Disease risk analysis for the reintroduction of the short-haired bumblebee (*Bombus subterraneus*) to the UK

NATURAL ENGLAND

Foreword

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Background

In 2007, Natural England proposed the re-introduction of the short-haired bumblebee (*Bombus subterraneus*) to the UK, a species declared regionally extinct in 2000. A reintroduction project for this bumblebee was formed in 2009 with partners Natural England, Hymettus, the RSPB and the Bumblebee Conservation Trust, with the intention being both to re-establish a sustainable population of this bee in the UK and to act as a flagship for bumblebee conservation more generally.

This report sets out the findings of a Disease Risk Analysis (DRA) conducted in 2011 for the reintroduction of the short-haired bumblebee to the UK, sourced from southern Sweden. The DRA is required as part of the evaluation process to secure a release license into the UK.

Neither the health and disease status, nor the parasites of the short-haired bumblebee population in Sweden have been extensively studied. As the proposed reintroduction would cross a geographic boundary, a detailed Disease Risk Analysis is a fundamental requirement, not least because epidemic disease from alien infectious agent introduction from Sweden to the UK could be catastrophic.

More broadly, it is important to consider parasites explicitly within reintroductions, if we want reintroductions to succeed and, at the same time, have minimal negative impacts to the areas where animals and plants are being taken from and introduced to.

This report considers how the risk of disease in native UK bee populations and in the reintroduced bees might be investigated through surveillance and mitigated through disease control and other measures.

It is important for Natural England:

- to be informed of potential alien infectious agents;
- to understand the risks that disease introduction may bring to native species; and
- to consider appropriate mitigation responses to such risks.

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Summary

- Reintroductions can play a key role in the conservation of endangered species. Bumblebees are in decline at regional and global scales, and reintroductions can be used to re-establish extinct local populations. The main driver of these declines is habitat loss through agriculture intensification but, more recently, emergent diseases have been demonstrated to be serious potential threats.

- The short-haired bumblebee, *Bombus subterraneus*, a widespread palearctic species is in decline across much of its range and was last seen in the United Kingdom in 1988, being declared regionally extinct in 2000. A reintroduction project partnership for this bumblebee was formed in 2009, with the intention being both to re-establish a sustainable population of this bee in the UK and to act as a flagship for bumblebee conservation more generally.

- The IUCN has recommended health monitoring of animals involved in translocation programmes, including reintroduction. Current opinion is that a disease risk analysis (DRA) should be conducted prior to translocation to address the significant disease risks of translocation and the potential detrimental effects to biodiversity conservation of a disease epidemic. This disease risk analysis ensures the costs and benefits of reintroduction from a disease perspective are considered.

- The report describes a qualitative disease risk analysis for the planned reintroduction of the short-haired bumblebee to the UK, specifically queens sourced from Sweden, based on the methodology described by Murray *et al.* (2004). The risk assessment is divided into four components namely, **(i)** release assessment, **(ii)** exposure assessment, **(iii)** consequence assessment and **(iv)** risk estimation, in which we consider source, carrier, transport, and destination and population hazards.

- In total 29 hazards are identified and described, including 12 source hazards, 14 destination hazards, one carrier hazard, one transport hazard and one population hazard. For each of the hazards a risk assessment was carried out and, for those hazards considered of low, medium or high risk, we considered ways in which the risk could be managed.

- The report considers how the risk of disease in native UK bee populations and in the reintroduced bees might be investigated through surveillance and mitigated through disease control and other measures. The following risk management options are recommended based on the results of the disease risk analysis including **(i)** quarantine, management, disease control and screening, and **(ii)** post-release health surveillance.

- Additional measures to prevent disease in the reintroduced bumblebees will be taken when new information on risks comes available. These will be compiled in a Disease Risk Management and Post-release Health Surveillance Protocol.

Acknowledgements

Short-haired Bumblebee Project website: http://www.bumblebeereintroduction.org/

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1   Introduction

1.1   In recent years, there has been a significant decline in the number of bumblebee species present in North America and Europe (Williams 1986; Goulson 2006; Fitzpatrick *et al*. 2007). The decline appears to be primarily driven by habitat loss (Williams 1986; Fitzpatrick *et al*. 2007; Goulson *et al*. 2008). Indeed, the intensification of agriculture and increased urbanization has led to the demise of bumblebee habitat and forage foods (Benton 2006; Carvell *et al*. 2006; Goulson *et al*. 2008). Evidence suggests that pathogens may also be important factors in bumblebee and other wildlife declines (Williams *et al.* 2002, Thorp & Shephard 2005; Colla *et al*. 2006, Brown 2011a, Cameron *et al*. 2011). In the USA, the movement and trafficking of commercial bumblebee hives may have resulted in disease introduction into native populations, although evidence for this is circumstantial at best (Brown 2011a). Bumblebees and their colonies have been reported to host a large number of parasites, and the epidemiology of these infections and their impact on their host have been subject to ongoing research and review (Schmid-Hempel 2001, Goulson 2003, Meeus *et al.* 2011).

1.2   The short-haired bumblebee *(Bombus subterraneus*) is now presumed extinct in the UK. It was last seen in 1988 at Dungeness, Kent. Prior to the 1970s, this species was abundant in Deal and Dover in Kent, in Suffolk and in other localities in Southern and Eastern England (Sladen 1912, cited in Benton 2006). Habitat loss and subsequent resource depletion are the primary factors implicated in their UK demise (Benton 2006; Goulson 2006; Goulson *et al.* 2008). In particular, it is likely that the removal of hedgerows from the UK may have reduced the available nesting and hibernation sites for short-haired bumblebees (Carvell *et al*. 2006; Goulson 2006). However, specific reasons why the short-haired bumblebee has suffered a greater decline due to habitat loss than other bumblebee species is unknown (Goulson *et al*. 2005, Williams 2005). Hypotheses proposed for its decline and extirpation include **(i)** the specialization of this long- tongued species to exploit diminishing native flora such as red clover (Goulson *et al*. 2005; Nagamitsu *et al*. 2007), **(ii)** a narrow niche range (Williams 2005; Williams *et al.* 2009) and **(iii)** late queen emergence from hibernation leading to a shorter reproductive season (Fitzpatrick *et al*. 2007; Williams *et al*. 2009).

Background

1.3   In 2007, Natural England proposed the re-introduction of the short-haired bumblebee to the UK from New Zealand (an introduced population of this species had been present in New Zealand since the 19th century). In 2010, a disease risk analysis (DRA) was conducted in connection with this proposal. The DRA was completed in October 2010 (Vaughan and Sainsbury 2010) and identified a total of 29 hazards comprising 11 source hazards, 15 destination hazards, two carrier hazards and one transport hazard. Owing to the scarcity of literature published on the diseases of bumblebees, we often extrapolated from our understanding of diseases in honeybees. Further screening of the source population prior to reintroduction was recommended for three source hazards, acute bee paralysis virus, *Nosema bombi*, and Kashmir bee virus given their medium or high risk status. The risk options for the other source hazards were relatively easily met through quarantine and screening. In 2010 males and queens from the nests established to rear queens were collected in New Zealand and sent to the UK for disease screening. There was no evidence of acute bee paralysis virus, *Nosema bombi*, or Kashmir bee virus in the sampled bumblebees

however 12 out of 16 of the screened bumblebees carried the tracheal mite (*Locustacaris buchneri*) (Brown, unpublished data). Of the 15 destination hazards, medium risk status was assigned to eight hazards. While the prevalence of these agents at the reintroduction site was unknown, we recommended simple surveillance techniques to monitor the impact of these hazards on the release population, for example through the post-mortem examination of carcasses. The two carrier hazards (deformed wing virus and Kashmir bee virus), were assigned a medium risk status. Risk management for these two viruses focussed on reducing stressors associated with reintroduction. The only identified transport hazard *Aspergillus candidus* was given a low risk status. In this case transporting the bees at temperatures to minimise *Aspergillus* growth, ensuring transport materials are clean and dry prior to loading and that the transport boxes are adequately ventilated according to stocking density should minimize the risk of disease. Risk management options for all identified hazards were then combined along with recommended quarantine and hygiene practices and post release health surveillance to create a Disease Risk Management and Post Release Health Surveillance protocol.
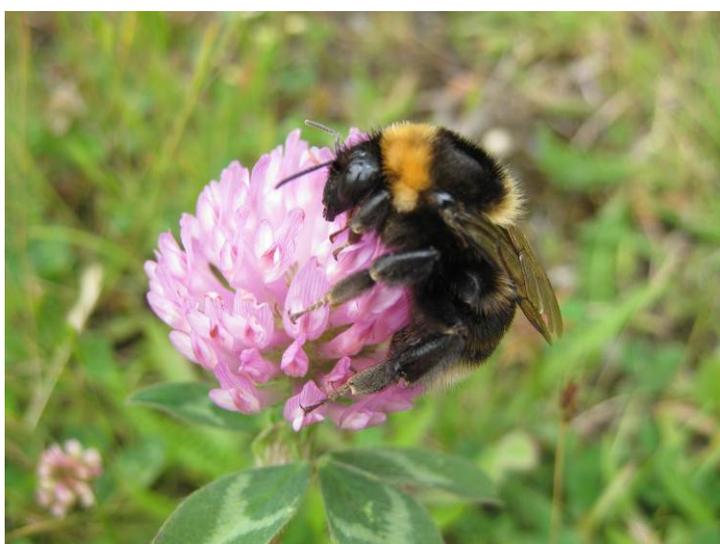
Plate 1 *Bombus subterraneus* queen feeding on red clover © Nikki Gammans

1.4   From 2009 to 2011 captive rearing and breeding with the aim of importing second generation queens from New Zealand for reintroduction occurred. In 2009 queens were imported but were dead on arrival. In 2010 no queens were reared for export from New Zealand. In a report on the reintroduction from New Zealand it was concluded that long-tongued bumblebee species including *B. subterraneus* are notoriously difficult to rear (Gammans 2011) and overall captive rearing of this species proved difficult. Gammans (2011) stated that 'high *B. subterraneus* queen mortality was observed, success was noted in larval production, but the queens often died before emergence.'

1.5   Despite the husbandry knowledge gained in undertaking captive rearing of this species, reintroduction from this source population was unsuccessful. Alternative source populations were discussed and a European source seemed favourable as wild queens could then be caught directly and reintroduced to the UK avoiding the need for captive rearing and hibernation. In addition recent genetic studies have suggested that a Swedish sourced population may be more genetically similar to the extinct UK population than a New Zealand sourced population presumably owing to genetic bottlenecking as the New Zealand population may have arisen from only two queens (Lye *et al*. 2011). Given the low genetic diversity of the New Zealand *B. subterraneus* population the long term sustainability of this

population was questionable. Ongoing research has shown (Gammans 2011) that *B. subterraneus* sourced from Sweden may be a more viable option (both genetically and logistically) as the populations in the south province of Skane and northern province of Stockholm and Uppsala are known to be self sustaining. Therefore a reintroduction sourcing *B. subterraneus* from Sweden has been proposed. A new DRA was undertaken in 2011 in connection with the revised proposal and completed in February 2012.

Disease Risk Analysis

1.6   The IUCN (1987 & 1988) has recommended health monitoring of animals involved in translocation programmes, including reintroductions. Current opinion is that a disease risk analysis (DRA) should be conducted prior to translocation (Davidson & Nettles 1992; Leighton 2002; Conservation Breeding Specialist Group 2003) to address the significant disease risks of translocation and the potential detrimental effects to biodiversity conservation of a disease epidemic (Griffin *et al.* 1993; Kirkwood & Sainsbury 1997). This disease risk analysis ensures the costs and benefits of reintroduction are considered from a disease perspective (Sainsbury *et al.* 2008a). In particular, an evaluation to ensure that the benefits of reintroduction will not be outweighed by potential importation of alien infectious agents (source hazards), which may affect existing bumblebee and other bee, and invertebrate populations, is important. Other potential hazards of significance to be assessed include destination and population hazards and transport hazards. In addition, the fate of parasites harboured by the reintroduced animal must be considered with reference to the health of animals and conservation of biodiversity.

1.7   Murray *et al.* (2004) described an advanced methodology for undertaking qualitative disease risk analysis for the importation of animals and animal products to protect domestic animal health and this methodology can potentially be extrapolated for use in a re-introduction undertaken for biodiversity conservation. Murray *et al.* (2004) confined their analysis to source hazards (infectious agents which could potentially be imported with the animal to be re-introduced). However, in a translocation carried out for conservation purposes we propose that it is also necessary to consider transport, carrier, and destination and population hazards. Translocated wild animals may be exposed to infectious agents while in transit which are potential threats to the destination ecosystem. Destination and population hazards may arise for three possible reasons:

   (a) because the reintroduction of animals alters the balance of community parasite dynamics giving rise to disease outbreaks facilitated by an immuno-suppresssed population;
   (b) non-infectious hazards, for example toxins, are present in the destination environment; and
   (c) the reintroduced animals may be exposed to novel pathogens not present in their former environment.

   Under scenario (a) the reintroduced population may be immuno-suppressed as a result of the stress of transport and their introduction to a new environment, or because non-infectious hazards reduce their ability to counter with an immune response. In addition, potentially commensal parasites (carrier hazards) in translocated animals could cause disease precipitated by the stress of translocation including the influence of management actions or because the reintroduction of animals alters the balance of community parasite dynamics, for example, due to changes in host density.

1.8   For a reintroduction carried out for conservation purposes to be truly successful in enhancing biodiversity, or returning a part of biodiversity to its pre-disturbed state, 'native' host parasites should perhaps be translocated with the host. Preservation of parasites during the stressful

reintroduction phase is likely to be managed best by minimising stress to the bumblebee hosts, to prevent the costs of parasitism overwhelming the health of the host. This component of the DRA will be discussed under the sub-heading of risk options and management.

1.9 The health and disease status, and the parasites of the short-haired bumblebee population in Sweden have not been extensively studied and the reintroduction is planned to cross a geographic boundary. Therefore a detailed disease risk analysis must be carried out because epidemic disease from alien infectious agent introduction from Sweden to England could be catastrophic. For example, the introduction into the United Kingdom of squirrelpox virus with the North American grey squirrel (*Sciurus carolinensis*) in the late 19th century led to substantial mortality in the red squirrel (*Sciurus vulgaris*) (Sainsbury *et al.* 2008b).

1.10 This report sets out the findings of a disease risk analysis for the reintroduction of the short-haired bumblebee to the UK, specifically queens sourced from Sweden, in which we consider source, carrier, transport, and destination and population hazards, and the need to conserve parasites where possible. We consider how the risk of disease in native UK bee populations and in the reintroduced bees might be investigated through surveillance and mitigated through disease control and other measures. These will be compiled in a Disease Risk Management and Post-release Health Surveillance Protocol.

2   Materials and methods

2.1   The methodology described by Murray *et al.* (2004) was used as a basis to carry out a disease risk analysis for the reintroduction of short-haired bumblebees to the UK. We extrapolate from their methods to consider, in addition, infectious transit, carrier and destination hazards and infectious and non-infectious population hazards. We also consider ways in which the parasites of short-haired bumblebees can be conserved through modification of therapeutic regimes and control measures to ensure that native parasites are maintained in the reintroduced population.

Reintroduction pathway

2.2   We defined our source environment as Sweden, and our destination environment the UK and assumed geographic isolation of Swedish bumblebees from UK bumblebees given several molecular analyses of population structure in declining bumblebee species have reported significant population structure and very low levels of migration (Darvill *et al*. 2006, Ellis *et al.* 2006). Lepais *et al.* (2010) showed that *B. pascuorum* and *B. lapidarius* queens typically disperse by at least 3 and 5 km, respectively, while analysis of the population genetic structure in the rare and declining *B. muscorum* in the Scottish islands demonstrated significant isolation by distance with significant genetic differentiation for populations 10 km apart (Darvill *et al.* 2006). Distances of this magnitude represented an efficient barrier to gene flow for this species, especially over water bodies (Darvill *et al.* 2006). In another rare species *B. sylvarum* genetic differentiation was significant between populations located more than 100 km apart (Ellis *et al.* 2006). The distance from Skane county in Sweden to Dungeness, Kent is approximately 1,400km and a water body (the English Channel) must also be crossed. The English Channel has been shown to present a significant barrier to migration (Widmer *et al.* 1998) and genetic isolation seems likely.

2.3   The bumblebee reintroduction project officer was consulted for the particulars of the proposed reintroduction pathway. Up to 100 queens will be collected from the wild immediately post-hibernation in late April or May along two transects in Skane County in Sweden, the first, on the south coast, from Trelleborg to Ystad, and the second, from the west coast, from Lund to Landskorna (see Plate 2). Once collected the bumblebees will be placed in separate vials and refrigerated. It is estimated that they will then be kept refrigerated for up to 9 days, until they reach the quarantine facility at Royal Holloway University of London, during which time they will be fed every evening with an artifical nectar solution. The bumblebees will be imported into the UK in individual glass vials in a refrigerator via road transport.

2.4   Upon arrival in the UK the queens will be transported to Royal Holloway University of London, where they will be transferred to new individual boxes for monitoring. Their transport vials and all material that accompanied the bumblebees will be destroyed as per Food and Environment Research Agency (FERA) guidelines on the import of bees. The bumblebee queens will be quarantined and monitored for two weeks then released to the Royal Society for the Protection of Birds (RSPB) reserve in Dungeness, Kent. Whilst in quarantine the bumblebees will be fed nectar and pollen. The pollen fed during quarantine will have been collected from both melanistic *B. subterraneus* queens at the collection sites in Sweden and also from long-tongued bumblebees at the release site in Dungeness. Dungeness is close to the last known recorded site of *B.subterraneus* and six of the UK's seven Biodiversity Action Plan (BAP)

priority species (indicating these species have declined) once occurred here. Now only two remain, *B. muscorum* and *B. humilis,* but with the exception of *B. subterraneus* the others are still present in Kent. This area is designated a Site of Special Scientific Interest (SSSI) and lies within a target area for the Environmental Stewardship Scheme (Gammans 2011) meaning the site is managed effectively for bumblebees.
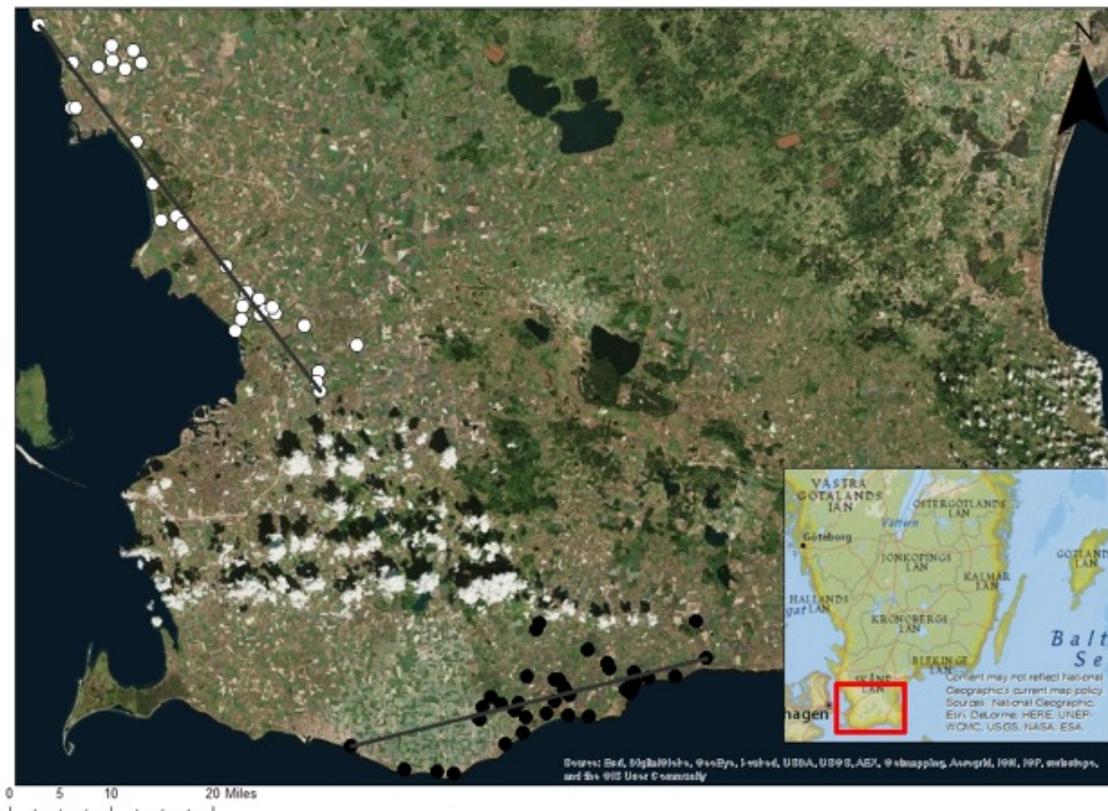
Plate 2 A map of southern Sweden including both the sites of collection of *B. subterraneus* queens for parasite pre-screening (see text below), shown by white and black dots, and the two transects that were subsequently developed for capturing queens for reintroduction to Dungeness (Kent), shown by grey lines.

Hazard identification

2.5   We identified parasites (micro- and macro-parasites) known to be present in the short-haired bumblebee, or other bee and invertebrate species, populations in the source geographic area, and potentially present in the destination geographic area, using the scientific literature. While we acknowledge that Swedish sourced short-haired bumblebee queens could have a similar evolutionary history to bumblebees in the UK, given the geographical isolation between Swedish and UK bumblebees and their lack of ability to disperse long distances (Darvill *et al.* 2006, Ellis *et al.* 2006, Lepais *et al.* 2010) e.g. from Sweden to the UK, it is likely that parasites possessed by Swedish bumblebees will have evolved strain differences from their UK counterparts (e.g. Imhoof & Schmid-Hempel 1998).

2.6   In 2011 to gain specific information on the current parasites of *B. subterraneus* in the source environment, 59 queens were captured in Sweden (see Plate 3), transported to the UK and screened for the following macro and microparasites of bumblebees, as well as six honeybee viruses: the tracheal mite *Locustacarus buchneri,* the microsporidian *Nosema bombi,* the neogregarine *Apicystis bombi*, the trypanosome *Crithidia bombi*, the parasitoid wasp *Syntretus spp.* and the nematode *Sphaerularia bombi*, in addition to the following viruses,

acute bee paralysis virus (ABPV), black queen cell virus (BQCV), deformed wing virus (DWV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV) and sac brood virus (SBV) (Brown 2011b). The sample numbers were chosen based on the maximum number we were able to take for destructive sampling by the Swedish authorities. *B. subterraneus* queens were collected along two transects in Skane County, Sweden (the intended source environment). Bees were collected whilst walking a standard transect, with bees being collected opportunistically as they were seen foraging. Any bees that carried pollen bags were not collected, as these bees would have already established a nest site. As a consequence, the prevalence of parasites that prevent colony founding (e.g. *A. bombi*, *C. bombi*, *N. bombi*, *Syntretus* spp. and *S. bombi*) would be overestimated for the sampled *B. subterraneus* populations. Of the 59 queens collected, two died during transport and were too autolysed for diagnostic testing of some micro- and macro-parasites, but were submitted for viral analyses.

Plate 3 *Bonbus subterraneus* queen collected in Skane Sweden in 2011 for disease screening © Nikki Gammans

2.7   In addition, 22 native UK bees including five *B. lapidarius* males, five *B. hortorum* workers, 10 *B. terraneus/lucorum* workers and two *B. pratorum* workers collected from the intended release site in Dungeness Kent were also screened at the National Bee Unit for viruses (ABPV, BQCV, DWV, IAPV, KBV and SBV). The sample size of 5 per species was selected based on Singh *et al.* (2010) who found positive viral screening results for DWV, BQCV, SBV, KBV, and IAPV using smaller samples, giving us confidence that if viruses were present in these bumblebee populations we would isolate them in our sample. A similar study across the UK found 1/3 of all *Bombus* tested to be positive for DWV (Evison *et al.* 2012), giving us further confidence in our approach.

2.8   When identifying hazards we considered not only known pathogens, but also apparent commensal parasites, because, although the host short-haired bumblebees may have immunity to such endemic, commensal parasites, these parasites might cause disease in naïve populations of the same or taxonomically-related species in other areas. The pathogenicity of many parasites of free-living invertebrates is unknown because of the paucity of disease investigations in invertebrates and so it is important that all potential parasites are considered as a part of the DRA.

2.9   For each parasite identified in the hazard identification, we assessed (i) whether short-haired bumblebees are a potential vehicle for the introduction of the parasite to the UK, and (ii) if the parasite was exotic to the UK (the destination country). If short-haired bumblebees were considered a potential vehicle and the parasite was exotic, the parasite was classified as a

source hazard for further consideration in a risk assessment. If a parasite was present in both the source and destination geographic area but was of a different strain, or potentially different strain, in the source area then it might still be classified as a hazard.

2.10 A list of infectious and non-infectious <u>destination</u> and <u>population</u> <u>hazards</u> was created using the scientific literature and confined to **(i)** infectious agents not present in Sweden but present in the UK (destination hazard), **(ii)** infectious and non-infectious agents present in the UK for which there is evidence of potential effects at the population level, including effects on small populations (population hazards). Under (ii) we looked for evidence of a potential effect on the population of short-haired bumblebees, bees or invertebrates of similar behaviour and ecology in the UK or other countries.

<u>Carrier hazards</u>: These are commensal infectious agents, and when the host is subjected to stressors, such as those associated with reintroduction, or factors which affect parasite dynamics, such as alterations in host density, cause disease in animals in the destination environment.

<u>Transport hazards:</u> might be infectious or agents of non-infectious disease present on route from Sweden to the UK, which are known to cause significant levels of disease for which there is evidence of an effect on the population of short-haired bumblebees, bees or invertebrates of similar behaviour and ecology in the UK, or other countries.

Risk assessment

2.11 The risk assessment was divided into four components as set out by Murray *et al*. (2004) namely, **(i) entry (release) assessment** (description of pathways necessary for the introduction of the hazard), **(ii) exposure assessment** (description of pathways necessary for the hazard to occur following introduction), **(iii) consequence assessment** (identification of the consequences of disease introduction and establishment, that is, the adverse human health, animal health, economic or environmental effects of interest) and **(iv) risk estimation**, and we carried out the risk assessment of source and transport hazards according to their methods (Murray *et al*. 2004). For destination and population hazards, the release assessment does not apply, but the remaining components of the risk assessment were carried out as for source and transport hazards.

2.12 In the release assessment we determined the likelihood that short-haired bumblebees will be infected or contaminated with a hazard and described the pathway necessary for the parasite or contaminant to be released into the destination environment. In the exposure assessment we described the biological pathway for invertebrates to be exposed to the hazard in the destination environment and estimated the likelihood of this occurring. In the consequence assessment we assessed the likelihood of significant biological, environmental and economic consequences occurring in association with the entry, establishment and spread of the hazard. In the risk estimation we combined the results of the release, exposure, and consequence assessments using the risk estimation decision steps described by Murray *et al.*, (2004) to characterize the level of risk associated with the hazard as negligible, very low, low, medium, or high. In each stage of the risk assessment the evaluation is made using a logical, reasoned, referenced, evidence-based approach using the terms advised by Murray *et al.* (2004) to classify risk. The relationships between the components of the risk assessment and management process are illustrated in Figure 1 below.
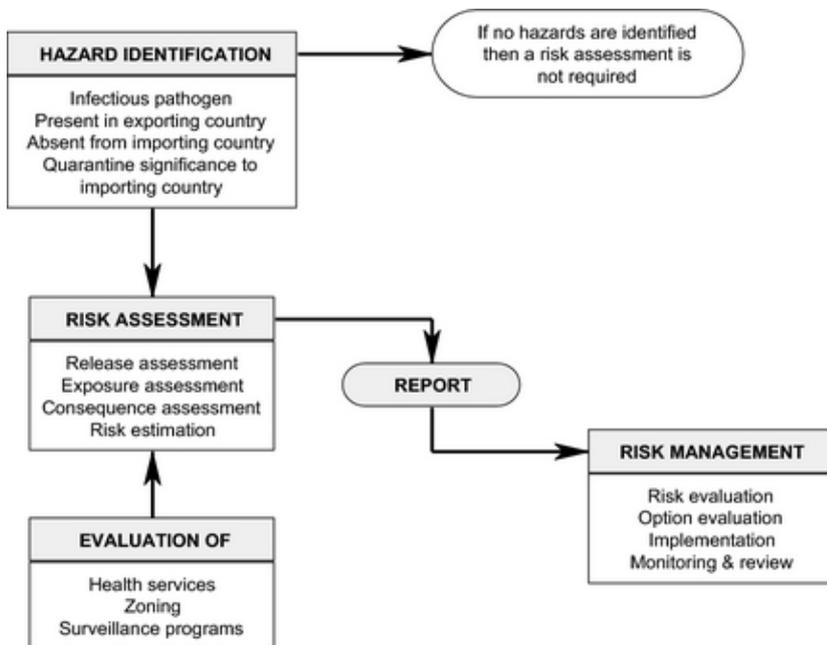
Figure 1 The relationship between hazard identification, risk assessment and the risk management process (after O.I.E., 2012)

Risk management

2.13 In undertaking risk management we identified and considered measures to reduce disease risks, for example through quarantine and disease screening (See section 3.15 for risk management options).

Risk communication

2.14 This paper forms one method by which the hazard identification, risk assessment and proposed risk management have been communicated to decision-makers and other stake-holders. The report will be updated in response to communications from stake-holders.

3   Results

Hazard identification

3.1   A list of all infectious agents present in Sweden which could potentially infect short-haired bumblebees was prepared from the literature. Infectious agents which were on the OIE list of notifiable diseases (OIE 2009) or were notifiable under the Bee Diseases and Pests Control (England) Order 2006 (Beebase 2006) were also considered. From this list, infectious agents were discounted as source hazards if they were known to currently occur in the UK, unless there were presumed to be strain differences between the agent in Sweden and that in the UK.

3.2   Literature review showed that most infectious agents of honeybees and bumblebees do not appear to cross-infect. For example, three species of *Nosema* sp. are known to infect honeybees or bumblebees. *Nosema apis* induces a serious disease in honeybees which is on the OIE list of notifiable diseases and does not infect bumblebees (Jilian *et al.* 2005). *Nosema ceranae* is a recent emergent parasite of honeybees (Paxton 2010) believed to cause colony death in meditteranean climates (Higes *et al.* 2009), and there is a single report of this parasite from bumblebees in South America (Plischuk *et al.* 2009). Bumblebees are infected by *Nosema bombi* which can kill entire colonies but is variable in expression, (Jilian *et al.* 2005) and is not an OIE notifiable infectious agent. Absence from the OIE list may be related to apparent lower commercial importance of bumblebees rather than the pathogenicity of the agent or the risk an agent represents to the health of bumblebees. The small hive beetle (*Aethina tumida*) is an exception because it can infect bumblebees and honey bees: bumblebee colonies placed in close contact to infested honeybee hives also became infested (Spiewok & Neumann 2006). The small hive beetle (*Aethina tumida*) is an OIE notifiable parasite but is currently not thought to be present in the UK (Defra 2009) or Sweden (Swedish University of Agricultural Sciences 2011) and therefore a disease risk analysis was not undertaken on this parasite. The deformed wing virus previously only reported in honeybees has now been identified in commercial *B. terrestris* and wild *B. pascuorum* in Europe (Genersch *et al.* 2006), and a recent survey of wild UK bumblebees isolated DWV in a third of *B. terrestris* sampled (Evison *et al.* 2012). It is possible therefore that this virus may also infect *B. subterraneus*. Kashmir bee virus (KBV) has recently been detected in honeybees in the UK and in *Bombus* sp. from New Zealand (Ward *et al.* 2007) but not in *Bombus* sp. in Sweden. This virus also infects wasps *Vespula germanica* (Anderson, 1991) and there appears to be a growing body of evidence that KBV is an insect virus, rather than being restricted to honeybees (Ward *et al.* 2007). Acute bee paralysis virus is genetically highly related to Kashmir bee virus (deMiranda *et al.* 2004) and has also been demonstrated in *Bombus* sp. (Bailey & Gibbs 1964).

3.3   American foulbrood (caused by *Paenibacillus* larvae) and European foulbrood (caused by *Melissococcus plutonius*) are both on the OIE list of notifiable diseases and Paenibacillus larvae is present in both the UK and Sweden (Beban *et al.* 2003, Defra 2009, Swedish University of Agricultural Sciences 2011). European foulbrood is notifiable and subject to statutory control in the UK, although it is not a notifiable disease in Sweden. Both American and European foulbrood have only been reported to affect honeybees at present (Beban *et al.* 2003, Defra 2009, Swedish University of Agricultural Sciences 2011), however statutory controls exist for both honeybee and bumblebee importation in regards to these diseases. In

July an outbreak of American foulbrood occurred in Halland county which is approximately at least 170km from the collection sites in Skane county in Sweden and was controlled as per current Swedish (and therefore EU legislation).

3.4   The mite *Varroa destructor* is present in the UK and Sweden and is under official control in Sweden, while in the UK it has now been deregulated in its notifiable status and is widespread throughout the UK. *Varroa destructor* was previously believed to only infect honeybees but one report exists of infection in the American bumblebee *Bombus pennsylvanicus* (Ongus, 2006). This parasite has not been reported in European bumblebees (G. Budge, personal communication October 2009). Tropilaelaps mites, also an OIE notifiable infectious agent, are not present in the UK or Sweden, are only reported in honeybees and therefore were not considered further in the disease risk analysis (Beban *et al.* 2003, Defra 2009, Swedish University of Agricultural Sciences 2011). The tracheal mite (*Acarapis woodi*) has only been reported in honey bees (OIE 2006) and has never been found in Sweden despite two nationwide surveys being conducted in 1993 and 2010. It therefore remains a notifiable parasite in Sweden. In the UK tracheal mite infection is not usually a serious disease, with relatively small numbers of colonies being affected (Beebase 2011) and it is not a notifiable disease in the UK. Given it has only been reported in honey bees it was not considered further in the disease risk analysis.

Parasite screening

3.5   Of the *B. subterraneus* queens sampled from Sweden in 2011, 3/57 (5.3%) were infected with *Crithidia bombi,* one from the first transect and two from the second transect. Four queens were infected with *Sphaerularia bombi,* (4/57; 7.1%) two from each transect. No other parasites or viruses were detected.

3.6   In the destination environment one *B. hortorum* worker was strongly positive for ABPV. This is the first report of ABPV in *B. hortorum* in the UK. These data are very useful when interpreted in conjunction with preliminary results from a study sampling wild-caught bumblebees across the UK for the viruses ABPV, CBPV, IAPV, SBV, BQCV and DWV. This UK wide study found DWV in a 1/3 of their *B. terrestris* samples, and a small number of *B. pascuorum* were also positive. *B. lapidarius, B. pratorum* and *B. hortorum* were virus free (Evison *et al.* 2012). These results were then used to inform the disease risk analysis.

Identified hazards assessed

3.7   In total 29 hazards (comprising 15 infectious agents) were identified for the proposed short-haired bumblebee reintroduction (**Table 1**). The 12 source hazards were *A. bombi* (Larsson 2007, Macfarlane 1995)*,* acute bee paralysis virus (ABPV) (Bailey & Gibbs 1964; Bailey 1976; Anderson 1988; Allen & Ball 1996; Pharo 2004), *Beauveria bassiana* (Leatherdale 1970; Alford 1975; Glare *et al.* 1993; Schmid-Hempel 1998; Yeo *et al.* 2003; Lozaro-Gutiérrez & Espana-Luńa 2008)*, Crithidia bombi* (Imhoof & Schmid-Hempel 1998; Schmid-Hempel *et al* 1999; Henson *et al.* 2009)*,* deformed wing virus (DWV) (Yue *et al.* 2007, Genersch *et al.* 2006), *Locustacarus buchneri* (Macfarlane 1975; Donovan 1980), *Nosema bombi* (Alford 1975; McIvor & Malone 1995; Tay *et al.* 2005; Larsson 2007; Rutrecht *et al.* 2007; Rutrecht & Brown 2008b)*, Metarhizium anisopliae* (Macfarlane *et al.* 1995; Yeo *et al.* 2003)*, Paecilomyces farinosus* (Leatherdale 1970; Yeo *et al.* 2003; Lang et al. 2005)*, Sphaerularia bombi* (Alford 1969; Macfarlane 1975; Donovan 1980)*, Verticillium lecanii* (Macfarlane *et al.* 1995; Yeo *et al.* 2003) and *Paenibacillus larvae* (Genersch 2010). None of these hazards are exotic to the UK but were considered as source hazards because strain and virulence

differences either exist or may have arisen between the UK and Swedish bee populations (**Table 1**).

3.8 The 14 destination hazards were: ABPV (Bailey & Gibbs 1964; Bailey 1976; Anderson 1988; Allen & Ball 1996; Pharo 2004), DWV (Yue *et al.* 2007, Genersch *et al.* 2006) and Kashmir bee virus (KBV) (Ward *et al.* 2007). *A. bombi, C. bombi, S. bombi* (Macfarlane 1995; Lippa & Triggiani 1996; Henson *et al.* 2009)*, B. bassiana, L. buchneri, M. anisopliae, N. bombi, P. farinosus, V. lecanii,* (Alford 1975; Donovan 1980; Corbet & Morris 1999)*, Melittobia* sp. and *P. larvae* (Genersch 2010). Two destination hazards (ABPV and KBV) are possibly exotic to bumblebees in Sweden. One carrier hazard, DWV (Allen & Ball 1996; Ball 2001; Beban *et al.* 2003; Todd & Ball 2003), and one transport hazard, *Aspergillus candidus* (Macfarlane *et al.* 1995), were identified (Table 1). Pesticides were considered a population hazard (Thompson & Hunt 1999).

3.9 Of the 12 identified source hazards, three were classified as medium risk, *C. bombi,* DWV and *S. bombi* and the remaining nine hazards were classified as low risk. Of the 14 identified destination hazards, one high risk hazard *C. bombi*, and five medium risk hazards, ABPV, *A. bombi*, DWV, *M. acasta* and *S. bombi* were identified and the remaining eight hazards were of low risk. The carrier hazard DWV was classified as medium risk and the transport hazard *A. candidus* was classified as a low risk. The population hazards, pesticides, were determined to be of low risk.

Table 1 Infectious agents identified as hazards in the risk analysis for the reintroduction of the short-haired bumblebee, *Bombus subterraneus*

Name of hazard	Non-native to UK	Found in Sweden	Strain differences considered	Type of hazard	Risk category
Acute bee paralysis virus (ABPV)	No - not species specific	Yes - not species specific	Potentially	Source hazard	Low
				Destination hazard	Medium
***Apicystis bombi* (Protozoan)**	No	Yes	Potentially	Source hazard	Low
				Destination hazard	Medium
***Beauveria bassiana* (Fungus)**	No	Yes	Potentially	Source hazard	Low
				Destination hazard	Low
***Crithidia bombi* (Trypanosome)**	No	Yes	Potentially	Source hazard	Medium
				Destination hazard	High
Deformed wing virus (DWV)	No	Yes	Potentially	Source hazard	Medium
				Destination hazard	Medium
				Carrier hazard	Medium
***Locustacarus buchneri* Tracheal mite**	No	Yes	Potentially	Source hazard	Low
				Destination hazard	Low
***Nosema bombi* (Microsporidian)**	No	Yes	Potentially	Source hazard	Low
				Destination hazard	Low
***Metarhizium anisopliae* (Fungus)**	No	Yes	Potentially	Source hazard	Low
				Destination hazard	Low
***Paecilomyces farinosus* (Fungus)**	No	Yes	Potentially	Source hazard	Low
				Destination hazard	Low
***Verticillium lecanii* (Fungus)**	No	Yes	Potentially	Source hazard	Low
				Destination hazard	Low
***Sphaerularia bombi* (Nematode)**	No	Yes	Potentially	Source hazard	Medium
				Destination hazard	Medium
Kashmir bee virus (KBV)	Yes (in *Apis mellifera*) not yet tested in bumblebees in UK *	No	Potentially	Destination hazard	Low
***Melittobia acasta* (Chalcidoid wasp)**	No	Yes	Potentially	Destination hazard	Medium
***Aspergillus candidus* (Fungus)**	No	Yes		Transport hazard	Low
***Paenibacillus* larvae (Bacterium)**	No	Yes	Potentially	Source hazard	Low

* does not appear to be species specific (Ward *et al,* 2007).

Identified hazards not assessed

3.10   Three potentially pathogenic agents have been isolated from bumblebees but have yet to be identified to species level: *Acrostalagmus* spp. (Dingley 1956; Chen & Dickson 2003), *Hirsutella* spp. (Glare *et al.* 1993; Macfarlane *et al.* 1995) and *Candida* spp. (Macfarlane *et al.* 1995) and were not taken forward to the risk assessment stage owing to the lack of firm evidence of pathogenicity to short-haired bumblebees. Two other infectious agents did not meet the criteria to be assessed as hazards as they had not been recorded in either the UK or Sweden and infected other species of *Bombus*: 1) Entomopox virus which was found in workers of the bumblebees *B. impatiens, B. pennsylvanicus* and *B. fervidus* in North America, with no adverse effects reported (Clark 1982), and 2) *Spiroplasma melliferum* (Beban *et al.* 2003; Bjornson and Schutte 2003; Fletcher *et al.* 2006) which was isolated from the haemolymph of *B. impatiens* and *B. pennsylvanicus* from North America and any pathogenic effects were not determined (Clark *et al.* 1985).

3.11   Two fungal species*, Doratomyces putredinis,* (Donovan 1980; Macfarlane 2005; Allen *et al*. 2007; Pavlovich 2008), and *Chrysosporium pannorum* (*Geomyces pannorum)* are reported to be opportunistic insect fungi commonly found in the soil and air which may result in infection of injured or weakened insects (Macfarlane *et al.* 1995), and, saprophytic, non-pathogenic insect associates (Hajek *et al.* 1997). These fungi were discounted as hazards owing to their variably, and sparsely reported pathogenicity.

Agents of disease in bees not considered hazards

3.12   The castrating parasite, the braconid wasp (*Syntretus splendidus)* has been reported to affect *B. lucorum, B. terrestris* and *B. pascuorum* sp. in the UK (Alford 1968, Baldock 2008) and similar parasitoid larvae have been reported in North America (Plath 2003) and Sweden (Hasselrot 1960, 2003) which suggests the wasp may be widely distributed. This wasp was therefore discounted as a hazard given it had been reported in both source and destination populations. Likewise the Conopid flies, *Physocephala rufipes* and *Sicus ferrugineus,* commonly reported to affect a number of bumblebee species in the UK (Schmid-Hempel *et al.* 1990), have also been commonly reported to affect Swedish bumblebees (Larsson 2007). *Physocephala rufipes* and *Sicus ferrugineus,* were therefore also discounted. Furthermore, conopid flies are not active until July/August and are therefore unlikely to constitute a hazard as the reintroduction is planned to occur in May.

Risk assessment and risk management

3.13   For each of the hazards we carried out a risk assessment and, for those hazards considered of low, medium or high risk, we considered ways in which the risk could be managed (see **Appendices** for details).

3.14   The complete disease risk analysis for all the source hazards are presented in tabular form and colour coded according to risk status: green – low risk hazard, orange – medium risk hazard, red – high risk hazard.

3.15   The following risk management options are recommended based on the results of this disease risk analysis including **(i)** quarantine, management, disease control and screening, and **(ii)** post-release health surveillance.

Quarantine, examinations, management and screening:

1. A clinical examination should be conducted prior to transport, and prior to release, by a veterinarian with bee expertise and only healthy bumblebees (i.e. showing no signs or suspicion of disease including infestations affecting bees) should be considered for reintroduction.

2. Transport containers must be cleaned, dried and disinfected prior to use, or new containers used. Use a bactericidal and virucidal disinfectant at the appropriate dilution rate (follow manufacturers' guidelines) such as Virkon® (DuPont Animal Health Solutions, Sudbury, Suffolk, UK). Avoid the use of hay or straw substrate in transport containers, and ensure boxes are well ventilated and maintained at constant temperatures.

3. The *B. subterraneus* quarantine facilities at Royal Holloway University (Surrey) should be separated from all insects by using a quarantine barrier and a strict quarantine regime must be enacted including (i) dedicated personnel (no contact with other insects), (ii) barrier clothing (overalls, boots, gloves) (iii) dedicated tools (iv) disinfectant footbath at the barrier and (v) disinfection of all tools used with a virucidal and bactericidal disinfectant such as Virkon® (DuPont Animal Health Solutions, Sudbury, Suffolk, UK).

4. Bumblebees should be held inside boxes that do not allow access to any other insect genera. Preferably the room in which they are housed should have sticky insect trap tape circumnavigating any potential entry points for insects such as windows and doors.

5. The individual plastic rearing containers should be kept at a constant temperature with ventilation provided to match the requirements of minimum air flow for a bumblebee. Precise guidelines should be developed in the Disease Risk Management and Post Release Health Surveillance Protocols (DRM & PRHS protocol).

6. Any bumblebee queens with clinical evidence of disease should be regularly assessed for welfare reasons, and a decision to euthanase for diagnostic *post-mortem* examination to assess the disease risks to the remaining bumblebees, and for early detection of alien parasites should be considered.

7. Any sick bumblebees must be placed in isolation or euthanased for diagnostic *post-mortem* examination

8. Isolation facilities should be available at the Royal Holloway facilities for suspected sick bees. Strict barrier isolation must be instituted, including (i) consideration of the use of dedicated personnel (ii) barrier clothing (overalls, boots, gloves) (iii) dedicated tools, (iv) disinfectant footbath at the barrier and (v) disinfection of all tools used with a virucidal and bactericidal disinfectant such as Virkon® (DuPont Animal Health Solutions, Sudbury, Suffolk, UK). Detailed plans for isolation will need to be set out in the DRM and PRHS Protocol.

9. The quarantine period should be a minimum of two weeks to enable effective screening for parasites. Any queens infected with *A. bombi* will likely die during quarantine as the average lifespan of infected queens is <7 days (Rutrecht & Brown 2007).

10. To minimise the potential for the development of disease associated with carrier hazards a complete and balanced diet, and suitable housing and husbandry guidelines must be formulated, which must be completed and reviewed prior to the import of the first bumblebees.

11. Faecal samples should be collected from all queens and tested for *C.bombi*, by microscopic examination of the faeces within 14 days of arrival in England. If any queens are infected they will be euthanased and not reintroduced.

12. If any bees are affected by fungal disease during the quarantine period, exposure to simulated sunlight could be considered in an attempt to reduce the fungal load of affected individuals and/or others deemed particularly susceptible. Sunlight (natural or artificial) (UV) has been shown to reduce the viability of fungal spores (Morley-Davies *et al.* 1995) and to reduce the prevalence of mycosis in invertebrates (Inglis *et al.* 1997) and so UV exposure could be trialed as a method of reducing the spore load on bees' carapaces. UV-B wavelengths (285 to 315 nm) are particularly damaging to fungi (and other micro-organisms) (Inglis *et al.* 1995). It would therefore be advisable to use a light with with high UV-B capabilities for 6.5 hours over one day (e.g. Zoo Med Reptisun 10® (Specified output: up to 15% UVA : 10% UVB); different fungal strains appear to differ in their susceptibility to UV light and in the duration of UV exposure required to significantly reduce conidial viability (Morley-Davies *et al.* 1995, Fargues *et al.* 1996).

13. Any bumblebees that die over the quarantine period should have a post-mortem examination undertaken by a veterinarian or pathologist. In particular the trachea should be incised and inspected for the eggs, nymphs and larval stages of the tracheal mite *L. buchneri*. Patchy discolouration or dark staining caused by mite feeding may also be evident. The haemocoel should then be examined for the nematode *S. bombi* (in queens) and the neogregarine *A. bombi*. *A. bombi* have characteristic sausage shaped bodies, and *S. bombi* larval stages may be evident. The abdomen should also be examined for the flagellate *C. bombi*, and the microsporidian *N. bombi*. A full post mortem examination methodology will be formulated in the DRM and PRHS protocol.

Post-release health surveillance:

14. Pathogen surveillance should be carried out on all dead *B. subterraneus* queens, and other bees, found in the field post reintroduction. Given post release population surveillance using defined transects will be undertaken, any dead queens or workers found during this surveillance should be collected and submitted for post-mortem examination. A full post mortem protocol will be formulated in the DRM and PRHS protocol which will outline specific testing requirements for identified hazards.

4   Discussion

4.1   We have described a qualitative disease risk analysis for the planned reintroduction of short-haired bumblebees from Sweden to the UK. In total 29 hazards have been detected and described, including 12 source hazards, 14 destination hazards, one carrier hazard, one transport hazard and one population hazard. All the source, and the majority of the destination, hazards are included on the basis of strain differences between species of parasite present in both Sweden and the UK. In some of these cases the evidence available on the pathogenicity of parasite strains is limited but the analysis is the best available given our current understanding. Pesticides were the only non-infectious agent considered a hazard. Some recommendations for disease risk management have been set out and are discussed in more detail in the Disease Risk Management and Post-release Health Surveillance Protocols. We would appreciate comments on our disease risk analysis and we recommend that the results are given detailed consideration as one component of a detailed cost-benefit analysis for this reintroduction programme.

4.2   Of the 12 source hazards, three were classified as medium risk, *Crithidia bombi,* DWV and *Sphaerularia bombi. Crithidia bombi* and *Sphaerularia bombi* can be detected during quarantine of arriving bumblebees from Sweden and we are confident that the risks from these two agents to native bumblebees, and the reintroduction programme, can be effectively ameliorated. The risk posed by DWV is harder to prevent or control, although measures to reduce the effect of stressors on the reintroduced bees are recommended, and therefore it is of interest to note that DWV was not detected when *B. subterraneus* from the source population was screened. The recommended risk options for the other identified source hazards are relatively easily met.

4.3   Of the 14 destination hazards, one high risk hazard was identified: *Crithidia bombi*. It will be important to enact a detailed and effective post-release disease surveillance programme in order to monitor the risk from *Crithidia bombi* to reintroduced bees. Five medium risk destination hazards were identified, ABPV, *Apicystis bombi*, DWV, *Melittobia acasta* and *Sphaerularia bombi.* ABPV has been detected at the reintroduction site in the first such record in the UK. Meticulous disease surveillance is recommended to monitor all five of these medium risk destination hazards (and DWV as a carrier hazard) to achieve early detection should an outbreak occur. We have set out some ideas on reducing stressors on the reintroduced bees, and recipient populations, but further comments on methods to reduce stress, and maintain bee health, from partners in the project would be valuable. Most viral infections are probably more likely to become evident when bees are stressed due to other diseases, weather conditions or management practices (Bakonyi *et al.* 2002). It is highly likely that the process of re-introduction will be stressful. Therefore simple practices such as (i) re-introducing in late spring so that the late emerging *B. subterraneus* can exit the nest in warmer weather and be exposed to less inclement weather, and (ii) providing an *ad-libitum* food sources to help reduce competition with native bumblebees, may help reduce disease incidence.

4.4   *Aspergillus candidus* was identified as a transport hazard and given a low risk status. In the case of *Aspergillus candidus*, transporting at temperatures to minimise *Aspergillus* growth, ensuring transport materials are clean and dry prior to loading and that the transport boxes are adequately ventilated according to stocking density should minimize the risk of disease. In

the case of *Paenibacillus larvae*, nectar and pollen fed to the bumblebees during quarantine should be sourced from areas free of AFB, and Swedish nectar and/or pollen should not be released at the reintroduction site (it should not be present in vials used for transport of bees to the reintroduction site and materials and over clothes used in the quarantine facility should not be brought to the reintroduction site).

4.5   It is possible that there are other, currently unknown, parasites present in short-haired bumblebees from Sweden which might cause disease in reintroduced short-haired bumblebees or other native species in the UK. These are particularly likely to cause disease epidemics in UK native species if they are alien to the UK. It will be important to monitor the short-haired bumblebees on arrival in England for these currently unknown hazards, through, for example, *post-mortem* examination of short-haired bumblebees and other *Bombus* and *Apis* sp. found dead in the vicinity of the reintroduction site. A full protocol for this monitoring can be developed closer to the time of reintroduction.

4.6   There are several newly emerging viral infections of bumblebees and honeybees which appear to infect multiple insect genera and developments in the distribution of insect virus hosts should be closely followed and regularly re-assessed during this reintroduction programme. For example KBV was identified in *Apis cerana* and *Apis mellifera* (Ball & Bailey 1997, Miranda *et al.* 2009) and then in bumblebees from New Zealand and European wasps *(Vespula germanica)* from Australia (Anderson 1991) and there is growing evidence to suggest it may be a pathogen of multiple insect genera (G. Budge, personal communication 10 Oct 2009). Therefore we included a comprehensive virus screen of a sample of the bumblebees from the source and destination sites to take account of these rapid developments in our understanding of viral distributions. This included testing for DWV, black queen cell virus (BQCV), sacbrood virus (SBV), and Israeli Acute Paralysis Virus (IAPV or IV). Ongoing screening for these viruses is recommended in any bumblebees that die during reintroduction or are found post-release.

4.7   In conclusion this disease risk analysis has identified some significant hazards to the reintroduction of short-haired bumblebees. Action can be taken to ameliorate some of these hazards and others closely monitored. The risk of disease should be carefully assessed as part of a cost-benefit analysis for this reintroduction programme.

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6 Appendices: Disease Risk Analysis

Table 2 Disease Risk Analysis for the source hazard acute bee paralysis virus (ABPV)

SOURCE HAZARD	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Acute bee paralysis virus (ABPV)	Assuming transmission pathways in bumblebees are the same as for honeybees, a 1st generation queen in Sweden may be infected with ABPV when foraging and being exposed to virus excreted on flowers previously visited by honeybees. APBV could then spread horizontally through direct transmission to larvae via salivary gland secretion or the mixing of infected saliva with pollen. Infected larvae (including the 2nd generation queen to be reintroduced) will either die before they are sealed in brood cells if large amounts of virus particles are ingested, or survive to emerge as inapparently infected adult bees (Chen & Siede 2007). Vector-borne transmission via mites is also possible and ABPV has been detected in *Varroa* sp. and their saliva (Ball 1989). *Varroa* sp. act as a virus vector transmitting ABPV from severely infected bees and brood via feeding activities. The mite damages bee tissues and releases viral particles into the hemolymph (Ball & Allen 1988). ABPV can activate from a latent infection to become a lethal infection especially in the presence of *Varroa* sp. mites, although other activators are also likely to exist (Chen & Siede 2007). *Varroa* has not been reported to parasitise the European bumblebee (G. Budge, personal communication October 2009) but it has been found to infect the American bumblebee *Bombus pennysylvanicus* (Ongus 2006).	Upon release, the *B. subterraneus* queens will forage for resources and establish their colonies. It is likely that any infected queens will initially transmit ABPV via horizontal transmission to larvae. The spread and establishment of infection is dependent on the prevalence of infection in the workers. Intensity of infection in colonies of at least one other bumblebee parasite, increases with the percentage of workers infected during larval development (Rutrecht & Brown 2007). ABPV could be passed from colony to colony through interactions at shared food resources (Durrer & Schmid-Hempel 1994) or through the drifting of infected workers to other colonies (Sakofski & Koeniger 1988; Schmid-Hempel 1998). Given that *B. subterraneus* is a long tongued bumblebee (Goulson *et al.* 2005), the flowers it visits and subsequently sheds virus on, are more likely to be visited by other native long tongued species. As such the limited foraging behaviour of *B. subterraneus* may reduce the probability of spread of ABPV. Late developing species, such as *B. subterraneus*, are also less efficient at widely disseminating the infection horizontally between colonies, as they probably have less time within a season to do so and therefore there is less likelihood of native UK early-emerging bumblebees, and honeybees being exposed to ABPV from late developing species. Vector-borne transmission is possible between imported bumble bees and honeybees but it is not certain that *Varroa* infect European bumblebees (G. Budge, personal communication October 2009). This combined with the limited foraging ability of *B.subterraneus* means there is a very low likelihood of exposure of other European bumblebees in the UK.	There is a low likelihood that at least one bumblebee/ and or honeybee at the destination site will be infected. Although, the majority of ABPV infections are sub-clinical (Bailey & Gibbs 1964) if the virus becomes clinical bees may tremble uncontrollably and are unable to fly. In addition, they lose the hair from their bodies and have a dark, shiny, or greasy appearance (Miranda *et al.* 2009). Paralytic bees are submissive to attack. When paralysis is serious, large numbers of afflicted bees can be found at the colony entrance, crawling up the sides of the hive and blades of grass, and tumbling to the ground. Affected bees also may be found on top bars or frames next to the hive cover with wings extended. These signs may occur 2-4 days post infection and death ensues over the following days (Bailey & Gibbs 1964). ABPV has been implicated in the mortality of honeybees from colonies infested with *Varroa* (Ball 1985) and is one of the viruses thought to be associated with colony collapse disorder in honeybees.
JUSTIFICATION OF HAZARD			Given country specific strains of ABPV have been found in honey bees (Bakonyi *et al.* 2002, Carreck 2007) significant impact on existing honeybee populations may occur, if strain differences exist between Swedish bumblebees and UK honeybees. If widespread dissemination did occur, which is unlikely as stated in the exposure assessment a reduction in the insect assisted pollination of plants and surrounding crops is likely and this would be of economic significance given that figures from Defra put a conservative estimate of £165m (in 2006) on the annual value of UK agricultural pollination provided by honey bees with the annual value of honey production in the UK fluctuating between £10m and £30m. It is likely that the introduced *B. subterraneus* are going to compete with native long-tongued bumblebees for food at the destination environment. The probable consequences of this are increased stress for both participants, which is likely to impose significant costs upon the fitness of the colony's individuals, which may result in activation of ABPV from its subclinical form.
Detected in *Apis mellifera* in Sweden (approximate 5% prevalence) (deMiranda *et al.* 2010) and the UK (Belton 2003). Has not been identified in *Bombus* sp. in UK (Allen & Ball 1996; WOH Hughes, pers comm. Oct 2011) and absent from 2011 tested Swedish *B. subterraneus* queens (n=59, estimated prevalence 0-7.1% [at the 95% confidence level]; we can estimate, with over 90% confidence, that the prevalence in the Swedish population is less than 5%). However following experimental inoculation, infection occurred in *Bombus* sp. (Bailey & Gibbs 1964). Therefore infection in *Bombus* sp. could occur and inter-country strain differences, including differences in virulence, may exist (Carreck 2007). ABPV is in the same family as Kashmir bee virus (KBV) and is serologically, biologically and pathogenically very closely related.	Overall there is a very low likelihood of ABPV infection being present in *B.subterraneus* queens in Sweden. However if present it could be acquired through exposure at shared food sources. If infection ensues there is a medium likelihood of horizontal transmission of a low level of virus at the larval stage from an infected *B. subterraneus* queen, &/or through digesting infected pollen. Yet given *B.subterraneus* has a foraging behaviour adapted to it being a long-tongued species, as compared to the short-tongued honeybee the likelihood of initial exposure combined with the lack of infection demonstrated in our sampled source population would make the likelihood of infection in reintroduced bumblebees very low.	Overall there is a low likelihood of disseminated infection because although the release site is a protected bumblebee reserve and hosts a variety of different bumblebee species living in high densities there is a low likelihood that susceptible bumblebee and honeybee species will be exposed when feeding owing to the limited foraging behaviour of *B.subterraneus* and its late emergence from hibernation.	

CONSEQUENCE ASSESSMENT cont.

Overall the likely biological consequences of infection could include mortality of: the reintroduced bumblebee colony under stressful conditions and given ABPV is not species-specific it is likely to be pathogenic to most bumblebee (experimentally proven) and honeybee species. However there is a low likelihood that a Swedish ABPV strain would spread to both bumblebee and honeybee colonies over a wide area given the limiting foraging behaviour of *B. subterraneus* and its late emergence from hibernation. As such the overall likelihood that ABPV will have significant economic and biological consequences on the native UK bumblebee and honeybee species is low unless these species are highly stressed and infection ensues.

SOURCE HAZARD	RISK ESTIMATION	RISK EVALUATION
Acute bee paralysis virus (ABPV)	The likelihood of release of a Swedish queen infected with ABPV is very low, however if infected the likelihood of the queen horizontally transmitting infection to larvae including the 2nd generation queen for reintroduction is medium. The likelihood of native honeybee and bumblebee exposure is low. There is a low likelihood of epidemic disease if native colonies are under stress. Therefore, because bees at the reintroduction site may be under stress, the overall risk level is considered to be LOW.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

There is no product available for ABPV control. Most viral infections become evident when bees are stressed due to other diseases, weather conditions or management practices including transport. It is highly likely that the process of re-introduction will be stressful.

To minimize the effect of ABPV and other viral infections: Control mite vectors, prior to export physically examine bumblebees for mites. Re-introduce in late spring, as given *B.subterraneus* are late emergers from hibernation they will then be actively foraging and nest-founding in warmer weather to minimize potential temperature stressors caused by temperature fluctuations.

The release environment has been managed for bumblebees therefore there should be adequate food availability to help reduce the host associated mortality of naive UK bumblebees owing to food shortage and competition at the release site.

Table 3 Disease Risk Analysis for the source hazard Apicystis bombi

SOURCE HAZARD	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Apicystis bombi	*A. bombi* is known to infect a wide variety of *Bombus* host species in Sweden (Larsson 2007) and therefore it is possible that one of the reintroduced *B. subterraneus* will be infected with *A. bombi*. *A. bombi.* typically infects adults and oocysts are likely to be ingested via faeco-oral transmission when feeding on flowers previously visited by infected European honeybees or *Bombus* sp. found in the source area. Subsequent infection of their colony through horizontal transmission (Schmid-Hempel 1998) can then occur. However given that honeybees have short tongues (6.5-8.5mm) and *B.subterraneus* have long tongues (approximately 11mm) the flowers these two species visit are likely to differ. Given that *B. subterraneus* is highly likely to be susceptible to infection, and that emerging queens post hibernation may be infected through horizontal colony transmission it is possible that at least one of the reintroduced short-haired bumblebees will be infected with *A. bombi*. However screening results of the source *B. subterraneus* found no evidence of infection therefore the likelihood of exposure seems low.	Upon release, the *B. subterraneus* queens will forage for resources and establish their colonies. These queens if infected could excrete *A bombi* via faeco-oral transmission when feeding on flowers and subsequently infect European honeybees or *Bombus* sp. including the long-tongued species *B. pascuorum, B. muscorum)* found in the release area which could subsequently infect their colony through horizontal transmission (Schmid-Hempel 1998). Given that honeybees have short tongues (6.5-8.5mm) and *B.subterraneus* have long tongues (approximately 11mm) the flowers these two species visit are likely to differ. However the likelihood of exposure to other native long-tongued species is medium. Overall there is a low likelihood of exposure owing to the limited foraging behaviour of *B.subterraneus*, and its late emergence from hibernation. If exposed it is likely that any infected reintroduced queen will disseminate *A. bombi* horizontally upon returning to the colony. However given their late emergence from hibernation there is a low likelihood that *A.bombi* will be widely disseminated through the destination population.	There is a low likelihood that at least one adult bumblebee in the destination environment will be infected, but if infected the ingested oocysts penetrate through the midgut wall into the body cavity and infect the fat body cells in which they grow develop and multiply. This results in a disintegrated fat body and infected colonies are often unable to grow and reproduce (Schmid-Hempel 1998). Energy reserves in bumblebees are stored in the fat body. The fat body reserves provide energy through the winter and into the spring when warm temperatures initiate adult emergence. If fat body reserves are inadequate in the spring, then the bumblebee will be lethargic. If the bee has enough energy to fly to nectar quickly, it may recover. However, if the weather is too cold or wet, or if flowers are scarce or too far, the bee may not survive (Australian hydroponic and greenhouse association 2008). Infection therefore effectively inhibits colony founding and infected queens often die in early spring (Rutrecht & Brown 2007). There is a low likelihood that a Swedish strain would spread to both bumblebee and honeybee colonies over a wide area given the limiting foraging behaviour of *B. subterraneus* and its late emergence from hibernation. As such the overall likelihood that *A.bombi* will have significant economic and biological consequences on native UK bumblebee and honeybee species is low.

JUSTIFICATION OF HAZARD

Reported to infect ten species of *Bombus* in Europe and North and South America, and also *A. mellifera* but bumblebees are the principal hosts (Lippa & Triggiani 1996).

Disease associated with *Apicystis bombi* has been reported in Sweden (Larsson 2007), Finland, France, Italy and Switzerland (Lippa & Triggiani 1996). *A. bombi* is likely to be in other EU countries including the UK (Lippa & Triggiani 1996, Brown, unpublished data 2011). This parasite was absent from sampled *B. subterraneus* queens in Sweden ((n=57, estimated prevalence 0-7.1% [at the 95% confidence level]; we can estimate, with over 90% confidence, that the prevalence in the Swedish population is less than 5%). *Apicystis bombi* is known to be present in the UK and Sweden (M.Brown, pers. comm. Dec 2011) however owing to geographical isolation, strain differences in the two countries may have occurred.

RISK ESTIMATION

There is a low likelihood of exposure owing to the limited foraging behaviour of *B. subterraneus*. However, if exposed, infection can result in severe disease which could inhibit colony founding of native long-tongued *Bombus sp.* and possibly honeybees. But the overall risk of this occurring is LOW given our inability to detect the *A. bombi* in Sweden in the sampled population.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

Any infected queens will likely die during quarantine as the average lifespan of infected queens is <7 days (Rutrecht & Brown 2007). If any bumblebees die in quarantine post-mortem examination should be conducted where characteristic sausage shaped spores may be present in the fat bodies, or mid gut when viewed under the light microscope (Cankaya & Kaftanogu 2006).

Table 4 Disease Risk Analysis for the source hazard Beauveria bassiana

SOURCE HAZARD	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Beauveria bassiana	Bumblebee queens are most likely to be infected by spores which are widespread in the environment, particularly the soil, attaching themselves to the bumblebee's cuticle, to invade or be vectored on its carapace (Kapongo *et al.* 2008a). Bumblebee queens for reintroduction may also acquire the spores when hibernating in the soil as *B. subterraneus* nests are created underground (Benton 2006). It is possible infection may be acquired through environmental contamination or when hibernating but the likelihood of this occurring is low.	Entomopathogenic fungi such as *B. bassiana* infect new hosts via conidia; spores adapted to withstand desiccation and attach to insect cuticles (Boucias *et al.* 1998; Wraight *et al.* 2000). Germination of the spore is temperature (optimum 25°C) (Fargues *et al.* 1997; Hallsworth & Magan 1999) and humidity (Gillespie & Crowford 1986; Hallsworth & Magan 1999) dependant Infection is acquired through spore deposition on an insect's cuticle (Ferron 1978), or in a moist cavity, such as the mouth (Tanada & Kaya 1993). This is followed by formation of a germ tube, which penetrates by enzymatic (Boucias & Pendland 1998) and mechanical action (Hajek & St. Leger 1994) to attach to a new host. This takes about 5 minutes (Boucias *et al* 1988). After a stage of mycelial growth, which eventually kills the host, externally borne conidiophores develop, which create conidia to infect further hosts (Hung *et al.* 1993; Sosa-Gómez & Alves 2000).

B. bassiana would be capable of infecting the queen's progeny, provided the larvae are exposed to a high enough concentration of conidia spores from the queen, however, if the concentration of spores on the queen is high enough to kill her progeny, she herself is also likely to succumb to infection. The queen, after translocation, hibernation, foraging and hive construction is also likely to have a higher susceptibility to infection leading to death, due to the effects of stress and suppression of the immune system, prior to egg laying. However a substantial proportion of the spore load, being vectored by an introduced queen, will have been cast off between her release and egg laying.

Imported queens could disseminate conidia in two ways: if infected with *B. bassiana* infection resulting in mycelial growth, can subsequently infect their progeny through horizontal transmission, or alternatively the queen could vector the agent to a more susceptible host when out feeding. With insects, higher concentrations of experimentally inoculated conidia led to a greater likelihood of mortality and a shorter time to death (Smith *et al* 2000; Kapongo *et al.* 2008a; Kapongo *et al.* 2008b).

The natural intensity of *B. bassiana* spores carried by bumblebees is unknown, but it is suspected to be low (Kapongo *et al* 2008a). | The risk of exposure of a detached vectored spore to native insects at the release site, is high, yet owing to the fungus's suspectibility to UV light which the queen will be exposed to upon release post hibernation it is unlikely the spore concentration will be high enough to cause infection. However if a reintroduced queen becomes infected and dies, and subsequently infects her progeny the likelihood of exposure at the release site will be high especially if strain differences in infectivity exist.

The biological consequences to arthropods are very high. The fungus, after penetrating the cuticle, proliferates as a walled hyphal body or a wall-less protoplast in the host's haemocoel. The host eventually dies of nutrient depletion, invasion of organs, toxicosis or physical obstruction (Hajek 1997; Butt & Goettel 2000). In heavy fungal infestations, bumblebee brood mortality can occur (Macfarlane *et al.* 1995).

Release of the fungus may increase crop yields around the release area, if the crop pests are significantly susceptible to the new strain. However, reduced insect assisted pollination of plants and surrounding crops is likely to be associated with the demise of any *Bombus* sp. hives. The economic consequences of *B. bassiana* introduction will be significant, should a fungal strain establish itself in the environment and prove to be highly infectious to native *Bombus* sp. as their hives provide ideal temperature and humidity conditions for fungal growth (Hokkanen *et al.* 2003). Honeybee hives are maintained at higher temperatures, as a self sterilization practice, preventing the growth and establishment of fungi (Hokkanen *et al.* 2003); therefore honeybees and crops visited by honey bees will not be affected.

Ecosystem dynamics have a low likelihood of being severely affected, even if the introduced strain causes significant mortality to other native insect species. Different strains of the fungus *B. bassiana* are used worldwide in pest management practices, as biological insecticides. A new foreign strain may cause no more damage to the native ecosystem, than use of a new laboratory developed strain. |

SOURCE HAZARD	EXPOSURE ASSESSMENT cont.	CONSEQUENCE ASSESSMENT cont.
Beauveria bassiana	Experimentally in the field, significantly more bumblebees died when administered a high concentration of *B. bassiana* (42-45%) inoculum that at the control inoculum concentration of 5-7% (Kapongo *et al.* 2008a). Therefore if a sufficient dose of *B. bassiana* attaches itself to the cuticle of a particularly susceptible insect host, it has a high likelihood of becoming infected. The risk of disease dissemination of *B. bassiana* to native species is very high as it is the most common fungus isolated from dead insects (Macleod 1954), Most insects are susceptible which explains its use as a insect biological control agent. Numerous bumblebee species have been reportedly infected with *B. bassiana* (Goettel *et al.* 1990) and given the release site is a protected bumblebee reserve site and hosts a variety of different bumblebee species living in high densities the potential for dissemination among native bumblebee species is high. However given conidia on leaves, exposed to sunlight for 24 hours, lose 50-100% of their virulence and viability (Gardner *et al.* 1977) the likelihood of vectoring the agent to a highly susceptible host in the release site is low; owing to the fungus's susceptibility to UV light given the release will occur in late Spring.	The environmental consequences are therefore likely to be negligible. If introduced, the likelihood of biological, economical and environmental consequences occurring is significant to *Bombus* sp. and *Bombus* assisted crop pollination but of little consequence to *Apis* sp. and *Apis* assisted crops.

RISK ESTIMATION	RISK EVALUATION
It is possible that one of the short-haired bumblebee queens upon importation will carry the hazard, *B. bassiana*. At the release site, the risk of disease dissemination to native insect species is high. However there is a low probability of being exposed to sufficient spores to cause infection owing to inactivation by UV light as the *B.subterraneus* will be released in late spring. Overall, the biological, economical and environmental consequences of novel fungus introduction are significant to *Bombus* sp. but the overall risk to the reintroduction is considered to be LOW.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

If any bees are affected by fungal disease during the quarantine period, exposure to simulated sunlight should be considered in an attempt to reduce the fungal load of affected individuals and/or others deemed particularly susceptible. Sunlight (UV) (natural or artificial) has been shown to reduce the viability of fungal spores (Morley-Davies et al. 1995). We recommend the use of a UV light with high UV-B capabilities to be used for 6.5 hours over one day. The recommended UV light to use would be the Zoo med Reptisun 10® (Specified output: up to 15% UVA : 10% UVB) it is the product with the highest UV-B light emission currently available. Wavelengths in the 285 to 315 nm range (UV-B) are the most damaging to fungi (Morley-Davies *et al.* 1995).

Table 5 Disease Risk Analysis for the source hazard Crithidia bombi

SOURCE HAZARD	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Crithidia bombi	*C. bombi* is known to infect a wide variety of *Bombus* host species (Schmid-Hempel 1998; Benton 2006, Larsson 2007) and screening results estimated a low prevalence of infection in Swedish *B. subterraneus* queens (1.2-15.7%).	*Crithidia* cells are passed out in the host's faeces; however 1000's are needed to be ingested for infection to ensue (Ruiz-Gonzalez & Brown 2006). Bees are infected through contact with infected cells on nest surfaces (Schmid-Hempel 2001). Inter-nest transmission is accomplished through the drifting of infected workers to other nests (Sakofski & Koeniger 1988; Schmid-Hempel 1998), or more importantly, through visitation to flower surfaces previously infected by a shedding forager (Durrer & Schmid-Hempel 1994). The success of floral transmission is between 20-40%. Short-haired bumblebee queens emerge from hibernation late in the season in June (Benton 2006). Therefore, *B subterraneus* queens with a low prevalence of *C bombi* infection will be released into an environment containing high densities of infected early season workers and if strain differences exist infection of the workers with *C* bombi from the queens may ensue. However, it is more likely the reintroduced queens will be infected with *Crithidia* from native bees as *B. subterraneus* are late emergers from hibernation and will be reintroduced in late May/early June by which time the annual *Crithidia* 'epidemic' will have already commenced at the destination site (M.Brown, pers comm. Dec 2011).	As far as the authors are aware *C. bombi* is genus-specific to bumblebees and is not communicable to other Hymenoptera (Ruiz-Gonzáles & Brown 2006). The exposure assessment showed that there is a medium likelihood that native species will be exposed and *C. bombi* will be disseminated and therefore the likelihood of at least one UK native bumblebee becoming infected is medium.

JUSTIFICATION OF HAZARD

Research has repeatedly confirmed the presence of the agent in Britain (Henson *et al.* 2009; M. Brown personal comm. 2011), Switzerland (Schmid-Hempel & Schmid-Hempel 1993) & Sweden (Larsson 2007). *C.bombi* was isolated from 3/57 *B.subterraneus* queens in the source population in 2011 (n=57, estimated prevalence 1.2-15.7% [at a confidence level of 95%]). However as *C. bombi* in Sweden has been geographically isolated from *C bombi* in the UK it is highly likely that genetic differences exist. Given that most strains of *C. bombi* differ in their infectivity to other colonies, through the genetic characteristics of the host line (Schmid-Hempel 2001) and that there is evidence from Switzerland that strain differences alter the intensity of infection (Schmid-Hempel & Schmid-Hempel 1993) and pathogenicity (Imhoof & Schmid-Hempel 1998): there is a high likelihood that *C. bombi* present in Sweden could be pathogenic to bumblebees in Britain.

Exposure of queens destined for reintroduction is likely to occur through contact with infected cells on nest surfaces (Schmid-Hempel 2001) or through visiting flower surfaces previously infected by a shedding forager (Durrer & Schmid-Hempel 1994). The likelihood of a queen becoming infected increases considerably as the season progresses, due to exponential dissemination of *C bombi* from an increasing number of infected colonies to uninfected colonies (Imahoof & Schmid-Hempel 1999; Schmid-Hempel 2001). The seasonal prevalence of *C. bombi*, means that infection prevalence is high when the late emerging *B.subterraneus* queens emerge and it is therefore likely that the *B. subterraneus* queens to be reintroduced will be exposed.

Given that *B. subterraneus* is susceptible to infection, that emerging queens are most likely to be infected and infection prevalence is low, it is highly likely that at least one of the short-haired bumblebees collected for reintroduction will be infected with *C. bombi.*

The likelihood that an infected *B. subterraneus* will encounter other bumblebee species at the release site is high, as the release site is a protected bumblebee reserve site and hosts a variety of different bumblebee species living in high densities. Exposure of native colonies to *C. bombi* is likely to occur through foraging behaviour. Given immune challenged bees perform poorly in memory tests (Riddell & Mallon 2005) it is possible that sick bees may have trouble navigating in their environment or remembering which flowers they have already visited: these alterations in the host's behaviour, may increase the chances of inter-colony drifting and subsequent pathogen transfer to other colonies or species.

After natural infection of *Bombus impatiens*, the average number of days needed for *C. bombi* to reach a stable faecal pathogen load was 12 (Otterstatter & Thomson 2006). However, *B. terrestris* faecal pathogen load can differ substantially between individuals, depending on the host's nutritional status (Logan *et al.* 2005). The mortality rate of *Bombus sp.* infected with *C. bombi* is condition dependent and can be up to 50% higher in infected *B. terrestris* workers under stressful starving conditions (Brown *et al.* 2000). Food shortage and other stressful conditions are likely to be more common in younger colonies (Schmid-Hempel 2001).

In the first crucial 25 days of a colony cycle, colonies naturally infected with *C bombi* grew at a slower rate and had a smaller worker force (Schmid-Hempel 2001). It has been proposed that pollen starved bees may re-allocate either: ovary development resources to combat infection (Moret & Schmid-Hempel 2000), or fat stores for post infection survival (Brown *et al.* 2003a). Furthermore, workers in infected colonies lay their own eggs 5 days later than in uninfected colonies, leading to later queen and drone emergence (Shykoff & Schmid-Hempel 1991a).

Delayed production of reproductive bumblebees, has significant implications on queen survival throughout hibernation (Schmid-Hempel 2001) and colony-founding. Hibernation is a stressful activity that depends on the utilization of limited fat stores for survival. Following the allocation of resources for hibernation, queens must then use their remaining reserves for foraging and other metabolically expensive activities necessary to found a colony (Brown *et al.* 2003b).

SOURCE HAZARD	EXPOSURE ASSESSMENT cont.	CONSEQUENCE ASSESSMENT cont.

SOURCE HAZARD

Crithidia bombi

EXPOSURE ASSESSMENT cont.

Bumblebees with a stimulated immune system also consume more nectar (Tyler *et al.* 2006) which extends the time an infected worker has to shed the infection at a flower. As the intensity of *C. bombi* infection increases in a foraging bumblebee's gut, the bumblebee spends 14% longer in contact with flowers but visits 10% fewer flowers per day, in comparison to a non-infected bumblebee (Otterstatter & Thomson 2006).

Severely infected bumblebees have been shown to contribute the most to horizontal transmission at flowers because they excrete larger quantities of *C. bombi* in their faeces (Otterstatter & Thomson 2006). Given that bee species present at the release site will potentially be naive to any genetically different reintroduced *C bombi* strain, these native colonies may develop a high intensity and prevalence of infection.

There is a high likelihood that infected *B subterraneus* queens will show altered behaviour, and increased drifting which will increase the likelihood of dissemination to other native colonies at the densely co-inhabited release site. These native bumblebees have a medium likelihood of developing a high intensity and prevalence of infection if strain differences exist further aiding transmission and dissemination.

CONSEQUENCE ASSESSMENT cont.

The likelihood that an introduced *C. bombi* strain will lead to the demise of at least one native bumblebee colony is medium. However, such a consequence might be natural for bumblebee species in the UK. Each year only a few bumblebee queens establish a colony, and these will have been selected through their response to parasitism or disease, and these bumblebee family lines dominate the population for the rest of the season. Over successive generations, the parasite will adapt to these hosts leading to a reduced ability to exploit the less dominant genotype of bee in the following season (Yourth & Schmid-Hempel 2006), a process known as negative frequency dependent selection. So although the release of the infectious agent into the environment will cause a drop in the amount of genetic variability across the population, it is likely that the effects will only last until the next season.

The biological consequences are the probable demise of native bumblebee colonies under stressful conditions and/or, a significant reduction in the probability that a queen will successfully hibernate and start a colony the next season. There is a high likelihood that both events will occur over a wide area because the infection will be disseminated and *C bombi* infects and is pathogenic to many bumblebee species. However once nests have been destroyed reduced competition for food will reduce stress and reduce the pathogenicity of *C. bombi* infection and therefore the biological consequences are low. Furthermore given native bumblebees are already exposed to differing strains of *C. bombi,* and have adapted to these, there is a high likelihood that they will adapt to new Swedish strains.

There is a low likelihood of environmental and economic consequences because the effects of the introduction of a new *C. bombi* strain will be brief, ecosystem stability is unlikely to be severely affected and it is highly unlikely that *C. bombi* infection will extirpate species as the current populations cope with existing *C. bombi* infections and will likely adapt to new strains from Sweden and the surviving populations will recover the following season. Furthermore as stated in the exposure assessment is more likely the reintroduced queens will be infected with *Crithidia* from native bees who will already be well into the *Crithidia* epidemic.

RISK ESTIMATION

It is of medium likelihood that one of the short-haired bumblebee queens upon importation will carry a novel strain of *C bombi* as the parasite is known to be present in the source population although the estimated prevalence is low (5%). There is a medium likelihood of exposure of susceptible bumblebee species at the release site. The biological, environmental and economic consequences are low principally because native bumblebees are probably already exposed to differing strains of *C bombi*. The overall risk level is considered to be MEDIUM.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

Faecal samples should be collected from all queens in quarantine and tested for *C.bombi* by microscopic examination of the faeces within 14 days of arrival in England. *C.bombi* is a protozoan that has been studied extensively in Europe, where it infects 10–30% of bumblebees on average (Schmid-Hempel, 1998, 2001). Faecal screening for *C. bombi* is a highly sensitive (proportion of infected individuals correctly identified=0.93) and specific (proportion of non-infected individuals correctly identified=0.94) test for an active infection (Otterstatter and Thomson 2006). Any infected individuals should not be

Table 6 Disease Risk Analysis for the source hazard Deformed Wing Virus

SOURCE HAZARD	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Deformed Wing Virus (DWV)	In Sweden the *B. subterraneus* queens destined for reintroduction will forage for resource. These bumblebee queens could be exposed via faeco-oral transmission when feeding at sites visited by honeybees, leading to subsequent horizontal transmission within an infected colony (Yue & Genersch 2005). Although both honeybees and bumblebees are susceptible, given that honeybees have short tongues (6.5-8.5mm) and *B.subterraneus* have long tongues with a tongue length of 11mm (the flowers these two species visit and subsequently shed virus on, are likely to differ. Furthermore given *B. subterraneus* are late developing species, they are less efficient at widely disseminating the infection horizontally between colonies, as they have less time within a season to do so.	Upon release, the *B. subterraneus* queens will forage for resources and establish their colonies. If the *B. subterraneus* queens are covertly or asymptomatically infected with DWV acquired in Sweden, and UK bumblebees and honeybees are possibly naïve to the Swedish strain, factors which may significantly increase stress on individuals over the course of the reintroduction may precipitate clinical disease in UK bumblebees and honeybees. In addition inclement weather, and unfavourable flying conditions for long periods of time will keep bees in their nests which may lead to in-nest faecal deposition, a major source of replicating viruses. All the above factors, including infestation with the *Varroa* mite may lead to the development of clinically symptomatic infection (Beelogics, 2010).	There is a low likelihood that at least one bee at the reintroduction site will be infected. DWV infection in honeybees following transmission by *Varroa destructor* has been associated with clinical symptoms including crippled wings, a bloated and shortened abdomen and discolouration (Ball & Allen 1988). Most importantly DWV has been associated with CCD (Yue *et al.* 2007). Honeybees infected with DWV have a reduction in life span (Kovac & Crailsheim 1988) symptomatic individuals live <67 hours post emergence from the pupa stage (Yang & Cox-Foster 2007). DWV is pathogenic to at least two bumble bee species (*B. terrestris* and *B. pascuorum*) causing wing deformity similar to clinically DWV-infected honey bees (Genersch *et al.* 2006).

<table>

JUSTIFICATION OF HAZARD

Deformed wing virus (DWV) is a viral pathogen of *Apis mellifera* associated with colony collapse disorder (CCD) when transmitted by the *Varroa destructor* mite (Yue *et al.* 2007). It is pathogenic to both honeybees and bumblebees (Genersch *et al.* 2006).

DWV has been found in Sweden (Nordstrom *et al.* 1999) and has been reported in southern England (Highfield *et al.* 2009). DWV was absent from *B.subterraneus* queens in the source population in 2011 (n=59, estimated prevalence 0-7.1% [at the 95% confidence level]; we can estimate, with over 90% confidence, that the prevalence in the Swedish population is less than 5%) however a UK wide study found DWV in a 1/3 of their *B. terrestris* samples, and a small number of *B. pascuorum* were also positive. *B. lapidarius, B. pratorum* and *B. hortorum* were virus free (WOH Hughes, pers. comm. November 2011). It is possible that strain differences exist between DWV in Sweden and DWV in England given the geographical isolation between the source and destination environments.

Venereal transmission via infected semen has also been reported (Yue *et al.* 2007). Once infected, vertical transovarial transmission will disseminate infection amongst the colony (Yue *et al.* 2007). DWV has been detected in all life stages of European honey bees and all workers drones and queens with wing deformities harbour the virus (Chen *et al.* 2005, Williams *et al.* 2009). Exposure to DWV via the mite vector *Varroa* is also possible. The *Varroa* mite is present in Sweden, and Swedish honeybees with DWV are in contact with *Varroa* sp. and cross-species transmission has been reported in one species of bumblebee, the American bumblebee (*Bombus pennsylvanicus*) (Ongus 2006). However *Varroa* has not been reported to infest European bumblebees (G. Budge, personal communication October 2009). Therefore there is a very low likelihood of exposure to DWV via the *Varroa* mite in Sweden.

As soon as elevated virus titers are reached, the virus becomes virulent and clinically symptomatic disease results (Genersch *et al.* 2010). There is a medium likelihood that significantly stressful events will occur during reintroduction which may lead to DWV disease.

If the above mentioned stress factors exist then it is likely that many of the reintroduced bumblebees will develop clinical disease. This may be lethal in which no further dissemination will occur. Alternatively, infected queens will transmit DWV through transovarial transmission to workers and then horizontal transmission from worker to larvae. The establishment of infection of at least one parasite in bumblebee colonies, and spread thereafter, increases in likelihood with the percentage of workers infected during larval development (Rutrecht & Brown 2007). DWV could most likely be passed from colony to colony through interactions at shared food resources (Durrer & Schmid-Hempel 1994).

There is a high likelihood that disseminated infection would occur if the virus is introduced in *B. subterraneus*, as the release site is a protected bumblebee reserve site and hosts a variety of different bumblebee species living in high densities. Honeybee species are also susceptible and found in the release area.

Yue *et al.* (2007) reported the importance of the *Varroa* vector for the development of overt (symptomatic) disease. If DWV was transmitted vertically within a colony in the absence of *Varroa,* individual honeybee fitness was unlikely to be affected. The infected colony even when harbouring covertly infected (asymptomatic) individuals will develop normally and eventually swarm to transmit the virus vertically to the next colony generation allowing long term population persistence. Overt (symptomatic) infection was reported when individuals were subjected to a strong immunosuppressive trigger, such as the *Varroa* mite. However there is a very low likelihood of *B. subterraneous* exposure to DWV via the *Varroa* mite in Sweden.

The potential biological consequences of DWV are severe. In the UK spring of 2007 increased honeybee mortality paralleled the effects of CCD in the US. In the winter of 2006-2007 between 651 000 and 875 000 of the US nation's estimated 2.4 million colonies were lost and over 25% of these deaths were consistent with CCD. DWV was one of the viral diseases associated with CCD (Underwood & vanEngelsdorp 2007). A subsequent UK survey examining honeybee loss in the spring of 2007 found the majority of honeybees contained DWV, which was attributed to be one of the causes of population loss (Budge 2007).

</table>

SOURCE HAZARD	RELEASE ASSESSMENT cont.	CONSEQUENCE ASSESSMENT cont.

SOURCE HAZARD

Deformed Wing Virus (DWV)

RELEASE ASSESSMENT cont.

Overall there is a low likelihood of exposure in Sweden as DWV was absent from tested *B.subterraneus* (estimated prevalence 0-7.1% [at the 95% confidence level]). *B. subterraneus* has a different feeding pattern to honeybees and is a late emerger from hibernation. However if exposed it is likely that any infected reintroduced queen will initially transmit DWV through transovarial transmission and then horizontal transmission from worker to larvae.

CONSEQUENCE ASSESSMENT cont.

Honeybees are essential for the pollination of over 90 fruit and vegetable crops worldwide, with the economic value of these agricultural products placed at more than $14.6 billion in the U.S. Like the US the UK honeybee industry is valued at an estimated £200m a year, and the retail value of pollination is valued closer to £1billion (Holland 2009). As such the economic consequences of infection could be severe.

However it is most likely that only the reintroduced queens and their offspring would be affected with covert (asymptomatic) infection. But this could lead to a failure of the reintroduction if the bumblebees were subjected to severe stressors on release especially if strain differences exist between the virus in Sweden and that found in the UK. This could considerably extend the reintroduction stage of the project and therefore significantly increase the economic cost of the introduction.

Ecosystem dynamics have a low likelihood of being severely affected, as the most likely individuals to be affected are the reintroduced queens. Although honeybees could be affected the different feeding pattern and late emergence from hibernation of *B. subterraneus* makes dissemination to honeybees of low likelihood.

RISK ESTIMATION

There is a low likelihood of exposure, but if exposed a high likelihood of covert (asymptomatic) infection and dissemination through vertical transmission. Evidence suggests that the likelihood of significant epidemic disease is high if severe stressors occur during the reintroduction, or if strain differences exist between Sweden and UK. Therefore the overall risk level is MEDIUM.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

There is no product available for DWV control. To minimize the impact of DWV and other viral infections: Release in late spring, as given *B.subterraneus* are late emergers from hibernation they will then exit the nest in warmer weather and release into an optimum environment managed for bumblebees to minimize competition at food sources.

Table 7 Disease Risk Analysis for the source hazard Locustacarus buchneri

SOURCE HAZARD	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Locustacarus buchneri **JUSTIFICATION OF HAZARD** *Locustacarus buchneri* is present in the UK (Donovan 1980) and Sweden (Larsson 2007). However *L. buchneri* was absent from the 57 *B.subterraneus* queens sampled from the source population in 2011 (n=57, estimated prevalence 0-7.1% [at the 95% confidence level]; we can estimate, with over 90% confidence, that the prevalence in the Swedish population is less than 5%). Whether differences in virulence exist between *L. buchneri* populations, is currently unknown (Goulson, D personal communication 1 June 2009). However, as the Swedish and British populations are geographically isolated there is a high likelihood that genetic differences between the populations have arisen and as a result, the agent is considered a potential hazard. However there is no causal evidence that *L. buchneri* has any negative impact on bumblebees.	*L. buchneri* is an internal mite that infests the trachea of the bumblebee host. Queens for reintroduction could acquire infection pre-hibernation from other infected bumblebee hosts in the source environment. The mite overwinters in queens and as a consequence, re-emerges in the spring (Otterstatter & Whidden 2004). Therefore mites infesting young queens at the end of the season will survive through hibernation (Husband & Sinha 1970). In Europe up to 100% of newly emerged queens can be parasitized by the mite (Schmid-Hempel 1998). However in screening 57 *B. subterraneus* queens from the source population we were not able to detect the mite.	*Locustacarus* sp. mites undergo all their developmental stages, within their bumblebee hosts: egg > larviform female or adult male > adult female (Husband & Sinha 1970). Larviform female and adult male mites hatch and mate inside the host (Otterstatter & Whidden 2004). The fertilized female larviform stage leaves the adult host through its spiracles (Benton 2006) & relocates to the trachea of other bumblebees (Otterstatter & Whidden 2004). There, after two weeks the larviform female molts and grows into a fully developed immobile egg laying adult female (Husband & Sinha 1970; Yoneda *et al.* 2008). After a further two weeks, the new female's eggs hatch into mobile larviform female and adult males (Yoneda *et al.* 2008) and thus mobile mites are produced at four week intervals. The larviform female mites usually move from adult bees, to 3rd and 4th instar bumblebee larvae. This occurs when the protective wax pollen wall is ruptured, during the developmental phase of regurgitative feeding by bee workers (Yoneda *et al.* 2008). Heavier larvae are reported to be infested with mites which suggests that the new queens can become infested at the larval stage, to promote and maximise the likelihood of transmission to next season's colonies. Adult bumblebees have significantly higher mite infestations, four to five weeks post emergence which suggests that some mite offspring, after hatching in the host's trachea, stay there and metamorphose, instead of seeking a new host (Yoneda *et al.* 2008). Thus, older bumblebees with higher intensity infections are more likely to disseminate the mites to other colonies when they forage, through drifting. The prevalence of infestation in workers is higher than in males (Otterstatter & Whidden 2004) and mites may preferentially infest workers because the workers are responsible for taking care of the brood increasing the chances of larval bumble bees being exposed to fertilized larviform mites.	It is of low likelihood that one native bumblebee will become infested by an adult female mite transmitted from an introduced *B subterraneus* at the release site owing to the absence of infection in the screened source population. However if infected the mite could adversely affect bumblebee host health (Husband & Sinha 1970): as adult female mites pierce the trachea of their hosts and suck haemolymph from inside the body cavity (Husband & Sinha 1970; Benton 2006). Heavy host infestation with *L. buchneri* has been associated with physical damage to the trachea and lethargy; this in turn is correlated with impeded or cessation of foraging and diarrhoea (Husband & Sinha 1970; Alford 1975). Field caught workers harbouring the mite, showed shorter lifespans in captivity than unparasitized bumblebees (Otterstatter & Whidden 2004). Consequently, at high levels of infestation, *L. buchneri* could jeopardise colony survival (Schmid-Hempel 2001). However, the studies above were correlational, and no causal evidence for a negative effect of *L. buchneri* exists. In addition, a positive correlation between infection and bee health exists (Rutrecht & Brown 2007). The tracheal mite *L. buchneri* appears to be species specific and preferentially parasitizes some bumblebee species, specifically the subgenus *Bombus sensu stricto* in Canada (Otterstatter & Whidden 2004), although no subgenus assocations exist in Europe (Stammer 1951, Shykoff & Schmid-Hempel 1991c). *Psithyrus*, non-colony forming parasitic cuckoo bumblebees, are less affected. *L. buchneri* is already present in the UK but native bumblebees may have weaker immune responses to a different genetic strain from Sweden. Therefore overall there is a low likelihood that dissemination of this strain could lead to significant biological, environmental and economic consequences through demise of native bee colonies, owing to the absence of infection in the source population and the lack of demonstrated negative causal effect.

SOURCE HAZARD	EXPOSURE ASSESSMENT cont.

SOURCE HAZARD

Locustacarus buchneri

EXPOSURE ASSESSMENT cont.

Field caught bumblebees have an average prevalence of infection of 10% (Macfarlane *et al.* 1995), although species differences do occur (Otterstatter & Whidden 2004). There is a high likelihood that an infected queen *B. subterraneus* will infest her brood and eventually infest other native bee colonies with *L. buchneri* through transmission by older drifting bumblebees. Dungeness, the release site, is an exceptionally fauna rich area, supporting 13 other bumblebee species (Williams 1986) and therefore once one native colony is infested the high density of new hosts provides appropriate conditions for dissemination and there is a high likelihood that *L. buchneri* recorded from a wide range of bumblebee species (Husband & Sinha 1970), will be disseminated and become established throughout the release area.

RISK ESTIMATION

It is of low likelihood that one of the reintroduced bumblebee queens, upon importation will carry the agent, because infestation was not detected during screening of the source population and therefore if present L buchneri is at low prevalence. However, if infected, queens have a high likelihood of disseminating the agent to susceptible hosts in the release area but the biological, economical and environmental consequences will be low owing to the lack of demonstrated causal effect, and the species-specificity of the mite. Therefore overall risk level is considered to be LOW.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

The queen bumblebees should be kept isolated from each other in quarantine, and periodic microscopic examination for mobile mites on the bumblebee queens and in the quarantine area, should be employed to detect mite infestation. Infested queens should not be reintroduced.

Table 8 Disease Risk Analysis for the source hazard Nosema bombi

SOURCE HAZARD	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Nosema bombi	Larsson (2007) studied 16 species of bumblebees in Sweden and Denmark and found *N.bombi* in all species including *B. subterraneus*. However only worker *B.subterraneus* were infected (n=21) with a 19.2% prevalence over a three year period. We found no evidence of infection in *B.subterraneus* queens collected from the source population in 2011.	If *B. subterraneus* queens are infected the likelihood of exposure and dissemination of *N. bombi* to native *Bombus* species is highly likely as 15 British species are known to be susceptible to *N. bombi* (Larsson 2007), Our understanding of *N bombi*'s transmission to other *Hymenoptera* spp. is inconclusive.	It is of low likelihood that a bumblebee at the reintroduction site will be infected by *N. bombi*. However if infected the malpighian tubules can become extremely enlarged by the parasite (Larsson 2007). In some cases they may be destroyed, releasing mature spores into the lumen (Otti & Schmid-Hempel 2007). Heavily infected bumblebees may lose their power of flight (Fantham & Porter 1914; Larsson 2007); become lethargic and clumsy (Larsson 2007; Otti & Schmid-Hempel 2007) or develop distended abdomens (Macfarlane *et al.* 1995; Otti & Schmid-Hempel 2007).

SOURCE HAZARD

Nosema bombi

JUSTIFICATION OF HAZARD

Nosema bombi is present in the source (Larsson 2007) and destination (Alford 1975) environment. However *N. bombi* was absent from *B.subterraneus* queens screened from the source population in 2011 (n=57, estimated prevalence 0-7.1% [at the 95% confidence level]; we can estimate, with over 90% confidence, that the prevalence in the Swedish population is less than 5%). Evidence suggests that the microsporidian may also have differing effects across host species; Otti & Schmid Hempel (2008) found infections to be severe in *B. terrestris* hosts, while Rutrecht & Brown (2009) found the results of infection to be negligible to fitness in *B. lucorum* hosts. This suggests that *N. bombi* strains may differ in their virulence to hosts. Alternatively variable host life-history may account for these differences. *N. bombi* is known to be transmissible to *B. subterraneus* (Tay *et al.* 2005; Larsson 2007) and it is possible that there may be strain differences between the UK and Sweden owing to geographical isolation, although Rutrecht & Brown (2009) suggested the absence of strain variation and genetic studies have identified no species- or geographica-specificity in *N. bombi* strains (Tay *et al.* 2005).

RELEASE ASSESSMENT

N. bombi could be acquired by a queen destined for reintroduction through interactions at shared food resources (Durrer & Schmid-Hempel 1994) or through the drifting of infected workers to the nest of a queen destined for reintroduction (Sakofski & Koeniger 1988; Schmid-Hempel 1998). The intensity of infection in a *N. bombi* infected nest increases exponentially, reaching its peak at the end of a season (Rutrecht & Brown 2008b). Given *B. subterraneus* are late colony developers (Benton 2006), their shorter exposure may allow more low-intensity infected hosts to survive the winter (Otti & Schmid-Hempel 2007). Consequently, the prevalence of *N. bombi* in *B. subterraneus* queens may be higher than in earlier emerging spring queens. The prevalence of *N. bombi* in *B. subterraneus* on account of the latter's late seasonal development imply there is a high likelihood that reintroduced *B. subterraneous* queens will be infected however the lack of infection demonstrated in our screening demonstrates there is a low likelihood of a reintroduced queen being exposed and infected.

EXPOSURE ASSESSMENT

N. bombi is passed from colony to colony through interactions at shared food resources (Durrer & Schmid-Hempel 1994) or through the drifting of infected workers to other nests (Sakofski & Koeniger 1988; Schmid-Hempel 1998). It is likely that the introduced *B. subterraneus* will compete with native bumblebees for food; the probable consequence of this struggle is increased stress for both participants. Bumblebees with a stimulated immune system show increased nectar consumption (Tyler *et al.* 2006), extending the time an infected worker spends shedding the agent at a flower. Immune challenged bees also perform poorly in memory tests (Riddell & Mallon 2005). Therefore, sick bees may have trouble navigating in their environment or remembering which flowers they have visited: which may increase the chances of inter-colony drifting and subsequent transfer of *N bombi* to other colonies or species.

It is likely that infected queens will then transmit the *N. bombi* infection vertically to larvae (Rutrecht & Brown 2008b) and then from larvae to worker. Spores are also released to, and acquired from the environment (Imhoof & Schmid-Hempel 1999) through the decay of a dead infected host or by shedding the spores in the bumblebee's faeces (Cali & Takvorian 1999). The spread of infection in bumblebee colonies is dependent on the percentage of workers infected during larval development (Rutrecht & Brown 2007) because larvae are significantly more susceptible than adults (van den Eijnde & Vette 1993). Most bumblebees spend the first few days after emergence in the nest, leading to an accumulation of infective material, through faecal shedding. Such faecal shedding increases the intensity of infection in the next generation of larvae (Rutrecht & Brown 2008b), as the agent may be horizontally transmitted when a larva/bumblebee ingests the parasite (McIvor & Malone 1995; Otti & Schmid-Hempel 2008).

CONSEQUENCE ASSESSMENT

Queens exhibit very little infection associated morbidity and mortality to *N. bombi* (Fisher & Pomeroy 1989) and queen survival is unaffected (Otti & Schmid-Hempel 2007). Infection with *N. bombi* may have no apparent impact on a queen's capacity to create a nest (Fisher & Pomeroy 1989; Otti & Schmid-Hempel 2007). However, controlled colony infections show the significant negative impact *N. bombi* can have on colony reproduction and growth (Otti & Schmid Hempel 2007). Uninfected *B. terrestris* worker survival is significantly better than that of *N. bombi* contaminated workers and males over 21 days old (Otti & Schmid-Hempel 2007). In the field, reduced worker survival significantly hinders an infected colony from being able to gather enough resources to produce gynes, sexual adults (Otti & Schmid-Hempel 2008). Furthermore the ability of infected gynes to produce their own offspring is considerably reduced (Otti & Schmid-Hempel 2007). However, similar experiments in *B. lucorum* found much lower impacts of *N. bombi* on host fitness (Rutrecht & Brown 2009). The net result is that *N. bombi* infection lowers colony fitness.

In America the collapse of commercial *B. occidentalis* populations are thought to be attributable to *N. bombi* infection (Whittington & Winston 2004; Velthius & van Doom 2006). *N. bombi* may have considerable effects on the size of bumblebee populations, especially if the introduced strain is highly virulent (Williams 1986). The loss of bumblebee nests will have environmental and economic consequences through reduced pollination. Thus the consequences of pathogen introduction are high, biologically, environmentally and economically.

SOURCE HAZARD

Nosema bombi

EXPOSURE ASSESSMENT cont.

A colony's susceptibility to infection also increases over its lifespan because closely related kin acquire parasitic infections more easily, as the parasite adapts to its). host (Schmid-Hempel & Crozier 1999; Shykoff & Schmid-Hempel 1991b). After experimental infection, 89% of *B. terrestris* workers derived from an infected queen harboured the *N. bombi* pathogen (Otti & Schmid-Hempel 2008). Prevalence, and infection intensity of, drones is significantly dependent on the prevalence and intensity of infection in the workers. Infection intensity in drones rises as a colony ages (Rutrecht & Brown 2008b). It is therefore highly likely that an infected queen, that establishes a colony, will propagate infected workers and drones, with both a high prevalence, and intensity of infection and will be capable of spreading *N. bombi* to other colonies.

Given that *B. subterraneus* is a long tongued bumblebee (Goulson *et al*. 2005), the flowers it visits and subsequently sheds spores on, are more likely to be visited by other native long tongued species. Therefore the limited foraging behaviour of *B. subterraneus* may reduce the speed of spread of *N. bombi*. Late developing species, such as *B. subterraneus*, are also less efficient at widely disseminating the infection horizontally between colonies, as they have less time within a season to do so. However, infected males can infect healthy queens during copulation (Otti & Schmid-Hempel 2007), potentially spreading the agent to secondary colonies of *B. subterraneus* that will be established by the progeny of the uninfected introduced queens. The agent is unlikely to be transmitted to native species via copulation: although trans-species coupling has been rarely observed (Benton 2006).

Given that *N. bombi* dissemination is more effective when early season species harbour infection, and almost every individual in a bumblebee colony is susceptible, there is a high likelihood that the agent will be disseminated locally, with a wider spread and more significant infection (higher intensity, higher prevalence) the following year because there is a high likelihood that the agent will be contracted by some of the early developing *Bombus* spp. queens. The culmination of these factors increases the likelihood of the development of a bumblebee host that is capable of shedding a large quantity of spores into the environment. As 15 British species are known to be susceptible to *N. bombi* (Larsson 2007), it is extremely likely that a native bumblebee will become infected from an infected reintroduced *B. subterraneus* queen, but the likelihood of a *B. subterraneus* queen being infected is low owing to the absence of infection in the screened source population.

RISK ESTIMATION

It is of low likelihood that one of the short haired bumblebee queens upon importation will carry the hazard, as *N.bombi* was not detected from the screened source population. However if exposed, 15 sp. of bumblebees at the release site are known to be susceptible. The biological consequences and the risk of environmental and economical consequences are substantial. However the reported absence of strain variation, and the lack of infection in the source population, means the overall risk level is considered to be LOW.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

Any bumblebees that die over the quarantine period should have a *post-mortem* examination undertaken by a veterinarian or pathologist. The abdomen should be examined for the microsporidian *N. bombi*. *N. apis* in honey bees is frequently treated successfully with fumagillin, however, this has not been found to work effectively against *N. bombi* in bumblebees (Whittington & Winston 2003).

Table 9 Disease Risk Analysis for the source hazards entomopathogenic fungi

SOURCE HAZARD *Paecilomyces farinosus, Verticillium lecanii, Metarhizium anisopliae*	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
JUSTIFICATION OF HAZARD *Paecilomyces farinosus, Verticillium lecanii, Metarhizium anisopliae* are not exotic to Britain, and both the source (Subinprasert 1987, Vanninen 1995) and destination (Leatherdale 1970; Macfarlane *et al.* 1995) environment harbour these pathogens. *Paecilomyces farinosus, Verticillium lecanii* and *Metarhizium anisopliae* are fungi and are known to be transmissible to bumblebees (Schmid-Hempel 1998; Goettel *et al.* 1990) and a wide variety of insect hosts (Leatherdale 1970). Isolates from different countries have varying levels of pathogenicity (Liu *et al.* 2002; Yeo *et al.* 2003) and it is possible that strain differences exist.	*Paecilomyces farinosus, Verticillium lecanii, Metarhizium anisopliae* have not been detected in *B. subterraneus*. However, bumblebees and other insects are known to vector entomopathogenic fungal spores (Dromph 2001; Kapongo *et al.* 2008a; Kapongo *et al.* 2008b). Spores are widespread in the environment, particularly in the soil, as this is where the decomposing bodies of fungal infected insects are situated (Mietkiewski & Tkaczuk 1998). Queens destined for reintroduction could be exposed to these agents when spores attach themselves to the queens's cuticle, to invade or be vectored on its carapace (Kapongo *et al.* 2008a). However the concentration of spores vectored naturally on a healthy queen's cuticle is likely to be very low (Kapongo *et al.* 2008a/b). Bumblebee queens may also acquire the spores when hibernating in the soil as *B. subterraneous* nests are created underground (Benton 2006). However the likelihood of infection through hibernating in the soil is unlikely as queens for re-introduction will be housed indoors. Overall there is a low likelihood of the release of *B subterraneus* infected with these fungi.	Entomopathogenic fungi infect new hosts via conidia which are spores adapted to withstand desiccation which attach to insect cuticles (Boucias *et al.* 1998; Wraight *et al.* 2000). Germination of the spores is a temperature (*Metarhizium anisopliae* (optimum 30°C), *Paecilomyces farinosus* (optimum 20°C) (Hallsworth & Magan 1999; Fariah & Wraight 2001)) & humidity (Gillespie & Crowford 1986; Hallsworth & Magan 1999; Chandler *et al.* 1994) dependant process. Insects become infected with entomopathogenic fungi when spores are deposited on their cuticle (Ferron 1978; Faria & Wraight 2001) or enter through body openings or are ingested (Schmid-Hempel 1998). Entry is followed by formation of a germ tube, which penetrates by enzymatic (Boucias & Pendland 1998; Askary *et al.* 1999; Lopes-Llorca *et al.* 2002) & mechanical action (Goettel *et al.* 1989; Hajek & St. Leger 1994). Infection can occur quickly, for example invasion of the cuticle of the potato aphid by *V. lecanii* occurred within 24 hours after contact with conidia (Askary *et al.* 1999). After a stage of mycelial growth, which eventually kills the host, externally borne conidiophores develop, which create conidia to infect further hosts (Ebert & Weisser 1997; Askary *et al.* 1999). With social insects, higher concentrations of conidia lead to a greater likelihood of mortality and a shorter time to death (Smith *et al* 2000). The natural intensity of entomopathogenic fungi carried by bumblebees is unknown, but it is suspected to be low (Kapongo *et al.* 2008a). If a sufficient dose of an entomopathogenic fungi such as *P. farinosus*, or *M. anisoplia* attaches itself to the cuticle of a particularly susceptible insect host, it has a high likelihood of becoming infected. Atypical of the other entomopathogenic fungi, *V. lecanii*, may concomitantly colonise as well as penetrate, the host's cuticle. This could increase the concentration of inoculum at the cuticle surface, leading to an enhanced probability of spores coming into contact with suitable sites for penetration (Askary *et al.* 1999). Thus, the probability of host death due to *V. lecanii* infection may be greater than for the other two fungi.	The impact of entomopathogenic fungi on bumblebee fitness in general is relatively unknown The likelihood that one native bumblebee becomes infected with the fungi *Paecilomyces farinosus, Verticillium lecanii, Metarhizium anisopliae* is low. However the biological consequences to arthropods of fungal infection are very high. The fungus, after penetrating the cuticle, proliferates as a walled hyphal body or a wall-less protoplast in the host's haemocoel. The host eventually dies of nutrient depletion, invasion of organs, toxicosis or physical obstruction (Gindin *et al.* 1994; Hajek 1997; Butt & Goettel 2000). In heavy fungal infestations, bumblebee brood mortality can occur (Macfarlane *et al.* 1995). The impact of *Paecilomyces farinosus, Verticillium lecanii, Metarhizium anisopliae* on bumblebee fitness and population dynamics of bumblebees is unknown. A study by Liu *et al.* (2002) demonstrated that the virulence of *P. farinosus* (56-62% mortality), *Verticillium lecanii* (<40%), against the tarnished plant bug (*Lygus lineolaris)* was less than that of *B. bassiana* (>80%) and *M. anisopliae* (>80%). Additionally *P. farinosus* & *M. anisopliae* grow at a slower rate than *B. bassiana* (Hallsworth & Magan 1999). A study by Hokkanen *et al.* (2003) demonstrated that *B. bassiana* may be more virulent to bumblebees than *M. anisopliae* (Hokkanen *et al.* 2003) & *B. bassiana* has a relatively small impact on bumblebee fitness, unless inoculated by a large dose of spores (Al-mazra'awi 2004; Kapongo 2008a; Kapongo 2008b). Thus if the growth rate of *V. lecanii* & *P. farinosus* is slower and causes a lower mortality to some insect species, than both *B. bassiana* and *M. anisopliae*, they may be less virulent to bumblebees. However, without definitive proof or study of the specific effects *V. lecanii* & *P. farinosus* have on bumblebees, it can not be assumed that this may be the case, as species differences exist between virulence of some strains to host species. For example, case mortality of social termites *Cryptotermes formosanus*, is 70% of the colony, when infected with *M. anisopliaei* (Yanagawa *et al.* 2009).

SOURCE HAZARD	EXPOSURE ASSESSMENT cont.	CONSEQUENCE ASSESSMENT cont.

SOURCE HAZARD

Paecilomyces farinosus, Verticillium lecanii, Metarhizium anisopliae

EXPOSURE ASSESSMENT cont.

Successful fungal colonization is dependent on environmental variables & host-parasite / genotype-genotype interactions, which are unpredictable & currently unknown in bumblebees.

Queen bumblebees spend most of their time post hibernation exposed to sunlight, while foraging and searching for suitable nesting sites, which can take up to two weeks and UV light is inhibitory to fungal growth (Heinrich 2004). Therefore, the likelihood of vectoring the agent to a highly susceptible host in the release site is low.

Should an imported *B. subterraneus* queen succumb to any of the fungal pathogens after reintroduction, spores (conidia) can be released to the environment from the cadaver (Faria & Wraight 2001). Infective conidia can be horizontally transmitted, by the wind or rain, leading to exposure of susceptible hosts to the agent (Schmid-Hempel 1998). Additionally, in optimal environmental conditions, fungal hyphae from germinated spores or fungus killed hosts can grow across substrates to contact new hosts (Faria & Wraight 2001). If exposure occurs, the dissemination of the agent is likely to be high, as the UK climate provides the necessary conditions for germination, the release environment is densely inhabited by insect fauna, and these fungi have a relatively broad arthropod host range (Schmid-Hempel 1998; Askary *et al.* 1999; Faria & Wraight 2001). However the likelihood of exposure occurring is low.

CONSEQUENCE ASSESSMENT cont.

Release of the fungi may increase crop yields around the release area, if the crop pests are significantly susceptible to the new strains. However, the demise of any *Bombus* sp hives will reduce insect assisted pollination of plants and surrounding crops. Honeybee hives are maintained at higher temperatures 32-36°C, as a self sterilization practice, preventing the growth and establishment of fungi (*Hokkanen et al.* 2003).

Crops visited by honey bees will not be affected by *V. lecanii* & *P. farinosus.* However, the optimal temperature for growth of *M. anisopliae* is higher than that of other entomopathogenic fungi at 30°C and thus this fungus may be more able to propagate in the honeybee hive.

The likelihood of an introduced bumblebee being infected and exposing native bumblebees and insects is low, and so although the likelihood of dissemination is high and these fungi can cause significant disease in arthropods, the overall likelihood of significant biological, economic and environmental consequences is low.

RISK ESTIMATION

It is possible that at least one of the short-haired bumblebee queens upon importation will carry the hazards, *P. farinosus, V. lacanii* or *M. anisopliae*. However, at the release site, susceptible animals have a low probability of being exposed to sufficient spores to cause infection and the likelihood of dissemination once infected is high. Overall, the biological, economical and environmental consequences of fungus introduction are low and overall risk level is considered to be LOW.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

All bumblebees in quarantine should be subject to a short duration of UV exposure (see 'Risk Options' for *Beauvaria bassiana* above for details)., to reduce the viability of any spores carried on the bees' carapace. Because most entomopathogenic fungi kill their hosts within a few days to a week (Askary *et al.* 1999; Liu *et al.* 2002; *Yanagawa et al.* 2009), recently translocated, and potentially immunocompromised bumblebees should be kept under observational quarantine for at least two weeks prior to translocation.

Table 10 Disease Risk Analysis for the source hazard Sphaerularia bombi

SOURCE HAZARD *Sphaerularia bombi*	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
JUSTIFICATION OF HAZARD *Sphaerularia bombi* is present in Britain (Alford 1969; Donovan 1980) and Sweden (Larsson 2007) and is known to be transmissible to the short-haired bumblebee *B. subterraneous* (Macfarlane 1975). We screened a sample of the source population *B. subterraneus* queens in 2011 and four queens were infected with *S. bombi,* (n=57; estimated prevalence 2.1-17.9% [at a confidence level of 95%]). It is not known whether differences in virulence exist between the *S. bombi* populations in each country (Goulson personal communication 1 June 2009). However, as the British and Swedish populations have been geographically separated from each other, genetic differences between both populations may have arisen.	*Sphaerularia bombi* prevalence varies, species to species, area to area & throughout the season (Alford 1973). A queen destined for reintroduction is only likely to be exposed during hibernation. Third larval stage, *S. bombi* nematodes, live, mate and moult twice in the soil (Poinar & Van Der Laan 1972; Macfarlane & Griffin 1990). Fertilized adult nematodes enter via the mouth of a hibernating queen bumblebee (Benton 2006) and start producing eggs. As many as 100,000 eggs are released during a period of two weeks, into the host's body cavity. Hatched third-stage larvae live in the haemocoel (Macfarlane & Griffin 1990; Benton 2006), until they are excreted from the queen's anus (Poinar & Van Der Laan 1972). Should the queen die, before the larvae are excreted, they can survive in a state of anabiosis in the cadaver until they are exposed to water (Poinar & Van Der Laan 1972). This survival trait allows nematodes to persist in high numbers in the environment (Macfarlane & Griffin 1990). Overall there is a medium likelihood of Swedish *B. subterraneus* queens being released with *S. bombi* given the estimated 2.1-17.9% prevalence of infection in the source *B. subterraneus* queens, and the fact that queens will be in hibernation in nests in the soil and so will be in close contact to the nematodes.	An infected reintroduced queen is likely to expose native bumblebees as the nematode causes alterations in host behaviour post emergence from hibernation to aid dissemination of larvae by faecal excretion. Instead of colony founding, an infested queen flies close to the ground, frequently alights, digs multiple shallow holes and crawl under fallen leaves. These behaviours increase the quantity of infective third stage larvae at potential hibernation sites of other bumblebees (Poinar & Van Der Laan 1972; Benton 2006). After ten weeks the third stage larvae mate, then continue their maturation into adults, ready to infect any new queens that hibernate in their vicinity (Macfarlane & Griffin 1990; Benton 2006). Given that, *S. bombi* makes behavioural alterations to the host to maximise its chances of infecting future hosts, the likelihood of a native queen being exposed to these nematodes on hibernation is high. The Dungeness release site, is an exceptionally fauna rich area, supporting 13 other bumblebee species (Williams 1986). The likelihood of one of approximately 100,000 released nematodes coming into contact with a naive native queen as she hibernates is high. On emergence from hibernation, that queen would then release a further 100,000 larvae in the release area, thus there is a high likelihood of nematode dissemination.	It is highly likely that at least one bumblebee at the release site will be infected. If infected the most common cause of death of infested bumblebees is reportedly due to exhaustion and fat reserve depletion (Poinar & Van Der Laan 1972). Some heavily infested queens are co-infested with fungal pathogens that probably hasten the death of the queen (Poinar & Van Der Laan 1972). Parasitized queens cannot form eggs and found colonies, as ovary development is inhibited (Macfarlane & Griffin 1990). In Canterbury, NZ, *S. bombi* associated mortality of *B. terrestris* & *B. hortum* queens was estimated to be between 3- 10% (Macfarlane & Griffith 1990). *S. bombi* may have considerable effects on the size of bumblebee populations, especially if the introduced strain is highly virulent (William 1986). Wasps (*Vespula*), are the only other susceptible genus, besides bumblebees (*Bombus*), that can harbour the nematode (Macfarlane & Griffin 1990) and could possibly also therefore be infected by an introduced strain. Introduction of a novel strain of *S. bombi* would have significant economic and environmental implications because an increase in the prevalence of infested queens reduces the number of colonies created in the field to pollinate crops and control agricultural pests. Environmental consequences associated with reduced pollination, only apply to the loss of bumblebee nests. Wasps are effective natural biological control agents and generally do not pollinate flowers as effectively as bees or bumblebees. An increase in agricultural pests controlled by wasps, such as caterpillars, may however cause some environmental change to crops and vegetation. Overall the likelihood of an individual becoming infested at the release site is high, as the nematode infests, not only a wide range of bumblebees, *Bombus* spp. (Donovan 1980), but also wasps, *Vespula* (Macfarlane & Griffin 1990). Therefore the likelihood of significant biological, environmental and economic consequences is also high.

SOURCE HAZARD	RISK ESTIMATION	RISK EVALUATION
Sphaerularia bombi	There is a medium likelihood that one of the short-haired bumblebee queens upon importation will carry the hazard, given its estimated prevalence in source *B. subterraneus* is 2.1-17.9%. If infected, queens have a high likelihood of exposing the agent to a susceptible host at the release site and a high likelihood of disseminating it. Overall, the biological, economic and environmental consequences of introduction are high. The overall risk level is MEDIUM.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

Quarantine on arrival in the UK involving monitoring of behaviour and possible diagnostic *post-mortem* examination for bumblebees exhibiting bizarre behaviours is recommended. On *post-mortem* examination the haemocoel should be examined for *S. bombi* larval stages.

Table 11 Disease Risk Analysis for the source hazard Paenibacillus larvae (causal agent of 'American foulbrood' in honeybees)

SOURCE HAZARD	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT
Paenibacillus larvae	_Paenibacillus larvae_ has not been isolated from bumblebees (_Bombus_ spp.) (Schmid-Hempel 2001; Benton 2006), including those reared in countries where _P. larvae_ is present (van der Steen & Blom 2010). Bumblebees have different complements of commensal bacteria to honeybees and do not appear to be susceptible to the most contagious bacterial pathogens of honeybees (such as _P. larvae_) (Pridal _et al._ 1997; van der Steen & Blom 2010). The prevalence of clinical American foulbrood in honeybee hives in Sweden has been reported to be low (<1%) (the paper did not investigate prevalence but gave this statistic [referencing a Swedish paper]) (Fries & Raina 2003). The recent AFB outbreak in Halland county was a minimum of 170km from the proposed collection sites, and the outbreak was controlled as per current Swedish (and EU legislation) including destruction of infected hives with the aim of preventing spread to other sites (Lindström 2006; Genersch 2010). Wild bumblebees at the collection site might have been exposed to _P. larvae_ spores through interactions at food resources shared with honeybees and collection of pollen contaminated with _P. larvae_ spores (Colla _et al._ 2006), however it is unlikely that queens from the collection sites would have been exposed to _P. larvae_ given its low prevalence in Sweden and the relatively long distance between the collection sites and the site of the outbreak (in a bumblebee-empty landscape, bumblebee queens can disperse up to 130km per year [Macfarlane & Griffin 1990], although distances are likely to be much lower where bumblebees are present [Lepais _et al._ 2010]), assuming the location of the outbreak was known with confidence. Bumblebees would, more likely, be exposed to _P. larvae_ through contamination of the pollen or nectar (from honeybees) fed to them during the course of the translocation and quarantine (Pridal _et al._ 1997; van der Steen & Blom 2010; Lindström _et al._ 2008). Eventhough Sweden has a low prevalence of clinical AFB in honeybee hives, a recent study found that 8 out of 12 composite Swedish honeys were positive for _P. larvae_ spores (Fries & Raina 2003). _Paemibacillus larvae_ would most likely be released at the reintroduction site if the reintroduced bumblebees had been fed contaminated pollen or nectar during transport, or if contaminated pollen or nectar was, itself, released inadvertently at the release site. There is a low likelihood of release of _Paenibacillus larvae_ at the release site.	Sympatric adult bees at the release site would be exposed to spores through use of shared food, or pollen, sources (Colla _et al._ 2006). Bumblebees that had been exposed to _P. larvae_ might excrete the spores in their faeces (Lindström _et al._ 2008), or possibly transport spores in contaminated pollen on their body/legs. In honeybees, the primary means of horizontal spread of _P. larvae_ between colonies appears to be through 'healthy' colonies 'robbing' other, infected, colonies that have succumbed to AFB (or by anthropogenic means, i.e. beekeepers moving contaminated brood or honey between hives) (Lindström _et al._ 2008). The likelihood of native honeybees at the release site collecting nectar or pollen contaminated with short-haired bumblebee faeces is low. The likelihood of bees at the release site encountering contaminated pollen, honey or nectar (including any that had been fed to the reintroduced bumblebees whilst they were in quarantine) is very low. If a native honeybees at the release site was exposed to _P. larvae_ the probability that they would transport spores back to their colonies is low, since the bacterium does not 'infect' adult bees (Genersch 2010), rather they would need to act as 'mechanical' vectors carrying spores on their body or via their gut. If, however, a larva in the bee's colony was exposed to contaminated pollen or nectar the risk of dissemination within the colony would be high because _P. larvae_ spores are highly infectious and are spread by direct transmission. The risk of dissemination between colonies is medium because there is a high density of bees at the reintroduction site. **CONSEQUENCE ASSESSMENT** The likelihood of one bumblebee being infected through transmission from a reintroduced bumblebee is negligible because bumblebees are not known to be susceptible to _P. larvae_ infection (Schmid-Hempel 2001; Benton 2006; van der Steen & Bloom 2010). Also, in honeybees, _P. larvae_ is only infectious to larval bees, not adults (although following exposure adult bees may excrete _P. larvae_ spores in their faeces) (Genersch 2010). The likelihood of a bumblebee being infected through transmission from imported pollen or nectar is very low. The likelihood of disease in bumblebees is therefore very low. The likelihood of one honeybee being infected is very low. The disease AFB can devastate honeybee hives if not controlled, with serious economic consequences for apiculturalists and potential implications for the wider ecosystem (Genersch 2010) and therefore the biological environmental and economic consequences are very severe. The likelihood of this occurring is very low.

SOURCE HAZARD	RISK ESTIMATION	RISK EVALUATION
Paenibacillus larvae	The risk of release of _P. larvae_ at the reintroduction site is low; and the risk of exposure of bees to _P. larvae_ as a consequence of the reintroduction is low and the probability of dissemination is medium. If colonies at the release site became infected with _P. larvae_ the potential consequences would be severe, but the likelihood of their occurrence is low. The overall risk level is therefore LOW.	Preventative measures should be employed to reduce the risk that honeybee pollen or nectar contaminated with _P. larvae_ spores is fed to bumble bees during the course of translocation and quarantine.

RISK OPTIONS

To minimize the risk of contaminated queens being collected, they must not be collected within a 130 km radius of a clinical outbreak of AFB since January 2011. This ruling also relates to the pollen which will be collected. In conjunction with this (with regard to England's statutory import controls [Fera 2011]) we must ensure that the collection site falls outside of any prohibition areas relating to AFB outbreaks. Although bumblebees do not appear to be infected, or affected, by _P. larvae_, all of the bees collected for reintroduction should appear healthy and should have no clinical signs of disease or abnormality.

Pollen or nectar to be fed to the bumblebee queens should be collected from an area in which AFB outbreaks have not recently occurred (the 'Attracker' solution, which it is planned will be fed to bees in addition to pollen during quarantine, is an artificial nectar solution so it is very unlikely to be contaminated with _P. larvae_). Good biosecurity should be employed during quarantine to reduce the likelihood of cross-contamination of bumblebees with pathogens (such as _P. larvae_) from captive or wild honeybees. Any pollen or nectar fed to the bumblebees during quarantine should subsequently be discarded in the quarantine area and materials and overclothes used in the quarantine area should not be taken to the reintroduction site.

Table 12 Disease Risk Analysis for the destination hazard acute bee paralysis virus

DESTINATION HAZARD	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Acute bee paralysis virus (ABPV)	Upon release, the *B. subterraneus* queens will forage for resources & ABPV could be passed from honeybee to bumblebee or from bumblebee to bumblebee through interactions at shared food resources (Durrer & Schmid-Hempel 1994) or through the drifting of infected bumblebee workers to other hives (Sakofski & Koeniger 1988; Schmid-Hempel 1998). However given that honeybees have short tongues (6.5-8.5mm) and *B.subterraneus* have long tongues (approximately 11mm) the flowers these two species visit are likely to differ. This reduces the likelihood of exposure. Furthermore given *B. subterraneus* are a late developing species, they will be less efficient at widely disseminating the infection horizontally between colonies, as they have less time within a season to do so.	It is highly likely that one reintroduced queen will be infected. If infection leads to clinical disease bees may tremble uncontrollably & are unable to fly. In addition, they lose the hair from their bodies and have a dark, shiny, or greasy appearance (Miranda *et al.* 2009). Paralytic bees are submissive to attack. When paralysis is serious, large numbers of afflicted bees can be found at the colony entrance, crawling up the sides of the hive and blades of grass, & tumbling to the ground. Affected bees also may be found on top bars or frames next to the hive cover with wings extended. These signs may occur 2-4 days post infection and death ensues over the following days (Bailey & Gibbs 1964). It has been presumed that ABPV plays a role in cases of sudden collapse of honeybee colonies infested with the *Varroa* mite (Bakonyi *et al.* 2002).

JUSTIFICATION OF HAZARD

Present in Sweden and UK in *Apis mellifera* (Belton 2003, DeMiranda *et al.*, 2010). ABPV has also been reported to experimentally infect *Bombus* sp. (Bailey & Gibbs 1964). Dungeness, the destination site, screening results for bumblebees in 2011 revealed the presence of infection in 1 *B. hortorum* worker (n=22, estimated prevalence 0.1-23.8% [at a confidence level of 95%]).

Inter-country strain differences of ABPV have been reported (Carreck 2007) therefore it is possible that a more virulent strain may exist in the UK compared to Sweden, and the reintroduced bumblebees could be naïve to this strain. ABPV is in the same family as Kashmir bee virus (KBV) and is serologically and biologically very closely related.

Varroa sp. mites, which are present in the UK and which infest honeybees, can horizontally transmit ABPV and might act as a vector for transmission between honeybees and bumblebees (Shen *et al.* 2005). However there is only one report of *Varroa* sp. infesting a bumblebee, the American bumblebee *Bombus pennysylvanicus* (Ongus 2006). *Varroa* has not been reported in infect European bumblebees (G. Budge, personal communication October 2009). Therefore there is a very low likelihood of exposure to ABPV via *Varroa* sp mites in the UK.

Overall there is a medium likelihood of exposure to ABPV occurring owing to its known presence in *B. hortorum* workers at the release site however the limited foraging behaviour of *B.subterraneus* and its late emergence from hibernation decrease the likelihood of exposure. If infected it is likely that any infected reintroduced queens will transmit ABPV through transovarial transmission and then horizontal transmission from workers to larvae. The spread and establishment of infection in bumblebee colonies is likely to increase with the percentage of workers infected, as has been shown for at least one other parasite of bumblebees (Rutrecht & Brown 2007).

There is a low likelihood that disseminated infection will occur if the virus is introduced from honeybees or from *B. hortorum* to *B. subterraneus.* The exposure assessment showed the risk of exposure and dissemination of ABPV to *B.subterraneus* is medium, but it is possible one animal will be exposed and disease will depend on the presence of stressors during the reintroduction. Although most infections in honeybees are sub-clinical, (Bailey & Gibbs 1964) infections can lead to clinical disease if stressors exist which lead to immune suppression these include mite infestation, bacterial infection, pollution, and contact with chemicals including insecticides (Bakonyi *et al.* 2002). The act of reintroduction and associated environmental change may also increase the likelihood of disease. It is likely that the introduced *B. subterraneus* are going to compete with native bees for food at the destination environment. The probable consequences of this are increased stress for both participants which is likely to impose significant costs upon the fitness of the colony's individuals. A combination of the above factors may result in subclinical infection progressing to clinical infection.

Given that the reintroduction of bumblebees will be stressful and ABPV disease is precipitated by stress there is a high likelihood of significant biological and economic consequences to the reintroduction programme because of a disease outbreak in the bumblebees.

The overall likelihood that ABPV will have significant biological and economic consequences on native UK honeybees and bumblebees is low. Ecosystem stability is unlikely to be severely affected as it is unlikely that the disease will cause significant mortality to native species, unless they are severely stressed.

DESTINATION HAZARD	RISK ESTIMATION	RISK EVALUATION
Acute bee paralysis virus (ABPV)	The likelihood of exposure to ABPV is medium because of its known presence in the release environment in bumblebees. In contrast, it has only been demonstrated in honeybees in the source environment. There is a high likelihood of significant consequences due to the stress of reintroduction precipitating disease. The overall risk level is considered to be MEDIUM.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

A single-step multiple-target (multiplex) reverse transcription-PCR (RT-PCR) was developed for the simultaneous detection and differentiation of ABPV in Austria (Grabensteiner *et al.* 2007) in honeybees. Pathogen surveillance should be carried out on all dead *B. subterraneus* queens found in the field, post reintroduction. Given post release population surveillance using defined transects will be undertaken, any dead queens or workers found during this surveillance should be collected and submitted for post-mortem examination and PCR testing.

Table 13 Disease Risk Analysis for the destination hazard Apicystis bombi

DESTINATION HAZARD *Apicystis bombi*	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT

DESTINATION HAZARD

Apicystis bombi

JUSTIFICATION OF HAZARD

Reported to infect ten species of *Bombus* in Europe and North and South America, and also *A. mellifera* but bumblebees are the principal hosts (Lippa & Triggiani 1996).

Disease associated with *Apicystis bombi* has been reported in Sweden (Larsson 2007), Finland, France, Italy and Switzerland (Lippa & Triggiani 1996). *A. bombi* is also likely to be in other EU countries (Lippa & Triggiani 1996). *A. bombi* is known to be present in the UK, but has not been studied at the release site (Brown, unpublished data) and is known to be present in Sweden however owing to geographical isolation, strain differences may have occurred.

EXPOSURE ASSESSMENT

Upon release, the *B. subterraneus* queens will forage for resources & establish their colonies. These queens could be exposed to *A bombi* via faeco-oral transmission when feeding on flowers previously visited by infected European honeybees (although infection has not been reported in Europe) or *Bombus* sp. (more likely) found in the release area and subsequently infect their colony through horizontal transmission (Schmid-Hempel 1998). However given that honeybees and some bumblebees have short tongues (6.5-8.5mm), this will minimise the risk of transmission as *B.subterraneus* have long tongues (approximately 11mm) the flowers these two groups visit are likely to differ. However, other long-tongued bumblebees are present at the release site and thus pose a significant risk of transmission. However if *B subterraneus* is exposed and infected, given its late development in the season it will be less efficient at widely disseminating the infection, because it has less time within a season to do so. Overall there is a medium likelihood of exposure owing to the limited foraging behaviour of *B.subterraneous*, and its late emergence from hibernation. If exposed it is likely that any infected reintroduced queen will disseminate *A. bombi* horizontally upon returning to the colony. However given their late emergence from hibernation there is a low likelihood that *A.bombi* will be widely disseminated through the reintroduced population.

RISK ESTIMATION

There is a medium likelihood of exposure owing to the foraging behaviour of *B. subterraneus*, and the presence of other long-tongued species at the release site. If exposed, infection can result in severe disease which could inhibit colony founding and lead to failure of the re-introduction. The overall risk of this occurring is MEDIUM.

CONSEQUENCE ASSESSMENT

It is highly likely that at least one reintroduced bumblebee will be infected post release given the release site is a protected bumblebee reserve and hosts a variety of long-tongued bumblebee species which live in high densities. If a reintroduced queen is infected the ingested oocysts will penetrate through the midgut wall into the body cavity and infect the fat body cells in which they grow develop and multiply. This results in a disintegrated fat body and infected colonies are often unable to grow and reproduce (Schmid-Hempel 1998). Energy reserves in bumblebees are stored in the fat body. The fat body reserves provide energy through the winter and into the spring when warm temperatures initiate adult emergence. If fat body reserves are inadequate in the spring, then the bumblebee will be lethargic. If the bee has enough energy to fly to nectar quickly, it may recover. However, if the weather is too cold or wet, or if flowers are scarce or too far, the bee may not survive (Australian hydroponic & greenhouse association 2008). Infection therefore effectively inhibits colony founding and infected queens often die in early spring (Rutrecht & Brown 2007).

Given *B.subterraneus* emerges late from hibernation it is unlikely to have a significant store in its fat body compared to earlier emerging bumblebees and therefore may be more susceptible to the effects of infection. If infected there is a high likelihood of disease which may inhibit colony founding and lead to failure of the reintroduction and therefore significantly increase the economic cost if further reintroductions are necessary. Ecosystem dynamics have a low likelihood of being severely affected, as the most likely individuals to be affected are the reintroduced queens.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

All *B. subterraneus* queens or workers found dead during post release population monitoring using transects should receive detailed post-mortem examination. On post-mortem examination characteristic sausage shaped spores may be present in the fat bodies, or mid gut when viewed under the light microscope (Cankaya & Kaftanoglu 2006).

Table 14 Disease Risk Analysis for the destination hazard Beauveria bassiana

DESTINATION HAZARD *Beauveria bassiana*	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
	Reintroduced queens could be exposed to *B. bassiana* spores deposited by bumblebees and other insects on the flowers and leaves in the top third of a plant's canopy (Al-mazra'awi 2004; Kapongo *et al.* 2008a). Conidia on leaves, exposed to sunlight for 24 hours, lose 50-100% of their virulence & viability (Gardner *et al.* 1977). Therefore the likelihood of the reintroduced *B. subterraneus* being exposed and infected through this route is low. *B. subterraneus* could also be exposed to *B. bassiana* from other infected or dead insects in close proximity releasing conidia; (spores adapted to withstand desiccation) attaching to their cuticles (Boucias *et al.* 1998; Wraight *et al.* 2000) or to a moist cavity, such as the mouth (Tanada & Kaya 1993). Higher concentrations of conidia lead to a greater likelihood of mortality (Kapongo *et al.* 2008b). However germination of the spore is temperature (optimum 25°C) (Fargues *et al.* 1997; Hallsworth & Magan 1999) and humidity (Gillespie & Crowford 1986; Hallsworth & Magan 1999) dependent. A germ tube forms, which penetrates (Boucias & Pendland 1998, Hajek & St. Leger 1994) to attach to the new host. After a stage of mycelial growth, which eventually kills the host, externally borne conidiophores develop, that create conidia to infect further hosts (Hung *et al.* 1993; Sosa-Gómez & Alves 2000). The queen, after translocation, hibernation, foraging & nest construction is likely to have a higher susceptibility to infection, due to the effects of stress and immunosuppression, prior to egg laying. However, if the concentration of spores on the queen is high enough to kill her progeny, she herself is also likely to succumb to infection which will inhibit colony founding and further dissemination. In addition, should UK *B. bassiana* prove highly infectious to reintroduced *B. subterraneus* colony infection is likely as nests provide ideal temperature and humidity conditions for fungal growth (Hokkanen *et al.* 2003) and could result in colony failure. The overall likelihood of exposure and infection is low. But if infected the likelihood of dissemination to progeny at the release site is high.	The risk of exposure of a detached vectored spore to a reintroduced queen at the release site, is high, yet owing to the fungus's susceptibility to UV light which the queen will be exposed to upon release post hibernation it is unlikely the spore concentration will be high enough to cause infection. However if a reintroduced queen becomes infected and dies, and subsequently infects her progeny the consequences of infection are high. The fungus, after penetrating the cuticle, proliferates as a walled hyphal body or a wall-less protoplast in the host's haemocoel. The host eventually dies of nutrient depletion, invasion of organs, toxicosis or physical obstruction (Hajek 1997; Butt & Goettel 2000). Higher concentrations of conidia, lead to a greater likelihood of mortality and a shorter time to death (Smith *et al* 2000; Kapongo *et al.* 2008a; Kapongo *et al.* 2008b). In heavy fungal infestations, bumblebee brood mortality can occur (Macfarlane *et al.* 1995). Therefore the biological consequences of an environment containing a high concentration of conidia could be severe. However given that the concentration of *B. bassiana* spores naturally carried by bumblebees is unknown, but is suspected to be low Kapongo *et al.* (2008a/b) epidemic disease in the reintroduced bumblebees is unlikely. Ecosystem dynamics have a low likelihood of being severely affected, as the most likely individuals to be affected are the reintroduced queens and given the high environmental load and therefore concentration of spores required for infection it is unlikely that epidemic infection would occur resulting in failure of the reintroduction.
JUSTIFICATION OF HAZARD The fungus *Beauveria bassiana* is known to be transmissible to bumblebees (Alford 1975; Schmid-Hempel 1998; Goettel *et al.* 1990) and a wide variety of insect hosts although it has not been recorded in *B. subterraneus*. It is found worldwide (Macleod 1963) however there may be strain differences of varying pathogenicity between the isolates in each country (Yeo *et al.* 2003; Lozano-Gutiérrez & Espana-Luńa 2008). Therefore reintroduced short-haired bumblebees may be naïve to the strain of *Beauveria bassiana* found in the UK.		

RISK ESTIMATION	RISK EVALUATION
It is highly likely that one of the honeybees or *Bombus* sp. in the release site will be infected with *B. bassiana*. Assuming *B. subterraneus* can be infected, reintroduced queens have a low likelihood of being exposed to sufficient spores to cause infection. However if infected mortality and dissemination to progeny which could cause colony failure is of medium likelihood. Overall, the biological, economic and environmental consequences of fungus introduction are medium. However the likelihood of infection is low therefore the overall risk to the reintroduction is considered LOW.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS
Pathogen surveillance should be carried out on all dead *B. subterraneus* queens found in the field, post reintroduction. Given that post release population surveillance using defined transects will be undertaken, any dead queens or workers found during this surveillance should be collected and submitted for post-mortem examination, microscopic examination and fungal culture. Infective conidia can be horizontally transmitted, by the wind or rain, leading to exposure of susceptible hosts to the agent. Therefore release should occur in summer (June) when warm conditions should prevail.

Table 15 Disease Risk Analysis for the destination hazard Crithidia bombi

DESTINATION HAZARD	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT

DESTINATION HAZARD

Crithidia bombi

JUSTIFICATION OF HAZARD

Research has repeatedly confirmed the presence of the agent in Britain (Henson *et al.* 2009; M Brown personal comm.) Ireland (Brown *et al.* 2003b), Sweden (Larsson 2007) and Switzerland (Yourth & Schmid-Hempel 2006). However as *C. bombi* in the UK has been igeographically solated from *C bombi* in Sweden it is highly likely that genetic differences exist between these strains.

Given that most strains of *C. bombi* differ in their infectivity to colonies, through the genetic characteristics of the host line (Schmid-Hempel 2001) and that there is evidence from Switzerland that strain differences alter the intensity of infection (Schmid-Hempel & Schmid-Hempel 1993) and pathogenicity (Imhoof & Schmid-Hempel 1998): there is a high likelihood that the *C.bombi* present in the UK is different to found in Sweden.

EXPOSURE ASSESSMENT

Reintroduced queens could be infected post release when they come into contact with *Crithidia* cells passed in UK bumblebee faeces on nest surfaces (Schmid-Hempel 2001) or when visiting flower surfaces previously infected by a shedding forager (Durrer & Schmid-Hempel 1994). 1000 parasitic cells are needed to establish infection in *B. terrestris* hosts (Ruiz-Gonzalez & Brown 2006) and severely infected bumblebees significantly contribute to horizontal transmission at flowers, as they excrete larger quantities of *C. bombi* in their faeces (Otterstatter & Thomson 2006).

Once infected inter-colony dissemination could occur through the drifting of infected workers to other nests (Sakofski & Koeniger 1988; Schmid-Hempel 1998). It is possible that sick bees may have trouble navigating in their environment as immune challenged bees perform poorly in memory tests (Riddell & Mallon 2005) and alterations to the host's behaviour, may increase the chances of inter-colony drifting and subsequent pathogen transfer.

Reintroduced bumblebee queens will emerge from hibernation in May (Benton 2006) and have a high likelihood of acquiring *C. bombi* as the release environment is likely to contain high densities of early season workers that may locally amplify disease transmission.

An infected queen that manages to found a colony will almost certainly infect her workers. As the season progresses, the colonies will grow and *C. bombi* will be transmitted both within the colonies and between colonies through the contamination of flowers by foraging workers (Yourth & Schmid-Hempel 2006).

Given that the reintroduced species will most likely be naive to the UK strain, the reintroduced colony may become severely infected at high prevalence.

The release site is densely co-inhabited by 13 other *Bombus* species. Therefore, dissemination of *C. bombi* is highly likely at the site. Any reintroduced bumblebees that are exposed are more likely to become severely infected and may develop an altered and prolonged foraging strategy to aid disease dissemination.

As such there is a high likelihood of exposure, infection and dissemination to workers and other *B.subterraneus* colonies.

CONSEQUENCE ASSESSMENT

The exposure assessment showed that there is a high likelihood that a reintroduced queen will be exposed and disseminate *C. bombi* to her colony. After natural infection of *Bombus impatiens*, *C. bombi* reached a stable faecal pathogen load on average 12 days post infection (Otterstatter & Thomson 2006). However, faecal pathogen load can differ substantially between individuals, depending on the host's nutritional status (Logan *et al.* 2005). The mortality rate caused by *C. bombi* can be up to 50% higher in infected *B. terrestris* workers under stressful starving conditions (Brown *et al.* 2000), furthermore food shortage and other stressful conditions are likely to be more common in younger colonies (Schmid-Hempel 2001) and therefore the reintroduced colonies may be more susceptible to mortality. It is likely that the introduced *B. subterraneous* will compete with native bumblebees for food at the destination environment. The probable consequences of this are increased stress for both participants. This competition is likely to impose significant costs upon the fitness of the colony's individuals, which may result in higher host mortality due to *C.bombi*.

In the first crucial 25 days of a colony cycle, *C. bombi* naturally infected colonies grew at a slower rate and had a smaller worker force (Schmid-Hempel 2001). It has been proposed that pollen starved bees may re-allocate either: ovary development resources to combat infection (Moret & Schmid-Hempel 2000), or fat stores for post infection survival (Brown *et al.* 2003a). Furthermore, workers in infected colonies lay their own eggs 5 days later than in uninfected colonies, leading to later queen and drone emergence (Shykoff & Schmid-Hempel 1991a). Delayed production of reproductive bumblebees, has significant implications for queen survival throughout hibernation (Schmid-Hempel 2001) and colony-founding. Hibernation is a stressful activity that depends on the utilization of limited fat stores for survival. Following the allocation of resources for hibernation, queens must then use their remaining reserves for foraging and other metabolically expensive activities necessary to found a colony, however if these have already been depleted owing to *C. bombi* infection colony founding will be delayed or less productive (Brown *et al.* 2003b).

The likelihood that *C. bombi* will lead to the demise of at least one reintroduced bumblebee colony is high. However, this mortality may not be higher than would be expected in natural populations. Each year only a few bumblebee queens establish a colony, most will have been selected through parasitism or disease, and these bumblebee family lines dominate the population for the rest of the season. Over successive generations in the season, the parasite will adapt to these hosts and these parasite strains will become more specialized. This leads to a reduced ability for them to exploit the less dominant genotype in the following season (Yourth & Schmid-Hempel 2006), a process known as negative frequency dependant selection. So although the release of novel strains of *C. bombi* into the environment will cause a drop in the amount of genetic variability across the population, it is likely that the effects will only last until the next season.

DESTINATION HAZARD	**CONSEQUENCE ASSESSMENT cont.**
Crithidia bombi	The likely biological consequences are: the probable demise of those colonies under stressful conditions and/or, a significant reduction in the probability of a queen to successfully hibernate and start a colony the next season. Given *C. bombi* is infectious to most bumblebee species, there is a high probability that the UK *C. bombi* strain would spread to all reintroduced bumblebee colonies in the release area. Therefore the overall likelihood that *C. bombi* will have biological consequences on the reintroduced population is high. However, once a few hives are destroyed, the reduced competition for food will alleviate some of the stresses on the remaining bumblebees. The environmental and economic consequences of *C. bombi* are likely to be brief, and low because it is most likely only the reintroduced bumble bees will be affected and therefore ecosystem stability is unlikely to be severely affected.

RISK ESTIMATION	**RISK EVALUATION**
It is highly likely that the reintroduced bumble bees will be exposed to *C. bombi* and will subsequently become infected. The biological consequences to the reintroduced bumblebees are high while environmental and economic consequences are low. The overall risk level to the reintroduction is considered to be HIGH.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS
Pathogen surveillance should be carried out on all dead *B. subterraneus* queens found in the field, post reintroduction. Given post release population surveillance using defined transects will be undertaken, any dead queens or workers found during this surveillance should be collected and submitted for post-mortem examination.

Table 16 Disease Risk Analysis for the destination hazard Deformed Wing Virus

DESTINATION HAZARD	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT

DESTINATION HAZARD

Deformed Wing Virus (DWV)

JUSTIFICATION OF HAZARD

Deformed wing virus (DWV) is a viral pathogen of *Apis mellifera* associated with colony collapse disorder (CCD) when transmitted by the mite *Varroa destructor* (Yue *et al.* 2007). It is pathogenic to both honeybees and bumblebees (Genersch *et al.* 2006).

DWV has been found in Sweden (Nordstrom *et al.* 1999) and has been reported in southern England (Highfield *et al.* 2009). DWV was absent from *B.subterraneus* queens in the source population in 2011 (n=59; estimated prevalence 0-7.1% [at the 95% confidence level]; we can estimate, with over 90% confidence, that the prevalence in the Swedish population is less than 5%) however a UK wide study found DWV in a 1/3 of their *B. terrestris* samples, and a small number of *B. pascuorum* were also positive. *B. lapidarius, B. pratorum* and *B. hortorum* were virus free (WOH Hughes, pers. comm. November 2011).

It is possible that strain differences exist between the source and destination population and as such the source population may be naïve to the destination population strain.

EXPOSURE ASSESSMENT

Upon release, the *B. subterraneus* queens will forage for resources and establish their colonies. These bumblebee queens could be exposed via faeco-oral transmission when feeding at sites visited by honeybees or bumblebees at the release site, leading to subsequent horizontal transmission within an infected colony (Yue & Genersch 2005). If resident bumblebees at the release site are covertly or asymptomatically infected with DWV, factors which may significantly increase stress on individuals over the course of the reintroduction may precipitate clinical disease. Such factors may include transport, lack of suitable food resources, adaptation to a new environment and exposure to novel parasites. In addition inclement weather, and unfavourable flying conditions for long periods of time will keep bees in their nests which may lead to in-nest faecal deposition, a major source of replicating viruses. All the above factors, including infestation with the *Varroa* mite may lead to the development of clinically symptomatic infection (Beelogics, 2010).

As soon as elevated virus titers are reached, the virus becomes virulent and clinically symptomatic disease results (Genersch *et al.* 2010). There is a medium likelihood that significantly stressful events will occur during reintroduction which may lead to DWV disease.

If the above mentioned stress factors exist then it is likely that many of the reintroduced bumblebees will develop clinical disease. This may be lethal in which no further dissemination will occur. Alternatively, infected queens will transmit DWV through transovarial transmission to workers and then horizontal transmission from worker to larvae.

There is a high likelihood that disseminated infection would occur in reintroduced *B. subterraneus*, as the release site is a protected bumblebee reserve site and hosts a variety of different bumblebee and honeybee species living in high densities. However, DWV was not found in tested bumblebees from the release site.

Overall there is a low likelihood of exposure in the UK as *B.subterraneus*, has a different feeding pattern to honeybees and short-tongued bumblebees, is a late emerger from hibernation, and DWV was absent from the sampled bumblebee populations at the release site. However if exposed it is likely that any infected reintroduced queen will initially transmit DWV through transovarial transmission and then horizontal transmission from worker to larvae.

The spread and establishment of infection in the bumblebee colonies founded by re-introduced queens may increase with the percentage of workers infected during larval development, as has been shown for at least one other parasite of bumblebees (Rutrecht & Brown 2007). Dissemination to workers from these colonies is most likely.

CONSEQUENCE ASSESSMENT

There is a low likelihood that at least one reintroduced queen will be infected. However if infected, DWV infection in honeybees following transmission by *Varroa destructor* has been associated with clinical symptoms including crippled wings, a bloated and shortened abdomen and discolouration (Ball & Allen 1988). Most importantly DWV has been associated with CCD (Yue *et al.* 2007). Honeybees infected with DWV have a reduction in life span (Kovac & Crailsheim 1988) symptomatic individuals live <67 hours post emergence from the pupa stage (Yaang & Cox-Foster 2007). DWV is pathogenic to at least two bumble bee species (*B. terrestris* and *B. pascuorum*) causing wing deformity similar to clinically DWV-infected honey bees (Genersch *et al.* 2006).

Yue *et al.* (2008) reported the importance of the *Varroa* vector for the development of overt (symptomatic) disease. If DWV was transmitted vertically within a colony in the absence of *Varroa,* individual honeybee fitness was unlikely to be affected. The infected colony even when harbouring covertly infected (asymptomatic) individuals will develop normally and eventually swarm to transmit the virus vertically to the next colony generation allowing long term population persistence. Overt (symptomatic) infection was reported when individuals were subjected to a strong immunosuppressive trigger, such as the *Varroa* mite. However there is a very low likelihood of *B. subterraneus* exposure to DWV via the *Varroa* mite. It is more likely that stressors associated with reintroduction will cause immunosuppression, increased viral concentration and overt (symptomatic) infection if a novel strain is present.

The potential biological consequences of DWV could be failure of the reintroduction, if the queens are exposed to a novel strain and become stressed leading to symptomatic infection. This could also considerably extend the reintroduction stage of the project and therefore significantly increase the economic cost of the introduction.

Ecosystem dynamics have a low likelihood of being severely affected, as the most likely individuals to be affected are the reintroduced queens. Although honeybees could be affected the different feeding pattern and late emergence from hibernation of *B. subterraneus* makes dissemination to honeybees of low likelihood.

DESTINATION HAZARD **Deformed Wing Virus (DWV)**	RISK ESTIMATION	RISK EVALUATION
	There is a low likelihood of exposure, but if exposed a high likelihood of covert (asymptomatic) infection and dissemination through vertical transmission. Evidence suggests that the likelihood of significant epidemic disease is high if the *B.subterraneus* queens are exposed to a novel strain of the virus and if severe stressors occur during the reintroduction. Therefore the overall risk level is MEDIUM.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

There is no product available for DWV control. To minimize the impact of DWV and other viral infections:

1. release in late spring, as given *B.subterraneus* are late emergers from hibernation they will then exit the nest in warmer weather and provide an *ad-libitum* food source to minimize competition at food sources.

2. all dead long-tongued bumblebee species *(B. subterraneus, B. pascourum* or *B. hortorum*) queens or workers found in the field during post release population monitoring using transects should receive detailed post-mortem examination and as a component of this be tested for DWV by real-time PCR (Genersch 2004).

Table 17 Disease Risk Analysis for the destination hazard Kashmir Bee Virus

DESTINATION HAZARD	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Kashmir Bee Virus (KBV)	Upon release, the *B. subterraneus* queens will forage for resources. KBV could be passed from honeybee to bumblebee and then from bumblebee colony to bumblebee colony through interactions at shared food resources (Durrer & Schmid-Hempel 1994) or through the drifting of infected workers to other hives (Sakofski & Koeniger 1988; Schmid-Hempel 1998) assuming the transmission pathways in bumblebees are the same as for honeybees. However given honeybees have short tongues (6.5-8.5mm) and *B.subterraneus* have long tongues (11mm) the flowers these two species visit and subsequently shed virus on, are likely to differ. These anatomical differences limit the foraging behaviour of *B. subterraneus* which may reduce their potential to be exposed to, and further disseminate KBV. Furthermore given *B. subterraneous* are late developing species, they will be less efficient at widely disseminating the infection horizontally between colonies, as they have less time within a season to do so. The *Varroa* sp. mite is present in the UK can horizontally transmit KBV (Shen *et al*. 2005). UK honeybees with KBV infection are also in contact with *Varroa* sp. and cross-species transmission has been reported with KBV. However, it is not known whether *Varroa* sp will infest native bumblebees in the UK as there is only one report of *Varroa* sp. infesting a bumblebee - the American bumblebee *Bombus pennsylvanicus* (Ongus 2006). *Varroa* has not been reported to affect the European bumblebee (G. Budge, personal communication October 2009). Therefore there is a very low likelihood of exposure to KBV via the *Varroa* mite.	A reintroduced queen has a low likelihood of becoming infected as KBV has only been reported in honeybees in the UK. However if infection did occur ongoing debate ensues about the pathogenicity of this virus (Ward *et al.* 2007). Honeybees infected with KBV have no described symptoms, even though Bailey & Ball (1991) & Allen & Ball (1996) suggest KBV is the most virulent of all known honeybee viruses.

Surveys in England and Wales (Ward *et al.* 2007) for KBV showed no obvious signs of virus infection, suggesting the virus was covert or latent. Although KBV can be covert it can become 'overt' and lethal (Ball 1997) especially when it exists with multiple other pathogens and when the individual is under stress (as may occur during the reintroduction). Reintroduced bumblebees are likely to be under increased stress adapting to their new environment and competing for food resources with native long-tongues species and, if exposed to a novel strain, covert or asymptomatic infection may lead to clinically symptomatic overt infection. However it is also possible that cross-protective antibodies are present in the reintroduced bumblebees which may provide protective immunity from the virus.

The likely biological consequences upon infection are: the potential for colony demise owing to exposure to a novel strain, and / or a significant reduction in the probability of a queen successfully hibernating and starting a colony the next season. This could extend the reintroduction stage of the project and therefore significantly increase the economic cost of the reintroduction.

Ecosystem dynamics have a low likelihood of being severely affected, as the most likely individuals to be affected are the reintroduced queens and their workers. |
| **JUSTIFICATION OF HAZARD**

KBV is in the same family as acute bee paralysis virus (ABPV) and is serologically and biologically closely related.

KBV has not been recorded in Sweden (deMiranda *et al.* 2010) and screening results from the source and destination environment showed an absence of KBV infection in the sampled bees. However given KBV has been detected in honeybees in the UK (Ward *et al.* 2007) and there is growing evidence to suggest it may be a pathogen of multiple insect genera it has been included as a hazard. | Overall there is a low likelihood of exposure owing to limited foraging behaviour of *B.subterraneus* and, its late emergence from hibernation. However, if exposed, it is likely that reintroduced queens will be infected and transmit KBV through transovarial transmission and then horizontal transmission from worker to larvae. The likelihood of establishment and dissemination of infection in reintroduced bumblebee colonies, would be high assuming *B. subterraneus* are susceptible. | |

RISK ESTIMATION	RISK EVALUATION
The likelihood of exposure and infection to KBV from honeybees is very low, as KBV has only been demonstrated in honeybees which have a different foraging behaviour to *B. subterraneus.* Evidence suggests the likelihood of significant epidemic disease is medium, as KBV will be novel to the reintroduced bumblebees, however given the likelihood of exposure is very low the overall risk level is considered to be LOW.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS
Pathogen surveillance should be carried out on all dead *B. subterraneus* queens found in the field, post reintroduction. Given post release population surveillance using defined transects will be undertaken, any dead queens or workers found during this surveillance should be collected and submitted for post-mortem examination. A KBV real-time PCR assay was developed by Ward *et al.* (2007) which has been shown to be diagnostic in *Apis* sp. *Bombus* sp. and *Vespula* sp.

Table 18 Disease Risk Analysis for the destination hazard Locustacarus buchneri

DESTINATION HAZARD *Locustacarus buchneri*	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT

DESTINATION HAZARD

Locustacarus buchneri

JUSTIFICATION OF HAZARD

Locustacarus buchneri is present in Britain (Donovan 1980) and Sweden (Larsson 2007). However *L. buchneri* was absent from *B.subterraneus* queens in the source population in 2011 (n=57, estimated prevalence 0-7.1% [at the 95% confidence level]; we can estimate, with over 90% confidence, that the prevalence in the Swedish population is less than 5%). Whether differences in virulence exist between *L. buchneri* populations, is currently unknown (Goulson, D personal communication 1 June 2009). However, as the Swedish and British populations are geographically isolated there is a high likelihood that genetic differences between the populations have arisen and as a result, the agent is considered a potential hazard. There is no causal evidence that *L. buchneri* has any negative impact on bumblebees.

EXPOSURE ASSESSMENT

Reintroduced queens could acquire mite infestation from inter-colony drifting of infected resident UK bumblebee workers (Otterstatter & Whidden 2004). Transmission is contact dependant.

If mites are transferred from UK bumblebees to reintroduced bumblebees, native larviform female and adult male mites may then hatch and mate inside the reintroduced bumblebees (Otterstatter & Whidden 2004). The fertilized female larviform stage mite will then leave the adult host through its spiracles (Benton 2006) & relocate to the trachea of reintroduced bumblebees (Otterstatter & Whidden 2004). There, after two weeks they moult and grow into fully developed immobile egg laying adult females (Husband & Sinha 1970; Yoneda *et al.* 2008). After a further two weeks, additional mobile larviform female and adult males are produced (Yoneda *et al.* 2008). The larviform females usually move from adult bumblebees to 3rd and 4th instar bumblebee larvae. This occurs when the protective wax pollen wall is ruptured, during the developmental phase of regurgitative feeding by workers (Yoneda *et al.* 2008). Adult bumblebees have high mite infestations four to five weeks post emergence as some mite offspring, after hatching in the host's trachea, stay there and metamorphose instead of seeking a new host (Yoneda *et al.* 2008). Thus, older worker bumblebees are more likely to forage and disseminate the disease to other colonies, through drifting.

Dungeness, the release site, is an exceptionally fauna rich area, supporting 13 other bumblebee species (Williams 1986). If native colonies are infected, the mite would have a high density of hosts to infest which would aid in dissemination to the reintroduced bumblebees. However the mite seems to show species specificity being most prevalent in *B. pratorum* and related species (M. Brown, pers comm. 2011). Thus, the agent is unlikely to disseminate and establish itself in the reintroduced population.

CONSEQUENCE ASSESSMENT

It is of low likelihood that one reintroduced queen will become infested by an adult female mite transmitted from a native bumblebee at the release site owing to the species specificity of the parasite. The tracheal mite *L. buchneri* appears to preferentially parasitise certain bumblebee species, specifically the subgenus *Bombus sensu stricto* in Canada (Otterstatter & Whidden 2004), although patterns of species-specificity in Europe are not linked to *Bombus* subgenera (Shykoff & Schmid-Hempel 1991c). *Psithyrus*, non-colony forming parasitic cuckoo bumblebees, are less affected. However if infected the mite could adversely affect bumblebee host health (Husband & Sinha 1970): as adult female mites pierce the trachea of their hosts and suck haemolymph from inside the body cavity (Husband & Sinha 1970; Benton 2006). Heavy host infestation with *L. buchneri* has been associated with physical damage to the trachea and lethargy; which itself has been associated with impeded or cessation of foraging & diarrhoea (Husband & Sinha 1970; Alford 1975). Field caught workers harbouring the mite, showed shorter lifespans in captivity than unparasitized bumblebees (Otterstatter & Whidden 2004). Consequently, at high levels of infestation, *L. buchneri* could jeopardise colony survival (Schmid-Hempel 2001). However, the studies cited above were correlational, and no causal evidence for an effect of *L. buchneri*. In addition, a positive correlation between infection and bee health exists (Rutrecht & Brown 2007).

Therefore, overall there is a low likelihood that dissemination of this mite could lead to significant biological, environmental and economic consequences and failure of the reintroduction owing to the species-specificity of the mite and the lack of demonstrated negative causal effect.

RISK ESTIMATION

It is unlikely that one of the reintroduced bumblebees will be infected from native bumblebees at the release site owing to the species specificity of the parasite. Therefore biological, economical and environmental consequences are unlikely. The overall risk to the reintroduction program is LOW.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

Pathogen surveillance should be carried out on all dead *B. subterraneus* queens found in the field, post reintroduction. Given post release population surveillance using defined transects will be undertaken, any dead queens or workers found during this surveillance should be collected and submitted for post-mortem examination. The trachea should be examined using a dissecting microscope with up to 40X magnification. In infected bumblebees the trachea will show patchy discolouration or dark staining caused by mite feeding. The eggs, nymphs and adult stages of the mite may also be seen in the trachea.

Table 19 Disease Risk Analysis for the destination hazard Melittobia acasta

DESTINATION HAZARD	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT

DESTINATION HAZARD

Melittobia acasta

JUSTIFICATION OF HAZARD

Mellitobia sp. are parasitoids of wasps and bees. *M. acasta* is the only species of *Mellitobia* in Europe (but is found on other continents) and has been reported in the UK: we are not aware of any reports of *Melittobia* sp. in Sweden. Reintroduced Swedish bumblebees might, therefore, be naïve to *Melittobia* sp.

EXPOSURE ASSESSMENT

Mellitobia females are active from late spring to late autumn and are multivoltine (i.e. they produce more than two broods per year) (Macfarlane & Donovan 1989). The release site is a protected bumblebee reserve and hosts a variety of bumblebee species living in high densities. A large variety of UK bee and wasp species are hosts of *M. acasta* (some acting as a 'reservoir hosts' for the pathogen), and hosts such as leafcutting bees (*Megachile* sp.), mason wasps (*Pison* sp.) and the mud-daubing wasp (*Scelriphon* sp.) (Dahms 1984) are present at the release site, therefore, when the reintroduced bumblebee queens emerge in late spring and begin nest building there is likely to be a high prevalence of *M. acasta* in the local area. Once a colony is infected *M. acasta* females can then attack nearby cells in the same nest, or fly to more distant hosts. They chew a hole in the host (final instar larva and/or prepupa and/or pupa) and sustain themselves on the host's body fluids, laying eggs for up to 36 days. This behaviour can be fatal to the host. "If parasitism occurs during the early growth of a bumblebee colony then no new queens are produced" (MacFarlane & Donovan 1989). Generational cycles of *Melittobia* sp. can be very short (egg-to-egg as little as 2-3 weeks). There is a high likelihood of reintroduced queens being exposed to *M. acasta* on release and of subsequent dissemination to their colonies. However given *B. subterraneous* is a late-emerging species they will have less time within a season to disseminate infection compared to the earlier-emerging bumblebees. This ecological factor may lower the prevalence of infection in *B. subterraneous* colonies.

CONSEQUENCE ASSESSMENT

It is highly likely that at least one nest established by a reintroduced queen will become infected and if parasitism occurs during the early growth of a bumblebee colony it is likely that no new queens will be produced (Macfarlane & Donovan 1989). This is likely to lead to nest destruction and failure of the reintroduction. Female *M. acasta* once emerged from their host are capable of attacking nearby cells in the nest or flying off to more distant hosts, including other susceptible bee and wasp species. *Melittobia* sp. have been shown to severely decrease the pollinating forces of e.g. leafcutting bees (which are present at the release site), with other potential losses in returns from pollination fees and/or seed returns and sales of surplus bees (Donovan & Read 1984).

The likely biological consequences are: the possible demise of the colony, a reduced probability that a new generation of queens will emerge from the colony to overwinter and so, in turn, a reduced likelihood of short-haired bumblebees establishing colonies the following year, and therefore reduced success of the reintroduction. Given that *M. acasta* is not species-specific, it has a wide host range which increases the likelihood of exposure. Therefore there is a high probability that *M. acasta* would spread to the reintroduced bumblebee colonies over the entire release area. Therefore the overall likelihood that *M. acasta* could have significant biological consequences on the reintroduced bumblebee population is high, especially if the reintroduced bumble bees are (as our literature search suggests) naïve to the parasite. This could considerably extend the reintroduction phase of the project and therefore significantly increase the economic cost of the introduction. However ecosystem dynamics have a low likelihood of being severely affected, as the most likely individuals to be affected are the reintroduced queens and their colonies.

RISK ESTIMATION

The likelihood of exposure is high owing to the parasite's wide host range, as is the likelihood of infection causing death of developing larvae/pupae within the colony followed by dissemination within and between colonies. Evidence suggests that there is a medium likelihood of significant disease. Therefore the overall risk level is considered MEDIUM.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

Post reintroduction, pathogen surveillance should, if feasible, be carried out on *B. subterraneous* nests if they appear to have failed or to be failing. Healthy bumblebees disperse at a rate of 130km a year (Macfarlane & Griffin 1990) and it would be impractical to survey such a wide area. Given that post release population surveillance will be undertaken using defined transects, any failed/failing nests found during this surveillance should be investigated and sampled as far as possible - larvae and/or pupae and/or cells should be inspected for *M. acasta*. However, a nest inspection risks precipitating colony failure, and therefore nests (if they can be detected) should only be inspected if there is a consensus that colony inspection is justified. Nest inspections will also be considered if a population decline occurs.

Table 20 Disease Risk Analysis for the destination hazard Nosema bombi

DESTINATION HAZARD

Nosema bombi

JUSTIFICATION OF HAZARD

Nosema bombi is present in the source (Larsson 2007) and destination (Alford 1975) environment. However *N. bombi* was absent from *B. subterraneus* queens screened from the source population in 2011 (n=57, estimated prevalence 0-7.1% [at the 95% confidence level]; we can estimate, with over 90% confidence, that the prevalence in the Swedish population is less than 5%). Evidence suggests that the microsporidian may also have differing effects across host species; Otti & Schmid Hempel (2008) found infections to be severe in *B. terrestris* hosts, while Rutrecht & Brown (2009) found the results of infection to be negligible to fitness in *B. lucorum* hosts. This suggests that *N. bombi* strains may differ in their virulence to hosts. Alternatively variable host life-history may account for these differences. *N. bombi* is known to be transmissible to *B. subterraneus* (Tay *et al.* 2005; Larsson 2007) and it is possible that there may be strain differences between the UK and Sweden owing to geographical isolation. However, Rutrecht & Brown (2009) suggested the absence of strain variation and molecular analyses by Tay *et al.* (2005) found no evidence for host-species or geographical strain specificity in *N. bombi* in Europe.

EXPOSURE ASSESSMENT

On release *B. subterraneus* queens are likely to be exposed when foraging prior to nest building. Spores are released to, and acquired from the environment (Imhoof & Schmid-Hempel 1999) through the decay of a dead infected host or by the shedding the spores in an infected bumblebee's faeces (Cali & Takvorian 1999). However given that *B. subterraneus* is a long tongued bumblebee (Goulson *et al.* 2005), the flowers it visits and feeds on, are more likely to be visited by other native long tongued species. As such the limited foraging behaviour of *B. subterraneos* may reduce its exposure to *N. bombi* as will the fact that the prevalence of infection is typically low in May, when the queens will be released.

Post exposure the *B. subterraneus* queens will forage for resources and establish their colonies. It is likely that any infected queens will initially transmit the *N. bombi* infection by vertical transmission, (Rutrecht & Brown 2008b) and then from worker to larvae. The spread and establishment of infection in bumblebee colonies will increase with the percentage of workers infected during larval development (Rutrecht & Brown 2007). This is because most bumblebees spend the first few days after emergence in the nest, leading to an accumulation of infective material, through faecal shedding. This increases the intensity of infection in the next generation of larvae (Rutrecht & Brown 2008b), as the agent may be horizontally transmitted when a larva/bumblebee ingests the parasite (McIvor & Malone 1995; Otti & Schmid-Hempel 2008). Larvae also have an increased susceptibility to infection compared to adults (van den Eijnde & Vette 1993) and a colony's susceptibility to infection increases with time because closely related kin acquire parasitic infections more easily, as the parasite adapts to its host (Schmid-Hempel & Crozier 1999; Shykoff & Schmid-Hempel 1991b).

Therefore there is a low likelihood of exposure, but if exposed a moderate likelihood of infection.

N. bombi is then disseminated from colony to colony through interactions at shared food resources (Durrer & Schmid-Hempel 1994) or through the drifting of infected workers to other hives (Sakofski & Koeniger 1988; Schmid-Hempel 1998). Infected males could also infect healthy queens during copulation (Otti & Schmid-Hempel 2007), potentially spreading the agent to secondary colonies of *B. subterraneus* that will be established by the progeny of uninfected introduced queens.

Late developing species, such as *B. subterraneus*, however will be less efficient at widely disseminating the infection horizontally between colonies, as they have less time within a season to do so. Given that *N. bombi* dissemination is more effective by early season species and almost every individual in a colony is susceptible, there is a medium likelihood that the agent will be disseminated locally, with a wider and more significant infection the following year. This is due to the high likelihood that the agent will be contracted by some of the early developing *Bombus* spp. queens.

CONSEQUENCE ASSESSMENT

There is a medium likelihood that a reintroduced queen will be exposed and infected. If infected Infected malpighian tubules can become extremely enlarged by the parasite (Larsson 2007). In some cases they may be destroyed, releasing mature spores into the lumen (Otti & Schmid-Hempel 2007). Heavily infected bumblebees may lose their power of flight (Fantham & Porter 1914; Larsson 2007); become lethargic and clumsy (Larsson 2007; Otti & Schmid-Hempel 2007) or develop distended abdomens (Macfarlane *et al.* 1995; Otti & Schmid-Hempel 2007).

Queens exhibit very little infection associated fitness losses to *N. bombi* (Fisher & Pomeroy 1989), and survival is unaffected (Otti & Schmid-Hempel 2007). However, controlled colony infections show the significant negative impact *N. bombi* can have on colony reproduction and growth (Otti & Schmid Hempel 2007). After experimental infection, 89% of *B. terrestris* workers derived from an infected queen harboured *N. bombi* (Otti & Schmid-Hempel 2008). Uninfected *B. terrestris* worker survival is significantly better than that of *N. bombi* infected workers and infected males over 21 days old (Otti & Schmid-Hempel 2007). In the field, reduced worker survival significantly hinders an infected colony from being able to gather enough resources to produce gynes, sexual adults (Otti & Schmid-Hempel 2008). Furthermore the ability of any infected gynes to produce their own offspring is considerably worse (Otti & Schmid-Hempel 2007). The net result is that *N. bombi* infection lowers colony fitness in *B. terrestris*. The impact of this parasite in *B. lucorum* is much lower (Rutrecht & Brown 2009). In America the collapse of the commercial *B. occidentalis* populations are thought to be attributable to *N. bombi* infection (Whittington & Winston 2004; Velthius & van Doom 2006).

The likelihood of a reintroduced bumblebee becoming exposed and infected towards the end of the season is high, as the agent will have increased its intensity that may allow it to pass between colonies, and through shared resources. Furthermore the infected bumble bees may stay longer at flowers and multiple passage of the agent through each generation will probably increase its ability to reproduce efficiently inside the host. The culmination of these factors increases the likelihood of the development of a bumblebee host that is capable of shedding a large quantity of spores into the environment.

Prevalence and infection intensity of drones is also significantly related to prevalence and intensity in the workers. Infection intensity in drones rises as a colony ages (Rutrecht & Brown 2008b). It is therefore highly likely that an infected queen, that establishes a colony, will propagate a large number of infected workers and drones, with both a high prevalence and intensity of infection that will be capable of spreading *N. bombi* to other colonies.

The biological consequences of infection could include the failure of reintroduced bees to produce viable colonies and therefore significantly increase the time and economic cost of the introduction. However ecosystem dynamics have a low likelihood of being severely affected, as the most likely species to be detrimentally effected are the reintroduced *B subterraneus* queens.

DESTINATION HAZARD *Nosema bombi*	RISK ESTIMATION	RISK EVALUATION
	There is a low likelihood of exposure, but if exposed a moderate likelihood of infection. The biological consequences of infection pose a medium risk to the reintroduced bumblebees. But given the low likelihood of exposure the overall risk level to the reintroduction is considered to be LOW.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

There is a lack of treatment options for this microsporidian in bumblebees. *N. apis* in honey bees is frequently treated successfully with fumagillin, however, this has not been found to work effectively against *N. bombi* in bumblebees (Whittington & Winston 2003). Therefore the emphasis on risk options should be on methods to prevent significant infection and monitor the parasite.

Pathogen surveillance should be carried out on all dead *B. subterraneus* queens found in the field, post reintroduction. Given post release population surveillance using defined transects will be undertaken, any dead queens or workers found during this surveillance should be collected and submitted for post-mortem examination These individuals should be examined for the presence of the microsporidian by light microscopic examination where white, rice shaped spores will be seen. For accurate species discrimination the real-time PCR developed by FERA is recommended.

Table 21 Disease Risk Analysis for the destination hazard entomopathogenic fungi

DESTINATION HAZARD	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT

DESTINATION HAZARD

Paecilomyces farinosus,
Verticillium lecanii,
Metarhizium anisopliae

JUSTIFICATION OF HAZARD

Paecilomyces farinosus, Verticillium lecanii, Metarhizium anisopliae are found in Sweden (Subiaprasent 1987, Vanninen 1995) and the UK (Leatherdale 1970; Macfarlane *et al.* 1995) *Paecilomyces farinosus*, *Verticillium lecanii, Metarhizium anisopliae* fungi are known to be transmissible to bumblebees (Schmid-Hempel 1998; Goettel *et al.* 1990) and a wide variety of insect hosts (Leatherdale 1970).

Isolates from different countries have varying levels of pathogenicity (Liu *et al.* 2002; Yeo *et al.* 2003) and it is possible that strain differences exist.

EXPOSURE ASSESSMENT

Reintroduced bumblebees could be environmentally exposed through spore deposition on their cuticle (Ferron 1978; Faria & Wraight 2001), through body openings or by ingestion (Schmid-Hempel 1998). This is followed by formation of a germ tube, which penetrates the cuticle by enzymatic (Boucias & Pendland 1998; Askary *et al.* 1999; Lopes-Llorca *et al.* 2002) & mechanical action (Goettel *et al.* 1989; Hajek & St. Leger 1994). After a stage of mycelial growth, which usually kills the host, externally borne conidiophores develop, which create conidia to infect further hosts (Ebert & Weisser 1997; Askary *et al.* 1999). Germination of the spores is a temperature *Metarhizium anisopliae* (optimum 30°C), *Paecilomyces farinosus* (optimum 20°C) (Hallsworth & Magan 1999; Fariah & Wraight 2001) and humidity (Gillespie & Crowford 1986; Hallsworth & Magan 1999; Chandler *et al.* 1994) dependant process. Infective conidia can be horizontally transmitted, by the wind or rain, leading to exposure of susceptible hosts (Schmid-Hempel 1998). Additionally, in optimal environmental conditions, fungal hyphae from germinated spores or fungus killed hosts can grow across substrates to contact new hosts (Faria & Wraight 2001) for example in a nest.

If an infected queen exposes her progeny to a high enough concentration of conidia spores the larvae may become infected but the likelihood of this scenario is low. If the concentration of spores on the queen is high enough to infect her progeny, she herself is likely to succumb to the infection, particularly since translocation, hibernation, foraging and hive construction are likely to make her more susceptible to infection. This could lead to death, due to the effects of stress and suppression of the immune system, prior to egg laying.

Reintroduced queens can disseminate conidia in two ways: through fungal related death resulting in mycelial growth, or through vectoring the agent to a more susceptible host. With social insects, higher concentrations of conidia, lead to a greater likelihood of mortality and a shorter time to death*; B. bassiana* in bumblebees (Smith *et al* 2000; Kapongo *et al.* 2008b), *M. anisopliae* in termites (Traniello *et al.* 2002). Atypical of the other entomopathogenic fungi, *V. lecanii*, may concomitantly colonise as well as penetrate, the host's cuticle. This could increase the concentration of innoculum at the cuticle surface, leading to an enhanced probability of spores coming into contact with suitable sites for penetration (Askary *et al.* 1999). Thus, the probability of host death due to *V. lecanii* infection may be greater than the other two fungi. However, successful penetration is dependent on environmental variables & host-parasite genotype-genotype interactions. Overall the likelihood of exposure to a sufficient number of spores to cause infection is low.

However, should a reintroduced *B. subterraneus* queen succumb to any of the fungal pathogens after reintroduction, spores (conidia) will be released to the environment from the cadaver (Faria & Wraight 2001). Dissemination to the queens progeny is likely as the UK climate provides the necessary conditions for germination. If the spore load is high, the progeny are not likely to survive and therefore the likelihood of further dissemination is low, however the likelihood of the progeny being infected by a sufficient spore load to cause disease is low.

CONSEQUENCE ASSESSMENT

The likelihood that a sufficient dose of an entomopathogenic agent attaches itself to the cuticle of a reintroduced bumblebee to infect it is low. However the biological consequences of infection are high. The fungus, after penetrating the cuticle, proliferates as a walled hyphal body or a wall-less protoplast in the host's haemocoel. The host eventually dies of nutrient depletion, invasion of organs, toxinosis or physical obstruction (Gindin *et al.* 1994; Hajek 1997; Butt & Goettel 2000). In heavy fungal infestations, bumblebee brood mortality can occur (Macfarlane *et al.* 1995).

The impact of *Paecilomyces farinosus, Verticillium lecanii, Metarhizium anisopliae* on the population dynamics of bumblebees is unknown. A study by Liu *et al.* (2002) demonstrated that the virulence of *P. farinosus* (56-62% mortality), *Verticillium lecanii* (<40%), against the tarnished plant bug (*Lygus lineolaris*) was less than that of *B. bassiana* (>80%) and *M. anisopliae* (>80%). Additionally *P. farinosus and M. anisopliae* grew at a slower rate than *B. bassiana* (Hallsworth & Magan 1999). A study by Hokkanen *et al.* (2003) demonstrated that *B. bassiana* may be more virulent to bumblebees than *M. anisopliae* (Hokkanen *et al.* 2003) & *B. bassiana* has a relatively small impact on bumblebee fitness, unless inoculated by a large dose of spores (Al-mazra'awi 2004; Kapongo 2008a; Kapongo 2008b).

Thus if the growth rate of *V. lecanii* & *P. farinosus* is slower and causes a lower mortality to some insect species, than *B. bassiana* and *M. anisopliae*, they may be less virulent to bumblebees. However, without definitive proof or study of the specific effects *V. lecanii* & *P. farinosus* have on bumblebees, it can not be assumed that this is the case, as species differences exist between virulence of some strains to host species (Yanagawa *et al.* 2009).

The biologic costs of infection are high and the infection could lead to reintroduction failure. There would be economic costs in failure but the environmental costs are low because the effect would be to reintroduced bumblebees.

DESTINATION HAZARD	RISK ESTIMATION	RISK EVALUATION
Paecilomyces farinosus, Verticillium lecanii, Metarhizium anisopliae	Reintroduced queens have a low probability of being exposed to sufficient spores to cause infection. The biological consequences of infection are high because the infection causes high mortality. The overall risk level is LOW.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS
Pathogen surveillance should be carried out on all dead *B. subterraneus* queens found in the field, post reintroduction. Given post release population surveillance using defined transects will be undertaken, any dead queens or workers found during this surveillance should be collected and submitted for post-mortem examination. Because most entomopathogenic fungi kill their hosts within a few days to a week (Askary *et al.* 1999; Liu *et al.* 2002; Yanagawa *et al.* 2009), any dead reintroduced bumblebees should be checked for signs of fungal growth. Fungal culture should be undertaken if any white growths are seen on the cuticular surface or internally.

Table 22 Disease Risk Analysis for the destination hazard Sphaerularia bombi

DESTINATION HAZARD *Sphaerularia bombi*	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT

EXPOSURE ASSESSMENT

Wasps (*Vespula*), and bumblebees (*Bombus*) can harbour the nematode (Macfarlane & Griffin 1990) but only queens can be infected (Goulson 2003). Only the gyne offspring of the reintroduced queens who were successful in producing colonies would be exposed. Third larval stage, *S. bombi* nematodes, live, mate & moult twice in the soil (Poinar & Van Der Laan 1972; Macfarlane & Griffin 1990). Fertilized adult nematodes enter a hibernating queen bumblebee (Benton 2006) & start producing eggs. Post-hibernation, nematodes evert their uteri and start producing eggs (Polnar & Van Der Laan 1972). As many as 100,000 eggs are released during a period of two weeks, into the host's body cavity. Hatched third-stage larvae live in the haemocoel (Macfarlane & Griffin 1990; Benton 2006), until they are excreted from the queen's anus (Poinar & Van Der Laan 1972). Should the queen die, before the larvae are excreted, they can survive in a state of anabiosis in the cadaver until they are exposed to water (Poinar & Van Der Laan 1972). This survival trait allows nematodes to persist in high numbers in the environment (Macfarlane & Griffin 1990). The host's behaviour is radically manipulated after emergence from hibernation. Instead of colony founding, an infested queen aids dissemination of the nematode larvae by faecal excretion. Queens fly close to the ground, frequently alight, dig multiple shallow holes and crawl under fallen leaves. These behaviours increase the quantity of infective third stage larvae at potential hibernation sites (Poinar & Van Der Laan 1972; Benton 2006). After ten weeks the third stage larvae mate, then continue their maturation into adults, ready to infect any new queens that hibernate in their vicinity (Macfarlane & Griffin 1990; Benton 2006).

Given that, *S. bombi* makes behavioural alterations to the host to maximise its chances of infecting future hosts, the likelihood of a second season queen being exposed is high but this will depend upon the hibernation site choices of *B. subterraneus* queens which are unknown.

Given the Dungeness release site, is an exceptionally fauna rich area, supporting 13 other bumblebee species (Williams 1986), the likelihood of exposure is high. On emergence from hibernation, that queen would then release a further 100,000 larvae in the release area, thus disease dissemination is highly likely.

CONSEQUENCE ASSESSMENT

It is highly likely that one second generation reintoroduced queen will be infected. If infected, the biological consequences on bumblebee health are extremely high. The most common cause of death is probably the result of exhaustion and fat reserve depletion, rather than the nematode infestation itself (Poinar & Van Der Laan 1972). Some heavily infested queens are also co-infested with fungal pathogens that probably hasten the death of the queen (Poinar & Van Der Laan 1972). Parasitized queens cannot form eggs and found colonies, as ovary development is inhibited (Macfarlane & Griffin 1990). In Cantebury NZ the *S. bombi* associated mortality of *B. terrestris* & *B. hortorum* queens was estimated to be between 3-10% (Macfarlane & Griffith 1990).

S. bombi may have considerable effects on the size of the reintroduced bumblebee population, especially if the UK strain is highly virulent compared to the Swedish strain (Williams 1986). This could considerably extend the reintroduction stage of the project and therefore significantly increase the economic cost of the reintroduction. However ecosystem dynamics have a low likelihood of being severely affected, as the most likely individuals to be affected are the reintroduced queens in the second year of the reintroduction.

JUSTIFICATION OF HAZARD

Sphaerularia bombi is present in the UK (Alford 1969; Donovan 1980) and Sweden (Larsson 2007) and is known to be transmissible to *B. subterraneus* (Macfarlane 1975). We screened a sample of the source population *B. subterraneus* queens in 2011 and four queens were infected with *S. bombi,* (n=57; estimated prevalence 2.1-17.9% [at a confidence level of 95%]).

It is not known whether differences in virulence exist between the *S. bombi* populations in the two countries (Goulson personal comm. 12 June 2009). However, as the British and Swedish populations are geographically isolated there is a high likelihood that genetic differences have arisen.

RISK ESTIMATION

It is highly likely that a reintroduced queen will be exposed and infected in hibernation given the biodiversity of the Dungeness release site and then go on disseminate the infection. The biological and economic consequences to the reintroduction are significant. The overall risk to the reintroduction program is MEDIUM.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

Pathogen surveillance should be carried out on all dead *B. subterraneus* queens found in the field, post reintroduction. Given post release population surveillance using defined transects will be undertaken, any dead queens or workers found during this surveillance should be collected and submitted for post-mortem examination. On post-mortem examination the fat body and mid-gut should be checked for the presence of larval stages of *Sphaerularia bombi* sp. under light microscopy.

Table 23 Disease risk analysis for the destination hazard Paenibacillus larvae (causal agent of American foulbrood in honeybees)

DESTINATION HAZARD

Paenibacillus larvae

JUSTIFICATION OF HAZARD

Paenibacillus larvae, a spore-forming bacterium*,* is the causal agent of 'American foulbrood' (AFB), a devastating disease of honeybees (Genersch 2010) which is notifiable in many countries, including Sweden and the UK (Defra 2009, Swedish University of Agricultural Sciences2011). The pathogen has a widespread distribution (Genersch 2010), and outbreaks of AFB have occurred in honeybees in both Sweden and the UK (Defra 2009, Swedish University of Agricultural Sciences 2011). There have been at least 15 outbreaks of AFB in honeybee colonies in Kent since 1999 (there have been no outbreaks in 2012 and no data is available specifically regarding 2011 [https://secure.fera.defra.gov.uk/beebase/public/BeeDiseases/afbSummary.cfm]). There are four known strains (genotypes) of *P. larvae* ('ERIC I-IV'), of which only two (ERIC I and II) have been isolated from field outbreaks "in recent years" (Genersch 2010). ERIC I has been isolated from a majority of AFB outbreaks internationally, and might have the greatest negative impact at the colony level (Genersch 2010). Both strains have been isolated from AFB outbreaks in Europe: ERIC II has been isolated from outbreaks in Germany and Austria but its distribution elsewhere is not known (Genersch 2010). Therefore, there might be strain differences between Swedish and British *P. larvae* isolates.

EXPOSURE ASSESSMENT

Released bumblebees would be exposed to *P. larvae* spores through use of food or pollen sources (Colla *et al.* 2006) shared with honeybees, if the honeybees with which they were sharing the resources were contaminated with *P. larvae* spores or shedding them in their faeces (which is unlikely). The primary means of horizontal spread of *P. larvae* between honeybee colonies appears to be through 'healthy' colonies 'robbing' other, infected, colonies that have succumbed to AFB (or by anthropogenic means, i.e. beekeepers moving contaminated brood or honey between hives) (Lindström *et al.* 2008).

Bumblebees that had been exposed to *P. larvae* might excrete the spores in their faeces (Lindström *et al.* 2008), or possibly transport spores in contaminated pollen on their body/legs. If a bumblebee was exposed to *P. larvae* the probability that it would transport spores back to its colony is low, since the bacterium does not 'infect' adult bees (Genersch 2010), rather they would need to act as 'mechanical' vectors carrying spores back to the colony on their body or via their gut. Since the bacterium does not appear to be a pathogen of bumblebees (Schmid-Hempel 2001; Benton 2006) the risk of dissemination within an exposed colony would be very low.

RISK ESTIMATION

The risk of exposure of bumblebees to *P. larvae* as a consequence of the reintroduction is low and the probability of dissemination is very low. If colonies at the release site were exposed to *P. larvae* the likelihood of them becoming infected, or adversely affected, by the bacterium is low. The overall risk level is therefore LOW.

CONSEQUENCE ASSESSMENT

The likelihood of one bumblebee being infected is negligible because bumblebees are not known to be susceptible to *P. larvae* infection (Schmid-Hempel 2001; Benton 2006; van der Steen & Blom 2010). The likelihood of disease in reintroduced bumblebees is therefore very low.

RISK EVALUATION

Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

Measures should be taken to minimize stress to the bumblebee queens during quarantine and post-release, to reduce their susceptibility to any pathogens which they could encounter on release.

Table 24 Disease Risk Analysis for the carrier hazard Deformed Wing Virus

CARRIER HAZARD	RELEASE ASSESSMENT	EXPOSURE ASSESSMENT

CARRIER HAZARD

Deformed Wing Virus (DWV)

JUSTIFICATION OF HAZARD

Deformed wing virus (DWV) is a viral pathogen of *Apis mellifera* associated with colony collapse disorder (CCD) when transmitted by the *Varroa destructor* mite (Yue *et al.* 2007). It is pathogenic to both honeybees and bumblebees (Genersch *et al.* 2006).

DWV has been found in Sweden (Nordstrom *et al.* 1999) and has been reported in southern England (Highfield *et al.* 2009).

Research on viral honeybee diseases in France indicated that DWV persistently infects bee populations in the absence of clinical signs, suggesting that colony disease outbreaks might result from environmental factors (for example nutritional stress, relocation and diseases of other aetiology) that lead to viral replication (Tentcheva *et al.* 2004). Therefore a shift from covert (asymptomatic or latent infection) to overt (symptomatic) infection is more likely when bumblebees are subjected to stressors, which may occur during the reintroduction.

If reintroduced short-haired bumblebees are infected with DWV the stress of reintroduction may precipitate disease.

RELEASE ASSESSMENT

In Sweden the *B. subterraneus* queens destined for reintroduction will forage for resources prior to their capture. These bumblebee queens could be exposed via faeco-oral transmission when feeding at sites visited by honeybees, leading to subsequent horizontal transmission within an infected colony (Yue & Genersch 2005). Although both honeybees and bumblebees are susceptible, given that honeybees have short tongues (6.5-8.5mm) and *B.subterraneus* have long tongues with a tongue length of 11mm the flowers these two species visit and subsequently shed virus on, are likely to differ. Furthermore given *B. subterraneus* are late developing species, they are less efficient at widely disseminating the infection horizontally between colonies, as they have less time within a season to do so.

Venereal transmission via infected semen has also been reported (Yue *et al.* 2007). Once infected vertical transovarial transmission will disseminate infection amongst the colony (Yue *et al.* 2007). DWV has been detected in all life stages of European honey bees and all workers drones and queens with wing deformities harbour the virus (Chen *et al.* 2005, Williams *et al.* 2009). Exposure to DWV via the mite vector *Varroa* is also possible. The *Varroa* mite is present in Sweden, and Swedish honeybees with DWV are in contact with *Varroa* sp. and cross-species transmission has been reported in one species of bumblebee, the American bumblebee (*Bombus pennsylvanicus*) (Ongus 2006). However *Varroa* has not been reported to affect European bumblebees (G. Budge, personal communication October 2009). Therefore there is a very low likelihood of exposure to DWV via the *Varroa* mite in Sweden.

Overall there is a low likelihood of exposure in Sweden as *B.subterraneus,* has a different feeding pattern to honeybees and is a late emerger from hibernation. However, if exposed it is likely that any infected reintroduced queen will initially transmit DWV through transovarial transmission and then horizontal transmission from worker to larvae.

EXPOSURE ASSESSMENT

Upon release, the *B. subterraneus* queens will forage for resources and establish their colonies. If the *B. subterraneus* queens are covertly or asymptomatically infected with DWV acquired in Sweden, factors which may significantly increase stress on individuals over the course of the reintroduction may precipitate clinical disease. Such factors may include transport, lack of suitable food resources, adaptation to a new environment and exposure to novel parasites. In addition inclement weather, and unfavourable flying conditions for long periods of time will keep bees in their nests which may lead to in-nest faecal deposition, a major source of replicating viruses. All the above factors, including infestation with the *Varroa* mite may lead to the development of clinically symptomatic infection (Beelogics, 2010).

As soon as elevated virus titers are reached, the virus becomes virulent and clinically symptomatic disease results (Genersch *et al.* 2010). There is a medium likelihood that significantly stressful events will occur during reintroduction which may lead to DWV disease.

If the above mentioned stress factors exist and bees are infected then it is likely that many of the reintroduced bumblebees will develop clinical disease. This may be lethal in which no further dissemination will occur. Alternatively, infected queens will transmit DWV through transovarial transmission to workers and then horizontal transmission from worker to larvae. The spread and establishment of infection in bumblebee colonies may increase with the percentage of workers infected during larval development, as has been shown for at least one other parasite of bumblebees (Rutrecht & Brown 2007). DWV could most likely be passed from colony to colony through interactions at shared food resources (Durrer & Schmid-Hempel 1994).

However it is most likely that only the reintroduced queens and their offspring would be affected with covert (asymptomatic) infection. But this could lead to a failure of the reintroduction if the bumblebees were subjected to severe stressors on release. This could considerably extend the reintroduction stage of the project and therefore significantly increase the economic cost of the introduction.

Ecosystem dynamics have a low likelihood of being severely affected, as the most likely individuals to be affected are the reintroduced queens. Although honeybees could be affected the different feeding pattern and late emergence from hibernation of *B. subterraneus* makes dissemination to honeybees of low likelihood.

| CARRIER HAZARD

Deformed Wing Virus (DWV)	RISK ESTIMATION	RISK EVALUATION
	There is a low likelihood of exposure, but if exposed a high likelihood of covert (asymptomatic) infection and dissemination through vertical transmission. Evidence suggests that the likelihood of significant epidemic disease is high if severe stressors occur during the reintroduction. This could include competition for food resources or secondary infectious disease whereby clinically symptomatic infection results leading to mass mortality. Therefore the overall risk level is MEDIUM.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS

There is no product available for DWV control. To minimize the impact of DWV and other viral infections: Release in late spring, as given *B.subterraneus* are late emergers from hibernation they will then exit the nest in warmer weather and release to an optimum environment managed for bumblebees to minimize competition at food sources.

All dead *B. subterraneus, B. pascourum* (long-tongued species) or *B. hortorum* (common species) queens or workers found in the field during post release population monitoring using transects should receive detailed post-mortem examination and as a component of this be tested for DWV by real-time PCR (Genersch 2004).

Table 25 Disease Risk Analysis for the transport hazard Aspergillus candidus

TRANSPORT HAZARD *Aspergillus candidus*	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
JUSTIFICATION OF HAZARD Pathogenic fungus reported worldwide (Kozakiewicz 1990) and has been isolated from dead *Apoidea* sp. (Batra *et al.* 1973) and *Bombus* sp. (Macfarlane 1976). *Aspergillus* sp. are ubiquitous and aspergillosis occurs when there is either a high environmental load, or is precipitated by stressors (e.g. transport and re-introduction).	*Aspergillus candidus* is ubiquitous. Exposure to reintroduced queens undergoing transport will occur via spore penetration through the body which is highly likely to occur if there is high stocking density in the transport container, damp conditions, poor ventilation and poor hygiene such as spoiled food. Batra *et al.* (1973) reported spoilage of transport provisions initiated by bacteria (*Lactobacillus* spp., and *Streptomyces* spp. and gram-negative rods), fungi (primarily yeasts and *Fusarium* spp) or the two groups acting together led to increased secondary fungal growth of *Aspergillus*. There is a high probability that at least one bumblebee will be exposed on the journey from Sweden to England. The disease is not considered contagious by horizontal or vertical transmission (Kearns 2003); however, more than one insect in a group is frequently affected due to exposure to the same stressors or other environmental conditions.	There is a low likelihood that a queen will be exposed and infected. However if infected, Macfarlane (1976) reported that overwintered *Bombus* sp. queens with *Aspergillus* sp. survived for only 9 (range 3-17) days compared to 50 (range 1-126) days (n=129). The abdominal contents of infected queens were solid and stiff with white mycelia within a few hours post death. The advanced colonization of the abdomen of the queens, and the reduction in their longevity indicated pathogenicity. In *Apoidea* sp. (Batra *et al.* 1973) *Aspergillus flavus* was the most common destructive filamentous fungus and destroyed the cell contents of 14.1% of 1733 cells (probably first invaded by yeasts and *Lactobacillus*) examined in 1968 in North America. Cells of alkali bees (*Nomia melanderi*) infested with *Aspergillus* were completely filled with mycelium extending up to and beyond the cell cap and sporulation was restricted to the apex of the bouquet-like growth (Batra *et al.* 1973). The presence of *Aspergillus* sp. will not necessarily result in an invasion of the larvae. Healthy prepupae are frequently seen completely surrounded by mycelium growing from the faecal material in a nest however a high environmental load and a compromised host will predispose to infection. Given *Aspergillus candidus* is found worldwide the environmental consequences of infection would be low and economic costs would be associated with improved hygiene which is likely to be labour intensive. Widespread infection is unlikely to occur which could lead to a failure of the reintroduction if management practices are sound.

RISK ESTIMATION	RISK EVALUATION
The likelihood of infection during transport is low, as it is unlikely that bumblebees will be exposed to a high environmental load of spores in their single use transport boxes or to moist conditions with poor ventilation. The likelihood of significant epidemic disease is low given the assumed low stocking density, but the likelihood of compromised immune status is medium given the stressors associated with transport. Infected bumblebees are likely to have a shortened life span and succumb to infection however the likelihood of infection occurring is low. Therefore the overall risk level is LOW.	Preventative measures should be employed to reduce the disease risks.

RISK OPTIONS
Optimal temperatures for *Aspergillus* growth range between 25-30'C (Belli *et al.* 2004). Bumblebees should be transported at temperatures to minimise *Aspergillus* growth. All transport materials should be clean and dry prior to loading. No food should be placed in the transport boxes and ventilation should be adequate according to the stocking density of the transport boxes (defined by an algorithm).

Table 26 Disease risk analysis for the population hazard, Pesticides

POPULATION HAZARD	EXPOSURE ASSESSMENT	CONSEQUENCE ASSESSMENT
Pesticides		

POPULATION HAZARD

Pesticides

JUSTIFICATION OF HAZARD

Pesticides are widely used in British agriculture (Fera 2009) and might have been a causal factor in the overall decline of bumblebee populations in Britain over the last 30 years (Thompson 2001). Reintroduced *B. subterraneus* are likely to be exposed to pesticides on crops and wild flowers on agricultural land bordering the Dungeness reserve. Pesticide exposure can negatively impact the health and foraging success of bumblebees (Thompson & Hunt 1999; Thompson 2001). The Wildlife Incident Investigation Scheme has detected pesticides (dimethoate, cyhalothrin or alphacypermethrin) at potentially toxic levels in at least three incidents of bumblebee mortality reported to the scheme since 1995 (Thompson & Hunt 1999; Health and Safety Executive 2011), however, it is likely that a larger number of such incidents have occurred and gone undetected, because, unlike in honeybees, bumblebee colonies are small and not closely monitored, and individuals forage in a variety of habitats where deaths are unlikely to be detected (Thompson & Hunt 1999; Thompson 2001).

EXPOSURE ASSESSMENT

The National Nature Reserve at Dungeness where short-haired bumblebees will be released comprises over 1,000 hectares. Pesticide levels in the reserve are likely to be low. However, as queens disperse following the release (in a bumblebee-empty landscape, bumblebee queens can disperse up to 130km per year [Macfarlane & Griffin 1990], although distances are likely to be much lower in the UK [Lepais *et al.* 2010]), as the population of released bumblebees expands year-by-year, and as next-generation queens disperse in the autumn, some of the population are likely to move out of the reserve and encounter areas of agricultural land where pesticides are used.

For *B. subterraneus* colonies that establish within Dungeness reserve, the likelihood of pesticide exposure will be low: studies have found that bumblebee workers commonly forage up to only 300m from their nest and workers from colonies in the reserve are therefore most likely to forage in the reserve (Thompson & Hunt 1999).

For *B. subterraneus* colonies that establish outside the reserve, it is likely that at least a low level of pesticide exposure will occur at some point during the course of their foraging season, given the common practice of pesticide spraying in agriculture (Fera 2009) and the large amount of agricultural land in Kent. Bees foraging on flowering plants that have been contaminated with pesticides will be exposed to the chemicals in two main ways: firstly, through drinking contaminated nectar, and secondly, through direct contact with pesticide residues on plants (Thompson & Hunt 1999). Short-haired bumblebees (and other long-tongued species) might be relatively specialist feeders compared with other *Bombus* species (Goulson *et al.* 2005), but even so, individuals of this species are still likely to forage from a broad range of flowering plants (Williams 2005, Thompson & Hunt 1999). These plants are likely to include: agricultural fruits that might have been sprayed with pesticide; arable weeds, which might have been exposed inadvertently during crop spraying; and flowering plants in field margins and hedgerows that might have been contaminated through spray drift (Thompson & Hunt 1999). Bumblebees are most active in the early morning and late evening (unlike honeybees, which forage most in the middle of the day), increasing their likelihood of direct exposure to pyrethroid pesticides which are applied specifically in the early morning or late evening (one reason being to avoid honeybee exposure) (Thompson & Hunt 1999; Thompson 2001). Pesticides are used on a wide variety of crops and could potentially be applied throughout the Spring or Summer, overlapping with months of *B. subterraneus* activity (Thompson & Hunt 1999).

We consider there to be a low risk of pesticide exposure for bumblebees within the reserve, whilst for bumblebees that disperse out of the reserve the risk of exposure is medium. The primary aim of the reintroduction is to establish a viable population of *B.subterraneus* at Dungeness, therefore for the purposes of this DRA we categorise the risk of exposure as low.

CONSEQUENCE ASSESSMENT

The likelihood of one bumblebee being exposed to pesticides at the release site is low, whilst the likelihood of exposure in the wider area surrounding the nature reserve is medium.

Bumblebee colonies are particularly susceptible to the effects of pesticides because their establishment is reliant on the survival of a single overwintering queen, and a smaller number of workers than in honeybee colonies. Individual bumblebees are likely to be just as susceptible to the effects of pesticides as honeybees taking into account their feeding behaviour (frequent foraging trips – up to 27 per day) and relatively large size (Thompson & Hunt 1999; Thompson 2001). Risk assessments for two permethrin pesticides in bumblebees found there was a "slight" to "high" risk of toxicity at levels of experimental exposure (Thompson & Hunt 1999). The level of pesticide exposure will determine how deleterious exposure is; the potential levels of exposure and the potential toxicities of pesticides are difficult to quantify (Thompson & Hunt 1999; Thompson 2001). Laboratory studies of *B. terrestris* have demonstrated that high doses of pesticide can cause mortality, and incidents of bumblebee mortality have been associated with misuse (and even recommended use) of pesticides (Thompson & Hunt 1999). Exposure to a high concentration of pesticide would most likely occur if a bumblebee was foraging in an area at the same time that it was being sprayed, or shortly after, and overall the risk of exposure to a high load of pesticide would be low. It is likely, however, that a low level of pesticide exposure would occur in bumblebees foraging outside the reserve, which could have a more insidious negative effect on their population.

Pesticides are likely to have other, more subtle, effects, such as those of insect growth regulators on brood development (Thompson & Hunt 1999) and the effects on individuals' memory and behaviour. Subtle negative impacts of pesticides on bumblebees might impact populations of the plants that they pollinate, and lead to wider ecosystem effects (Thompson & Hunt 1999).

The primary aim of the reintroduction is to establish a viable population of *B.subterraneus* at Dungeness, and at this site the risk of adverse consequences from pesticides is low.

POPULATION HAZARD	RISK ESTIMATION	RISK EVALUATION AND RISK OPTIONS
Pesticides	The overall risk to *B.subterraneus* from pesticides at the release site is LOW. However, pesticide exposure might pose a MEDIUM risk to longer-term range expansion of *B. subterraneus* provided a population at Dungeness is successfully established.	It will not be possible to control the movement and dispersal of reintroduced bumblebees, and those that disperse away from the reserve might be exposed to pesticides. We are not aware of any methods to prevent the adverse effects of exposure to pesticides in free-living bumblebees.