclei receiving eggs or recently hatched larvae) have been examined and found to be free of A. woodi (honey bee tracheae dissection or ELISA). All original imported material should be destroyed.

For queen cells, semen or eggs

No sanitary measures required.

TROPILAELAPS

32.3.2.3 Recommended sanitary measures

For queen bees

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from tropilaelaps (see Appendix I) or

2) Be from hives treated with a miticide effective against tropilaelaps (e.g., Apistan) beginning three weeks before shipment and through to the time of shipment; and
3) Be from hives sampled and found free of tropilaelaps (Apistan and sticky boards) within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) Queen bees and accompanying workers should be held away from other adult bees and brood for a period twice the recognised longest period for survival of the mite away from brood (i.e., six days).
   b) No recipient nuclei or separation of confined areas is required.

If the original consignment is found to contain tropilaelaps during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted at the end of the withholding period. All remaining imported material should be destroyed.

For queen cells

Each consignment meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from tropilaelaps (see Appendix I), or

2) Be from hives treated with a miticide effective against tropilaelaps (e.g., Apistan) beginning three weeks before shipment and through to the time of shipment; and
3) Be from hives sampled and found free of tropilaelaps (Apistan and sticky boards) within seven days of shipment; and
4) Be placed in post-arrival quarantine (see Appendix II); where
   a) All queen cells should be emerged into nuclei colonies containing no brood, and remaining broodless for 6 days; and

b) All recipient nuclei colonies should consist of bees from New Zealand; and
c) All recipient nuclei colonies should be treated with a miticide effective against tropilaelaps (e.g., Apistan).
d) Separate confined areas do not need to be used.

If the original consignment is found to contain tropilaelaps during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted at the end of the withholding period. All remaining imported material should be destroyed.

For semen or eggs

No sanitary measures required.

VARROA

33.3.2.3 Recommended sanitary measures

For queen bees and queen cells

Each consignment should meet the following criteria:

1) Be from hives from a country or part of the territory of a country free from *V. destructor* (see Appendix I), or

2) Be from hives sampled and found free of *V. destructor* (alcohol wash) within seven days of shipment; and
3) Be in new cages or containers not previously in contact with bees; and
4) Be placed in post-arrival quarantine (see Appendix II); where
   a) All accompanying worker bees should be killed and examined (alcohol wash) for *V. destructor*; and
   b) Each queen bee/cell should be placed into a nucleus hive consisting of varroa-free bees from New Zealand; and
   c) Separate confined areas should be used for holding the importing material and rearing the progeny, with transfer of genetic material to the second confined area via eggs.

If the original consignment is found to contain *V. destructor* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the nuclei in the second confined area (nuclei receiving eggs) have been examined and found to be free of *V. destructor* (alcohol wash). All remaining imported material should be destroyed.

For semen or eggs

No sanitary measures required.

VARROA OTHER THAN V. DESTRUCTOR

34.3.2.3 Recommended sanitary measures

For queen bees and queen cells

Each consignment should meet the following criteria:

1) Be from hives either from a country or part of the territory of a country free from *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*, or

2) Be from hives treated with a miticide effective against varroa (e.g., Apistan) beginning 24 days before shipment and through to the time of shipment; and
3) Be from hives sampled and found free of *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* (Apistan/sticky boards and alcohol wash) within seven days of shipment; and
4) Be in new cages or containers not previously in contact with bees; and
5) Be placed in post-arrival quarantine (see Appendix II); where
   a) All accompanying worker bees should be killed and examined (alcohol wash) for *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*; and
   b) Each queen bee/cell should be placed into a nucleus hive consisting of bees from New Zealand; and
   c) All recipient nuclei colonies should be treated with a miticide effective against varroa (e.g., Apistan); and
   d) Separate confined areas should be used for holding the importing material and rearing the progeny, with transfer of genetic material to the second confined area via eggs.

If the original consignment is found to contain *V. underwoodi, V. rindereri, E. sinhai* or *E. wongsirii* during inspection in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once the nuclei in the second confined area (nuclei receiving eggs) have been examined (Apistan/sticky boards and alcohol wash) and found to be free of *V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii*. All remaining imported material should be destroyed.

For semen or eggs

No sanitary measures required.

UNWANTED BEE GENETICS

AFRICANISED HONEY BEES

39.3.2.3 Recommended sanitary measures

For honey bee queens

Consignments must:

1) Be from a country or part of the territory of a country free from Africanised honey bees (see Appendix I), or

2) Be from hives sampled and found free of Africanised honey bees (morphometric analysis or PCR using nuclear DNA) within seven days of shipment; and
3) Be placed in post-arrival quarantine (see Appendix II) where:
   a) Each queen bee should be placed into a nucleus hive consisting of bees from New Zealand; and
   b) All accompanying worker bees should be killed and examined for Africanisation (morphometric analysis or PCR using nuclear DNA); and
   c) Adult bee progeny should be examined for Africanisation (morphometric analysis or PCR using nuclear DNA).
   d) Because the genetic material of Africanised bees is transferable in eggs, there is no need to separate the confined area holding the imported material and the confined area where progeny are reared (see Appendix II).

If the original consignment is found to contain africanised genes during testing in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once testing shows those progeny to have an acceptably low probability of being Africanised. The original imported material should be destroyed.

<u>For honey bee queen cells and eggs</u>

Consignments must:

1) Be from a country or part of the territory of a country free from Africanised honey bees (see Appendix I), or

2) Be from hives sampled and found free of Africanised honey bees (morphometric analysis or PCR using nuclear DNA) within seven days of shipment; and
3) Be placed in post-arrival quarantine (see Appendix II), where:
   a) Each queen cell or egg should be placed into a nucleus hive consisting of bees from New Zealand; and
   b) Adult bee progeny should be examined for Africanisation (morphometric analysis or PCR using nuclear DNA); and
   c) Because the genetic material of Africanised bees is transferable in eggs, there is no need to separate the confined area holding the imported material and the confined area where progeny are reared (see Appendix II).

If the original consignment is found to contain africanised genes during testing in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once testing shows those progeny to have an acceptably low probability of being Africanised. The original imported material should be destroyed.

<u>For honey bee semen</u>

Consignments must:

1) Be from a country or part of the territory of a country free from Africanised honey bees (see Appendix I), or

2) Be from hives sampled and found free of Africanised honey bees (morphometric analysis or PCR using nuclear DNA) within seven days of shipment; and
3) Be placed in post-arrival quarantine (see Appendix II), where:
   a) the semen is examined for Africanisation (PCR using nuclear DNA)
   b) the adult progeny reared from the inseminated queen should be examined for Africanisation (morphometric analysis or PCR using nuclear DNA).
   c) Because the genetic material of Africanised bees is transferable in semen, there is no need to separate the confined area holding the imported material and the confined area where progeny are reared (see Appendix II).

If the original consignment is found to contain africanised genes during testing in post-arrival quarantine, the consignment should be destroyed. The biosecurity clearance for release of progeny from post-arrival quarantine should only be granted once testing shows those progeny to have an acceptably low probability of being Africanised. The original imported material should be destroyed.

CAPE HONEY BEE

40.3.2.3 Recommended sanitary measures

Each consignment must be from hives from a country or part of the territory of a country free from Cape honey bees (see Appendix I).

Table 2 is a summary of the analysis carried out in this document.

Assessments (release, exposure and consequence) found to have a non-negligible risk are marked with a *plus* (+). Those assessments found to have a negligible risk are marked with a *minus* (–).

Table 2: Results of Risk Analysis

	Common Name/ Disease	Scientific Name	Potential Hazard	Release	Exposure	Consequence	Sanitary Measures
3	Acute paralysis virus	Acute paralysis virus	No	n/a	n/a	n/a	n/a
4	*Apis* iridescent virus	*Apis* iridescent virus	Yes	–	–	–	No
5	Arkansas bee virus	Arkansas bee virus	Yes	+	+	–	No
6	Bee paralysis	Chronic paralysis virus	No	n/a	n/a	n/a	n/a
7	Bee virus X	Bee virus X	No	n/a	n/a	n/a	n/a
8	Bee virus Y	Bee virus Y	No	n/a	n/a	n/a	n/a
9	Berkeley bee virus	Berkeley bee virus	Yes	+	+	–	No
10	Black queen cell	Black queen cell virus	No	n/a	n/a	n/a	n/a
11	Chronic paralysis associate virus	Chronic paralysis associate virus	No	n/a	n/a	n/a	n/a
12	Cloudy wing virus	Cloudy wing virus	No	n/a	n/a	n/a	n/a
13	Deformed wing virus	Deformed wing virus	Yes	+	+	+	E,Q/C
14	Egypt bee virus	Egypt bee virus	Yes	+	+	–	No
15	Filamentous virus	Filamentous virus	No	n/a	n/a	n/a	n/a
16	Kashmir bee virus	Kashmir bee virus	No	n/a	n/a	n/a	n/a
17	Sacbrood	Sacbrood virus	No	n/a	n/a	n/a	n/a
18	Slow paralysis virus	Slow paralysis virus	Yes	+	+	–	No
19	Thai sacbrood	Thai sacbrood virus	Yes	–	–	–	No
20	American foulbrood	*Paenibacillus larvae larvae*	Yes	+	+	+	Q/C
21	European foulbrood	*Melissococcus plutonius*	Yes	+	+	+	E,Q/C
22	*Paenibacillus alvei*	*Paenibacillus alvei*	No	n/a	n/a	n/a	n/a
23	Powdery scale disease	*Paenibacillus larvae pulvifaciens*	Yes	+	+	–	No
24	Septicaemia	*Pseudomonas aeruginosa*	No	n/a	n/a	n/a	n/a
25	Spiroplasmas	*Spiroplasma melliferum, S. apis*	Yes	+	+	–	No
26	Chalkbrood	*Ascosphaera apis*	Yes	+	+	–	No
27	Stonebrood	*Aspergillus* spp.	No	n/a	n/a	n/a	n/a
28	Bee louse	*Braula coeca*	Yes	+	+	+	Q/C
29	External acarine mites	Acarapis dorsalis, A. externus	No	n/a	n/a	n/a	n/a
30	Small hive beetle	*Aethina tumida*	Yes	+	+	+	Q/C
31	Tracheal mite	*Acarapis woodi*	Yes	+	+	+	Q

32	*Tropilaelaps* spp	*Tropilaelaps clareae, T. koenigerum*	Yes	+	+	+	Q/C
33	*Varroa*	*Varroa destructor*	Yes	+	+	+	Q/C
34	*Varroa* spp	*Varroa jacobsoni, V. underwoodi, V. rindereri, Euvarroa sinhai, E. wongsirii*	Yes	+	+ (2)	+ (2)	Q/C (2)
35	Wax moth (greater & lesser)	*Galleria mellonella; Achroia grisella*	No	n/a	n/a	n/a	n/a
36	Amoeba disease	*Malpighamoeba mellificae*	No	n/a	n/a	n/a	n/a
37	Gregarine disease	Gregarinidae	Yes	+	+	–	No
38	*Nosema*	*Nosema apis*	No	n/a	n/a	n/a	n/a
39	Africanised honey bee	*Apis mellifera scutellata* and its hybrids	Yes	+	+	+	E,S,Q/C
40	Cape honey bee	*Apis mellifera capensis*	Yes	+	+	+	E,S,Q/C
41	Honey bee races (1)	*Apis mellifera carnica, A. m. caucasica*	Yes	+	–	–	No
42	Honey bees	*Apis spp.* other than *A. mellifera*	Yes	–	–	–	No

n/a = where the hazard identification process concludes that an organism is not a potential hazard, the risk assessment is not carried out
E = eggs
S = semen
Q/C = queens/queen cells
Note 1 – other than Africanised honey bees and the Cape honey bee
Note 2 – not *Varroa jacobsoni*

APPENDIX I – DEFINITIONS USED IN SANITARY MEASURES

The following definitions have been used in developing the recommended sanitary measures sections of this analysis. The definitions appear in somewhat abridged form in the *OIE International Animal Health Code* (OIE, 2002).

Country or part of the territory of a country free from a bee disease

(Called "Free Zone" in the OIE Code)

Means a clearly defined territory within a country in which no case of a disease included in the Code has been reported during the period stated for such a disease in the Code, and within which and at the borders of which official veterinary control is effectively applied for animals and animal products, and their transportation.

Country or part of the territory of a country with a statutory control programme for the disease

(Called "Official Control Programme" in the OIE Code)

Means a programme which is approved, and managed or supervised by the Veterinary Administration of a country for the purpose of controlling a pathogen or disease by specific measures applied throughout that country or within a zone or zones of that country.

Official veterinary control

Means that the Veterinary Authority knows the location of the animals and the identity of their owner or responsible keeper and is able to apply appropriate animal health measures, as required.

References

OIE. *International Animal Health Code*. Office International des Epizooties, Paris, 2002

APPENDIX II – DEFINITION OF 'POST-QUARANTINE FACILITIES

This definition is based on the successful post-quarantine facility used for the import of honey bee genetic material into Australia (White and Rhodes, 1988):

Post-quarantine facilities should consist of:

confined areas, so that the imported honey bees, and hives and materials used to keep these bees, do not come into contact with honey bees or materials from outside the post-quarantine facility;

hives and materials inside the confined area for the rearing of queen honey bees produced from imported eggs or eggs of the imported queens;

hives and materials to hold the queen bees reared from those eggs, housed in a confined area separated from the area containing the imported bees and associated materials.

References

White B, Rhodes JW. Importation of queen bees - quarantine facility and procedures [in Australia]. In: White B, Rhodes JW (eds*). Bee Keeping in the Year 2000. Proceedings of the Second Australian and International Beekeeping Congress, Surfers Paradise, Gold Coast, Queensland, Australia, July 21-26* Pp 65-67. Federal Council of Australian Apiarists' Assn., Brisbane, 1988