Technical Report

Risk Profile on Edible Insects

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Science, Research and Evidence Directorate

Summary

Edible insects have been available to consumers in the UK market for several years. The Novel Food legislation covering edible insects was updated in 2018. Since then, all companies placing insects on the market that did not have a history of consumption in the EU prior to May 1997, must submit an application for authorisation of their products. Several applications have been received. Given the future responsibility held by the FSA to conduct the risk assessment of Novel Food applications, FSA risk managers commissioned the production of a risk profile looking to identify the hazards to humans from consumption of edible insects.

This risk profile has been informed by the 2015 EFSA risk profile, aiming to serve as an update to that report with relevant information identified in the scientific literature between 2015 and 2020. To this end, a systematic search protocol was designed, to identify studies concerning microbiological, antimicrobial resistance, toxicological, allergenicity and composition variability. The insect species of study include those identified by the 2015 EFSA risk profile, as well as those identified by the UK Advisory Committee for Novel Foods and Processes (ACNFP) from a previous horizon scanning exercise. Out of the 1759 publications initially obtained, 98 remained after sifting to agreed criteria. The relevant information was compiled and summarised in this report, extracting conclusions regarding risk to consumers and control measures.

Several hazards have been identified. Edible insect products can present high levels of microbial contamination if the animals are not reared in appropriate conditions or if the product is not processed by heating to high temperatures for several minutes. Insects also have the potential to accumulate toxic compounds, particularly heavy metals, when fed contaminated substrate. Ensuring hygienic rearing practices and minimising the levels of contamination of the substrate can help avoid accumulation of toxic compounds, but more research is necessary to inform the identified knowledge gaps in this area.

This review has updated the evidence on the allergic cross-reactivity between

shellfish and insects, therefore consideration may be given to informing consumers accordingly. De-novo sensitisations are likely to occur in the future, but estimates of the risk are likely to be highly uncertain based on the existing literature. Lastly, there is a high composition variability of insect larvae depending on the substrate. Standardisation of substrates can minimise composition variability.

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1. Statement of purpose

1.1. Background

Human consumption of insects and insect products can be considered a growing trend in the Western World. In the UK, various insect species have been available on the market to consumers for several years. The Novel Food legislation covering edible insects was updated in 2018 to reflect the need for all companies placing insects on the market, that did not have a history of consumption in the EU, to submit an application for authorisation of their products. Under the current regulation, products already present in the EU market needed to submit their application before the 1st of January 2019 to be able to continue to market their products, pending a decision on their application.

The UK has received several applications for validation from the Commission under the traditional foods route, and many more have been submitted to the EU under the full authorisation route. The two most relevant species to this end are larvae of *Tenebrio molitor* (mealworm) and adults of *Acheta domesticus* (house crickets), but other relevant species were also considered in this report.

The Novel Foods authorisation process requires that anyone wishing to commercialise a product with less than 25 years of proven consumption in the region must present an application for authorisation for that product. This application must contain all the relevant information as detailed in the relevant guidance by EFSA, including the identification, safety, and usage of the food to be authorised. There are two routes through which an applicant can seek authorisation:

- The **full authorisation** process for foods for which there is not sufficient evidence of safety of consumption in other countries.
- The **traditional foods** authorisation process for those foods for which a long history of safe consumption can be proven.

Within the authorisation process in the EU, EFSA carries out one initial validation

stage, in which the dossier is evaluated on the basis of scientific information completeness, and a risk assessment stage, in which a group of experts in the field evaluate and assess the dossier before formalising an opinion on its safety. The FSA currently mirror this process in GB

FSA risk managers commissioned the production of a risk profile that would look to identify the hazards to humans from consumption of insects as food. In addition, it is recognised that edible insects pose additional questions in relation to wider regulatory frameworks, namely hygiene requirements and their use as feedingstuffs for food producing animals. Work is ongoing towards updating the hygiene regulations to establish the principles that should be applied to insects, and this risk profile is intended to assist in the next phase of this work.

1.2. Risk question

This risk profile aims to identify hazards to human health following consumption of edible insects using the report by EFSA in 2015 as a basis.

The Scope section describes what was included as an objective of study in the review and what was excluded. The Methodology section describes the species of insect chosen for the literature review and the reasoning behind this selection, as well as the systematic search methodology followed for the identification of the relevant literature. The Risk profile section summarises the information found through the literature review, some of which has also been compiled in the form of tables in the Appendices section.

1.3. Legislation

Edible insects do not currently fall under their own specific EU legislation. Instead, a series of regulations apply:

- Principle of General Food Law: Reg. 178/2002
- Food Hygiene Regulation: Reg. 852/2004
- Food of Animal Origin: Reg. 853/2004
- Official Controls Regulation: Reg. 2017/625
- Novel Food Regulation: Reg. 2015/2283
- Commission Implementing Regulation (EU) 2017/2468
- Commission Implementing Regulation (EU) 2017/2469
- Commission Implementing Regulation (EU) 2017/2470
- Commission Implementing Regulation (EU) 2018/456
- General requirements for feed hygiene and animal health: Reg. 183/2005
- Animal by-products and Derived Products: Reg. 1069/2009
- Animal Welfare Directive 98/58/EC
- Placing on the Market and Use of Feed: Reg. 767/2009
- Transmissible Spongiform Encephalopathies Regulation: Reg. 999/2001
- Processed Animal Protein Amendments: Reg. 2017/893
- Undesirable Substances in Animal Feed: Directive 2002/32/EC

1.4. Previous reports and reviews

Several reports from other competent authorities have been previously produced identifying the available evidence to evaluate the risk to consumers from eating edible insects.

- EU
- France
- Belgium

The literature review performed as part of this risk profile identified several previous comprehensive reviews covering some of the areas within the scope of this document:

- Microbiology and AMR – **Garofalo et al. 2019**
- Toxicology – **Schrögel et al. 2019**
- Allergenicity – **De Gier et al. 2018**
- Edible insects in general - **Van der Fels-Klerx et al. 2018, Ferri et al. 2019**

2. Scope

2.1. Microbiology and antimicrobial resistance

Various factors, including the presence of microorganisms in ingredients, their introduction via cross- contamination during harvesting or preparation, inadequate cooking of the food and/or the improper treatment and storage of products, affect the number and type of microorganisms present in food products at the point of purchase. Such microorganisms may themselves be pathogenic to consumers or they can cause faster spoilage, increasing food waste.

Following the Novel Food authorisation process, levels of microbiological contaminants must be tested and then stated as part of the specification of the Novel Food (EFSA, 2016). The most common indicators of microbiological contamination of food include, amongst others, counts of mesophilic aerobes, *Enterobacteriaceae*, *Escherichia coli* and *Listeria monocytogenes* (HPA, 2009). Maximum microorganism count levels for different types of insect product have not been yet specified in legislation. In the UK, guidelines from the Health Protection Agency (HPA) and Public Health England (PHE) are used to interpret the enumeration of pathogens and hygiene indicators in ready to eat food (HPA, 2009).

Other than bacterial contamination, The EFSA Risk Profile from 2015 (EFSA, 2015) concluded that, based on the information available at the time, infection with viruses, parasites and prions from edible insects would not constitute a risk unless inadequate rearing practices allowed for external contamination. This was primarily due to the lack of presence of such pathogens in insects with the ability to infect humans, and the evidence showing the incapability of prions to multiply within insects. This evidence was re-evaluated as part of this literature review to incorporate new scientific evidence published from 2015 to 2019.

Antimicrobial resistance is a global growing problem that poses a risk to the capability of treating infectious diseases. As a consequence of antibiotic use in several sectors, including animal husbandry and agriculture, infectious agents

become resistant to the action of antibiotics, sometimes at a higher rate than new antibiotics can be produced (WHO, 2020). It is therefore necessary to consider the impact on AMR from using antibiotics in insect-rearing.

No specific pathogenic agents or AMR genes were identified or excluded prior to the execution of the review, in order to allow for a wider research scope. During the search and analysis process, those agents non-pathogenic to humans and those not capable of altering the hygienic quality of the food were not compiled as part of the results.

2.2. Toxicology

Toxicological safety evaluation is a requirement of the Novel Food authorisation process, including genotoxicity, subchronic and chronic toxicity, carcinogenicity, and reproductive and developmental toxicity (EFSA, 2016). Toxic chemicals may accumulate in edible insects from the substrate they feed on or by direct contact with contaminants during rearing. These chemicals can also form through the processing of the insects after harvesting, although there is a significant knowledge gap in the literature concerning this topic.

The guidance for the authorisation of Novel Foods specifies that for insects, consideration must be given to the hazards identified in the EFSA risk profile for edible insects of 2015, which includes heavy metals, toxins, pesticides, and toxic by-products of the processing (EFSA, 2015).

The literature review included a wide search to avoid the exclusion of relevant toxic compounds or potentially new risks discovered, given the lack of studies identified by the 2015 EFSA document. The scope of the review does not cover toxins produced by the insects themselves. This review is not a full evaluation of toxicological risk to human health, as it does not include additional information currently unavailable, such as exposure and consumption data, as well as referring those to health-based guidance values.

2.3. Allergenicity

One of the main characteristics of the average insect-based food is their high content in protein, and hence, the risk that some of these proteins act as allergens for the consumer. Historically there have been reports of allergic reactions to the consumption of insects and the cross-reactivity between mealworm and crustaceans has been repeatedly confirmed. However, the exact mechanism and potential for both cross-reactivity and de novo sensitisation to insect proteins is still an area with a lot of unknowns (EFSA, 2015).

Both cross-reactivity and de-novo sensitisation were considered for the scope of the review.

2.4. Composition variability

As part of the Novel Food authorisation process, applicants must specify the nutritional values that characterise their product. The final product must meet these specifications in order for it to be commercialised (EFSA, 2016). Therefore, a high variability in composition within samples of the same type of product may cause it to fail to comply with the specifications and not be able to be commercialised. Furthermore, if edible insect composition variability is not taken into account, the product could mislead customers by believing they are consuming a food with a proportion of nutrients that cannot actually be guaranteed to be the way it is described.

The scope of the review aims to identify potential hazards to misleading consumers or placing them at a nutritional disadvantage. No other nutritional considerations were taken into account for this review.

2.5. Exclusions

Physical hazards that are part of the structure of the edible insect, such as spines or stingers, were not considered as part of the scope of this review. No nutritional considerations other than those previously mentioned were taking into account in the review. Other than microbiological contamination and toxic compound accumulation, the scope of the review does not include further packaging, storage, or transport considerations. Risk related to feed, food for pets, insects captured in the wild or

reared under uncontrolled conditions were not evaluated, as these are considered irrelevant or unacceptable for human consumption.

2.6. Other legitimate factors

The ‘other legitimate factors’ described in the next section are not within the remit of this report, which aims to identify hazards to humans from consumption of insects as food. However, they are important to consider within wider strategic work on regulating edible insects and their impact on food safety. These factors could form the basis to future complementary pieces of work to be undertaken.

Term	Definition
Consumer acceptance:	Social acceptance of insects as food should be considered to support risk management decisions. Questions include whether the public will react positively to the inclusion of insect products in the market, and what the overall consumer perception will be like.
Environmental impact:	The industry claims that insect farming has a significantly lower negative impact on the environment than other farmed animals. The aim of this review is not to identify this evidence, but it should be considered as a potential future line of work in order to inform risk management decisions.
Animal welfare:	Currently there is no legislation defining or covering welfare of farmed insect populations, and it is not likely to be developed in the near future. Risk managers will take these factors into consideration.
Impact of trade:	Introduction on the market of edible insects and, particularly, products derived from animal protein, could have a positive impact in trade relationships with other nations that already trade with insect products, but could also pose a risk to the introduction of products with lower standards of hygiene and safety, as well as competing with other products as a source of protein for human diets.

3. Methodology

3.1. Literature review

A literature review was performed in order to identify the available evidence in the scientific literature since the report was produced by EFSA in 2015. The review was carried out with a systematic approach to ensure an objective selection process of the existing evidence. The retrieval and summarisation of results was carried out through a flexible, narrative approach, given the wide variability of topics and study designs predicted to be encountered through the search.

3.2. Research questions

The research questions that this review sought to answer, as written by risk managers, and agreed upon by risk assessors at the FSA, are:

- What are the hazards to human health from consumption of edible insects or their products based on the insects' natural microbial flora and microbiological contamination potential?
- What is the risk of negatively contributing to antibiotic resistance by rearing insects in a farm environment?
- What is the risk to human health from consumption of insects or their products based on their potential to produce or accumulate toxic chemicals in the rearing and production processes?
- What are the allergenicity risks to human health from consumption of the selected insect species or their products?
- What is the risk to human nutrition from consumption of insects or their products?
- What new evidence is available from 2015 to 2019 regarding safety of consumption of edible insects?

3.3. Objectives

The main objectives of the review are:

- To identify new hazards associated with human consumption of insects and

insect products relating to the questions stated above, since 2015.

- To identify knowledge gaps related to these areas.

3.4. Search protocol

A systematic search protocol was designed for the retrieval of relevant papers. The protocol includes information on the databases and search terms used, the selection criteria for papers and the type of information retrieved, as well as a description of how this information would be compiled and presented.

While the protocol aimed to provide a systematic structure to the search, the review is not strictly a systematic review and therefore does not necessarily abide by all the relevant rules and principles.

3.5. Databases

Three databases were selected to perform the search:

- PubMed
- Food Science Source
- Web of Science

These were selected based on the nature of the articles expected to be found in them. PubMed provides a source of biomedical scientific articles and acts as a generalist database for the search. Food Science

Source specialises in scientific articles relating to food safety, and acts as the specialist database for the search. Web of Science was chosen following common use by EFSA in literature review searches for food safety related literature reviews.

Other databases were considered but discarded:

- PMC
- ScienceDirect

PMC was discarded due to the high number of non-relevant results obtained in the

search when using the chosen Boolean operators, given the low retrieval specificity that this database has. ScienceDirect was discarded as the search tool would not allow for the extensive Boolean term combination to be used.

3.6. Search terms

The first pool of search terms was selected based on the insect species selected to be the subject of this risk profile, and both the scientific and common names were used. The word “insect” was not used due to the lack of specificity of results obtained. The species were selected based on those present in the EFSA risk profile of 2015, as well as those identified by the ACNFP in a past horizon scanning exercise, as the most likely species to be commercially produced for human consumption:

Musca domestica – Common house fly *Hermetia illucens* – Black soldier fly *Tenebrio molitor* – Mealworm *Zophobas atratus* – Giant mealworm *Alphitobius diaperinus* – Lesser mealworm *Galleria mellonella* – Greater wax moth *Bombyx mori* – Silkworm

Acheta domesticus – House cricket

Gryllodes sigillatus – Banded cricket

Locusta migratoria migratorioides – African migratory locust *Schistocerca americana* – American grasshopper *Schistocerca gregaria* – Desert locust

Mesobuthus martensii – Chinese yellow scorpion (included despite not being an ‘insect’ per se)

Atta laevigata – Leaf cutter ant

Gonimbrasia belina – Mopane moth

Other terms were identified as they related to human consumption practices and potential risks of this consumption. The terms were distributed into three pools and combined to produce a broad search while maintaining an acceptable level of specificity.

Table of terms

Species	**Food**	**Risk**
Musca domestica	Food	Bacter*
Hermetia illucens	Consumption	Allerg*
Tenebrio molitor	Edible	Microbiol*
Zophobas atratus	Market	Substrate
Alphitobius diaperinus	Process*	Vir*
Galleria mellonella	Roast	Accumulat*
Achroia grisella	Human*	Contamin*
Bombyx mori	Boil	Chemical
Acheta domesticus	Eat*	Parasit*
Gryllodes sigillatus	Entomophag*	Toxi*
Locusta migratoria migratorioides	Ate	Antibiotic*
Schistocerca americana		Risk
Mesobuthus martensii		Safety
Atta laevigata		Hazard
Gonimbrasia belina		Intake
Schistocerca gregaria		Expos*
Common house fly		
Black soldier fly		
Mealworm		
Giant mealworm		
Lesser mealworm		
Greater wax moth		
Lesser wax moth		

Species	Food	Risk
Silkworm		
House cricket		
Banded cricket		
African migratory locust		
American grasshopper		
Chinese yellow scorpion		
Leaf cutter ants		
Mopani moth		
Desert locust		

Boolean search

((musca domestica) OR (hermetia illucens) OR (tenebrio molitor) OR (zophobas atratus) OR (alphitobius diaperinus) OR (galleria mellonella) OR (achroia grisella) OR (bombyx mori) OR (acheta domesticus) OR (gryllodes sigillatus) OR (locusta migratoria migratorioides) OR (Schistocerca americana) OR (mesobuthus martensii) OR (atta laevigata) OR (gonimbrasia belina) OR (schistocerca gregaria) OR (common house fly) OR (black soldier fly) OR (mealworm) OR (giant mealworm) OR (lesser mealworm) OR (greater wax moth) OR (lesser wax moth) OR (silkworm) OR (house cricket) OR (banded cricket) OR (African migratory locust) OR (American grasshopper) OR (Chinese yellow scorpion) OR (leaf cutter ants) OR (mopane moth) OR (desert locust)) AND ((food) OR (edible) OR (process*) OR (market) OR (human*) OR (consum*) OR (roast*) OR (boil*) OR (eat*) OR (entomophag*) OR (ate)) AND ((risk) OR (allerg*) OR (toxi*) OR (safety) OR (microbiol*) OR (hazard*) OR (substrate) OR (parasite*) OR (chemical) OR (accumulat*) OR (contamin*) OR (antibiotic) OR (bacter*) OR (vir*) OR (intake) OR (expos*))

The search was capped to show articles published between January 2015 and November 2019

3.7. Selection criteria

This section of the protocol served as a guide to researchers rather than a set of rules. Adherence to these guidelines was kept flexible to allow for the inclusion of articles that could provide valuable information.

Articles obtained through the search were screened for relevance in two different stages:

- In the first stage, all titles and abstracts obtained were sifted on three different criteria:
    - The article mentions a relevant insect species or insects in general.
    - The article mentions a **food-related** or **human safety** component.
    - The article was published in English or Spanish language.

The articles included after the screening in stage 1 were obtained and read for further selection:

- In the second stage, the selected articles were read and kept for analysis and inclusion in the review as long as they presented information that could be relevant to evaluate the risk of human consumption of edible insects.

3.8. Extraction form

No formal extraction form was used in the production of this review, as the lack of specificity of the research questions found that such forms were not useful. However, a series of common themes of information were sought out, including:

Insects	Consumption	Risks
• Species • Stage of development • Rearing characteristics • Substrate • Place of rearing	• Alive/Dead • Raw/Cooked • Whole/Pulverised • Processed/Processing type • Quantity/Daily intake	• Allergenicity • Viral/bacterial/parasitical infection potential • Toxic compounds • AMR bacteria

Not all the information extracted was reported in the review's narrative synthesis. A summary of some of the data retrieved can be found distributed in tables in the Appendices section.

3.9. Synthesis

Due to the lack of typified information that was obtained through the inclusion of different types of study, as well as time constraints for the production of the review, synthesis was performed narratively, grouping studies of the same type where appropriate.

3.10. Results

After entering the search terms, PubMed retrieved 1431 articles, of which 91 were selected in the first sift. Food Science Source retrieved 312 articles, of which 16 were selected in the first sift. Web of Science retrieved 25 articles, with only one being selected after the first sift. Of the total 108 articles selected, 10 were discarded for duplication, for a final 98 articles to be analysed individually.

After analysis, 39 articles were discarded for lack of relevant data, and a further 17 where added retrieved from reviews identified in the systematic process or to complement the information presented. These additional articles did not appear in the search terms for not being present in the selected databases or for being published outside of the 2015-2019 selected period of time.

The following sections of the report summarise the results from the analysis of all obtained studies. Only those results claimed to be significant by the authors were reported. It is structured with the scientific evidence retrieved first, followed by the identified risks and the potential control measures as derived from the literature.

4. 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 (Gałęcki and Sokół, 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 (Gałęcki and Sokół, 2019).

Insect 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 a*l., 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 150°C 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 (Milanović *et al*., 2016, Milanović *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-), β-lactams (bla-; mec-), vancomycin (van-) and aminoglycosides (aac-aph).

In their effort to evaluate the existence of AR-coding genes in edible insects, Milanović *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 (Milanović 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* (Milanović 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 β-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 a*l., 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 a*l., 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.

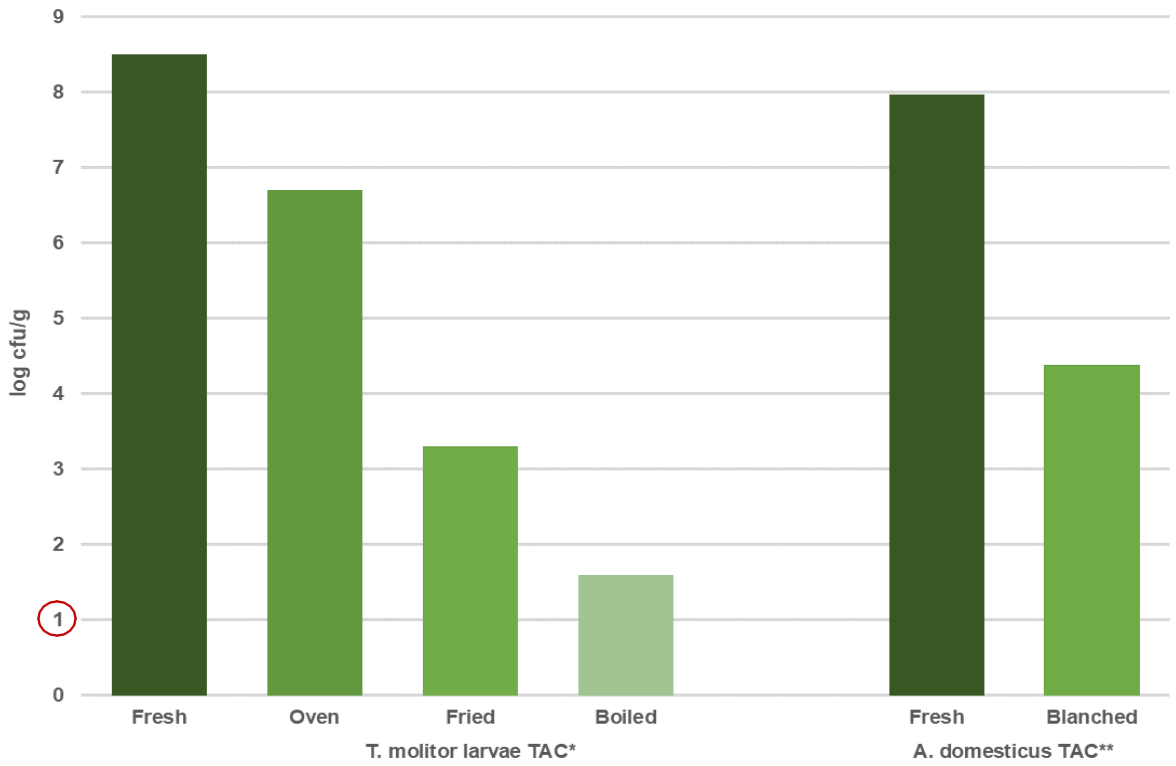

Figure 1: Total Aerobic Count before and after heat treatment. *Data from Caparros et al. 2018.

** 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 a*l. (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 Świątek (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 Świątek, 2016).

Similarly, van der Fels-Klerx *et al*. (2016) studied the bioaccumulation factor[1] 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±0.05[a]	5.8±1.0[a]
0.5 ML	0.58±0.12[a]	1.2±0.30[a]	9.5±3.6[a]
1.0 ML	0.56±0.13[a]	1.4±0.20[a]	6.1±1.9[a]
2.0 ML	0.49±0.10[a]	1.2±0.40[a]	6.9±0.92[a]

Tenebrio *molitor*

Quantity	As	Pb	Cd
Control	n/a	n/a	0.43±0.039[a]
0.5ml	1.4±0.045[a]	0.043±0.013[a]	0.71±0.083[b]
1.0ml	1.6±0.11[a]	0.046±0.032[a]	0.65±0.037[b]
2.0ml	2.6±0.23[b]	0.051±0.022[a]	0.69±0.056[b]

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 µg/kg), aflatoxin B1 (13.3 µg/kg), aflatoxin B2 (2.6 µg/kg), aflatoxin G2 (7 µg/kg), ochratoxin A (39.4 µg/kg) and zearalenone (130.4 µg/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 α-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 50°C and pH 7.0. Thermal treatment of locust extract occurred at temperatures between 80-100°C for 10 minutes, as well as an autoclaving process at 121-138°C 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 180°C or lyophilised at -50°C and 150 Pa. In vitro digestion was performed through the use of porcine pepsin, pancratin and lipase as well as bovine α-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.

5. Conclusions

Between 2015 to 2019 there were numerous studies published looking at the safety for consumption of edible insects, of which this review identified 98 relevant publications. However, some knowledge gaps still remain unaddressed. Table D, in the Appendices section, provides a summary of the key hazards identified in this review for each insect species.

5.1. Microbiology and AMR

Edible insects have a highly variable microbial profile across, and within species. There exists a risk for edible insect products to present high levels of microbial contamination. This may be caused by excessive microbial growth as a consequence of inadequate refrigeration and storage of raw materials, by cross-contamination throughout the food chain, or if the rearing conditions fail to comply with the hygienic described in Regulations, as mentioned in section 1.3. The literature shows that treating the product with high temperatures for several minutes can reduce the microbial load significantly although the exact time and temperature of exposure has not been determined. As with other food types, consideration must be given to the appropriate storage of both raw and processed ingredients and products, both to minimise the growth of microorganisms present and to reduce the risk of cross-contamination.

Further studies are required to determine precisely the nature of the microflora of relevant edible insect species, in order to better understand the effectiveness of control measures and the microbial populations that need to be included as part of the product's composition and specification analyses.

Production of insects in large quantities will increase the risk of disease within the insect population and between insect farms. Treating the population with antibiotics would have a potential negative impact on antimicrobial resistance. Optimisation of hygienic rearing practices may be considered to avoid the need to use antimicrobials to control disease transmission. More research will be required to better understand

disease transmission mechanism within and across farmed insect populations, as well as the most effective ways of control.

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.

5.2. Toxicology

Edible insects, larvae in particular, have the potential to accumulate toxic compounds, particularly heavy metals, when fed contaminated substrate. The evidence regarding accumulation of mycotoxins and pesticides is contradictory across the literature. Ensuring hygienic rearing practices and minimising levels of contamination of the substrate can help avoid accumulation of all toxic compounds that are not produced by the insect.

There are numerous knowledge gaps regarding insect's bioaccumulation potential and the impact of metabolism of toxic compounds. More research would also clarify whether heat processing could cause the formation of toxic substances such as acrylamide, furan or polycyclic aromatic hydrocarbons. Future studies could help identify if toxic metabolites may form as a result from the metabolisation of mycotoxins within the insect, if these remain in the final product, and in which quantities they may be found.

5.3. Allergenicity

Edible insects pose a risk to consumers allergic to shellfish, therefore consideration may be given to informing consumers accordingly through labelling. There is a risk of insects causing de-novo sensitisations in the future, but this information cannot be predicted with precision based on the existing literature. Future monitoring of cases and further research will be necessary to fully understand the de-novo sensitisation potential of the different species of edible insects, and how their processing, stage of development and feed may affect this potential.

5.4. Composition variability

Edible insects' larvae present variable compositions depending on the composition of the substrate fed to them and the time of harvest. This poses a risk of misleading consumers. Standardisation of substrates and batch-mixing can minimise composition variability. None of the articles identified studied the variability within batches fed with the same feed. Further research is needed to identify how standardised insect feeds affect composition variability across and within populations. This would allow to relate the feed and insect composition to the final product's specification.

5.5. Human consumption and exposure data

The review did not retrieve articles or official documents studying human consumption or exposure analysis data in the United Kingdom. Understanding how edible insects are consumed by the UK's population would allow to identify species preferred by consumers, the role of the food in the diet, and throw light into the allergic sensitivity profile of the UK population to edible insects. This, paired with exposure data collected systematically, would help narrow the risks posed by the food and identify ways to manage such risks, as well as optimise research efforts.

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Acronyms

Acronym	Definition
AMR/AR	Antimicrobial resistance
DM	Dry matter
EFSA	European Food Safety Authority
FSA	Food Standards Agency
ACNFP	Advisory Committee on Novel Foods and Processes
CFU	Colony forming units
EU	European Union
UK	United Kingdom
TAC	Total aerobic count
YMC	Yeast and moulds count
PCBs	Polychlorinated biphenyls
DDT	Dichlorodiphenyltrichloroethane
AF	Aflatoxin
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
ELISA	Enzyme-linked immunosorbent assay
MALDI	Matrix-assisted laser desorption/ionization
TOF	Time of flight
MS	Mass spectrometry

Appendices

Table A: Microbial levels for different **unprocessed** edible insects (* potential EU food, †potential EU feed, ‡ potential pet food refer to EFSA, 2013?) measured the harvest stage (larval or adult).

Insect species	Hazard	Load (log cfu/g)	Reference
*Tenebrio molitor**‡	Total aerobic count	8.6	(Caparros Megido et al., 2017)
*Tenebrio molitor**‡	Yeast and mould count	4.7	(Caparros Megido et al., 2017)
*Tenebrio molitor**‡ (non-starved)	Total aerobic count	6.4 - 7.8	(Mancini et al., 2019a)
*Tenebrio molitor**‡ (non-starved)	*Enterobacteriaceae*	5.8 - 6.4	(Mancini et al., 2019a)
*Tenebrio molitor**‡ (non-starved)	*Staphylococci*	3.8 - 5.9	(Mancini et al., 2019a)
*Tenebrio molitor**‡ (non-starved)	Lactic acid bacteria	5.2 - 6.2	(Mancini et al., 2019a)
*Tenebrio molitor**‡ (non-starved)	Bacterial endospores	0.0 - 5.3	(Mancini et al., 2019a)
*Tenebrio molitor**‡ (starved)	Total aerobic count	6.4 - 7.6	(Mancini et al., 2019a)
*Tenebrio molitor**‡ (starved)	*Enterobacteriaceae*	4.9 - 6.4	(Mancini et al., 2019a)
*Tenebrio molitor**‡ (starved)	*Staphylococci*	3.9 - 4.9	(Mancini et al., 2019a)
*Tenebrio molitor**‡ (starved)	Lactic acid bacteria	4.9 - 6.1	(Mancini et al., 2019a)
*Tenebrio molitor**‡ (starved)	Bacterial endospores	0.0 - 3.6	(Mancini et al., 2019a)
*Tenebrio molitor**‡	*Enterobacteriaceae*	6.1 - 7.1	(Osimani et al., 2018a)
*Tenebrio molitor**‡	Lactic acid bacteria	7.7 - 8.2	(Osimani et al., 2018a)
*Tenebrio molitor**‡	Mesophilic aerobes	8.2 - 8.5	(Osimani et al., 2018a)
*Tenebrio molitor**‡	Spore-forming bacteria	3.6 - 3.7	(Osimani et al., 2018a)
*Tenebrio molitor**‡	Total aerobic count	7.7 - 8.3	(Stoops et al., 2016)
*Tenebrio molitor**‡	*Enterobacteriaceae*	6.8 - 7.6	(Stoops et al., 2016)
*Tenebrio molitor**‡	Lactic acid bacteria	7.0 - 7.6	(Stoops et al., 2016)

Insect species	Hazard	Load (log cfu/g)	Reference
*Tenebrio molitor**‡	Bacterial endospores	<1.0 - 3.5	(Stoops et al., 2016)
*Tenebrio molitor**‡	Yeast and mould count	5.2 - 5.7	(Stoops et al., 2016)
*Tenebrio molitor**‡	Total aerobic count	8.0 - 9.3	(Vandeweyer et al., 2017a)
*Tenebrio molitor**‡	*Enterobacteriaceae*	6.8 - 8.3	(Vandeweyer et al., 2017a)
*Tenebrio molitor**‡	Lactic acid bacteria	7.3 - 8.2	(Vandeweyer et al., 2017a)
*Tenebrio molitor**‡	Aerobic bacterial endospores	1.7 - 5.0	(Vandeweyer et al., 2017a)
*Tenebrio molitor**‡	Bacterial endospores	4.8 - 9.1	(Vandeweyer et al., 2017a)
*Tenebrio molitor**‡	Yeast and mould count	4.2 - 7.5	(Vandeweyer et al., 2017a)
Hermetia illucens†	*Bacillus cereus*	3.8^{2}	(Wynants et al., 2019)
*Acheta domesticus**‡	Total aerobic count	7.9	(Caparros Megido et al., 2017)
*Acheta domesticus**‡	Yeast and mould count	4.8	(Caparros Megido et al., 2017)
*Acheta domesticus**‡	Total aerobic count	8.1 - 8.8	(Vandeweyer et al., 2017a)
*Acheta domesticus**‡	*Enterobacteriaceae*	7.2 - 8.3	(Vandeweyer et al., 2017a)
*Acheta domesticus**‡	Lactic acid bacteria	7.4 - 8.8	(Vandeweyer et al., 2017a)
*Acheta domesticus**‡	Aerobic bacterial endospores	2.6 - 4.9	(Vandeweyer et al., 2017a)
*Acheta domesticus**‡	Bacterial endospores	<3.0 - 5.5	(Vandeweyer et al., 2017a)

Insect species	Hazard	Load (log cfu/g)	Reference
*Acheta domesticus**‡	Yeast and mould count	5.6 - 7.2	(Vandeweyer et al., 2017a)
*Locusta migratoria**‡	Total aerobic count	7.8 - 8.6	(Stoops et al., 2016)
*Locusta migratoria**‡	*Enterobacteriaceae*	7.1 - 7.6	(Stoops et al., 2016)
*Locusta migratoria**‡	Bacterial endospores	3.3 - 3.8	(Stoops et al., 2016)
*Locusta migratoria**‡	Lactic acid bacteria	7.6 - 8.5	(Stoops et al., 2016)
*Locusta migratoria**‡	Yeast and mould count	5.0 - 5.4	(Stoops et al., 2016)

[2] Bacterial levels in both fresh insects and substrate were consistent with those reported by other authors other than *Bacillus cereus* levels. Authors concluded there existed no exclusive correlation between the substrate's microbial composition and the microbial composition of the BSF larvae, and argue that these differences are caused by multiple other factors, including type of substrate, rearing practices and parental origin of the larvae.

Table B: Microbial levels for different **processed** edible insects (* potential EU food, ‡ potential pet food refer to EFSA, 2013).

Insect species	Hazard	Processing	Load (log cfu/g)	Reference
*Tenebrio molitor** ‡	Total aerobic count	1-min water (80-100°C)	4.64	(Caparros Megido et al., 2017)
*Tenebrio molitor** ‡	Yeast and mould count	1-min water (80-100°C)	<1.0	(Caparros Megido et al., 2017)
*Tenebrio molitor** ‡	Total aerobic count	Freeze dried	4.47	(Caparros Megido et al., 2017)
*Tenebrio molitor** ‡	Yeast and mould count	Freeze dried	<1.0	(Caparros Megido et al., 2017)
*Tenebrio molitor** ‡	Moulds	Boiled and dried	2.21 - 2.30	(Garofalo et al., 2017)
*Tenebrio molitor** ‡	Mesophilic aerobes	Boiled and dried	2.6 - 4.8	(Osimani et al., 2017c)
*Tenebrio molitor** ‡	Enterobacteriaceae	Boiled and dried	<1.0	(Osimani et al., 2017c)
*Tenebrio molitor** ‡	Sulphite-reducing clostridia	Boiled and dried	1.5 - 4.0	(Osimani et al., 2017c)
*Tenebrio molitor** ‡	*Staphylococcus aureus*	Boiled and dried	<1.0	(Osimani et al., 2017c)
*Tenebrio molitor** ‡	*Bacillus cereus*	Boiled and dried	<1.0	(Osimani et al., 2017c)
*Tenebrio molitor**	Lactic acid bacteria	Boiled and dried	1.7-2.8	(Osimani et al., 2017c)

Insect species	Hazard	Processing	Load (log cfu/g)	Reference
‡				
*Tenebrio molitor** ‡	Yeasts and moulds	Boiled and dried	<1.0 - 2.4	(Osimani et al., 2017c)
*Acheta domesticus** ‡	Total aerobic count	4-min blanched	4.39	(Caparros Megido et al., 2017)
*Acheta domesticus** ‡	Yeast and mould count	4-min blanched	<1.0	(Caparros Megido et al., 2017)
*Acheta domesticus** ‡	Total aerobic count	Freeze dried	4.05	(Caparros Megido et al., 2017)
*Acheta domesticus** ‡	Yeast and mould count	Freeze dried	<1.0	(Caparros Megido et al., 2017)
*Acheta domesticus** ‡ (whole)	*Enterobacteriaceae*, *Clostridium perfringens* spores and moulds	Boiled and dried	<2.00	(Garofalo et al., 2017)
*Acheta domesticus** ‡ (whole)	Mesophilic aerobes	Boiled and dried	4.01 - 4.50	(Garofalo et al., 2017)
*Acheta domesticus** ‡ (whole)	Yeasts	Boiled and dried	4.52 - 5.10	(Garofalo et al., 2017)

Insect species	Hazard	Processing	Load (log cfu/g)	Reference
*Acheta domesticus** ‡ (powdered)	*Enterobacteriaceae*, lactic acid bacteria, *Clostridium perfringens* spores and yeasts	Boiled and dried	<2.00	(Garofalo et al., 2017)
*Acheta domesticus** ‡ (powdered)	Mesophilic aerobes	Boiled and dried	3.91 - 4.80	(Garofalo et al., 2017)
*Acheta domesticus** ‡ (powdered)	Moulds	Boiled and dried	2.92 - 3.10	(Garofalo et al., 2017)
*Acheta domesticus** ‡ (whole)	Mesophilic aerobes	Boiled and dried	4.2	(Osimani et al., 2017c)
*Acheta domesticus** ‡ (whole)	*Enterobacteriaceae*, sulphite-reducing clostridia, *Staphylococcus aureus* and yeasts and moulds	Boiled and dried	<1.0	(Osimani et al., 2017c)
*Acheta domesticus** ‡ (whole)	*Bacillus cereus*	Boiled and dried	3.6	(Osimani et al., 2017c)
*Acheta domesticus** ‡ (whole)	Lactic acid bacteria	Boiled and dried	2.1	(Osimani et al., 2017c)
*Acheta domesticus** ‡ (powdered)	Mesophilic aerobes	Boiled and dried	5.0	(Osimani et al., 2017c)
*Acheta domesticus** ‡ (powdered)	*Enterobacteriaceae*	Boiled and dried	3.1	(Osimani et al., 2017c)
*Acheta domesticus** ‡ (powdered)	Sulphite-reducing clostridia	Boiled and dried	2.8	(Osimani et al., 2017c)
*Acheta domesticus** ‡ (powdered)	*Staphylococcus aureus*	Boiled and dried	<1.0	(Osimani et al., 2017c)

Insect species	Hazard	Processing	Load (log cfu/g)	Reference
*Acheta domesticus** ‡ (powdered)	*Bacillus cereus*	Boiled and dried	5.1	(Osimani et al., 2017c)
*Acheta domesticus** ‡ (powdered)	Lactic acid bacteria	Boiled and dried	5.5	(Osimani et al., 2017c)
*Acheta domesticus** ‡ (powdered)	Yeasts	Boiled and dried	2.0	(Osimani et al., 2017c)

Insect species	Hazard	Processing	Load (log cfu/g)	Reference
*Acheta domesticus** ‡ (powdered)	Moulds	Boiled and dried	3.3	(Osimani et al., 2017c)
*Gryllodes sigillatus** ‡	Total viable counts	Boiled	2.6	(Vandeweyer et al., 2018)
*Gryllodes sigillatus** ‡	Total viable counts	Frozen (-20°C)	2.4	(Vandeweyer et al., 2018)
*Gryllodes sigillatus** ‡	Total viable counts	Oven dried (10h, 80°C)	4.3	(Vandeweyer et al., 2018)
*Gryllodes sigillatus** ‡	Total viable counts	Smoked and dried	7.9[3]	(Vandeweyer et al., 2018)
*Schistocerca gregaria**‡ (whole)	Mesophilic aerobes	Boiled and dried	4.1	(Osimani et al., 2017c)
*Schistocerca gregaria**‡ (whole)	*Enterobacteriaceae*, sulphite-reducing clostridia, *Staphylococcus aureus*	Boiled and dried	<1.0	(Osimani et al., 2017c)
*Schistocerca gregaria**‡ (whole)	*Bacillus cereus*	Boiled and dried	2.1	(Osimani et al., 2017c)
*Schistocerca gregaria**‡ (whole)	Lactic acid bacteria	Boiled and dried	2.4	(Osimani et al., 2017c)
*Schistocerca gregaria**‡ (whole)	Yeasts	Boiled and dried	2.0	(Osimani et al., 2017c)
*Schistocerca gregaria**‡ (whole)	Moulds	Boiled and dried	2.2	(Osimani et al., 2017c)

Authors associate this higher count to external contamination after treatment.

Table C: Composition variability for insects (DM= dry matter; * potential EU food, †potential EU feed, ‡ potential pet food refer to EFSA, 2013).

Species	Feed	Fibre	Fat	Protein	Ash	Moisture	Reference
Hermetia illucens† (Larvae day 1)	Chicken feed	-	4.8%	56.2%	-	-	(Liu et al., 2017)
Hermetia illucens† (Larvae day 14)	Chicken feed	-	28.4%	39.2%	-	-	(Liu et al., 2017)
Hermetia illucens† (Pupa)	-	-	7.2%	43.8%	-	-	(Liu et al., 2017)
Hermetia illucens† (Adult)	Chicken feed	-	21.6%	57.6%	-	-	(Liu et al., 2017)
Hermetia illucens† (Larvae)	Vegetables	-	2%	14%	-	78%	(Jucker et al., 2017)
Hermetia illucens† (Larvae)	Fruit	-	21%	12%	-	62%	(Jucker et al., 2017)
Hermetia illucens† (Larvae)	Fruit + Veg	-	12%	18%	-	62%	(Jucker et al., 2017)
Hermetia illucens† (Larvae)	Plant-based	-	33.8%	-	5.1%	63.4%	(Liland et al., 2017)
Hermetia illucens† (Larvae)	*Asophyllum nodosum*	-	8.1%	-	15.8%	76.9%	(Liland et al., 2017)
Hermetia illucens† (Larvae)	Fruit + Veg (7:3)	17.0% DM	26.8 % DM	41.8 % DM	12.9 % DM	-	(Meneguz et al., 2018)
Hermetia illucens† (Larvae)	Fruit	19.7% DM	40.7 % DM	30.7 % DM	7.2 % DM	-	(Meneguz et al., 2018)
Hermetia illucens† (Larvae)	Winery by-products	17.7% DM	32.2 % DM	34.4 % DM	14.5 % DM	-	(Meneguz et al., 2018)
Hermetia illucens† (Larvae)	Brewery by-products	8.7%	29.8 % DM	52.9 % DM	7.3 % DM	-	(Meneguz et al., 2018)
Hermetia illucens† (Larvae)	Poultry feed	8.75%	4.02%	14.7%	-	66.5%	(Nguyen et al., 2015)
Hermetia illucens† (Larvae)	Pig liver	13.7%	8.39%	21%	-	55.3%	(Nguyen et al., 2015)

Species	Feed	Fibre	Fat	Protein	Ash	Moisture	Reference
Hermetia illucens† (Larvae)	Fruit + Veg	8.38%	2.2%	12.9%	-	71.8%	(Nguyen et al., 2015)
Hermetia illucens† (Larvae)	Rendered fish	12.7%	11.6%	19.4%	-	53.4%	(Nguyen et al., 2015)
*Tenebrio molitor**‡ *(Larvae)*	Brewery spent grains	12.54 %	6.46%	13.22%	-	66.66%	(Mancini et al., 2019a)
*Tenebrio molitor**‡ *(Larvae)*	Bread	6.09%	14.82%	10.73%	-	67.38%	(Mancini et al., 2019a)
*Tenebrio molitor**‡ *(Larvae)*	Cookies	6.72%	17.77%	10.15%	-	64.45%	(Mancini et al., 2019a)
*Tenebrio molitor**‡ *(Larvae)*	Grains + Cookies	11.26 %	11.77%	13.44%	-	62.47%	(Mancini et al., 2019a)
*Tenebrio molitor**‡ *(Larvae)*	Bread + Cookies	6.12%	17.48%	10.72%	-	64.66%	(Mancini et al., 2019a)

Table D: Key hazards identified in this literature review by insect species

Species	Hazards identified
Acheta domesticus	**Microorganisms (other than parasites)** • Aerobic bacteria (Caparros Megido et al., 2017, Vandeweyer et al., 2017a, Garofalo et al., 2017, Osimani et al., 2017c) • Yeasts and moulds (Caparros Megido et al., 2017, Vandeweyer et al., 2017a, Garofalo et al., 2017, Osimani et al., 2017c) • Lactic acid bacteria (Vandeweyer et al., 2017a, Garofalo et al., 2017, Osimani et al., 2017c) • Bacterial endospores (Vandeweyer et al., 2017a, Garofalo et al., 2017, Osimani et al., 2017c) • *Enterobacteriaceae* (Vandeweyer et al., 2017a, Garofalo et al., 2017, Osimani et al., 2017c) • *Staphylococcus aureus (*Osimani et al., 2017c) • *Cronobacter sakazakii* (Walia *et al*. 2018) **Parasites** • *Isospora spp.* (Gałęcki and Sokół, 2019) **AMR genes** • tet(M) (Milanović et al., 2016) • tet(K) (Milanović et al., 2016) • tet(O) (Milanović et al., 2016) • tet(S) (Roncoli *et al*. 2019) **Allergenicity: Cross-reactivity** • Tropomyosin (Pali-Schöll *et al*., 2019)
Hermetia illucens	**Microorganisms (other than parasites)** • *Bacillus cereus* (Wynants et al., 2019) **Parasites** • *Eimeria nieschulzi (*Muller *et al.* 2019) • *Eimeria tenella* (Muller *et al.* 2019) • *Ascaris suum* (Muller *et al.* 2019) **Heavy metals accumulation** • Cadmium (Biancarosa *et al*. 2018, Purschke *et al*. 2017) • Arsenic (Biancarosa *et al*. 2018) • Mercury (Biancarosa *et al*. 2018) • Lead (Biancarosa *et al*. 2018, Purschke *et al*. 2017) • Mycotoxins accumulation • Aflatoxin B1 (Bosch *et al.* 2017)

Species	Hazards identified
Locusta migratoria	**Microorganisms (other than parasites)** • Aerobic bacteria (Stoops *et al*., 2016) • Yeasts and moulds (Stoops *et al*., 2016) • Lactic acid bacteria (Stoops *et al*., 2016) • Bacterial endospores (Stoops *et al*., 2016) • Enterobacteria (Stoops *et al*., 2016) **Parasites** • *Isospora spp.* (Gałęcki and Sokół, 2019) • *Balantidium spp.* (Gałęcki and Sokół, 2019) • *Entamoeba spp.* (Gałęcki and Sokół, 2019) **AMR genes** • tet(M) (Osimani *et al*., 2017b) • tet(K) (Osimani *et al*., 2017b) • bla(Z) (Osimani *et al.*, 2017b) • erm- (Osimani *et al*., 2017b) • aac-aph (Osimani *et al*., 2017b)
Tenebrio molitor	**Microorganisms (other than parasites)** • Aerobic bacteria (Caparros Megido et al., 2017, Mancini et al., 2019a, Osimani et al., 2017c, Osimani et al., 2018a, Stoops et al., 2016, Vandeweyer et al., 2017a) • Yeasts and moulds (Caparros Megido et al., 2017, Mancini et al., 2019a, Stoops et al., 2016, Vandeweyer et al., 2017a, Osimani et al., 2017c) • Lactic acid bacteria (Mancini et al., 2019a, Osimani et al., 2017c, Osimani et al., 2018a, Stoops et al., 2016, Vandeweyer et al., 2017a) • Bacterial endospores (Mancini et al., 2019a, Osimani et al., 2018a, Stoops et al., 2016, Vandeweyer et al., 2017a) • *Staphylococci* (Mancini et al., 2019a, Osimani et al., 2017c) • *Enterobacteriaceae* (Mancini et al., 2019a, Stoops et al., 2016, Vandeweyer et al., 2017a, Osimani et al., 2017c) **Parasites** • *Isospora spp.* (Gałęcki and Sokół, 2019) • *Balantidium spp.* (Gałęcki and Sokół, 2019) • *Entamoeba spp.* (Gałęcki and Sokół, 2019) **AMR genes and substances** • tet(M) (Milanović et al., 2016) • tet(S) (Milanović et al., 2016) • tet(K) (Milanović et al., 2016)

Species	Hazards identified
	• erm(B) (Osimani *et al*., 2017a) • aac-aph (Osimani *et al*., 2017a) • Chitosan (Shin *et al*., 2019) **Heavy metals accumulation** • Cadmium (Bednarska and Świątek 2016, van der Fels-Klerx *et al*. 2016) • Zinc (Bednarska and Świątek, 2016) • Arsenic (van der Fels-Klerx *et al*. 2016) • Lead (van der Fels-Klerx *et al*. 2016) **Mycotoxins accumulation** • Aflatoxin B1 (Bosch *et al.* 2017) **Pesticides accumulation** • Diflufenican (Houbraken *et al*., 2016) • Fenpropimorph (Houbraken *et al*., 2016) • Isoproturon (Houbraken *et al*., 2016) • Linuron (Houbraken *et al*., 2016) • Mefenoxam (Houbraken *et al*., 2016) • Pendimethalin (Houbraken *et al*., 2016) • Pyrimethanil (Houbraken *et al*., 2016) • Tebuconazole (Houbraken *et al*., 2016) **Allergenicity: Cross-reactivity** • Apolipophorin-III (Barre *et al*., 2019) • Larval cuticular protein (Barre *et al*., 2019) • Hemolymph protein (Barre *et al*., 2019) • Tropomyosin (Pali-Schöll *et al*., 2019, Broekman et al., 2016)
Schistocerca gregaria	**Microorganisms (other than parasites)** • Aerobic bacteria (Osimani et al., 2017c) • Yeasts and moulds (Osimani et al., 2017c) • *Bacillus cereus* (Osimani et al., 2017c) • *Enterobacteriaceae* (Osimani et al., 2017c) • *Staphylococcus aureus* (Osimani et al., 2017c) **Allergenicity: Cross-reactivity** • Tropomyosin (Pali-Schöll *et al*., 2019)
Blattodea spp.	**Parasites** • *Isospora spp.* (Gałęcki and Sokół, 2019) • *Balantidium spp.* (Gałęcki and Sokół, 2019)

Species	Hazards identified
	• *Entamoeba spp.* (Gałęcki and Sokół, 2019)
Musca domestica	**Microorganisms (other than parasites)** • *Salmonella enterica* (Pava-ripoll *et al*. 2015) • *Cronobacter sakazakii* (Pava-ripoll *et al*. 2015) • *Escherichia coli* 0157:h7 (Pava-ripoll *et al*. 2015) • *Listeria monocytogenes* (Pava-ripoll *et al*. 2015)
Bombyx mori	**AMR genes** • tet(S) (Milanović et al., 2016) • tet(K) (Milanović et al., 2016) • bla(Z) (Milanović et al., 2016) • BmGlv2 (Shin *et al*., 2019) **Heavy metals accumulation** • Arsenic (Feng *et al*. 2019) • Cadmium (Feng *et al*. 2019) • Lead (Feng *et al*. 2019)