



# Small hive beetle diagnosis and risk management options

European Food Safety Authority (EFSA)

## Abstract

Visual inspection of a bee hive or commodity combined with the use of traps is recommended to screen for small hive beetle (SHB) presence. Any observation or result of a screening test suggesting the presence of SHB should be confirmed. Treatments like heating, freezing and/or irradiation can be applied to eradicate SHB from non-living bee products and from used beekeeping equipment, but cannot be applied to living material as these will kill bees and brood along with the SHB. Prevention, control and/or reduction of an SHB infestation in a honey bee hive while keeping the bees and/or brood alive, could be achieved using mechanical control, chemical and biological treatments or applying good beekeeping practices. It is feasible and effective to conduct surveillance in SHB-affected zones and control for pest freedom during transport of commodities and at the place of destination via internationally recognised systems of health certificates. This strongly depends on the delay between the health checks and the departure from the place of origin, because the free-flying pest may infest the bees and/or products between these two steps if adequate precautions are not taken to avoid the infestation. If SHB has been detected very early after its arrival and is not yet widespread, it is recommended applying an eradication approach rigorously and immediately after SHB detection to prevent further spread of the pest since none of the available risk mitigation methods can be applied to fully control the pest outside of managed bee colonies and/or commodities. Implementation of all available methods to prevent, control and reduce SHB infestation is recommended when eradication is considered not to be a feasible option anymore in the considered zone. Screening for the presence of SHB in swarms and feral colonies will inform risk managers on the feasibility to eradicate the pest in the considered zone.

**Keywords:** small hive beetle, *Aethina tumida*, diagnosis, risk reduction option, bees

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# 1. Introduction

## 1.1. Background and Terms of Reference as provided by the European Commission

The recent EFSA opinion on small hive beetle (SHB) and *Tropilaelaps*<sup>1</sup> has addressed comprehensively the risk of entry of these pests into the European Union (EU). Since the publication of the opinion in early September 2014, SHB has been detected in Calabria, Italy, with dozens of infected apiaries within an area of 20 km radius. Surveillance outside this area but within and beyond a radius of 100 km has not detected other occurrences. However, the tracing of colonies practicing transhumance, within the area and having left the area, were later confirmed infected, leading to the discovery of SHB in Sicily in early November.

These areas are a major source of queen bees produced in large quantities for shipment to many places in the EU, as well as for mobile apiaries or transhumant hives moving in from elsewhere and leaving afterwards for 3 flowering seasons from spring to late autumn.

Italy has implemented regional and national measures to contain, survey and if possible to eradicate SHB.<sup>2</sup> This involves destruction of infected apiaries and restriction of movement of colonies and certain apiculture products, by-products and beekeeping equipment. A Commission Decision has also been adopted covering aspects related to intra-EU trade.<sup>3</sup> In particular colonies and queens must not leave restricted areas. However, it should be noted that anecdotal evidence suggests that intra-EU movements of live bees are liable to illegal activities, which are difficult to control, particularly in the case of queen bees that can be easily hidden or sent (e.g. by post). This exposes the rest of the EU to a risk of introduction of the SHB, despite sound rules, especially if those are perceived unnecessarily restrictive.

While the current aim of the Italian veterinary services is to eradicate the SHB, it is uncertain whether this is possible and if not which are the best method to mitigate against its spread as well as the damage caused in apiaries. It is also unclear whether SHB is capable of surviving various European winter conditions, to spread and to establish permanently either in the already infected areas or beyond or to become endemic. There are uncertainties as to whether it would have a major impact on the bee population and on the beekeeping activities implying serious socio-economic consequences for the beekeeping sector that are disputed at least by some, e.g. by a certain Italian beekeeper's organisation.

In North America, the introduction of the SHB caused damages to the beekeeping sector, mainly in the southern States of the USA, while in the northern States damage was more limited and survival of SHB is less clear. In Canada its survival, spread and damage remained low, raising the question of its ability to become established.

Very few animal health requirements for SHB in the usual intra-EU trade context have been established, based on the fact that SHB has been hitherto exotic in the EU. The relevant Directive 92/65/EEC<sup>4</sup> lays down animal health requirements for intra EU movements of bees and the model health certificate for such movements. It should be noted that these requirements are simply meant to create in an initial phase an automatic block for movements of bees in case an outbreak would be notified in a Member State. They are not suitable to handle trade between infected areas and free areas.

In order to avoid the introduction into the EU of the SHB (and *Tropilaelaps* spp.) with imports of live bees, Regulation (EU) No 206/2010<sup>5</sup> contains the requirements and the model certificate for import of live queen bees and queen bumble bees. These requirements have been assessed favourably by the previous EFSA opinion. Nevertheless these requirements still stipulate freedom from SHB within an area of 100 km radius. This is a condition that large parts of Italy are unlikely to be able to fulfil, should similar rules apply to them as to third countries, unless SHB is completely eradicated.

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In order to support the Commission and the Member States in improving the control, eradication and trade measures as regards the SHB, scientific advice from EFSA is required in this area. The Commission therefore considers it opportune to request EFSA to assess all the available scientific information and to evaluate the risk of survival, establishment and spread of the SHB in the EU.

In view of the above, in accordance with Article 31 of Regulation (EC) No 178/2002, the Commission asks EFSA to provide scientific and technical assistance concerning:

1. the currently employed diagnostic methods for the detection of SHB and the risk mitigation measures applied worldwide in relation to SHB in apiaries and in controlled establishments producing queens, as well as measures applied to domestic movements of colonies, queens and other honeybee products and by-products
2. the best practices or strategies to be applied in an infected area in order respectively to eradicate or to control the spread of the SHB.

## 1.2. Interpretation of the Terms of Reference

The Terms of Reference of this scientific opinion relate to currently used diagnostic methods for the detection of SHB and risk mitigation measures that are in place to prevent or reduce the risk of SHB survival, spread and/or establishment. In this scientific report, the use of diagnostic methods and the implementation of risk mitigation measures were considered in a bee hive or in commodities consisting of queens, colonies or swarms, bee products to be used in apiculture (e.g. bee-collected pollen, fresh royal jelly and propolis with beeswax), non-extracted honey combs and used beekeeping equipment. The risk pathways regarding natural movement (e.g. wind, flying beetle) is not included in this scientific report since it will be assessed in detail in the scientific opinion that will be adopted by end 2015. The risk pathways regarding non-bee products (e.g. fruits) and soil were not considered as major pathways of spread and therefore not included in this scientific report. Only honey bees (*Apis mellifera*) are taken into account in this scientific report since an in depth assessment of the role of *Bombus* spp. in survival, spread and establishment of SHB will be done in the scientific opinion that is requested to be generated by end 2015.

## 2. Methodologies

Relevant scientific publications were identified by searching the Web of Science using the search string 'small hive beetle' OR '*Aethina tumida*'. Publications in English published from January 2000 to January 2015 were included. Screening the titles and abstracts identified 39 publications on diagnostic methods or risk mitigation measures. Research papers from before 2000 and identified by the experts to be relevant to the Terms of Reference, were also included.

Identification of currently applied diagnostic methods to screen for the presence of SHB was done based on the available scientific literature and consultation of OIE Terrestrial Manual (2014b). Only reported methods were considered in this scientific report. Each method is briefly described and the experts discussed the technical feasibility of each method based on what has been reported in the scientific literature. Information on detection levels and/or recovery rates was retrieved from the scientific literature. The sample size needed to detect SHB in an area, when it is present, was calculated to illustrate the scale of sampling that would be required in practice.

In order to calculate the sample size needed (number of hives to be sampled, considering to sample one hive per apiary given that presence of SHB between hives within an apiary are potentially correlated) to detect SHB in an area, when it is present (assuming that the population it is sampled from is finite), the following formula was used (Cannon, 2001 and 2002):

$$n = \frac{1}{1 - \left( \frac{1 - \frac{1}{N}}{1 - \frac{1}{N}} \right)^{\frac{1}{1 - \frac{1}{N}}}} \quad (1)$$

Where:

$n$  is the required sample size

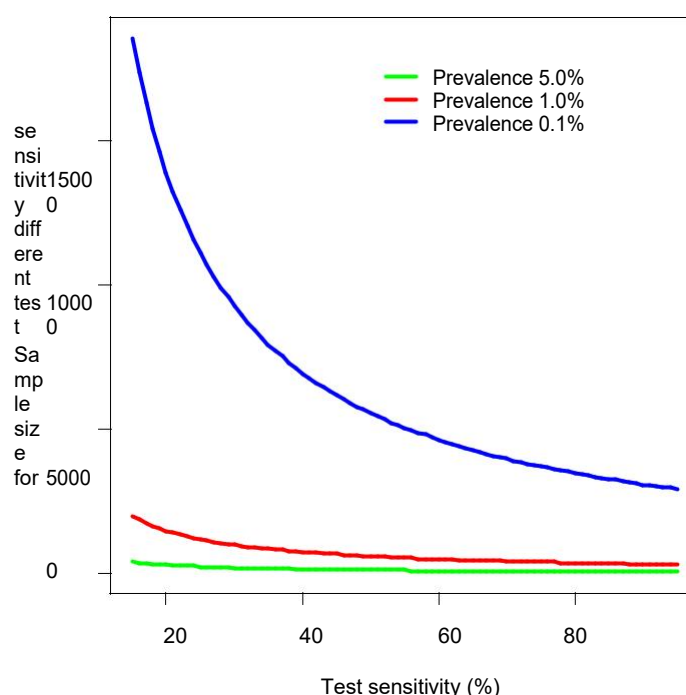
$N$  is the pre-specified Confidence Level

is the prevalence to be detected

is the total population size

is the test sensitivity

Equation (1) assumes a test specificity to be 100%, but it is acceptable given that the purpose of the surveillance is to detect SHB. In order to estimate the sample size required it is needed to specify the prevalence to be detected, here three scenarios were considered (0.1%, 1% and 5%). The confidence level used for the calculations was 95% and the population size considered was 20,000 (Figure 1). However, when specifying test sensitivity to be used, a panel of four experts were consulted and asked to provide their educated guesses for each of the detection methods considered relevant. Two rounds of consultations were performed, in which lower and upper quantiles were asked. After the first round of consultation, results of detection test sensitivity ranges were provided and discussed with the experts. In the discussion, all potential factors that could be influencing the performance of the test were considered, and a second round of individual consultation was carried out. After the second round, the precautionary principle was considered, reporting the minimum sensitivity lower quantile and the maximum of the upper quantile values reported by the working group experts. This approach considered maximum uncertainty, reflecting the lack of information. It is important to highlight that the approach has been followed in the absence of data, but should not be considered as definitive. Experiments should be conducted in order to explore deviations with respect to expert opinions regarding test sensitivity to detect SHB and calculation of sample size should be redone when more information becomes available.



**Figure 1:** Graphical representation of the sample size needed for different detection method sensitivities (ranging from 15% to 95%) and prevalence to be detected (5%, 1% and 0.1%).

The risk mitigation measures identified in the scientific opinion on the risk of entry of SHB into the EU (EFSA, 2013) were evaluated by the experts and reformulated to clarify them and to make them applicable to the survival, spread or establishment of SHB within the EU. The experts scored the effectiveness, technical feasibility and uncertainty based on the definitions described in the previous scientific opinion (EFSA, 2013) (Appendix A). For scoring the effectiveness of the risk mitigation measures, it was assumed that the risk mitigation measure was implemented in an optimal manner. The rationale used as basis for the given scores, are described in the scientific report.

### 3. Assessment

In this scientific report, no distinction is made between bee hives in apiaries or in controlled establishments producing queens since there is no scientific evidence available that there might be a difference in biosecurity levels between them. Some measures could be taken by queen breeders to improve the biosecurity level, for instance better control of the health status of living honey bees that are introduced, use of new equipment, use of new queen mailing cages, careful selection of the 6–8 worker bees to be placed in the queen mailing cages, visual screening of the queens, loaded cages kept separate so that no beetles can pass through the mesh screens. However, it is impossible to implement a closed system as used to rear bumble bee colonies due differences in the biology of honey bees. For instance, honey bee queens naturally mate in flight outside of the hive. 'Instrumental insemination' can be performed in specialised facilities, but the whole process still cannot all be done entirely inside a protected system. Another limitation is the cost for construction and maintenance of these biosecurity plants and their efficacy from the point of view of queen rearing with freedom from SHB infestation.

#### 3.1. SHB diagnostic methods

Only diagnostic methods currently used for screening are considered in this chapter of the scientific report. These methods rely on detection of eggs, larvae and/or adult beetles by visual inspection of all components of a hive, use of traps, visual inspection after killing the bees and the pest, or use of PCR on samples of hive debris. Digging and sieving soil around infested hives<sup>6</sup> is the only method available to screen for SHB pupae, but this method is not included since soil as a risk pathway is not considered in this scientific report, owing to the confounding factors such as soil type, moisture retention capacity, slope of the landscape and prevailing weather conditions. In the event of pest detection inside the hive, a confirmatory diagnostic test (e.g. morphological analysis or PCR) will have to be performed to eliminate the possibility of false positive screening results and to avoid unnecessary destruction of bee colonies. The reported information on detection levels or recovery rates, sensitivity estimates provided by the working group experts and technical feasibility for each of the methods are presented in Table 1.

##### 3.1.1. Visual inspection of all components of a hive

This procedure is often accomplished with two people,<sup>7</sup> one to work the colony and the second to collect the beetles. They proceed as follows: remove the lid from the hive and meticulously examine the inner part of the lid for the presence of adult beetles. Place it at the side of the bee hive. Lightly smoke the colony, remove the outermost frame in the super and/or in the hive (e.g. Dadant–Blatt type), and quickly examine both faces of the framed comb for the presence of adult SHB bearing in mind the SHB have aversion to light and therefore are expected to swiftly move to dark areas. The outermost frame is then placed at the side of the hive and all the other frames undergo the same visual inspection one by one. Once inspected, each frame is reintroduced in the super or in the hive in the same order using the room left available by the outermost frame in order to prevent robbing. Then the inside faces of the hive and the bottom board are carefully examined. When all frames have been inspected, they are placed again, in the original position as is the outermost frame and the hive is closed. When present, the frames of both the super and the hive should be thoroughly examined (Mutinelli et al., 2014). This procedure was adapted from those described by Spiewok et al. (2007), Neumann and Hoffmann (2008) and (Neumann et al., 2013; OIE, 2014a) and has been further improved to limit robbing. Solution for extreme satiation would be to use a second box with a lid to protect screened frames from robbing bees. A video is available on the IZSve website.<sup>8</sup>

Visual inspection is currently used to screen for the presence of SHB in bee hives or commodities of, for example, queens, colonies or swarms, bee products to be used in apiaries, non-extracted comb honey or beekeeping equipment (e.g. OIE, 2014a; Spiewok et al., 2007). Visual inspection can also be applied outside managed bee colonies such as facilities like honey houses or fruits (e.g. Mutinelli et al., 2014).

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<sup>6</sup> Majority of wandering larvae and pupae within 2 meters around the hive if suitable soil is present, distance can increase drastically (50 meters or more) if there is no suitable soil around the hive.

<sup>7</sup> Usually the beekeeper and the official veterinarian or bee inspector, or two beekeepers.

<sup>8</sup> <https://www.youtube.com/watch?v=wFb9EZeIIzc&list=UUZ5EUOfj2fHpKU-H0ZtJPw>, last accessed 10 February 2015.





Spiewok et al. (2007) reported an experiment to estimate the numbers of SHB that might have been missed during visual inspection. Specific numbers of adult SHB (42, 88, 98, 112, 135 or 172) were introduced into six SHB-free colonies and SHB were given one hour to disperse inside the colonies before the control inspection started. The results showed that on average 9 (8–10) SHB were not detected, which corresponds to an average failure rate of 8.4% (6.4–11.5). Neumann and Hoffmann (2008) reported that  $14.06 \pm 10.53\%$  of the adult SHB remained undetected during visual inspections compared to visual inspection after killing the bees and the pest. However, there are no data reported on the detection limit and/or sensitivity of this diagnostic method. The sensitivity ranges given by experts when using the precautionary principles (see section methodology) is 25% to 90% (Table 1). The number of samples (bee hives) required to detect SHB in a bee hive at a prevalence of 5%, 1% and 0.1% ranges from 65–238, 329–1,188 and 3,090–11,128 respectively when considering 20,000 bee hives in the considered zone. It is clear that when the prevalence to be detected decreases the required number of samples increases. It should be noted that these results should be considered as indicative rather than definitive, prevalence of SHB and only aim to guide risk managers in defining what is feasible to apply in the field to monitor prevalence of SHB in bee hives. The wide sensitivity ranges provided by the experts also indicate the level of uncertainties, indicating the need for experiments specifically designed to estimate sensitivity of the visual inspection method. The experts indicated that it was not possible to provide meaningful informed guesses on the sensitivity of visual inspections to detect SHB in non-living commodities due to very high uncertainties.

The proposed procedure for bee hive visual inspection should be standardized as it cannot be replaced by any other method. The intensity of this method, as practised globally, seems to vary from visual inspection of debris collected in the bottom tray of the hive for the presence of eggs, larvae and adult beetles to meticulous dissection and inspection of individual combs for the detection of eggs and/or larvae. Owing to this, the sensitivity of this method may also vary. Visual inspection is however time consuming and requires trained people both in terms of honey bee manipulation and SHB detection. It is advisable that it is carried out by the beekeeper together with a veterinarian or a bee inspector according to the national/local organization.<sup>9</sup> Since beehive inspection implies its opening, it is not possible to apply it during winter or bad weather conditions (e.g. low temperatures, rain or snow). Therefore, visual inspection may not be feasible some time, particularly during winter season. The sensitivity of visual inspection of all components of a bee hive to screen for SHB might increase when this is done in combination with traps.

### 3.1.2. Use of traps

Different traps are currently available on the market or home made by beekeepers.<sup>10</sup> The detection level depends upon the design and construction of traps, in particular, their ability to trap and retain beetles and prevent bees from entering the trap and killing or eating the pest. Owing to this, the sensitivity of this method also varies. In addition, the shape, size, colour of material and location of traps inside the hive are confounding factors. A diagnostic trap (Schäfer et al., 2008) and a control-type trap (Beetle Blaster<sup>11</sup>) are examples of commonly used ones.

The diagnostic trap is inserted into the beehive through the hive entrance and left for at least two days (Schäfer et al., 2008). It is then removed and thoroughly inspected (each flute is examined or the corrugated plastic is shook against the sides of a bucket possibly containing water to immobilize adult SHB or is inserted in a plastic bag, sealed, shook and directly examined for SHB). If negative, the trap is inserted again into the hive; if positive, 24 hours at  $-12^{\circ}\text{C}$  or lower temperature is required before re-use in order to kill the SHB beetles possibly still present in the trap.<sup>12</sup>

The control-type trap (Beetle Blaster) is placed between the top bars of two frames adding some mineral or vegetal oils and vinegar as bait. The latter type of bait is used because of its presumed

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attractive effect on Nitidulidae beetles, although there are no clear data underpinning it (Bernier et al., 2014). This trap can be used for both diagnostic and control purposes. Since it is applied between frames, it requires the opening of the beehive for both the placement and removal of traps. Propolis of trap openings has to be checked and propolis removed if present. Trap efficacy could be compromised if propolis is not removed (Bernier et al., 2014).

Traps are currently used to screen for the presence of SHB in bee hives or commodities of, for example, colonies or swarms (e.g. OIE, 2014a).

There are some data on the efficacy of traps although the results are difficult to compare with each other due to many confounding factors. Schäfer and co-workers (2008 and 2010) tested diagnostic

strips made of corrugated plastic (75 × 500 × 4 mm), which created rows of narrow tunnels, placed on bottom boards over two nights. The overall strip efficacy was determined as  $35.4 \pm 20.6\%$  (N = 54 colonies) by comparing total SHB numbers in the hives (visual inspection) with the numbers in the strips. Comparison of a transparent d-strip (75 × 500 × 4mm) and a black d-strip (100 × 478 × 4 mm) revealed similar efficacies,  $28.3\% \pm 29.6\%$  versus  $29.9\% \pm 24.8\%$  (Schäfer et al., 2010). A study on the efficacy of bottom board traps using corrugated plastic sheets (15 × 15 × 1.5 cm, with a gauge of 0.5 cm) and CheckMite+ strips containing coumaphos as active ingredient for SHB diagnosis and control revealed that  $14.06 \pm 10.53\%$  SHB remained undetected in comparison to inspection of the hives after killing the bees and the pest (Neumann and Hoffmann, 2008). Since the majority of SHB was found on the bottom boards (Neumann and Hoffmann, 2008) a trap located there presumably has a better chance of catching beetles compared to other locations in the hive (in case of non-screened bottom boards). Comparing Beetle Barn<sup>TM</sup>, Hood<sup>TM</sup> and AJ's Beetle Eater<sup>TM</sup> traps did not reveal significant differences in efficacy of traps over different sampling dates (Bernier et al., 2014), probably because this study did not estimate the non-detection errors of any of the tested traps. The use of yeast-induced pollen dough as a bait in bottom board traps has been reported to capture significantly more beetles than unbaited traps (Torto et al., 2007), but again also this study failed to deliver a convincing sensitivity. In a study analysing out-hive pole traps made of a polyvinyl chloride (PVC) pipe containing fermented pollen dough bait, the average catch in white traps (mean ± SE,  $2.47 \pm 0.30$ ) was statistically significantly higher than that of black traps ( $1.53 \pm 0.29$ ) (de Guzman et al., 2011). However, another study showed that SHB prefer shaded colonies over those which are sun-exposed (Arbogast et al., 2009) and field experience strongly suggests that adult SHB are negative photo tactic. Among the heights evaluated, there were more beetles caught when traps were positioned at 46 cm (the same height as the entrance of the hives) with  $3.07 \pm 0.51$  beetles compared with beetles captured at 1 m ( $1.88 \pm 0.30$ ) or 3 m ( $1.06 \pm 0.18$ ) high. However, the relationship between the numbers of beetles in colonies and capture rates in traps was very poor and did not provide a basis to evaluate trap efficiency. The traps used in this study are not SHB-specific since a high number of non-SHB Nitidulid were caught. Arbogast and colleagues (2012) described a trap designed to intercept post-feeding larvae as they reach the end of the bottom board on their way to the ground for pupation. Trap efficiency was estimated by releasing groups of 100 larvae into empty brood boxes and counting the numbers trapped. Some larvae escaped, but mean efficiency ranged from 87.2 to 94.2%. It was mentioned that the traps also detected small numbers of larvae leaving honey bee colonies, even when no larvae were observed in the hives.

There are no data reported on the sensitivity of using traps to screen for the presence of SHB. The sensitivity ranges given by experts when using the precautionary principles (see section methodology) is 15% to 85% (Table 1). The number of samples (bee hives) required to detect SHB in a bee hive at a prevalence of 5%, 1% and 0.1% ranges from 69–398, 349–1981 and 3,272–18,547 respectively when considering 20,000 bee hives in the considered zone. It is clear that when the prevalence to be detected decreases the required number of samples increases. It should be noted that these results should be considered as indicative rather than definitive and only aim to guide risk managers in defining what is feasible to apply in the field to monitor prevalence of SHB in bee hives. The wide sensitivity ranges provided by the experts indicate the level of uncertainties, indicating the need for experiments specifically designed to estimate sensitivity of the detection by using traps. The experts indicated that it was not possible to provide meaningful informed guesses on the sensitivity of traps to detect SHB in non-living commodities due to very high uncertainties.

The procedure for the use of traps should be standardized, particularly the duration of the application (at least 2 days). Usually, only adults are detected by traps. Sensitivity is affected (lower sensitivity)

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by weather conditions (<20°C, Schäfer et al., 2008). Traps can be implemented easily in wide areas and application does not require special expertise, particularly traps applied through hive entrance. Personnel are required to check traps over time. The sensitivity of using traps to screen for SHB will increase when combining with visual inspection of all components of a bee hive (i.e. at the moment when the trap is placed as well as when the trap is inspected). The use of UV light, as suggested by one publication (Duehl et al., 2012), is not suitable in the field (outdoor conditions) but may be relevant to indoor conditions.

### 3.1.3. Visual inspection after killing the bees and the pest

An accurate method to detect SHB is to conduct a visual inspection after killing the pest and the colony (Neumann and Hoffmann, 2008; Neumann et al., 2013). Bees and SHB can be killed by using for example petrol fuel vapours or sulphur dioxide fumes. All the bee hive entrances have first to be sealed. Honey bees are killed within 10–45 seconds of injection of one of these agents. However, it is recommended that one has to wait for 5 minutes and check if all honey bees are dead. If still alive, repeat the treatment and prolong the exposure time. This operation should be done when bees are not actively foraging, e.g. in the evening or early morning. Then all the frames and each part of the bee hive can be carefully inspected on site without the presence of moving bees or SHB. The operator should seek information regarding health and safety risks as well as legal requirements before selecting the product to be used.

Visual inspection after killing the bees and the pest is currently used to screen for the presence of SHB in bee hives or commodities of, for example, queens, colonies or swarms for research purpose but cannot be applied systematically in the field (Neumann and Hoffmann, 2008). In field conditions, this method could nevertheless be applied for wild swarms. Accurate visual inspection is indeed difficult to perform in the absence of wood frames and without destroying the colony.

There are no data reported on the detection limit and/or sensitivity of this diagnostic method. The sensitivity ranges given by experts when using the precautionary principles (see section methodology) is 35% to 95% (Table 1). The number of samples required to detect SHB in a bee hive at a prevalence of 5%, 1% and 0.1% ranges from 62–170, 312–849 and 2,928–7,948 respectively when considering 20,000 bee hives. It is clear that when the prevalence to be detected decreases the required number of samples increases. It should be noted that these results should be considered as indicative rather than definitive and only aim to guide risk managers in defining what is feasible to apply in the field to monitor prevalence of SHB in bee hives. The wide ranges provided by the experts indicate the level of uncertainties, indicating the need for experiments specifically designed to estimate sensitivity of the detection method. The experts indicated that it was not possible to provide meaningful informed guesses on the sensitivity of visual inspections to detect SHB in non-living commodities to very high uncertainties.

The procedure should be standardized. It cannot be widely applied because it requires destruction of the colony but is very useful for detection method validation (could be considered the 'gold standard'). It is time consuming and requires appropriate training. All live stages except pupae could be detected.

### 3.1.4. Use of PCR on samples of hive debris

A method to screen hive debris for the presence of SHB using real-time PCR in conjunction with an automated DNA extraction protocol has been described (Ward et al., 2007). Primers were designed to amplify the SHB cytochrome oxidase I gene (COI) from mitochondrial DNA and an *Apis mellifera* 18S rRNA real-time PCR assay was used as an internal positive control. The method was able to detect DNA from SHB eggs, larvae and adult specimens collected from Africa, Australia and North America and no cross-reaction was observed with DNA from nine genera of insects (some insects being from the same family as SHB). The method was used to successfully detect SHB DNA extracted from spiked and naturally infested debris (Ward et al., 2007) and has been used to detect SHB in hive debris collected from apiaries in a Spanish surveillance study (Cepero et al., 2014) and from sentinel apiaries being used as part of contingency exercises in the United Kingdom (personal communication Mike Brown, National Bee Unit, United Kingdom, 04 February 2015).

This diagnostic method has been proposed to screen for the presence of SHB in bee hives (Ward et al., 2007). There are currently no reports available on the use of PCR to detect SHB in commodities of live bees or non-living products.

Reported detection level is  $17.28 \pm 2.84$  mg/30 g of spiked hive debris;  $29.69 \pm 2.55$  mg/10 g of naturally infested hive debris (Ward et al., 2007). There are no data available on the sensitivity of the method. The sensitivity ranges given by experts when using the precautionary principles (see section methodology) is 30% to 95% (Table 1). The number of samples required to detect SHB in a bee hive at a prevalence of 5%, 1% and 0.1% ranges from 62–198, 312–990 and 2,928–9,273 respectively when considering 20,000 bee hives. It is clear that when the prevalence to be detected decreases the required number of samples increases. It should be noted that these results should be considered as indicative rather than definitive and only aim to guide risk managers in defining what is feasible to apply in the field to monitor prevalence of SHB in bee hives. The wide ranges provided by the experts indicate the level of uncertainties, indicating the need for experiments specifically designed to estimate sensitivity of the detection method.

Standardization and validation of a PCR method is not done so far. Implementation of PCR to screen for the presence of SHB requires specialized laboratory and trained personnel. The procedure is quicker than inspection after killing the bees and the pest, but its application on a wide area or surveillance program have never been tested (Ward et al., 2007). Hives equipped with bottom board or mesh floor are required to collect debris. Sensitivity might be compromised by weather conditions and season because of the biological cycle of the SHB (as mentioned for the other diagnostic methods).

**Table 1:** Characteristics of currently used detection methods to screen for the presence of SHB in bee hives

Detection method	Reported information on detection levels or recovery rates	Sensitivity ranges given by experts <sup>(a)</sup>	Number of samples required given a design prevalence of <sup>(b)</sup>			Technical feasibility
			5%	1%	0.1%	
Visual inspection of all components of a hive	An average of 9 (8-10) SHB were not found corresponding to 8.4% (6.4-11.5) (Spiewok et al., 2007); 14.06 ± 10.53% of the adult SHB remained undetected during visual inspections (Neumann and Hoffmann, 2008)	25%–90%	65–238	329–1,188	3,090–11,128	No standardization, time consumption depends on the level of detail of the inspection, training required, not possible to apply during winter or bad weather, sensitivity might be compromised by cold weather conditions
Use of traps	The efficacy of some traps is reported (see main text) but it is impossible to compare these with each other.	15%–85%	69–398	349–1,981	3,272–18,547	No standardization, eggs cannot be detected, sensitivity might be compromised by open bottom boards and cold weather conditions, quick approach which can be applied in a wide area, should be applied in association with visual inspection
Inspection after killing the bees and the pest	No information reported	35%–95%	62–170	312–849	2,928–7,948	No standardization, cannot be widely applied because it requires destruction of the colony but very useful for detection method validation (gold standard), time consuming, training required, all live stages could be detected
Use of PCR on samples of hive debris	17.28 ± 2.84 mg/30 g of spiked hive debris; 29.69 ± 2.55 mg/10 g of naturally infested hive debris (Ward et al., 2007)	30%–95%	62–198	312–990	2,928–9,273	No standardization, sophisticated equipment required, quicker than inspection after killing the bees and the pest, trained staff required, debris cannot be collected in absence of a bottom board, sensitivity might be compromised by cold weather conditions

(a): Based on lower and upper quartile sensitivities (see methodology section)

(b): Population size of 20,000 bee hives and 95% confidence level

## 3.2. SHB risk mitigation measures

The risk mitigation measures identified in the previous scientific opinion on the risk of SHB entry into the EU (EFSA, 2013) were reformulated to be applicable to prevent and/or control the survival, spread or establishment of SHB in Europe. In contrast to the previous scientific opinion, SHB is now present in the EU and only the context within the EU is taken into account.

The effectiveness and technical feasibility of each risk mitigation measure was assessed, as described in the methodology chapter, for living bees (queens, colonies (can contain sealed brood and/or honey/empty frames) or swarms (no brood present)) and non-living materials (bee products used in apiculture (e.g. bee-collected pollen, fresh royal jelly and propolis with beeswax), non-extracted comb honey or used beekeeping equipment (trade of sealed brood comb was not considered as trade in this commodity was expected to be very limited)). Honey-bee semen and honey-bee venom are considered as safe commodities (OIE, 2014b), whereas packaged extracted honey, refined or rendered beeswax, propolis and frozen or dried royal jelly are subjected to treatment that would kill SHB (Mutinelli, 2011).

The risk mitigation measures could be applied in protection and/or surveillance zone, during transport or at the place of destination. The protection zone and the surveillance zone are defined by the Italian competent authorities (in absence of an EU standard) as the territory within a radius of 20 km and 100 km, respectively, around an SHB confirmed apiary. No movement of honey bees and bumblebees or commodities (unprocessed apiculture by-products, beekeeping equipment and comb honey intended for human consumption) are allowed from the whole territory of Calabria and Sicily to other zones in the EU (Commission Implementing Decision 2014/909/EU of 12 December 2014). In addition, the Decision provides an obligation for Italy to carry out surveillance in the 20 km zone, mirroring what the competent authority is already doing. According to the order issued by the region of Calabria (number 94, 19th September 2014), movements of bees and commodities in the protection zone are only allowed from 30 days after the last confirmed positive result (detection of SHB) in the protection zone<sup>13</sup> and after inspection of the apiary whereas movements of bees and commodities within the surveillance zone is allowed after two consecutive health inspections carried out 21 days apart with negative results demonstrating absence of SHB. A restriction on the movement of hives was not effectively implemented when SHB was introduced in Australia and is considered to have facilitated the fast spread of SHB throughout the country (personal communication Diana Leemon, Department of Agriculture and Fisheries, Brisbane, Australia, 18 February 2015).

Transport is considered to start with collection of living bees or non-living materials before packaging and shipment and ends at the arrival of the package at the place of destination (which can be located in the protection zone, the surveillance zone or zones considered to be SHB-free within the EU). Border controls are not taken into account when living bees or non-living materials are transported within the EU. Therefore, when shipping live bees or non-living materials, screening for the presence of SHB at the place of origin just before departure and then at place of destination immediately upon arrival is very important since it is the last checkpoint before possible release of the pest, if present, in the environment. This double inspection procedure is included in the current EU legislation on imports of live bees and bee products from third countries into the EU and is recommended to implement also for intra-EU trade.

The effectiveness, feasibility and uncertainty levels associated with the risk mitigation measures applicable to living bees and non-living commodities are presented in Table 2.

### 3.2.1. Applicable in a protection and/or surveillance zone

The implementation of risk mitigation measures in a protection and/or surveillance zone is not applicable to queen honey bees since implementation of measures will be done at colony or swarm level.

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<sup>13</sup>No movements have been allowed since last September in the protection zone until early March 2015 (personal communication, Franco Mutinelli, IZSVE, Italy, 02 March 2015).



## Monitoring the pest status

This risk mitigation measure means the implementation of a passive monitoring system, e.g. the compulsory notification and the relevant legislative framework for SHB throughout the whole territory of the country.

Monitoring the pest status will be done at colony or swarm level in the protection and/or surveillance zone and is therefore not applicable to queens only. In colonies and swarms, monitoring is feasible in practice using a range of different diagnostics methods with limited technical difficulties (visual inspection: Spiewok et al., 2007; traps: Neumann and Hoffmann 2008; Schäfer et al., 2010; PCR: Ward et al., 2007; Cepero et al., 2014), but the sensitivity/non-detection error is currently not known, resulting in a moderate uncertainty. In any case, beetles are likely to remain undetected in managed colonies and swarms due to a low infestation level. Moreover, detection of SHB outside of managed apiaries is at present extremely unlikely, hence efficacy is low. For bee products to be used in apiculture, non-extracted comb honey and used beekeeping equipment, no study has been published on SHB detection or survival. Based on prior experience, visual inspections appear to have moderate efficacy, can be easily implemented in practice but have a high uncertainty.

## Prevent, control or reduce infestation by the pest

This risk mitigation measure means that best practices and/or active monitoring programmes without certification (e.g., private initiative) are performed to ensure that the pest is absent or infection is controlled or reduced but keeping the bees alive or commodities intact. Monitoring can be done, for instance, by being observant for signs of prior infestation on bees hives such as slime trails on dead bee hives (Neumann and Elzen, 2004), using in-hive traps for early detection of beetle presence (Elzen et al., 2001; Neumann and Hoffmann, 2008), using pieces of live bee brood (pupal stage) as a bait in honey houses to detect early presence of SHB (SHB adults will locate brood and lay eggs making early detection possible), monitoring brood bait weekly for signs of SHB larvae. There are no treatment methods available to eradicate the infestation in any material containing living organisms (e.g. live bees and/or brood) without killing them, although some measures could be applied to control or reduce the infestation while keeping the bees and/or brood alive, for instance by maintaining good hygiene around the apiary/honey house (Sanford, 1999; Hood, 2011), extracting the honey within two to three days (Hood, 2011), avoiding the use of contaminated equipment, restrict the movement of hives, using mechanical control such as commercially available or home made in-hive traps (Hood and Miller, 2003; Bernier et al., 2014) or harbourage,<sup>14</sup> chemical treatments like coumaphos, fluvalinate or fipronil (Elzen et al., 1999; Mostafa and Williams, 2002; Levot and Haque, 2006a, b and c), biological treatments using fungi or nematodes (e.g. Ellis et al., 2004 and 2010; Mürrle et al., 2006; Cuthbertson et al., 2012 and 2013), possible alternative treatment such as slaked lime and diatomaceous earth product (Buchholz et al., 2009); ). Most of these control methods have been tested experimentally, but there are no conclusive results assessing their effectiveness in field conditions. Also the narrowing of hive entrances to repress beetle excess has been suggested (Ellis et al., 2003) although it could lead to increased temperatures in the hive. The use of organic acids has been shown to be effective in laboratory conditions (Schäfer et al., 2009) but not in field conditions (Buchholz et al., 2011). Nevertheless, at the moment, there is no veterinary treatment approved in the EU legislation to treat colonies for SHB but this could be made available via derogation (The cascade) of the current rule according to article 11 of Directive 2001/82/EC of the European Parliament and of the Council as amended by Directive 2004/28/EC of the European Parliament and of the Council by the Member State veterinary services. The use of insecticides raises also questions of innocuity for honey bees, of problems of residues in products dedicated for human consumption, of the development of resistant populations of beetles and of environmental toxicity (e.g. de Guzman et al., 2011; New South Wales website<sup>19</sup>). Chemical control in honey frames will not be granted approval by competent authorities.

Prevent, control or reduce infestation by the pest will be done at colony or swarm level in the protection and/or surveillance zone and therefore, this risk mitigation measure is not applicable to queens only. In colonies and swarms, prevention, control of reduced infestation is feasible in practice via active diagnostic monitoring or using best management practices (e.g. integrated pest management; Hood, 2011) and a range of different diagnostic methods (e.g. Baxter et al., 1999;



Elzen et al., 2001; Neumann and Hoffmann, 2008; Hood, 2011), and the efficacy appears to be moderate (Neumann and Hoffmann, 2008). However, detection systems rely mainly on training individuals to carry out visual inspection. Even with trained staff, there is the possibility of missing infestation. Reinfestation from outside of apiaries is likely (e.g. Spiewok and Neumann 2006; Mutinelli et al., 2014). In any case, beetles are likely to remain undetected/controlled outside non-managed bee hives (e.g. wild swarms, bumblebee nests and possibly on other food sources).

It is clear that several treatments can reduce and/or control SHB infestations while keeping bees and/or brood alive, but they often do not succeed in eradicating the pest and further optimization of the treatments is required. Treatments to control and reduce SHB infections have been reviewed in a DEFRA document,<sup>15</sup> of which the main findings and conclusions are summarised in this paragraph. Coumaphos is the active ingredient in commercially available products and is widely used for control of SHB in the USA. Elzen et al. (1999) demonstrated that SHB can be successfully controlled using 10% coumaphos-containing strips in trapping devices. Neumann and Hoffmann (2008) demonstrated that although mortality at the colony level was limited, the strips can be effectively used for estimating SHB infestation levels when applied in bottom board traps. *Bacillus thuringiensis* (*Bt*) is used for pest control in a range of commercial products but strain-dependant insect selectivity of *Bt*, indicates the need for further investigation into the testing of more *Bt* strains against SHB (Buchholz et al., 2006). Studies by the Australian government have shown the potential of a trapping device treated with dilutions of the insecticide fipronil as an in-hive control measure against SHB. Laboratory experiments identified fipronil from eight insecticides as the most potent to SHB. Tests demonstrated a 98.4% mortality of SHB compared to controls (Levot and Haque, 2006) and further development of the trap has shown favourable results in honey residue trails (Levot, 2007). Interestingly, of the other insecticides tested, temephos, imidacloprid and methomyl showed effectiveness similar to or better than coumaphos. Buchholz et al. (2009) tested the potential of dry slaked lime, powdered limestone and diatomaceous earth to control SHB. While slaked lime hindered pupation, treatment with high doses of diatomaceous earth significantly increased SHB mortality. No significant differences of SHB mortality were observed in laboratory treatments of powdered limestone. This indicates that diatomaceous earth products have potential as alternative in- hive chemical control of SHB. Mürrle et al. (2006) and Ellis et al. (2004) showed potential for fungal control of SHB. Pest mortality was significantly higher when larvae had been exposed to fungi post feeding, but further investigation is needed to establish if the identified *Aspergillus flavus* and/or *A. niger* are causative agents of SHB mortality. *Beauveria bassiana* significantly increased SHB mortality above all other fungal treatments. However, preliminary studies on SHB larvae produced a lower mortality. This confirms previous studies that suggest infectivity is linked to specific insect developmental stages. Cabanillas and Elzen (2006) and Ellis et. al. (2010), demonstrated that entomopathogenic nematodes could be an effective component of an integrated pest management scheme for SHB. In both the general persistence test and field trails, *Heterorhabditis indica* and *Steinernema riobrave* significantly increased SHB mortality. Location had a significant effect on SHB mortality with more SHB emergence from the forested site than the clear field site. The nematode *S. feltiae* has been shown to have little impact from work in the USA.

Improving beekeeping management practices after introduction of SHB in the US was able to reduce the impact of the infestation (Hood, 2011) via: keeping strong and queenright colonies (e.g. hygienic stocks, strong honey bee colonies harass beetles and keep them at bay, selection of good apiary sites: in sunshine/direct sun which lowers moisture and not in the shade); good sanitation in the honey house, at the apiary and in storage rooms; no storage of old combs, if required: cool storage (+4° C) or SHB proof; immediate (within 2–3 days) honey processing after harvest, if not possible: cool storage (+4° C) or SHB proof; keeping a high bee density in the hives relative to total comb area; providing bee access to all parts of the hive (e.g. appropriate size to control nest periphery, SHB adults are dangerous if they get access to the combs since the beetles are opportunists and will exploit any weakness); implementing even stricter control of other pathogens (e.g. *Varroa destructor*). Some useful advisory leaflets are available, illustrating nicely the key practices to follow (e.g. see websites of Clemson University,<sup>16,17</sup> University of Arkansas<sup>18</sup> or New South Wales.<sup>19</sup>

There are also bad or poor hive management practices that should be avoided since they can lead to SHB infestations in the apiary, for instance dead hives and catch boxes where infestations are allowed to establish and become a source of SHB in the apiary, weak/disorganised hives which are less able to attack SHB adults or replace brood and stores lost to SHB predation, expanding hives such as transferring nucleus hives to singles, brood manipulations where SHB adults and/or larvae can be transferred between hives, hive manipulations that leave bees disorganized such as splitting hives and leaving hives open for too long during mobile extraction, supplementary protein feed where feed is placed outside the brood nest is unprotected or cannot be consumed within two to three days, supplementary sugar feeding resulting in fermented syrup that provides a stimulating food source for both SHB adults and larvae, undefended frames in particular where supers/lids contain holes that allow access to SHB from outside the hive, placement of infested sticky hive inserts on hives, pollen traps where trapped pollen is left in the trap drawers for too long (Stedman, 2006).

Several treatments are described that could be used to control and reduce SHB infestation in bee products to be used in apiculture, non-extracted comb honey and used beekeeping equipment, while keeping the product or material intact. However, data are lacking to confirm this and the required infrastructure is not widely available in apiaries. Reported methods are:

(i) freezing: The precise thresholds values for each developmental stage are unknown. It is reported that adult beetles will die below 0°C, although no data were found (Frazier and Steinhauer, 2000; Somerville, 2003; Hood, 2011; New South Wales website<sup>19</sup>). Freezing at core temperature of minus 12°C or less for at least 24 hours is recommended by OIE (2014b). It is important that core temperature readings are taken under various loading capacities to establish both the minimum temperatures achievable by the unit and the time taken for all material to reach the target temperature (Stedman, 2006). Also cold rooms can be used to reduce SHB infestation on equipment as it will prevent SHB reproduction. This can be achieved with core temperatures of 10°C or below (New South Wales website<sup>19</sup>).

(ii) heating: Heating to 50°C core temperature and holding at that temperature for 24 hours is recommended for non-living materials by OIE (2014b). SHB development can also be prevented by maintaining a low relative humidity of 40% or below. This can be achieved using dehumidifiers in closed rooms, the use of fans to provide air movement through the equipment or by storing equipment to allow good air flow through it. (New South Wales website<sup>19</sup>) It is important to reduce relative humidity below 50% otherwise elevating the temperature might result in increased larval activity and damage (Stedman, 2006).

(iii) irradiation: irradiation with 400 Gy is recommended for non-living materials by OIE (2014b). There are some data available supporting the implementation of irradiation (100% adult male SHB mortality after six days when using 75 Gy personal communication Peter Neumann, Institute of Bee Health, Switzerland, 06 February 2015) but no published data were found in an initial screen of the scientific literature.

(iv) fumigation (e.g. aluminium phosphide <sup>20</sup> (Levot and Haque, 2006), carbon disulphide (Lundie, 1940) or paradichlorobenzene (Mostafa and Williams, 2002)),

(v) household bleach treatment (Park et al., 2002; New South Wales website<sup>19</sup>).

### **Conduct surveillance programmes aiming to achieve guarantees of pest freedom**

This risk mitigation measure means that a surveillance programme is in place aiming to achieve guarantees of pest freedom and a certificate is provided by an authority in case of a negative result for pest presence. An official pest-free status is given for a country or zone, for instance as described in the OIE International Animal Health Code (2014b).

This risk mitigation measure is not applicable to queens only since implementation of measures will be done at colony or swarm level. In colonies and swarms, surveillance is feasible in practice (OIE, 2014b; Spiewok et al., 2007 and 2008; Bernier et al., 2014) and efficacy may be high pending non-detection error and timing of the used diagnostic methods. However, reinfection from outside of apiaries is possible (e.g. Spiewok and Neumann, 2006). For bee products to be used in apiculture, non-extracted comb honey and beekeeping equipment, visual inspection can be implemented but the effectiveness of this risk mitigation measures is influenced by variation in awareness of bee pests and the available diagnostic capacity. However, data are lacking to confirm this.

### **Apply any treatment to eradicate the pest**

This risk mitigation measure means the application of a chemical (eg. acaricides, organophosphates, bleach or fumigants) or physical (e.g. irradiation, freezing or heating) treatment to eradicate SHB (e.g. Elzen et al., 2002; Park et al., 2002; Hood, 2004; Mürrle et al., 2006; Levot and Haque, 2006a and 2006b; Ellis and Delaplane, 2007; Buchholz et al., 2009; Schäfer et al., 2009; Cuthbertson et al., 2010; Buchholz et al., 2011). Killing of bees and destruction of the commodity is possible by implementation of this risk mitigation measure.

This risk mitigation measure is not applicable to queens only since implementation of measures will be done at colony or swarm level. For colonies and swarms, bee products to be used in apiculture, non-extracted comb honey and beekeeping equipment, burning can be performed to eradicate the pest (Mutinelli et al., 2014). Although soil as a risk pathway is not considered in this scientific report, it is worthwhile to mention that soil can be treated with permethrin (Baxter et al., 1999; White, 2003; Pettis and Shimanuki, 2000; Mutinelli et al., 2014). It has been recommended to kill the residual soil-burden of SHB pupae in treated apiary sites after beetle infested colonies have been removed (Delaplane, 1998). There are indications for an external traps application in order to intercept larvae when leaving the hive (Arbogast et al., 2012). However, very limited information is available on this topic.

## **3.2.2. Applicable during transport**

### **Control pest freedom of bee or product**

This risk mitigation measure means that a consignment is controlled for SHB presence at the moment of packaging and/or during a later stage of the transport process. This could for example be done by using internationally recognised procedures and the release of an official health certificate (OIE Terrestrial Animal Health Code, 2014b; Commission Regulation (EU) No 206/2010;<sup>21</sup> Council Directive 92/65/EEC<sup>22</sup>). Positive cases will be destroyed.

When this risk mitigation measure is applied, it minimises the probability of SHB spread. However, the effectiveness of this risk mitigation measure is influenced by variation in awareness of bee pests and the available diagnostic capacity (sensitivity of the method used). Health inspection should be done as close as possible to the initiation of the transport procedure. Inspection of honeybee queen consignments is effective because the way they are packaged is easy to be controlled (queens are in cages with a small number of associated attendants, e.g. usually 6-10 attendants but it can be up to twenty). Battery cages are more problematic, as in this system worker bees are not in individual cages but shared across all the queens in the box, and shaken in. These battery cages are not compliant with the current regulation on queen import from third countries. The control can be done during packaging by visual inspection. After inspection, the place where the consignments are stored before transport must be insect-proof. For colonies and swarms, effectiveness and feasibility of the control measures are lower due to the high number of bees in a colony or swarm commodity. They are

scored 'moderate' if the control is done before the transport. Visual inspection can be done and traps can be used for the control. Traps can be put in place during 48h and visual inspection can be conducted when the traps are inserted in the hive and when they are checked 48h later (double inspection). The effectiveness and feasibility scores are 'low' if the control is done during the transport: considering the way of packaging, control can be very difficult or even impossible to conduct. Inspection of non-living products is highly effective and feasible. The uncertainty level is low for all the commodities, except for colonies and swarms.

### **Apply any treatment to eradicate infestation during transport**

This risk mitigation measure is identical to the 'application of any treatment to eradicate infestation in an SHB protection and/or surveillance zone' (see above). Destroying living commodities is an effective and feasible measure to eradicate the pest if detected during transport. For the non-living products, the eradication can be done by freezing or irradiation (OIE, 2014b). They are effective and feasible measures, although specific infrastructures are required. The uncertainty level is low for all the commodities, published data being available concerning the way to kill SHB.

### **Isolate the bee or product to avoid exchange of the pest with the environment**

This risk mitigation measure means the application of any measure to prevent escape or entrance of the pest from the consignment (or from transport material) from the place of origin to the arrival at the final destination. This measure is applied in order to prevent contact with the environment.

An example is covering a consignment of honey bees with fine mesh through which a live SHB cannot enter (OIE, 2014b).

This risk mitigation measure is already applied for commodities of queens. The choice of the material (insect-proof mesh, for instance) is important. Consignments of bees, bee products and beekeeping equipment could be made insect-proof. Therefore, this risk mitigation measure would have a high effectiveness. The feasibility is high for living material and or non-living material. Covering honeybee colonies or swarm with a mesh is not a problem for their survival during transport (ventilation is sufficient). Although no specific publications exist concerning this topic, the uncertainty level is low. If the appropriate material is used (for instance, mesh fine enough considering the size of the adult small hive beetle and larvae<sup>23</sup>), these measures is efficient and feasible.

### **Hold bee or product under quarantine to guarantee pest freedom**

This risk mitigation measure means that the consignment is placed under quarantine to detect clinical signs of an SHB infestation and/or maintain quarantine until the pest is killed.

In the particular case of SHB, quarantine can allow time for eggs to hatch (incubation period between two to six days, depending on temperature and humidity conditions: Lundie, 1940; Stedman, 2006; Somerville, 2003). Eggs are indeed difficult to detect by visual inspection and difficult to detect by trapping unless oviposition slides are used (Neumann et al., 2013). It is usually easier to detect larvae than eggs. Quarantine is difficult to implement for living bees because the beetles will survive longer than bees (there are no exact data on the survival time of SHB but it has been reported to be more than six months (Lundie, 1940) and up to 13 months (personal communication Jeff Pettis, Bee Research, USDA, 06 February 2015)). It is a problem in particular for queen consignments. In theory, it could be done under quarantine laboratory conditions (e.g. Cuthbertson et al., 2008) but is not feasible to implement widely and hence is not done so far. Non-living materials can be kept under quarantine until the pest is killed. This option has a potential high effectiveness when applied on used beekeeping equipment, but there are practical issues in applying it systematically. For non-extracted honey combs, it could be possible to observe destructive effects of the pest (honey and comb destruction, honey fermentation for instance). In general, the time required to make quarantine effective is unknown and would need to be defined considering the biological characteristics of the pest.

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<sup>23</sup> Adult beetle and larvae are the life stages of SHB with the capacity to move and/or fly (OIE, 2013).

### **3.2.3. Applicable at place of destination**

#### **Control pest freedom on bee or product**

This risk mitigation measure means that a consignment is checked for SHB presence at the place of destination or screening of existing or sentinel hives at high-risk locations. Positive cases will be destroyed and notified to the competent authorities.

The same methods can be applied as described under 'control pest freedom on bee or product' during transport (see above), hence the same scores are given. Health inspection should be done as close as possible after arrival of the living bees or non-living material in the apiary at the place of destination and the beekeeper should remain vigilant by checking colonies and reporting any findings.

#### **Apply any treatment to eradicate infestation at the place of final destination**

This risk mitigation measure is identical to the 'application of any treatment to eradicate infestation in an SHB protection and/or surveillance zone' (see above). Queen cages with low number of attending workers and food can be visually screened which is likely to have a high efficacy (Murilhas, 2004; Valerio da Silva, 2014), has a high feasibility and a low uncertainty. Destroying living bees is an effective and feasible measure to eradicate the pest if detected at the place of final destination. For the non-living products, the eradication can be done by freezing or irradiation whereas burning after killing could be applied for living bees (Mutinelli et al., 2014). They are effective and feasible measures, although specific infrastructures are required. The uncertainty level is low for all the commodities, published data being available concerning the way to kill SHB.

#### **Reduce illegal trade**

This risk mitigation measure means the implementation of any action to reduce illegal trade from a protection and/or surveillance zone to other zones in the EU, for instance via awareness campaigns to increase the awareness on SHB. Scoring of effectiveness, technical feasibility and uncertainty was not possible.

**Table 2:** Scoring of effectiveness (Eff.), technical feasibility (Feas.) and uncertainty (Unc.) of risk mitigation measures applicable to bees or non-living materials

Risk mitigation measure	Queens			Colonies and swarms			Bee products to be used in apiculture			Non-extracted comb honey			Used beekeeping equipment		
	Eff.	Feas.	Unc.	Eff.	Feas.	Unc.	Eff.	Feas.	Unc.	Eff.	Feas.	Unc.	Eff.	Feas.	Unc.
<b>Applicable in SHB affected area</b>															
Monitor the pest status	NA	NA	NA	L	M	M	M	H	H	M	H	H	M	H	H
Prevent, control or reduce infestation by the pest	NA	NA	NA	M	M	M	H	M	L	H	L	L	H	L	L
Conduct surveillance programmes	NA	NA	NA	H	H	L	H	H	L	H	H	L	H	H	L
Apply any treatment to eradicate the pest	NA	NA	NA	H	H	L	H	H	L	H	H	L	H	H	L
<b>Applicable during transport</b>															
Control pest freedom of bee or product	H	H	L	M(a) /L(b)	M(a)/ L(b)	M(a)/ M(b)	H	H	L	H	H	L	H	H	L
Apply any treatment to eradicate infestation	H	H	L	H	H	L	H	H	L	H	H	L	H	H	L
Isolate the bee or product to avoid exchange of the pest with the environment	H	H	L	H	H	L	H	H	L	H	H	L	H	H	L
Hold bee or product under quarantine	N	N	L	N	N	L	H	M	M	H	L	M	H	M	M
<b>Applicable at place of destination</b>															
Control pest freedom on bee or product	H	H	L	M	M	M	H	H	L	H	H	L	H	H	L
Apply any treatment to eradicate infestation	H	H	L	H	H	L	H	H	L	H	H	L	H	H	L
Reduce illegal trade	No scoring possible														

(a): Before collecting bees.

(b): After collecting bees.

NA: not applicable; L: low; M: moderate; H: high; N: negligible.



## 4. Conclusions

**TOR1** – *the currently employed diagnostic methods for the detection of SHB and the risk mitigation measures applied worldwide in relation to SHB in apiaries and in controlled establishments producing queens, as well as measures applied to domestic movements of colonies, queens and other honeybee products and by-products.*

- Visual inspection of all components of a bee hive, use of traps, inspection after killing the bees and the pest, use of PCR on samples of hive debris are currently used to screen for the presence of SHB. However, standardization and validation of these diagnostic methods is lacking at present. The sensitivity of the methods will also depend on the timing, e.g. during winter or bad weather and the test sensitivity is likely to be compromised.
- Screening for the presence of SHB outside of and in a bee hive could be done using various traps. This could be applied on a wide scale (high coverage possible) given good weather conditions and sensitivity will increase when combining with visual inspection of all components of a bee hive.
- Screening for the presence of SHB in most commodities or facilities like honey houses could be done via visual inspection. The sensitivity might increase when this is done in combination with traps.
- Screening for the presence of SHB using inspection after killing bees and the pest can only be applied for research purposes.
- Any observation or result of a screening test suggesting the presence of SHB should be confirmed using morphological analysis and/or PCR method.

**TOR2** – *the best practices or strategies to be applied in an infected area in order respectively to eradicate or to control the spread of the SHB.*

- It is feasible and effective to conduct surveillance in SHB affected zones and control for pest freedom during transport of commodities (of queens, bee products to be used in apiaries, non-extracted comb honey and used beekeeping equipment) and at the place of destination via internationally recognised systems of health certificates. This strongly depends on the delay between health checks and departure from the place of origin and the preventative measures used to keep out the pest from entering the consignment, because the free-flying pest may infest the shipped bees and/or products immediately prior to departure.
  - Risk mitigation measures such as avoiding the use of contaminated equipment, maintaining good hygiene around the apiary/honey house, extracting the honey within two to three days, restrict movement of hives and implementing mechanical control, chemical or biological treatments can be applied to prevent, control or reduce an SHB infestation in a honey bee hive while keeping the bees and/or brood alive. However, these methods often do not succeed in eradicating the pest.
  - Risk mitigation measures considered in this report to prevent, control or reduce infestation by the pest in prevention and/or surveillance zones cannot completely eliminate the chances of survival, spread or establishment of this beetle due to its high mobility and possibility to reproduce outside of managed honey bee colonies.
  - There are no risk mitigation measures available to eradicate an SHB infestation in any material or product containing living organisms (e.g. life bees and/or brood), without killing them.
  - Treatments such as heating, freezing and/or irradiation can be applied to eradicate SHB from non-living bee products and used beekeeping equipment.
  - It is feasible and effective to isolate the bee or product during transport to avoid exchange of the pest with the environment.
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## 5. Recommendations

- If SHB has been detected very early after its arrival and is not yet widespread in a zone, it is recommended applying an eradication approach rigorously and immediately after SHB detection, to prevent further spread of the pest since none of the available risk mitigation methods can be applied to fully control the pest outside of managed bee colonies and/or commodities.
  - Implementation of all available methods to prevent, control and reduce SHB infestation is recommended when eradication is considered not to be feasible anymore in the considered zone due to the widespread distribution of the pest in the given zone.
  - Screening for the presence of SHB in swarms and feral colonies, for instance via visual inspection, will inform risk managers on the feasibility to eradicate the pest in the considered zone.
  - Diagnostic methods to detect SHB should be standardized and validated to allow rapid detection of the pest and to improve the design of monitoring activities.
  - Enhancing the education and awareness training in the detection and control of SHB and in the implementation of good beekeeping practices for beekeepers and officials is recommended to improve the awareness, skills and expertise required to prevent or control survival, spread and establishment of SHB.
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