Risk profile on edible insects

4.1 Microbiological hazards

4.1.1 Scientific evidence

As stated by Garofalo et al. (2019) in their review, “understanding of the microbial dynamics during insect rearing is limited, and is an important knowledge gap that needs to be filled”. Despite this, the literature review retrieved evidence showing the great variability of microbial profiles that can be observed between species, companies, and even batches within the same rearing company (Vandeweyer et al., 2017a). The level of microbial contamination and microbial variability in edible insects occurs not only due to the microbial profile of the substrate, but due to a variety of causes, including variabilities between insect generations, the stage of harvest of the animals, and the physico- chemical conditions to which they are submitted during processing (Osimani et al., 2018a, Wynants et al., 2019).

In the following paragraphs, data on microbial levels for different edible insects are presented, as well as the effect observed after the food was exposed to treatment. The presentation of quantitative information on main microbial groups is relevant for verifying the efficacy of heat-treatments and hygiene conditions of edible insect rearing (Camargo et al., 2019).

Bacteria and fungi

Several studies looking at the microbial profile of different species of edible insects were identified by this literature review. The majority of the studies focused on Tenebrio molitor and Acheta domesticus as species. Markers used by authors to describe the microbial profile included total aerobic counts (TAC), yeast and moulds count (YMC), Enterobacteriaceae, lactic acid bacteria, mesophilic aerobes, and species such as Bacillus cereus and Staphylococcus aureus. A summary of bacterial and fungal levels can be found in Tables A (unprocessed) and B (processed) in the Appendices. While the tables provide a comprehensive summary and overview of the bacterial levels in edible insects, numbers reflect averages and ranges as reported by authors, who used different methods of analysis across studies. Referring back to the original study is recommended for an in-depth understanding on how each level was obtained.

Parasites, viruses and prions

A prevalence study in 300 small-scale European insect farms detected three parasite families that are known to be harmful to humans in 88 farms, including Isospora spp., Balantidium spp. and Entamoeba spp. Results are summarised in Table 1. No parasitic load or incidence data was reported by the authors (Ga??cki and Sokó?, 2019). As of 2019, the parasitic transmission potential of insects used for food and feed has never been demonstrated (Eilenberg et al., 2015). Another study by Muller et al. (2019) evaluated the potential for transmission of parasitic infection of Eimeria nieschulzi, Eimeria tenella and Ascaris suum by black soldier fly larvae, and found that either the faeces used as fertilizer or the larvae used as feed for animals for human consumption, could contaminate or transmit the parasites further into the food chain reaching humans. The

authors recommend applying some proven form of antiparasitic treatment, (such as heating, drying or UV light), to either element before giving them any further use (Muller et al., 2019).

Table 1: Proportion of farms of various insect species in which *Isospora* spp., *Balantidium* spp. and *Entamoeba* spp. were identified (Ga??cki and Sokó?, 2019).

Inspect species farmed	*Isospora* spp.	*Balantidium* spp.	*Entamoeba* spp.
Tenebrio molitor	*7/75 (9.33%)*	*11/75 (14.67%)*	*9/75 (12%)*
Acheta domesticus	*4/75 (5.33%)*	-	-
Locusta migratoria	*8/75 (10.67%)*	*9/75 (12%)*	*4/75 (5.33%)*
Blattodea spp.	9/75 (12%)	13/75 (17%)	14/75 (18.67%)

Regarding viruses, as reported by EFSA in 2015, the majority of viruses associated with reared insects will be pathogenic only to them and not to humans (EFSA, 2015). Those human viruses that are related to insect viruses cannot replicate within the insect (Eilenberg et al., 2015). As of the date of this report, as recorded by van der Fels-Klerx et al. (2018), no peer-reviewed publications were found showing the capability of the edible insects' species selected for this review to act as vectors for human-pathogenic viruses known to replicate in insects (Eilenberg et al., 2015). However, this may need to be considered in the future if the consumption of known vectors of human or other mammalian pathogens is proposed.

In relation to prions, the evidence compiled by EFSA in 2015 (EFSA 2015) remains relevant today, as no new evidence was located during this review suggesting that prions can multiply within insects, and that any prion contamination existing in an insect food product would have its origin in the substrate fed to said insect (van der Fels?Klerx et al., 2018). Under current regulations, insects need to be raised on safe substrates, equally to all other farmed animal species. Contaminated substrates would not be permitted to be used in insect rearing.

Highly pathogenic microorganisms

Several families and species of microorganisms can cause significant illness in humans when present in food in sufficient quantities. Various studies identified in this review looked into the presence of these highly pathogenic microbes, with various results. Salmonella spp. and Listeria monocytogenes were found to be absent in 25g of product in five studies identified in this review in which the insects had been reared for commercial purposes (Garofalo et al., 2017, Grabowski and Klein, 2017b, Osimani et al., 2017c, Osimani et al., 2018a, Grabowski and Klein, 2017a). Salmonella spp. was found in the residue (frass, exuviae and leftover substrate) of larvae of Hermetia illucens, despite not being found in the larvae themselves (Wynants et al., 2019).

Pava-ripoll et al. (2015) produced a study of four relevant pathogens (Salmonella enterica, Cronobacter sakazakii, Escherichia coli 0157:h7 and Listeria monocytogenes) that showed their potential for transmission to eggs and to persist transstadially to larvae and first-generation adults when previously fed contaminated feed. Cronobacter sakazakii, was described by Walia et al. (2018) as a hazard that could be present in cricket powder. This species of bacterium has been associated with a high fatality rate in neonates. The authors argue that this powder should be checked for the presence of Cronobacter spp., particularly if it is to be used for fortified foods for undernourished infants.

According to Garofalo et al. (2019), previous descriptions of the presence of these pathogenic species in edible insects have not resulted in outbreaks reported in the scientific literature. The search produced by the FSA for this review has also not identified reported outbreaks in the literature.

Efficacy of treatment of edible insects

As previously mentioned in several examples, applying the appropriate kind of treatment to insect products, depending on the species and time of harvest, has been shown to lower microbial levels and help improve the hygiene of the product. Heat treatments have proven to be the most effective ones in minimising microbial levels while ensuring the elimination of Salmonella spp. and Listeria monocytogenes (Garofalo et al., 2017, Grabowski and Klein, 2017b, Osimani et al., 2017c, Osimani et al., 2018a, Schlüter et al., 2017). The use of techniques such as degutting and water rinsing have been shown not to be effective for lowering microbial counts without the inclusion of a heat-treatment step (Wynants et al., 2017). These results are consistent with those reported by Mancini et al.(2019b), although fasting T. molitor larvae for 24 hours was found to be effective in lowering counts by 2 log cfu/g. Cooking at 150oC for 10 minutes was the most effective way to kill Listeria monocytogenes in larvae fed on contaminated substrate (Mancini et al., 2019b). A study by Vandeweyer et al. (2017b) in commercial T. molitor larvae showed how a blanching step of between 10 and 40 seconds was effective at lowering naturally-occurring microbial counts by 4.4-6.4 log cfu/g (from max counts of 3.1-7.9 log cfu/g), but not aerobic endospores. The resistance of bacterial endospores would require specific treatment in order for them to be reduced, however, it has been described how subsequent heat-treatment stages would deteriorate the nutritional quality of the food (Grabowski and Klein, 2016).

A paper by Caparros Megido et al. (2018) described the effects of different heat treatments applied to T. molitor larvae in nutritional quality and microbial levels. Boiling at 100oC and oven-cooking at 70oC for 15 and 30 minutes produced very small variations in the basic nutritional composition of the mealworms. Aerobic counts in raw mealworm were calculated to be 8.5 log cfu/g, which were reduced to 6.7 and 6.1 when oven-cooked for 15 and 30 minutes respectively, 3.3 for mealworms fried for one minute in 15 ml of olive oil, and 1.6 after boiling at 100oC for one minute (Caparros Megido et al., 2018). Relating to the loss of nutritional quality of edible insects, a study in black soldier fly revealed that the killing method can also alter the protein profile of the product. Freezing was deemed to be a less preferable killing method than blanching, as it activates various enzymatic pathways that lead to the consumption of lysine and cysteine and reduces the extractability of the insect’s protein fraction, reducing the nutritional quality of the food product (Leni et al., 2019a).

In a study by Grabowski and Klein (2016) three different levels of heat treatment were applied to adult Gryllus bimaculatus crickets and larvae of Zophobas atratus intended for pet consumption, and bacterial counts were measured using standard ISO food microbiology techniques. After 10 minutes of nucleate boiling, followed by 24 h of 60oC drying, total bacterial count for Zophobas atratus larvae was 7.1 log cfu/g. No pre-treatment levels were measured by the authors. Raising the drying temperature to 80oC lowered bacterial counts to 3.5 log cfu/g. In the case of the crickets, the previous two treatment described did not show differences in bacterial counts (8.3 and 8.4 log cfu/g, respectively). A treatment based on 30- minute nucleate boiling, followed by 80oC drying for 12 hours and a further 12h of 100oC drying was needed to lower counts to 4.5 log cfu/g (Grabowski and Klein, 2016). Another study by Grabowski and Klein (2017b) compared microbiological counts on different insect species based on different treatments. The exact temperature and time conditions at which the insects and products were exposed were not provided. Deep fried insects showed lower microbiological counts than dried or powdered products. Canned silkworms showed the highest bacterial counts on average, particularly for Enterobacteriaceae and bacilli. Salmonella spp., Listeria monocytogenes and E. coli were absent. No other Listeria species were analysed to obtain bacterial counts. However, the authors placed homogenized samples onto blood agar plates to identify other pathogenic species. Results retrieved species such as Bacillus cereus, Aspergillus spp. and Listeria ivanovii, showing the potential for contamination of the product and the importance of good hygienic practices throughout the production chain. Endospore-forming species like B. cereus have a higher potential to contaminate the insect after the application of heat treatment has reduced the competition from other microbial species (Grabowski and Klein, 2017b). These results show how different insect species and life stages require different treatments for achieving similar hygiene conditions, coinciding with the conclusions reached by the study by Caparros et al. (2017).

Applying heat treatment to edible insects is not sufficient to prevent all microbiological risks. A case of histamine poisoning occurred in Thailand after a group of students consumed fried grasshoppers and silkworm pupae. This was caused by the poor conditions in which the insects were kept through the food chain, which resulted in bacterial contamination that converted the histidine of the insects into histamine. Histamine is heat-resistant, and hence the frying process did not alter the concentration of the chemical within the insects, which accumulated to high levels within the insects (Chomchai and Chomchai, 2018). The study authors point out that clinical signs of histamine poisoning are very similar to those of a hypersensitivity reaction, and that if only one patient had been poisoned in this scenario, a wrong diagnosis of hypersensitivity to the food would have been concluded as the cause. According to both this study and another one cited within it (Mungaomklang et al., 2011), histidine conversion can be carried out by naturally occurring bacteria in the insects and the excess conversion occurred due to deficiencies in refrigeration in the transport chain, causing bacterial overgrowth (Chomchai and Chomchai, 2018).

Antimicrobial resistance

Several studies, as identified by the comprehensive review by Garofalo et al. in 2019 and by the review presented in this report, looked into detecting the presence of transferrable AR genes in edible insects (Milanovi? et al., 2016, Milanovi? et al., 2018, Osimani et al., 2017a, Osimani et al., 2017b, Osimani et al., 2018b, Roncolini et al., 2019, Vandeweyer et al., 2019). The main genes studied in these papers were those coding for resistance against tetracyclines (tet-), erythromycin (erm-), ?-lactams (bla-; mec-), vancomycin (van-) and aminoglycosides (aac-aph).

In their effort to evaluate the existence of AR-coding genes in edible insects, Milanovi? et al. (2016) studied powdered and whole A. domesticus, L. migratoria, T. molitor and B. mori pupae amongst other insect species reared for commercial purposes. They tested for the presence of 11 different AR genes, of which the most prevalent across species was tet(K), in 90.9% of samples, followed by tet(S) and bla(Z) with 54.5% prevalence and erm(B) at 45.4%. When comparing insect species, there is great variability in the frequency of detection of the different AR genes. The sample of cricket powder showed no presence of AR genes in the present microflora, contrasting with the sample of winged termites, carrying five AR genes (Milanovi? et al., 2016). This is consistent with the variability reported on the microbial profile of edible insects with results from the study by Vanderweyer et al. (2019) that showed a large difference in the AR profile between mealworms and crickets, but concluded that the AR risk posed by edible insects is unlikely to be higher than other foods. (Vandeweyer et al., 2019). Roncoli et al. (2019) also reported a high prevalence of tetracycline- and erythromycin- resistant genes in samples of A. domesticus, with prevalences of 62.5% for tet(M) and tet(O), 59.4% for tet(S), 34.4% for tet(K), 34.4% for erm(B), 25% for erm(C) and 21.9% for blaZ.

Similar results were reported in a study in L. migratoria that looked at the presence of 12 AR genes, and found a prevalence of 70% for tet(M) and 83.3% for tet(K) and bla(Z). Lower prevalences were found for erm-, and aac-aph genes. No van- and mec- genes were detected (Osimani et al., 2017b). In contrast, another study showed the presence of vancomycin-resistant genes in T. molitor samples, with very disparate frequencies of 90% for samples coming from France and 10% for those coming from Belgium. Other genes found in high frequencies and reporting similar results to the previously mentioned studies, include tet(K) between 80-100% prevalence, erm(B) at 57.5% and aac-aph at 40% (Osimani et al., 2017a). Both of these groups of samples were subjected to a qPCR analysis in an effort to quantify five carbapenem resistance genes (blaNDM?1, blaVIM, blaGES, blaOXA?48, and blaKPC) in the insects' microflora, and a significant difference was found in the OXA-48 and NDM- 1 genes, with prevalence of 57% and 27% in L. migratoria and 3% and 10% in T. molitor (Milanovi? et al., 2018). The authors discarded the geographical origin as the reason for the variability in AR gene prevalence, and defend in another paper how transmission of AR genes combines several factors such as vertical transmission and contamination of the insect feed (Osimani et al., 2018b).

Insects have been proposed to be natural carriers of AR, coinciding with studies in different species of fly that confirmed the presence of tetracycline-resistant genes (Zhang et al., 2017) and the role of the insect as a source of sustained AR transmission in the farm environment (Fukuda et al., 2018). Other studies supporting that idea show how substances naturally produced by insects possess antimicrobial qualities, such as gloverin2 produced by silkworms (BmGlv2), which inhibits the growth capacity of gram-negative bacteria, and chitosan obtained from T. molitor, which proved to have antimicrobial activity against S. aureus, B. cereus, L. monocytogenes, and E. coli (Shin et al., 2019). It can be concluded that edible insects possess microbiota containing antimicrobial resistance genes of which the most prevalent are those for tetracycline, erythromycin and ?-lactams. Coincidentally with the microbial profile of edible insects, the AR profile varies across species and geographical location.

4.1.2 Hazard evaluation

The high variability of the microbial profile and microbial counts of edible insects, even when reared under similar conditions, makes it difficult to estimate the risk for the consumer if the product is not treated in order to reduce microbial levels. There exists a potential risk of bacterial endospores persisting after the food is submitted to heat treatment. There are knowledge gaps that require further investigation to fully understand microbial dynamics during the rearing process.

The report of presence of parasites pathogenic to humans in fresh insects does not allow evaluation of the risk of parasitic infection following consumption of contaminated insects. Application of a heating step in the processing of the insect product is expected to lower the parasitic load, but this information could not be retrieved from the literature search.

No evidence has been found of a risk of viral transmission from consuming edible insects.

There is no increased risk of prion infection expected when consuming edible insects, as these do not have the potential to replicate prions and can only carry whichever prions are present in the substrate, which must comply with hygiene standards.

There exists a potential hazard of bacterial overgrowth in fresh insects if these are not stored and refrigerated appropriately throughout the production chain. This overgrowth can lead to bacteria converting histidine into histamine, creating a risk of histamine poisoning for consumers. The use of thorough HACCP protocols in line with good microbiological practices, as well as traceable substrates for rearing insects may wish to be considered to reduce the impact of microbiological contamination of edible insects.

There is a potential hazard that the rearing of edible insects on a large scale may incur the use of antibiotics to minimise the negative impact of bacterial diseases in the production chain, contributing to AMR. The exact impact of this practice is not possible to determine with the available information.

4.1.3 Control measures

The most effective methods of reducing microbial counts are heat-based treatments. Boiling, blanching, or drying at high temperatures for a sufficient amount of time has been proven by the literature to reduce bacterial counts significantly, but the temperature and time required vary by insect species, life stage and microbial target. Given the variability between treatments and species described in the literature, general guidelines on minimum temperature and time of treatment cannot be identified. Cold-based treatments can also lower microbial counts. Two studies identified by this review showed how freeze- drying yellow mealworm and house crickets and freezing banded crickets (Vandeweyer et al., 2018) lowered aerobic counts and YMC as effectively as boiling. Other methods vary in effectiveness, with degutting and washing proven to

not be effective, and fasting 24-48 hours proving effective, if less so than heat treatment (Mancini et al., 2019). Treating insects with heat can cause an increased presence of endospore-forming bacteria, whose levels could be checked as a way of evaluating the microbiological safety of the food. Other authors mention irradiation as possible way of reducing bacterial counts in edible insects (Ferri et al., 2019, Wynants et al., 2017), but no studies looking specifically into this process were identified in this review.

Most authors, as compiled in this review and by the review by Garofalo et al. (2019), recommend a combination of adequate hygienic conditions of the rearing premises and the substrate, the application of a heating step, and adequate storage conditions (moderate temperature and moisture) for ensuring the microbiological safety of edible insects. This combination of measures as shown in the literature was effective in minimising the presence of Salmonella spp. and Listeria monocytogenes. In the case of powdered insect products, a cooking step is also recommended by authors in the literature, as pulverisation of insects as part of the processing process shows an increase in microbial loads by 1.6–2.2 log cfu/g, probably because of the release and dispersion of the gut microbiota (Garofalo et al., 2017, Schlüter et al., 2017, Vandeweyer et al., 2017a).

In the UK there are no defined maximum accepted microbial levels specific to edible insects' products. For ready-to-eat foods in the UK, including insects, regulatory requirements are defined by the Health Protection Agency guidelines (HPA, 2009). Figure 1 shows the results of two studies by Caparros et al. (2017 and 2018) in which different types of heat treatment lowered the Total Aerobic Counts of the samples. In order to mitigate the impact of antimicrobial resistance, optimisation of hygienic rearing practices and biosafety will reduce the need for using antibiotics on the insect population to combat disease.

Figure 1: Total Aerobic Count before and after heat treatment. *Data from Caparros et al. 2018

** Data from Caparros et al. 2017. Red ring around 1 log cfu/g marks the minimum TAC allowed by the United Kingdom for ambient stable canned, bottled, cartoned and pouched foods immediately. after removal from container.

4.2 Toxicological hazards

4.2.1 Scientific evidence

Some studies looked broadly into the toxicology of insects and detected no apparent risks. Poma et al. (2017) evaluated the presence of several chemical compounds, potentially toxic for humans, in a low number of samples from commercially available insect products of greater wax moth, migratory locust, yellow mealworm, and lesser mealworm. Samples were analysed for flame retardants, PCBs, DDT, dioxin compounds, pesticides, and heavy metals such as arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), tin (Sn) and zinc (Zn). The authors concluded that samples showed the presence of some of the studied chemicals in low quantities and in similar or lower concentrations than other animal products (Poma et al., 2017). In another study, Han et al. (2016) performed a 90-day oral study in rats to evaluate the toxicological potential of freeze-dried, powdered T. molitor larvae, administered daily. No clinical, histopathological, or allergic effects were observed up to a dose of 3000 mg/kg/day, (Han et al., 2016). However, other examples in the literature, as presented in the following paragraphs, show that data regarding toxicological safety of edible insects are still contradictory.

Heavy metals

A study by Bednarska and ?wi?tek (2016). measured concentrations of cadmium (Cd) and zinc (Zn) in three cellular fractions of T. molitor larvae when fed contaminated wheat flour with concentrations of 0, 100, 300 and 600 mg Cd/kg of flour and 0, 1000 and 2000 mg Zn/kg of flour. The fractions were S1 (microsomal and cytosolic components), S2 (comprising tissue and cell membranes) and G (granules). Fraction S1 was unaltered by increased concentration levels of both heavy metals in the substrate. In contrast, for substrate concentrations of 100, 300 and 600 mg Cd/kg, levels of S2 fraction (10.4, 34.1 and 46.3 mg/kg) and G fraction (8.5, 14.1 and 22.0 mg/kg) increased in correlation, compared to the control level of ~0mg/kg. In the case of zinc, the S2 and G fractions remained unaltered for a substrate concentration of 1000mg/kg but increased at a substrate concentration of 2000mg/kg, to ~29mg/kg and ~31mg/kg, in contrast to a control level of ~1 mg/kg (Bednarska and ?wi?tek, 2016).

Similarly, van der Fels-Klerx et al. (2016) studied the bioaccumulation factor1 for black soldier fly and yellow mealworm when administered feed spiked with arsenic, cadmium, and lead. The results from this study are shown in Table 2. The capability of insect larvae to absorb these metals was also shown by Feng et al. (2019) in a study in silkworm (B.mori) in which pupae developed from larvae fed with leaves from mulberry trees grown in contaminated soil. For soil concentrations of As (52.63 mg/kg), Cd (1.03 mg/kg) and Pb (258 mg/kg), silkworm pupae accumulated concentrations of 1.52, 0.02, and 0.34 mg/kg of As, Cd and Pb, respectively. Cadmium and lead were also found at concentrations of 0.03 and 0.34 mg/kg, respectively. No control was used in this experiment due to the natural contamination of the soil (Feng et al., 2019).

Table 2: Bioaccumulation factor (BAF) for black soldier fly and yellow mealworm for three treatments calculated on a dry weight basis (n/a = not applicable due to concentrations below the limit of detection of 0.1 mg/kg). No superscripts in common within a column indicates significant differences (ANOVA followed by Turkeys HSD post hoc text, with $p < 0.05$). Based on EC maximum levels (ML): As (ML 0.5=1 mg/kg, ML 1=2 mg/kg and ML 2=4 mg/kg); Cd (ML 0.5=0.25 mg/kg, ML 1=0.5 mg/kg and ML 2=1

1 Bioaccumulation factor: ratio of metal concentration in plant biomass to that in the soil mg/kg); Pb (ML 1=2.5 mg/kg, ML 2=5 mg/kg, and ML 2=10 mg/kg). Results taken from van der Fels-Klerx et al. (2016).

Hermetia illucens

Quantity	As	Pb	Cd
Control -	n/a	1.1±0.05a	5.8±1.0a
0.5 ml	0.58±0.12a	1.2±0.30a	9.5±3.6a
1.0 ml	0.56±0.13a	1.4±0.20a	6.1±1.9a
2.0 ml	0.49±0.10a	1.2±0.40a	6.9 ± 0.92^{a}

Tenebrio *molitor*

Quantity	As	Pb	Cd
Control -	n/a	n/a	0.43±0.039a
0.5 ml	0.58±0.12a	0.043±0.013a	0.71±0.083b
1.0 ml	0.56±0.13a	0.046±0.032a	0.65±0.037b
2.0 ml	0.49±0.10a	0.051±0.022a	0.69±0.056b

In another study conducted by Biancarosa et al. (2018) H. illucens larvae were raised on substrates containing eleven different percentages (0% to 100% with 10% increases) of seaweed

(Ascophyllum nodosum). Heavy metal (Cd, Pb, Hg, and As) concentrations in control feed (0% seaweed) and the corresponding control larvae were all below 0.1mg/kg, except for Cd, measured at 0.41 mg/kg in larvae. When fed the substrate with 50% seaweed composition (in which concentrations of 0.21 (Cd) 0.15 (Pb), 0.011 (Hg), and 14 (As) mg/kg were measured), concentrations of 1.6 (Cd), 0.16 (Pb), 0.012 (Hg) and 9.2 mg/kg (As) were measured in larvae. When fed on a substrate with 90% seaweed, concentrations of Cd, Pb and Hg peaked, at around 2.2, 0.3, and 0.02 mg/kg, respectively. Arsenic levels kept rising up to 23 mg/kg for substrate made entirely out of seaweed. The authors concluded that H. illucens larvae are capable of accumulating heavy metals, particularly cadmium and arsenic, from the substrate they are fed, in this case comprising A. nodosum (Biancarosa et al., 2018).

More evidence of metal bioaccumulation in H. illucens larvae was described by Purschke et al. (2017) by feeding them pre-contaminated substrate at concentration levels of chromium (15.2 mg/kg), nickel (15.2 mg/kg), arsenic (3.0 mg/kg), cadmium (1.5 mg/kg), mercury (0.2 mg/kg), and lead (15.2 mg/kg). The authors highlighted the high accumulation of cadmium (13.7 mg/kg) and lead (35.6 mg/kg), indicating the potential for bioaccumulation of these two heavy metals in other larval species (Purschke et al., 2017).

Mycotoxins

In a study conducted by Bosch et al. (2017) larvae of H. illucens and T. molitor were fed poultry feed spiked with aflatoxin B1 at tiered concentrations of 0.01, 0.025, 0.05, 0.10, 0.25, and 0.5 mg/kg of dry feed under laboratory conditions. Aflatoxin B1 (AFB1) and M1 (AFM1) levels stayed below 0.10 µg/kg for black soldier fly larvae. In T. molitor larvae, AFM1 stayed below 0.10 µg/kg, whereas AFB1 levels were <0.10, 0.16, 0.34, 0.59, 1.29 and 1.44 µg/kg for the different batches of contaminated feed previously described. Feed without additions or with just solvent added served as controls. The authors noted a high tolerance to aflatoxin B1 in both species, and remark that more studies would be required to evaluate the presence of other AFB1 metabolites (Bosch et al., 2017). These results were similar to those found in a study by Camenzuli et al. (2018) looking into mycotoxin accumulation in lesser mealworm (A. diaperinus) and black solider fly larvae. Feed was spiked with mycotoxins at three concentrations, aflatoxin B1 (0.02, 0.2 and 0.5 mg/kg dry feed), zearalenone (ZEN: 0.5, 5, 12.5 mg/kg dry feed), deoxynivalenol (DON: 5, 50, 125 mg/kg feed), and ochratoxin A (OTA: 0.1, 1, 2.5 mg/kg feed). The non-spiked feed served as a control. Larvae of either species did not accumulate aflatoxin B1. Lesser mealworm larvae showed concentrations for all mycotoxins below the limit of quantification (0.001 mg/kg for AFB1 and OTA, and 0.1 mg/kg for DON and ZEN). ZEN, DON and OTA were detected in black soldier fly larvae marginally above the limit of quantification, but 10 to 1000 times lower than the concentration in feed, showing a low capability of accumulation of mycotoxins in larvae from contaminated feed (Camenzuli et al., 2018).

In another study conducted by Purschke et al. (2017), A. diaperinus larvae were fed corn gluten feed that was naturally contaminated with deoxynivalenol (DON: 1207 µg/kg), fumonisin 1 and 2 (FB1: 727 µg/kg, FB2: 294 µg/kg), and zearalenone (ZEN: 173 µg/kg). DON was found in A. diaperinus larvae at a concentration of 726 ± 164 µg/kg. FB1 was measured at a concentration level of 127 µg/kg. FB2 and ZEN did not reach the limit of detection of 25 µg/kg and 10 µg/kg, respectively. The authors did not use the non-contaminated substrates as a control for their study. When analysing the residual fractions, the overall mass balance did not exceed 60%, and the authors noted that this indicated larval metabolism of the mycotoxins into unknown compounds (Leni et al., 2019b). Similarly, Purschke et al.(2017) studied H. illucens larvae whose feed was spiked with deoxynivalenol (697.7 ?g/kg), aflatoxin B1 (13.3 ?g/kg), aflatoxin B2 (2.6 ?g/kg), aflatoxin G2 (7 ?g/kg), ochratoxin A (39.4 ?g/kg) and zearalenone (130.4 ?g/kg), but found no accumulation of mycotoxins in the larvae after harvesting.

Pesticides

Houbraken et al. (2016) performed a study on T. molitor larvae that showed their capacity to bioaccumulate pesticides from contaminated carrots and the risk associated with rearing larvae with vegetal waste streams. The larvae were fed a substrate consisting of wheat bran and flour 50/50 plus contaminated carrots immersed for one minute in a pesticide cocktail with twelve active agents (2,4-D, bentazone, bifenthrin, clopyralid, diflufenican, fenpropimorph, isoproturon, linuron, mefenoxam, pendimethalin, pyrimethanil, tebuconazole) at a concentration of 1000 mg/l, on which they fed for 48 hours. Larvae were analysed in two control groups and two exposed groups, both starved and non-starved. Concentrations of 2,4-D, bentazone and bifenthrin were under the limit of detection (0.15, 1.2, and 5.1 ng/g), and clopyralid was under the limit of quantification (2 ng/g) for both exposed groups. The results are summarised in Table 3 (Houbraken et al., 2016).

Table 3: Pesticide residues measured on homogenised carrots (mg/kg) and in unstarved and starved

T. molitor larvae (ng/g). (LOD: limit of detection, LOQ: limit of quantification). Results taken from Houbraken et al., (2016).

-	Residue untreated carrots (mg/kg)	T. *molitor* unstarved (ng/g)	T. *molitor* starved (ng/g)	Residue treated carrots (mg/kg)	T. *molitor* unstarved (ng/g)	T. *molitor* starved (ng/g)
2,4-D	<LOD	<LOD	<LOD	8.36	<LOD	<LOD
Bentazone	<LOD	<LOD	<LOD	0.919	<LOD	<LOD
Bifenthrin	<LOD	<LOD	<LOQ	0.808	<LOD	<LOD
Clopyralid	<LOD	<LOD	<LOD	1.75	<LOQ	<LOQ
Diflufenican	0.000219	0.667	0.774	3.41	7.92	3.81
Fenpropimorph	<LOQ	<LOD	<LOD	13.81	47.2	9.21
Isoproturon	<LOD	<LOD	<LOD	2.17	1.65	0.552
Linuron	0.0026	0.208	0.149	11.8	23.1	17.4
Mefenoxam	<LOD	<LOD	<LOD	2.67	1.43	<LOD
Pendimethalin	<LOD	<LOQ	<LOQ	6.09	6	4.47
Pyrimethanil	<LOD	<LOQ	<LOQ	22.71	72.2	42.9

Tebuconazole

Residue untreated carrots (mg/kg)	T. *molitor* unstarved (ng/g)	T. *molitor* starved (ng/g)	Residue treated carrots (mg/kg)	T. *molitor* unstarved (ng/g)	T. *molitor* starved (ng/g)
0.0236	<LOD	<LOD	8.37	3.45	0.813

Using different pesticides, the study by Purschke et al. (2017), showed that chlorpyrifos, chlorpyrifos-methyl and pirimiphos-methyl did not bioaccumulate in H. illucens larvae. In this study, 100 g of corn flour was spiked with the aforementioned pesticides at a concentration of 2.5 mg/kg each. After 10 days, larval pesticide concentrations were 0.006, <0.001 and 0.001 mg/kg respectively, confirmed when compared to the control (Purschke et al., 2017). The absence of bioaccumulation in this study may be due to differences between larval species or the pesticide itself. Variability between species has been reported before in accumulation of heavy metals (van der Fels-Klerx et al., 2016). In the case of pesticide rac-furalaxyl, Yin et al (2017) studied the potential for bioaccumulation in T. molitor larvae, observing the enantiomerisation of the chemical into enantiomers S- and R-furalaxyl, but showing low bioaccumulation factors of 0.058 and 0.042, respectively. Larvae were fed wheat bran spiked with rac- furalaxyl at a concentration of 10 mg/kg for 21 days. Enantiomerisation was not observed in the feed (Yin et al., 2017).

Summary

The reviewed data on the capacity of edible insects to bioaccumulate toxic compounds varies between species, chemicals and stages of development. Evidence suggests that insects have a higher tendency to accumulate heavy metals, particularly cadmium and arsenic than any other type of toxic compound. The literature reports a low capacity of larvae of edible insects to accumulate mycotoxins, but does point to the metabolism of these mycotoxins into metabolites, some of which may be potentially harmful, although this would have to be studied further. No studies identified in this review looked into the capability of mycotoxin-producing organisms to proliferate and contaminate food products derived from edible insects when stored after processing. It is evident that more studies are required to better characterise the capability of edible insects to bioaccumulate toxic substances, and the differences in bioaccumulation between species and stages of development.

4.2.2 Hazard evaluation

It can be concluded there is a risk of insects accumulating heavy metals from contaminated substrates. According to the scientific literature, the likelihood of bioaccumulation is slightly higher for cadmium and arsenic versus other heavy metals.

Evidence suggests insects are unlikely to accumulate mycotoxins from contaminated substrates. These mycotoxin levels could metabolise into compounds that may, or may not be harmful to consumers, which cannot be concluded with the available evidence.

Evidence of insects accumulating pesticides from substrates is contradictory. The extent of bioaccumulation depends on various factors such as the insect species.

4.2.3 Control measures

Given the limited available evidence retrieved through the systematic search, it is difficult to point to specific methods of control to limit bioaccumulation of each specific toxic compound. It can be deducted from the literature that avoiding contamination of insect feed is the most effective way of mitigating the risk of bioaccumulation of toxic compounds. A study showed that insects starved for 24 hours bioaccumulated lower in vivo pesticide concentrations (Houbraken et al., 2016). Thus, starvation of insects may be an approach that can be used to reduce their in vivo pesticide concentrations. The recommendations present in the 2015 EFSA risk profile focusing on maintaining hygienic conditions of the substrate and the rearing enclosure, are therefore still relevant.

4.3 Allergenicity hazards

4.3.1 Scientific evidence

Several efforts have been conducted in recent years to clarify the mechanism and the specific proteins that can trigger allergic reactions in consumers of edible insects and derived products. A systematic review by Ribeiro et al. (2018) confirmed cross-reactivity between crustaceans and edible insects, as well as tropomyosin and arginine kinase acting as the major cross-reacting allergens (Ribeiro et al., 2018). This review identified further specific examples that throw light onto the current knowledge status on edible insect allergenicity. Several studies were identified focusing on shellfish cross-reactivity and aiming to identify new allergens and trigger mechanisms. It is apparent that further research will be needed in order to fully understand the allergenic potential of edible insects, particularly when introducing a variety of insect species into the diet of a non-sensitised population such as the UK.

Shellfish cross-reactivity

Based on previous knowledge about the cross-reactivity between shellfish and yellow mealworm, several authors looked into identifying the specific proteins that cause allergenicity and the potential cross-reactivity with other insect species. A study by Barre et al. (2019) looked into identifying proteins with allergenic potential in T. molitor larvae. The soluble protein content showed protein fractions with pH ranging between 3.0 and 9.0, and molecular weight between 10-100 kDa. The authors pointed out that this protein-content diversity suggests that a large number of soluble protein fractions could behave as potential cross-reacting IgE-binding allergens. Through an SDS-PAGE and a mass spectrometry characterization, 106 distinct protein fractions with allergenic potential were identified. The amino acid structure was then compared to that of other similar proteins in other insect species concluding there exists close similarity with those of other insects and arthropods, including dust mites and shrimps, and molluscs such as mussels and oysters. Proteins identified as new potential allergens included apolipophorin-III, larval cuticular protein and hemolymph protein (Barre et al., 2019).

Another study assessed the cross-reactivity of shrimp, house dust mite and flies with edible insects such as A. domesticus, desert locust, yellow mealworm and L. migratoria. The immunoblots performed confirmed the already well-described cross-reactivity of mealworm and crustacean allergens through tropomyosin. Cross-reactivity with what appears to be ?-amylase also occurred between crustacean and migratory locust. S. gregaria and A. domesticus both showed cross- reactivity with proteins within the 35-38 kDa band, corresponding to tropomyosin. In the case of flies, cross-reactivity occurred at the 35-38 kDa band, but also 72 kDa and between 12-14 kDa. Sera from patients allergic to stable flies, as well as those allergic to house dust mites, showed cross-reactivity to house cricket, desert locust and migratory locus). That same study looked into the effect of processing on allergic reactions to migrant locust. Locusta migratoria extracts were processed in two different ways. Enzymatic hydrolysis was performed using a mix of alcalase, neutrase, flavourzyme and papai at 50oC and pH 7.0. Thermal treatment of locust extract occurred at temperatures between 80-100oC for 10 minutes, as well as an autoclaving process at 121-138oC for 20 minutes. Samples treated through heat or enzymatic hydrolysis showed no reactions in five patients allergic to crustaceans (Pali-Schöll et al., 2019). In another study by van Broekhoven et al. (2016) the authors showed how thermal and enzymatic processing of larval extract from T. molitor, Z. atratus and A. diaperinus caused the IgE-binding cross-reaction to diminish in intensity when using sera from crustaceans-allergic patients. Samples were treated through boiling for 5 minutes, fried for 5 minutes at 180oC or lyophilised at -50oC and 150 Pa. In vitro digestion was performed through the use of porcine pepsin, pancratin and lipase as well as bovine ?-chymotrypsin (van Broekhoven et al., 2016).

In another example on the cross-reactivity of tropomyosin, 15 patients with shrimp allergies participated in a double-blind, placebo-controlled food challenge trial, in which they were fed blanched mealworm. Participants were fed 7 portions per challenge day, in quantities of 2.16 mg, 21.6 mg, 216 mg, 648 mg,
2.16 g, 6.48 g and 13.0 g of mealworm protein. In 13 cases, mealworm (T. molitor) allergy was confirmed, with IgE binding occurring to tropomyosin and arginine kinase as well as other unidentified proteins. Symptoms on 11 patients were moderate to severe (Broekman et al., 2016).

De novo sensitisation and insect cross-reactivity

In the future, understanding the intrinsic allergenic potential of edible insects beyond shellfish cross- reactivity will be very important to map the allergenicity of edible insects as a whole. In a subsequent study to the one previously cited, Broekman et al. (2017a) studied the cross-reactivity between different edible insects and concluded that the differences in cuticle proteins, protein binding profiles and variations in the basophil activation test show that different proteins can cause sensitisation to different insect species. This would mean that mealworm allergy is not indicative for insect allergy and that it is possible to develop species-specific insect allergies (Broekman et al., 2017a). Similarly, Francis et al. (2019) evaluated the profile of the allergenic protein arginine kinase in T. molitor larvae and A. domesticus samples and the cross-reactivity

between these two species. Results did not show similar immunoblotting responses, indicating a lack of cross-reactivity across species, but authors do not discard the possibility of arginine kinase showing cross-reactivity with other species of insect (Francis et al., 2019).

Bombix mori is one of the most studied species in the literature, as the pupa stage of its life cycle is commonly consumed in Asia. A paper identified tropomyosin from silkworm pupa as a 285-amino acid protein of 32.8 kDa which was found to be 73.5% identical with shrimp and crab tropomyosins. Eight out of fifteen samples showed IgE binding to recombinant silkworm tropomyosin in an ELISA with sera of silkworm allergic patients, the same proportion that reacted to shrimp and crab tropomyosin (Jeong et al., 2017). A different piece of work looking into identifying allergens of silkworm pupae performing a 2-DE Western blot and a MALDI-TOF-MS analysis, found that chitinase and paramyosine caused a strong IgE bind from sera of silkworm-allergic patients. The authors proceeded to compare the amino acid sequences with those of existing allergies. They found that silkworm chitinase resembles Der f 18 of Dermatophagoides farina, with a 24.8% amino acid identical and 57.4% similarity scores, and that silkworm paramyosin closely resembles Der p 11 of Dermatophagoides pteronyssinus, with a 62.8% amino acid identical and 90.0% similarity scores. They argue that more investigation would help identify the specific epitopes of the potentially allergenic proteins of B. mori (Zhao et al., 2015). Another protein of 30 kDa, Bom m 9 was identified and deemed to be a strong allergen for silkworm-allergic patients (Zuo et al., 2015).

In another effort to understand the specific allergic potential of edible insects as opposed to the cross- reactivity with previous shellfish sensitisation, a study on four patients with long-term mealworm allergy developed through occupational exposure showed a different binding pattern on immunoblot test to mealworm when compared to shellfish. The basophil activation to mealworm was stronger than to shrimp, suggesting that mealworm can trigger allergic sensitisation in humans even if they have not previously been sensitised to shellfish. On top of arginine kinase and tropomyosin, larval cuticle proteins were identified as allergenic proteins in mealworm. According to the authors, results indicate that a long-term exposure to mealworm may be needed to development allergy to the larvae (Broekman et al., 2017b). An example of de novo exposure allergic reaction to edible insects was reported in New Zealand, where a father and son without previously reported allergies had severe reactions to the ingestion of silkworm pupae (Gautreau et al., 2017). This evidences the difficulty of predicting a future pattern of de novo sensitisation to edible insects in the UK, and, as Barre et al. (2019) pointed out in the conclusions of their study, it is likely that we will know more about specific allergenic proteins as new cases appear after the introduction of insects in the diet.

4.3.2 Hazard evaluation

There is very strong evidence of the high potential risk of patients allergic to shellfish experiencing cross-reactivity with edible insects. Shellfish (crustaceans and molluscs) are part of the 11 named allergens that require precautionary labelling in the UK, but the exact prevalence of shellfish allergy in the UK is difficult to determine, and varies depending on the sector of the population studied. Several systematic reviews have estimated prevalence values for shellfish at the European and Global scale. The estimated prevalence for shellfish allergy in Europe was 1.3% for self-reported based studies and 0.1% in food- challenge based studies (Nwaru et al., 2014). At the global level, shellfish allergy reported prevalences varied from 0% to 10.3%, where food challenges showed prevalences between 0% and 0.9% (Moonesinghe et al., 2016).

There is a risk that some consumers may develop de novo sensitisation to insect-specific allergens. An estimation of the proportion of consumers or the exposure required to the allergens to develop sensitisation has not been identified in the literature.

4.3.3 Control measures

There is some scientific evidence suggesting that processing insects through high temperatures or enzymatic digestion lowers the allergic reactivity to their principal allergens, but the specifics as to how these mechanisms may be effective are still unknown.

The high variability of edible insect composition, together with how much is still unknown on their de novo allergenic potential make it highly difficult to predict how the introduction of edible insects in the Western diet will affect the population beyond shellfish-allergic consumers.

Given the allergic potential of edible insects, consumer education, labelling and monitoring may be considered to minimise risk, based on what authors report.

4.4 Composition variability

4.4.1 Scientific evidence

Insects can undergo significant changes in their composition throughout their life cycle, as demonstrated by Liu et al. (2017) in their study in black soldier fly, in which differences were shown in the nutritional composition at the different stages from the egg to the adult stage. The same feed was used throughout the cycle, in which at first larvae increased their crude fat percentage from 4.8 to 28.4% in 14 days, and reduced their protein content from 56.2 to 39.2%. The following pupa stage showed higher levels of protein, at 43.8% and the lowest fat quantity at 7.2%, and finally, the post-mortem adult tests showed a protein proportion of 57.6% and a crude fat of 21.6% (Liu et al., 2017). This review retrieved several articles outlining the composition variability of edible insects in their early development stages based on the different diets they were reared on. A summary of several obtained results can be found on Table C in the Appendices section.

Larvae variability

A study by Dreassi et al. (2017) looking at the fatty acid composition of T. molitor larvae and pupae when fed on six different diets showed a significant variability for some fatty acids. Results indicated variations in fat percentage between 34.42-48.17% in larvae and from 30.18 to 42.52% in pupae, however, these variations where not associated with diets with a higher fat percentage. More significant differences were found in the fatty acid profiles. Larvae fed on the diet with a higher fat percentage showed higher monounsaturated fatty acids and lower polyunsaturated fatty acids than those fed on the no-fat diet (Dreassi et al., 2017). Another study in yellow mealworm fed on five different substrates (brewery spent grains, bread, cookies, 50% grains plus 50% cookies and 50% bread plus 50% cookies), showed that insects reared on cookies showed a fat percentage of 17.77% of their total composition, as opposed to the 6.46% of those reared on spent grains. Contrarily, the carbohydrate proportion was greater for spent grain-fed larvae (12.54%) compared to bread-reared (6.09%) and cookie-fed (6.72%) insects. Variability in protein content and dry matter were not as pronounced as the other values (Mancini et al., 2019a).

Ewald et al. (2020) showed how Z. atratus larvae fed on different waste sources developed significantly different compositions. Dry matter varied from around 27% for larvae fed on fish and rotten mussels to 35% for those fed solely on bread. Crude fat showed levels of 58% for larvae fed on bread as opposed to those fed on rotten and fresh mussels, at about 31%. Crude protein was measured at 40% for larvae fed on fish, as opposed to 28% for larvae fed on food waste. Ash content was higher in larvae fed on mussels (16-22%) than larvae fed on bread or fish (4-6%) (Ewald et al., 2020).

Several of the retrieved articles were performed on black soldier fly (H. illucens). Jucker et al. (2017) studied the composition of larvae fed on three different diets based on fruit, vegetables and mixed fruit and vegetables. Insects fed on the vegetable diet showed a moisture content of

78%, compared to the 62% of fruit and mixed diet. Fat levels were 21% for larvae fed on fruit, compared to 2% and 12% for larvae fed on vegetables and mixed feed, respectively. Although closer in proportion, significant differences in protein levels were reported, with the fruit diet resulting in a 12% protein content, the vegetable diet in a 14% and the mixed diet showing the highest levels at 18%. Looking at the fatty acid profile of the larvae, those fed with the mixed feed showed the higher n-6/n-3 ratio (7.3) compared to only fruit (4.6) and only vegetables (1.2). Other significant differences can be found on the saturated fatty acid content at 86.0% for fruit diet compared to the 56.5% of the vegetable diet, which in turn showed higher monounsaturated (27.2%) and polyunsaturated fatty acid (16.2%) content than the fruit diet (11.2% and 2.8% respectively). The mixed feed resulted on the highest levels of polyunsaturated fatty acid content of the larvae at 24.1% (Jucker et al., 2017).

Liland et al. (2017) evaluated the impact on the nutritional properties of black soldier fly larvae when fed brown algae (Asophyllum nodosum) at increasing concentrations from 0-100%. The total larvae lipid composition fed on a purely plant-based diet (33.8%) decreased significantly when fed on a purely algae diet (8.1%). On the contrary, the ash and moisture quantity increased by 10% and 13.5% respectively. Vitamin E concentration also increased in the larvae as more seaweed was incorporated to the diet (Liland et al., 2017). Another study in black soldier fly compared larvae composition when fed a mix of vegetables and fruit at a 7:3 proportion, a pure fruit feed, winery by-products and brewery by-products. Larvae fed on brewery by-products showed the highest amount of crude protein at 52.9% dry matter (DM), which contrasts with the 30.7% DM protein of those fed exclusively on fruit. The latter also showed the highest proportion of fats (40.7% DM) and fibre (19.7% DM). The lowest fat content was shown by larvae fed on the vegetable and fruit mix (26.8% DM), and the lowest fibre content corresponded to the group fed with brewery by-products (8.7%) (Meneguz et al., 2018). Similarly, Nguyen et al. (2015) reported the nutritional composition of black soldier fly larvae when reared on poultry feed, pig liver, fruit and vegetable mix and rendered fish. Calories per 100g of larvae varied form 105 for those fed the fruit and vegetable mix to 233 for those reared on fish. Coincidentally, levels of fats were lowest for the fruit and vegetable mix (2.22g) and highest for the fish-fed group (11.6). The maximum protein content corresponded to larvae that were fed pig liver at 21g, and the lowest were those fed on the fruit and vegetable mix at 12.9g (Nguyen et al., 2015).

Kazek et al. (2019) reported significant differences in G. mellonella larvae fatty acid composition when fed on two different diets. One consisted of a mix of wheat flour, wheat bran, dry milk, corn flour, dry yeast, glycerine, honey and water. The other was pure natural beeswax. Larvae fed on the mix diet showed higher quantities of short-chain fatty acids and stearic acid, as well as lower quantities of margaric acid and long-chain fatty acids than larvae fed on beeswax (Kazek et al., 2019).

4.4.2 Hazard evaluation

There is a potential risk of consumers being misled due to the potential of insects (particularly insect larvae), of presenting high composition variability, if this composition is not appropriately batch-tested before commercialisation.

4.4.3 Control measures

Based on the available evidence, standardisation of feed composition and rearing practices, together with consumer awareness, appear to be reliable options to minimise edible insects' composition variability. Internal batch-testing can help identify whether there are inconsistencies across product specifications.