BIOSECURITY NEW ZEALAND

Import risk analysis: Honey bee products

15 December 2004

Ministry of Agriculture and Forestry
Te Manatū Ahuwhenua, Ngāherehere

Import risk analysis: Honey bee products

Biosecurity New Zealand
Ministry of Agriculture and Forestry
Wellington
New Zealand

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Animal Biosecurity
Biosecurity Authority

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Approved for general release

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TABLE OF CONTENTS

BACTERIA

FUNGI

ARTHROPOD PARASITES

PROTOZOA

UNWANTED BEE GENETIC MATERIAL

SUMMARY AND APPENDICES

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Since this risk analysis is based to a large extent on MAF's risk analyses on Honey bee hive products and used equipment (2002) and on Honey bee genetic material (2003), this project does not have a 'primary author'.

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1. EXECUTIVE SUMMARY

The initial risk analysis on honey bee hive products and used beekeeping equipment, written by a consultant on behalf of MAF Biosecurity Authority, was released for public consultation in July 2002.

Public consultation raised a number of issues of concern, and MAF decided that it was necessary to re-write the risk analysis, in line with current procedures and processes. The honey bee genetic material risk analysis of 2003 was used as the template for the re-write, and for consistency the same hazard list was adopted. The original commodity definition was modified by excluding used beekeeping equipment, on the grounds that there was too much uncertainty regarding the risks of disease transmission by that pathway.

The amended commodity definition included the following products :

- Honey
- Propolis
- Pollen
- Royal jelly
- Beeswax
- Bee venom

In this risk analysis, these commodites were considered only in pure form. That is, because of the vast range of manufactured products that contain small amounts of various mixtures of honeybee products, and the diversity of specific manufacturing processes used for such products, it was considered that a general risk analysis of this kind could not address such products, and decisions on these will made by MAF on a case by case basis, applying the principle of equivalence.

Since honey bee pathogens are highly adapted to *Apis* species, the likelihood of any of the organisms on the hazard list causing unwanted harm to New Zealand native insects is considered to be negligible.

The risk analysis concluded that the risk was non-negligible, and that safeguards were justified, for the following organisms:

- *Paenibacillus larvae* subsp. *larvae*, the cause of American foulbrood
- *Melissococcus pluton*, the cause of European foulbrood
- *Braula coeca*, the bee louse
- *Aethina tumida*, the small hive beetle
- Parasitic mites of the family *Varroidae*

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2. INTRODUCTION

2.1 BACKGROUND

The risk analysis entitled "*Honey Bee Hive Products and Used Beekeeping Equipment*", written by a consultant on behalf of MAF, was released for public submission in August 2002.

MAF received 19 submissions on that risk analysis, one from Australia and 18 from New Zealand. The Review of Submissions was released in June 2003, and summarised the major concerns of stakeholders as follows :

- There was concern that honey bee viruses had been inadequately covered in the 2002 risk analysis.
- There was concern about the adequacy of recommended measures for a number of diseases, particularly European foulbrood.
- There was general concern that the risks associated with the importation of used beekeeping equipment were too high or too uncertain to justify their inclusion in the risk analysis.
- There was concern about the risk of introducing small hive beetle, which was discovered in Australia soon after the risk analysis was released for public consultation.

MAF decided to re-write the risk analysis, to expand the sections on bee viruses, and to adopt the standard MAF format for the risk analysis that had in the meantime been used for the risk analysis on honey bee genetic material. In view of stakeholders' concerns about the uncertainty of risks associated with used beekeeping equipment, MAF consulted further with stakeholders and found that there was currently little demand for its importation. Therefore, in view of the stakeholder concerns about this commodity, MAF considered it prudent to remove it from the scope of this risk analysis, and to leave its consideration for the future if demand warrants it.

For consistency, the initial hazard list was based on that used in the bee genetic material risk analysis.

2.2 COMMODITY DEFINITION

This document is an analysis of the biosecurity risks posed by the importation of the following honey bee products :

- honey
- propolis
- pollen
- royal jelly
- beeswax
- bee venom

As in the 2002 bee products risk analysis, this document considers the above products only in pure form. That is, their use in various concentrations in manufactured products that have been subjected to specific manufacturing processes cannot be assessed in a general risk analysis of this kind, and decisions on such commodities will have to be made by MAF on a case by case basis.

Attractiveness to bees is an important consideration in this risk analysis, as the likelihood of some commodities harbouring particular agents is unclear, but the presence or absence of an exposure pathway to susceptible species in this country (see section 2.3 for a discussion of the risk analysis methodology) depends on whether the commodity is or is not attractive to bees, which has been shown to depend essentially on sugar content. In what was considered to be a worst case scenario trial (autumn, the time of year when bees are most likely to feed on non-flower food sources, and using bees that had been trained to feed on sugar syrup at a feeding station), it was shown that bees would collect syrup with 10% and 5% (w:v) sugar, but not 2.5% (Goodwin, pers. comm)[1]. In another trial, the lowest concentration of honey and sugar that bees collected in preference to water was 2% (Goodwin and Cox, 2004). This matter is further discussed in section 2.2.7 of this document.

The following account of the production of these commodities concludes that honey, propolis and pollen are botanical in origin and are collected and processed by bees rather than being produced *de novo*. However, royal jelly, venom and beeswax are manufactured by honey bees themselves (Blum, 1992; Gary, 1992; Schmidt and Buchmann, 1992; Snodgras and Erickson, 1992; White, 1992).

2.2.1 Honey

The definition of honey in the Codex Alimentarius[2] is:

> *"Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature. Blossom honey or nectar honey is the honey which comes from nectars of plants. Honeydew honey is the honey which comes mainly from excretions of plant*

[1] M Goodwin, Agresearch Ruakura, unpublished report, May 2003.
[2] The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. http://www.codexalimentarius.net/web/index_en.jsp

sucking insects (Hemiptera) on the living parts of plants or secretions of living parts of plants."

Nectar is a sweet liquid secreted by plant nectaries that are typically located inside flowers, and is one of the 'rewards' offered to bees and other insects in return for their pollination services for plants. Nectar is composed almost entirely of sugars and water, in widely differing proportions. During the conversion of nectar into honey, two distinct processes are involved. One brings about a chemical change in the sugar and the other is a physical change, in particular the elimination of surplus water. Honey bees collect nectar by means of the proboscis, sucking it out of flowers and into a part of the gut known as the 'honey stomach'. When a bee has collected a full nectar load, after visiting however many flowers are necessary according to the prevailing conditions, she returns to the hive. There she passes the nectar to a house bee, who deposits it into a cell where it is transformed into honey over a period of several days through the action of a number of enzymes that are added by bees. Water removal is achieved by bees manipulating the nectar to optimise evaporation, and once the honey is 'ripe' (i.e. reduced in water content to about 19% or less) workers seal the cell with a beeswax cap. More than 95% of the solids of honey are carbohydrates, mostly simple sugars or monosaccharides (White, 1992). 'Comb honey' is honey that is still sealed in the wax comb in which it was stored by the bees, whereas 'extracted honey' is that which has been removed from the comb and separated from wax cappings.

The first step in extracting honey is to cut the wax cappings of honey combs with a heated vibrating knife or a chain flail system. Honey is separated from wax cappings by spinning, pressing or, in small operations, by draining. If a spinner or centrifuge is used, before separation the honey and wax mixture is usually pumped (by means of various mechanical pumps) through a heat exchanger and heated to 40-45°C for a short period. At some stage of the process the honey is also strained before being put into drums. Instead of a spinner or centrifuge, a high force press may be used to separate the honey and the wax.

Most honeys stored in drums will granulate and will need to be melted to get it out. This is usually done by placing the drums in a hot room at about 63°C for 3-5 days or by putting heat coils under each drum.

Commercially packed honey is often heated at various stages of the packing process to assist pumping and straining. Creamed or granulated honey is kept at 12-14°C for 3-6 days to achieve granulation and then another week at 12°C to set firm, after which it may be stored at 17-20°C for months. Liquid honey is usually flash heated to 150°C for a few minutes, bottled hot and then left to cool in a stack of jars over many hours.

This risk analysis considers comb honey and various forms of extracted honey. Honey as an additive in processed foods, cosmetics and other products is not considered.

2.2.2 Propolis

Propolis is a resinous and often sticky plant-derived material used by bees for caulking, sealing, lining, strengthening and preserving inside the hive and around the entrance. Propolis and some of its constituents exhibit a variety of biological and pharmacological activities, and it has had an ancient history as a curative agent in human health.

Although it is collected from locally available plants, its composition from a variety of sources is remarkably similar, indicating that bees select particular plant resins for its production. Excluding beeswax, which is an additive produced by the bees, propolis consists of a mixture of resins, terpenes and volatile oils. Propolis resins appear to be mixtures of natural polymers that, due to their insolubility or inertness, are likely to be important only for their structural properties. The pharmacologically active constituents of propolis are found in fractions soluble in solvents such as alcohols. Most important are the flavones, flavonols, and flavonones (collectively called the flavonoids) and various phenolics and aromatics. Propolis has antimicrobial properties, and this is an important part of the chemical arsenal within the hive for combating contamination and pathogen invasion. The flavone pinocembrin is active against a variety of bacteria, fungi and molds, and along with glangin, 3-acetyl pinobanksin, and caffeic and ferulic acids it is probably responsible for much of the biological activity of propolis (Schmidt and Buchman, 1992).

To harvest the highest grade of propolis special ‘inserts’ are placed in hives – these provide spaces that mimic holes or cracks in the hive, thereby encouraging bees to fill them with propolis. Lower grades of propolis can be obtained from hive scrapings, but these contain wood chips, paint and wax (Schmidt and Buchman, 1992).

Propolis is traded internationally mainly in three forms. Most is traded as a powder, which is produced by extraction in 70% alcohol or propylene glycol followed by recovery in a retort system. Another form is the tincture itself, which can be in consumer-ready bottles or can be repackaged into a variety of supplements and herbal medicines. Flakes or disks of unprocessed propolis are also traded. These three forms of propolis are considered in this risk analysis. All imported propolis is chemically extracted for manufacture into powder or tincture.

Raw propolis, and the small quantities of propolis that may be included in manufactured products such as skin lotions, beauty creams, soaps, shampoos, lipsticks, chewing gums, toothpastes, mouthwashes and sunscreens are not considered in this risk analysis.

2.2.3 Pollen

Pollen consists of the male reproductive cells of flowering plants, with which bees have co-evolved over 90 million years. Pollen supplies all the bees' nutrients (proteins, fatty acids, minerals, vitamins) for brood rearing, and for adult growth and development. Honey bees collect pollen with their mouthparts, using the tongue and mandibles for licking and biting the anthers with the result that pollen grains stick to the mouthparts and become thoroughly moistened with saliva containing nectar and honey. By crawling over the flowers the face, thorax and abdomen of the bee also become covered with pollen, and this is brushed off with the front and middle legs and is transferred to the pollen baskets located on the rear legs. On returning to the hive, the foraging bee deposits the collected pollen into a cell. There it is manipulated by other worker bees and further moistened by saliva forming a moist dark mass to which nectar and honey are added. A phytocidal acid is added to prevent germination and bacterial degradation. Pollen stored in this way undergoes chemical changes and becomes what is known as 'bee bread', which can last for many months. About 24% of pollen is protein, and 27% carbohydrates, mostly in the form of the simple sugars fructose and glucose, which is added by foraging bees in the form of nectar and honey.

Pollen is collected commercially by a device known as as a pollen trap, of which there are many designs. The trap essentially dislodges up to about 60% of the pollen pellets off the legs of returning bees. The pollen falls into a collection drawer, and is therefore known as "bee-collected pollen" or "trapped pollen" (Schmidt and Buchmann, 1992).

The international market for pollen is mainly for human nutritional supplements, feeding to bees, and as an animal food (especially race horses). Pollen for human consumption is formulated into a wide range of products, including tablets, granules, oral liquids (which are usually a pollen extract in a honey base), candy bars, tonics etc.

This risk analysis will consider dry or frozen bee-collected pollen that is imported either in bulk or in capsules for human consumption.

2.2.4 Royal jelly

Royal jelly is a creamy-white secretion of the hypopharyngeal glands of young workers that is fed to larval queens. It is about two-thirds water, about 13% protein and about 11% sugars and also contains a number of unique short chain carbon-free fatty acids, vitamins and minerals. It typically has a pH of about 3.8. Royal jelly is commonly used as a human dietary supplement, and is also used in cosmetics. In Asian countries it has medical uses.

Royal jelly is usually produced in colonies maintained specifically for that purpose, without a queen. Worker larvae are inserted into artificial queen cups and worker bees are allowed to feed these for 3 days, whereupon the larvae are removed and the royal jelly is harvested with a spoon or by gentle suction (Schmidt and Buchmann, 1992).

The international market for royal jelly is mainly the cosmetic industry and the health food market. In the health food market royal jelly is often added as a supplement to other ingredients and vitamins and can be taken in capsules, in beverages, in confectionaries, or mixed with honey as a spread. Small amounts of royal jelly are included in a range of cosmetics.

This risk analysis will consider only 'pure' royal jelly imported either in capsules for human consumption or in bulk, either as a powder or in 'fresh' form (liquid or frozen).

2.2.5 Beeswax

Beeswax is a complex mixture of lipids and hydrocarbons that is produced by the wax glands of honey bees. The wax glands are specialised parts of the body wall epidermis, and they are located in pairs on either side of the base of the rear four adbominal segments. The wax is produced as a liquid, but it rapidly hardens to form small flakes on contact with air. Each scale weighs about 1.1 mg. Beeswax is produced by quiescent bees around 14 days of age, and it is subsequently processed by workers into comb, which involves incorporating salivary gland secretions. The average honey frame contains about 2-3 kg of honey, but the wax necessary to construct the 7,100 cells weighs only about 100 g.

Wax is commercially produced from three sources – wax cappings, bits of burr comb scrapings from hive bodies and frames, and old combs that are to be recycled. The highest quality wax comes from cappings that have been melted in a variety of ways and then formed into blocks in molds. The melting point of beeswax ranges from 62°C to 65°C. A ton of

extracted honey yields about 9-11 kg of wax from the cappings (Schmidt and Buchmann, 1992).

The major international market for beeswax is in the cosmetics and related industries, where it is used in a myriad of products including facial creams, ointments, lotions and lipsticks. The largest industry using beeswax as a raw material is the candle industry. There are a range of other minor industrial uses, including the dental industry, waterproofing materials, floor and furniture polishes, grinding optical lenses, childrens crayons, sweets and chewing gum, musical instruments, ski and ironing wax and wax for archery bow strings. None of these finished products are considered in this risk analysis.

The beeswax commodity that is considered in this risk analysis is raw beeswax that has been melted and formed into blocks, foundation and candles.

There are many time/temperature treatments employed in recovery of beeswax and making it into foundation. At the lower end of the scale, raw wax may be recovered by simply melting in hot water and allowing the wax to settle on top of the water. Such forms of beeswax will contain various impurities, but on account of the negligible sugar content, it will not be attractive to bees. Even 'slumgum', which is the waxy residue that remains after rendering old bee combs, is only marginally attractive to bees (Tew, 1992), and it is not traded internationally. However, the higher the level of wax purity required, the more heating and microfiltration is likely to be used. Wax purity is likely to be more important in foundation manufacture, particularly when making foundation from dark-stained wax derived from brood combs. Rendering of such material may involve temperatures up to 120°C for at least 2 hours followed by microfiltration, perhaps combined with holding at 100°C for 24 hours (Lyttle, pers.comm.)[1].

The minimum treatment standards for beeswax considered in this risk analysis will be that it has been produced by melting the raw wax in hot water and holding it in molten form for at least 2 hours i.e. at least 60°C for 2 hours.

2.2.6 Venom

Honey bee venom is synthesised by the venom glands of workers and queens, stored in the venom reservoir, and injected through the sting apparatus during the stinging process. The sting apparatus of bees is in fact a modified egg-laying organ (ovipositor), which is present in many other female insects. Venom is a bitter hydrolytic blend of proteins with basic pH, and it has long been used for treating various human ailments in many cultures, particularly for its alleged anti-inflammatory and anti-arthritic effects. The venom gland is located in a chamber at the end of the abdomen, and is completely separate from all other glands and organs of the bee.

Bee venom is commercially collected by means of a special apparatus that uses electrical current to force worker bees to sting a rubber mat or synthetic membrane and the venom is collected on a glass plate positioned below the membrane. The device is inserted into each hive and operated for only a few minutes (Schmidt and Buchmann, 1992).

[1] Peter Lyttle, NZ Beeswax Ltd., http://www.beeswax.co.nz/, telephone conversation with H Pharo, 23 July 2004.

The international market in bee venom is small and is almost entirely confined to medical use for desensitising patients allergic to bee stings and also for folk medicines for arthritis. It is mostly traded in capsules, vials and tablets for human consumption. It may also be traded in bulk powder form. Both forms are considered in this risk analysis.

2.2.7 Attractiveness to bees

As a result of diverse opinions on the attractiveness of bee products to bees that arose during public consultation on the first MAF risk analysis on bee products, in 2004 MAF commissioned a study into this (Goodwin and Cox, 2004), the results of which are incorporated into the following conclusions.

Honey, either in combs or extracted, is highly attractive to bees, and the lowest concentration of honey and sugar collected in preference to water appears to be about 2%, which means that there is probably no concentration of honey that could be added to another product that would not make it attractive to bees, especially if the material was placed in a position that allowed evaporation of whatever water it contained. Therefore, all forms of honey are considered to be attractive to bees.

Propolis (powdered or tincture) was found to be unattractive to bees, and it is concluded that the internationally traded forms of propolis are not attractive to bees.

Pollen is recognised as being potentially attractive to bees, on account of both its protein content and its relatively high sugar content (about 27%). Therefore pollen is considered to be attractive to bees, but capsules of pollen for human consumption will not be considered attractive to bees.

Fresh royal jelly is considered to be attractive to bees. As powdered royal jelly absorbs moisture, it is reasonable to consider it to be potentially as attractive to bees as the fresh form. However, royal jelly powder in capsules for human consumption are not considered to be attractive to bees.

Internationally traded forms of beeswax (blocks, candles) are not considered to be attractive to bees. However, if imported beeswax is made into foundation which is subsequently placed in hives, or used to coat plastic bee frames, then the wax will come into close contact with bees regardless of its attractiveness to bees.

Bee venom, in the form that it is traded internationally, is not considered to be attractive to bees.

Notwithstanding that the major internationally traded forms of beeswax, venom and propolis are considered unattractive to bees, these products may imported and added to various honey mixtures in the preparation of health products, and as indicated above, this would make them attractive to bees. Moreover, some beekeepers may deliberately alter the attractiveness of some imported forms of these commodities in order to feed them directly to their bees; as an extreme example of this, imported royal jelly capsules could be opened and the contents placed inside the hive for the bees to consume. These issues risks will not be directly addressed in this document, but they will be considered when developing any import health standards that may result from this risk analysis.

2.3 RISK ANALYSIS METHODOLOGY

The methodology used in this risk analysis follows the guidelines in Section 1.3 of the *International Animal Health Code* ("the 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 risk analysis process used by the MAF is shown in Figure 1.

The Hazard Identification process begins with the collation of a list of organisms associated with honey bees. The initial hazard list used in this risk analysis is the same as was used in the bee genetic material risk analysis, which was completed in June 2003.

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, 17 viruses were included for consideration. In addition a range of other bee-disease-causing organisms were added.

For each organism in the initial hazard list, the epidemiology is discussed, including a consideration of the following questions:

1) whether the various commodities 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.

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).

Under the OIE methodology, for each potential hazard, the following analysis is carried out:

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.

Not all of the above steps may be necessary in all risk assessments. The OIE methodology makes it clear that if the likelihood of release is negligible for a certain potential hazard, then the risk estimate is automatically negligible and the remaining steps of the risk assessment need not be carried out. The same situation arises where the likelihood of release is non-negligible but the exposure assessment concludes that the likelihood of exposure to susceptible species in the importing country is negligible, or where both release and exposure are non-negligible but the consequences of introduction are concluded to be negligible. However, in this risk analysis for each organism that is considered to be a potential hazard, all steps in the risk assessment are carried out for the sake of transparency.

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 considered in the hazard identification for each organism.

Further details, including the full hazard identification, and where appropriate the risk assessment and the recommended risk management measures, can be found in the chapters on the individual agents.

Figure 1. The risk analysis process.

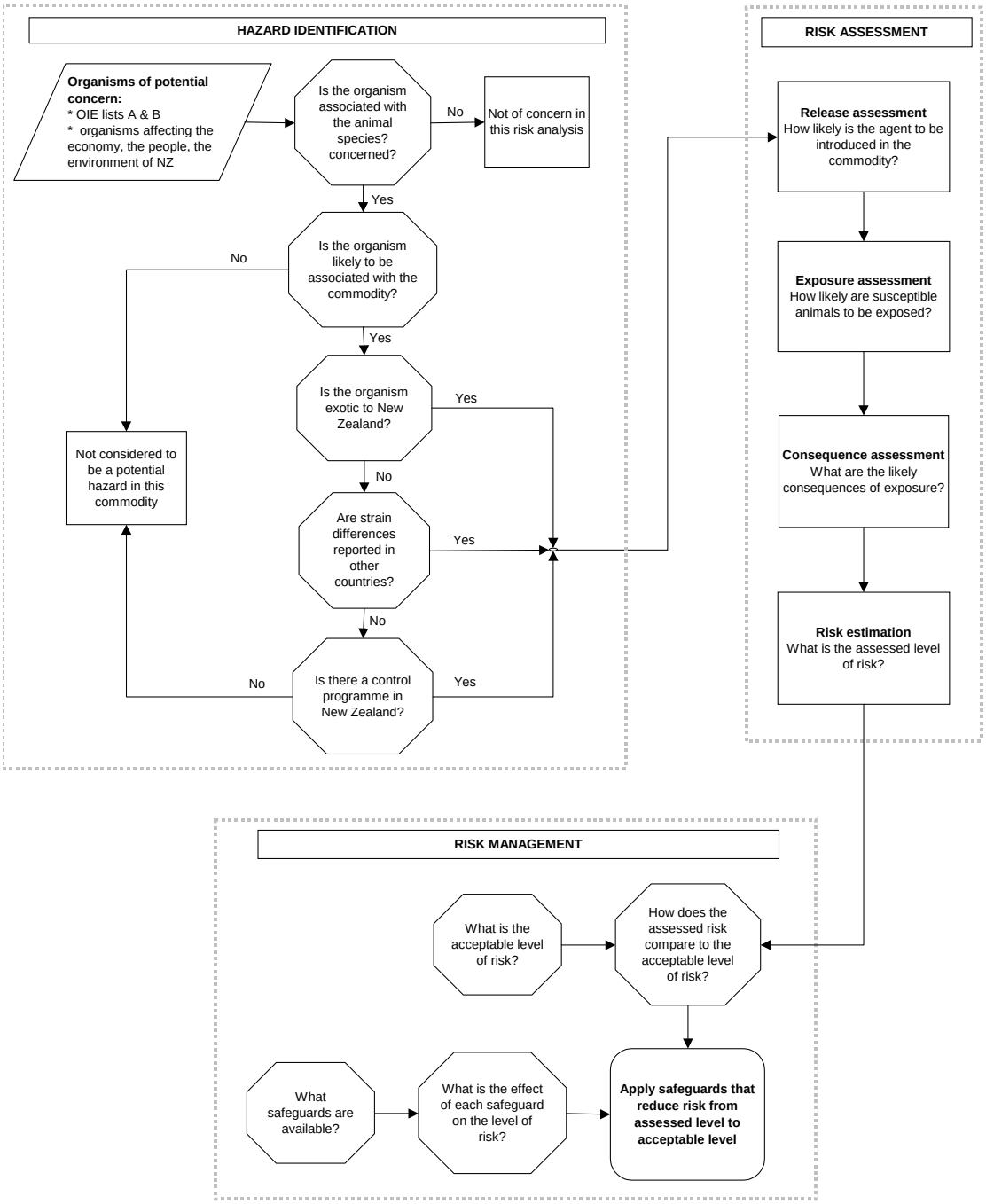

Table 1. Organisms Considered in this risk analysis

Ch.	Common Name/ Disease	Scientific Name	Present in NZ?	OIE List?	Under official Control or Unwanted?	More Virulent Strains Overseas?
VIRUSES						
3	Acute paralysis virus	Acute paralysis virus	Yes	No	No	No
4	*Apis* iridescent virus	*Apis* iridescent virus	No (2)	No	No	n/a (1)
5	Arkansas bee virus	Arkansas bee virus	No (2)	No	No	n/a
6	Bee paralysis	Chronic paralysis virus	Yes	No	No	No
7	Bee virus X	Bee virus X	Yes	No	No	No
8	Bee virus Y	Bee virus Y	Yes	No	No	No
9	Berkeley bee virus	Berkeley bee virus	No (2)	No	No	n/a
10	Black queen cell	Black queen cell virus	Yes	No	No	No
11	Chronic paralysis virus associate	Chronic paralysis virus associate	Yes	No	No	No
12	Cloudy wing virus	Cloudy wing virus	Yes	No	No	No
13	Deformed wing virus	Deformed wing virus	No (2)	No	No	n/a
14	Egypt bee virus	Egypt bee virus	No (2)	No	No	n/a
15	Filamentous virus	Filamentous virus	Yes	No	No	No
16	Kashmir bee virus	Kashmir bee virus	Yes	No	No	No
17	Sacbrood virus	Sacbrood virus	Yes	No	No	No
18	Slow paralysis virus	Slow paralysis virus	No (2)	No	No	n/a
19	Thai sacbrood	Thai sacbrood virus	No (2)	No	No	n/a
BACTERIA						
20	American foulbrood	*Paenibacillus larvae larvae*	Yes	Yes	Official control	No (4)
21	European foulbrood	*Melissococcus pluton*	No (3)	Yes	Unwanted	n/a
22	*Paenibacillus alvei*	*Paenibacillus alvei*	No? (2)	No	No	n/a
23	Powdery scale disease	*Paenibacillus larvae pulvifaciens*	No (2)	No	No	n/a
24	Septicaemia	*Pseudomonas aeruginosa*	Yes	No	No	No
25	Spiroplasmas	*Spiroplasma melliferum, S. apis*	No (2)	No	No	n/a
FUNGI						
26	Chalkbrood	*Ascosphaera apis*	Yes	No	No	No (5)
27	Stonebrood	*Aspergillus* spp.	Yes	No	No	No
ARTHROPOD PARASITES						
28	Bee louse	*Braula coeca*	No (2)	No	Unwanted	n/a
29	External acarine mites	*Acarapis dorsalis, A. externus*	Yes	No	No	No
30	Small hive beetle	*Aethina tumida*	No (2,3)	No	Unwanted	n/a
31	Tracheal mite	*Acarapis woodi*	No (3)	Yes	Unwanted	n/a
32	*Tropilaelaps* spp.	*Tropilaelaps clareae, T. koenigerum*	No (3)	No	Unwanted	n/a
33	*Varroa destructor*	*Varroa destructor*	Yes	Yes	Official control	No (6)
34	Other *Varroa* species	*Varroa jacobsoni, V. underwoodi, V. rindereri, Euvarroa sinhai, E. wongsirii*	No (3)	No	Unwanted	n/a
35	Wax moths	*Galleria mellonella; Achroia grisella*	Yes	No	No	No

PROTOZOA						
36	Amoeba disease	*Malpighamoeba mellificae*	Yes	No	No	No
37	Gregarine disease	*Gregarinidae*	No [(2)]	No	No	n/a
38	*Nosema* disease	*Nosema apis*	Yes	No	No	No
OTHER HONEY BEES & RACES						
39	Africanised bee	*Apis mellifera scutella*	No	No	Unwanted	n/a
40	Cape honey bee	*Apis mellifera capensis*	No	No	Unwanted	n/a
41	Other honey bee races	*Apis mellifera carnica and Apis mellifera caucasia*	Yes	No	No	n/a
42	Honey bees other than *A. mellifera*	*Apis* spp. other than *A. mellifera*	No	No	Unwanted	n/a

Note 1 : n/a = for exotic organisms, the question of more virulent strains overseas does not arise
Note 2 : not reported in New Zealand
Note 3 : not found during surveys
Note 4 : strains resistant to oxytetracycline are present overseas
Note 5 : while there is limited evidence of strain variation in virulence of *Ascosphaera apis* under experimental conditions, there is no evidence of this being linked to severity of chalkbrood under natural conditions
Note 6 : strains resistant to various miticides are present overseas

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3. ACUTE PARALYSIS VIRUS

3.1 Hazard Identification

3.1.1 Aetiologic Agent: Acute paralysis virus, a small (particle diameter 30 nm) single-stranded RNA virus morphologically and physiochemically resembling picornaviruses. It is now considered to be a cricket paralysis-like virus and is classified as a *Cripavirus* in the family *Dicistroviridae* (Mayo, 2002).

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.* It is about 70% identical to Kashmir bee virus over the whole genome (de Miranda et al., 2004), and there is good evidence that both viruses persist as inapparent infections in nature and are probably transmitted in a similar manner in the absence of *Varroa destructor*– that is, via the salivary gland secretions of adult bees and the food to which these secretions are added (Bailey, 1976; Anderson, 1991). It appears that bees may be infected with both viruses simultaneously (Evans, 2001), and it has been suggested that they occupy the same ecological niche (Anderson, 1991).

It is generally present as an inapparent infection in adult bees (Bailey et al., 1963). However, it has been shown to kill both adults and brood in colonies infested with *V. destructor* (Ball and Allen, 1988). It appears that the mite induces replication of the virus when the mite feeds on in apparently infected bees. It is not known what activates the latent infection of acute paralysis virus when it is 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 bees 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 (also known as invertebrate iridescent virus 24), a large (particle diameter 120-130 nm) double stranded DNA virus, tentatively classified in as a member of the genus *Iridovirus*, in the family *Iridoviridae* (van Regenmortel et al., 2000).

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

Iridoviruses have been found occurring naturally in insects of the orders Diptera, Coleoptera and Lepidoptera, but *Apis* iridescent virus is the only one that has been found in the order Hymenoptera (Bailey et al., 1976). It has been found only in dead and dying *A. cerana* from Kashmir and Northern India (Ball and Bailey, 1997), in which it apparently causes causes 'clustering disease' (Bailey and Ball, 1978). However, little is known of the epidemiology of the virus and its seasonal prevalence appears to vary over its known range (Mishra et al., 1980; Shah, 1985; Verma and Phogat, 1982; Verma and Joshi, 1985). Nothing has been published in the scientific literature on this virus in the past 20 years.

In laboratory trials, *Apis* iridescent virus readily grows when injected into or fed to individual adult *A. mellifera* where it formed crystalline aggregates in several tissues, especially the fat body and the hypopharyngeal glands. It also grows when injected into young larvae. However, disease was not reported as a result of these experiments. In addition, unlike a number of other iridoviruses tested it failed to multiply in larvae of the greater wax moth, *Galleria mellonella* (Bailey et al., 1976). Neither the disease nor the virus has been reported in *A. mellifera* in nature, even in areas where it is sympatric with *A. cerana* (Allen and Ball, 1991).

4.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.3, *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

Apis iridescent virus has only been found in dead and moribund *A. cerana* in Kashmir and Northern India, and there does not appear to be any publication more recently than 1985, which can be taken to mean that it either has a very restricted range or it is not of interest elsewhere. Nevertheless, the likelihood of contamination of the commodities from Northern India at the time of their formation cannot be discounted.

Although no work has been done on degradation and loss of infectivity of *Apis* iridescent virus *per se*, the survival of most bee viruses outside the body of their host is very limited

(Ball, 1999). For example, in hives with clinical signs consistent with the parasitic mite syndrome, while most samples of adult bees taken from brood combs within the colony were positive for viruses, most samples from crawling and dead bees outside the hive were negative for viruses (Calderon et al., 2003). This suggests that viruses survive away from live bees for at most a day or two. In the case of Kashmir bee virus, the coat proteins of virus particles rapidly degrade and the particles lose their infectivity when removed from the host (Bailey et al., 1979; Anderson, 1986).

Therefore, it is considered unlikely that honey bee products stored away from honey bees would carry infective levels of the virus.

Further, iridoviruses are relatively sensitive to heat – the iridovirus causing epizootic haematopoetic necrosis in freshwater fish is inactivated within 24 hours at 40°C (Langdon, 1989), and iridoviruses in general are inactivated in about 15-30 minutes at 55°C (van Regenmortel et al., 2000). While survival is likely to be somewhat longer at lower temperatures, it is considered unlikely that honey bee products stored at ambient temperatures away from honey bees would carry infective levels of *Apis* iridescent virus at the time of importation even if they came from areas where *Apis* iridescent virus was present.

Therefore, it is concluded that the likelihood of any of the commodities carrying *Apis* iridescent virus is negligible.

4.2.2 Exposure Assessment

In the absence of *Varroa destructor*, bee viruses are usually present at very low levels in bee colonies, and the mechanism of transmission from bee to bee is unknown. Assuming that either the oral route or direct contact is likely under natural conditions, the attractiveness of the commodities to bees is considered to be a good indicator of the likelihood of exposure if the commodities did carry the virus. Attractiveness to bees is discussed in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. Although beeswax that is made into foundation or applied to plastic frames comes into close contact with bees, considering the processing involved in the production of beeswax, the likelihood of this pathway resulting in exposure of bee viruses to susceptible species in New Zealand is considered to be negligible.

4.2.3 Consequence Assessment

Honey bee viruses are generally highly species-specific (Ball, 1999), and unlike many of the irridescent viruses from other insects, *Apis* iridescent virus is unable to grow in larvae of the greater wax moth, indicating that this virus is more species-specific than many other insect iridoviruses (Bailey et al., 1976). Therefore it is considered that the likelihood of this virus growing in native insects is negligible.

Although *Apis* iridescent virus has been grown in *A. mellifera* in the laboratory (Bailey et al., 1976), the virus and the disease caused by it have been reported only in *A. cerana* under natural conditions. Therefore, the consequences of the virus being introduced into New Zealand are considered to be negligible, as *A. cerana* is not present in this country.

In view of the restricted international distribution of this virus, its introduction into New Zealand would probably result in a loss of export markets for live bees.

In view of the likely international market reaction the consequences of introduction are considered to be non-negligible.

4.2.4 Risk Estimation

The likelihood of *Apis* iridescent virus being present in the imported commodities is considered to be negligible. If the virus were present in imported commodities, the likelihood of exposure is considered to be non-negligible for some commodities and the consequences would probably be significant. However, since the likelihood of *Apis* iridescent virus being present in the commodities is considered to be negligible, the risk estimate is 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, an unassigned small (particle diameter 30 nm) single-stranded RNA virus (van Regenmortel et al., 2000).

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 of *Apis mellifera* that 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 show no outward signs of disease, but they die in about 14 days (Bailey and Woods, 1974).

While the precise method of spread is not known, other viruses that are also found in pollen loads (sacbrood virus, chronic and acute paralysis viruses) are thought to be present in the hypopharyngeal glands of infected adult bees, and spread from these to larvae probably occurs when the latter are fed by protein-rich secretions from these glands (Bailey, 1976).

5.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.3, 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 commodities would have to come from a colony that is infected with Arkansas bee virus, which has so far been reported only in the United States.

Since the virus has been shown to be present in pollen loads taken from foraging bees, it is reasonable to assume that it is present in the hypopharyngeal and/or salivary glands of infected bees. In the absence of information to the contrary, it is assumed that honey, pollen and royal jelly could potentially carry the virus, at least at the point of formation. Although their initial production does not involve secretions from the hypopharyngeal and/or salivary glands, propolis and beeswax are masticated by bees, and so may be assumed to be potentially contaminated. Venom would not be expected to carry the virus at any stage.

Although no work has been done on degradation and loss of infectivity of Arkansas bee virus *per se*, the survival of most bee viruses outside the body of the bee is very limited, particularly in the case of the small non-occluded single stranded RNA viruses like Arkansas bee virus (Ball, 1999). For example, in hives with clinical signs consistent with the parasitic mite syndrome, while most samples of adult bees taken from brood combs within the colony

were positive for viruses, most samples from crawling and dead bees outside the hive were negative for viruses (Calderon et al., 2003). This suggests that viruses survive away from live bees for at most a day or two. In the case of Kashmir bee virus, also a single stranded RNA virus, the coat proteins of virus particles rapidly degrade and the particles lose their infectivity when removed from the host (Bailey et al., 1979; Anderson, 1986).

Therefore, it is considered unlikely that honey bee products stored away from honey bees would carry infective levels of the virus.

In view of the limited international distribution of the virus and the low likelihood that it would remain viable in honey bee products, it is concluded that the likelihood of any of the commodities carrying Arkansas bee virus is negligible.

5.2.2 Exposure Assessment

In the absence of *Varroa destructor*, bee viruses are usually present at very low levels in bee colonies, and the mechanism of transmission from bee to bee is unknown. Assuming that either the oral route or direct contact is likely under natural conditions, the attractiveness of the commodities to bees is considered to be a good indicator of the likelihood of exposure if the commodities did carry the virus. Attractiveness to bees is discussed in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. Although beeswax that is made into foundation or applied to plastic frames comes into close contact with bees, considering the processing involved in the production of beeswax, the likelihood of this pathway resulting in exposure of bee viruses to susceptible species in New Zealand is considered to be 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. Therefore it is considered unlikely that the virus would have any adverse effects if introduced into New Zealand. In view of the restricted international distribution of this virus, its introduction into New Zealand would probably result in a loss of export markets for live bees.

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

In view of the likely international market reaction the consequences of introduction are considered to be non-negligible.

5.2.4 Risk Estimation

The likelihood of Arkansas bee virus being present in the imported commodities is considered to be negligible. If the virus were present in imported commodities, the likelihood of exposure is considered to be non-negligible for some commodities and the consequences would probably be significant. However, since the likelihood of Arkansas bee virus being present in the commodities is considered to be negligible, the risk estimate is 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

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6. BEE PARALYSIS

6.1 Hazard Identification

6.1.1 Aetiologic Agent: Chronic paralysis virus, an unclassified polymorhic (particles 20 x 30 to 60 nm) single-stranded RNA virus (van Regenmortel et al., 2000).

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 chronic paralysis virus. Although the condition has been recognised for over 100 years, the cause of the disease was not identified until 1963 (Bailey et al., 1963).

The virus appears to cause two distinct clinical syndromes (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. Severely infected colonies can suddenly collapse, with large numbers of dead bees found at the entrance (Bailey, 1969).

The second syndrome 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). There is no evidence in the literature that strains of the virus vary in pathogenicity. Susceptibility to bee paralysis has been shown to be linked to hereditary factors (Kulincevic and Rothenbuhler, 1975), and the prevalence of paralysis is usually 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 MF, Ball BV (1996). The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162.

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7. BEE VIRUS X

7.1 Hazard Identification

7.1.1 Aetiologic Agent: Bee virus X, an unassigned small (particle diameter 35 nm) RNA virus (van Regenmortel et al., 2000). As with several other bee viruses, its morphology is similar to that of picornaviruses (van Regenmortel et al., 2000).

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*. It 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). It is only transmissible *per os* to adult honey bees (van Regenmortel et al., 2000). 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 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 reported in Europe, Australasia, Argentina, Canada and Iran (Allen and Ball, 1996), and in New Zealand (Anderson, 1988). There are no reports in the literature of strain variation in virulence.

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 (1996). The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162.

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8. BEE VIRUS Y

8.1 Hazard Identification

8.1.1 Aetiologic Agent: Bee virus Y, an unassigned small (particle diameter 35 nm) RNA virus (van Regenmortel et al., 2000).

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.* It 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 is only transmissible *per os* to adult honey bees (van Regenmortel et al., 2000), and it multiplies only 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 from Europe, North America and Australasia (Allen and Ball, 1996) including New Zealand (Anderson, 1988). There are no reports in the literature of strain variation in virulence.

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 MF, Ball BV (1996). The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162.

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9. BERKELEY BEE VIRUS

9.1 Hazard Identification

9.1.1 Aetiologic Agent: Berkeley bee virus, an unassigned small (particle diameter 30 nm) single-stranded RNA virus (van Regenmortel et al., 2000).

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 isolated from the original isolate of Arkansas bee virus and has also been found in Californian bees (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 2.3, 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 commodities would have to come from colony that is infected with Berkley bee virus, which has so far been reported only in the United States.

The epidemiology of Berkley bee virus is unknown, but since the virus was discovered in association with Arkansas bee virus, for the purposes of this risk analysis it is reasonable to assume that the viruses behave similarly. Therefore, it can be reasonably assumed that honey, pollen and royal jelly could potentially carry the virus, at least at the point of formation. However, since their production does not involve secretions from the hypopharyngeal and/or salivary glands, propolis, beeswax and venom would not be expected to carry the virus at any stage.

Although no work has been done on degradation and loss of infectivity of Berkley bee virus *per se*, but the survival of most bee viruses outside the body of the bee is very limited, particularly in the case of the small non-occluded single stranded RNA viruses like this (Ball, 1999). For example, in hives with clinical signs consistent with the parasitic mite syndrome, while most samples of adult bees taken from brood combs within the colony were positive for viruses, most samples from crawling and dead bees outside the hive were negative for viruses (Calderon et al., 2003). This suggests that viruses survive away from live bees for at most a day or two. In the case of Kashmir bee virus, also a single stranded RNA virus, the coat proteins of virus particles rapidly degrade and the particles lose their infectivity when

removed from the host (Bailey et al., 1979; Anderson, 1986). In view of the above, it is considered unlikely that honey bee products stored away from honey bees would carry infective levels of the virus even if they came from infected colonies.

Therefore it is concluded that the likelihood of any of the commodities carrying Berkley bee virus is negligible.

9.2.2 Exposure Assessment

In the absence of *Varroa destructor*, bee viruses are usually present at very low levels in bee colonies, and the mechanism of transmission from bee to bee is unknown. Assuming that either the oral route or direct contact is likely under natural conditions, the attractiveness of the commodities to bees is considered to be a good indicator of the likelihood of exposure if the commodities did carry the virus. Attractiveness to bees is discussed in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. Although beeswax that is made into foundation or applied to plastic frames comes into close contact with bees, considering the processing involved in the production of beeswax, the likelihood of this pathway resulting in exposure of bee viruses to susceptible species in New Zealand is considered to be 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. In view of the restricted international distribution of this virus, its introduction into New Zealand would probably result in a loss of export markets for live bees.

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

In view of the likely international market reaction the consequences of introduction are considered to be non-negligible.

9.2.4 Risk Estimation

The likelihood of Berkeley bee virus being present in the imported commodities is considered to be negligible. If the virus were present in imported commodities, the likelihood of exposure is considered to be non-negligible for some of the commodities and the consequences would probably be significant. However, since the likelihood of Berkeley bee virus being present in the commodities is considered to be negligible, the risk estimate is negligible.

9.3 Risk Management

9.3.1 Risk Evaluation

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

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10. BLACK QUEEN CELL VIRUS

10.1 Hazard Identification

10.1.1 Aetiologic Agent: Black queen cell virus, a small (particle diameter 30 nm) single-stranded RNA virus that is now classified as a *Cripavirus* in the family *Dicistroviridae* (Mayo, 2002).

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 virus causes a disease of *Apis mellifera* queen larvae. The infected queen dies in the prepupal or pupal stage, and the wall of the infected queen cell changes colour to dark brown or black. The dead larva contains many virus particles (Bailey and Ball, 1991).

In contrast to sacbrood virus, 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 that are also infected with *Nosema apis* (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), including New Zealand (Anderson, 1988). There are no reports in the international literature of variation in virulence for different isolates.

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

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11. CHRONIC PARALYSIS VIRUS ASSOCIATE

11.1 Hazard Identification

11.1.1 Aetiologic Agent: Chronic paralysis virus associate, a very small (particle diameter 12 nm) unassigned single-stranded RNA satellite virus (van Regenmortel et al., 2000).

11.1.2 OIE List: None.

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

11.1.4 Epidemiology

Chronic paralysis virus associate is always associated with chronic paralysis virus, on which it depends for genetic information for its replication, but it is serologically distinct. It does not multiply when injected alone into bees, and is probably a satellite of chronic paralysis virus, inhibiting or interfering with 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).

Chronic paralysis virus associate is present in New Zealand (Todd and Ball, 2003).

Since chronic paralysis virus associate depends on the chronic paralysis virus for its replication, its distribution probably mirrors that of chronic paralysis virus (Allen and Ball, 1996). Nothing in the international literature suggests that different isolates have different levels of virulence.

11.1.5 Conclusion

Chronic paralysis virus associate 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 it is not classified as a potential hazard for the purposes of this analysis.

References

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12. CLOUDY WING VIRUS

12.1 Hazard Identification

12.1.1 Aetiologic Agent: Cloudy wing virus, a very small (particle diameter 17 nm) unassigned RNA virus which is a candidate species for the newly created family *Dicistroviridae* (Mayo, 2002).

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.* It causes mortality in heavily infected adult bees together with opaqueness in their wings (Bailey and Ball, 1991). Heavy infection can cause colony death (Bailey and Ball, 1991). There is no seasonal incidence of infection (Bailey et al., 1983).

Transmission of the virus is uncertain, but it appears not to be transmitted by *Varroa* mites (Todd and Ball, 2003), and infection may be airborne short distances between adult bees confined together (Bailey et al., 1980). Experimental attempts to infect adult bees by feeding them the virus or injecting it into their haemolymph were not successful (Ball and Bailey, 1997).

Cloudy wing virus has been reported in Europe, North America and Australasia (Allen and Ball, 1996), including New Zealand (Anderson, 1988), where has been found to be the most common virus isolated from dead bees in one survey (Todd and Ball, 2003). Nothing in the international literature suggests that different isolates can have different levels of virulence.

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 MF, Ball BV (1996). The incidence and world distribution of honey bee viruses. *Bee World* 77, 141-162.

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

13. DEFORMED WING VIRUS

13.1 Hazard Identification

13.1.1 Aetiologic Agent: Deformed wing virus, an unassigned small (particle diameter 30 nm) RNA virus that is serologically distantly related to Egypt bee virus (van Regenmortel et al., 2000). It has been provisionally assigned to the "floating genus" *Iflavirus*.

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 (DWV) is a virus most commonly found in *Apis mellifera*, although it has also been detected in *Apis cerana* from China (Ball, 1989). The appearance of wing deformity due to DWV apparently depends on the stage at which individuals are infected. The introduction of the virus and establishment of overt infection in developing honey bee brood is closely linked to the feeding activities of *Varroa destructor*. Pupae infected with the virus at the white-eye stage of development survive to emergence but may have poorly developed wings and soon die (Bailey and Ball, 1991). However, most young bees emerging from infested cells in an infected colony appear normal, although they can contain as much virus as deformed individuals and their productivity and lifespan are similarly reduced (Ball, 1993). It appears that virus titres have to reach certain levels in pupae before the infection is manifested by deformed wings (Bowen-Walker et al., 1999; Chen et al., 2004). Bees infected as adults appear normal but it is not known whether their longevity may also be affected (Ball and Bailey, 1997).

Although DWV was known as an overt infection of adult bees in Britain before the arrival of *V. destructor* (Ball and Evans, 1989) it has significantly increased in prevalence in recent years. DWV is now found in *Apis mellifera* colonies infested with *V. destructor* in many countries (Bailey and Ball, 1991). It has also been detected in individual mites and the ability of *V. destructor* to transmit the virus has been demonstrated experimentally (Bowen-Walker et al., 1999). Mites apparently overcome the normal mechanisms that limit virus transmission because they innoculate the virus directly into the haemolymph of pupae; a life stage that would not otherwise naturally become infected. However, there is no evidence that mites become infected with the virus. Rather, mites merely act as mechanical vectors of the virus from adult bees to other adults or pupae (Ball, personal communication[1]). DWV has been detected serologically in 69% of dead bee samples collected from mite-infested colonies in midsummer in Poland (Topolska et al., 1995), and in over 90% of infested colonies in England (Ball, 2001). Thus, DWV is considered to be an emerging disease of bees and *V. destructor* is considered to play a major role in its spread (Ball, personal communication[2]). There is little information on DWV in the absence of *V. destructor*, although the virus has been detected in dead adult honey bees before the mite arrived in Britain and South Africa (Bailey and Evans, 1989; Allen and Ball, 1996). The detection of DWV in honey bee eggs

[1] Brenda Ball, Rothamsted Research, UK, email to H Pharo dated 11 November 2004.
[2] Brenda Ball, Rothamsted Research, UK, email to H Pharo dated 2 February 2004.

and larvae suggest that other transmission pathways such as nursing bees and transovarial transmission may be possible (Chen et al., 2004).

Although DWV has been recorded in *A. mellifera* from many European, Middle Eastern, North African and Asian countries, as well as from South Africa, like many honey bee viruses, surveillance for this virus is hampered by the lack of a readily useable diagnostic test. It is generally only diagnosed when the characteristic clinical signs of deformed wings are seen in bees, which in turn is usually associated with severe infestations of *V. destructor*. Testing individual bees by ELISA indicates that the amount of DWV in bees from non infested cells from an overtly infected mite-infested colony is below the threshold for detection. However, similar large amounts of virus were detected in both mite-infested non-deformed bees. Large amounts of virus can also be detected in individual mites from such colonies (Bowen-Walker et al., 1999). This observation strongly supports the view that in the absence of *V. destructor*, the level of DWV in honey bees remains low and is very unlikely to be detected serologically.

DWV has not been reported from South America, the South Pacific, Australia or New Zealand (Allen and Ball, 1996). However, since DWV is not notifiable in New Zealand, there is no obligation on beekeepers to report clinical signs that could indicate its presence, and since it is consequently not included in the MAF laboratory standard for honey bee surveillance, there is no routine testing for its presence in bees submitted to diagnostic laboratories for other reasons.

Nevertheless, since the discovery of the varroa mite in New Zealand, two limited surveys have failed to detect DWV in dead bees taken from colonies with severe *V. destructor* infestations. In a preliminary investigation in 2002, 79 samples of bees from 32 New Zealand honey bee colonies on two apiary sites around Tauranga (samples comprised about 30 bees each) were tested at the Rothamstead Research in the UK using both the immunodiffusion test and a more sensitive ELISA. No DWV was detected (Todd and Ball, 2002). Further, from November 2002 to July 2003, 63 samples of dead bees were taken from 13 Auckland hives on one site (Mt Albert) and these were also negative for the virus by the immunodiffusion test and ELISA at the same laboratory. Several of the bees tested in the 2002 survey did have deformed wings, but the cause of that deformity is not known (Todd, personal communication[1]).

13.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.3, DWV must be classified as a potential hazard for the purposes of this analysis.

13.2 Risk Assessment

13.2.1 Release Assessment

As with most viruses of bees, DWV is thought to persist at very low levels inside the body of the host and very rarely replicates sufficiently to cause any pathology. The virus is present at highest concentrations in *V. destructor* from dead and deformed bees and in such bees

[1] Jacqui Todd, Hortresearch, Auckland, New Zealand, emails to H Pharo dated 27 January 2004 and 10 February 2004.

themselves, and very much lower levels are present in normal bees with or without mite infestation (Bowen-Walker et al., 1999).

Clinically affected bees die quite quickly after emergence (Bailey and Ball, 1991) and since they therefore do not participate significantly in honey collection or other hive tasks it is reasonable to assume that they do not contaminate hive products to any significant extent. However, a much greater proportion of clinically normal bees in infected colonies contain large amounts of virus, and these apparently normal bees could contaminate hive products since if they are infected post emergence their longevity does not seem to be significantly reduced.

Although little work has been done on degradation and loss of infectivity of DWV *per se*, the survival of most bee viruses outside the body of their host is very limited, particularly in the case of the small non-occluded RNA viruses like DWV (Ball, 1999). For example, in hives with clinical signs consistent with the parasitic mite syndrome, while most samples of adult bees taken from brood combs within the colony were positive for Egypt bee virus and DWV, most samples from crawling and dead bees outside the hive were negative for viruses (Calderon et al., 2003). This suggests that these viruses survive away from live bees for at most a day or two. In the case of Kashmir bee virus, also a single stranded RNA virus, the coat proteins of virus particles rapidly degrade and the particles lose their infectivity when removed from the host (Bailey et al., 1979; Anderson, 1986).

Researchers working with DWV in the USA have found that the virus is quite difficult to work with owing to its fragile nature when it is isolated from bees. One such researcher has commented to MAF as follows (de Miranda, personal communication[1]):

> *"DWV is pretty unstable when we extract it from bees. The particles fall apart during extraction and it does not keep long in the fridge or freezer. As a consequence, the viral RNA also degrades rapidly outside bee tissues, even in extraction buffers used in research laboratory"*

Therefore, it is considered unlikely that honey bee products stored away from honey bees would carry infective virus.

MAF's risk analysis on honey bee genetic material concluded that the likelihood of DWV being present in bee semen was negligible[2], a conclusion that is strongly supported by the observations by the above researcher in the USA, and by the fact that although the virus was been present in the UK for some time, it was only after the introduction of *V. destructor* that it spread and became a problem. It is reasonable to conclude that the likelihood of the DWV being in honey bee products is lower than the likelihood of it being in living material such as semen.

In view of the above, it is concluded that the likelihood of any of the commodities carrying deformed wing virus is negligible.

13.2.2 Exposure Assessment

In the absence of *Varroa destructor*, bee viruses are usually present at very low levels in bee colonies, and the mechanism of transmission from bee to bee is unknown. Assuming that either the oral route or direct contact is likely under natural conditions, the attractiveness of

[1] Joachim de Miranda, Penn State University, USA, email to H Pharo dated 28 January 2004
[2] Import Risk Analysis: honey bee (Apis mellifera) genetic material. MAF Biosecurity Authority, June 2003.

the commodities to bees is considered to be a good indicator of the likelihood of exposure if the commodities did carry the virus. Attractiveness to bees is discussed in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. Although beeswax that is made into foundation or applied to plastic frames comes into close contact with bees, considering the processing involved in the production of beeswax, the likelihood of this pathway resulting in exposure of bee viruses to susceptible species in New Zealand is considered to be negligible.

13.2.3 Consequence Assessment

Deformed wing virus has been associated with bee mortality, particularly in the presence of *V. destructor*. Reports from several countries indicate that there is a significant link between deformed wing virus, *V. destructor*, and honey bee colony collapse (Martin et al., 1998; Nordstrom et al., 1999). There are currently no published studies verifying a causal relationship between deformed wing virus and colony death, but in the United Kingdom, where almost all mite samples contain the deformed wing virus (Ball, 2001), long term research has suggested that the virus is the cause of the majority of honey bee colony deaths ascribed to the mite (Martin et al., 2003). Colony collapse obviously has a significant negative impact on both beekeeper incomes and the profitability of providing proper strength colonies for paid pollination services (Tew, 1999).

The above notwithstanding, it is difficult to predict the consequences of introduction of deformed wing virus into New Zealand, since there are clearly many other causes of colony collapse following varroa infection already in this country. At one extreme, it might be argued that the introduction of deformed wing virus would cause far more rapid colony collapse in mite-infested hives, and at the other extreme it might be argued that there are already many honey bee viruses in this country that are almost certainly involved in the parasitic mite syndrome, and that another virus would make very little difference. There is no basis for picking between these or determining what intermediate position to take.

Although DWV has a widespread distribution and there are no official control programmes in place for it anywhere in the world, its introduction into New Zealand would probably result in a loss of export markets for live bees.

However, honey bee viruses are unusually specific and cannot be cultivated in other insects or in insect cell tissue culture (Ball, 1999). This, together with the fact that native bees are solitary insects and there is no contact between generations (Matheson, 1997) supports the

conclusion that the likelihood of DWV virus having any effect on New Zealand native insects is negligible.

In view of the likely international market reaction the consequences of introduction are considered to be non-negligible.

13.2.4 *Risk Estimation*

The likelihood of DWV being present in the imported commodities is considered to be negligible. If DWV were present in imported commodities, the likelihood of exposure is considered to be non-negligible for some of the commodities and the consequences would probably be significant. However, since the likelihood of DWV being present in the commodities is considered to be negligible, the risk estimate is negligible.

13.3 Risk Management

13.3.1 Risk Evaluation

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

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14. EGYPT BEE VIRUS

14.1 Hazard Identification

14.1.1 Aetiologic Agent: Egypt bee virus, an unassigned small (particle diameter 30 nm) RNA virus that is serologically distinct from all other bee viruses except deformed wing virus (van Regenmortel et al., 2000) to which it is distantly serologically related.

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.* It is distantly related serologically to deformed wing virus, but virtually 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 has not been detected anywhere else in the world (Allen and Ball, 1996).

14.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.3, 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

As with most viruses of bees, Egypt bee virus is thought to persist at very low levels inside the body of live individuals and very rarely replicates sufficiently to cause any pathology (Ball, personal communication[1]). Although there is no evidence that *Varroa destructor* can transmit Egypt bee virus, in view of the distant serological similarity between Egypt bee virus and deformed wing virus, in the absence of contrary information similar assumptions have been made about the likelihood of the commodities carrying this virus. Thus, it is reasonable to conclude that the likelihood of the commodities being contaminated with Egypt bee virus is low, even at the time of their production by bees.

Although no work has been done on degradation and loss of infectivity of Egypt bee virus *per se*, the survival of most bee viruses outside the body of their host is very limited, particularly in the case of the small non-occluded RNA viruses like Egypt bee virus (Ball, 1999). For example, in hives with clinical signs consistent with the parasitic mite syndrome, while most samples of adult bees taken from brood combs within the colony were positive for viruses, most samples from crawling and dead bees outside the hive were negative for viruses (Calderon et al., 2003). This suggests that viruses survive away from live bees for at most a day or two.

In the case of Kashmir bee virus, also a single stranded RNA virus, the coat proteins of virus particles rapidly degrade and the particles lose their infectivity when removed from the host (Bailey et al., 1979; Anderson, 1986).

In view of the above, it is concluded that the likelihood of any of the commodities carrying Egypt bee virus is negligible.

14.2.2 Exposure Assessment

In the absence of *Varroa destructor*, bee viruses are usually present at very low levels in bee colonies, and the mechanism of transmission from bee to bee is unknown. Assuming that either the oral route or direct contact is likely under natural conditions, the attractiveness of the commodities to bees is considered to be a good indicator of the likelihood of exposure if the commodities did carry the virus. Attractiveness to bees is discussed in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. Although beeswax that is made into foundation or applied to plastic frames comes into close contact with bees, considering the processing involved in the production of beeswax, the likelihood of this pathway resulting in exposure of bee viruses to susceptible species in New Zealand is considered to be negligible.

14.2.3 Consequence Assessment

Egypt bee virus has a very limited distribution internationally, and in those countries where it is established it 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 *V. destructor* or other mites. It is therefore unlikely that the virus would have any adverse effects if it were introduced into New Zealand. However, in view of the restricted international distribution of this virus, its introduction into New Zealand would probably result in a loss of export markets at least for live bees.

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

In view of the likely international market reaction the consequences of introduction are considered to be non-negligible.

14.2.4 Risk Estimation

The likelihood of Egypt bee virus being present in the imported commodities is considered to be negligible. If the virus were present in imported commodities, the likelihood of exposure is considered to be non-negligible for some commodities and the consequences would probably be significant. However, since the likelihood of Egypt bee virus being present in the commodities is considered to be negligible, the risk estimate is 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, a large (particle size 300 x 400 nm) unclassified DNA virus (Bailey et al., 1981).

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 severely 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 both 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, a small (particle diameter 30 nm) single-stranded RNA cricket-paralysis-like virus that has recently been assigned to the genus *Cripavirus* in the family *Dicistroviridae* (de Miranda et al., 2004).

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 in *Apis cerana* from India (Bailey and Woods, 1977). It is thought to be the most virulent of all known honey bee viruses; few particles are required for infection and the virus can multiply rapidly when introduced into the haemolymph of both adult bees and pupae, causing death within three days (Allen and Ball, 1995). However, in *A. mellifera* the virus is usually found as an inapparent infection in adult bees (Anderson and Gibbs, 1988), and it is thought that the virus may be activated to multiply to lethal levels by the injection of foreign proteins in a similar manner to that reported for acute paralysis virus (Dall, 1985; Bailey and Gibbs, 1964).

Kashmir bee virus is very closely related to acute paralysis virus, the two being about 70% identical over the whole genome (de Miranda et al., 2004). There is good evidence that both viruses persist as inapparent infections in nature and that in the absence of *Varroa destructor* they are probably transmitted in a similar manner, that is, via the salivary gland decretions of adult bees and the food to which these secretions are added (Bailey, 1976; Anderson, 1991). It appears that bees may be infected with both viruses simultaneously (Evans, 2001), and it has been suggested that occupy the same ecological niche (Anderson, 1991). Larvae can survive after they ingest Kasmir bee virus and some of them become inapparently infected adults (Bailey and Ball, 1991).

Kashmir bee virus has been identified in Canada and the USA, Spain, India, Fiji, Australia and New Zealand (Allen and Ball, 1995; 1996; Anderson, 1990; Hung et al., 1996).

Serological differences exist between isolates of Kashmir bee virus from different countries, the relevance of this is not clear because all strains of the virus are highly virulent (Ball and Bailey, 1997). Isolates found in Canada and Spain more closely resemble acute paralysis virus than previously identified isolates (Allen and Ball, 1995). Thus, the serological differences reported between isolates reported between isolates from the US (Bruce et al., 1995) and Australia (Bailey et al., 1979) do not support the existence of strains of different virulence.

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, a, small (particle diameter 30 nm) single-stranded RNA virus that has recently been assigned to the "floating genus" *Iflavirus* (Gosh et al., 1999).

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. The virus may be present and multiply in young adult bees without causing obvious disease (Bailey, 1969). Infected bees pass sacbrood virus in food to young larvae, which then become infected and die in the prepupal stage (Bailey, 1969). Fluid accumulates between the larva and the unshed skin of the final moult, resulting in a distinct watery sac, and 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 that are fed the virus when more than two days old can 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 are also reduced by the infection (Bailey and Ball, 1991).

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). The disease has a seasonal occurrence – outbreaks are common in the spring and it normally disappears spontaneously during summer (Ball, 1999). Sacbrood has been reported in every continent where honey bees are kept and it 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, an unassigned small (particle diameter 30 nm) single-stranded RNA virus which, in common with several other honey bee viruses, is morphologically similar to the picornaviruses (van Regenmortel et al., 2000).

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

Until the arrival of *Varroa destructor* in Britain, slow paralysis virus was only known as a laboratory infection of *Apis mellifera*; it was originally detected in the UK as an inapparent infection of adult bees during studies on another bee virus, and when it was injected into the haemolymph of adult bees it killed them in 12 days (Bailey, 1976).

Initially nothing was known of the natural history of this virus, but with the establishment of *V. destructor* in the UK, the virus began to be seen in association with adult bee mortality in varroa-infested colonies (Ball and Bailey, 1997). The virus became prevalent late in the season as the brood nest contracted and multiple mite infestations of brood cells was common. Often all of the brood produced late in the year was killed by slow paralysis virus infection and colonies dwindled and died because of the lack of young bees to carry them through the winter. In the initial stages of mite colonisation in Britain slow paralysis virus was detected as an established infection in about a quarter of infested colonies, but virus incidence appears to have declined over time, and it is now detected only in about 4% of mite samples submitted by beekeepers from all over Britain (Ball, personal communication[1]).

Slow paralysis virus has been identified only in Britain, Fiji and Western Samoa, but this probably reflects the the lack of surveillance for it internationally (Allen and Ball, 1996).

18.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.3, 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

As with most viruses of bees, slow paralysis virus is thought to persist at very low levels inside the body of live individuals. However, in contrast to most other honey bee viruses in nature it normally never replicates sufficiently to cause any pathology. With the arrival of *V. destructor* in Britain, overt fatal infections were observed and transmission of slow paralysis

[1] Brenda Ball, Rothamsted, UK. External review of MAF's Honey bee genetic material risk analysis, September 2002.

virus from severely infected to healthy bee pupae and mites has been demonstrated in the laboratory (Ball, personal communication[1]). Infection with slow paralysis virus is rare even in colonies with large mite populations, so it is assumed that the likelihood of the commodities being contaminated with the virus is low, even at the time of their production by bees.

Although no work has been done on degradation and loss of infectivity of slow paralysis virus *per se*, the survival of most bee viruses outside the body of their host is very limited, particularly in the case of the small non-occluded single stranded RNA viruses like slow paralysis virus (Ball, 1999). For example, in hives with clinical signs consistent with the parasitic mite syndrome, while most samples of adult bees taken from brood combs within the colony were positive for viruses, most samples from crawling and dead bees outside the hive were negative for viruses (Calderon et al., 2003). This suggests that viruses survive away from live bees for at most a day or two. In the case of Kashmir bee virus, also a single stranded RNA virus, the coat proteins of virus particles rapidly degrade and the particles lose their infectivity when removed from the host (Bailey et al., 1979; Anderson, 1986).

Therefore, it is considered unlikely that honey bee products stored away from honey bees would carry sufficient virus to initiate infection.

In view of the above, it is concluded that the likelihood of any of the commodities carrying the virus is negligible.

18.2.2 Exposure Assessment

In the absence of *Varroa destructor*, bee viruses are usually present at very low levels in bee colonies, and the mechanism of transmission from bee to bee is unknown. Assuming that either the oral route or direct contact is likely under natural conditions, the attractiveness of the commodities to bees is considered to be a good indicator of the likelihood of exposure if the commodities did carry the virus. Attractiveness to bees is discussed in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. Although beeswax that is made into foundation or applied to plastic frames comes into close contact with bees, considering the processing involved in the production of beeswax, the likelihood of this pathway resulting in exposure of bee viruses to susceptible species in New Zealand is considered to be negligible.

[1] Brenda Ball, Rothamsted, UK, emails to H Pharo dated 2 February 2004 & 11 November 2004..

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 in most situations the effects of the virus have not been isolated from effects of varroa and its associated parasitic mite syndrome (Hung et al., 1996). However, the evidence from the UK suggests that even if slow paralysis virus did become established in the honey bee population in New Zealand, its effect would be transitory and would not be severe. (Ball personal communication[1]). Therefore, it is likely 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 in the wake of varroa infestation and the parasitic mite syndrome. In view of the restricted international distribution of this virus, its introduction into New Zealand would probably result in a loss of export markets for live bees.

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

In view of the likely international market reaction the consequences of introduction are considered to be non-negligible.

18.2.4 Risk Estimation

The likelihood of slow paralysis virus being present in the imported commodities is considered to be negligible. If the virus were present in imported commodities, the likelihood of exposure is considered to be non-negligible for some commodities and the consequences would probably be significant. However, since the likelihood of slow paralysis virus being present in the commodities is considered to be negligible, the risk estimate is 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, an unassigned small (particle diameter 30 nm) single-stranded RNA virus morphologically similar to the picornaviruses, as are several other honey bee viruses (van Regenmortel et al., 2000). Thai sacbrood virus is closely related to sacbrood virus (Ball and Bailey, 1997; Ball, 1999a).

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 only in *Apis cerana.* The virus is widely distributed throughout Southeast Asia (Ball and Bailey, 1997) and it 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 (Allen and Ball, 1996), it has not been reported to cause disease signs in *A. mellifera* in localities where the colonies were in close proximity to *A. cerana* colonies (Allen, 1995). The transmission of Thai sacbrood under experimental conditions has been demonstrated to be possible by feeding adult bees with freshly purified virus suspensions in sugar syrup (Verma et al., 1990). This material is then fed to larvae which then exhibit the typical clinical signs of failure to pupate and death.

In the absence of information to the contrary it is reasonable to assume that the epidemiology of Thai sacbrood virus is similar to the closely related sacbrood virus, they key aspects of which are summarised below.

Sacbrood virus persists in adult bees without causing obvious disease. The youngest bees are the most susceptible and probably become infected when they remove larvae killed by the virus. During this activity they ingest liquid constituents, especially the virus-laden ecdysial fluid, and within a day of ingesting this material, the virus begins to collect in their hypopharyngeal glands. Infected nurse bees probably transmit sacbrood virus when they feed larvae with secretions from their hypopharyngeal glands (Bailey and Ball, 1991). Adult bees detect and remove most larvae in the early stages of viral infection, thereby limiting spread, and infected adult bees are prevented from transmitting the virus by behavioural changes (Bailey and Fernando, 1972). In particular, infected young bees cease to eat pollen (their only source of protein) and cease to feed and tend larvae. Although they continue to fly and forage, most infected bees fail to gather pollen. The few that do collect pollen bring back the virus in their pollen loads, each load containing about 10^6 virus particles, which is probably secreted by the bees from their glands into the liquid they add to pollen when they collect it (Bailey, 1976). Thus, an important route of transmission of sacbrood virus is by pollen, which is quickly fed to young susceptible individuals. The virus put into nectar by infected bees is far less important as a source of infection because incoming nectar is much diluted among the rest and quickly and widely distributed withing the bee colony, whereas pollen loads remain entirely within the honeycomb cell where they are placed, meaning any virus in the cells

would remain concentrated and more likely to infect a bee than virus in nectar (Bailey, 1976). However, since most infected bees fail to collect pollen this may not be the primary route of transmission.

19.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.3, 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

Thai sacbrood virus is widely distributed throughout Asia in *A. cerana* populations. Assuming that, as with sacbrood, infectivity is largely confined to pollen, the major commodity of potential concern with regard to the likelihood of release would be pollen imported from countries with commercial *A. cerana* colonies.

The level of Thai sacbrood virus present in pollen derived from *A. cerana* colonies would depend on the stability of the virus outside the body of the bee. In general terms bee viruses do not remain infective for long outside the body of their host (Ball, 1999b). For example, in hives with clinical signs consistent with the parasitic mite syndrome, while most samples of adult bees taken from brood combs within the colony were positive for Egypt bee virus and deformed wing virus, most samples from crawling and dead bees outside the hive were negative for these viruses (Calderon et al., 2003). This suggests that these viruses survive away from live bees for at most a day or two. In the case of Kashmir bee virus, also a single stranded RNA virus, the coat proteins of virus particles rapidly degrade and the particles lose their infectivity when extracted from the host (Bailey et al., 1979; Anderson, 1986). In the case of sacbrood virus, White found that infectivity in larval remains was lost after a few weeks (Bailey, 1976). White showed in 1913 that sacbrood virus is killed by heating at 58°C for 10 minutes or by prolonged exposure to 30-35^{o}C (Bailey, 1976) – this latter temperature range is very commonly encountered in all honey bee colonies.

Therefore, in view of the fragility of the virus outside the host, that likelihood of the virus being present in these commodities at the time of importation can be considered to be negligible.

19.2.2 Exposure Assessment

In the absence of *Varroa destructor*, bee viruses are usually present at very low levels in bee colonies, and the mechanism of transmission from bee to bee is unknown. Assuming that either the oral route or direct contact is likely under natural conditions, the attractiveness of the commodities to bees is considered to be a good indicator of the likelihood of exposure if the commodities did carry the virus. Attractiveness to bees is discussed in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to

bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Although honey, pollen and royal jelly are considered to be attracted to bees, and beeswax that is made into foundation or applied to plastic frames can be expected to come into close contact with bees, there is no evidence that *A. mellifera* are susceptible to natural infection with Thai sacbrood virus. It is reasonable to conclude that the likelihood of establishment in *A. mellifera* is low. However, in view of the ability of the virus to grow in *A. mellifera* under experimental conditions, it is concluded that the likelihood of exposure and establishment to susceptible species in New Zealand is non- negligible.

19.2.3 Consequence Assessment

Since disease caused by Thai sacbrood virus has been reported only in *Apis cerana*, the consequences of its introduction as far as New Zealand honey bees (*A. mellifera*) are concerned would be negligible. Thai sacbrood virus is unlikely to have any effects on New Zealand native insects since honey bee viruses cannot be grown in other insects or in insect cell tissue culture (Ball, 1999b). However, because of the restricted international distribution of this virus, its introduction into New Zealand would probably result in a loss of export markets for live bees.

In view of the likely international market reaction the consequences of introduction are considered to be non-negligible.

19.2.4 Risk Estimation

The likelihood of Thai sacbrood virus being present in the imported commodities is considered to be negligible. Taking a precautionary approach, it is assumed that the likelihood of exposure to susceptible species in New Zealand is non-negligible, and if the virus were found in New Zealand the international market reaction would probably be significant. However, since the likelihood of Thai sacbrood virus being present in the commodities is considered to be negligible, the risk estimate is 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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20. AMERICAN FOULBROOD

20.1 Hazard Identification

20.1.1 Aetiologic Agent: Family *Bacillaceae, Paenibacillus larvae* subsp. *larvae.*

20.1.2 OIE List: B.

20.1.3 New Zealand's Status: Present in New Zealand. Under official control (a National Pest Management Strategy under the Biosecurity Act 1993). Listed on the unwanted organisms register as a reportable organism.

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* (Heyndrickx et al., 1996; formerly *Bacillus larvae*). Larvae may become infected in two ways. Up to 20% of transmission occurs by larvae being reared in a cell that previously contained infected larvae, but most larvae become infected by ingesting food contaminated with spores that have been picked up by cell-cleaning bees that later assume nurse bee duties (Ratnieks, 1992).

Under natural conditions, nurse bees reduce the concentration of spores fed to larvae by a filtering action of the proventriculus – up to 80% of spores contaminating nectar are removed in this way. The spores germinate soon after they enter the larval gut and the vegetative form of the bacteria penetrate the body cavity through the gut wall (Davidson, 1973). Infected individuals usually die nine days or more after hatching in either the larval or pupal stage, but recent in vitro studies have shown that larvae can die at a younger stage before the capping of the cell (Brodsgaard et al., 2000). Most spores are formed 11 days after hatching in propupae and each infected larva produces about 2500 million spores (Sturtevant, 1932). *P. l. larvae* spores can remain viable for over 35 years (Haseman, 1961).

The number of spores required to infect larvae increases with larval age. As few as ten spores can infect 24 hour old larvae under experimental conditions, but larger numbers are needed to infect older larvae (Woodrow, 1942; Brodsgaard et al., 1999). While the LD_{50} for a one-day-old larva is estimated at 20 spores, several million spores are necessary to infect a 4-5-day-old larva (Ratnieks, 1992). At the colony level, spores of *P. l. larvae* have a low level of infectivity. The lowest concentration of spores that have been fed to colonies and reported to become infected is 50 million spores/litre of syrup – 100 mls of such a solution, comprising 5 million spores is the minimum amount necessary to infect a colony(Sturtevant, 1932; Goodwin et al., 1994).

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 may quickly result in colony death; infection may disappear without recurrence; or infection may disappear and then recur in about three weeks, resulting in colony death (Goodwin and Van Eaton, 1999).

Control of American foulbrood disease is generally by the destruction (burning) of colonies found with clinical symptoms, or the destruction of individual combs found with clinical symptoms (Matheson and Reid, 1992).

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, and until recently sub-Saharan Africa was thought to be free (Matheson, 1997a), but it has recently been reported from South Africa and Guineau Bissau (Hansen et al., 2003). It has been recognised on the Indian subcontinent since 1959 (Singh, 1961).

Different isolates of *P. l. larvae* show marked genetic, protein and antigenic homogeneity, as determined by SDS polyacrilamide gel electrophoresis, immunoblot and rep-PCR analyses (Djordjevic et al., 1994; Hornitzky and Djordjevic, 1992; Alippi et al., 2004b). However, there are no reports of strains of *P. l. larvae* with differing pathogenicity, although isolates have been reported with varying resistance to oxytetracycline (Alippi, 1999b; Miyagi et al., 2000, Cox, 2000; Evans, 2003).

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*, only a few media that are rich in organic growth factors will induce germination or sporulation of *P. l. larvae* (Alippi, 1999a; Dingman and Stahly, 1983). An inoculum of many millions of spores is needed to start growth on these media (Bailey and Ball, 1991).

20.1.5 Conclusion

P. l. larvae is present in New Zealand, but 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

20.2.1.1 Honey

P. l. larvae spores are frequently found in honey. In Australia, 63 of 505 honey samples (12.5%) were positive for *P. l. larvae* (Hornitzky and Clark, 1991). It has also been demonstrated in Australia that *P. l. larvae* spores in honey did not always relate to American foulbrood disease signs in the hive of origin, but the higher the concentration of spores in honey the greater was the likelihood that disease would be detected in the hives of origin (Hornitzky and Clark, 1991). Positive tests for spores have been reported for 8% of 82 honey samples from USA and Canada (Steinkraus and Morse, 1992), 55% of 394 Argentinian honey samples (Alippi et al., 2004b), 62% of 68 Austrian honey samples (Derakhshifar, 1994) and 56% of 131 honey samples from a range of countries (Hansen, 1984). Honey extracted from combs of diseased colonies contained about 25 million spores per g (Gochnauer, 1981).

20.2.1.2 Propolis

Since small numbers of spores may be found on the inside surface of the hive – 300,000 spores per 100 sq cm of hive surface (Gochnauer, 1981) – it is reasonable to assume that a similarly low level of contamination of propolis is also possible. In addition, propolis scrapings often contain a certain amount of wax, which may contain quite high concentrations of *P. l. larvae* spores – up to 9 million spores per g in wax from diseased hives (Gochnauer,

1981). There are no published reports of propolis being contaminated with *P. l. larvae*, although spores have apparently been found in a propolis extract used in herbal medicine (Brodsgaard, personal communication[1]).

20.2.1.3 Pollen

P. l. larvae spores have been reported in pollen trapped from honey bee colonies. It has been suggested that the pollen may become contaminated by bees regurgitating contaminated honey to aid the packing of pollen in their pollen baskets and by housekeeping bees dropping small pieces of diseased larvae or dried scales into the pollen trap drawer (Hitchcock and Revell, 1963). In diseased hives, pollen was reported to harbour 4.5 million *P. l. larvae* spores per g (Gochnauer, 1981). Contaminated pollen has been shown to cause infections when fed to other colonies (Gochnauer and Corner, 1974; Hitchcock and Revell, 1963)

20.2.1.4 Royal jelly

There are no reports of royal jelly harbouring *P. l. larvae* spores. Since commercial royal jelly is generally harvested from specialised artificial queen cells from which larvae have been removed before they have a chance to die from American foulbrood, it is considered that the likelihood of royal jelly containing significant levels of *P. l. larvae* spores is negligible. However, if royal jelly is produced from worker cells, the likelihood of it containing spores is considered to be non-negligible. It is also possible that nurse bees could become contaminated with *P. l. larvae* spores and that this could result in spores being present in royal jelly.

20.2.1.5 Beeswax

P. l. larvae spores have been reported from air-dried comb wax from diseases hives at levels of 9 million spores per g (Gochnauer, 1981). Wax that was recovered from such combs by boiling in water for 20 minutes was found to have very low levels of spores, most being washed off into the water or slumgum. Moreover, the viability of remaining spores was reduced to about 0.001% of that of unheated spores (Gochnauer, 1981). Several attempts to culture *P. l. larvae* from commercial wax foundation delivered negative results (Gochnauer, 1981; Hansen and Rasmusen, 1991), and although the bacterium could be cultured from one of six batches of wax from infected colonies that was melted using steam at 120°C, it could not be cultured from foundation made with wax from diseased colonies (Hansen and Rasmusen, 1991). To inactivate all spores on beekeeping equipment it was necessary to immerse it in paraffin wax at 160°C for 10 minutes (Goodwin and Haine, 1998), but beeswax that is melted at temperatures over 120°C is generally considered to be sterile (Smirnov and Tsivilev, 1969). Therefore, for the minimum standard of traded beeswax considered in this risk analysis (i.e. 60°C for 2 hours, as discussed in section 2.2.5) the likelihood of release is considered to be very low but non-negligible.

20.2.1.6 Bee venom

There are no reports of *P. l. larvae* spores being detected in bee venom. In view of the transmission mechanisms for the organism in hives, and considering the location and isolation from other organs of the venom gland, the likelihood of bee venom being contaminated with significant numbers of *P. l. larvae* spores may be assumed to be negligible.

[1] Camilla Brodsgaard, letter to H J Pharo dated 3 September 2004.

20.2.1.7 Release assessment conclusion

The likelihood of release of *P. l. larvae* is considered to be negligible in the case of bee venom, regardless of the infection status of the colony. However, for honey, pollen, royal jelly, beeswax and propolis produced from infected colonies, the likelihood of release of *P. l. larvae* is non-negligible. The likelihood of release in beewax foundation is considered to be negligible.

20.2.2 Exposure Assessment

For *P. l. larvae* spores in imported honey, wax, pollen and propolis to come into contact with susceptible species in New Zealand, these commodities would have to be harvested by worker bees and taken back to hives and fed to young larvae, or be fed directly to the colony by beekeepers.

The attractiveness of these commodities to bees is discussed as part of the commodity definition in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly. In addition, since beeswax may be made into foundation or applied to plastic frames, the likelihood of exposure is considered to be non-negligible. For propolis and bee venom, the likelihood of exposure is considered to be negligible.

20.2.3 Consequence Assessment

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).

Since the organism is already present in New Zealand, the consequences of importing commodities contaminated with *P. l. larvae* into this country would be whatever additional disease resulted from exposure to a consignment of contaminated commodities. The American Foulbrood National Pest Management Strategy requires colonies infected with the disease to be destroyed, at an estimated cost in 1997 of $325 per colony, comprising the cost of the hive, destruction costs and lost production (NBA, 1997).

There are no reports of strains of *P. l. larvae* with differing pathogenicity, but there are strains that are reported to have varying resistance to oxytetracycline. This is of little relevance to New Zealand, however, because for the last 50 years there has been 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 highly unlikely that oxytetracycline will be fed in New Zealand for American foulbrood control, and as long as New Zealand does not allow this form of treatment, the importation of oxytetracycline-resistant strains are not expected to have any consequences, and indeed without the continuous selection pressure for resistance in the form of antibiotic use, the persistence of these strains could reasonably be expected to be limited.

Therefore, the consequences of introduction of *P. l. larvae* are considered to be non-negligible, but the consequences of introduction of oxytetracycline-resistant strains of this organism are no different to the introduction of non-resistant strains.

Although American foulbrood has been present in New Zealand for a long time, it has never been reported in species other than *Apis mellifera.* The disease causes problems in commercial beekeeping because of the social nature of *A. mellifera*, which forms colonies comprising many thousands of individuals in close contact with one another, quite unlike native bees which are solitary insects where there is no contact between generations (Donovan, 1980; Donovan et al, 1984; Matheson, 1997b). The consequences for native insects are therefore considered to be negligible.

20.2.4 Risk Estimation

The release assessment conclusions for the commodities are summarised in Table 2.

For honey, pollen, royal jelly and beeswax, the likelihood of release and exposure are non-negligible, and the consequences of introduction are also considered to be non-negligible. Therefore, for these commodities the risk of *P.l. larvae* is considered to be non-negligible and the organism is considered to be a hazard.

For propolis, although there is a non-negligible likelihood of introduction, the nature of the imported commodities means that the likelihood of exposure is negligible. As is discussed under the risk analysis methodology in Section 2.3 of this document, this means that the risk estimate is automatically negligible and the consequence assessment is not applicable for these commodities. Thus, for propolis the risk of *P.l. larvae* is considered to be negligible and the organism is not considered to be a hazard.

For bee venom, both the release and exposure assessments are negligible, meaning that the risk estimate is automatically negligible and the consequence assessment is not applicable for this commodity. Thus, for this commodity the risk of *P.l. larvae* is considered to be negligible and the organism is not considered to be a hazard.

Table 2. Release assessment conclusions for *P.l.larvae*

	Release	**Exposure**	**Consequence**	**Risk Estimate**
Honey	Non-negligible	Non-negligible	Non-negligible	Non-negligible
Propolis	Non-negligible	Negligible	n.a.	Negligible
Pollen	Non-negligible	Non-negligible	Non-negligible	Non-negligible
Royal jelly	Non-negligible	Non-negligible	Non-negligible	Non-negligible
Beeswax	Non-negligible	Non-negligible	Non-negligible	Non-negligible
Bee venom	Negligible	Negligible	n.a.	Negligible

It is important to note that these risk estimates are not based on the risk of introduction of oxytetracycline-resistant strains of *P.l. larvae,* as in the absence of antibiotic feeding in this country, such strains would not have any different effect than non-resistant strains.

20.3 Risk Management

20.3.1 Risk Evaluation

Since the risk estimate for American foulbrood in honey, royal jelly, pollen and beeswax is non-negligible, and since *P. l. larvae* is under official control in New Zealand, risk management measures are justified for these commodities, to reduce the risks to an acceptable level.

20.3.2 Option Evaluation

20.3.2.1 Risk management objective

Although risk management measures against American foulbrood are warranted as a result of there being an official control program in place in New Zealand, 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. In other words, the acceptable level of risk is signalled by the rules of the control program. The relevant rules under the National Pest Management Strategy are rules 29 (1) and 31(1) which prohibit the sale or use of bee products from hives known or suspected to be clinically affected by American foulbrood. 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 on New Zealand hives.

20.3.2.2 Options available

Although the latest OIE *Code* (OIE, 2004) for the first time includes recommendations for honey, pollen, beeswax, propolis and royal jelly in regard to American foulbrood, the treatments and their technical justification for the measures are not yet included in the *Code.* Moreover, when they are completed, the treatment recommendations for the destruction of *P. l. larvae* in these commodities will apply only to importing countries that are officially free from American foulbrood, and therefore will not be applicable for New Zealand.

Culture testing of adult bees (Hornitzky and Karlovskis, 1989) may be used to predict whether a honey bee colony is likely to have clinical signs of American foulbrood. More than 85% of honey bee colonies that produced more than 100 *P. l. larvae* colonies per plate exhibited clinical symptoms of American foulbrood (Goodwin et al., 1996). Further work is necessary to develop this sampling technique into a surveillance programme that enables the certification at a given confidence level that hives are not showing clinical signs of this disease. Specific PCR-based detection tests have been developed for fast and reliable diagnosis of American foulbrood (Govan et al., 1999: Alippi et al., 2004a). Commercial ELISA kits for the detection of diseased larvae are available[1], but not for the detection of spores in bee products.

[1] VITA® (Europe) Limited, Basingstoke, Hampshire,, UK. http://www.vita-europe.com/company.htm

Although the inactivation of *P. l. larvae* spores on hive parts has been demonstrated by immersion in paraffin wax at 160°C for 10 minutes (Goodwin and Haine, 1998), such a treatment is obviously not suitable for honey bee products. *P. l. larvae* spores on hive parts and in bee products have also been shown to be killed by 10 kGy gamma radiation (Hornitzky and Wills, 1983; Hornitzky, 1994; Ratnieks, 1989).

For comb wax, treatment in boiling water for 20 minutes was found to have removed the vast majority of spores, and the viability of remaining spores was reduced to about 0.001% of the viability of unheated spores (Gochnauer, 1981). This level of reduction in germination capability is similar to that produced by gamma irradiation at 0.2 Mrad (i.e. 2 kGy) (Gochnauer and Hamilton, 1970) and is almost as great a reduction as that produced by fumigation with ethylene oxide for 18 h (Gochnauer and Corner, 1976). A method to detect *P. l. larvae* spores in wax has been developed by Ritter (2003). However, to ensure complete inactivation of spores, wax must be melted at a temperature of 120°C or higher and held at that temperature for 24 hours (Smirnov and Tsivilev, 1969, cited in Matheson and Reid, 1992).

A sterilisation process has been developed in the USA that uses a combination of hydrostatic pressure and temperature to inactivate spores of *Clostridium botulinum* in honey that is used for baby food (Omahen, 2004). The standard US industry practice for pasteurisation of honey is heating to 76°C and holding for about 5 minutes. This is adequate to destroy fungi and yeasts but it is not adequate for bacterial spores, such as *C. botulinum*, which requires heating to at least 120°C under pressure and holding at this temperature for at least 3 minutes. However, heating honey to such high temperatures causes unacceptable changes to its flavour and texture which this new sterilisation process is designed to overcome. The process pressurises honey to about 2,400 bar (35,000 psi) and passes it very quickly through a heat exchanger to raise its temperature to 82°C within a few seconds. When the pressure is dropped, the temperature instantly spikes to about 135°C, after which the honey is cooled within seconds. Since bacterial spore survival at that high temperature is measured in seconds, the result is a sterile product that has not been physically changed. Researchers at the University of Georgia have patented this process and are about to evaluate it for the inactivation of honey bee pathogens in extracted honey (Toledo, personal communication[1]). It is possible that this process might be commercially applicable in the near future.

An equivalent level of protection to that achieved under the New Zealand National Pest Management Strategy (PMS) for American foulbrood could be achieved by requiring official veterinary certification from the country of origin that the bee products were not derived from hives that were known or suspected to be clinically affected by American foulbrood. For equivalence to the PMS, such certification would have to be backed by an annual inspection of hives by a person certified as competent to make the diagnosis of American foulbrood, following the guidelines set out in Appendix 3.4.2 of the OIE *Code*.

However, few countries have either control programmes for American foulbrood or tracing systems to allow certification of the origin of bee products. It is possible to test honey for *P. l. larvae* to estimate spore concentration (Hansen, 1984; Hornitzky and Clark, 1991; Alippi, 1995), and to dilute contaminated honey with other honey so that the final concentration is less than the lowest reported to cause infection when fed to bees – that is, 50 million spores per litre (Sturtevant, 1932). If an additional safety margin of two orders of magnitude were

[1] Dr Romeo Toledo, Food Scientist, College of Agricultural and Environmental Sciences, University of Georgia. Email to HJ Pharo dated 8 July 2004.

applied for spore levels in honey, then honey with less than 500,000 spores per litre could be considered safe. PCR protocols have been developed for the direct detection of *P. l. larvae* spores in honey samples, although these do not differentiate between *P. l. larvae* and *P. l. pulvifaciens* (Bakonyi et al., 2003; Lauro et al., 2003).

20.3.2.3 Recommended sanitary measures

For venom and propolis

No sanitary measures required.

For honey, pollen, royal jelly, and beeswax

Each consignment must be either:

(i) from a country or part of the territory of a country free from American foulbrood

or

(ii) from hives that were inspected for American foulbrood within the previous 12 months, by a person certified as competent to diagnose the disease (following appendix 3.4.2 of the OIE *Code*), and found not to be clinically infected or suspected to be clinically affected by American foulbrood .

or

(iii) tested and found to have a *P.l. larvae* spore count of less than 500,000 per litre.

or

(iv) irradiated with 10 kGy

or

(v) heated to 120°C and then held at that temperature for 24 hours.

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21. EUROPEAN FOULBROOD

21.1 Hazard Identification

21.1.1 Aetiologic Agent: Family *Enterococcaceae, Melissococcus pluton*[1].

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 larvae of *Apis mellifera*, *Apis cerana* and *Apis laboriosa* caused by a fastidious anaerobic bacterium, *Melissococcus pluton* (Bailey and Collins, 1982a, 1982b). Larvae become infected by being fed contaminated brood food by nurse bees. The bacteria begin to grow vigorously within the midgut, and by the time the larva is 5 days old, the area of the midgut that is normally occupied by the food mass is packed by the bacteria, creating an abnormal demand for food. If there are too many young larvae for the available brood food nurse bees normally eject such larvae because they are the first to show signs of starvation. If the population of nurse bees is such that surplus food is available, infected larvae can survive and in this way most colonies can keep the infection contained (Shimanuki, 1997). Thus, European foulbrood is not a disease in the classical sense as it does not cause pathology in the honey bee larvae and those larvae that die do so from starvation. Infected larvae that survive discharge *M. pluton* bacteria with larval faeces onto the wall of the brood cells (Bailey, 1959), where they appear to survive better in the thin smears than in dead larval remains. Most *M. pluton* are removed from cells by house-cleaning worker bees, which then act as passive vectors to contaminate larval food, but it appears that *M. pluton* does not grow in the gut of adult bees (Bailey, 1957), probably because there is too much oxygen.

Clinical symptoms of European foulbrood are only likely when the ratio of nurse bees to diseased larvae decreases for some reason, such as when nurse bees are recruited away from larval feeding by the demands of a high 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 larvae, the disease usually subsides (Bailey and Ball, 1991). Therefore, honey bee colonies are usually more seriously affected during the spring and early summer (Tarr, 1938; White, 1920).

A number of secondary opportunistic bacteria can be found in larvae killed by *M. pluton*, including *Lactobacillus eurydice*, *Paenibacillus alvei*, *Paenibacillus apiarius*, *Brevibacillus laterosporus* and *Enterococcus faecalis*. These do not cause the disease, but they do affect the odour and appearance of dead brood, which can make them of some diagnostic importance (Bailey, 1959).

In a series of classic experiments carried out early in the 20th century, *M. pluton* was shown to survive for about 12 months in an incubator and at normal room and outdoor temperatures. In

[1] This bacterium has previously been referred to in the literature as *Bacillus pluton* and *Streptococcus pluton*, and more recently as *Melissococcus plutonius*. However, the current OIE standard terminology is used here.

water, the organism resisted direct sunlight for about 30 hours, while in honey exposed to direct sunlight the organism was destroyed after 3-4 hours. However, in honey stored away from direct sunlight the organism survived up to 7 months (White 1920).

Control of European foulbrood, once signs become apparent, is generally through the feeding of oxytetracycline in sugar syrup or in powdered sugar dusted on combs (Shimanuki, 1997).

There is no evidence of significant strain variation among isolates of *M. pluton* (Bailey and Gibbs, 1962), and although one isolate from Brazil was less closely related to most other isolates (Bailey, 1984), REA typing of Australian isolates showed minimal genetic diversity (Djordjevic et al., 1999).

European foulbrood is found on all continents, including Australia (Matheson, 1997a), 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. pluton* is classified as a potential hazard for the purposes of this analysis.

21.2 Risk Assessment

21.2.1 Release Assessment

Notwithstanding the lack of evidence that this fastidious anaerobic organism grows anywhere other than in the lumen of the larval midgut (Bailey, 1959), it is generally accepted that *M. pluton* can be present and can survive for a significant period in honey, pollen, beeswax, propolis and royal jelly (OIE, 2004).

21.2.1.1 Honey

M. pluton can be found in honey from infected hives, albeit in relatively low numbers. In one study, 6% of bulk honey samples from endemic areas were culture positive for *M. pluton* (Hornitzky and Smith, 1998). The concentration of *M. pluton* reported in honey under natural conditions is up to 3.3×10^3 organisms per ml (Wootton et al., 1981).

Recently published work (McKee et al., 2003) has demonstrated that the polymerase chain reaction (PCR) assay is a more sensitive tool for the detection of *M. pluton* in honey than culture. In a study of 80 honey samples from different states in Australia that 22/80 (27.5%) were positive by culture whereas 57/80 (71.3%) were positive using PCR. This work demonstrates that *M. pluton* is more common in honey than previously demonstrated.

The likelihood of release in honey is therefore non-negligible.

21.2.1.2 Royal jelly

M. pluton can be found in royal jelly, although there is little indication from the literature of the likelihood of this occurring. Royal jelly imported into New Zealand was routinely tested

for the presence of *M. pluton* until December 2001 (Giacon and Malone, 1995), when imports were suspended following the isolation of *M. pluton* in quarantine from three out of 10 samples of freeze-dried, bulk royal jelly imported from China (Jamaludin et al., 2002). The likelihood of release in royal jelly is therefore non-negligible.

21.2.1.3 Pollen

Bee-collected pollen does not appear to have been tested for the presence of *M. pluton*. However, in view of the ability of the organism to survive on the walls of cells that have contained infected larvae (Bailey, 1959), it appears reasonable to assume that bee-collected pollen is likely to be contaminated with the organism when collected by bees from infected colonies. It has been speculated that the feeding of bee-collected pollen to colonies was one factor contributing to the spread of EFB in western Canada (MacDonald, 1981). The likelihood of release in pollen is therefore non-negligible.

21.2.1.4 Propolis

There are no reports of propolis being contaminated with *M. pluton,* and the antimicrobial properties of propolis (Ghisalberti, 1979; Grange and Davey, 1990) may indeed limit the survival of this organism. However, since *M. pluton* is thought to be able to overwinter on the sides of cell walls or in faeces and wax debris on the bottom of the hive (Shimanuki, 1997), and since propolis, whether from scrapings or propolis mats, often has a wax component, it is considered that the likelihood of *M. pluton* being present in propolis is non-negligible.

21.2.1.5 Beeswax

Although wax has not been tested for *M. pluton,* since the organism is known to persist on the walls of cells, it is reasonable to assume that wax may be contaminated by the organism, at least at the time of its formation. Therefore, the likelihood of *M. pluton* being present in raw beeswax cappings and comb wax can be considered to be non-negligible. The likelihood of *M. pluton* being present in processed beeswax as defined in section 2.2.5 or in beeswax foundation will depend on the time/temperature parameters of the manufacturing process and it is therefore reasonable to consider the likelihood to be non-negligible unless a critical time/temperature has been achieved.

21.2.1.6 Bee venom

There is no evidence that bee venom can harbour *M. pluton* and, considering the collection methods for bee venom, and the anaerobic conditions required for growth of the organism, there is no good reason to assume that significant contamination of bee venom is likely. The likelihood of the organism being present in venom is therefore considered to be negligible.

21.2.1.7 Release assessment conclusion

The likelihood of release of *M. pluton* is considered to be non-negligible for honey, propolis, pollen, royal jelly and beeswax, but it is considered to be negligible for bee venom.

21.2.2 Exposure Assessment

For *M. pluton* bacteria in imported commodities to come into contact with susceptible species in New Zealand, these commodities would have to be harvested by worker bees and taken back to hives and fed to young larvae, or be fed directly to the colony by beekeepers. The attractiveness of these commodities to bees is discussed as part of the commodity definition in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly. In addition, since beeswax may be made into foundation or applied to plastic frames, the likelihood of exposure is considered to be non-negligible. For propolis and bee venom, the likelihood of exposure is considered to be negligible.

21.2.3 Consequence Assessment

Although in areas with uninterrupted nectar flows the level of infection usually remains low, in which case colonies can cope with the infection without assistance (Shimanuki, 1997; Alippi, 1999), under some circumstance honey bee colonies may be destroyed or seriously crippled by European foulbrood (Bailey and Ball, 1991). Since European foulbrood can be a major problem for bee colonies used for pollination (Shimanuki, 1997), it would be likely to have significant implications for the more than 70,000 colonies in New Zealand used for kiwifruit pollination and for the many thousands of colonies used for pollinating pip and stone fruits, berry fruits and small seeds.

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 to be 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 mask 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 e.g. Japan.

European foulbrood is unlikely to have any effects on New Zealand native insects since this fastidious anaerobic organism is restricted to honey bees and it appears able to grow only in the honey bee larval midgut. European foulbrood causes problems in commercial beekeeping because of the social nature of *A. mellifera,* which forms colonies comprising many thousands of individuals in close contact with one another, quite unlike native bees which are solitary insects and where there is no contact between generations (Donovan, 1980; Donovan et al, 1984; Matheson, 1997b).

Therefore, the introduction of European foulbrood is likely to cause significant negative effects on bee colonies 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. These consequences are considered to be significant.

21.2.4 Risk Estimation

The release assessment conclusions for the six commodities are summarised in Table 3.

Table 3. Release assessment conclusions for *M. pluton*

	Release	**Exposure**	**Consequence**	**Risk Estimate**
Honey	Non-negligible	Non-negligible	Non-negligible	Non-negligible
Propolis	Non-negligible	Negligible	n.a.	Negligible
Pollen	Non-negligible	Non-negligible	Non-negligible	Non-negligible
Royal jelly	Non-negligible	Non-negligible	Non-negligible	Non-negligible
Beeswax	Non-negligible	Non-negligible	Non-negligible	Non-negligible
Bee venom	Negligible	Negligible	n.a.	Negligible

For honey, pollen, royal jelly and beeswax the likelihood of release and exposure are both non-negligible, and the consequences of introduction are also non-negligible. Therefore, for these commodities the risk of *M. pluton* is considered to be non-negligible and the organism is considered to be a hazard.

For propolis, although there is a non-negligible likelihood of introduction, the nature of the imported commodity means that the likelihood of exposure is negligible. As is discussed under the risk analysis methodology in Section 2.3 of this document, this means that the risk estimate is automatically negligible and the consequence assessment is not applicable for this commodity. For these commodities, the risk of *M. pluton* is considered to be negligible and the organism is not considered to be a hazard.

For bee venom, both the likelihood of release and the likelihood of exposure are negligible, meaning that risk estimate is automatically negligible, the consequence assessment is not applicable, and the organism is not considered to be a hazard.

21.3 Risk Management

21.3.1 Risk Evaluation

Since the risk estimate for European foulbrood in honey, royal jelly, pollen and beeswax is considered to be non-negligible, risk management measures are justified for these commodities to reduce the risks to an acceptable 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 that imported honey, royal jelly, pollen and beeswax do not harbour *M. pluton* when given a biosecurity clearance in New Zealand.

21.3.2.2 Options available

The OIE Terrestrial Animal Health Code (the *Code*) (OIE, 2004) recommends that honey, pollen, beeswax, propolis and royal jelly from countries that are not free from European foulbrood should have been processed to ensure the destruction of *M. pluton.* However, the *Code* does not specify what treatments are considered appropriate for the destruction of *M. pluton*[1], so this is reviewed in the following section of this risk analysis.

Heat

A number of studies have investigated the thermal stability of *M. pluton* in honey, but no studies have been carried out on other bee products. In the absence of information to the contrary, it will be assumed that the thermal inactivation of *M. pluton* is similar in different bee products.

A study from the USA in the early 1900s estimated the thermal death point[2] of *M. pluton* suspended in honey to be 79°C for 10 minutes (White, 1914). Further estimates were made as a result of later trials carried out in Australia in 1980 (Wootton et al., 1981) and in the UK in 2001 (Ball et al., 2001), the results of which are shown in Table 4. The viability of M. pluton was assessed by White (1914) in terms of its ability to infect honey bee larvae, while in the more recent studies by Wootton et al. (1981) and Ball et al. (2001), viability was assessed by bacterial culture.

The 'thermal death times' estimated by Wootton et al. (1981) showed quite significant variation between the five different honeys tested – the value shown in Table 4 is the maximum value. It was suggested that different pH and water content of the honeys might be responsible for the different 'thermal death times' observed, but no simple relationship was apparent (Wootton et al., 1981), and this has not been further investigated.

Ball et al. (2001) carried out a series of trials over a wider range of temperatures, and fitted a first order kinetic model to the data assuming a linear relationship between the logarithm of the number of survivors and time. The linear regression model of $\log_{10}$ (cfu + 1) against time for each temperature treatment, was used to predict D values (i.e. time for one $\log_{10}$ reduction in organism count, or in other words the time for a 90% reduction) and 'extinction times' which are shown in Table 4. In addition to the temperatures shown in the table, Ball et al. (2001) included treatments at temperatures of 90°C and 100°C in their experiments, but when fitting regression lines and determining D values, the data for these two higher temperatures

[1] In the 13th edition of the Terrestrial Animal Health Code, 2004, article 2.9.3.8 refers to measures that are in Appendix XXX (under study).

[2] "Thermal death point" is defined in food processing as the temperature at which an organism dies in a given time. The "thermal death time" is the time it takes for an organism to die at a given temperature.

were not used because inactivation was so rapid (somewhere between 2 and 5 minutes) that insufficient data points were available to calculate the regression line.

Table 4. Thermal inactivation of *M. pluton* in honey – results of two recent studies.

Temperature (°C)	Wootton et al., 1981 Maximum 'thermal death time'	Ball et al., 2001 'Extinction time'
50	48 h	47 h 30 min
60	8 h	12 h 38 min
70	3 h 30 min	1 h 53 min
80	1 h	17 min

Comparing the results of Wootton et al. (1981) and Ball et al. (2001) is difficult, as the methods of the experiments differ in several fundamental ways. For example, Wootton et al. (1981) used 5 distinct honeys each with a single predominant floral source, and found considerable variation between these, but no variation within each honey type, as identical results were achieved for each of two replicates for each honey. By contrast, Ball et al. (2001) used a single blended honey derived from several European countries, as they considered there was no simple relationship between bacterial death time and moisture and acidity, and the blended honey could be taken to represent a "middle range" of values for moisture content and acidity.

Notwithstanding these differences, the estimates of thermal death point in the two recent studies are in reasonable agreement for temperatures 50 – 70°C.

It is worthy of note that the concentration of organisms at the start of the Wootton et al. (1981) study was 1.25×10^5 per ml (about 5 log10 CFU/ml), which is substantially higher than the 3.3×10^3 per ml (3.5 log10 CFU/ml) found in honey under natural conditions (Wootton et al., 1981). Similarly, Ball et al. (2001) used a greater concentration of organisms than has been reported naturally in honey – the starting concentration used was between 10^6 and 10^7 CFU per ml. Since these trials started with a concentration of organisms in honey that was between 40 and 3000 times the concentration that is found in honey under natural conditions, the effect is that the predicted inactivation times from both studies are erring strongly on the side of caution.

In order to explore the assumption of linearity and to overcome a mathematical difficulty associated with the calculation of 'extinction time' used by Ball et al. (2001), Cox and Domijan (2004) applied an alternative empirical fit model to their data. This model and the results arising from it are included as Appendix 1 to this risk analysis. The approach of Cox and Domijan (2004) was to develop a predictive model that allows any D value to be calculated for any temperature, and the values shown in Table 5 were produced using that model.
Although the Cox and Domijan (2004) model does allow the prediction of required treatment times for temperatures above 80 °C, the authors are in agreement with Ball et al. (2001) that there is insufficient data for accurate predictions above that temperature. This is demonstrated by the spreading confidence intervals for predictions at temperatures higher than 80°C in Appendix 1. This notwithstanding, the fact that the time predicted for a 6D reduction as calculated by Cox and Domijan (2004) is more or less the same as the 'extinction time' calculated by Ball et al. (2001), provides a high degree of confidence that these time-temperature combinations provides a high level of protection for honey.

Table 5. Comparison of predictions from two models using the data of Ball et al. (2001).

	Ball et al (2001)		**Cox and Domijan (2004)***		
Temperature (°C)	D value (min)	Extinction time (min)	1D value (min)	4D value (min)	6D value (min)
50	373.5	2851	534.0	2153.4	3237.7
60	99.1	758	65.6	352.5	576.3
70	17.3	113	6.9	57.8	107.5
80	3.2	17	0.5	9.6	22.0
90			0.02	1.6	5.8
100			<0.01	0.3	4.7

* note that

- 1D reduction is a 90% reduction in numbers
- 4D reduction is a 99.99% reduction in numbers
- 6D reduction is a 99.9999% reduction in numbers

Just how safe a 6D reduction is can be considered in view of the normal concentration of organisms in honey being about 3.5 log10 CFU/ml. A treatment giving a 6D reduction would result in a final concentration of organisms of -2.5 log10 CFU/ml or 0.003 CFU/ml. At that concentration, a bee would have to consume on average about 300 ml of honey in order to pick up a single CFU. By contrast, a 4D reduction would result in a final titre of -0.5 log10 CFU/ml or 0.3 CFU/ml, at which concentration a bee would have to consume on average approximately 3 ml of contaminated honey to pick up a single CFU. There is no completely objective way of determining at what level honey becomes 'safe'. It could be argued that a 4D reduction would be adequate because a single bee would be very unlikely to consume 3ml of honey from a single imported source, but it could also be argued that a colony of bees could relatively easily consume 300 ml of honey from such a single source. A 6D reduction is therefore justified.

Heat and hydrostatic pressure

A sterilisation process has been developed in the USA that uses a combination of hydrostatic pressure and temperature to inactivate spores of *Clostridium botulinum* in honey that is used for baby food (Omahen, 2004). Standard US industry practise for pasteurisation of honey is heating to 76°C and holding for about 5 minutes, which is adequate to destroy fungi and yeasts, this is not adequate for bacterial spores, such as *C. botulinum,*.which requires heating to at least 120°C under pressure and holding at this temperature for at least 3 minutes. However, heating honey to such high temperatures causes unacceptable changes to its flavour and texture which this new sterilisation process is designed to overcome. The process pressurises honey to about 2,400 bar (35,000 psi) and passes it very quickly through a heat exchanger to raise its temperature to 82°C within a few seconds. When the pressure is dropped, the temperature instantly spikes to about 135°C, after which the honey is cooled within seconds. Since bacterial spore survival at that high temperature is measured in seconds, the result is a sterile product that has not been physically changed. Researchers at the University of Georgia have patented this process and are about to evaluate it for the inactivation of honey bee pathogens in extracted honey (Toledo, personal communication[1]). It is possible that this process might be commercially applicable in the near future.

[1] Dr Romeo Toledo, Food Scientist, College of Agricultural and Environmental Sciences, University of Georgia. Email to HJ Pharo dated 8 July 2004.

Gamma irradiation

The use of gamma radiation to kill *M. pluton* has been investigated in a number of different commodities (Hornitzky, 1981, 1994). Although 0.8 Mrad (800,000 rad, or 8kGy) was insufficient to kill *M. pluton* (Pankiw et al., 1970), 1.0 to 1.5 Mrad (10 – 15 kGy) was used to eliminate the organism from honey (Hornitzky, 1981). In honey that had a starting concentration of 1.23×10^5 organisms per ml, no organisms survived 14 kGy, and this appears to be a generally recommended treatment level (Hornitzky, 1994).

Irradiation of honey may result in a slight intensification of flavour as well as a distinct change from a dark to light colour (Kaznelson and Robb, 1962). It may also result in some surface foam, but it appears that overall irradiation causes no significant deterioration of honeys as measured by colour and taste (Hornitzky, 1994)

Testing for the presence of *M. pluton* in bee products

Although it is relatively difficult to culture *M. pluton* in the laboratory, as it is a fastidious anaerobe, cultivation has been used to detect the organism in a range of bee products (Bailey, 1984; Giacon and Mallone, 1995; Hornitzky and Smith, 1998).

The polymerase chain reaction (PCR) assay can also be used for the detection of *M. pluton* in honey (Djordjevic et al., 1998; Govan et al., 1998; McKee et al., 2003). Commercial ELISA kits for the detection of diseased larvae are available[1] (Pinnock and Featherstone, 1984), but not for the detection of infection in bee products. The use of PCR for testing honey that had been heat-treated to kill *M. pluton* may be of questionable value, as PCR can detect the DNA of dead organisms.

21.3.2.3 Recommended sanitary measures

For venom and propolis

No sanitary measures required.

For honey, royal jelly, pollen and beeswax

Each consignment must be

either

(i) from a country or part of the territory of a country free from European foulbrood

or

(ii) gamma irradiated with 15 kGy

or

[1] VITA® (Europe) Limited, Basingstoke, Hampshire,, UK. http://www.vita-europe.com/company.htm

(iii) Heated to achieve a 6D reduction in organism numbers according the model of Cox and Domijan (2004), as shown in Table 6. Agitation suitable to ensure the even distribution of heat is required, and automatic temperature tracing must demonstrate that the core temperature has been reached before timing begins.

An alternative to the above measures, for royal jelly and pollen only, is to import the pollen in a form that is not considered to be attractive to bees, such as consumer-ready capsules or tablets packaged for direct retail sale.

Table 6. Time-temperature treatments required to reduce the risk of *M. pluton* by 6D.

Temperature (°C)	Time
50	54 h
60	10 h
70	1 h 48 min
80	22 min
90	5 min
100	5 min

Note : intermediate temperatures can be considered in discussion with MAF using the predictive model developed by Cox and Domijan (2004) for this purpose.

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22. PAENIBACILLUS ALVEI

22.1 Hazard Identification

22.1.1 Aetiologic Agent: Family *Bacillaceae, Paenibacillus alvei.*

22.1.2 OIE List: None.

22.1.3 New Zealand's Status: Not listed on the unwanted organisms register. Has been isolated in New Zealand on one occasion.

22.1.4 Epidemiology

Paenibacillus alvei, formerly known as *Bacillus alvei* (Ash et al., 1993) is an aerobic, spore-forming bacterium that in the late 19th century was thought to be the cause of European foulbrood. By the middle of the 20th century the true cause of European foulbrood was determined to be *Melissococcus pluton,* and it became apparent that *P. alvei* was a saprophyte that lived on the remains of dead honey bee larvae, particularly those from colonies infected with European foulbrood (Shimanuki, 1997).

P. alvei is one of a number of secondary invader bacteria can be found in larvae killed by *M. pluton* including *Lactobacillus eurydice*, *Paenibacillus apiarius, Brevibacillus laterosporus* and *Enterococcus faecalis.* These bacteria do not cause disease, but they do affect the odour and appearance of dead brood (Bailey, 1959; 1963).

Unlike *M. pluton* 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 (Munkundan et al., 1979), from soil in Egypt (Hafez and El-Mohandes, 1999) and from ewes' milk in Spain (Roman-Blanco et al., 1999).

Two Russian reports indicate that under laboratory conditions apparently designed to mimic weak colonies, *P. alvei* was found to be pathogenic to 2-4-day-old honey bee larvae (Skrypnik, 1984; Kardakov and Movsum-Zade, 1975). But in other laboratory trials the feeding 10^5 *P. alvei* cells to individual 2-day-old larvae caused no mortality (Bailey et al., 1973). Under field conditions in the rest of the world *P. alvei* is not considered to be a primary pathogen of honey bees, and there is now widespread agreement that *P. alvei* is an opportunist secondary invader of *Apis mellifera* larvae that have been killed by other pathogens, particularly European foulbrood (Bailey, 1963; Alippi, 1997; Bailey et al., 1973; Bailey and Ball, 1991; Djordjevic et al., 2000). This viewpoint appears to be supported by the high degree of genetic heterogeneity and biochemical variability exhibited by *P. alvei* isolates, while the marked genetic homogeneity of *M. pluton* and *P. l. larvae* suggests that they have evolved to form close host-parasite relationships with honey bee larvae (Djordjevic et al., 2000).

The role of *P. alvei* in bee colonies infected with diseases other than European foulbrood has not been well investigated under field conditions (Djordjevic et al., 2000), but there is some evidence that this saprophyte can infect larvae killed by other pathogens. For example, when fed to larvae together with sacbrood virus, *P. alvei* was found in some of the larvae that died

of sacbrood (Bailey et al., 1973). *P. alvei* has been reported in larvae killed by foulbrood in Argentina, and when it is isolated from colonies it is difficult to be certain which foulbrood disease (American or European) was responsible for killing the larvae prior to the secondary invasion by *P. alvei* (Alippi, 1991; 1997).

In Australia, *P. alvei* has bee reported from 83% of colonies that had clinical signs of American foulbrood, but the organism was also found in 35% of non-diseased colonies that had a history of American foulbrood in the previous 6 months, and in 43% of hives that had no history of disease in the previous 6 months (Hornitzky and Karlovskis, 1989). Since European foulbrood is endemic in both Argentina and Australia, the precise role of *P. alvei* in these bee colonies cannot be determined, as the possibility that colonies are commonly simultaneously infected with European foulbrood and American foulbrood cannot be excluded. In addition, since *P. alvei* is resistant to oxytetracycline, it is likely to be more frequently isolated from hives in countries where antibiotics are fed to bees for the control of foulbrood.

The world distribution of *P. alvei* is not well documented (Bailey and Ball, 1991). However, since the presence of *P. alvei* spores in *A. mellifera* larvae is used in many countries as an indicator of European foulbrood, it is reasonable to consider its distribution in honey bees to be similar to that of European foulbrood (Alippi, 1997). *P. alvei* has not been detected in Western Australia, where *M. pluton* is also absent (Hawkins, 2001). However, its apparently widespread distribution in many different environments suggests that this saprophyte is likely to be ubiquitous, but its detection in bees requires the presence of European foulbrood.

P. alvei has been found in 56% of honey samples in Argentina (Alippi, 1995; Alippi, 1997) and in 16% of 505 honey samples in Australia (Hornitzky and Clark, 1991).

Laboratory records at the Institute of Arable Crops Research at Rothamsted in the UK indicate that *P. alvei* was once isolated from a sample of dead honey bee brood submitted for diagnosis from New Zealand in 1980 (Ball, personal communication[1]), but no further isolations of *P. alvei* have been reported from this country. In New Zealand, surveillance for European foulbrood in honey bee larvae is based on microscopy, culture and PCR, and although many suspect samples have been processed by official diagnostic laboratories, none have been found to contain *P. alvei*. Diagnosis of American foulbrood is made based on microscopy and bacterial culture, and laboratories in New Zealand have not experienced problems with *P. alvei* overgrowing plates used to culture *P. l. larvae* such as is reported in Australia (Hornitzky and Nicholls, 1993). Although no dedicated surveys for *P. alvei* have been carried out in New Zealand, the lack of any recent detection of the organism during routine 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. pluton* the presence of *P. alvei* would remain undetected unless active surveillance and laboratory testing is undertaken. No information is available on the presence of the organism in other (non-honey bee) niches in the New Zealand environment.

[1] Brenda Ball, Rothamsted Research UK, letter to H Beban, 15 May 2002.

22.1.5 Conclusion

Under field conditions, *P. alvei* is a saprophyte, not a primary pathogen of *Apis mellifera. P. alvei* has been isolated in this country on one occasion. There is insufficient evidence to suggest that this organism would interfere with the diagnosis of foulbrood in the absence of *Melissococcus pluton*, the causative organism of European foulbrood. 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*.

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*, (Heyndrickx et al., 1996) formerly named *Bacillus pulvifasciens* (Katznelson, 1950).

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. *P. l. pulvifaciens* is commonly found on honey bees but it is thought to become pathogenic only under conditions of high stress (Shimanuki, 1997), or that it may be a saprophyte that is a fortuitous and ill-adapted pathogen of bees (Bailey and Ball, 1991).

There is little information available on the distribution of either the disease or the organism. 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 and Spain (Hansen, 1984; Alippi, 1999). Neither powdery scale disease nor *P. l. pulvifaciens* has been reported in New Zealand.

23.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.3, *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

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

Assuming that transmission of *P. l. pulvifaciens* is similar to *P. l. larvae* (the cause of American foulbrood), it may be concluded that the commodities could potentially be contaminated with spores of the organism. Further, assuming that the likelihood of introduction in the commodities is similar to *P. l. larvae*, it can be concluded that the

likelihood of release is considered to be negligible in the case of bee venom regardless of the infection status of the colony. However, for honey, royal jelly, wax, pollen and propolis produced from infected colonies, the likelihood of release of *P. l. larvae* is non-negligible.

23.2.2 Exposure Assessment

Assuming that the epidemiology of *P. l. pulvifaciens* is similar to *P. l. larvae*, for *P. l. pulvifaciens* spores in imported honey, wax, pollen and propolis to come into contact with susceptible species in New Zealand, contaminated imported commodities would have to be harvested by worker bees in this country and taken back to hives and fed to young larvae, or be fed directly to the colony by beekeepers.

The attractiveness of these commodities to bees is discussed as part of the commodity definition in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. However, for beeswax that is made into foundation or applied to plastic frames, and for any bee product that is subsequently mixed with honey, the likelihood of exposure is non-negligible.

23.2.3 Consequence Assessment

P. l. pulvifaciens is probably a saprophyte that is poorly adapted to honey bees, and that only manifests clinically in colonies of *A. mellifera* that are highly stressed (Bailey and Ball, 1991; Shimanuki, 1997).

There are no reports of powdery scale disease in insects other than honey bees, which are social insects, forming colonies comprising many thousands of individuals in close contact with one another. By contrast, native bees are solitary insects that do not have any contact between generations (Donovan, 1980; Donovan et al, 1984; Matheson, 1997). For these reasons, the likelihood of *P. l. pulvifaciens* having any effect on native bees is considered to be negligible.

Since powdery scale disease is rare and of little economic importance, it is unlikely that *P. l. pulvifaciens* would have significant effects if introduced or established in New Zealand. However, because of its limited international distribution, the finding of this saprophyte might result in a short-term disruption to exports of live bees or bee products from New Zealand, but it would be unlikely to result in long-term restrictions since there are no official control programmes for saprophytes such as this anywhere in the world.

Therefore the consequences of introduction are likely to be negligible.

23.2.4 Risk Estimation

For honey, royal jelly, wax, pollen and propolis produced from infected colonies, the likelihood of release of *P. l. pulvifaciens* is assumed to be non-negligible. The likelihood of exposure of *P. l pulvifaciens* spores to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and venom. However, the consequences of introduction are negligible, and therefore the risk 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*.

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 ubiquitous environmental bacterium *Pseudomonas aeruginosa*. This organism, which is endemic in New Zealand (Young, 2000), is found commonly in stagnant water, where it can grow over a wide range of temperatures, and it is present in soil and in intestinal contents of man and animals (Anonymous, 1979). It is considered to be an opportunist pathogen of plants animals, and in New Zealand it causes a range of diseases of many animals besides honey bees, including cattle (Vermunt and Parkinson, 2000), sheep (Julian, 2003), deer (Smits, 2003), horses (Thornton, 2002), dogs and cats (Orr, 1995).

Septicaemia occurs occasionally in adult honey bees when the colony is weakened due to other factors. Infection is thought to occur through the bee's spiracles (Shimanuki, 1997). Symptoms include a change in colour 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 occurs within 24 hours of infection (Alippi, 1999).

Streptomycin has been used in some countries to control septicaemia in honey bee colonies, but development of resistant strains of the bacteria has limited its usefulness (Shimanuki, 1997).

The distribution of septicaemia in honey bees is somewhat uncertain, but 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, Spiroplasma apis.*

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 members of the genus *Spiroplasma* in the class *Mollicutes*. This class includes several other genera of very small bacteria that lack cell walls – mycoplasmas, ureaplasmas, acholeplasmas, anaeroplasmas and thermoplasmas. Spiroplasmas have long been known to be present in, and cause serious disease of, various species of plants. Some spiroplasmas that cause plant diseases are vectored by specific insects (e.g. leafhoppers), in which the spiroplasma infection may actually be beneficial (Anonymous, 2003). The discovery in 1977 that a spiroplasma was the cause of a disease of honey bees in the USA (Clark, 1977) was followed by discovery of about 30 spiroplasmas isolated from a variety of sources – honey bees, bumblebees, solitary bees, other insects and surfaces of plants (Clark, 1978). Although 12 spiroplasmas have been isolated from bees (Clark et al., 1985), very few have been associated with bee mortality. The finding that a spiroplasma in the nectar of the tulip poplar (*Liriodendron tulipifera*) can kill honey bees has led to the suggestion that many or most of the spiroplasmas affecting insects may in fact be plant-derived (Clark, 1977; 1978).

S. melliferum has been found to severely infect workers and drones in the US (Clark et al., 1985). After it is ingested, the spiroplasma apparently multiplies in the haemolymph until the bee dies (Clark, 1977). Infected bees become sluggish and die within a week (Clark, 1982), resulting in up to 40% of foraging bees dying during the nectar flow (Clark, 1978).

S. apis causes a lethal infection called 'May disease' in France (Mouches et al., 1982; Mouches et al., 1984). The signs of May disease are similar to bee paralysis – infected bees cannot fly, and crawl about on the ground near the hive with shaky movements. However, unlike paralysis, bees with May disease have swollen and hard abdomens (Mouches et al., 1982, 1984). Notwithstanding sometimes significant losses of foraging bees, colonies recover spontaneously in mid-summer (Bailey and Ball, 1991).

The mechanism of spread of spiroplasmas between bees and between colonies is unknown, although it has been speculated that infection occurs by the oral route, through faeces of infected insects deposited on plant surfaces (Clark et al., 1985). However, the close association of disease with particular flowering plants suggests an interaction between insects and certain plants, which means that there may be relatively few sources of infection in nature; this is supported by well-defined seasonal peaks of incidence of spiroplasma infections in both France and the USA (Bailey and Ball, 1991). However, the finding of spiroplasmas in a shipment of bees from Hawaii, where trees of the type associated with the disease in North America do not exist, suggests that there may be other floral sources in nature (Clark, 1978). Spiroplasma-infected bees have also been reported in Peru (Shimanuki,

1997). However, all the primary literature on spiroplasmas in bees dates from the early 1980s, suggesting that it has not been deemed necessary to further research the interesting relationships between specific plants, spiroplasmas and these particular insects.

25.1.5 Conclusion

Since spiroplasmas have not been reported in New Zealand, under the criteria presented in Section 2.3, they must be classified as a potential hazard for the purposes of this analysis.

25.2 Risk Assessment

25.2.1 Release Assessment

Since the mechanism of spread of spiroplasmas is unknown, the likelihood of the commodities covered in this risk analysis harbouring these organisms is unclear. But if faecal contamination does indeed play a role, it may be assumed that the hive environment, including the commodities, could be contaminated at least at the time of their storage or production in the hive.

Moreover, since spiroplasmas have been shown to be present in plant nectar, it is reasonable to assume that these organisms may be present at least in honey and pollen of hives whose bees are foraging on those plant sources. On the other hand, the distinct seasonality of occurrence of clinical signs in both France and the US suggests that the source of the organism is limited to a few specific plants, and that persistence within the hive is insignificant.

However, if spiroplasmas could be assumed to be present in some commodities in certain seasons at least at the time of their formation, then the likelihood of spiroplasmas being present in imported commodities would depend on the ability of these organisms to survive in the environment. In the absence of other information, it is reasonable to assume that their survival would be similar to other Mollicutes, as they all lack cell walls. Thus, it can be expected that spiroplasmas, like mycoplasmas, are highly sensitive to dessication, and their survival in air is restricted to a few days (Mitscherlich and Marth, 1984), and even then only if protected from sunlight (Quin et al., 1994).

There is no evidence in the literature that honey bee products can be vehicles for transmission of spiroplasmas. However, the fact that the disease does not appear to have spread from France to neighboring countries over the past two decades suggests that they are not. Moreover, the available evidence suggests that bees are infected only by particular floral sources in early spring. Therefore, the likelihood of the imported commodities harbouring spiroplasmas is considered to be negligible.

25.2.2 Exposure Assessment

The attractiveness of these commodities to bees is discussed as part of the commodity definition in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly,

beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. However, for beeswax that is made into foundation or applied to plastic frames, and for any bee product that is subsequently mixed with honey, the likelihood of exposure is non-negligible.

25.2.3 Consequence Assessment

Spiroplasmas are reported to cause disease symptoms in bees each year in France, with resulting large numbers of dead or moribund adult bees appearing in the front of hives. Although, in extreme cases, up to 40% of foraging bees die during the nectar flow, affected colonies recover spontaneously.

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 (Bailey and Ball, 1991). Leading beekeeping texts all regard spiroplasmas as causing a disease of only minor importance, and this is reflected by the absence of any addition to the scientific literature on this subject in more than 20 years. Because of their limited international distribution, the finding of spiroplasmas in New Zealand might result in a short-term disruption to exports of live bees and bee products, but it would be unlikely to result in long-term restrictions since there are no official control programmes for spiroplasmas anywhere in the world.

There are no reports of *S. apis* or *S. melliferum* causing pathogenicity in insects other than *Apis mellifera*, although Clark (1982) reported that spiroplasmas from five species of Hemiptera were unable to infect honey bees.

Although the method of spread of spiroplasmas is not known, it is suspected to be via the faeces of infected insects. Considering that *A. mellifera* is a social insect, forming colonies comprising many thousands of individuals in close contact with one another, whereas native bees are solitary insects that do not have any contact between generations (Donovan, 1980; Donovan et al, 1984; Matheson, 1997), there is nothing to suggest that spiroplasmas could be expected to have any effect on native bees.

Nevertheless, in view of the possible short term disruption to exports of bees and bee products if these organisms were found in New Zealand, the consequences of introduction are considered to be non-negligible.

25.2.4 Risk Estimation

The likelihood of spiroplasmas being present in the imported commodities is considered to be negligible. If spiroplasmas were present in imported commodities, the likelihood of exposure

is considered to be non-negligible and the consequences might be significant, at least in the short term. However, since the likelihood of spiroplasmas being present in the commodities is considered to be negligible, the risk estimate is 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*.

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*. Honey bee larvae are most susceptible when they ingest spores of *A. apis* at about 3-4 days of age, and then are chilled briefly 2 days later, immediately after they are sealed in their cells to pupate (Gilliam et al., 1978). The spores germinate in the larval gut and mycelia invade the larvae, which eventually die and desiccate, taking on a white or greyish 'mummified' appearance. Infective spores are formed when the larvae dies (Bailey, 1963).

The fungus has been isolated from the midgut of adult bees, and also from the honey sac in summertime (Heath, 1982a), but ingested spores do not appear to germinate and grow in adults. Transmission between adult bees within the colony appears to be via food sharing while transmission between colonies has been demonstrated to result from transfers of bees, including requeening, and a range of natural and human-factored pathways have been suggested (Gilliam and Vandenberg, 1997). Spores remain viable for long periods in honey, and they can survive under dry conditions for 12 months (Heath, 1982b). Spores require an anaerobic environment for germination, but the fungal mycelium requires more aerobic conditions for growth (Heath and Gaze, 1987).

Although the infective dose of *A. apis* is not known, the ability of chalkbrood infection to spread is believed to be low (Bailey, 1963), and experimentally it is difficult to induce chalkbrood infection by inoculation of honey bee colonies with spores (Puerta et al., 1999). Nevertheless, feeding pollen that is naturally or artificially contaminated with spores has been shown to transmit chalkbrood infection (Mehr et al., 1976), and it is reasonable to conclude that feeding contaminated honey to bees would also be able to initiate infection in colonies.

The effects of chalkbrood have been reported to range from 'transient and not serious' to "persistent and damaging" (Bailey, 1963). The disease manifests mostly as the colony expands in early summer (Heath, 1982a). Losses of honey production of 5% (Heath, 1982a) to 37% (Yacobson et al., 1991) have been recorded. Although chalkbrood has been reported to sometimes kill colonies overseas (Anderson, 1938), this has not been reported 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. *A. 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 (Milne, 1983;

Spivak and Gilliam, 1991). Breeding programmes in several countries have shown that strains of bees with good hygienic behaviour (uncapping and removal of dead larvae) have less clinical symptoms of the disease (Gilliam and Vandenberg, 1997). 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), including the following :

- chilling of larvae immediately after being sealed in their cells, most likely in spring
- poor ventilation e.g. long grass around the hive
- partial occupation of the hive, such as commonly occurs during winter
- rainy summers when hives are in cool, wet locations
- feeding highly hydrated honey (>17% water)
- hives weakened by other diseases (tracheal mites, European foulbrood, American foulbrood, nosema, septicaemia, rickettsiosis, sacbrood)
- use of antibiotics, which may upset the equilibrium of intestinal flora, increasing the likelihood of infection of larvae
- feeding excessive amounts of pollen.

There are a few reports of different levels of virulence between strains of *A. apis* under laboratory conditions (Glinski and Chmielewski, 1989; Sawathum and Ritter, 1995). One such report claimed up to 18–fold differences in virulence (Glinski and Chmielewski, 1982). However, this appears to have little practical significance in the field, where the effects of managemental and environmental factors and bee genetics appear to dominate in terms of seriousness of disease (Gilliam and Vandenberg, 1997).

Chalkbrood in honey bees has been reported from Europe, North and South America, Asia, the Middle East, north Africa, the Indian Subcontinent, and Australasia (Gilliam and Vandenberg, 1997; Matheson, 1997a). The route of international spread is not known, but it is suspected that imported pollen or live bees is the most likely route, at least in the case of the USA (Gilliam and Vandenberg, 1997).

A. apis was first identified in New Zealand in 1957 (Palmer-Jones, 1964; Seal, 1957) but it was not until the mid 1980s that the disease was reported in commercial hives (Reid, 1993). By 1987 it was detected over most of New Zealand, especially in colonies of black bees, and there were reports of some hives with half the brood affected with chalkbrood. Since then the level of chalkbrood has stabilised, and it is difficult to determine the ongoing effects on hive productivity (Reid, 1993). The possibility that the outbreak was due to the introduction of a virulent strain of *A. apis* in imported pollen has been mooted, and although this cannot be discounted there is also no evidence to support it.

Chalkbrood was first reported in Israel in 1984, and up to 1990 it was present at a very low level. The next year, however, there was a substantial increase in infection rate without any apparent link between apiaries (Yacobson et al., 1991). Although a number of theoretical possibile explanations were considered at the time, it was not possible to determine the major reason for this increase. The most likely cause of the outbreak was considered to be either the increased use of fumagillin to control widespread *Nosema apis* infection, or the simultaneous infection with sacbrood virus. On morphological grounds, the possibility of the emergence of a more virulent strain of *A. apis* was considered unlikely (Yacobson et al., 1991).

26.1.5 Conclusion

Chalkbrood is present in New Zealand, but is not under official control. Although *A. apis* is not listed on the unwanted organisms register, more virulent strains have been reported under laboratory conditions abroad. Therefore *A. apis* must be classified as a potential hazard for the purposes of this analysis.

26.2 Risk Assessment

26.2.1 Release Assessment

26.2.1.1 Honey

Although *A. apis* spores in honey can be inactivated by heating for 8 hours at 65°C or for 2 hours at 70°C, spores have been frequently detected in retail packs of honey from New Zealand, Australia, France, Germany, Greece, the USA and Argentina (Anderson et al., 1997; Reynaldi et al., 2003). The fungus can survive for long periods in honey, as demonstrated by its isolation from honey after 2 years of storage at both 20°C and 30°C.

26.2.1.2 Royal jelly

There are no reports of *A. apis* spores being detected in royal jelly. However, since the fungus is widespread and persistent in infected colonies, it is reasonable to assume that the spores can be present in royal jelly.

26.2.1.3 Pollen

A. apis has been isolated from bee-stored pollen (Heath, 1982a). Chalkbrood mummies have been found in imported pollen in the USA, and spores remain viable in pollen for at least 12 months (Gilliam and Vandenberg, 1997).

26.2.1.4 Propolis

Although there are no reports of *A. apis* being isolated from propolis, since spores are widespread on surfaces within the hive (Puerta et al., 1999) it is reasonable to assume that propolis may be contaminated with spores if the colony is infected.

26.2.1.5 Beeswax

Although there are no reports of *A. apis* being isolated from propolis, the fungus has been isolated from the surface of combs (Puerta et al., 1999), so it is reasonable to assume that beeswax may be contaminated with spores if the hive is infected.

26.2.1.6 Bee venom

There is no evidence that bee venom can harbour *A. apis* and, considering the collection methods for bee venom, there is no good reason to assume that it occurs.

21.2.1.7 Release assessment conclusion

The likelihood of release of *A. apis* is considered to be non-negligible for honey, propolis, pollen, royal jelly and beeswax, but it is considered to be negligible for bee venom.

26.2.2 Exposure Assessment

For *A. apis* spores bacteria in imported honey, wax, pollen and propolis to come into contact with susceptible species in New Zealand, these commodities would have to be harvested by worker bees and taken back to colonies and fed to young larvae, or be fed directly to the colony by beekeepers.

The attractiveness of these commodities to bees is discussed as part of the commodity definition in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. However, for beeswax that is made into foundation or applied to plastic frames, and for any bee product that is subsequently mixed with honey, the likelihood of exposure is non-negligible.

26.2.3 Consequence Assessment

The major consequence of chalkbrood is reduced honey production. For example, on one apiary in Israel, colonies with clinical chalkbrood signs produced 37% less honey than hives with no clinical signs (Yacobson et al., 1991). However, the severity of consequences varies considerably as a result of a many factors, including the genetics of bee stocks (Milne, 1983) and a range of environmental conditions (Bamford, 1989).

Any adverse effects from increases in severity of chalkbrood are likely to be transitory, since susceptibility to chalkbrood infection is strongly influenced by honey bee genetics (Gilliam and Vandenburg, 1997), and beekeepers usually control chalkbrood simply by requeening of colonies with more resistant stock (Heath, 1982a).

Since the virulence of *A. apis* in New Zealand has not been compared to the virulence of the organism in other countries, it is not possible to precisely determine the consequences of introducing *A. apis* from abroad. However, there is no evidence that ‘strains’ of *A. apis* more virulent than those already in New Zealand are present overseas. The introduction *A. apis* from abroad would be unlikely to be detected, and it would not result in any 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 *A. apis* has been isolated from species of solitary bees overseas (Gilliam and Vandenberg, 1997), there are no reports of chalkbrood disease in insects other than *A. mellifera*. Moreover, chalkbrood is a disease of stressed *A. mellifera* colonies, usually in early summer as the colony expands, and despite being endemic in this country for at least 50 years, there is no evidence of any effects on native insects. Whereas *A. mellifera* is a highly social insect, forming colonies comprising many thousands of individuals in close contact with one another, native bees are solitary insects that do not have any contact between generations (Donovan, 1980; Donovan et al, 1984; Matheson, 1997b). For these reasons, the likelihood of chalkbrood having any effect on native bees is considered to be negligible.

Therefore the consequences of introduction are considered to be negligible.

26.2.4 Risk Estimation

With the exception of bee venom, the likelihood of release in the commodities is considered to be non-negligible. The likelihood of exposure for honey, royal jelly, pollen and beeswax is considered to be non-negligible. However, the likelihood of significant consequences is considered to be negligible, and 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* 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 Vandenburg, 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 conidiospores. 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 bee colonies 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: Class *Insecta*, Order *Diptera*, Infraorder *Muscomorpha*, Superfamily *Carnoidea*, Family *Braulidae*, *Braula coeca*.

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 not a true louse, but a highly modified 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 cells just before the cells are capped. Upon hatching, larvae construct tunnels of wax that act as a shelter for the pupal stage. Adults die within six hours of hatching if they do not attach themselves to an adult bee (Herrod-Hempsall, 1931). The life cycle takes about 3 weeks, the same as honey bees themselves (Caron, 1981).

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). Large numbers of *B. coeca* adults on queen bees have also been suggested as a cause of supersedure (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 it has incorrectly been reported as being present in New Zealand as a result of misinterpretation by Smith and Caron (1985)of the world-wide distribution maps of Nixon (1982), in fact the bee louse has not been recorded in New Zealand (Matheson, 1997a).

28.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.3, 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

Since the adult bee louse is only a pest of adult bees and it survives for a very short period away from adult bees, the likelihood of adults being present on the commodities at any time is very low. There are no reports of any life stages of bee louse being present in extracted honey, pollen, propolis, venom, royal jelly or in processed beeswax. Considering the processes that

these products that are subjected to during their manufacture and the life cycle of the bee louse, the likelihood of these commodities carrying the bee louse is considered negligible.

However, the bee louse lays eggs in honeycomb cells just before the cells are capped, and after hatching the larvae construct wax tunnels in the comb for their pupal stage. In view of this, the likelihood of eggs or larvae being in comb honey is considered to be non-negligible.

28.2.2 Exposure Assessment

Once adults emerged from eggs or larvae in imported honeycomb, they would have to attach to an adult bee within six hours to survive. This would be likely if bees were able to forage on imported comb honey, since this commodity is attractive to bees, as discussed as part of the commodity definition in section 2.2 of this document. If a bee louse were to attach to a foraging bee, it would subsequently be introduced to the colony where the likelihood of establishment would be high. Therefore, the likelihood of exposure and establishment for bee louse in imported comb honey is 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). Although it has been suggested that severe infections could 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 appear to be any published data to support these suggestions.

Although the bee louse is found in most countries, the introduction of the organism in New Zealand would be likely to result in restrictions on the export of bees or bee products from New Zealand to some countries that do not reporting its presence.

The bee louse has only been reported on honey bees. Whereas *A. mellifera* is a highly social insect, forming colonies comprising many thousands of individuals in close contact with one another, native bees are solitary insects that do not have any contact between generations (Donovan, 1980; Donovan et al, 1984; Matheson, 1997b). For these reasons, the likelihood of the bee louse having any effect on native bees is considered to be negligible.

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

28.2.4 Risk Estimation

The likelihood of release of bee louse in all commodities apart from comb honey is considered to be negligible. The likelihood of exposure and the likelihood of negative consequences arising from exposure are considered to be non-negligible, so bee louse is classified as a hazard for comb honey, but it is not classified as a hazard for the other commodities.

28.3 Risk Management

28.3.1 Risk Evaluation

Since the risk is considered to be non-negligible for comb honey, risk management measures are justified for this commodity to reduce the risks to an acceptable level.

28.3.2 Option Evaluation

28.3.2.1 Risk management objective

The objective is to effectively manage the risk of bee louse by ensuring that imported comb honey does not harbour *B. coeca* when given a biosecurity clearance in New Zealand.

28.3.2.2 Options available

Since the life cycle of *B. coeca* takes about 3 weeks, and since adults die within 6 hours of hatching if they do not attach themselves to adult bees, storage of bee products away from bees for 4 weeks or longer would ensure that no life stages of the bee louse are surviving on or in the product.

Chemical treatment of bee colonies for *B. coeca* has been advocated, for example by the use of Perizin®, but the efficacy of this treatment has not been accurately determined (Kessler, 1987).

Most insects have a high lethal temperature from 38 to 44 °C, and the most heat-resistant species die at temperatures of 47 to 52°C (Ross et al., 1982). Time/temperature treatments for various stages of a range of tropical fruitflies in fruit have been developed, the most rigorous of which is 47.2°C for 10 minutes (Sales et al., 1997; Foliaki and Armstrong, 1997; Vueti et al., 1997). Lethal low temperatures for insects vary as much as lethal high temperatures (Ross et al., 1982), but freezing is lethal for most insects. Considering the close taxonomical relationship between the bee louse and fruitflies (bee louse is in the superfamily Carnoidea, and fruit flies are in the superfamily Tephritoidea, both of which are grouped under the rank Acalyptratae), it can reasonably be assumed that heating to 47°C or higher for 20 minutes or freezing for up to 48 hours would kill the bee louse.

Fumigation with methyl bromide will kill most insect pests and international standards have been developed for a range of plants and plant products (Bond, 1989). Although no fumigation schedule has been developed specifically for bee louse, it is reasonable to assume that the schedule for fresh flowers and folliage (which covers mites and other insects), would be effective against bee louse if the gas were able to come into contact with the eggs, larvae, pupae or adult forms of this insect. However, since it is not known whether methyl bromide is able to penetrate the beeswax of honey combs to make such contact with the non-adult forms, even in a vacuum, fumigation cannot be recommended as a treatment option for this insect.

Ionizing radiation is known to be an effective phytosanitary treatment for a range of insects, and international standards have been developed for minimum doses required for certain outcomes, among which are insect mortality, preventing successful development, inability to

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reproduce or inactivation (IPPC, 2003). The bee louse is classified in the same superfamily (Carnoidea) as the frit flies, one species of which , *Hippleates pusio* (the eye gnat), is reported to be sterilised by 50 Gy of radiation (IAEA, 2004). Irradiation is a standard treatment for fruitflies, which are classified in the same suborder (Brachycerca) as the bee louse. The dose of radiation required to prevent adult fruitfly emergence from the 3rd instar is 50-250 Gy (IPPC, 2003).

28.3.2.3 Recommended sanitary measures

For extracted honey, propolis, pollen, beeswax, royal jelly and bee venom

No sanitary measures required.

For comb honey

Each consignment must

either

(i) be from a country or part of the territory of a country free from *B. coeca*

or,

(ii) after packing, be treated by one of the following measures

(a) holding for 4 weeks away from bees

or

(b) heating to 50°C and holding at that temperature for at least 20 minutes

or

(c) irradiation with 250 Gy

or

(d) freezing for 24 hours

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29. EXTERNAL ACARINE MITES

29.1 Hazard Identification

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

29.1.2 OIE List: none.

29.1.3 New Zealand's Status: Acarapis externus, *A. dorsalis* and *A. vagans* mites are present in New Zealand and are not under official control.

29.1.4 Epidemiology

Acarapis externus and *A. dorsalis* are external mites parasitic on adult *Apis mellifera*. Both of these mites are restricted to honey bees as hosts and feed externally on adult bees, by means of modified mouthparts that they use to pierce the cuticle of bees and suck blood (Delfinado-Baker and Baker, 1982). Although a third species of external mite, *A. vagans*, has been proposed, it is not clearly separated from *A. externus* or *A. dorsalis* (Bailey and Ball, 1991) and is regarded by some as '*nomen dubium*' (Delfinado-Baker and Baker, 1982).

A. externus is found generally on the neck of the bee and on pits on the back of the head, and *A. dorsalis* occupies a groove across the top of the thorax. A. vagans is found primarily on the base of the hind wings of bees (Bailey and Ball, 1991). None of these mites appear to affect bees adversely (Eickwort, 1997; De Guzman et al., 2001).

External acarine mites have a worldwide distribution (Bailey and Ball, 1991) and *A. externus*, *A. dorsalis* and *A. vagans* are endemic in New Zealand (Clinch and Ross, 1970).

29.1.5 Conclusion

External acarine mites 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 *A. externus*, *A. dorsalis* and *A. vagans* 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: Class *Insecta*, Order *Coleoptera*, Family *Nitidulidae*, *Aethina tumida*.

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

Small hive beetle (*Aethina tumida*) is a pest of honey bee combs. Most adult beetles live for about two months, but some may live up to six months (Lafreniere, 2000), and they can survive for five days without food or water (Pettis and Shimanuki, 1999). Newly-emerged adults invade hives after mating, flying further than 10 miles (Brown and Morton, 2003). Inside the hive, female beetles lay eggs that hatch within six days. The beetle larvae predominantly feed on stored honey, wax and pollen (Lundie, 1940), but they are also reported to eat bees, eggs and brood. The larval stage lasts between 10 and 20 days (Taber, 1999a), and when feeding is complete larvae leave the hive and burrow into the soil in front of the hive to pupate (Fore, 1999). Pupation usually takes between two weeks and two months (Taber, 1999a).

The reproductive potential of the small hive beetle is enormous, with each female being able to produce up to a thousand eggs over a lifespan of 4-6 months. In South Africa as many as five generations per year are possible (Brown and Morton, 2003).

Small hive beetle was first described in the mid 19th Century in South Africa, where it is considered a minor pest of honey bees (Lundie, 1940), and until recently it was thought to be restricted to that continent. However, in 1998 it was detected in Florida and it is now widespread in the USA (Fore, 1999; Hood, 2000; Mostafa and Williams, 2002). It was first detected in Egypt in 2000, and since then it has been found in a number of apiaries along the Nile Delta (Neumann and Elzen, 2004). Small hive beetle has also been found once in Manitoba, Canada (Brown and Morton, 2003). In October 2002[1], it was discovered in Australia, initially in the Sydney region (Fletcher and Cook, 2002), soon after in Queensland (CSIRO, 2002), and more recently it was reported from Victoria (Anderson, pers comm.)[2]. In October 2004 small hive beetle was reported to have been introduced into Portugal, apparently as a result of the illegal importation of queen bees from Texas (NBU, 2004).

It is not known how small hive beetle reached the USA or Australia, but swarms on shipping containers are considered to be one of the most likely routes (Brown and Morton, 2003). Other likely routes are imported live bees (package bees and colonies), used beekeeping equipment, crude beeswax, and there has been speculation that the beetle might have been carried in certain imported fruits or soil with containers, imported plants or agricultural

[1] Small hive beetle had not been reported in Australia at the time of writing the first version of this risk analysis, which was released for public consultation in July 2002.
[2] Denis Anderson, CSIRO Entomology Unit, email to H Pharo dated 19 July 2004.

machinery (Lafreniere, 2000). However, most spread in the USA has apparently been the result of the seasonal movement of colonies from infested areas (Brown and Morton, 2003). Speculation about the ability of fruits, particularly rockmelons, to act as an alternative food supply, and therefore to be potential vehicles for spread (Eischen et al., 2000), is based on laboratory trials on the attractiveness of the beetle to fermenting fruits, in which it was determined that rockmelons were the most attractive (Eischen et al., 1999).

Small hive beetle was found in Canada in August 2002 in beeswax cappings that was shipped in barrels from Texas, USA, for rendering (Dixon and Lafreniere, 2002). However, it appears that it has not established in Canada. Close inspection of a large number of colonies on and around the location of the original find in 2003 and again in 2004 have failed to find any evidence of small hive beetle infestation, which suggests that it was unable to survive the Canadian winter (Brown and Morton, 2003; Lafreniere, pers. comm.[1]).

In Africa the damage caused by the small hive beetle is relatively mild, essentially because of the behaviour of African bees. Observations of the pest-host relationship of the Cape honey bee, *A. mellifera capensis* in South Africa indicated that it responds to the beetle in an aggressive manner, grasping the beetles and attempting to sting them. The bees chased the adult beetles, corralling them into areas were they could not lay eggs (Elzen et al., 1999b). The bees also consume any exposed beetle eggs and are capable of removing the larvae from the hive (Taber, 1999a, 1999b). The African honey bee, *A. mellifera scutellata* also exhibits different behaviour from that of the European bee *A. mellifera.* They readily abscond in the presence of predation or perturbation of the hive, they block hive entrances with propolis to protect the hive from beetle entry, they keep the bottom boards clean, thus providing the beetles with few places to hide and lay eggs (Taber, 1999a). However, even in South Africa there are pronounced regional variations.

The impact of small hive beetle in the United States has been much greater than in South Africa (Eischen et al., 1999), but the counties that have been most affected are those where the climate is similar to the subtropical and warm temperature zone of South Africa (Lafreniere, 2000). In severely affected areas, larvae tunnelling in sealed honey can render it unfit for human consumption by virtue of the direct damage through tunnelling and indirect damage resulting from contamination by larval faeces, fermentation, and abandonment of hives (Elzen et al., 1999a; Fell, 1999).

The most vulnerable part of the beekeeping operation is equipment in storage and products containing wax, honey and/or pollen. In beetle infested areas, full honey supers stored in the honey house or on hives above bee escapes are most vulnerable to attack because there are no or fewer bees to protect the stored honey. Environmental conditions generally associated with honey houses such as high temperature and humidity provide optimal conditions for beetle development. This fact alone underscores the need for the protection of supers in the honey house both before and after extraction. Severe beetle infestation in the honey house can also quickly result in beetle infestations in the bee yards through the transportation of contaminated equipment (Lafreniere, 2000).

In Australia, despite initial fears, its impact appears to have been limited so far (Anderson, pers comm.)[2].

[1] Rheal Lafreniere, Provincial Apiarist, Manitoba, Canada. Email to H Pharo dated 21 July 2004.
[2] Denis Anderson, CSIRO Entomology Unit, email to H Pharo dated 19 July 2004.

The rate of development of the small hive beetle is directly related to temperature, with the hottest months of the year being associated with the most dramatic increases in beetle populations. At 30°C, about 98% of larvae will successfully pupate in about 13 days, while at 20°C, the pupation rate is only about 50% and the time required is about 25 days. At 10°C, beetles require about 100 days for pupation, but the survival rate is very low (Lafreniere, 2000). Soil type is also very important for pupation, with loose sandy or sandy loam soils being best (therefore in the US the beetle has caused most serious losses in Florida, Georgia and South Carolina); heavy clay soils least suitable. Larvae in honey houses are usually unable to complete the life cycle as they cannot find suitable places to pupate, particularly when the honey house has a concrete floor (Lafreniere, 2000).

Survival of all stages of small hive beetle is highly dependent on moisture, and in the absence of water all life stages die in 1-3 days. However, larvae have been observed to survive in extracted honey for more than a week provided they are able to swim to the surface. Larvae and adult beetles died in 2-3 days on dry pollen (Pettis, pers. comm.[1]).

Control of the small hive beetle usually requires the use of insecticides – typically 40% permethrin as a soil drench in front of the beehive, and 10% coumaphos strips (Check-Mite+) within the colony (Elzen et al., 1999b). Other control methods, such as positioning hives on large pieces of black plastic, or breeding for better housekeeping, are possibilities (Taber, 1999a). Non-chemical control strategies have been developed to decrease or eliminate beetle damage in honey houses. The strategy relies on maintaining the humidity below 50%, under which conditions small hive beetle eggs do not hatch (USDA, 2003).

30.1.5 Conclusion

Since it has not been reported in New Zealand, under the criteria presented in Section 2.3, 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

It is assumed that in countries where small hive beetle is present the commodities could potentially be infested with *A. tumida* adults or be contaminated with eggs or larvae, either as a result of colony infestation or contamination in the honey shed.

30.2.1.1 Honey

It is considered that the likelihood of *A. tumida* adults being present in the honey shed is non-negligible for any operator that has any apiaries infested with this beetle.

However, in view of the size of the beetle and the production processes involved in the extraction and preparation of honey for direct consumer sale, in particular the application of heat (see section 2.2.1 for further details), it is considered that the likelihood of any stages of *A. tumida* surviving in extracted honey packaged for direct consumer sale is negligible.

[1] Jeff Pettis, Research Entomologist, USDA-ARS Bletsville, USA. Email to H Pharo dated 28 July 2004.

The likelihood of adult *A. tumida* beetles or eggs surviving in a drum of honey is considered to be very low, but larvae might survive for some weeks in such an environment provided they were able to swim to the surface (Pettis, pers. comm.). It is considered that honey filtered by standard processes is highly unlikely to contain any life forms of *A. tumida.* Further, in view of the several heat steps involved in the processing of extracted honey (see section 2.2.1 for further details) it is considered that contamination of honey in bulk drums would be possible only after processing and only in a honey shed that was infested with small hive beetle. In such a facility, beetles may fly into filled drums or they might remain inside recycled drums under the lid and above the honey layer. Thus, live small hive beetles may be present in drums of honey that were not heated prior to opening after importation. Although the likelihood of this is low, it is considered to be non-negligible.

Since it is known that adult beetles can survive for months in comb honey, and since the larval stage of 10-20 days takes place in the honey comb, it is considered that the likelihood of any of the stages of *A. tumida* being present in or on honey comb from an infested colony is non-negligible.

30.2.1.2 Propolis

There are no reports of propolis being contaminated with *A. tumida,* and in view of the processes involved in the production of internationally traded forms of this commodity, and in particular the absence of water in the final products, the likelihood of release of *A. tumida* in propolis is considered to be negligible.

30.2.1.3 Pollen

Since small hive beetle survives for a very short period on dry pollen, the likelihood of small hive beetle being present in internationally traded forms of pollen is considered to be negligible.

30.2.1.4 Royal jelly

While the likelihood of small hive beetle being present in fresh royal jelly can reasonably be assumed to be non-negligible, since it survives for a very short period in the absence of moisture, the likelihood of small hive beetle being present in powdered royal jelly is considered to be negligible. The likelihood of any life stages surviving in frozen royal jelly is also negligible.

30.2.1.5 Beeswax

Since insects are killed rapidly at temperatures above 45°C, any of the processes involved in the recovery of solid wax would ensure the destruction of all life stages of small hive beetle, and the attractiveness of such beeswax to small hive beetle can be considered to be negligible. Therefore the likelihood of the forms of beeswax traded internationally harbouring small hive beetle is considered to be negligible.

30.2.1.6 Bee venom

Considering the production methods involved, the likelihood of small hive beetle being present in bee venom is considered to be negligible.

30.2.1.7 Release assessment conclusion

The likelihood of introduction of small hive beetle is considered to be non-negligible for extracted bulk honey in drums and for comb honey.

The likelihood of introduction of small hive beetle in extracted honey in consumer-ready packs and in the internationally traded forms of propolis, pollen, royal jelly, beeswax and bee venom is considered to be negligible.

30.2.2 Exposure Assessment

Since small hive beetle is able to fly considerable distances and it is attracted to bee colonies, bee products and equipment that is commonly found in honey sheds, if adult beetles were introduced into New Zealand, it is considered very likely that they would establish.

The likelihood of effective exposure for eggs or larvae is less certain. Eggs will not hatch if the humidity is less than 50%, so the likelihood of eggs in any of the imported commodities hatching is low. Moreover, larvae require soil in which to pupate after living about 20 days in the honey comb, and it is considered that the likelihood of successful pupation would be low, but non-negligible.

Therefore it is concluded that the likelihood of exposure is considered to be non-negligible for adult beetles, eggs and larvae.

30.2.3 Consequence Assessment

The spread of *A. tumida* in temperate climates of North America after its initial introduction to Florida in the spring of 1998 suggests that it could become established in New Zealand (Lafreniere, 2000). However, the extent to which the New Zealand climate would suit the beetle is unclear. Most affected counties in the USA are those with climate similar to the subtopical and warm temperate zone of South Africa, while in colder northern areas of North America the beetle is considered unable to survive outside the hive, and therefore reproduce, over winter (Lafreniere, 2000; Brown and Morton, 2003).

New Zealand honey bees are likely to show a similar vulnerability to *A. tumida* as bees in parts of the eastern United States and Australia, since New Zealand bees are more closely related to strains present in these countries than to African honey bees. Notwithstanding the considerably colder climate in New Zealand, it is considered that significant colony losses in New Zealand are possible, and beekeepers might need to use pesticides to control the beetles. It is likely that there would be considerable regional variation in the impact of small hive beetle in New Zealand, depending on temperature and soil type. Because of the limited distribution of small hive beetle throughout the world, its presence in New Zealand would be likely to result in restrictions being imposed on exports of queens and package bees and bee products.

Small hive beetle has been shown to be able to complete its life cycle in colonies of a species of bumble bee, *Bombus impatiens*, under laboratory conditions (Ambrose et al., 2000). Although bumble bees are not native to New Zealand, they are significant feral pollinators and are used for specific agricultural pollination tasks (e.g. glasshouse tomatoes), so if bumble

bees could be infested with small hive beetle under natural conditions, the consequences might be significant.

However, it can reasonably be assumed that small hive beetle would be unlikely to have any effect on New Zealand native bees, which, in contrast to the highly social *A. mellifera*, are solitary insects that do not produce wax or store honey, and which do not have any contact between generations (Donovan, 1980; Donovan et al, 1984; Matheson, 1997). Similarly, it is considered highly unlikely that small hive beetle would have any effects on any other New Zealand native insects.

In view of the above, the likely consequences of establishment of the small hive beetle in New Zealand are considered to be high.

30.2.4 Risk Estimation

The likelihood of release is considered to be non-negligible for bulk extracted honey and for comb honey, but negligible for the other commodities. The likelihood of exposure and establishment of the organism in New Zealand is considered to be high. 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 for bulk extracted honey and comb honey, and *A. tumida* is classified as a hazard in these two commodities.

30.3 Risk Management

30.3.1 Risk Evaluation

Since the risk estimate for *Aethina tumida* is non-negligible for bulk extracted honey and comb honey, risk management measures are justified for these commodities to reduce the risks to an acceptable 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 imported bulk extracted honey and comb honey do not carry the organism when given a biosecurity clearance in New Zealand.

30.3.2.2 Options available

The OIE *Code* does not recommend measures for small hive beetle.

Since small hive beetle has a reasonably well understood international distribution, countries or zones may be certified as free, provided adequate monitoring or surveillance programs are in place.

A number of options are possible for countries or zones that are not free from small hive beetle. Pre-export measures might include certified processes that manage the risk according to quality systems for maintaining the environment of the packing house or for specific heat and filtration processes for production of commodities.

Most insects have a high lethal temperature from 38 to 44 °C, and the most heat-resistant species die at temperatures of 47 to 52°C (Ross et al., 1982). Nitidulid beetles are rapidly killed by temperatures higher than 50°C, the treatment of dried fruit by exposure to 55°C for 3 hours provided good control of storage beetles (Navarro et al., 2003).

Freezing honey in the comb has also been shown to kill small hive beetle eggs and larvae (Sandford, 1999).

Ionizing radiation is known to be an effective phytosanitary treatment for a range of insects, and international standards have been developed for minimum doses required for certain outcomes, among which are insect mortality, preventing successful development, inability to reproduce or inactivation (IPPC, 2003). An International Atomic Energy Agency database includes measures for 79 members of the order Coleoptera (IAEA, 2004). Doses up to 400 Gy are recommended to sterilise stored product beetle adults (IPPC, 2003).

Although methyl bromide fumigation, which is commonly used for stored product beetles in the order Coleoptera (Bond, 1989), might be used to ensure that no viable small hive beetles remained on the outside of the commodities, this treatment would not affect anything that was inside (e.g. inside containers of honey or within honey combs).

30.3.2.3 Recommended sanitary measures

<u>For pollen, royal jelly, beeswax, propolis and bee venom</u>

No sanitary measures required.

<u>For extracted honey packaged for direct retail sale</u>

No sanitary measures required.

<u>For bulk extracted honey</u>

Each consignment must

either

(i) be from a country or part of the territory of a country free from small hive beetle

or

(ii) after packing and ensuring that the outside of packaging is free of substances that will be attractive to small hive beetles, be treated by one of the following measures:

(a) heating to 50°C and holding at that temperature for 24 hours.

or

(b) irradiation with 400 Gy

or

(c) freezing for 24 hours

For comb honey

Each consignment must

either

(i) be from a country or part of the territory of a country free from small hive beetle

or

(ii) after packing and ensuring that the outside of packaging is free of substances that will be attractive to small hive beetles, be treated by one of the following measures:

(a) heating to 50°C and held at that temperature for 24 hours.

or

(b) irradiation with 400 Gy

or

(c) freezing for 24 hours

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31. TRACHEAL MITE

31.1 Hazard Identification

31.1.1 Aetiologic Agent: Class *Arachnida,* 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 the respiratory system of adult honey bees, particularly *Apis mellifera,* but also *Apis cerana* and *Apis dorsata*.. The mite infests the respiratory system of adult honey bees (Wilson et al., 1997). 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 et al., 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 2.3, *A. woodi* must be classified as a potential hazard for the purposes of this analysis.

31.2 Risk Assessment

31.2.1 Release Assessment

Since all stages of the mite live exclusively in the trachea of adult bees, apart from mated females that can live outsider the host for a few hours while moving between bees, the likelihood of *A. woodi* being present in any of the commodities is considered negligible.

31.2.2 Exposure Assessment

Considering the very short period that *A. woodi* can survive away from adult bees, the likelihood of exposure to susceptible species in New Zealand is considered to be negligible for all commodities.

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.

Since the tracheal mite is only known to parasitise honey bees, it is considered unlikely to have any effects on New Zealand native insects.

In view of the above, the consequences of introduction are considered to be high.

31.2.4 Risk Estimation

The likelihood of release and exposure in the commodities is considered to be negligible. Therefore, notwithstanding that the consequences of introduction would be high, the risk is considered to be negligible, and *A. woodi* is not classified as a hazard in the commodities.

31.3 Risk Management

31.3.1 Risk Evaluation

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

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32. *TROPILAELAPS* SPECIES

32.1 Hazard Identification

32.1.1 Aetiologic Agent: Class *Arachnida*, Family *Laelapidae, Tropilaelaps clareae, Tropilaelaps koenigerum*.

32.1.2 OIE List: None.

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

32.1.4 Epidemiology

Tropilaelaps clareae is a mite that was originally parasitic on *Apis dorsata* in Asia, but over the past 40 years it has also been know to parasitise *A. mellifera* in tropical Asia (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* spp. feed on the haemolymph of honey bee larvae. The mite is carried by 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. Mating takes place inside the cell. Adult females leave the cell when the bee emerges and stay on adult bees for a few days before entering another cell to begin reproduction (Kitprasert, 1984). Adult 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 kill the colony quite rapidly (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* spp. 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* spp. are not able to survive for more than three days without brood.

T. clareae has been found in southeast Asia, Afghanistan, China and Kenya and New Guinea (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 2.3, these mites must be classified as potential hazards for the purposes of this analysis.

32.2 Risk Assessment

32.2.1 Release Assessment

Since *Tropilaelaps* spp. cannot survive away from brood for more than two or three days, the likelihood of any of the commodities carrying these mites is considered to be negligible.

32.2.2 Exposure Assessment

In view of the very short survival period for these mites away from brood, the likelihood of exposure to susceptible species in New Zealand is considered to be negligible for all commodities.

32.2.3 Consequence Assessment

The establishment of *Tropilaelaps clareae* would likely cause severe consequences for the New Zealand beekeeping and horticultural industries, as it is considered to be a serious pest in southeast Asia (Woyke, 1989). The presence of *T. clareae* would probably have a major negative effect on the export of queens and package bees from New Zealand.

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*.

However, *Tropilaelaps* spp. are unlikely to have any effects on New Zealand native insects since these mites are restricted to honey bees.

In view of the above, the consequences of introduction are considered to be high.

32.2.4 Risk Estimation

The likelihood of release and exposure in the commodities is considered to be negligible. Therefore, notwithstanding that the consequences of introduction and establishment would be high, the risk is considered to be negligible, and *Tropilaelaps* spp. are not classified as a hazard in the commodities.

32.3 Risk Management

32.3.1 Risk Evaluation

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

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33. *VARROA DESTRUCTOR*

33.1 Hazard Identification

33.1.1 Aetiologic Agent: Class *Arachnida*, Family *Varroidae*, *Varroa* destructor.

33.1.2 OIE List: B.

33.1.3 New Zealand's Status: Present in New Zealand. Under official control control (a National Pest Management Strategy under the Biosecurity Act 1993). Listed on the unwanted organisms register as a notifiable organism.

33.1.4 Epidemiology

Varroa destructor (Anderson and Trueman, 2000), known until recently as *V. jacobsoni* (Oudemans, 1904), is a mite that was originally parasitic on *Apis cerana.* However, it is believed to have successfully first parasitised *A. mellifera* last century in Japan and eastern Siberia (Ritter, 1981), and it is now recognised worldwide as the most significant parasite of *A. mellifera,* in which is causes varoosis, a disease of honey bee brood and adults.

Adult female *V. destructor* 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). But the mites 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).

The mites survive for a limited period away from live bees. At a relative humidity of about 74%, the mean survival time at 13°C was 18 hours on pollen and 35 hours on comb, while at 26°C it was about 40 hours for both substrates. The longest survival time on comb was 102 hours, and 132 hours on pollen (De Guzman et al., 1993).

V. destructor has caused the death of hundreds of thousands of honey bee colonies in all areas where it has become established in *A. mellifera* (Chun, 1965; Ritter, 1981; De Jong, 1997; Tew, 1999). The mite 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 *V. destructor* 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 the mite in some overseas countries have developed resistance to various control products (Milani, 1995). *V. destructor* is found in all significant beekeeping countries with the exception of Australia (Matheson, 1997). In New Zealand, the South Island remains free of this mite.

33.1.5 Conclusion

Although *V. destructor* is present in New Zealand, it is under official control and the South Island remains free. Moreover, there is evidence that 'strain variation' exists between populations of mites in other countries regarding their resistance to chemical control products. Since acaricides are vital to beekeepers in New Zealand, the introduction of resistant strains of *V. destructor* would have a significant negative impact on control efforts in this country. Therefore under the criteria presented in Section 2.3, it must be classified as a potential hazard for the purposes of this analysis.

33.2 Risk Assessment

33.2.1 Release Assessment

Since *V destructor* survives less than 6 days away from honey bees, and since the entire life cycle takes place in brood, it is extremely unlikely to be present on any of the commodities at the time of their importation.

33.2.1.1 Honey

In view of the production processes involved, it is considered that the likelihood of any life stage of *V. destructor* being present in extracted honey, either in bulk or packed for retail sale, is negligible.

In view of what is known about the survival of mites away from bees, it is considered that the likelihood of *V. destructor* being present on comb honey sourced from varroa infested hives less than 6 days before importation is non-negligible.

33.2.1.2 Pollen

In view of what is known about the survival of mites away from bees, it is considered that the likelihood of *V. destructor* being present in pollen that is harvested from varroa-infested hives less than 6 days before importation is non-negligible.

33.2.1.3 Propolis, royal jelly, beeswax and venom

While they might be present on raw propolis, royal jelly and beeswax at the time of harvesting from the hive, considering the production processes involved, it is considered that the likelihood of *V. destructor* being present on these commodities at the time of importation is negligible. The likelihood of the mites being in venom is considered to be negligible at any stage.

33.2.2 Exposure Assessment

If mites were present on or in imported comb honey or pollen, to become established in a colony in New Zealand they would have to become attached to an adult foraging bee and be taken back to the colony where they would then be able to complete their life cycle by invading brood cells. Since both honey and pollen are considered to be attractive to bees (as discussed see the commodity definition in section 2.2 of this document), the likelihood of exposure is considered to be non-negligible for these two commodities.

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 for the mite 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 the mite could have a positive effect on the prevalence of American foulbrood through the death 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 *V. destructor*, 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).

Some populations of *V. destructor* overseas have developed resistance to a number of chemical control products, including fluvalinate, flumethrin and acrinathrin (Milani, 1995). Trials conducted when *V. destructor* was identified in New Zealand demonstrated that the population introduced was not resistant to fluvalinate or flumethrin (Goodwin and McBrydie, 2000; Taylor and Goodwin, 2001), and the introduction of resistant strains would have a negative effect on control of *V. destructor* in New Zealand, since fluvalinate and flumethrin are the most commonly used chemical control products in this country.

It is possible that varroa mites from other countries could also harbour exotic viruses that could be damaging to honey bee health in New Zealand.

Therefore the consequences of introduction are considered to be high.

However, varroa is unlikely to have any effects on New Zealand native insects since it is highly adapted to live and reproduce only on *Apis mellifera*.

33.2.4 Risk Estimation

The likelihood of release of any life stage of *V. destructor* in all imported commodities apart from comb honey and pollen is considered to be negligible. Since the likelihood of exposure for honey and pollen is non-negligible and the consequences are considered to be high, the risk of *V. destructor* is considered to be non-negligible and the mite is classified as a hazard in these two commodities. For the other commodities, since the likelihood of release is considered to be negligible, the risk is negligible, and *V. destructor* is not classified as a hazard.

33.3 Risk Management

33.3.1 Risk Evaluation

Since the risk estimate for extracted honey, propolis, royal jelly, beeswax and bee venom is negligible, no sanitary measures are required for these commodities. However, the risk

estimate for *V. destructor* is non-negligible for comb honey and pollen, so risk management measures are justified for this commodity to reduce the risk to an acceptable level.

33.3.2 Option Evaluation

33.3.2.1 Risk management objective

New Zealand is justified in imposing risk management measures against *V. destructor* as a result of there being an official control program for varroa in this country, and since there is evidence that miticide-resistant strains of varroa exist abroad, it is reasonable to consider that such strains would be more harmful in New Zealand, if introduced, than the strain already present in this country. In this situation the principle of non-discrimination covered in article 2.3 of the WTO Sanitary and Phytosanitary agreement does not limit the measures that can be imposed to those that exist under the rules of the official control program.

Therefore, the risk management objective is to effectively manage the risk of *V. destructor* by ensuring that imported comb honey and pollen do not carry the organism when given a biosecurity clearance in New Zealand.

33.3.2.2 Options available

The OIE *Code* does not recommend measures for *V. destructor* in honey bee products.

Freezing, fumigation or immersion of frames in warm water (55 °C for 1 hr) are generally-recognised methods of killing *V. destructor* on drone comb (SBA, 2004; Anonymous, 2004).

Most insects have a high lethal temperature from 38 to 44 °C, and the most heat-resistant species die at temperatures of 47 to 52°C (Ross et al., 1982).

Heating is used in a number of ways to kill *V. destructor*, as the mites begin to die at temperatures above 38°C. Hot air at 42-48°C has been shown to kill *V. destructor* on brood, although above 44°C the brood is also injured (de Jong, 1997). Heating to 44°C for 4 hours has been shown to kill all mites on capped brood (Goodwin and Van Eaton, 2001).

Lethal low temperatures for insects vary as much as lethal high temperatures (Ross et al., 1982). Freezing honey in the comb is known to kill small hive beetle eggs and larvae (Sandford, 1999), and it has long been recognised that freezing kills *V. destructor* quickly; indeed, the ‘freezing drone brood’ method (freezing brood combs for up to 48 hours) is one of the oldest methods of *V. destructor* control in bee hives (Reddell, 2004).

Fumigation with methyl bromide is known to kill most insects and international standards have been developed for a range of plants and plant products (Bond, 1989). Although no fumigation schedule has been developed specifically for *V. destructor*, it is reasonable to assume that the schedule for fresh flowers and folliage (which covers mites as well as other insects), would be effective against this species of mite. MAF Quarantine Process Procedure 38 comprises a methyl bromide concentration of 48 g/m^3 at atmospheric pressure and at a temperature of 10-15°C for a period of 2 hours, which can reasonably be considered adequate to kill *V. destructor*.

Ionizing radiation is known to be an effective phytosanitary treatment for a range of arthropods, and international standards have been developed for minimum doses required for certain outcomes, among which are insect mortality, preventing successful development, inability to reproduce or inactivation (IPPC, 2003). An International Atomic Energy Agency database includes measures for 29 members of the class Arachnida (IAEA, 2004). Irradiation at doses up to 350 Gy are recommended as adequate for stored product mites in the family Acaridae (IPPC, 2003), and it is assumed that this would also be effective for *V. destructor*.

However, since these mites can survive less than 6 days away from bees or brood, simply holding commodities away from bees prior to export for at least 6 days would ensure that no live *V. destructor* remain.

33.3.2.3 Recommended sanitary measures

<u>For extracted honey, propolis, royal jelly, beeswax and bee venom</u>

No sanitary measures required.

<u>For comb honey and pollen</u>

Each consignment must either

(i) be from a country or part of the territory of a country free from *V. destructor*

or

(ii) be treated by one of the following measures:

(a) holding for 2 weeks in a bee-free environment prior to export

or

(b) heating to 50°C and holding at that temperature for 20 minutes

or

(c) freezing for 48 hours

or

(d) fumigation with with methyl bromide at a rate of 48 g per cubic metre at atmospheric pressure and at a temperature of 10-15°C for a period of 2 hours

or

(e) irradiation with 350 Gy

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34. OTHER *VARROA* SPECIES

34.1 Hazard Identification

34.1.1 Aetiologic Agent: Class *Arachnida*, Family *Varroidae, Varroa jacobsoni, Varroa underwoodi, Varroa rindereri, Euvarroa sinhai, Euvarroa wongsirii*.

34.1.2 OIE List: None.

34.1.3 New Zealand's Status: Exotic to New Zealand. *Varroa underwoodi* and *Euvarroa sinhai* are listed on the unwanted organisms register as notifiable organisms.

34.1.4 Epidemiology

Varroa jacobsoni is a parasitic mite of the Asian honey bee, *Apis cerana*. This mite was originally thought to be the one responsible for varroasis in the European honey bee, *Apis mellifera*, worldwide, but recent genetic research has shown that the mite parasitising *A. mellifera* is a different species, *V. destructor* (Anderson and Trueman, 2000). *V. jacobsoni* is present in Indonesia, Malaysia and New Guinea (Anderson and Trueman, 2000), and it is considered unable to reproduce on *A. mellifera* (Anderson, 1994).

V. underwoodi is a parasitic mite of *A. cerana* (Delfinado-Baker and Aggarwal, 1987). It is considerably smaller than *V. destructor* and is found at low numbers in drone cells of *A. cerana* in Nepal, South Korea, Papua New Guineau and Indonesia (De Jong, 1997; Anderson et al, 1997).

V. rindereri is a parasitic mite of *A. koschevnikovi*, the Asian red honey bee. This mite appears to be confined to the island of Borneo, although the range of of *A. koschevnikovi* includes peninsular Malaysia and Sumatra as well (Otis and Kralj, 2001). The ability of *V. rindereri* to parasitise *A. mellifera* is uncertain, because colonies of *A. mellifera* are rarely maintained within the range of *A. koschevnikovi* (Otis and Kralj, 2001).

In summary, at least 30 genotypes of *Varroa* spp. have been identified in on the Asian honey bee *A. cerana*, the natural host of *Varroa* spp. mites. However, recent evidence suggests that only two of these genotypes can reproduce on the European honey bee *A. mellifera* – these are the Korean and Japanese genotypes of *V. destructor*. All other *Varroa* spp. mites, including *V. jacobsoni, V. rindereri* and *V. underwoodi*, will attempt to reproduce on *A. mellifera* if given the opportunity, but these genotypes seem to lack the ability to lay eggs on the *A. mellifera* brood at a critical stage of the infection cycle. The mechanism for this is not understood (Anderson, 2004).

Euvarroa sinhai is a parasitic mite of the Asian dwarf honey bee *A. florea*. It has been found in Thailand, India, Sri Lanka (Koeniger et al., 1993) and in Iran (Mossadegh, 1990). The mite appears to have a similar lifecycle to *V. destructor*, and it parasitises only drone brood (Akrantanakul and Burgett, 1976), as does *V. destructor* on its natural Asian bee host, *A. cerana*. Adult mites are phoretic on adult worker bees and drones, but are present in very low numbers – from 3 to 6 females per 1000 workers over the course of the year in Iran (Mossadegh, 1991). Experimental infestations of *E. sinhai* on *A. mellifera* and *A. cerana* have been demonstrated (Mossadegh, 1990; Koeniger et al., 1993), but the significance of this is unclear.

E. wongsirii is a parasitic mite of another dwarf honey bee, *A. andreniformis*. It has a biology similar to *E. sinhai*,.but there are no reports of it being found in association with *A. mellifera* (Otis and Kralj, 2001). It 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

V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai and *E. wongsirii* are exotic to New Zealand and two of these species are listed on the unwanted organisms register as notifiable organisms. Therefore under the criteria presented in Section 2.3, these mites must be classified as potential hazards for the purposes of this analysis.

34.2 Risk Assessment

34.2.1 Release Assessment

Varroa destructor is known to survive less than 6 days on *Apis mellifera*, and it is probably reasonable to assume that the survival of *V. jacobsoni, V. underwoodi* and *V. rindereri* away from their respective hosts is of a broadly similar duration (Anderson, personal communication[1]). However, for *E. sinhai* and *E. wongsirii* the situation is less clear, and the fact that they parasitise migratory bees suggests that the mites must have some way of surviving away from brood for longer periods. Indeed, in Iran *E. sinhai* adults are known to be able to survive for long periods (4-10 months) between cycles of brood production (Mossadegh, 1991).

Notwithstanding the apparent similarity between different species of *Varroa*, the uncertainty about the biology of *V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* justifies a conclusion that it is possible for these mites to survive away from brood of their host bees for a period of several months or more.

34.2.1.1 Honey

Given the processing involved in its production, it is considered that the likelihood of the mites being present in extracted honey, either in bulk form or packed for retail sale, is negligible.

Since they may be able to survive for significant periods of time away from brood, the likelihood of these mites being present on imported comb honey is considered to be non-negligible.

34.2.1.2 Pollen

Assuming that these mites are similar in this regard to *V. destructor*, the likelihood of *V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* being present in pollen is considered to be non-negligible.

[1] Dr D Anderson, Principal Research Scientist, CSIRO, Australia. Email to HJ Pharo dated 2 November 2004.

34.2.1.3 Propolis, royal jelly, beeswax and venom

While *V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* mites might be present on propolis, royal jelly and beeswax at the time of harvesting from the hive, in view of the processes involved in the production of these commodities, the likelihood of these mites being present on the defined commodities at the time of importation is considered to be negligible. The likelihood of the mites being in venom is considered to be negligible at any stage.

34.2.2 Exposure Assessment

If mites were present on or in imported comb honey or pollen, to become established in a colony in New Zealand they would have to become attached to an adult foraging bee and be taken back to the colony where they would have the opportunity to complete their life cycle. Since both honey and pollen are considered to be attractive to bees (as discussed see the commodity definition in section 2.2 of this document), the likelihood of exposure is considered to be non-negligible for these two commodities.

34.2.3 Consequence Assessment

If *V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* were able to establish in *A. mellifera* in New Zealand, it can be assumed that the consequences would be similar to those resulting from *V. destructor.* However, the likelihood of these mites establishing in *A. mellifera* is uncertain. *A. mellifera* is on balance relatively resistant to *Varroa* spp. and, of the more than 30 genotypes of *Varroa* that are known to exist, only two can reproduce in colonies of *A. mellifera*; one of these (the Japan genotype of *V. destructor*) does so only with difficulty (Anderson, 2000). Although *V. jacobsoni* has been found in association with *A. mellifera* adults and brood, it appears unable to reproduce in *A. mellifera* colonies (Anderson, 1994). Neither *V. underwoodi* nor *V. rindereri* have been reported on adult bees or brood of *Apis mellifera.* Although *E. sinhai* is capable of infesting *A. mellifera* experimentally, neither that species nor *E. wongsirii* have ever been seen in *A. mellifera* brood cells in localities in India, Thailand and Vietnam where *A. florea* or *A. andreniformis* are sympatric (Anderson, personal communication[1]). Thus, all the available evidence suggests that *V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* would not be able to establish in *A. mellifera* colonies if they were introduced into New Zealand (Anderson ,1994; 2004). None of these species of mite are likely to have any effects on New Zealand native insects since they are highly adapted to live and reproduce only on *Apis* spp.

However, in view of the remaining uncertainty concerning the likelihood of these mites being able to establishm in *A. mellifera*, and given the assumption that the resulting consequences if they did establish would be significant, the likelihood of adverse consequences for *V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* is considered to be non-negligible.

34.2.4 Risk Estimation

The likelihood of release of the mites *Varroa jacobsoni, V. underwoodi, V. rindereri, Euvarroa sinhai* and *E. wongsirii* is considered to be non-negligible for comb honey and

[1] Dr D Anderson, Principal Research Scientist, CSIRO, Australia. Email to HJ Pharo dated 8 November 2004.

pollen, but for all other commodities it is considered to be negligible. The likelihood of exposure for these commodities is considered to be non-negligible, and the consequences of introduction are assumed to be non-negligible.

Therefore the risk posed by these mites is considered to be non-negligible for comb honey and pollen, and the mites *V. jacobsoni , V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* are classified as a hazard in those commodities. For all other commodities, since the likelihood of release is considered to be negligible, the risk is negligible, and these mites are not classified as a hazard.

34.3 Risk Management

34.3.1 Risk Evaluation

Since the risk estimate for *V. jabsoni, V. underwoodi, V. rindereri, Euvarroa sinhai* and *E. wongsirii* in comb honey and unprocessed pollen is non-negligible, risk management measures are justified to reduce the risks to an acceptable level.

34.3.2 Option Evaluation

34.3.2.1 Risk management objective

The objective is to effectively manage the risk of *V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* by ensuring that imported comb honey does not carry these mites when given a biosecurity clearance in New Zealand.

34.3.2.2 Options available

The OIE Code does not recommend any measures for managing the risk of *V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii* in bee products.

Freezing, fumigation or immersion of frames in warm water (55 °C for 1 hr) are generally-recognised methods of killing *Varroa destructor* on drone comb (SBA, 2004; Anonymous, 2004). In the absence of information to the contrary, it is reasonable to assume that the same measures will be effective against *V. jacobsoni, V. underwoodi, V. rindereri, E. sinhai* and *E. wongsirii.*

Most insects have a high lethal temperature from 38 to 44 °C, and the most heat-resistant species die at temperatures of 47 to 52°C (Ross et al., 1982).

Heating is used in a number of ways to kill *V. destructor*, as they begin to die at temperatures above 38°C. Hot air at 42-48°C has been shown to kill *V. destructor* on brood, although above 44°C the brood is also injured (de Jong, 1997). Heating to 44°C for 4 hours has been shown to kill all *V. destructor* on capped brood (Goodwin and Van Eaton, 2001). There is no reason to suspect that these mites would not be similarly sensitive to heating.

Lethal low temperatures for insects vary as much as lethal high temperatures (Ross et al., 1982). Freezing honey in the comb is known to kill small hive beetle eggs and larvae (Sandford, 1999), and it has long been recognised that freezing kills *V. destructor* quickly; indeed, the ‘freezing drone brood’ method (freezing brood combs for up to 48 hours) is one of

the oldest methods of *V. destructor* control in bee hives (Reddell, 2004). There is no reason to suspect that these mites would not be similarly susceptible to freezing.

Fumigation with methyl bromide is known to kill most insects and international standards have been developed for a range of plants and plant products (Bond, 1989). Although no fumigation schedule has been developed specifically for *Varroa*, it is reasonable to assume that the schedule for fresh flowers and folliage (which covers mites as well as other insects), would be effective against *Varroa* spp. MAF Quarantine Process Procedure 38 comprises a methyl bromide concentration of 48 g/m^3 at atmospheric pressure and at a temperature of 10-15°C for a period of 2 hours – this can be considered adequate to kill *Varroa* spp.

Ionizing radiation is known to be an effective phytosanitary treatment for a range of arthropods, and international standards have been developed for minimum doses required for certain outcomes, among which are insect mortality, preventing successful development, inability to reproduce or inactivation (IPPC, 2003). An International Atomic Energy Agency database includes measures for 29 members of the class Arachnida (IAEA, 2004). Irradiation at doses up to 350 Gy are recommended as adequate for stored product mites in the family Acaridae (IPPC, 2003), and it is assumed that this would be effective on *Varroa* spp, which are classified in the same taxonomic group.

34.3.2.3 Recommended sanitary measures

<u>For extracted honey, royal jelly, beeswax, propolis and bee venom</u>

No sanitary measures required.

<u>For comb honey and pollen</u>

Each consignment must be

either

(i) From a country or part of the territory of a country free from *Varroa jacobsoni, V. underwoodi, V. rindereri, Euvarroa sinhai* and *E. wongsirii.*

or

(ii) be treated by one of the following measures:

(a) heating to 50°C and holding at that temperature for 20 minutes

or

(b) freezing for 48 hours

or

(c) fumigation with with methyl bromide at a rate of 48 g per cubic metre at atmospheric pressure and at a temperature of 10-15°C for a period of 2 hours

or

(d) irradiation with 350 Gy

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35. WAX MOTHS

35.1 Hazard Identification

35.1.1 Aetiologic Agent: Class *Insecta*, Family *Pryalidae, Galleria mellonella, Achroia grisella*.

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 and temperature. 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 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 life history 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 and methyl bromide, or by spraying stored combs with formulations of *Bacillus thuringiensis.* There are no reports of strain variation among greater and lesser wax moths abroad. (Williams, 1997).

35.1.5 Conclusion

The greater and lesser wax moths 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*.

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 intestine of adult honey bees and then travel through the alimentary canal to lodge in the Malpighian tubules (Crane, 1990). The amoeba encysts in the Malpighian tubules, and the cysts are then deposited in faeces. The cysts are ingested by adult bees 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* was first reported in New Zealand more than 50 years ago (Cumber, 1948), and more recently it has been found in honey bees from a number of locations on both islands of 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, destroying epithelial cells (Stejskal, 1955).

Although it is known that the organism produces cysts that are passed through the gut of the bee into its faeces (Hitchcock, 1948), the precise mechanism of spread of gregarine disease is not known. Spread between colonies has been suggested to possibly involve package bees (Stejskal, 1965), contaminated water (Stejskal, 1965), bumble bees (Hitchcock, 1948), cockroaches (Stejskal, 1955), and contaminated comb (Hitchcock, 1948).

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). While the economic importance of gregarines has not been determined (Oertel, 1965), it is thought that bees infected by gregarines may not be able to work efficiently and may die prematurely (Stejskal, 1965). Warm climates are probably more favourable to gregarine disease, since the organism is killed by freezing (Hitchcock, 1948; Stejskal, 1955). Thus, it has been suggested that there is little reason to control gregarine infections in temperate climates (Fries, 1997).

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 2.3, these organisms must be classified as a potential hazard for the purposes of this analysis.

37.2 Risk Assessment

37.2.1 Release Assessment

There is little information regarding the mode of transmission of the disease, and no information regarding the number of gregarine cysts needed to establish infection in a colony. Since the disease occurs only rarely, cyst transfer and germination are unlikely to be highly efficient.

However, in view of the lack of information, it is assumed that the likelihood of gregarines being in the commodities is non-negligible.

37.2.2 Exposure Assessment

Assuming that infection takes place by the oral route, and that contamination of the commodities is likely, then the likelihood of exposure is considered to depend on the attractiveness of the commodities to bees. This is discussed as part of the commodity definition in section 2.2 of this document. In summary, honey, pollen and some forms of royal jelly are considered to be attractive to bees, while propolis, beeswax and bee venom, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible for honey, pollen and royal jelly, and negligible for propolis, beeswax and bee venom. However, for beeswax that is made into foundation or applied to plastic frames, and for any bee product that is subsequently mixed with honey, the likelihood of exposure is 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 problems for bees in the more sub-tropical areas of the North Island of New Zealand. Although gregarines have a wide distribution internationally, the finding of thses organisms in New Zealand might result in a short-term disruption to exports of live bees or bee products from New Zealand, but it would be unlikely to result in long-term restrictions since there are no official control programmes for gregarines anywhere in the world.

Gregarine disease has only been reported in *Apis* spp. and there is no information to suggest these organisms would have any effects on New Zealand native insects.

Therefore the consequences of introduction are considered to be negligible.

37.2.4 Risk Estimation

Although the likelihood of release is assumed to be non-negligible, and the likelihood of exposure for some of the commodities is also non-negligible, the likelihood of significant consequences is negligible, and the risk is therefore considered to be negligible for all commodities.

37.3 Risk Management

37.3.1 Risk Evaluation

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

References

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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: None[1].

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 Microsporidian *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).

Individual infected bees can carry from 30 to 50 million spores in the midgut (Bailey and Ball, 1991), while the entire gut may contain more than 200 million spores if bees do not defaecate (Fries, 1997). 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 the lowest prevalence in the summer and the highest in late winter and early spring (Bailey, 1955b).

Despite the absence of overt clinical symptoms, the effects of nosemosis on honey bee colonies 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 *N. apis* 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.

[1] As a result of a vote by members at the 72nd General Session of the International Committee of the OIE held in Paris in May 2004, Nosema was removed from List B, in line with the recommendation of the ad hoc group on diseases of bees.

References

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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

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. These types are known as races (Crane, 1990).

Apis mellifera scutellata is a race of honey bee naturally occurring in an extensive range of eastern and southern Africa from Ethiopia to the Cape (Ruttner, 1986). It 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 (Kerr, 1957).

Since its introduction, *A. m. scutellata* 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). It 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 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

This discussion on *A. m. scutellata* is included in this risk analysis only for completeness, as it is listed as an unwanted organism in New Zealand. However, since genetic material of bees cannot be carried in the defined commodities, Africanised bee is not classified as a potential hazard for the purposes of this analysis.

References

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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

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. These types are known as races (Crane, 1990).

The Cape honey bee (*Apis mellifera capensis*) is a race of *A. mellifera* found in the Cape region of southern Africa. The bee is notable for its exceptionable ability to produce diploid (female) adults from unfertilised eggs generates from laying workers (Verma and Ruttner, 1983), a trait called thelytoky. Thelytoky does exist in other subspecies of *A. mellifera*, but is very rare. The laying workers of these other subspecies almost invariably produce haploid adults, 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 colonies 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

This discussion on *A. m. capensis* is included in this risk analysis only for completeness, as it is listed as an unwanted organism in New Zealand. However, since genetic material of bees cannot be carried in the defined commodities, the Cape honey bee is not classified as a potential hazard for the purposes of this analysis.

References

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41. OTHER HONEY BEE RACES

41.1 Hazard Identification

41.1.1 Scientific Name: Family *Apidae, Apis mellifera caucasica*.

41.1.2 OIE List: None.

41.1.3 New Zealand's Status: A. m. caucasica has been introduced into New Zealand on several occasions. 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. These types are known 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).

The main honey bee races used worldwide in commercial beekeeping are *A. m. ligustica* (the 'Italian bee'), *A. m. carnica* (the 'Carniolan bee'), and to a lessor extent *A. m. caucasica* (the 'Caucasian bee') (Dietz, 1992). *A. m. mellifera* (the 'European black bee' or the 'German black bee')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.

New Zealand is known to have three honey bee races – *A. m. ligustica, A. m. mellifera,* and *A. m. carnica*. The Italian bee is the predominate race in this country, and until 2004 the European black bee was the only other race used (Matheson, 1997). Carniolan semen was imported from Europe in the autumn of 2004 and has been inseminated into Italian queens. Husbanded colonies in New Zealand are often hybrids of the Italian and European black bees.

41.1.5 Conclusion

Since genetic material of bees cannot be carried in the defined commodities, *A. m. caucasica* is not classified as potential hazards for the purposes of this analysis.

References

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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 and 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 the species most commonly managed by humans. The other three species are *A. cerana* (the Asian honey bee), *A. florea* (the dwarf honey bee) and *A. dorsata* (the giant honey bee), none of which are present in 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* is found in Borneo and *A. nuluensis* is found in Sulawesi and Mindanao (Takahashi et al., 2002).

42.1.5 Conclusion

This discussion on honey bees other than *A. mellifera* is included in this risk analysis only for completeness, as they are listed as unwanted organisms in New Zealand. However, since genetic material of bees cannot be carried in the defined commodities, honey bees other than *A. mellifera* are not classified as a potential hazard for the purposes of this analysis.

References

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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.

20. AMERICAN FOULBROOD

For venom and propolis

No sanitary measures required.

For honey, pollen, royal jelly, and beeswax

Each consignment must be either:

(i) from a country or part of the territory of a country free from American foulbrood

or

(ii) from hives that were inspected for American foulbrood within the previous 12 months, by a person certified as competent to diagnose the disease (following appendix 3.4.2 of the OIE *Code*), and found not to be clinically infected or suspected to be clinically affected by American foulbrood .

or

(iii) tested and found to have a *P.l. larvae* spore count of less than 500,000 per litre.

or

(iv) irradiated with 10 kGy

or

(v) heated to 120°C and held at that temperature for 24 hours.

21. EUROPEAN FOULBROOD

For venom and propolis

No sanitary measures required.

For honey, royal jelly, pollen and beeswax

Each consignment must be

either

(i) from a country or part of the territory of a country free from European foulbrood

or

(ii) gamma irradiated with 15 kGy

or

(iii) Heated to achieve a 6D reduction in organism numbers according the model of Cox and Domijan (2004), as shown in Table 6. Agitation suitable to ensure the even distribution of heat is required, and automatic temperature tracing must demonstrate that the core temperature has been reached before timing begins.

An alternative to the above measures, for royal jelly and pollen only, is to import the pollen in a form that is not considered to be attractive to bees, such as consumer-ready capsules or tablets packaged for direct retail sale.

Table 6. Time-temperature treatments required to reduce the risk of *M. pluton* by 6D.

Temperature (°C)	Time
50	54 h
60	10 h
70	1 h 48 min
80	22 min
90	5 min
100	5 min

Note : intermediate temperatures can be considered in discussion with MAF using the predictive model developed by Cox and Domijan (2004) for this purpose.

28. BEE LOUSE

For extracted honey, propolis, pollen, beeswax, royal jelly and bee venom

No sanitary measures required.

For comb honey

Each consignment must

either

(i) be from a country or part of the territory of a country free from *B. coeca*

or,

(ii) after packing, be treated by one of the following measures

(a) holding for 4 weeks away from bees

or

(b) heating to 50°C and holding at that temperature for at least 20 minutes

or

(c) irradiation with 250 Gy

or

(d) freezing for 24 hours

30 SMALL HIVE BEETLE

For pollen, royal jelly, beeswax, propolis and bee venom

No sanitary measures required.

For extracted honey packaged for direct retail sale

No sanitary measures required.

For bulk extracted honey

Each consignment must

either

(i) be from a country or part of the territory of a country free from small hive beetle

or

(iii) after packing and ensuring that the outside of packaging is free of substances that will be attractive to small hive beetles, be treated by one of the following measures:

(a) heating to 50°C and holding at that temperature for 24 hours.

or

(b) irradiation with 400 Gy

or

(c) freezing for 24 hours

For comb honey

Each consignment must

either

(i) be from a country or part of the territory of a country free from small hive beetle

or

(ii) after packing and ensuring that the outside of packaging is free of substances that will be attractive to small hive beetles, be treated by one of the following measures:

(a) heating to 50°C and held at that temperature for 24 hours.

or

(b) irradiation with 400 Gy

or

(c) freezing for 24 hours

33 *VARROA DESTRUCTOR*

For extracted honey, propolis, royal jelly, beeswax and bee venom

No sanitary measures required.

For comb honey and pollen

Each consignment must either

(i) be from a country or part of the territory of a country free from *V. destructor*

or

(ii) be treated by one of the following measures:

(a) holding for 2 weeks in a bee-free environment prior to export

or

(b) heating to 50°C and holding at that temperature for 20 minutes

or

(c) freezing for 48 hours

or

(d) fumigation with with methyl bromide at a rate of 48 g per cubic metre at atmospheric pressure and at a temperature of 10-15°C for a period of 2 hours

or

(e) irradiation with 350 Gy

34. OTHER *VARROA* SPECIES

For extracted honey, royal jelly, beeswax, propolis and bee venom

No sanitary measures required.

For comb honey and pollen

Each consignment must be

either

(i) From a country or part of the territory of a country free from *Varroa jacobsoni, V. underwoodi, V. rindereri, Euvarroa sinhai* and *E. wongsirii.*

or

(ii) be treated by one of the following measures:

(a) heating to 50°C and holding at that temperature for 20 minutes

or

(b) freezing for 48 hours

or

(c) fumigation with with methyl bromide at a rate of 48 g per cubic metre at atmospheric pressure and at a temperature of 10-15°C for a period of 2 hours

or

(d) irradiation with 350 Gy

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	–	+	+	No
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	+	+	+	Yes
21	European foulbrood	*Melissococcus pluton*	Yes	+	+	+	Yes
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	+	+	+	Yes

29	External acarine mites	Acarapis dorsalis, A. externus	No	n/a	n/a	n/a	n/a
30	Small hive beetle	*Aethina tumida*	Yes	+	+	+	Yes
32	*Tropilaelaps* spp	*Tropilaelaps clareae, T. koenigerum*	Yes	-	-	+	No
33	*Varroa destructor*	*Varroa destructor*	Yes	+	+	+	Yes
34	Other *Varroa* spp	*Varroa jacobsoni, V. underwoodi, V. rindereri, Euvarroa sinhai, E. wongsirii*	Yes	+	+	+	Yes
35	Wax moths	*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* disease	*Nosema apis*	No	n/a	n/a	n/a	n/a
39	Africanised bee	*Apis mellifera scutellata* and its hybrids	No	n/a	n/a	n/a	n/a
40	Cape honey bee	*Apis mellifera capensis*	No	n/a	n/a	n/a	n/a
41	Other honey bee races	*Apis mellifera carnica, A. m. caucasica*	No	n/a	n/a	n/a	n/a
42	Honey bee races other than *A. mellifera*	*Apis spp.* other than *A. mellifera*	No	n/a	n/a	n/a	n/a

Note :
n/a = where the hazard identification process concludes that an organism is not a potential hazard, a risk assessment need not be carried out
- = negligible; + = non-negligible likelihood

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APPENDIX 1. MODELLING THERMAL DESTRUCTION OF BACTERIAL CELLS.

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Abstract

Thermal inactivation of organisms is most commonly modelled by the first order kinetic model which assumes a linear relationship between the logarithm of the number of survivors and time. However, the microbial survival curves observed in practice often deviate from this assumption. There is much debate on the origins of these deviations from the model, but because relatively little is actually known of what happens to the microbial target during the processing, the potential interpretations have not gained any consensus. Consequently, the basis for constructing a definitive mechanistic model is still fairly inadequate. In addition, it is often difficult to ascertain whether the apparent nonlinearity is just an artefact due to the methods employed in the process, or a feature bound to the mechanism of inactivation. Many empirical models exist, but they are usually considered to be too complicated to be of practical utility. The general method almost universally adopted by the food industry is to fit a series of the first order kinetic models for each temperature and subsequently model the relationship between the estimated 'thermal death times' and temperature.

The work in this paper was motivated by a data set from an experiment investigating the eating time required to kill a harmful organism in a food product.
We explore an alternative empirical fit that incorporates the data from all temperatures into one equation and allows the whole domain of the data to be fitted simultaneously. Furthermore, the model has the advantage of simplicity and interpretability. Due to its empirical nature, it is mathematically flexible and can be extended to deal with various types of departure from the simple first order kinetic model.

Introduction

In modelling the destruction of micro-organisms by lethal treatments, the most commonly used mathematical model is based on the analogy with first order kinetics, i.e. the death rate is proportional to the number of molecules present:

$$dN/dt=-kt$$

where N is the number of living cells after an exposure time t, and k is a rate constant. The model was originally proposed by Chick (1908) and is generally employed for the kinetic analysis of microbial inactivation through thermal, chemical, radiative or other treatments (Reichart 1994). In particular it has played a fundamental role in defining the safety systems in foods. Since the model assumes that the plot of the logarithm of the number of survivals against the exposure time will result in a linear relationship, it is also referred to as the 'log-linear model'.

The general method adopted by the food industry for determining *thermal* processing time is to calculate the time needed for a 90% reduction in survivor numbers by taking the inverse of the temperature dependent death rate coefficient k (Kormendy and Kormendy, 1997; Peleg and Cole, 2000; Ball *et al*., 2001; Jagannath *et al*., 2003; Lambert, 2003). At a given

temperature, this 'decimal reduction time' is constant and is often given the symbol D. The 'thermal death time' is a multiple of the decimal reduction time, the coefficient depending on the type of bacterial cells or spores that are being treated (Peleg and Cole, 2000; Kormendy and Kormendy, 1997). The relative effects of different temperatures are then quantified by plotting the logarithm of D-values against temperatures, which is also assumed to result in a linear relationship (Stumbo *et al*., 1950; Ball, 1943).

There has been growing evidence that the microbial survival curves are not always linear, which means that the standard model of first order mortality kinetics is inappropriate (Peleg and Cole, 2000). Two types of deviation commonly occur; the shoulder, or lag, which is generally thought to be a combination of some activation mechanism with inactivation, and the tail, or the upward concavity, the interpretation of which remains very contentious (Cerf, 1977). Peleg and Cole (2000) list three common hypotheses: one is based on the idea that the microbial population is a mixture of two or more homogenous populations of dissimilar resistance, caused by either genetic heterogeneity or microbial injury. The so called 'Vitalistic hypothesis' states that the microbial population has a spectrum of resistance to the inactivation treatment within the population. Note that the first order kinetic model follows the cumulative form of the exponential distribution, but one can assume that the spectrum of resistance should be modelled by a different distribution, perhaps with a sigmoidal cumulative shape. The alternative is to assume that the spores (or cells) have an inactivation pattern governed by a different kind of kinetics altogether. Elsewhere in the literature, tailing deviations are seen as a result of experimental difficulties when thermal inactivation assessments are made. Cerf (1977) gives an exhaustive review on the subject. While much of the debate has been focused on how to interpret the deviation from the model (Kilsby *et al*., 2000), and considerable effort has been focussed on the engineering aspects of thermal (or other) processing, relatively little is actually *known* of what happens to the microbial target (Peleg and Cole, 2000). Hence, the biological theory that underpins some aspects of the inactivation process is relatively tentative and forms an insufficient base for constructing a definitive and undisputable mechanistic model.

Consequently, there is a plethora of published mathematical inactivation models with successful applications to food processing, ecology, medicine (disinfection and hygiene) etc. A common approach is to adopt the assumption that the microbial population is a mixture of populations with different death rates for each component and fit a multi-compartment (usually biphasic) model to the microbial survival curve. The 'Vitalistic' approach is to replace the first order kinetic model by the cumulative form of some known distribution (other than exponential). Peleg and Cole (2000) list logistic, log normal, beta and log beta distributions as the most commonly used alternatives to describe the distribution of resistances within the microbial population. In addition, one can disregard both of these approaches and fit an empirical mathematical model. A general comment is that these models are often 'too complicated to be of practical utility' (Lambert and Johnston, 2000; Prokop and Humphrey, 1970). As a result, the simple first order kinetic model, or its modified version that allows a transformation on time, are preferentially used (Lambert and Johnston, 2000).

The work in this paper was motivated by a data set from an experiment investigating the heating time required to kill a harmful organism in a food products. The source is an unpublished study done by Ball *et al.* (2001). The organism is currently not present in New Zealand, but is widely distributed in other parts of the world and hence, presents a potential risk if imported. Prior to importation, the food source is processed with a series of treatments, one of which involves a heat treatment that provides a means of eliminating viable bacterial

cells. The study was undertaken to determine time-temperature combinations necessary to kill the organism.

An alternative empirical fit to the thermal death data at hand is explored. It assumes that microbial inactivation is a monomolecular reaction; hence the suggestion that within bacteria there might exist more than one key target volume is ignored. In addition, an assumption is made that the 'tail survivors' do not constitute a genetically distinct population. The model incorporates the data from all temperatures into one equation and allows the whole domain of the data to be fitted simultaneously as opposed to individual independent series. This enables us to model any between-temperature trends. Furthermore, the model has the advantage of simplicity and interpretability. Due to its empirical nature, it is very flexible and can be built up to deal with various types of departure from the first order kinetic model. We have not found an example of it in the literature, although, due to sheer volume of material published on the matter and the broad range of the application it is virtually impossible to examine every published model for microbial inactivation.

Data

The data are expressed as the number of colony forming units (CFUs) surviving after heating a suspension of bacterial cells in the food source for different periods of time (in minutes) at temperatures that spanned from 50^{o}C to 100^{o}C. Each temperature was tested 2 or 3 times. The length of the heating time-periods varied between temperatures and sometimes between experiments at a single temperature. In all experiments, duplicate tubes were used for each time point at each temperature. For the purposes of the analysis the duplicates were averaged. Additionally, for each temperature, a corresponding pair of tubes maintained at room temperature (24^{o}C) was included in the experiments, with the purpose of serving as control for estimating the total viable cell count.
No initial colony counts were measured, as a substitute, room temperature values were used as an estimate. The data were log transformed for all statistical analyses in order to produce approximately normally distributed data with no extreme outliers. The information on the level of dilution for each data point was not available, so 10 was used as the minimum observable value for all the zeros, hence an increment of 5 was added to the average of the duplicate readings before taking logs.
Plots of the log-transformed data against time are given in Figure 1. After the first zero count, all trailing zero counts were excluded from the analysis. From the graphs, the relationship between the log (CFU+5) and time is not necessarily linear, especially in the tail-end areas of the curves where the survivor numbers become scarce. This is particularly evident in larger temperatures, say, 90^{o}C and 100^{o}C, for which the viable cell count is determined at very few time points, hence making it difficult to get a good estimate of the relationship.

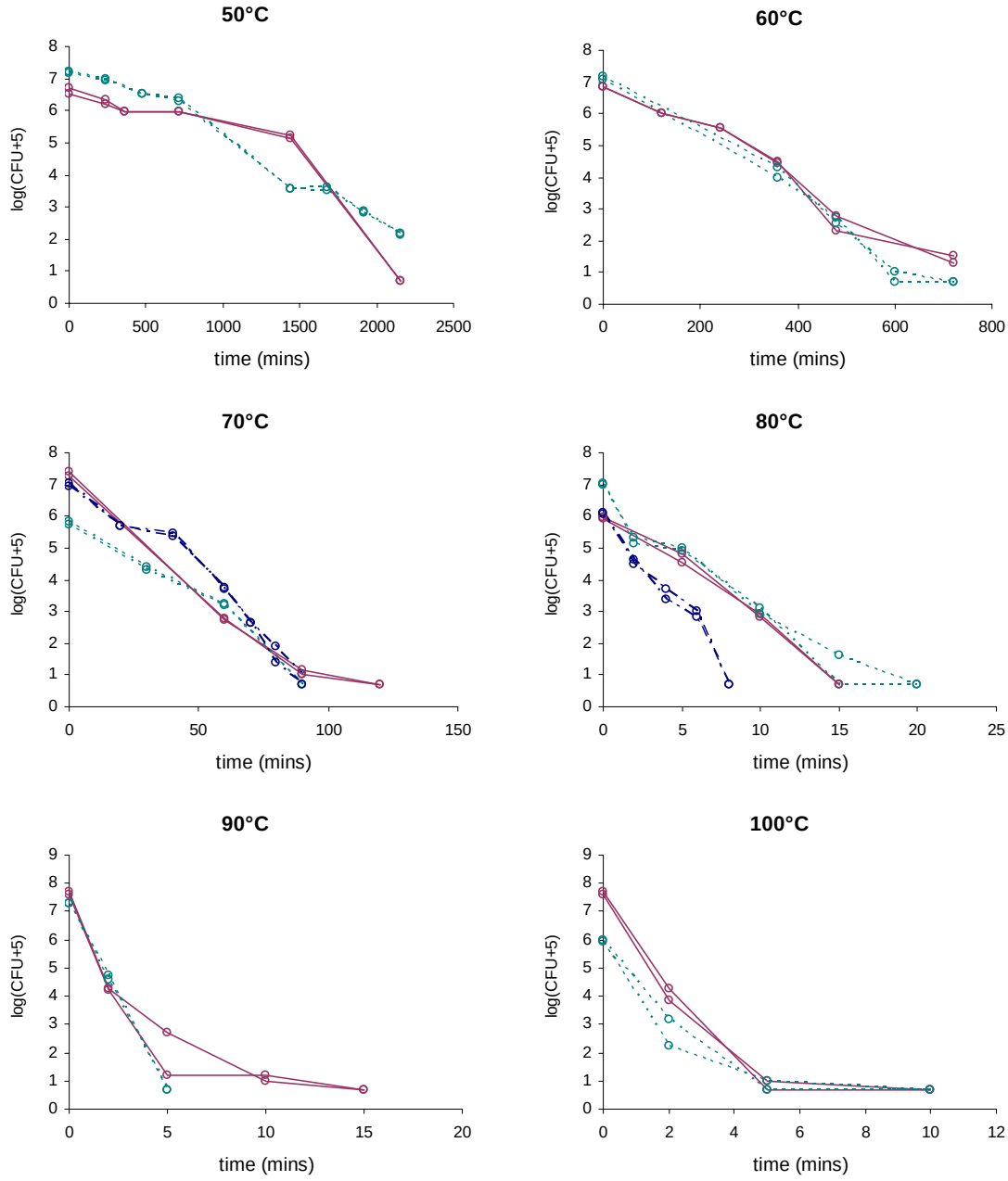

Figure 1 Plots of the log-transformed CFU on time for temperatures of 50°C, 60°C, 70°C, 80°C, 90°C and 100°C.

Modelling the effects of cooking times and temperatures on the reduction in CFU

The original analysis by Ball *et al.* (2001) models the relationship between the counts and heating time separately for each temperature. The temperatures of 90°C and 100°C were excluded from the analysis due to insufficient data.

After fitting a straight line to log transformed counts versus elapsed time at each temperature, the time to decimal reduction D, was estimated by taking the reciprocal of the slope. Subsequently, these estimates of D across various temperatures were used to model the relationship of the time required to decimal reduction and temperature, which leads to an exponential model (see figure 2).

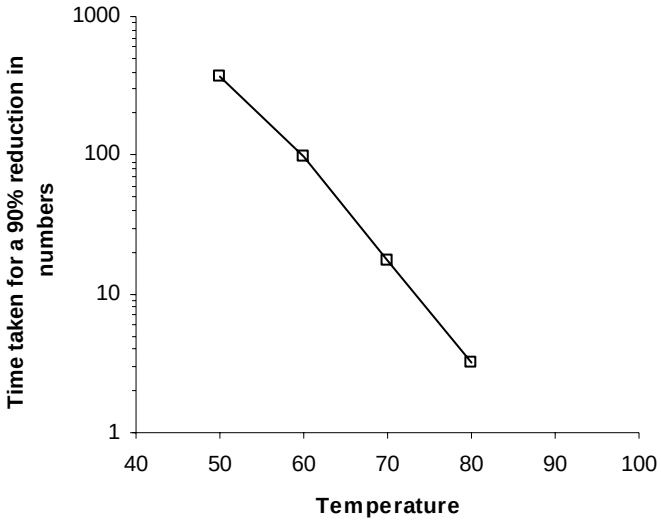

Figure 2 Time taken for a 90% reduction in numbers, as estimated in the Ball *et al.* (2001) study, is plotted against temperature on a logarithmic scale.

This analysis does not make it possible to draw reliable inferences about thermal death time for temperatures above 80°C, even though experiments were run at 90°C and 100°C. In fact, extrapolation of the line fitted to the data in figure 4 leads to estimated Ds clearly inconsistent with the data collected at 90°C and 100°C.

The data was re-examined to investigate if it is possible to make use of all the data to get reasonable estimates also at temperatures of up to 100°C.

The whole model can be fitted in one stage, rather than in the 2 steps. We assume that the relationship between log (CFU+ 5) and time t can be described by a linear model for some power transformation t^c of t, say

$$\log (CFU+ 5) = \log (CFU_o+ 5) - \text{slope } t^c,$$

where the slope depends on the temperature. In table 1, the initial population counts averaged over experiments are given for each temperature.

Table 1 *Average viable cell counts before the thermal treatment for each temperature.*

temperature	log (CFUo + 5)
50	7.204
60	7.190
70	7.415
80	7.041
90	7.703
100	7.703

At time t=D (the time to decimal reduction),

$$\log (CFU_D+ 5) = \log (CFU_o+ 5) - \text{slope } D^c.$$

This can be rewritten as:

$$\log (CFU_o + 5) - \log(CFU_D + 5) = \text{slope } D^c,$$

Hence, slope=$1/D^c$. The model thus becomes

$$\log(CFU + 5) = \log (CFU_o + 5) - t^c/D^c.$$

In addition, assume that D, the time to reduce log(CFU+ 5) to $\log(CFU_D + 5)$, can be modelled by:

$$D = A \exp(-B \text{ temp}),$$

where A and B are parameters that need to be estimated from the data (Stumbo *et al*., 1950; Ball, 1943). Combining the two formulae:

$$\log (CFU + 5) = \log (CFU_o + 5) - t^c/(A \exp(-B \text{ temp}))^c,$$

from which it follows that

$$\log (CFU_o + 5) - \log (CFU + 5) = t^c/(A \exp(-B \text{ temp}))^c,$$

or

$$\log(\log (CFU_o + 5) - \log (CFU + 5)) = c \log(t) - a + b \text{ temp},$$

where a and b are parameters that need to be estimated from the data. This is a simple multiple regression of the log of the drop in log (CFU+ 5) against log (t) and temperature.

An extra term in the model can be added to account for nonlinearity in the temperature which is indicated by plot in Figure 3. Furthermore, the plots in figure 1 imply that the power transformation t^c of time t needed to fit a linear model is dependent on temperature. This can also be easily built into the model by adding another extra parameter.

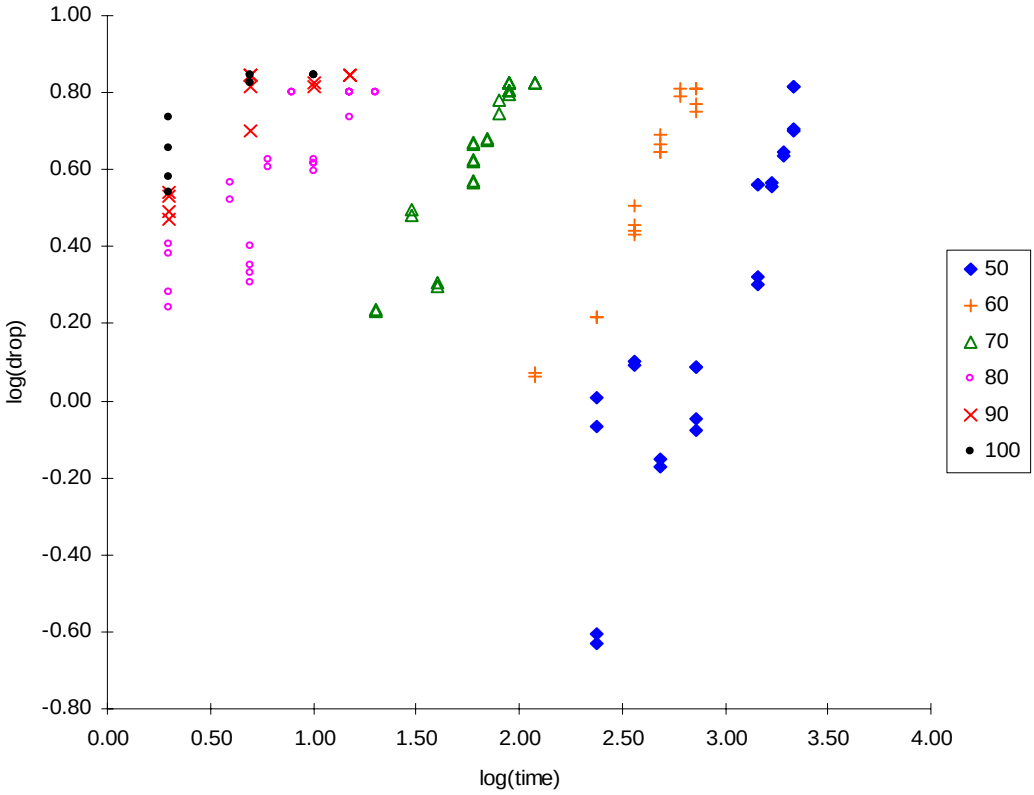

Figure 3 Nonlinearity in the temperature

The model now becomes

$$\log(\log(CFU_o + 5) - \log(CFU + 5)) = a + b\ temp + c\ \log(t) + d\ temp^2 + e\ temp\ \log(t),$$

a multiple regression model which was subsequently fitted in R (version 1.7.1). The parameter estimates and their standard errors are given in table 2. The coefficient for temp square term is very close to zero, but is still highly significant. The power transformations for time t are given for each temperature in table 3.

Table 2 *Regression model parameters*

parameter	estimate	se	t-value	Pr(>|t|)
a	0.654	0.019	34.718	< 2e-16
b	0.048	0.002	23.085	< 2e-16
c	0.628	0.033	19.019	< 2e-16
d	-0.001	0.000	-12.808	< 2e-16
e	-0.017	0.002	-8.039	1.18E-12

The predictive model is thus

$$\log(\log(CFU_o + 5) - \log(CFU + 5)) = 0.65 + 0.048\ temp + 0.628\ \log(t) - 0.001 temp^2 - 0.017\ temp\ \log(t).$$

(logs are base 10)

Table 3 *Power transformation for time t*

temp	c + e temp
50	0.99
60	0.82
70	0.65
80	0.49
90	0.32
100	0.15

This model allows us to predict the log (CFU+ 5) for any given time, temperature and initial number of bacterial cells. The log-transformed colony forming unit counts are plotted against time for each temperature in figure 4 with fitted values superimposed.

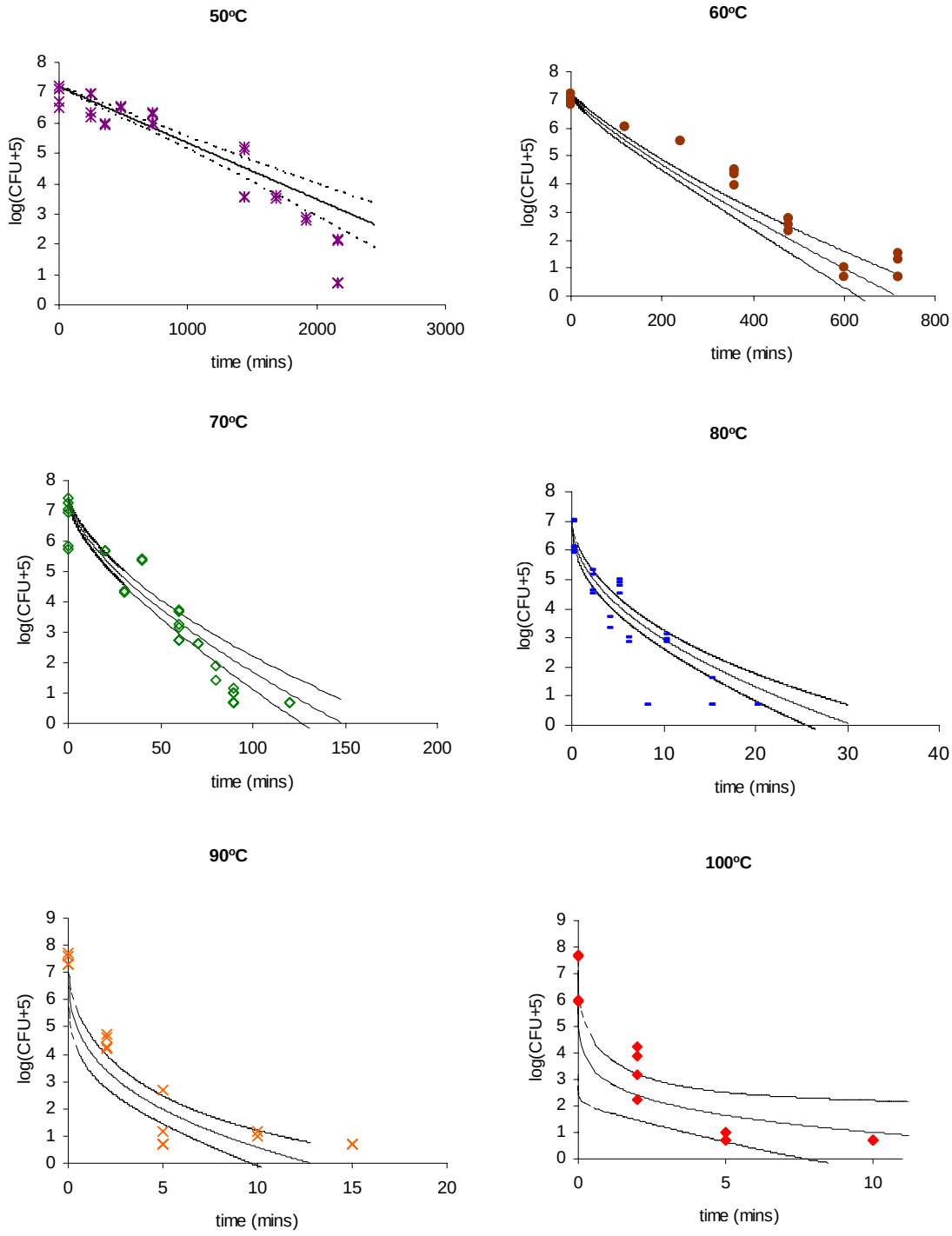

Figure 4 Log -transformed CFU counts are plotted against time for different temperatures. Fitted values are superimposed with 95% confidence intervals for the mean.

The original analysis also involved a computation of the 'extinction times' for each temperature, which, as an estimate of thermal death times, is the variable of practical interest. When determining time-temperature combinations necessary to kill the organism, in particular the 'thermal death times', it is important to consider whether the extrapolation to, say, 12 decades of reduction in the microbial population is justified. Cerf (1977) states that:

due to the lack of precision of the enumeration of survivors at low levels, no confidence should be given to the data corresponding to less than 10^2 survivors per ml, unless a number of experiments are done.

Hence, he recommends that the possible number of reductions that the curve can be studied over should depend on the size of the initial population regardless of the model employed. Others have also suggested that achieving the log reductions necessary to estimate the industry standard 'thermal death times' may not be possible from their data (Peleg and Cole, 2000; Kilsby *et al*., 2000; Lambert, 2003). Moreover, if the analysis assumes the first order kinetic model, and there appears to be some upward concavity, the extrapolation to 'thermal death time' will result in an underestimation, and potentially inadequate food processing.
Time taken for a 90% and 6D reduction in numbers on temperatures are given in table 4 and plotted in figures 5 and 6.

Table 4 *Time taken for D and 6D reductions in number of viable bacterial cells*

Temp	Time to 90% reduction	95% CIs	Time to 6D reduction	95% CI for 6D reduction
50	534.05	(469.3, 607.7)	3237.72	(2683.6, 3906.2)
60	65.61	(55.3, 77.8)	576.29	(509.5, 651.8)
70	6.97	(5.5, 8.8)	107.48	(92.5, 124.9)
80	0.55	(0.3, 0.9)	22.06	(18.5, 26.2)
90	0.02	(0, 0.1)	5.81	(4.4, 7.6)
100	<0.01	(0, 0.8)	4.70	(1.7, 13.3)

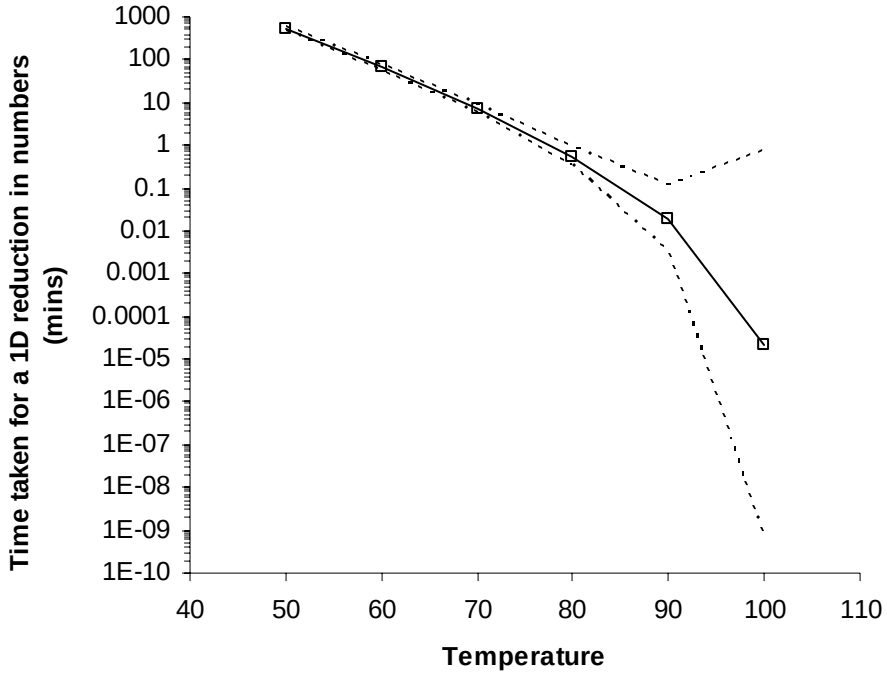

Figure 5 Time taken for a 90% reduction in numbers on temperatures with 95% confidence intervals.

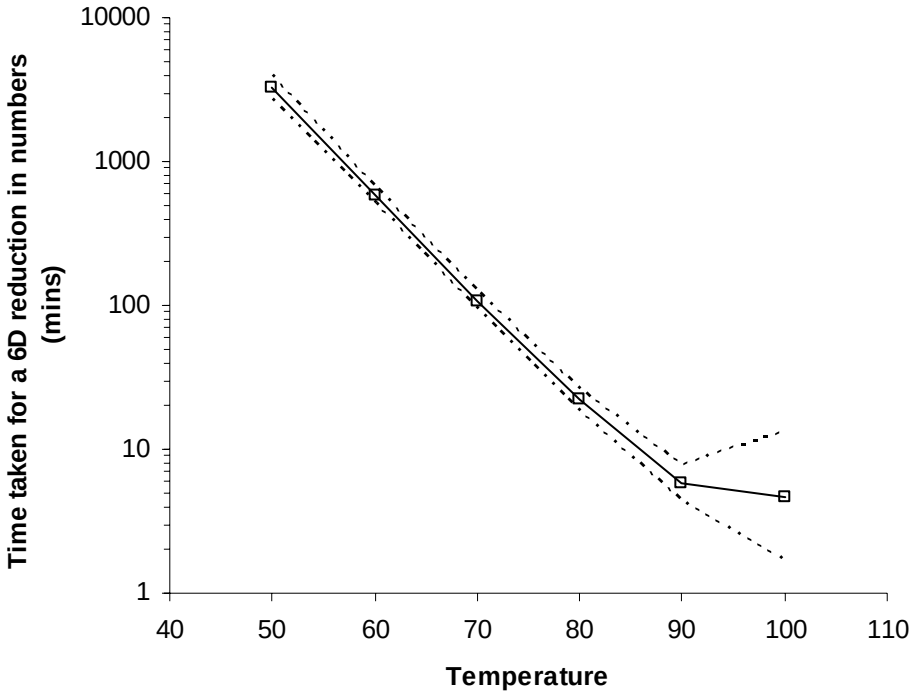

Figure 6 Time taken for a 6D reduction in numbers on temperatures with 95% confidence intervals.

Discussion

The first order kinetic model is unsuitable for the dataset of Ball *et al*. (2001). However, it is difficult to determine whether the observed nonlinearity was a result of artefactual causes or biological reasons. A two compartment model might be appropriate, but given the scarcity of data at higher temperatures, it would have been impossible to fit.
Humpheson *et al*. (1998) discuss a number of biological hypotheses that support the mechanistic foundation behind a biphasic model, mainly based on the assumption of microbial injury of intra cellular components (proteins, membranes, ribosomes, nucleic acid material), and cite a number of other previously published reports which suggested that the tail survivors are not a genetically distinct population. At present, there is no consensus on the possible biological reasons of tailing and there doesn't appear to be an adequate mechanistic model describing the kinetics of the inactivation of the microbial target.

Stumbo *et al*. (1950) discuss the nonlinearity in the microbial survival curves and observe that this seems to occur more often for the temperatures higher than those commonly employed. They remark that there exist a number of publications reporting the same trend, but suggest that this is likely to be a consequence of the inaccuracy in the data at higher temperatures caused by the methods employed in the processing. There are at least two recent papers that note the same trend; Humpheson *et al*. (1998) and Peleg and Cole (2000) both observed a relationship between nonlinearity and processing temperatures in thermal inactivation of *Salmonella enteritidis* PT4 and *Clostridium Botulinum* spores respectively. This would suggest that the trend might be an effect intrinsic to the mechanism of cell inactivation rather than just an artefact, in which case a thermal destruction model should reflect this.

Humpheson *et al*. (1998) report increased nonlinearity in the form of tailing at higher temperatures, linearity as temperatures decrease, and an appearance of a lag, or shoulder at the lowest temperatures. This is very similar to the trend observed in the bacterial data of Ball *et al*. (2001). Humpheson *et al*. (1998) hypothesise that the data forms two distinct populations with different death rates and fit a two compartment model for each individual temperature. Hence, no attempt is made at modelling the observed temperature-nonlinearity dependence.

In this paper, the time variable is transformed in order to model the nonlinearity in the microbial survival curves. Instead of forcing this transformation, the model allows it to be estimated from the data. Peleg and Cole (2000) show that this is analogous to the cumulative form of the Weibull distribution. The whole model is fitted in one stage rather than in two steps. The model equation is easily interpreted, and due to its empirical nature, it is easily expanded to include other trends observed in the data, such as the potential temperature - power transformation on time relationship seen in graphs in figure 2. Furthermore, we were able to add an extra term to model the observed nonlinearity in the temperature.

Peleg and Cole (2000) pre-emptively anticipate a relationship between temperature and nonlinearity in the microbial survival curves and put forward a mechanistic explanation: the survival curves are expected to be concave downward at higher temperatures in response to the increase in the survivors' sensitivity as a result of damage accumulation. However, this hypothesis is unsatisfactory since it conflicts with the observed trend. The approach taken by Peleg and Cole (2000) in order to deal with the nonlinearity of the survival curves is also to the fit the first order kinetic model amended to allow for a transformation on time. Each temperature is modelled separately and afterwards, the relationships between the processing temperatures and the estimated death rate coefficients and transformation of time coefficients are examined. Subsequently, Peleg and Cole (2000) model the estimated transformation of time on temperature with an empirical equation and discuss fitting the model in one step. Note that this is not dissimilar from the work described in this paper.

Other models in the literature have employed a similar approach. Kilsby *et al*. (2000) model is based on the cumulative distribution form of the Normal or Prentice distribution and is also fitted in one stage. Lambert (2003) expands this idea further and in his strictly empirical model estimates the maximum log reductions achievable from the data. However, it is a fair comment to state that the model described in this paper is of a much simpler construction. Since it is empirical, it has the advantage of flexibility and it can be built up to deal with more complex relationships, while still retaining its mathematically simple and transparent form.

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