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Risk profile related to production and consumption of insects as food and feed

EFSA Scientific Committee

Abstract

The present opinion has the format of a risk profile and presents potential biological and chemical hazards as well as allergenicity and environmental hazards associated with farmed insects used as food and feed taking into account of the entire chain, from farming to the final product. The opinion also addresses the occurrence of these hazards in non-processed insects, grown on different substrate categories, in comparison to the occurrence of these hazards in other non-processed sources of protein of animal origin. When currently allowed feed materials are used as substrate to feed insects, the possible occurrence of microbiological hazards is expected to be comparable to their occurrence in other non-processed sources of protein of animal origin. The possible occurrence of prions in non-processed insects will depend on whether the substrate includes protein of human or ruminant origin. Data on transfer of chemical contaminants from different substrates to the insects are very limited. Substrates like kitchen waste, human and animal manure are also considered and hazards from insects fed on these substrates need to be specifically assessed. It is concluded that for both biological and chemical hazards, the specific production methods, the substrate used, the stage of harvest, the insect species and developmental stage, as well as the methods for further processing will all have an impact on the occurrence and levels of biological and chemical contaminants in food and feed products derived from insects. Hazards related to the environment are expected to be comparable to other animal production systems. The opinion also identifies the uncertainties (lack of knowledge) related to possible hazards when insects are used as food and feed and notes that there are no systematically collected data on animal and human consumption of insects. Studies on the occurrence of microbial pathogens of vertebrates as well as published data on hazardous chemicals in reared insects are scarce. Further data generation on these issues are highly recommended.

Keywords: insects, food, feed, microbes, allergenicity, chemicals, safety, production, consumption

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Summary

The European Commission (EC) asked the European Food Safety Authority (EFSA) to assess the microbiological, chemical and environmental risks arising from the production and consumption of insects as food and feed and to cover the main steps from the production chain up to consumption by pets, food producing animals and humans. EFSA was requested to provide an overall conclusion based on the above assessment, on the potential risks posed by the use of insects in food and feed, relative to such risks posed by the use of other protein sources used in food or feed.

In agreement with the EC, this opinion has the format of a risk profile including considerations of hazards associated with insects as food and feed, placed in the context of hazards associated with other sources of protein. The mandate also considers potential risks arising from importation of insects and products of insects from countries outside the EU, but not the importation of live insects. Health or welfare of insects, hazards related to insects harvested from the wild, nutritional value of insects as food and feed and occupational hazards are outside the scope of this opinion.

This opinion is based on data from peer reviewed scientific literature, assessments performed at Member State level and information from relevant stakeholders that were invited to provide information as hearing experts at a working group meeting. All data and information are compiled in the format of a risk profile. The risk profile addresses biological hazards (bacteria, viruses, parasites, fungi, prions), chemical hazards (heavy metals, toxins, veterinary drugs, hormones and others) as well as allergens and hazards related to the environment.

It is concluded that for both biological and chemical hazards, the specific production methods, the substrate used, the stage of harvest, the insect species, as well as the methods used for further processing will all have an impact on the possible presence of biological and chemical contaminants in insect food and feed products.

The opinion addresses the potential occurrence of hazards in non-processed insects, grown on different substrate groups, in comparison to the occurrence in other non-processed sources of protein of animal origin.

When currently allowed feed materials are used as substrate to feed insects, the possible occurrence of microbiological hazards is expected to be comparable to their occurrence in other non-processed sources of protein of animal origin. The possible occurrence of prions in non-processed insects will depend on whether the substrate includes protein of human or ruminant origin. Data on transfer of chemical contaminants from different substrates to the insects are very limited. Other relevant substrates and the possible occurrence of hazards are considered and summarised in the opinion. Substrates like human and animal manure are also considered. For both biological and chemical hazards their possible occurrence in non-processed insects fed on such substrates needs to be specifically assessed.

The environmental risk of insect farming is expected to be comparable to other animal production systems. Insect waste may contain insects and insect material. The adoption of existing waste management strategies should be applicable for managing waste from insect production. Assessment of the individual production systems will determine the precise strategy to be adopted on a case by case basis.

The opinion also notes the knowledge gaps and uncertainty related to possible hazards when insects are used as food and feed and concludes that there are no systematically collected data on animal and human consumption of insects. Also, there are only a few studies on the occurrence of microbials potentially pathogenic for vertebrates as well as published data on hazardous chemicals in reared insects.

Further research for better assessment of microbiological and chemical risks from insects as food and feed including studies on the occurrence of hazards when using particular substrates, like food waste and manure is recommended.

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1. Introduction

During the last years, there has been an increased interest in using insects for food and feed. Insects are considered as suitable alternatives to mainstream animal sources of food such as chicken, pork, beef and fish as well as an alternative feed. The specific nutritional value of insects depends on the species (see Appendix A) as well as on their breeding and processing, and is outside the scope of this assessment.

Farming of insects when optimised is suggested to lead to lower emission of greenhouse gases and ammonia than cattle or pigs and higher efficiency in converting feed to protein. Insect rearing/farming may be a low-tech activity and requires low-capital investment (FAO, 2013).

More than 2 000 insect species have been documented in the literature as edible, most of them in tropical countries. The most commonly eaten insects are members of the *Coleoptera* (beetles), *Lepidoptera* (caterpillars of butterflies and moths), *Hymenoptera* (bees, wasps, ants), *Orthoptera* (grasshoppers, locusts, crickets, termites), *Hemiptera* (cicadas, leaf and plant hoppers, true bugs, scale insects), *Odonata* (dragonflies) and *Diptera* (flies) families. At present in the EU, insects as food for human consumption represent a niche market and the use of insects as feed is limited to feed for certain companion animals.

There are several legislative requirements that impact the use of insects as food and feed. Currently, the feed ban provisions of Regulation (EC) No 999/2001[1] (TSE Regulation) do not allow insect Processed Animal Protein (PAP) to be fed to farmed animals due to lack of a safety profile. With respect to feed/substrate for insects, Annex III to Regulation (EC) No 767/2009[2] prohibits the feeding of faeces and separated digestive tract content even though these materials are used in other parts of the world as substrates in insect production. The legislative framework specifically related to insects used as food and feed, however, is still under development.

The processing and storage of insects and their products should follow the same health and sanitation regulations as for any other traditional food or feed items in order to ensure food safety. Issues to be considered include microbial, chemical and allergic hazards. In addition, general animal (vertebrate) health and welfare rules should also apply for insects.

The EFSA Focal Points of Member States and EFTA countries were asked to share existing and on-going risk assessments on the safety of insects as food and feed performed in their country (see Appendix B). Four countries (Belgium, France, Iceland and Netherlands) replied that they have performed risk assessments related to insects as food or feed.

With reference to the mandate, the present opinion will have the format of a risk profile and presents the potential hazards associated with using insects as food and feed taking into account of the entire chain, from farming to the final product. The opinion also includes the hazards arising from import of farmed (not live) insects from outside Europe and also covers hazards arising from procedures which are not in compliance with existing EU legislation.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

At present, insects represent a very small niche market in the EU. Several Member States indicated sporadic human consumption of certain types of insects. Nonetheless, the use of insects as a source of food and feed is seen as bringing important environmental, economic and food security benefits. Insects are powerful bio-converters, which can transform low quality biomass into nutritionally valuable proteins. Interest in production of insects for food and feed in the EU is growing. Furthermore, import of insects and insect products into the EU as food and feed is an issue that needs to be addressed as the use of insects is more common outside the EU.

[1] Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ L 147, 31.5.2001, p. 1–40.

[2] Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. OJ L 229, 1.9.2009, p. 1–28.

A number of organisations have started studying the prospect of using insects for food and feed. The Food and Agricultural Organization of the United Nations (FAO) published a paper ('Edible Insects – Future prospects for food and feed security') (FAO, 2013) summarising the opportunities for, and constraints on, the use of insects as food and feed. DG Research is currently co-financing a research project to explore the feasibility of using insect proteins for feed (PROteINSECT)[3].

DG for Health and Food Safety is currently reflecting on the development of its policy in the area of insects in the framework of legislation on novel foods, animal feed and on the need for further risk/policy measures to ensure the safety of the food and feed chain.

In support of that, the Commission has decided to ask the European Food Safety Authority (EFSA) to elaborate an initial scientific opinion on the microbiological, chemical and environmental safety risks arising from the consumption and production of insects as food and feed.

To build upon already existing or on-going risk assessments done at Member State level, EFSA is invited to collaborate with national risk assessment bodies. The Commission is aware that there are a high variety of insect species that would need to be considered, each with their specific characteristics of production and possible hazards. As guidance for the assessment and with a view to restrict the scope, a list of insect species which were reported to have the biggest potential to be used as food and feed in the EU is hereby presented (see supporting documentation (Section 1.1.2)).

1.1.2. Terms of Reference

In accordance with Article 29 of the Regulation (EC) No 178/2002[4], the European Commission asks the European Food Safety Authority to assess the microbiological, chemical and environmental risks arising from the production and consumption of insects as food and feed. The assessment of those risks should cover the main steps from production chain up to consumption:

1) Production (farming of insects): production process including substrates (feedstock) for the insects;
2) Processing: manufacturing of insects to insect products;
3) Consumption of the products by pets, food producing animals and humans considering the composition of the products and potential microbial and chemical contamination.

EFSA is requested to provide an overall conclusion based on the above assessments, on the risks posed by the use of insects in food and feed, relative to such risks posed by the use of other protein sources used in food or feed.

The list of insect species should serve as guidance in the overall assessment and should not be considered definitive or exhaustive.

Supporting documentation

List of insect species which were reported to have the biggest potential to be used as food and feed in the EU:

Musca domestica: Common housefly

Hermetia illucens: Black soldier fly

Tenebrio molitor: Mealworm

Zophobas atratus: Giant mealworm

Alphitobus diaperinus: Lesser mealworm

Galleria mellonella: Greater wax moth

Achroia grisella: Lesser wax moth

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3 http://www.proteinsect.eu/

4 Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety OJ L 31, 1.2.2002, p. 1–24.

Bombyx mori: Silkworm

Acheta domesticus: House cricket

Gryllodes sigillatus: Banded cricket

Locusta migratora migratorioides: African migratory locust

Schistocerca Americana: American grasshopper

1.2. Interpretation of the Terms of Reference

After discussion with the requestor (European Commission), the Terms of Reference were further clarified as follows:

- 'Initial scientific opinion' indicates that this opinion would be more in line with a risk profile approach. More detailed mandates from the Commission on specific insects/uses may follow at a later date, most probably related to feed dossiers and to novel food applications.
- The list of insect species provided is indicative and not exhaustive. EFSA is currently not expected to perform a risk assessment for each insect species.
- The sentence on providing an overall conclusion 'relative to such risks posed by the use of other protein sources used' refers to presenting a list of hazards associated with insects as food and feed, placed in the context of hazards associated with other sources of protein.
- Assessment of environmental risks will consider the upstream and downstream impacts of insect production. Accidental release of insects from production facilities is not to be considered at this time;
- The mandate will consider potential risks arising from importation of insects and products of insects from countries outside the EU, but not the importation of live insects.

These issues are understood to be outside the terms of reference:

- Health or welfare of insects;
- Plant health risk of insect use for food and feed;
- Products from apiculture;
- Contamination of insects or products thereof intended for food/feed by pesticide residues, as existing regulatory provisions would apply to insects as food and feed;
- Hazards related to insects harvested from the wild (as opposed to farmed);
- Occupational hazards.

2. Data and Methodologies

2.1. Data

The evidence base used for this opinion stems primarily from expert knowledge gathered by a working group of the EFSA Scientific Committee dedicated to the work for this opinion, consultations with members of the EFSA Panels on Biological Hazards (BIOHAZ), on Contaminants in the Food Chain (CONTAM), on Dietetic Products, Nutrition and Allergies (NDA) and on Additives and Products or Substances used in Animal Feed (FEEDAP), from published EFSA Scientific Opinions, Guidance Documents, assessments performed at Member State level (Appendix B) and from data retrieved from the scientific literature. In addition, relevant stakeholders were invited to provide information as hearing experts at a working group meeting (Annex A).

2.2. Methodologies

The methodology used for this opinion was to aggregate the information from the diverse EFSA areas and external experts, discuss them in a working group of the EFSA Scientific Committee and extract from such discussions principles and proposals for adoption by the EFSA Scientific Committee. EFSA followed its specific standard operating procedure detailing the steps necessary for establishing, updating or closing the working group of the Scientific Committee that prepared this opinion. The standard operating procedure implements the Decision of the Executive Director on the selection of experts of the Scientific Committee, Panels and working groups[5].

3. Farming and processing of insects

3.1. Insect species and substrates applied in primary insect production

3.1.1. Species

The insect species within the scope of this risk profile were identified from the assessments performed by national authorities in Belgium (FASFC, 2014), in the Netherlands (NVWA, 2014) and in France (ANSES, 2015), from the websites of European companies active in the area of farmed insects and from information provided by relevant stakeholders that were invited to provide information as hearing experts at a working group meeting (Table 1). 'Edible insects' include arthropods such as spiders and scorpions, which are not *Insecta sensu strictu*. In the context of utilization for food and feed, some other arthropods are also included as 'insects'. The insects considered are only those that are farmed and produced on a commercial basis both within and outside Europe. The list of insects for food and feed (Table 1) is not exhaustive.

There are many commercial products of farmed insect species outside Europe, but most species are similar to the ones produced by companies in the European Union. Table 1 includes the most important insects produced within and outside Europe, which are crickets, mealworms, flies and silkworms. The insects listed are grouped according to the developmental stage (larvae, pupae, adults), at which they are commonly harvested for processing and made available as food or feed. It is also indicated whether the insects are considered for human consumption or as feed for animals and where the species is produced. Additional information is included as to whether the insects are produced as pet food or as feed for food-producing animals, such as fish, chicken or pigs.

[5] See http://www.efsa.europa.eu/en/keydocs/docs/expertselection.pdf

Table 1: Examples of insect species known to be farmed on commercial basis both within and outside Europe, grouped by life-stage (adult, larvae or pupae) of utilizations and, secondarily, by closely related species if several are farmed. Note: the insect species within the scope of this risk profile were taken from the assessments performed by national authorities in Belgium (FASFC, 2014), in the Netherlands (NVWA, 2014) and in France (ANSES, 2015), from the websites of European companies active in the area of farmed insects and from information provided by relevant stakeholders that were invited to provide information as hearing experts at a working group meeting.

Groups and scientific names	English name	Farmed for human consumption	Farmed for feed	Additional information, including estimated volumes
Species utilized in full grown (adult) stages				
Crickets				
Acheta domesticus	house cricket	X	X (pets)	Farmed for live pet feed in many countries, also in Europe. In Netherlands farmed to be marketed for human consumption. Widely farmed in Thailand, and neighbouring countries. Farming promoted in Kenya. Production in USA.
Gryllodus sigillatus	banded cricket		X (pets)	Farmed for live pet feed.
Gryllus assimilis	field cricket		X (pets)	Field cricket native in Asia.
Gryllus bimaculatus	black cricket or field cricket	X		Widely farmed in Thailand, and also in Laos and Cambodia. Farmers change between *Gryllus bimaculatus* and *Acheta domesticus.*
Teloegryllus testaceus (Gryllus testaceus)	common or field cricket	X		Field cricket native in Americas.
Grasshoppers/locusts				
Orthoptera group, such as: *Oxya spp.; Melanoplus spp.; Hieroglyphus spp.; Acridia spp.* *Locusta migratora; Schistocerca Americana*		X	X (pets)	Various grasshopper/locusts species are produced as live pet feed in and outside Europe. Some species are marketed for human consumption in Netherlands. Worldwide grasshoppers are consumed from wild collection. Some tropical countries hesitate to promote farming due to crop pest risks if released.
Species utilized in larvae stages				
Mealworms				Mealworms are easy to rear and are produced for live pet feed in many countries.
Alphitobius diaperinus	lesser mealworm (larvae of lesser meal beetle/darkling beetle)	X	X (pets)	Produced as pet food and in some countries also for human consumption
Tenebrio molitor	mealworm (larvae of yellow meal beetle)	X	X (pets)	Same as above

Groups and scientific names	English name	Farmed for human consumption	Farmed for feed	Additional information, including estimated volumes
Zophobas atratus	superworm, zophobas (larvae of darkling beetle)	X	X (pets)	Same as above
Zophobas Morio (Tenebrio molitor)	giant mealworm		X (pets)	Mealworm treated with juvenile hormone (an insect hormone delaying metamorphosis)
Other species used as larvae				
Musca domestica	house fly		X	House fly larvae are known to be produced on industrial scale in South Africa and China. Experimental or emerging commercial production in other countries. Reared for feed for food-producing animals (fish, chicken, pigs), either fresh or as dried and powdered protein supplement.
Chrysomya chloropyga	blow fly		X	Experimental in South Africa for animal feed production
Rhynchophorus ferrugineus	palm Weevil	X		Traditionally collected in SE Asia. Recently farming systems have developed in Thailand.
Species utilized in pupae or pre-pupae stages				
Hermetia illucens (harvested as larvae, pre-pupae or pupae)	black soldier fly		X	Black soldier fly is known to be produced on industrial scale in USA, South Africa and China, for feed for food-producing animals (fish, chicken, pigs). Emerging commercial production in other countries. Experimental production in European countries.
Bombyx mori	silkworm	X	X	By-product from silk production. Traditionally used for human consumption in some countries. Experimental use in processed products.

3.1.2. Substrates

A wide range of organic materials can be used as source of nutrients or as substrates for rearing of insects; substrate is the overall term that will be applied for these materials as insect feed throughout the opinion. The substrates that will be included in the production will depend on the legislative framework, availability, the applicability in the specific farming system and the cost. Due to the different requirements, the substrate preference will differ among the different insect species.

Substrates in use in the EU

Animals in the EU may be fed only with safe feed (Commission Regulation (EC) No 68/2013[6], Regulation (EC) No 178/2002 and Regulation (EC) No 767/2009). With respect to substrate for insects, Annex III to Regulation (EC) No 767/2009 prohibits the use of faeces and separated digestive tract content even though these materials are used in other parts of the world for insect production.

According to Regulation (EC) No 1069/2009[7], insects are considered as 'farmed animals' and thus, for their feeding the use of certain substrates such as manure, catering waste or former foodstuff containing meat and fish, are not allowed.

According to the hearing experts who participated in a working group meeting (see Annex A), the main substrates currently applied in the European insect production include commercial animal feed, former foodstuffs not containing meat and fish (i.e. production surplus, misshapen products or foods with expired best-before-date that had been produced in compliance with EU food law) and co-products from primary production of food of non-animal origin.

Substrates used outside the EU

In addition, outside the EU, commercial feed for food-producing animals (in particular commercial chicken feed) as well as specifically developed insect feed are used. In Thailand, the growing insect production sector uses chicken feed or feed manufactured specifically for crickets and co-products from production of vegetables (Durst and Hanboonsong, 2015). In addition, a wide range of bio-waste products as well as animal manure are readily available and used as substrates for insect rearing in some countries and the organic materials are typically composted or heat treated before use. In Kenya, rearing of insects has been included in an overall waste management project, where human manure after composting is applied as substrate for rearing of insects (*Hermetia illucens*; black soldier fly) (Banks, 2014). A global overview of utilization of specific substrates is not available (van Huis et al., 2015; Vantomme, 2015).

Substrates considered within the assessment

The increased focus on insect rearing is very much based on the potential of insects to convert organic material of low quality into high quality food and feed. Therefore, there is an interest for potential use of types of organic materials as substrate other than those approved by the current legislation.

For the purpose of this opinion, substrates are categorized as follows:

A. Animal feed materials according to the EU catalogue of feed materials (Commission Regulation (EU) No 68/2013) and authorized as feed for food producing animals.

B. Food produced for human consumption, but which is no longer intended for human consumption for reasons such as expired use-by date or due to problems of manufacturing or packaging defects. Meat and fish may be included in this category.

C. By-products from slaughterhouses (hides, hair, feathers, bones etc.) that do not enter the food chain but originate from animals fit for human consumption.

[6] Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29, 30.1.2013, p. 1–64.

[7] Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). OJ L 300, 14.11.2009, p. 1–33.

D. Food waste from food for human consumption of both animal and non-animal origin from restaurants, catering and households.

E. Animal manure and intestinal content.

F. Other types of organic waste of vegetable nature such as gardening and forest material.

G. Human manure and sewage sludge.

For some of the substrates it may be relevant to distinguish between materials of ruminant vs. non-ruminant origin when assessing the risks in relation to the production of insects for feed.

It is stressed that the categorization described above is only relevant as a tool for this opinion and does not reflect the applicable legislative framework in the EU.

3.2. Overview of the production chain

The general structure of the insect production chain is described in Figure 1. This includes farming of insects (Section 3.2.1) including harvesting and processing and utilization (Section 3.2.2) for the purpose of either feed or food.

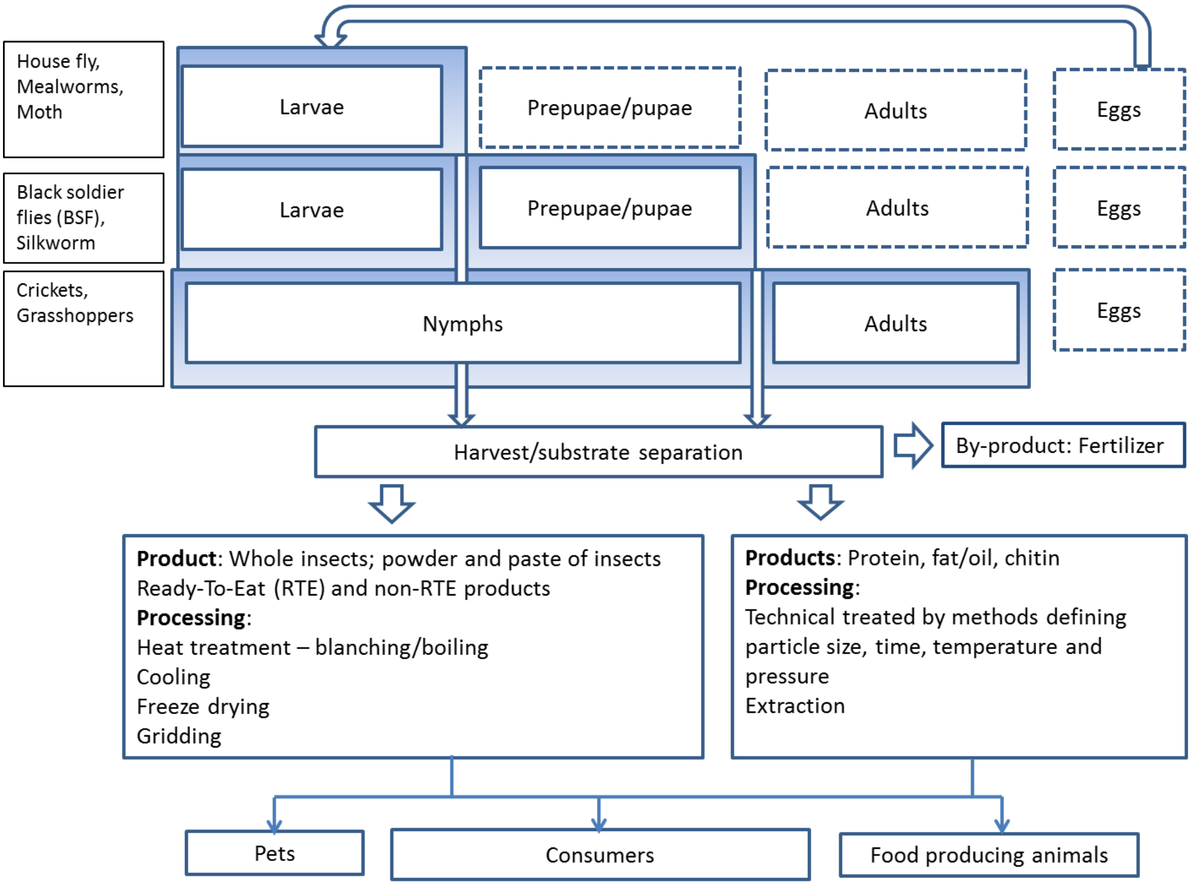

Figure 1: Overview of the chain from production to consumer. The main farmed species are grouped by the life-stage of harvesting. The shaded area in the lifecycle from larvae to adults indicates where exposures to feed substrates are carried over between early life-stages to the point of harvest.

3.2.1. Farming systems

Insect farming in EU is incipient and knowledge on existing and relevant farming and processing practices has been obtained from relevant stakeholders that were invited to provide information as hearing experts at a working group meeting (see Annex A). Thus, the information in Sections 3.2.1 and 3.2.2 below is based on this meeting rather than on practices described in the scientific literature.

Insect farming has the same characteristics as other animal production systems: The insects need access to water and feed (substrate) to supply energy and nutrients for growth and excrete intestinal content (frass). The production is impacted by the physical conditions (small scale/large scale, low or high level of technological management solutions etc.) and the level of biosecurity in place to prevent introduction of e.g. microorganisms from the surrounding environment (wild life, neighbouring animal farms, waste management units etc.).

In principle, there are no differences in the farming system regarding rearing of insects for feed or for food although they have to comply with different legislative frameworks. The existing differences among systems described by the hearing experts were related to different combinations of insect species and substrates applied and to the level of automation/industrialization of the farming (and processing) system.

In European insect farms, insects are kept in a closed environment, in boxes/cages, where the atmosphere, substrate, water etc. can be controlled. The hearing experts present at the meeting of the working group informed that no hormones, antibiotics or chemicals are used for the existing insect farming systems except biocides for disinfection of the production environment in between batches of insects. However, in intensive insect production systems, antibiotics may be used to treat or prevent diseases, as for example in the case of apiculture.

Species produced for harvest as larvae are grown on a substrate in which the animal waste/frass is also excreted. The physical conditions and time for developing the eggs to be harvested as larvae depend on the species. Eggs are introduced onto the substrate either manually or mechanically or by natural oviposition directly from adult flies. The larvae are maintained on the substrate for 1–2 weeks depending on species and also temperature. For instance, it takes 8 to 10 weeks at a temperature of 28–30 °C and 60 % relative humidity for mealworms (*Tenebrio molitor*) and lesser mealworms (*Alphitobius diaperinus*) to reach the size for harvest (NVWA, 2012; cited in FASFC, 2014). For the black soldier fly (BSF; *H. illucens*), the time will be approximately 12 days.

Harvesting of the larvae of the farmed species is in general characterized by a need for an active process to separate the larvae from the substrate. In less industrialized farming systems, this can be done manually by sieving whereas for industrialized systems, automatic systems of different types for the harvesting process have been developed. In less industrialized farming systems, the food substrates are often removed from the insects hours/day(s) before harvest in order to let the insect empty their intestinal tract. This procedure is not always included in the more industrialized farming systems where the insects normally undergo extensive processing after harvest.

Crickets and locusts/grasshoppers are produced to the adult stage before harvest. The production is a two-step production system, one for egg laying and hatching and one for growth to harvesting size. Cricket species are mainly for human consumption and live for pet food. The commercial production systems are characterized by a production phase for egg laying, hatching and growth of nymphs, which are transferred to grow out containers where the adult crickets are raised to harvest size. The overall production time is 3–4 months. During the growth period the adult crickets actively move for feeding and drinking. Substrates and waste can largely be kept separated. Substrates are sometimes totally removed before harvesting for gut emptying.

Insects (e.g. crickets) in the adult stage move around, which is reflected by different areas in the cages for feeding and for excretion of waste. This natural behaviour gives a physical separation of the insects and the waste enabling harvest of the insects leaving the waste/frass in the production by picking up the insects. In larger farming systems, harvesting can also be done by a more or less automatic sieving procedure.

After harvest, the insects may or may not be killed before further processing. As an example, freezing has been mentioned by the hearing experts for killing of crickets and mealworms.

In addition to the production of insects either as whole insects and products thereof or as raw material for the production of protein, fat/oil and chitin, these farming systems will also generate a by-product in terms of insect excreta and remains of substrates. According to the hearing experts, this by-product is used and marketed as fertilizer for plant production (see section on environmental risks).

3.2.2. Manufacturing of insects to insect products

Processing is an important part of food or feed manufacturing. One aim of processing food and feed ingredients is to reduce health risks. It is common practice in the feed industry to apply heat and pressure to compound feed which reduces the risk of viable pathogens entering the food chain. Similarly, fractionation of food and feed ingredients ensures more effective risk management strategies to be employed.

In general, there are three different ways of distributing insects for human or animal consumption; as whole insects, as whole insects processed into e.g. a powder or paste, and as an extract such as a protein isolate, fat/oil or chitin. As shown in Figure 1, the processing steps will differ among production systems depending on the kind of products produced.

Whole insects

Where whole insects are distributed for use in food and feed it is understood that some processing will have taken place before distribution. Blanching, chilling and drying are the most common processes that have been encountered in this respect each with the aim of extending shelf life and also reducing microbial load. It is also understood that labelling of whole insects aims to advise the consumer of appropriate 'in home' practices that are aimed at reducing risks. This may include instructions for cooking and 'wash before use' types of message. In some cases, specific parts of the insect are advised to be removed such as the wings and legs of crickets, to improve the eating experience and reduce choking risks. These practices are broadly in line with those for other foods such as preparation instructions provided with poultry and generally accepted practices when eating shellfish, such as peeling prawns.

Insect meal/paste

Insects may be prepared into ground powders or pastes by milling either after drying (powder) or milling when frozen or without treatment (paste). These products are then further used as ingredients in food or feed. In both cases, the final products are largely intended for use in further processed food/feed.

Fractionation

Many methods for fractionating insects are conceivable and this approach is increasingly common within the emerging insect production industry. However, precise details of the processes used are difficult to obtain as these (combined with insect rearing practices) are the intellectual property of the manufacturing companies. Mechanical separation and solvent extraction is used for the production of fractionated insect products. An example of this may be the mechanical separation of large insoluble chitin particles from ground insects using water or steam extraction technologies. Within the same process line or otherwise, organic solvent extraction using for example hexane will allow the separation of fats/oils from protein. More advanced technologies such as super critical fluid extraction using CO_2 or microwave assisted accelerated solvent extraction are all feasible technologies for the large-scale production of protein and fat isolates. A major goal of the industry appears to be the development of solvent-free protein/fat extraction technologies.

3.3. Consumption of insects and products thereof

3.3.1. Consumption by humans

Insects can be considered as valuable sources of protein. Because of their high content of protein/amino acids and other nutrients (see Appendix A), they could be an alternative to traditional food of animal origin, such as milk, meat, fish and eggs in human nutrition (Shockley and Dossey, 2014).

Insects for human consumption have received increasing attention during the past years, but the current contribution to the total food intake in Europe is still limited. There are no systematically collected data available on insect consumption in European countries. National consumption surveys are conducted based on national priorities and methodologies, and by the use of national food composition tables. EFSA included insects as a food category into the standardised food classification and description system called FoodEx2[8] that is used for the identification and characterisation of food in order to provide a common link to all the diverse food databases. However, no data on consumption of insects as food (FoodEx2 code A02LQ) existed in the EFSA Comprehensive European Food Consumption database until the end of 2014. National consumption surveys do not distinguish insects as a food option. Also the global database on food supply hosted by FAO (FAO, 2015) does not include insects as a food item. Worldwide, the most rapid increase in commercial availability of farmed insects has occurred in South-East (SE) Asia by the development of a cricket farming sector, centred in Thailand and spreading to neighbouring Laos and Cambodia. Also food consumption data in Thailand do not distinguish edible insects. Some insects are included in national food composition tables in SE Asia and in some African countries. The food composition unit at FAO in Rome, INFOOD, has compiled a list of nutrient composition of indigenous foods in various countries which includes a number of insects.

At present, the pattern of consumption may only be estimated through sales data of insect products. The data can only be available from the producers or the retailers as long as insects are not covered by national or international food supply data. Consumption pattern based on media coverage and other communications is that insects mainly have gained a position in some countries as a snack. Insects have also gained attention in menus of high-cuisine restaurants. How and to what extent the inclusion of insects in gastronomy can impact the general consumption pattern in the population is unclear but holds the potential for a rapid change in future consumption patterns. Surveillance of insect consumption in national consumption surveys would support documentation of changes in the pattern of intake.

Once insects may be approved for human food, the hearing experts anticipated that consumption of insects and products thereof could increase significantly, especially among future generations. This is based on considerable interest of consumers and a growing number of small emerging companies that want to market insects or processed foods with insects.

Concluding remarks

At present, it is not known whether insects and/or insect based foods might bear nutritional advantages or disadvantages with regard to an adequate supply of nutrients (amino acid and fatty acid profiles, micronutrients) or if insects have any at present unknown negative nutritional properties which e.g. can impair nutrient absorption or interfere with digestive processes if consumed in substantial amounts.

3.3.2. Consumption by food producing animals and pets

There is currently a lack of consolidated information relating to the magnitude and frequency of feeding of insects to farm animals. Whilst the feeding of farmed insects to livestock in Europe is currently restricted by legislation which make it economically and legally challenging to do so, information provided by relevant stakeholders that were invited to provide information as hearing experts at a working group meeting and through on-going research indicates that fly larvae and mealworms are being fed particularly to chickens and fish in Africa, China and other parts of Asia such as Thailand.

According to the nutritional composition (see Appendix A) and feeding studies, insects can be a component of the feed in the same way as other protein sources such as fish meal or soya. Insects would not replace 100 % of traditional feed ingredients, but make up a proportion of the feed (see Appendix A – nutritional composition).

Some early papers (e.g. Ravindran and Blair, 1993) reviewed studies conducted on insects in animal feed and recent publications have followed up with summaries on animal feeding studies with insects

[8] http://www.efsa.europa.eu/it/datex/datexfoodclass.htm

(e.g. Veldkamp et al., 2012; FAO, 2013; Makkar et al., 2014; Riddick, 2014; Sanchez-Muros et al., 2014).

Some results of feeding studies are summarised under special consideration of safety of food and feed of animal origin. Most of the studies were done in developing countries, mainly by using bio-waste and animal manure as substrates for growth.

Feeding studies with laboratory animals demonstrated the nutritive value of insect protein. Finke et al. (1989) fed mormon cricket (*Anabrus simplex*) and house cricket (*Acheta domesticus*) to weaning rats and evaluated the response (weight or nitrogen gain) as a fraction of nitrogen intake. They found that these protein sources were equivalent or superior to soy protein as a source of essential amino acids, respectively. Amino acids found in relatively low amounts in insects compared to fishmeal and soybean are histidine, lysine and tryptophan (Sanchez-Muros et al., 2014).

Goulet et al. (1978) fed mealworms (*T. molitor*) to rats and found similar data as measured with soybean meal.

Insects are natural feed sources for poultry and to a certain extent, also for pigs. Feeding studies have been carried out in different animal species. Insects and processed insects are used as complementary feed sources for poultry in developing countries (Ravindran and Blair 1993). Termites (*Kalotermes flavicollis*) have reportedly been used as feed for chickens and guinea fowl in some African countries (Iroko, 1982; Farina et al., 1991). In previous studies, dried black soldier prepupae (*H. illucens)* and mealworm larvae (*T. molitor*) were fed to chicks (Hale, 1973; Ramos-Elorduy et al., 2002) and pigs successfully (Newton et al., 2005; Gamboa, 1997).

When given a choice, pigs did not discriminate against a diet containing ground soldier fly larvae (*H. illucens*) compared with soybean meal (Newton et al., 2005).

Some authors replaced under field conditions soybean meal by black soldier flies (*H. illucens*) and housefly pupae (*Musca domestica*) in poultry diets (e.g. Ravindran and Blair, 1993).The same authors replaced fishmeal with by-products of silk manufacturing in diets for layers or chickens. Furthermore, grasshoppers (*Acrida cinerea*) and mormon crickets (*Anabrus simplex*) were also able to replace fishmeal and soybean meal in poultry diets (Wang et al., 2007).

Munyuli Bin Mushambanyi and Balezi (2002) replaced meat meal, a 20 % feed ingredient in poultry farming, with flour derived from cockroaches (*Blatta orientalis*) and termites (*K. flavicollis*) and did not observe any negative effect. Similar results are described by Ramos-Elorduy et al. (2002). The authors replaced soybean meal by 0, 5 or 10 % dried larvae of mealworms (*T. molitor*) grown on low-nutritive waste products in broiler diets and did not observe any negative effect.

Similar results were described by Ramos-Elorduy et al. (2002) with other insects in broiler feeding (e.g. *A. simplex, A. domesticus, Bombyx mori, A. diaperinus, Tribolium castaneum, K. flavicollis*), but also by other authors (DeFoliart, 1982, Finke et al., 1985; DeFoliart et al. 1987; Nakagaki et al., 1991; Sonaiya, 1995) and by Despins (1994) in growing turkey poults.

Larvae of the common housefly (*M. domestica*) contain 54 % crude protein in dried matter and could successfully replace fishmeal in broiler diets (Teguia et al., 2002; Awoniyi et al., 2004; Hwangbo et al., 2009). Ijaiya and Eko (2009) replaced in isocaloric and isonitrogenous diets (3000 kcal/kg and 20 % crude protein) 25, 50, 75 and 100 % of fishmeal by silkworm meal in broiler feeding and did not find any significant effect on body weight gain, feed intake, feed conversion, slaughtering results and meat quality. Similar results are reported by Kumar et al. 1992) in broilers and Aduku et al. (1991) in breeding and Ijaiya and Fasanya (1999) in growing rabbits.

Makkar et al. (2014) reviewed studies with various insect products and found 24 feeding experiments (17 from Africa, four from Asia and three from the US) with broilers fed with various portions of house fly larvae meal (maggot meal). Twelve broiler studies (9 from India) are described with silkworm meal. Fishmeal, soybean meal and groundnut cake could be successfully replaced up to 100 % with insect protein.

Aquaculture is the fastest-growing animal-food producing sector and sustainable expansion of this sector is required to meet the increasing demand for fish (FAO, 2012). Insects are natural feed sources for many fish species. Insect protein is a suitable replacement for fishmeal in diets for juvenile fish and crustaceans (Riddick, 2014; van Huis et al, 2015). Insects in the form of meal or pellets can provide adequate protein to replace standard fishmeal in feed for omnivorous fish such as carp and catfish. However, in carnivorous fish such as salmon and trout, only some of the fishmeal can be replaced by insect products (Riddick, 2014).

There are several reports (Riddick, 2014; FAO, 2013) which proposed to use insects as protein sources for various animal species, mainly for fish. The most promising species for feed production are larvae of black soldier flies (*H. illucens*), common housefly larvae (*M. domestica)*, silkworms (*Anaphe panda*), yellow mealworms, grasshoppers (*Acridids* spec.) and termites (*K. flavicollis*). These and other insects were successfully fed to rainbow trout (Ramos-Elorduy et al., 1989; St-Hilaire et al., 2007; Danieli et al., 2011), red sea bream (*Pagrus major*) (Iwai et al., 2015) and catfish (Ng, 2001; Pimentel et al., 2004) and replacing a proportion of fishmeal and fish oil in the feed. Sealey et al. (2011) replaced 25 and 50 % of dietary fishmeal with two sources of black soldier fly prepupae (*H. illucens*) in feed of rainbow trout and found a lower weight gain in some cases, but no influence on sensory parameters. Lock et al. (2015) replaced up to 100 % of the fish meal with two different insect meals in feed for Atlantic Salmon (*Salmo salar*) for 15 weeks, and found that fish fed one insect meal performed as well as the fish raised on a fishmeal-based diet, whereas fish fed the other insect meal did not.

Makkar et al. (2014) analysed in their review studies with catfish, tilapia, rainbow trout, Atlantic salmon, turbot and crustaceans, where fishmeal was replaced by dried black soldier fly larvae meal, housefly maggots, dried mealworms, locust meal, grasshoppers or silkworm pupae meal. Most studies demonstrated that about 50 % replacement of fishmeal had no adverse effect on animal performances.

Insects are fed to various pets in living form or prepared in different ways. Crickets are an important food source for many insectivorous reptiles and amphibians, but the calcium content is too low to meet the requirements of these animals (Allen and Oftedal, 1989; Barker et al., 1998; Finke, 2002).

Concluding remarks

Nutritional data indicate that insects provide protein similar to soybean meal and fishmeal and feeding trials show that insect products may partially replace traditional protein sources in animal feeding.

4. Risk profile of insects used as food and feed

4.1. Microbiological hazards

There are two types of microbiota to be considered as potential hazards in insects for food and feed, those that are intrinsically associated with insects as part of their life style and those that are introduced during farming and processing and carried forward.

The microbiota (including bacteria, viruses, fungi) present in the gut of the insects is essential for the metabolism, behaviour and survival of the insects. This microbiota is normally a reflection of the lifestyle of insects in the wild as well as under rearing conditions. The gut content passage time varies depending on the species of insects and their kind of diet, e.g. up to 24 hours in drosophila (El-Tabey, 1951). Insects are processed both as food and feed with their intestinal content, and even if the intestine is emptied before harvesting, frass will remain in the substrate and can contaminate the insects. Some of the microbiota may become pathogenic to the insects under stress circumstances. Also, like other animals, insects will have a microbiota on their surface and some of these are pathogenic to insects. The questions here are whether any of these microbes (including viruses) are pathogenic to other than insects, for example, to humans and animals and, if so, whether they could be transferred through food and feed containing insects or products thereof.

4.1.1. Bacteria

Specific studies on the microbiological safety of insects specifically reared or harvested for food or feed production are rare in the scientific literature.

The microbial flora of insects is composed of bacteria of different genera: *Staphylococcus, Streptococcus, Bacillus, Proteus, Pseudomonas, Escherichia, Micrococcus, Lactobacillus* and *Acinetobacter* (Agabou and Alloui, 2010; Amadi et al., 2005; Braide et al., 2011; Giaccone, 2005).

Pathogenic bacteria of insects (entomopathogenic) are regarded as harmless to animals and humans due to the fact that the hosts are so phylogenetically different (FAO, 2013). Bacterial hazards (and their toxins) for humans and animals related to insects will therefore mainly originate from a residential microbiota (natural or accidental) related to the rearing conditions (substrates and feed), handling, processing and preservation (ANSES, 2015). Many pathogens are used in biocontrol of insect pests and are either generally regarded as safe (GRAS) in North America or are QPS (qualified presumption of safety) in Europe, when intentionally added to food or feed for this purpose (Sundh et al., 2012; Leuschner et al., 2010). Those, that are not, are tested specifically and individually for the presence of toxins or other metabolic compounds and their safety for humans and animals prior to use. Safety of the use of invertebrate pathogens is an intrinsic part of the registration of these agents as biocontrol agents prior to commercialization (Eilenberg et al., 2015).

In fresh, farmed insects (*T. molitor*, *A. domesticus* and *Brachytrupes* sp.), spore-forming bacteria and *Enterobacteriaceae* were isolated (Klunder et al., 2012). The results suggest that substrate and insect species do influence gut microbial composition. Substrate and taxonomy did not, however, uniformly influence gut bacterial profiles across all insect guilds analysed here. Of the different host orders analysed, hymenopterans, termites and, to a lesser extent, beetles were significantly similar within their group of species (Colman et al., 2012).

Recently both in Belgium and in the Netherlands, risk assessments have been developed concerning the farming of insects for food production. The report from the Scientific Committee of the Belgian Federal Agency for the Safety of the Food Chain (FASFC, 2014) provides some data about the microbiological status of insects specifically farmed for food production. High values of 10^7 cfu/g for the total aerobic bacterial count, but also for the total anaerobic bacterial count and *Enterobacteriaceae* were found in a preliminary Belgian study on mealworms (*T. molitor*), locusts (*Locusta migratoria*) and morio worms (*Zophobas atratus*). Lower values (less than 10 cfu/g) for *Enterobacteriaceae* were measured on raw silk worms (*B. mori*). In another exploratory Belgian study on raw and frozen mealworms and locusts, similar high values (10^7 – 10^9 /g) for the aerobic bacterial count and 10^4 cfu/g for the aerobic spores were measured.

In the risk assessment from the Netherlands, the results of a small-scale survey on the microbiological status of 55 insect products (locusts, lesser mealworms, mealworms and a mealworm snack) that had undergone no treatment apart from freeze-drying, are reported. In this study it was found that 59 % of the insect products tested exceeded the process hygiene criterion for aerobic bacteria in raw materials used in meat preparation (10^6 cfu/g), while the concentration of *Enterobacteriaceae* in 65 % of the samples exceeded the criterion for raw materials used in meat preparations (10^3 cfu/g). The study investigated the presence of *Clostridium perfringens*, *Salmonella* and *Vibrio* and none of these were detected. In 93 % of the samples, the concentrations of the spore-forming bacterium *Bacillus cereus* were less than 100 cfu/g (NVWA, 2014).

There is more literature concerning the ability of insects to act as vehicles of known zoonotic pathogens like *Salmonella*, *Campylobacter* etc. in farmed animals, providing information about their presence, prevalence, and survival ability in different insect hosts. The subjects generally investigated are beetles, cockroaches and flies.

Although zoonotic pathogens are found in the substrates used to grow insects, active replication of the pathogens in the intestinal tract does not seem to happen in insects, while it is widely demonstrated in farmed animals, such as poultry or pigs, where bacteria such as *Salmonella* have their niche.

Campylobacter is easily isolated from insects in contact with infected poultry, as in flies which can also be vectors of infection for healthy poultry (Strother et al., 2005; Templeton et al., 2006; Wales et al., 2010). However, from experimental conditions it has been suggested that *Campylobacter* can survive only for a short period in insects (Hazeleger et al., 2008; Strother et al., 2005; Templeton et al., 2006).

The role of arthropods also as vectors of *Salmonella* is clearly demonstrated in many studies (Agabou and Alloui, 2010; Davies and Breslin, 2003; Goodwin and Waltman, 1996; Holt et al., 2007). Insects such as flies can easily acquire and spread *Salmonella* (Holt et al., 2007; Leffer et al., 2010; Roche et al., 2009) also as far as serovars relevant for public health are concerned, such as *Salmonella Enteritidis* (Leffer et al., 2010).

The feeding of contaminated larvae *of Lucilia sericata* to chickens has been shown to be a potent means of establishing *Salmonella* infection (Davies and Wray, 1993). Houseflies (*M. domestica*) are commonly found to be carrying *Salmonella* in cage layer houses, and have also been demonstrated to undergo transovarian transmission (Davies and Breslin, 2003).

Houseflies (*M. domestica*) released into rooms containing hens challenged with *S. Enteritidis* rapidly became contaminated. Feeding contaminated flies to hens resulted in gut colonization of a third of the birds, but release of contaminated flies in a room containing previously unchallenged hens failed to result in colonization of any of the subject birds. These results indicate that flies exposed to an environment containing *S. Enteritidis* can become colonized with the organism and might serve as a source of transmission of *S. Enteritidis* within a flock situation (Holt et al., 2007)

Different studies have therefore demonstrated the role of insects in maintaining the presence of zoonotic agents, such as *Salmonella* and *Campylobacter*, in infected farms, mostly in poultry, but in these studies the pathogens were re-isolated from insects for only a few days after challenge, indicating that, for the flies to remain positive over time, they needed a re-exposure (Greenberg et al., 1970; Holt et al., 2007; Shane et al.; 1985). Kobayashi et al. (1999) challenged houseflies with *E. coli* O157, and showed that the ingested bacteria were harboured in the intestine of flies and continued to be excreted for 3 days after feeding. No *E. coli* O157 was detected in the alimentary canals of the flies four days after challenge.

Re-exposure could occur, in farmed insects, as a consequence of the contamination of the substrate used which can contain pathogens and allow their multiplication to an extent which depends on the characteristics of the substrate itself. Contaminated substrates could be treated before use, but the risk of recontamination should be considered.

Antimicrobial resistance

Antimicrobial resistance is a natural and widespread phenomenon, which is amplified by the use of antibiotics in all the sectors involved: human medicine, agriculture, animal husbandry. All organisms that can cause disease in humans and/or animals (bacteria, viruses, fungi, parasites) reveal a remarkable ability to adapt, to evolve and to survive by developing resistance to each and every therapeutic compound administered (Acar and Moulin, 2012). As far as the bacteria are concerned, both pathogens and non-pathogens are submitted to the same selective pressure when antimicrobials are used, and they can develop resistance to all classes of drugs.

A recent and comprehensive report by ECDC, EFSA and EMA (2015) has assessed associations between the consumption of selected combinations of antimicrobials and the occurrence of resistance in bacteria. Such associations were observed for most of the combinations addressed in humans and animals, even if the epidemiology of resistance is complex, and several factors aside from the amount of antimicrobial consumption influence the level of resistance. In insect farming, the use of antimicrobials is reported in the scientific literature for emergency treatment in case of diseases caused by bacteria, fungi or microsporidia which can seriously damage farmed insects (Eilenberg et al., 2015).

In mass-rearing of silkworms on an artificial diet, diseases from bacterial infections are frequent causes of total loss of cocoon harvest, which can be avoided through supplementation of artificial diet with antibiotics. For the latter, one of the most used antibiotics which can be added to artificial diet is chloramphenicol, which has been used for many years in sericulture, both in rearing on the leaves and on the diet to counteract epidemic outbreaks (Cappellozza et al., 2011).

Finally, some authors report the use of antimicrobials in order to accelerate nymphal development, increase survival and adult longevity in insects such as *Nezara viridula* (L.) (*Heteroptera*: Pentatomidae), concluding that streptomycin has potential in rearing *N. viridula*, especially in improving quality of field-collected adults, by mitigating the introduction of pathogenic bacteria, and improving the quality of the population (Hirose et al., 2006).

Owing to these considerations and the possibility for development of antimicrobial resistance, bacteria monitoring and control of the use of antimicrobials in insect farming is recommended.

Concluding remarks

Studies on the occurrence of human and animal bacterial pathogens in farmed insects used as food and feed are very scarce in the scientific literature.

Pathogenic bacteria (such as *Salmonella*, *Campylobacter* and verotoxigenic *E. coli*) may be present in non-processed insects depending on the substrate used and the rearing conditions. Most likely, the prevalence of some of these pathogens for example campylobacter, will be lower compared to other non-processed sources of animal protein, since active replication of the pathogens in the intestinal tract does not seem to happen in insects.

Furthermore, the risk of transmission of these bacteria could be mitigated through effective processing.

4.1.2. Viruses

Insects contain a plethora of viruses and many of these are pathogenic to insects, i.e. they cause disease and may lead to mortality and colony collapse (Eilenberg et al., 2015; King et al., 2012). Most viruses in insects are specific at the family or species level and are therefore only pathogenic for invertebrates (Table 2) and not for humans or other vertebrates such as farm animals and birds. However, these insect-specific viruses are a major concern to producers farming insects for food and feed since they can cause loss in production (Eilenberg et al., 2015). All of these insect-specific viruses used for insect biocontrol are considered safe for vertebrates including humans and include those that are intentionally added to food or feed crops (Gröner, 1986; Laird et al., 1990); Leuschner et al., 2010; Sundh et al., 2012).

Of special note is that some of these insect viruses have taxonomically related viruses in vertebrates (Table 2) (King et al., 2012), such as poxviruses, parvoviruses (B19), picornaviruses (e.g. polio, foot-and-mouth disease), orthomyxoviruses (influenza), rhabdoviruses (rabies) and reoviruses (diarrhoea virus) in mammals and iridoviruses in fish and amphibians. However, these taxonomically related viruses remain restricted to vertebrates. These vertebrate viruses do not replicate in insects and are not actively transmitted by insect vectors to vertebrates. Therefore, these viruses are not further considered in this report.

Members of a few virus families with representatives in insects (Iridoviridae, Parvoviridae, Iflaviridae, Dicistroviridae and Reoviridae) require further assessment, as they may be present in insects related to farmed insect species for food and feed (Table 2). Vertebrate iridoviruses, only occurring in fish and amphibians, do not infect insects (and vice versa), whereas reoviruses of insects are very different in structure and pathology from the vertebrate taxa of these families. There are no reports of invertebrate iridoviruses and reoviruses in vertebrates. The most frequent infection in crickets is caused by densoviruses (Parvoviridae) (Weissman et al., 2012; Szelei et al., 2011) and by viruses of the order Picornavirales (cricket paralysis virus, Discistroviridae). Since both virus types have close relatives in humans (human parvovirus B19, polio, hepatitis A), the question is whether these viruses can cross the vertebrate/invertebrate border. Experiments using densovirus of crickets have demonstrated that this virus, although taken up, cannot replicate in vertebrate cells, which are a proxy for virus infection in vertebrate organisms (El Far et al., 2004). The same is true for entomopoxviruses (Li et al., 1997). There are no reports of other viruses from these taxa (genus Densovirus, family Dicistroviridae) infecting vertebrate hosts.

In some cases, however, insects are replicative vectors of viruses infecting vertebrates. These arthropod-borne viruses are called arboviruses and they cause disease in humans (such as dengue, West Nile disease, rift valley fever, haemorrhagic fever, Chickungunya) or farm animals (e.g. Schmallenberg) (King et al., 2012). They also successfully replicate in their vectors, for instance mosquitoes, midges or ticks. As such, these viruses can cross the species barrier and replicate effectively in both vertebrates and invertebrates. However, there is no evidence that such viruses occur in insects used for food and feed (Table 2).

Insects can also be passive vectors of human and livestock viral diseases. Sawabe et al. (2006) suggested that blowflies carrying highly pathogenic avian influenza H5N1 were responsible for the outbreak of avian influenza in Japan in 2004. Nielsen et al. (2011) reported that low pathogenic avian influenza H5N7 and H7N1 remained viable in the digestive tract of houseflies. There is a single reported case of passive transfer of the high pathogenicity avian influenza (H5N1) by the housefly *M. domestica*. Artificially inoculated houseflies were able to transmit the virus and cause disease in chicken under experimental feeding conditions (Wanaratana et al., 2013). In this case, the virus does not replicate in its invertebrate host, but is a 'passenger', the host being a mere carrier of the virus. These observations highlight the potential of insects produced for food or feed to transmit vertebrate viral diseases.

Two contrasting studies are available on the survival of animal viruses in animal manure, substrate of *A. domestica* culturing (Lalander et al., 2015; Wei et al., 2009). *A. domestica* flies were grown on pig manure and human faeces in closed plastic bins for about 4 weeks, when the manure/faeces was inoculated with reovirus type 3, canine adenovirus 1 and porcine enterovirus 1. At various times after inoculation, the fermented manure/faeces was dried at 105 °C) for 14 h and stored at -70 °C until analysis (Lalander et al., 2015). After 14 days, the virus concentrations were below detection level, suggesting that these viruses did not survive their physical environment and/or treatment (heating). Unfortunately, the transmission potential of these viruses via *A. domestica* was not assessed. Wei et al. (2009) added human adenovirus 41 to poultry litter, dairy and swine manure and bio-solids before storage at 20 and 4 °C, respectively. These authors analysed for up to 60 days for the presence of Ad41 infectivity. At both temperatures, Ad41 was reduced about 2 logs in infectivity in 20–40 days, but a low concentration of viruses remained even beyond 60 days.

Although the literature is still inconclusive on the potential survival of vertebrate viruses in insects, it remains possible that these viruses can be passively transmitted by flies. This is a potential hazard, when flies are reared on adenovirus-contaminated manure/faeces, but depends on the ability of the virus to survive and be maintained in the fly over longer periods of time and their sensitivity for downstream treatment. Wei et al. (2010), using a similar approach, found that known food-borne viruses such as norovirus and hepatitis A virus could survive in untreated manure and litter for at least 60 days at both 20 °C and 4 °C.

The vertebrate viruses discussed above, are non-enveloped (no lipoprotein membrane, only proteins plus genetic information). Viruses with a lipid envelope, such as some arboviruses (vertebrate viruses that are transmitted by insects), such as West Nile, Chikungunya, Dengue, and influenza viruses, are less likely to survive the processing of the insects for food and feed, as they are very fragile and quickly inactivated. However, most foodborne viruses mentioned above are non-enveloped, which provides more stability to the virus, and they may escape manure treatment and insect processing practices.

There is also a lack of information relating to the likelihood of human viruses such as norovirus, rotavirus, Hepatitis E and Hepatitis A being passively transferred from feedstock through residual insect gut contents. Processing technologies and cooking practices may help to reduce risks of passive transfer to an acceptable level (e.g. the proper cooking of bivalves such as mussels).

In conclusion, insect pathogenic viruses occurring in insects produced for food and feed are specific for insects and therefore are not regarded as a hazard for vertebrate animals and humans. The current collective evidence shows that viruses of vertebrates can survive in substrates and be picked up by insects produced for food or feed via the substrate. The key issue here is the risk of transmission. This risk could be mitigated through proper choice of substrate and effective processing.

Table 2: Viruses infecting insects and presence of vertebrate relatives.

	Genetic information					Vertebrate relatives	Comments
	dsDNA	**ssDNA**	**ssRNA(-)**	**ssRNA(+)**	**dsRNA**		
Poxviruses	**x**					**x**	rare in caterpillars
Ascoviruses	x						frequent in caterpillars
Asfarviruses	x					x	rare case
Baculoviruses	x						common in caterpillars
Bracovirus	x						common in wasps
Herpesviruses	x						rare case
Ichnoviruses	x						common in wasps
Iridoviruses	**x**					**x**	common in insects/ only in fish
Parvoviruses		**x**				**x**	in crickets
Bunyaviruses			x			x	arbovirus
Orthomyxoviruses			x			x	rare cases
Rhabdoviruses			x			x	mainly in aphids
Dicistroviruses*				**x**		**x**	in all insect species
Flaviviruses				x		x	arbovirus/ mosquito and tick
Iflaviruses*				**x**		**x**	in all insect species
Nodaviruses				x		x	only in fish
Tetraviruses				x			rare in caterpillars
Togaviruses				x		x	arbovirus/ mosquito and tick
Reoviruses					**x**	**x**	unique genus in caterpillars

Notes: Virus data from King et al., 2012; *picorna-like; bold/shaded: in species related to farmed insects.

4.1.3. Parasites

Information in the literature refers to non-European areas (mostly Asia) and to insects harvested in the wild, and so the risk can be very different from what is found in farmed insects, with strict control of environmental conditions and substrates applied.

The presence of parasites in insects is well documented in a review about foodborne intestinal flukes in Southeast Asia where the isolation of six different species from insects was discussed; in this area there is a long, widespread tradition of insect consumption. Evidence from human autopsies and insect analysis suggested the possible foodborne transmission of parasites (trematodes) belonging to the family *Lecithodendridae* and *Plagiorchidae* (Chai et al., 2009).

An important case in which insects show their potential as a biological vector is trypanosomiasis. The World Health Organization (2010) has estimated that about 10 million people are infected with Chagas in the Americas, two million of them in Brazil alone. More than 10 000 people die each year. Historically, transmission occurs predominantly in rural areas of Latin America, where poor housing conditions promote contact with infected vectors. Cases have been reported in the literature linking infection with the accidental ingestion of insects or consumption of contaminated food (Pereira et al., 2010).

The trematode *Dicrocoelium dendriticum* (family *Dicrocoeliidae*) is another parasitic zoonotic agent potentially infecting humans through insect consumption. The infection is due to the ingestion of ants containing metacercariae whereas pseudo-infections (presence of *D. dendriticum* eggs in stool in the absence of adult worms) are due to the consumption of infected animal liver (Jeandron et al., 2011).

Among potential foodborne and waterborne pathogens also Protozoa, such as *Entamoeba histolytica* and *Giardia lamblia,* have been isolated in cockroaches. These insects can also harbour *Toxoplasma* spp. and *Sarcocystis* spp.*,* but only for a limited time as demonstrated for *Toxoplasma* spp. in *Periplaneta americana* and *Blatella germanica* (Graczyk et al., 2005)*.*

Despite the documented occurrence of parasites in the insects in general and the linkage between sporadic human parasitic disease and insect consumption there are no data on the occurrence of parasites in farmed insects. A properly-managed closed farm environment would lack all the hosts necessary for the completion of parasite life cycles and proper management before consumption, relying on freezing and cooking, can further eliminate potential risks.

4.1.4. Fungi

Insects carry or are sensitive to entomopathogenic fungi. They produce insect-specific toxins causing mortality in insects. Some of these fungi are used as biocontrol agents of pest insects, often on vegetable crops for animal and human consumption. However, in the latter situation, these fungi have received no QPS (Qualitative Presumption of Safety) status at the species level, because of the lack of sufficient information on the effect(s) of the produced fungal toxins on humans and animals (EFSA BIOHAZ Panel, 2014). In order for a fungus to be registered as a commercial product, safety experiments, i.e. effects on animals and humans of a particular strain have to be done on each individual fungal species/variety. Occasionally, diseases associated with entomopathogenic fungi are seen in immunocompromised individuals (Roberts and St. Leger, 2004; Strasser et al., 2000; Jani et al., 2001; Goettel et al., 2001). In general, though, these insect-pathogenic specific fungi have a very good safety record both for vertebrate animals as well as for the environment (Zimmerman 2007a and b; Mudgal et al., 2013).

Insects may also be carriers of fungi and yeasts with potential hazards to animals and humans. Yeasts and fungi were found in considerable amounts in fresh, freeze-dried as well as in frozen insects (*T. molitor* and *L. migratoria*) (FASFC, 2014).

The importance of proper processing, handling, drying and storage was further stressed by a study from Botswana, after unacceptable levels of aflatoxins were documented in some commercial lots of mopane worms (*Gonimbrasia belina*: *Saturniidae*) (Schabel, 2010).

From the same species, dried under laboratory conditions, some fungi were isolated (Aspergillus spp., Penicillium spp.), among which are also mycotoxigenic species (Simpanya et al., 2000).

In general, any risk from fungi associated with insects produced for food and feed or introduced during farming, processing and storage could be mitigated by hygienic measures in the entire production chain.

4.1.5. Prions

Prion-related risks from insects could be potentially linked to three main issues: insect-specific prions, insects as mechanical vectors of animal/human prions, and insects as biological vectors of prions (i.e. involving replication of animal/human prions within insects).

Risks related to insect-specific prions

No genes encoding for a prion or prion-related protein have been reported in insects and prion proteins are hence not naturally expressed in insects (Post et al., 1999; Thackray et al., 2012). Therefore, it is expected that no specific prion diseases can develop in insects.

Risks related to insects as mechanical vectors of animal/human prions

Various studies suggested the possible role of insects (e.g. flies) as mechanical vectors of infectious prions.

Post et al. (1999) fed larvae of *Sarcophaga carnaria* (a meat-eating fly causing animal/human myiasis) with brains of scrapie-infected hamsters, and assayed larvae every two days for the presence of the pathological form of the prion protein (PrP^{Sc}). They demonstrated detection of PrP^{Sc} as soon as two days post-eating in living flies. Several days later, they did not detect PrP^{Sc} in living larvae, but detected PrP^{Sc} as late as 14 days post-eating in dead flies. They subsequently fed different groups of hamsters with inner organs of larvae and pupae and observed clinical signs and detection of PrP^{Sc} (by Western Blot) in 50 % or more of the hamsters of each group. The authors concluded that the contents of larvae and pupae from flies having eaten infectious hamster brains could transmit scrapie, and that prion diseases might be transmitted by flies in different development stages, even after death of the fly.

Lupi (2006) reviewed available scientific papers in relation to myiasis as a possible risk of human prion diseases, and discussed the possibility that ocular, cutaneous, intracerebral or spinal myiasis (caused for example by ectoparasites *Hypoderma bovis* or *Oestrus ovis*) could contribute to the dissemination of prions to animals and humans, due to ectoparasites acting as vectors of the agents. The author suggested that this possibility should be further investigated. Similar considerations were also expressed for the potential role of ectoparasites in the transmission of Chronic Wasting Disease (CWD) (Lupi, 2005). Corona et al. (2006) investigated the role of *O. ovis* in the pathogenesis of scrapie. The authors collected larvae from the nasal cavities of scrapie positive and negative sheep, and reported the detection of PrP^{Sc} in larvae of the parasites in three scrapie-affected sheep originating from two different scrapie outbreaks. They also observed a link between positive larvae and positive olfactory mucosa in sheep.

Risks related to insects as biological vectors of animal/human prions

As discussed above (risks related to insect-specific prions), given the absence of PrP-encoding genes in insects, prion proteins cannot be naturally expressed in insects. Therefore, mammalian prions cannot replicate in insects. Therefore, insects are not considered to be possible biological vectors and amplifiers of prions.

To explore the possibility of using insect models to study the transmission of mammalian prion diseases, Thackray et al. (2012, 2014a, b) investigated the transmission of ovine prions originating from brains of sheep affected with classical and atypical scrapie to the fly *Drosophila melanogaster*. The authors successfully transmitted ovine prions to different *D. melanogaster* models transgenic for ovine PrP (i.e. expressing the gene encoding for ovine PrP), observing accelerated neurotoxic effects compared to non-inoculated transgenic flies. Exposure of non-transgenic control flies to ovine prions did not result in neurotoxicity, arguing against transmission in wild-type flies. In one of these studies (Thackray et al., 2014a), proteinase K-resistant PrP^{Sc} was detected by protein misfolding cyclic amplification (PMCA) in transgenic *Drosophila* exposed to prions. Other studies reported expression of mammalian PrP in transgenic *Drosophila* flies (Bujdoso and Thackray, 2013; Fernandez-Funez et al., 2009, 2010; Gavin et al., 2006; Raeber et al., 1995; Sartori et al., 2010), but did not investigate transmission of prion diseases to flies.

The role of the substrates used on prion-related risks posed by insects

Since, replication of prions is not considered possible in insects, the total prion infectivity carried by insects would depend on the amount of infectivity present in the substrate used and can only be equal to or smaller than this.

The substrate used to feed insects strongly influences the possible risks posed by insects in the dissemination of prions. When assessing those risks, the different end-use of insects as food or feed should also be taken into consideration, since the risk posed to humans or animals can be different.

- With regard to insects farmed for use as food:
    - In general, the use of food- and feed-grade substrates (substrate groups A, B and D), animal manure and intestinal content (substrate group E) and organic waste of vegetable nature (substrate group F) should not pose any additional risk compared to other food.
    - The risk related to the use of other substrates (substrate groups C – by-products from slaughterhouses and G – human manure and sewage sludge) should be specifically evaluated. Some of this material is currently excluded from the food chain because of prion-related concerns (e.g. certain tissues from ruminants because of BSE-related risks), and material of human origin such as human manure may pose risks of transmission of human prion diseases.

- With regard to insects farmed for use as feed:
  - In general, the use of food- and feed-grade substrates of non-ruminant origin (subcategories of substrate groups A, B, C and D not containing products of ruminant origin), non-ruminant animal manure and intestinal content (subcategory of substrate E) and organic waste of vegetable nature (substrate group F) should not pose any additional risk compared to other feed.
  - The risk related to other substrates (substrate groups A, B, C D and E containing products of ruminant origin, and substrate group G) should be specifically evaluated. Some of this material is currently excluded from the feed chain because of prion-related concerns (e.g. certain tissues from ruminants because of the risk of spread of bovine spongiform encephalopathy (BSE) and other animal transmissible spongiform encephalopathies (TSE)), and material of human origin such as human manure may pose a risk of transmitting human prion diseases to animals (e.g. vCJD to cattle).
- When assessing the risks posed by certain substrates both for humans and animals as indicated above, the following aspects would influence the level of risk and should be considered:
  - the species from which the material used as substrate originates (e.g. the risk would be much higher for material of ruminant origin).
  - the tissues used as substrate (e.g. certain tissues from TSE-positive animals carry much higher levels of infectivity than others).
  - in the case of insects to be used as feed, the species of destination of the feed (e.g. sheep would be susceptible hosts to cattle BSE; and in general the risk would be much higher when the species, from which the substrate originates, coincides with the species of destination of the feed).
  - prior submission of the material used as substrate to thermal treatments (e.g. thermal hydrolysis can inactivate prions).

Concluding remarks

- Normal cellular prion proteins are not naturally expressed in insects. Therefore, no relevant risks exist in relation to insect-specific prions. For the same reason, mammalian prions cannot replicate in insects, and therefore insects are not considered to be possible biological vectors and amplifiers of prions.
- Various studies suggested the possible role of insects as mechanical vectors of infectious prions. Insects farmed on a substrate or in an environment in which infectious prions are present could act as mechanical vectors of infection and represent a potential risk of transmission of prion diseases through food and feed.
- The total prion infectivity carried by insects would depend on the amount of infectivity present in the substrate used and can only be equal to or smaller than this.
- In general, insects fed on substrates of non-human and non-ruminant origin should not pose any additional risk compared to the use of other food or feed, while the risk posed by insects fed on other substrates should be specifically evaluated.

4.1.6. Concluding remarks

The substrate used and the farming environment strongly influence insects' microbiota, and therefore the foodborne risk is influenced by the nature and the hygienic conditions of the substrate and the farming environment. In general, food and feed-grade substrates (substrates groups A, B and C as defined in Section 3.1.2), if maintained in good hygienic conditions, should not pose any additional risk when fed to insects as compared with other approved foods or feeds.

The risk related to other kind of substrates should be specifically evaluated, taking into account of the kind of treatment applied, which can minimize, as in the case of treatment with high temperatures, the microbial contamination. In this case, the possible presence of spore-forming bacteria, which can survive heat treatment, must be carefully considered, as well as the risk of cross-contamination after treatment.

The harvesting process may be of critical importance since it will impact the risk of transfer of hazards from the substrates to the insects and further on to the products thereof.

The risk of infection posed by human or animal consumption of insects and products thereof is modulated by a combination of the substrates used and the processing steps applied between farming and consumption.

4.2. Chemical hazards

Like products from other animals, insect derived food and feed products may contain hazardous chemicals. Some of these chemicals may be present in the substrates for insects, such as environmental contaminants, e.g. heavy metals (Lindquist, 1992; Merrington et al., 1997; Green et al., 2001; Vijver et al., 2003; Handley et al., 2007; Zhuang et al., 2009), organochlorines such as dioxins (Hunter et al., 1987; Jamil and Hussain 1992; Devkota and Schmidt 2000), polybrominated diphenyl ethers (Gaylor et al. 2012), mycotoxins and plant toxins. Insects may also contain elevated levels of trace elements e.g. selenium (Hogan and Razniak, 1991), which may accumulate in the insect from the feeding substrate. Other chemicals are likely to be used during rearing of insects, e.g. biocides to clean facilities and equipment or veterinary drugs to treat certain diseases. Furthermore, some insect species produce toxins (venoms) themselves.

When contaminants accumulate in insects reared for food and feed, they may pose a threat to animal and human health. Published data on hazardous chemicals in reared insects are, however, scarce. More data are available for insects collected in the wild. Reviews on the chemical safety of insect products have been published by food safety authorities including Belgium (FASFC, 2014), France (ANSES, 2015) and The Netherlands (NVWA, 2014) as well as by Belluco et al. (2013) and van der Spiegel et al. (2013). The following section gives a brief overview of hazardous chemicals that may be present in insect food and feed products.

4.2.1. Heavy metals and arsenic

Concentrations of heavy metals and arsenic in insects depend on the characteristics of the elements and their concentrations in the substrates, the insect species and their growth stage.

In a study of the chemical safety of farmed insects (Charlton et al., 2015) house fly (*M. domestica*), blue bottle (*Calliphora vomitoria*), blow fly (*Chrysomya spp.*) and black soldier fly (*H. illucens*) reared using a variety of substrates and production methods at different geographical locations were analysed for metals. Cadmium was present in some insect samples at concentrations that exceeded the maximum residue limit for complete animal feed, whereas this was not the case for the other metals measured. Table 3 presents the metal concentrations found in farmed insects, and gives the EU maximum limits for these metals in feed materials and complete feed. House fly larvae (five samples) contained cadmium concentrations ranging from 0.33–0.72 mg/kg, two of which were below the EU maximum limit for cadmium in complete animal feed (0.5 mg/kg 88 % dry matter), and three above (Charlton et al., 2015). However, the concentration of cadmium in the substrate was not measured and the maximum limit for cadmium is higher in complete feed for certain animal categories: 1 mg/kg complete feed for cattle (except calves), sheep (except lambs), goats (except kids) and fish; 2 mg/kg in complete feed for pet animals). There is limited data available regarding the influence of different substrates on the metal content in farmed insects. The EU maximum content for cadmium in feed materials of animal origin is 2 mg/kg (88 % dry matter); all of the nine insect samples analysed by Charlton et al. (2015) had concentrations below this limit.

Table 3: Concentrations of cadmium, lead, mercury and arsenic (mg/kg dry weight) in farmed insects (MD1 to MD5: house fly (*M. domestica*); CV1 and CV2: blue bottle (*C. vomitoria*); CH: blow fly (*Chrysomya spp.*); HI: black soldier fly (*H. illucens*) reared using a variety of production methods at different geographical locations.

Element	Feed material max. limit[(a)]	Complete feed max. limit[(a)]	MD1	MD2	MD3	MD4	MD5	CV1	CV2	CH	HI
Cadmium[(b)]	2	0.5	0.334	0.625	0.348	0.711	0.723	0.02	0.018	0.370	0.120
Lead[(b)]	10	5	0.46	1.16	0.249	0.058	0.333	<0.001	<0.001	<0.001	<0.001
Mercury[(c)]	0.1	0.1	0.004	0.035	0.038	0.002	0.004	<0.002	<0.002	0.008	0.007
Arsenic[(b)]	2	2	0.191	0.408	0.094	0.161	0.079	0.009	0.004	0.734	0.142

(a): EU maximum limit in feed material and complete feed for farm animals based on 88% dry matter.
(b): Commission Regulation (EU) No 1275/2013 of 6 December 2013 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for arsenic, cadmium, lead, nitrites, volatile mustard oil and harmful botanical impurities.
(c): Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

Source: Charlton et al. (2015) with supplementary information from the authors (available at: http://www.proteinsect.eu/index.php?id=63)

Cadmium accumulation in insects has previously been described by Diener et al. (2011) and uptake of cadmium and lead from different types of soil by *T. molitor* was documented by Vijver et al. (2003). Levels of cadmium and lead in edible insect larvae (*Rhynchophorus phoenicis*, snout beetle and *Analeptes trifaciata*, Rhinoceros beetle) bought at Nigerian markets were 0.02 – 0.03 mg Cd/kg dry weight and 0.03–0.06 mg Pb/kg dry weight, respectively (Banjo et al. 2010). EC maximum concentrations for cadmium in animal products range from 0.05 µg/kg wet weight for meat (excl. offal) of bovine animals, sheep, pig and poultry, to 1.0 mg/kg for kidneys, bivalve molluscs and cephalophods. For lead, these maximum concentrations range from 0.1 mg/kg wet weight for meat (excluding offal) of bovine animals, sheep, pig and poultry, to 1.5 mg/kg wet weight of bivalve molluscs and cephalopods (without viscera) (see EC/1881/2006).

Concentrations of cadmium and lead ranged from 0.02–0.07 mg/kg and from 0.03–0.10 mg/kg dry weight, respectively, for five different types of edible arthropods (Banjo et al., 2010). Dried grasshoppers (Chapulines) were suspected to be the cause of elevated blood lead levels in people from a community in California (Handley et al., 2007). The latter authors investigated sources of an outbreak of lead poisoning in Monterey County, California. They aimed to investigate risk factors for elevated blood lead levels (> 10 µg/dL) among children and pregnant women in three county health department clinics between 2001–2003. Home-prepared dried grasshoppers (chapulines) sent from Oaxaca (Mexico) were considered to be one source of lead poisoning. These chapulines contained significant amounts of lead. From the total number of 10 chapuline samples originating from Zimatlan, Oaxaca, a large variation in lead concentrations was seen, from not detected to levels as high as 2 500 mg/kg (Handley et al., 2007).

Bogong moths (*Agrotis infusa*), historically a traditional food item in Australia, were shown to take up arsenic from arsenic polluted soils (Green et al. 2001) and selenium has been shown to accumulate in *T. molitor* (Hogan and Razniak 1991). Aphids (*Sitobion avenae*) feeding on wheat grown in soils fertilised with sewage sludge containing cadmium and zinc, accumulated these metals (Merrington et al., 1997). Aphids contained cadmium and zinc levels up to 0.386 mg Cd/kg and 319 mg Zn/kg zinc, which was eight and ten, respectively, times higher than the concentrations in the wheat (Merrington et al., 1997). Cadmium bioaccumulation was also observed in grasshoppers, *Calliptamus italicus*, *Oedipoda caerulscens*, *O. germanica*, and *Chortippus crassiceps* (Devkota and Schmidt, 2000) and mottled water hyacinth weevil, *Neochetina eichhorniae* (Jamil and Hussain, 1992). The latter authors investigated the biotransfer of heavy metals from contaminated aquatic plant *Eichhornia crassipes* and the insect *N. eichhornae* feeding on them. Plants were exposed to known concentrations (25, 50, 100 mg/L) of cadmium or mercury for one week, before being fed to the insects. Percentages of metal accumulations in insects from the leaves were 6.3–10 % for cadmium and 3.5–7.5 % for mercury.

Results showed that the water hyacinth weevils accumulated large quantities of cadmium at all three concentrations, but bioaccumulation of mercury was low (Jamil and Hussain, 1992).

Devkota and Schmidt (2000) investigated concentrations of three different heavy metals (mercury, cadmium, lead) in grass in four species of acridid crassicepts (*C. italicus*, *O. caerulscens*, *O. germanica*, and *C. crassiceps*) feeding on grassland. In both the grass and insect samples, concentrations of heavy metals were in the order of lead > cadmium > mercury. In all four grasshopper species investigated, cadmium concentrations were in the range 2–4 times higher than the concentrations in the grass. Lead concentrations in grass were lower than in the four species of grasshoppers. Mercury concentrations were two times higher in *C. crassiceps* than in the grass, but mercury concentrations were lower than that of cadmium and lead. Female *O. caeruslescens,* but not the males showed a 1.3 times accumulation of mercury whereas male *O. caeruslescens* and the two other grasshopper species had lower concentrations than the grass.

Cadmium accumulation is dependent on the developmental stage of phytophagous insect species, with larvae containing higher concentrations than adult insects (Lindqvist, 1992). In a field study with phytophagous larvae of a Noctuid moth (*Spodoptera litura*) no bioaccumulation of cadmium and lead was found, whereas larval faeces contained high concentrations of these metals (Zhuang et al., 2009). In conclusion, transfer of heavy metals from substrates (e.g. organic matter, plants) for insects is apparently the most important route of contamination. Accumulation is dependent on insect species, growth stage, and metal in question.

4.2.2. Toxins produced by or accumulated in insects

Insects can contain naturally noxious compounds or 'insect toxins', which may either be synthesized (autonomous production) or accumulated from their substrate. Some species of insects which produce toxins warn their predators through vivid coloration and patterns (Zagrobelny et al., 2009). In general, the accumulation of toxins from substrate is a survival strategy in the evolutionary process. Some of these toxins can lose their properties through cooking processes (Berenbaum, 1993). Some insect species are capable of sequestering plant toxins (Berenbaum, 1993; Duffey, 1980; Nishida, 2002) making them less palatable to predators (Brower, 1969), such as glucosinolates in the harlequin bug, *Murgantia histrionica* (Aliabadi et al., 2002). Other insect species can produce toxins (Blum, 1994), such as insects of the Tenebrionidae family that produce (benzo)quinones and alkenes (Brown et al. 1992; Crespo et al., 2011) or moths of the genus Zygaena that produce cyanogenic glucosides, which release cyanide upon degradation (Zagrobelny et al., 2009).

More specifically, poisonous insects can be divided into two categories, phanerotoxics and cryptotoxics. Phanerotoxics have organs for the synthesis and delivery of poisons, as in the case of bees and ants. These substances are generally inactivated in the digestive tract. Insects which are cryptotoxic do not possess an external secretary apparatus and are toxic only after being ingested. Cryptotoxic insects may contain toxins as a consequence of synthesis or accumulation. These substances can be localized in specific structures or diffused in different body areas.

For the insects listed in the Terms of Reference, there are no immediate indications that they excrete reactive, irritating or toxic substances in the life stage used for consumption (FASFC, 2014). Toxicological tests conducted on whole insects or insect proteins are almost non-existent (NVWA, 2012) and up to now there are no risk evaluations available that verify the 'toxic dose' of insects (that are poisonous or that are only to be consumed under certain conditions) (FASFC, 2014).

Mycotoxins in insects may originate from those produced by pathogenic fungi, such as Aspergillus spp., Penicillium spp. and Fusarium spp. in the substrates and also from mycotoxin production in the gut of insects (Schabel, 2010; FAO, 2013). Mycotoxins can affect insect viability (McMillian et al., 1981).

The presence of mycotoxins in insects (house fly, blue bottle, blow fly and black soldier fly) farmed using various production methods at different geographical locations has been investigated (Charlton et al., 2015). The presence of 69 mycotoxins was analysed, and the mycotoxins detected were beauvericin (6.9 µg/kg dry weight), enniatin A (12.5 µg/kg dry weight) and enniantin A1 (7.3 µg/kg dry weight) in house flies.

Van Broekhoven (2014) investigated the potential transfer of deoxynivalenol (DON) from wheat as substrate to insects. Mealworms larvae were reared with naturally contaminated wheat flour, at concentration of 4 900 µg DON/kg flour, or with non-contaminated wheat flour for 14 days. Frass was collected and substrate was given to allow *ad libitum* intake. Concentrations of DON and its derivatives DON-3G, 3-Acetyl-DON and 15-Acetyl-DON were determined, both in the mealworms after harvest, and after fasting for 24 hours, in both the control and experimental group. The mean DON concentration in frass of the reared mealworms was 1 140 µg DON/kg dry weight. Concentrations of DON and its derivatives in the exposed and control mealworms and frass were below Limits of Quantification (LOQ: 100 µg/kg for DON, 3-Acetyl-DON and 15-Acetyl-DON, and 500 µg/kg for DON-3G. In a similar second experiment, mealworm larvae were fed wheat spiked with 8 000 µg DON/kg, and in this case, the mean concentration of DON in the frass was 4 980 µg/kg dry weight (van Broekhoven, 2014).

4.2.3. Veterinary drugs and hormones

Residues of veterinary drugs in substrates like manure may end up in insects and products thereof. This is also the case for the use of antimicrobials or other veterinary drugs during insect production.

Antimicrobial substances are sometimes added to formulated diets to prevent bacterial infections, for example, in silkworm farming (Cappellozza et al., 2011; Inglis and Sikirowski, 2009). Furthermore veterinary drugs may be sprayed or mixed with substrates during rearing of insects to combat infections with a variety of insect pathogens, and this use may also result in residues in the insects. In addition, other substances, such as hormones, if used in farming could result in residues (Belluco et al., 2013).

The EU veterinary (residue) drug legislation does not currently contain provisions for insects. The only insect product in which MRLs have been set for a few veterinary drugs is honey (Commission Regulation (EU) 37/2010[9]).

Farmed insects (house fly, blue bottle, blow fly and black soldier fly) were screened for veterinary medicines using 175 certified analytical standards to assess recovery of the analytical method (Charlton et al., 2015). Quantitative data were obtained for 68 compounds and qualitative data was obtained for 492 compounds. Insects were also analysed for chloramphenicol. Nicarbazin was found in one sample of house fly, all other compounds tested were below the limit of detection (100 µg/kg dry matter).

In insect farming, the use of antimicrobials is reported in the scientific literature, for emergency treatment in case of diseases caused by bacteria, fungi or microsporidia which can seriously damage farmed insects (Eilenberg et al., 2015).

Testing for veterinary drugs and hormones in insects intended for use as food or feed can be managed in accordance with current practices for other animal products in the food chain.

4.2.4. Other contaminants in insects

Pesticide residues

Substrates for insect rearing may contain residues of pesticides. The concentrations of 393 pesticide residues were measured in farmed insects (house fly, blue bottle, blow fly and black soldier fly) using UPLC-MS/MS with levels of detection (LOD) ranged from 10 to 50 µg/kg (Charlton et al., 2015). The pesticide residues detected were chlorpyrifos (800 µg/kg dry weight) in one sample of house fly larvae reared on milk powder and sugar in Wuhan, China and piperonyl butoxide was present in one sample of blue fly farmed in the UK (200 µg/kg dry weight). All other pesticide concentrations in the farmed insects were below the respective LODs.

In terms of feed safety, the Codex Alimentarius recommends that the concentrations of chlorpyrifos and piperonyl butoxide in animal feed (alfalfa and pea fodder, respectively) should be below 5 000 µg/kg and 2 000 µg/kg, respectively. The limited data on levels of these contaminants in feed for insects were below these proposed maximum concentrations.

[9] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1–72.

Dioxins and dioxin-like PCBs

Dioxin and dioxin-like PCB (DL-PCB) levels were analysed in farmed insects (house fly, blue bottle, blow fly and black soldier fly) by Charlton et al. (2015). Dioxin concentrations (sum of PCDD/F) ranged from 0.11–0.39 ng WHO-PCDD/F-TEQ/kg dry weight in the insect species tested, and sum dioxins and DL-PCB ranged from 0.23–0.63 ng WHO-PCDD/F-PCB-TEQ/kg dry weight (see Table 4). The EU maximum content for dioxins in feed materials of animal origin (‘Other land animal products including milk and milk products and eggs and egg products’) is 0.75 ng WHO-PCDD/F-TEQ/kg (88 % dry matter), and 1.25 ng WHO-PCDD/F-PCB-TEQ/kg (88 % dry matter), for DL-PCB (Commission Regulation (EU) No 277/2012[10]). There are currently no EU maximum levels for dioxins and dioxin-like PCB in insects as food or feed, for comparison the maximum levels for dioxins and sum dioxins and DL-PCB in fishery products are 3.5 ng WHO-PCDD/F-TEQ/kg wet weight and 6.5 ng WHO-PCDD/F-PCB-TEQ/kg wet weight, respectively (Commission Regulation EU 1259/2011[11]).

The sum of PCB ICES (International Council for the Exploration of the Sea) 6, also called indicator PCB-6 (PCB 28, 52, 101, 138, 153 and 180) ranged between 0.05–4.28 µg/kg dry weight in *M.domestica* larvae samples, and between 0.31–1.13 µg/kg in the other insect species tested (blue bottle, blow fly and black soldier fly; Charlton et al., 2015). The EU maximum content for PCB6 in feed materials of animal origin (‘Other land animal products including milk and milk products and eggs and egg products’) is 10 µg/kg (Commission Regulation (EU) No 277/2012). No maximum level for PCB6 has been set for insects as food, however for comparison the maximum level for fishery products is 75 µg/kg wet weight (Commission Regulation EU 1259/2011).

Table 4: Upper bound concentrations of dioxin (ng WHO-PCDD/F-TEQ$_{2005}$/kg), dioxin-like PCB (non-ortho and mono-ortho ng WHO-PCB-TEQ$_{2005}$/kg), sum dioxins and dioxin-like PCB ng PCB TEQ$_{2005}$/kg), and sum PCB ICES 6 (µg/kg) in farmed insects (MD1-MD5: house fly (*M. domestica*); CV1 and CV2: blue bottle (*C. vomitoria*); CH: blow fly (*Chrysomya spp.*); HI: black soldier fly (*H. illucens*)) reared using a variety of production methods at different geographical locations.

Contaminant	MD1	MD2	MD3	MD4	MD5	CV1	CV2	CH	HI
PCDD/F (ng TEQ/kg dry matter)[(a)]	0.18	0.30	0.14	0.14	0.14	0.44	0.39	0.11	0.13
Non-ortho PCB (ng TEQ/kg)[(a)]	0.07	0.31	0.32	0.10	0.14	0.03	0.04	0.13	0.09
Mono-ortho PCB (ng TEQ/kg)[(a)]	0.01	0.02	0.09	0.01	0.01	0.01	0.01	0.01	0.01
Sum TEQ (ng/kg)[(a)]	0.26	0.63	0.55	0.25	0.29	0.48	0.44	0.25	0.23
ICES-6[(b)] (µg/kg)	1.08	1.68	4.28	0.05	0.24	0.31	0.62	1.13	0.69

(a) Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES-6)
(b) Data transformed to ng WHO-TEQ$_{2005}$/kg from Charlton et al. (2015) raw data in µg/kg

Source: Charlton et al. (2015) with supplementary information from the authors (Available at: http://www.proteinsect.eu/index.php?id=63).

Accumulation of PCBs from polluted soils in wild insects, especially ground dwelling species has previously been shown (Davis et al., 1981; Blankenship et al., 2005; Walters et al., 2009).

Limited data are available on the levels of organic pollutants in insects. Levels of dioxins, DL-PCB and indicator PCBs (ICES6) that have been found in insects are below the maximum limits established in the EU for other food/feed and feed ingredients.

[10] Commission Regulation (EU) No 277/2012 of 28 March 2012 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels and action thresholds for dioxins and polychlorinated biphenyls. OJ L 91, 29.3.2012, p. 1–7.

[11] Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. OJ L 320, 3.12.2011, p. 18–23.

Polycyclic aromatic hydrocarbons

The EU has established maximum levels for polycyclic aromatic hydrocarbons (PAH) in food which include the sum of four substances (PAH4) (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) and a separate maximum level for benzo(a)pyrene (BaP). The maximum levels for PAH4 vary from 1 µg/kg (in baby food) to 35 µg/kg in smoked bivalve molluscs (and 1–6 µg BaP/kg respectively; Commission Regulation (EU) No 835/2011[12]). No maximum limit has been established for PAH in animal feed. Charlton et al. (2015) reported that the concentrations of PAH in farmed insects (house fly, blue bottle, blow fly and black soldier fly) varied between 0.28–9.82 µg PAH4/kg and <0.05–2.2 µg BaP/kg dry matter.

Packaging migration contaminants

When insects are reared on substrates which may contain packaging materials, like plastic and paper, e.g. as derived from former foodstuffs, they may be exposed to chemical contaminants that migrate from the packaging materials. Such compounds, e.g. ink, bisphenol A and phthalates, may migrate from the packaging material into the substrate, and may be eaten by the insects. However, to date, data on such migration compounds is not available.

4.2.5. Concluding remarks

Insect species, stage of harvest, production methods, substrate, as well as processing methods of insect (products) will all impact the occurrence and accumulation of contaminants in insect food and feed products. The greatest influence may be from the substrate, in relation to the insect species reared on it.

For insects with a short life cycle and, thus limited repeated feeding, bioaccumulation is less likely to occur than in insects that are reared over a longer time period.

The presence of most chemical contaminants in insects, except for natural toxins which are formed by fungi e.g. mycotoxins, can thus be controlled by controlling the levels of contaminants in the substrate. Data on transfer of contaminants from different substrate to the insects are, however, very limited. From the limited data available, it is seen that insects may accumulate heavy metals, in particular cadmium, from their substrates. Several chemicals may accumulate, but data is lacking to conclude on the extent of accumulation in comparison with accumulation in food producing animals.

4.3. Allergens

Insects can cause allergic reactions like eczema, rhinitis, conjunctivitis, angioedema and bronchial asthma. The most familiar allergic reactions to insects are those caused by insect bites or stings, by for instance bees or wasps. Other allergic reactions are caused by inhalation (e.g. dust with cockroach faeces) and contact (e.g. caterpillar hairs) and primarily occur with people who regularly come into contact with insects (e.g. entomologists, fish bait breeders, etc.) (FAO, 2013; Panzani and Ariano, 2001). Allergic reactions to mealworms (*T. molitor*) and lesser mealworms (*A. diaperinus*) due to contact have been documented (Siracusa et al., 2003; Schroeckenstein et al., 1990 and 1988). A number of cases have also been documented in which the consumption of insects has caused an allergic reaction and even anaphylactic shock in humans (FAO, 2013; Ji et al., 2009). A study by Freye et al. (1996) demonstrated that people were sensitized after eating the larvae of mealworm beetles (*T. molitor*) and darkling beetles (*Zophobas morio*).

Whereas like in humans, allergies occur in pet and farm animals, no indications of allergy caused by consumption of insect-derived feed are reported in pet and farm animals in the literature.

For any novel food for human consumption, hence also for insects or insect-derived foods, the potential elicitation of allergic reactions should be explored. Allergic reactions may occur through i) the elicitation of a reaction in individuals already sensitized to the insects or to a cross-reacting allergen and/or ii) the de novo sensitization of individuals and potential elicitation of an allergic reaction, constituting a 'new' food allergy. The allergenicity assessment should consider the likelihood of these

[12] Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. OJ L 215, 20.8.2011, p. 4–8.

events taking into account of the composition food or ingredient, its source, information from available literature and experimental data. Food allergens are usually (glyco)proteins. It may be assumed that foods produced from insects will always contain proteins, as the insects will serve as novel sources for proteins. Hence, food produced from novel sources such as insects may cause allergic reactions either by de novo sensitization or by cross reactivity. Foods produced from insects exhibiting allergenic properties may retain the allergenic activity, while the production process may also enhance this activity by revealing hidden allergens or concentrating allergens already present.

The source of the food is an important determinant of the likelihood of allergenicity of the food. A literature review of the allergenicity of the food or ingredient and the insect used as its source should be performed. In cases where the food or ingredient is already present in the diet in Europe or elsewhere (e.g. as a constituent of conventional food sources), case reports and allergenicity assessment may be available in the literature.

In case of known allergic properties of insects, as a general principle of assessment, the immunological reactivity of individuals who react to the insects should be tested *in vitro* and *in vivo* against the insect-derived food. Sera of people with confirmed allergies to that insect source can be subjected to specific immunological tests, e.g. Western Blotting or radioallergosorbent test (RAST). If *in vitro* tests are negative, *in vivo* skin prick tests may be performed. In case of negative outcomes, clinically supervised double blind placebo controlled challenges in these people may be performed to confirm the absence of allergenic potential. In addition to specific allergic responsiveness to the insect in question, potential cross-reactivity with other known allergens should be assessed.

Especially in the case that the insect in question has hitherto not been used, and in case allergenicity to the insect is unknown, potential allergenicity is difficult to negate. If the origin belongs to a family of species with known allergenic species, cross reactivity may be an issue and should be considered. Comparison of the amino acid sequence of proteins present in the insect to known allergens could be done according to the EFSA Scientific Opinion on the assessment of allergenicity (EFSA Panel on Genetically Modified Organisms, 2010). High homology with a known allergen may indicate potential allergenicity or cross reactivity. A cut off of 35 % identified in a window of 80 amino acids is used as the criterion for cross reactivity. Such proteins should be further investigated using in vitro or in vivo methods as indicated above.

Foods that are derived from insect sources without known allergenic activity, or where new proteins do not show cross reactivity with known allergens, molecular weight, heat stability, sensitivity to pH, digestibility by gastrointestinal proteases, and detectable amounts in plasma, may provide information on the likelihood of allergenicity of foods produced from insects. Additional evidence might emerge from pre-marketing human results and reports of workers' sensitizations.

The risk of allergies to insects in the case of insects as a source of food or feed proteins is plausible, and may be based on the existence of common allergens (pan-allergens) of arthropods such as arachnids, crustaceans (lobster, shrimp, crab), myriapods and insects. Similarly, allergens of molluscs and helminths are often very similar to those of insects and may lead to cross-allergies (Barre et al. 2014). The more or less close phylogenetic relationships between the different classes of arthropods may explain sequence homologies and similarities in structure constituting B cell epitopes in common allergens (pan-allergen), responsible for possible cross allergy between edible insects and other arthropods, mites (arachnids), crustaceans and non-edible insects (cockroaches). Insect consumption by individuals allergic to e.g. dust mites or shrimp could therefore well trigger allergic reactions associated with this cross-reactivity.

In a recent study (Verhoeckx et al. 2014), the cross reactivity between mites (tropomyosin 'Der p 10' of House dust mite) and the mealworm (*T. molitor*) was demonstrated. The cross reactivity is based on the pan-allergen tropomyosin, but also on other allergens such as arginine kinase, triose phosphate isomerase and tubulin. Tropomyosin belongs to a family of highly conserved proteins, with multiple isoforms (due to variations of amino acids) and found in both the muscle cells and non-muscle of all species of the animal kingdom (Leung et al., 1996; Reese et al., 1999). Tropomyosin is a thermostable allergen of 32–39 kDa, consisting of two alpha helices wound around each other, giving the protein a helical structure (Metz-Favre et al., 2009). Arginine kinase is an enzyme often found in invertebrates and allergic cross-reaction is already known between different crustaceans, mites, the Indian meal moth (Lepidoptera - Pyralidae), *B. mori*, *B. germanica* (blattoptères - Blattidae) and *P. americana* (blattoptères - Blattidae) (Liu et al., 2009; Verhoeckx et al., 2014). For these reasons, the risk of allergic reactions caused by consumption of products made from *T. molitor* may be comparable to the risk of allergic reactions to house dust mite or crustaceans.

In another recent study (Srinroch et al., 2015), allergens cross reactive with allergens from the crustacean, *Macrobrachium* spp, were identified in another edible insect, the cricket *Gryllus bimaculatus*. The allergens identified were arginine kinase, in addition to glyceraldehyde 3-phosphate dehydrogenase (Srinroch et al., 2015), hence the risk of consumption of these crickets may be comparable to the risk of consumption of crustaceans.

An insect component that has been suspected of causing allergic reactions is chitin. Chitin is a naturally occurring polysaccharide of glucosamine, which can be found in the cell walls of fungi and the exoskeleton of crustaceans (e.g. crabs, lobsters and shrimp) and insects. Chitin and its derivative chitosan (produced industrially via de-acetylation of chitin) are not allergenic themselves, but have immune modulatory properties, depending on the administration route and the size of the chitin particles (FAO, 2013; Muzzarelli, 2010; Lee et al., 2008). Such immunomodulatory properties may have consequences for the expression of allergic reactions to other allergens. An EFSA-opinion in 2010 stated that the intake of 5 g of chitin-glucan from crustaceans does not raise a public health concern (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2010).

Concluding remarks

A number of cases have been documented in which the consumption of insects has caused an allergic reaction and even anaphylactic shock in humans. Although allergies occur in pet and farm animals, no information on allergy caused by consumption of insect-derived feed is reported in pet and farm animals in the literature.

In the case of allergenic proteins, or of proteins cross reacting with allergens such as tropomysin or arginine kinase from crustacean or mites, a potential measure may be to indicate presence of the insect protein and the possible allergenicity or cross reactivity on the label of the product.

It is advisable that pet animals or food-producing animals fed on insect proteins are monitored for allergic reactions, in order to gain more insight into the relevance of potential allergenicity for animals.

4.4. Impact of processing and storage

Contamination of insects and products thereof can also occur after farming and before consumption, as happened when five individuals in Kenya died of botulism following the consumption of termites (Nightingale and Ayim, 1980). In this case, the insects had been stored in plastic bags, in anaerobic conditions during four days of transportation. *C. botulinum* has also been considered the cause of three lethal cases in Namibia, following the ingestion of caterpillars (Schabel, 2010).

However, a relevant aspect in a food perspective is not only the microbiota composition of live animals but also the possibility to safely process, store and preserve derived products. In this context, Klunder et al. (2012) recently evaluated the microbiological content of fresh, processed, and stored edible insects. The study focused on mealworms (*T. molitor*) and crickets *(A. domesticus* and *Brachytrupes sp.*) that were analysed as fresh, boiled, roasted, fresh, and stored (refrigerated and kept at room temperature). Results indicated that in fresh insects *Enterobacteriaceae* and spore-forming bacteria can be isolated, but generally they do not belong to pathogenic species. Crushing of the mealworm larvae resulted in higher counts of viable bacteria, which may have resulted from the release of microbiota from the insects' intestines, which can then be distributed throughout the product. Boiling insects for five minutes is an efficient process for eliminating *Enterobacteriaceae* but not spore forming bacteria. Thus, storage at refrigeration temperature (5 to 7 °C) is suggested. Moreover, keeping boiled insects at 5 to 7 °C prevents spoilage, while it is not efficient against spoilage of fresh ones. Roasting alone did not kill all *Enterobacteriaceae*; therefore, a few minutes of boiling is recommended before roasting. The authors also showed that lactic acid fermentation was able to inactivate *Enterobacteriaceae* and keep remaining spore-forming bacteria stable at acceptable levels where they were unable to germinate and grow (Klunder et al., 2012).

In a study performed on *T. molitor* and *L. migratoria*, oven drying (11 minutes at 90 °C) reduced the aerobic count by 2–3 log units and the *Enterobacteriaceae* count by 3–5 log units. Boiling at 100 °C for eight minutes reduced both the aerobic and the *Enterobacteriaceae* count to less than 10 cfu/g (FASFC, 2014).

The use of modern techniques such as plasma and hydrostatic pressure treatments appear to be effective in reducing microbial load during processing of *T. molitor*. The more effective treatment among the ones tested was the combination of high hydrostatic pressure (600 MPa) and high temperature (90 °C) (Rumpold et al., 2014).

For the production of protein, oil and chitin, the raw insect materials may be required to undergo a heat treatment process in accordance with the legislation on animal-by-products (Regulation (EC) No 1069/2009). In this Regulation different technical treatment methods are defined by particle size, time, temperature and pressure in order to obtain sufficient reduction of biological hazards in the product. In addition, criteria for evaluation of alternative treatment methods are given.

Processing conditions like heating and freeze-drying have minimal effect on concentrations of most chemical contaminants. However, fractionation of insect products can control the levels of specific chemical contaminants. For example, highly lipophilic dioxins may be largely removed by defatting insect meal. The fate of contaminants may therefore be influenced by concentration or dilution during processing insects into insect derived products.

Food processing can also lead to the formation of toxic substances such as heterocyclic aromatic amines (HCAs), acrylamide, chloropropanols, and furans. HCAs are potent mutagens and possible human carcinogens that are formed during the heating of creatine-containing foods, whereas acrylamide (which is genotoxic and carcinogenic in animals) is formed from carbohydrates and amino acids in some foods during high-temperature cooking processes, such as frying, roasting, and baking. The formation and concentration of processing contaminants is dependent on ingredients and processing conditions, like temperature-time of heating and pH (Dolan et al., 2010). To date, no information is available on the potential formation of such food-processing contaminants during processing insects.

4.5. Environmental hazards

Environmental Risk Assessment (ERA) considers the impact on the environment caused by, for example, the introduction of GM plants, and the use or presence of certain substances in food, feed and plant protection products. The sustainability of food production practices has increasingly become a topic of concern. The present assessment considers the environmental hazards which may be associated with insect farming.

4.5.1. Contained harvesting - farming system

Currently, insects for biocontrol, honey and sericulture (silk production) are already mass reared in contained facilities (e.g. Cáceres et al., 2012). This existing experience and scale of mass rearing of insects for other activities than for food and feed provides detailed information on the environmental risks associated with insect waste. In traditional agriculture, the environmental compartments of concern are the soil, ground water and surface water, resulting from the spread of manure from farm animals. The concentration of chemical contaminants in manure depends on the concentration in the feed, the nature of the contaminant and its metabolism in the farm animal. When the by-product from farmed insects (mixture of faeces (frass) and substrates) is spread on arable land as fertilizer (Figure 2.1), the same potential environmental risks will arise as from traditional farming practices. Knowledge is required concerning the nitrogen content and contaminant (and additives, for example copper) content in insects and insect frass in order to assess the environmental hazards which may be associated with insect farming.

Contaminants and additives (such as copper and zinc) present in insect substrate will unavoidably be released into the environment. When present in substrate, contaminants and trace elements excreted in the faeces and exoskeleton will enter the soil environment if the faeces and shed exoskeletons are applied as fertiliser to the land in the form of manure, slurry or litter. This may present two main potential risks: accumulation within the topsoil to concentrations posing potential toxic risk to soil organisms and leaching of contaminants from the soil to surface waters at concentrations posing potential toxic risk to organisms resident in the water column and bottom sediments.

When farmed insects are used in aquaculture feed, potential contaminants (such as cadmium) and trace elements (such as copper) may be released directly to the broader aquatic environment around an aquaculture facility, in uneaten substrate, or be taken up by fish and then excreted into the environment. In this sense, use of insects as feed ingredient does not differ from other feed in aquaculture. As stated in the EFSA technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008), the compartment of concern for fish farmed in cages is assumed to be the sediment, whereas for fish farmed in land-based systems the effluent flowing to surface water is considered to pose the main environmental risk.

Open cultivation of insects for food and feed

The management of insect habitat could be implemented for the (semi) cultivation of insects for food and feed. Semi-cultivation practices are used when insects are reared in open habitats and not in contained environment. This practice involves the use of skill and labour to increase the availability of particular species of insects for food and feed. Semi-cultivated populations are well established for insects (e.g. palm weevil larvae) in South America and the Indo-Pacific regions (FAO, 2013).

The increase in the population size of a species has potential environmental risks. Many edible insects are pests and pose threats to agricultural crops. The harvesting of these pests for food and feed might mitigate these risks through the reduction in population sizes. However, the wide-scale semi-cultivation may levy increased risks to biodiversity/crops and hence increases use of conventional insect control methods (e.g. insecticides).

Increased semi-cultivation may have wider consequences to biodiversity by altering species interactions through competition (more competitors) and/or predation (availability of more prey). This semi-cultivation also has consequences for focal population (genetic stability). Increased cultivation of insects may exacerbate inbreeding effects and influence the genetic stability/diversity of wild population.

Intensive production of animals also increases the risk of zoonosis (diseases shared between humans and wild/domestic animals). Environmental risks of this might be enhanced by poor biosecurity (waste-management) agricultural practice.

Concluding remarks

The environmental risk of insect farming is expected to be comparable to other animal production systems. Insect waste may contain some insects and insect material. The adoption of existing waste management strategies should be applicable for managing waste from insect production and assessment of the individual production systems will determine the precise strategy necessary (on a case by case basis).

4.5.2. Energy use and general environmental impact

The production and processing of insects demand energy, water, land and further resources. This requires a risk profile on environmental impacts and mitigation. Compared with conventional livestock rearing, such studies with insects are very scarce.

Some authors analysed the feed conversion, the land use (Oonincx and de Boer, 2012; van Huis 2013) and the GHG emissions of mealworms (*T. molitor*; Oonincx et al. 2010; Oonincx and de Boer, 2012) as a protein source for humans of food producing. The mealworms were kept for periods of three days under laboratory conditions, fed with chicken mash and carrots. Results were compared with values of traditional livestock animals. The feed conversion of insects under laboratory conditions was better than in 'traditional' animals and the Carbon Footprints (CF) of insects were lower. Production of one kg of edible protein from mealworms requires similar amounts of energy and less land compared with traditional animals (Oonincx et al. 2010; Oonincx and de Boer, 2012). Recently, Lundy and Parrella (2015) suggest that the laboratory scale rearing of crickets (*A. domestica*) was influenced by the type of diets used to rear the insects. The authors concluded that crickets reared on poultry feed showed similar feed conversion and emissions as poultry. More studies under field conditions are necessary to allow a conclusive evaluation of the sustainability of insects as protein rich food and feed sources (e.g. FAO, 2013; Windisch et al. 2013; Mlcek et al. 2014; Lundy and Parrella 2015).

Concluding Remarks

The environmental impact of insect farming in terms of resource use and emissions depends on insect species, substrate use as feed and other influencing factors but is expected to be comparable to other animal production systems. More evidence, through environmental life cycle analyses of mass-rearing insects is needed to evaluate more thoroughly the insect species most likely (Table 1) to be adopted for food and/or feed.

4.6. Summary of hazards per substrate group

According to the Terms of Reference, EFSA is requested to provide an overall conclusion based on the above assessments on the risks posed by the use of insects in food and feed, relative to such risks posed by the use of other protein sources used in food or feed.

Hazards were considered for the entire chain including both primary production and processing, but when comparing the hazards occurring in insects for food and feed with hazards in other protein sources used in food or feed the comparison was performed at the raw material level before further processing and taking into account of the use of different substrates.

Table 5 summarises the biological and chemical hazards, as well as prions, in insects grown on different substrate groups in comparison with the occurrence of these hazards in other protein sources of animal origin.

Table 5: Summary of the expected occurrence of hazards in non-processed insects, grown on different substrate groups, in comparison to the occurrence in other protein sources of animal origin.

Substrate (as described in Section 3.1.2) on which insects are reared	**Occurrence of hazards in non-processed insects compared to the occurrence in other protein sources of animal origin**		
	Biological hazards (see Sections 4.1.1-4.1.4)	**Prions (see Section 4.1.5)**	**Chemical hazards (see Section 4.2.5)**
Group A: Animal feed materials according to the EU catalogue of feed materials (Regulation (EU) No 68/2013) and authorized as feed for food producing animals.	Equal or lower	- Equal or lower, if the substrate does not include material of ruminant origin -Unknown, if the substrate includes material of ruminant origin	Unknown if equal, lower or higher
Group B: Food produced for human consumption, but which is no longer intended for human consumption for reasons such as expired use-by date or due to problems of manufacturing or packaging defects. Meat and fish may be included in this category.	Equal or lower	- No expected occurrence, if the substrate does not include material of ruminant origin -Unknown, if the substrate includes material of ruminant origin	Unknown if equal, lower or higher
Group C: By-products from slaughterhouses (hides, hair, feathers, bones etc.) that do not enter the food chain originating from animals fit for human consumption	Equal or lower	- No expected occurrence, if the substrate does not include material of ruminant origin -Unknown, if the substrate includes material of ruminant origin	Unknown if equal, lower or higher
Group D: Food waste from food for human consumption of both animal and non-animal origin from restaurants, catering and household	Equal or lower	- No expected occurrence, if the substrate does not include material of ruminant origin -Unknown, if the substrate includes material of ruminant origin	Unknown if equal, lower or higher

Substrate (as described in Section 3.1.2) on which insects are reared	Occurrence of hazards in non-processed insects compared to the occurrence in other protein sources of animal origin		
	Biological hazards (see Sections 4.1.1-4.1.4)	**Prions (see Section 4.1.5)**	**Chemical hazards (see Section 4.2.5)**
Group E: Animal manure and intestinal content	Unknown	- No expected occurrence, if the substrate does not include material of ruminant origin -Unknown, if the substrate includes material of ruminant origin	Unknown if equal, lower or higher
Group F: Other types of organic waste of vegetable nature such as gardening and forest material	Equal or lower	No expected occurrence	Unknown
Group G: Human manure, and sewage sludge	Unknown	Unknown	Unknown

5. Overall Conclusions

This opinion has the format of a risk profile that identifies hazards. Specific risk assessments should be performed taking into account of the whole production chain from farming to consumption including the species and substrate to be used as well as methods for farming and processing.

5.1. Biological hazards

The substrate used and the farming environment strongly influence insects' microbiota, and therefore the occurrence of hazards on insects is influenced by the nature and the hygienic conditions of the substrate and the farming environment.

Published studies on the occurrence of bacterial vertebrate pathogens in insects or products thereof for food and feed are very limited.

Pathogenic bacteria (such as *Salmonella*, *Campylobacter* and verotoxigenic *E. coli*) may be present in non-processed insects depending on the substrate used and the rearing conditions. Most likely the prevalence of some of these pathogens, for example *Campylobacter*, will be lower compared to other non-processed sources of animal protein, since active replication of the pathogens in the intestinal tract does not seem to occur in insects.

Insect pathogenic viruses occurring in insects produced for food and feed are not pathogenic for vertebrate animals and humans. However, viruses pathogenic for vertebrates can be transmitted by insects via contaminated substrates. The risk of transmission of vertebrate viruses could be mitigated through effective processing and adequate detection techniques for these viruses.

Despite the documented occurrence of parasites in insects and the linkage between sporadic human parasitic disease and insect consumption, a properly-managed closed farm environment would lack all the hosts necessary for the completion of parasite life cycles and proper management before consumption, relying on freezing and cooking, can eliminate risks.

Cellular prion proteins are not naturally expressed in insects. Therefore, no relevant risks exist in relation to insect-specific prions. Similarly, mammalian prions cannot replicate in insects, and therefore insects are not considered to be possible biological vectors and amplifiers of prions.

Various studies suggested the possible role of insects as mechanical vectors of infectious prions. Insects farmed on a substrate or in an environment in which infectious prions are present could act as mechanical vectors of infection and represent a potential risk of transmission of prion diseases through food and feed.

The total prion infectivity carried by insects would depend on the amount of infectivity present in the substrate used and can only be equal to or smaller than this.

The expected occurrence of hazards in non-processed insects, grown on different substrate groups, in comparison to the occurrence in other non-processed sources of protein of animal origin can be summarized as follows:

Microbiological hazards:

- For microbiological hazards, the occurrence in non-processed insects is expected to be equal or lower when insects are fed on substrate groups A, B, C, D, F (see Section 3.1.2).
- The risk related to substrate groups E and G (manure, sewage, sludge from animals/humans) should be specifically evaluated, taking into account of the kind of treatment applied, which can minimize, as in the case of treatment with high temperatures, the microbial contamination. In this case, the possible presence of spore-forming bacteria, which can survive heat treatment, must be carefully considered.

Prions:

- For prions, the occurrence in non-processed insects is expected to be equal or lower when insects are fed on substrates, which do not harbour material of ruminant or human (manure) origin.
- In general, the use of substrates of non-human and non-ruminant origin should not pose any additional risk compared to the use of other food or feed, while the risk posed by the use of other substrates should be specifically evaluated.

The risk of infections posed by human or animal consumption of insects and products thereof is modulated by a combination of the substrates used and the processing steps applied between farming and consumption.

The harvesting process may be of critical importance since it will impact the risk of transfer of hazards from the substrates to the insects and further on to the products of these.

5.2. Chemical hazards

Production methods, substrate, stage of harvest and insect species (potential for accumulation of chemicals) in insect rearing will all have an impact on the presence of chemical contaminants in insect food and feed products.

The expected occurrence of hazards in non-processed insects, grown on different substrate groups, in comparison to the occurrence in other sources of protein of animal origin can be summarized as follows:

- When substrates A to E are used, the possibility of contamination could be equal, lower or higher compared to the contamination in other non-processed sources of protein of animal origin. Chemical contaminants in the substrate may accumulate in the insects, but there is a lack of data on all specific chemicals and the extent of accumulation of each of these in insects. Possible accumulation of chemicals from substrate to insects should be further investigated per chemical, insect type with its life stage, and substrate type with its level of contamination.
- No data are available for the possibility of contamination from substrates groups F and G.

5.3. Allergens

A number of cases have been documented in which the consumption of insects has caused an allergic reaction and even anaphylactic shock in humans. Although allergies occur in pet and farm animals, no information of allergy caused by consumption of insect-derived feed are reported in pet and farm animals in the literature.

In case of allergenic proteins, or in case of proteins cross reacting with allergens such as tropomysin or arginine kinase from crustacean or mites, a potential measure may be to indicate presence of the insect protein and the possible allergenicity or cross reactivity on the label of the product.

It is advisable that pet animals or food-producing animals fed on insect proteins are monitored for allergic reactions, in order to gain more insight in the relevance of potential allergenicity for animals.

5.4. Processing

For the production of protein, fat/oil and chitin, to be used in animal feed, the raw insect materials may be required to undergo a heat treatment process as is described in the legislation on animal-by-products (Regulation (EC) No 1069/2009). In this Regulation, different technical treatment methods are defined by particle size, time, temperature and pressure in order to obtain sufficient reduction of biological hazards in the product. In addition, criteria for evaluation of alternative treatment methods are given.

In contrast to microbiological hazards, processing conditions like heating and freeze-drying have minimal effect on concentrations of chemical contaminants. The fate of contaminants may be influenced by concentration or dilution during processing of insects into insect derived proteins. Concentrations of chemical hazards will differ in the protein fraction and oil fraction. For example, highly lipophilic dioxins will be removed from the protein fraction and move into the oil fraction.

5.5. Environmental hazards and impact

The environmental risk of insect farming is expected to be comparable to other animal production systems. Insect waste may contain insects and insect material. The adoption of existing waste management strategies should be applicable for managing waste from insect production. Assessment of the individual production systems will determine the precise strategy adopted on a case by case basis.

The production and processing of insects has demand for energy and water resources: this requires a risk profile on environmental impacts and mitigation.

6. Uncertainties

The uncertainties which arise due to the lack of knowledge in the following areas are listed and not characterised further as this opinion has the format of a risk profile.

Human consumption

- There are no systematically collected data available on insect consumption in European countries;
- The pattern of consumption may only be estimated through sales data of insect product;
- How and to what extent the inclusion of insects in gastronomy and in the product range of food suppliers can impact the general consumption pattern in the population is unclear but holds the potential for a rapid change in future consumption patterns.

Animal/pet consumption

- There is lack of consolidated information relating to the magnitude and frequency of managed feeding of insects to farm animals.

Bacteria

- There is a lack of studies on the occurrence of human and animal bacterial pathogens in insects processed for food and feed are very scarce in the scientific literature.

Viruses

- Insect virus infections do induce major metabolic changes in insects and may produce substances toxic to humans, but there is no scientific evidence for such a case;
- There is lack of information relating to the likelihood of human viruses such as norovirus, rotavirus, Hepatitis E and A being passively transferred from feedstock through residual insect gut contents.

Parasites

- Information in the literature refers to non-European areas (mostly Asia) and to insects harvested in the wild, and so the risk can be very different from what is found in farmed insects, with strict control of environmental conditions and substrates applied.

Prions

- There is lack of information on the extent to which insects act as mechanical vectors of prions.

Allergens

- There is lack of pre-marketing human results and reports of workers' sensitizations.

Chemicals

- Published data on hazardous chemicals in reared insects in scientific literature are scarce;
- Data on accumulation/excretion of chemical contaminants from the substrates are very limited;
- To date, there is lack of information on the use of veterinary medicines for the treatment of insects to be used for food and feed;
- No information is available on the potential formation of food-processing contaminants during processing insects.

Processing

- There is a lack of information relating to precise details of the processes used.

Environmental

- There is lack of information on the environmental impact of different mass-rearing insect productions systems.

7. Recommendations

It is recommended to initiate research on the issues mentioned in Section 6, Uncertainties.

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Abbreviations

DON	Deoxynivalenol
ERA	Environmental Risk Assessment
FAO	Food and Agriculture Organization of the United Nations
GHG	Greenhouse Gas
HCAs	Heterocyclic Aromatic Amines
ICES	International Council for the Exploration of the Sea
LOD	Limit Of Detection
MRL	Maximum Residue Limit
PAH	Polycyclic Aromatic Hydrocarbons
PAP	Processed Animal Protein
PCB	Polychlorinated Biphenyl

Appendix A – Nutritional composition

About 1 900 insect species in various development stages (van Huis, 2013) are eaten worldwide. Apart from the species and the developmental stages (eggs, larvae, pupae, adults), the nutritional composition depends on the feed of the insects (Bukkens, 1997; Finke, 2005; Oonincx and van der Poel, 2011, Oonincx and Dierenfeld, 2012; Belluco et al., 2013; Rumpold and Schlüter, 2013; Makkar et al., 2014; Mlcek et al., 2014; Morales-Ramos et al., 2014; Sanchez-Muros, 2014). Handling, preparing and processing of insects may also influence the composition of insects (Babiker et al., 2007; Kinyuru et al., 2010; Falade and Omojola, 2010). Thus, the nutritional values of insects are highly variable (see Table 6) and a comprehensive description is beyond the scope of this opinion. The main components of insects are protein and fat and lower proportions of fibre and ash (see Table 6), but they contain also variable amounts of micronutrients.

Crude protein and amino acids

The crude protein content of a variety of insect orders has been determined between 13 and 77 % of dry matter (Xiaoming et al., 2010) while the average protein content was reported to range between 7 and 48 g/100 g fresh weight (FAO, 2012).

The digestibility of insect proteins and their utilization in vivo has been considered as good, e.g. the apparent faecal digestibility of black soldier fly larvae and soymeal in male growing pigs was 76 and 77 %, respectively. Studies using housefly meal in broilers yielded apparent faecal digestibility of 69 % (Pretorius, 2011) and 98.5 % (Hwangbo et al., 2009). The former study (Pretorius, 2011) reported amino acid digestibility of > 90 % while the digestibility of crude protein was much lower which might be attributed to the indigestibility of chitin-N and/or of acid detergent fibres (lignin, cellulose) (Makkar et al., 2014).

Besides digestibility, the amino acid content determines the protein quality. Tryptophan and/or lysine are the amino acids most commonly mentioned as first limiting amino acids of insect protein (Ramos-Elorduy et al., 1997; Finke, 2005; Bukkens, 2005). Sanchez-Muros (2014) considered histidine, lysine and tryptophan as the most frequent limiting amino acids in insects compared to fishmeal and soybean protein. Overall levels of essential amino acids in insect meals are considered as good, e.g. most essential amino acid concentrations in silkworm pupae meal and black soldier fly larvae being higher than in soymeal or the FAO Reference Protein (Makkar et al., 2014).

Crude fat (ether extract) and fatty acids

Insects are a considerable source of fat. Reported values range between < 5 and more than 50 % crude fat of dry matter in edible insects (Xiaoming et al., 2010; Rumpold and Schlüter, 2013; Mlcek et al., 2014; Makkar et al., 2014). There is a high variation in fatty acids profiles across insect species (Thompson, 1973; Lu et al., 1992). Womeni et al. (2009) reported fatty acids of several edible insect species consumed in Cameroon to contain quantities of palmitic (8–38 %), oleic (9–48 %), linoleic (7–46 %) and α-linolenic acid (15–38 %) as percentage of oil content. Although the nutritional importance of linoleic and α-linolenic acid as essential amino acids is well recognized, the presence of high amounts of unsaturated fatty acids will also give rise to rapid oxidation of insect food products during processing, causing them to go rancid quickly (FAO, 2013).

Carbohydrates

Information on carbohydrate content of insects is scarce. Estimates of crude fibre content in different insect species ranged between 0 and 86 % of dry matter as reported by various authors (Bukkens, 1997; Finke, 2005; Grabowski et al., 2008; Rumpold and Schlüter, 2013; Sanchez-Muros et al., 2014; Makkar et al., 2014). Most carbohydrates in insects are formed by chitin. Chitin is considered as indigestible for man and animals (FAO, 2013) and may decrease the insect protein digestibility (DeFoliart, 2002). Chitin in medium and large-sized particles is also considered to induce allergic inflammation (Muzzarelli, 2010).

Minerals

Finke (2005) summarized the mineral content of 32 species (mainly larvae) and found the ranges, given in g/kg dry matter for Ca: 0.4–24.8; P: 1.2–14.3; Mg: 0.3–27.4; and given in mg/kg dry matter Cu: 9–265; Mn: 3–39; Zn: 21–390; Se: 0.3–400. Most insects contain higher levels of phosphorus compared with calcium. The phosphorus availability from insects in non-ruminants is nearly 100 %

(Mlcek et al., 2014). The iron content in many edible insects is equal or higher than in beef, e.g. ranging between 8 and 20 mg/100 g dry matter in locusts (*Locusta migratoria*) and between 31 and 77 mg/100 g in the mopane caterpillar (Oonincx et al., 2010). Most insects appear to be good sources of the trace elements iron, zinc, copper, manganese and selenium (Barker et al., 1998; Belluco et al., 2013; Rumpold and Schlüter, 2013) but not for calcium (Finke, 2013).

Vitamins

Finke (2013) analysed the vitamin content of four species of feeder insects (soldier fly larvae, Turkestan cockroach, tebo worms and adult house flies) and results were as follows: < 300 μg/kg retinol; < 2 μg/kg vitamin D_2; 2.5 μg/kg vitamin D_3; 6–30 mg/kg α-tocopherol; < 10–23 mg/kg vitamin C, < 0.01–11 mg/kg thiamin (B_1); 16–77 mg/kg riboflavin; 27–45 mg/kg pantothenic acid; 34–91 mg/kg niacin; 1.7–6.1 mg/kg pyridoxine; 0.8–2.7 mg/kg folic acid; 0.35–0.68 mg/kg biotin, 5–237 μg/kg vitamin B_{12}; and 625–1 100 mg/kg choline. Thus, the vitamins considered most likely to be deficient when these species of insects are used as food for insectivores were the vitamins A, D, E, B_1 and B_{12}.

In general, studies on vitamin content in edible insects are considered as insufficient by some authors (Chen et al., 2010; Mlcek et al., 2014).

Table 6: Examples for ranges of proximate body composition (Crude nutrients by Weende analysis; in % of dry matter) of different insect species by various authors)

Authors	No. of species or species (e.g. Makkar et al. 2014)	Crude protein (N x 6.25)	Crude fat (Ether extract)	Carbohydrates (NFE, fibre, NDF)	Crude ash
Bukkens (1997)	50	7.5–79.6	2.2–61.1	0–11.4	1.1–15.8
Finke (2005)	75	22.5–80.0	2.2–48.0	5.1–34.8	1.0–28.9
Rumpold and Schlüter (2013)	234 (samples)	4.9–74.8	0.7–67.2	3.0–86.3	0.6–26.0
Sanchez-Muros et al. (2014)	72	9.5–70.1	1.5–56.1	1.8–77.7	0.6–26.0
Makkar et al. (2014)	Black soldier fly larvae (1–5)[(a)]	41.1–43.6	15.0–34.8	7.0	14.6–26.8
	Housefly maggot meal (19–29)	42.3–60.4	9.0–26.0	1.6–8.6	6.2–17.3
	Tenebrio molitor (2–10)	47.2–60.3	31.1–43.1	7.4–15.0	1.0–4.5
	Locust or grasshopper meal (7–9)	29.2–65.9	4.2–14.1	2.4–14.0	4.4–10.0
	House cricket (2–4)	55.0–67.2	9.8–22.4	15.7–22.1	3.6–9.1
	Silkworm pupae meal (6–11)	51.6–70.6	6.2–37.1	2.5–5.8	3.3–10.6

(a): Number of samples

For references, see list in main text.

Appendix B – Risk assessments at Member State level

The EFSA Focal Points of Member States and EFTA countries were asked to share existing and on-going risk assessments on the safety of insects as food and feed performed in their country. Four countries (Belgium, France, Iceland and Netherlands) replied that they have performed risk assessments related to insects as food or feed.

Belgium

A common advice of the Scientific Committee of the Federal Agency for the Safety of the Food Chain (FASFC) and of the Superior Health Council (SHC) was published on food safety aspects of insects for human consumption in September 2014 (FASFC, 2014). In the opinion, the potential microbiological, chemical, physical and parasitological risks associated with the consumption of insects are evaluated. The evaluation only pertains to insects intended for human consumption, insects that are consumed as a whole or as a 'preparation of the whole insect' (e.g. grinding of whole worms), and insects that are reared under standardized conditions. Thus, safety aspects of insects that are bred for feed, the human consumption of fractions of insects (protein preparations or other extracts obtained from insects), and insects harvested in the wild are not considered.

France

ANSES carried out a scientific and regulatory literature review on risks associated to the consumption of insects and derived products in food and feed. As a follow-up, in June 2014, ANSES launched an internal request to develop an opinion on the health risks linked to the use of insects for food and feed. The opinion includes biological, physical, chemical and allergic risks by the production and the consumption of insects (ANSES, 2015).

Iceland

The authorities performed an environmental risk assessment for black soldier fly that was imported for research purposes in Iceland. It was suggested that the fly poses no threat to local insect fauna as it is a tropical species and wild populations are highly unlikely to form, due to the cold climate of the country.

Netherlands

NVWA published an opinion on the 'risks associated with the consumption of mass-reared insects' (NVWA, 2014). The assessment includes chemical, microbiological and parasitological risks from consumption of heat-treated and non-heat-treated insects.

The following countries replied that they have not performed a risk assessment on insects: Austria, Bulgaria, Croatia, Cyprus, Czech Republic, Estonia, Finland, Greece, Latvia, Lithuania, Luxembourg, Norway, Poland, Portugal, Hungary, Slovak Republic, Slovenia, Switzerland and UK.

For references, see list in main text.

Annex A – Minutes of the 5th meeting (first day; 19 February 2015) of the Working Group on the Safety risks arising from the production and consumption of insects as food and feed[1]

Held on 19 February 2015 (10:00–18:00) in Brussels, Belgium

Hearing Experts:

- Paul Vantomme; Food and Agricultural Organization (FAO).
- Venik (the Dutch insect producers association): Margot Calis (Kreca, The Netherlands) and Jonathan Koppert (Koppert Biological Systems, The Netherlands).
- International Producers of Insects for Feed and Food (IPIFF): Tarique Arsiwalla (Protix, The Netherlands) and Antoine Hubert (Ynsect, France).
- GREEiNSECT research consortium: David Drew (Agriprotein; South Africa) by teleconference and Prof. Yupa Hanboonsong (Khon Kan University, Thailand).
- PROteINSECT research consortium: Geert Bruggeman (Nutrition Biosciences; Belgium) and Richou Han (Guangdong Entomological Institute (GEI), Guangzhou, China) by teleconference.

The following questions[2] were proposed to the hearing experts and a summary of the answers is reported below:

1. Which are the major insect species used for food and feed production?

The major commercial insect species are mealworm (*Tenebrio molitor*), lesser mealworm (Alphitobus diaperinus), house cricket (*Acheta domesticus*), field cricket (*Gryllus bimaculatus*), black soldier fly (*Hermetia illucens*), locust (*Locusta migratoria*), silkworm (*Bombyx mori*), superworm (*Zophobas morio*), common housefly (*Musca domestica*), grasshopper (*Orthoptera group,* such as*: Oxya spp.; Melanoplus spp.; Hieroglyphus spp.; Acridia spp.*), palm weevil or sago larvae (Rhynchophorus ferrugineus Olivier) and blow fly (*Chrysomya chloropyga*).

The above list is non-exclusive; other species are or may be used as food and feed. All these species can theoretically be used for the production of both food and feed, but for practical reasons such as nutrition, the type of substrate available to rear the insects, economics, life cycle times and consumer acceptance some species are more likely to be used as food and/or feed.

2. a. Which substrates are presently used and which have greatest potential to be used as feed for insect production?

The substrates currently used are the ones permitted by the legislation of each country. In general terms, the following substrates are used in different countries or could potentially be used in the future in other countries (depending on the criteria that are described in question 2b below):

- Exclusively traceable vegetative materials, such as, pre-consumer products (e.g. vegetable residues, fruit residues), cereal based material wheat bran, chaff bran, grass and brewery by products, hay and powder made of plants.

[1] Annex A has been produced exclusively by the industry hearing experts who attended the working group meeting on 19 February 2015 and who are indicated as authors of the document. Annex A is published as annex to this opinion in accordance with the transparency principle to which the European Food Safety Authority is subject. It presents exclusively the opinion and positions of those concerned and it may not be considered an output adopted by EFSA or by the working group developing the Opinion or anyway as representing EFSA's views or positions on the subject. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in Annex A.

[2] Note: The questions referred to the state-of-the-art and forecasting, both inside and outside the EU and covered insects and products thereof for food and feed.

- Post-consumer waste including organic kitchen waste and catering waste. It should be noted that this material does not comply with the traceability standards and is therefore not allowed to be used by EU insect producers. Such material may contain undesirable substances as well.
- Commercial feed e.g. chicken feed, cricket feed.
- Livestock manure; however, this is currently not allowed nor being used in EU; IPIFF insect producers are not intending to consider the use of manure as insect substrate.
- Former foodstuff i.e. food approved for human consumption, but beyond their expiry date. According to the current EU legislation, these products shall not contain meat and fish or residues of packaging material in order to be used as feed (except fishmeal). However, the use of former foodstuff with packaging residues (such as glass, paper, wood, plastics and bio-plastics) may be an alternative for the future, however risks such as the insects eating the packaging or risks from packaging components (e.g. colorants, glues, inks etc.) that may be migrated to the substrate need to be assessed.

b. What are the criteria for selecting substrates? What are the properties of the substrates?

- Legislation that permits and prohibits certain substrates for insect breeding; as an example: IPIFF insect producers use only pre-consumer traceable vegetative materials as substrate for insects. This is a practice that is foreseen to be followed in short term. In the medium term, pre-consumer former foodstuffs (incl. meat and fish) may be considered.
- The insect species that are going to be used. Substrates have to be: (i) nutritious for these specific species in order to ensure speed of growth and weight gain. Also, fatty acids profile of the insects may be controlled via the substrate composition; (ii) easy to remove during harvesting of the insects; and (iii) what the insects normally prefer.
- Easy availability and processing.
- Reasonable cost.
- Perception of breeders.

c. What kind of processing these substrates are subject to prior to their use?

- Wheat bran, chaff bran, and hay powder, as well as chicken feed, can be used directly.
- Former foodstuffs may pass the steps of grinding and possible sieving for size reduction or partial removal of packaging.
- Kitchen waste is subject to grinding and sometimes to heating at 60–100 °C.
- Leafy vegetables and fruits are not heat treated and may pass the steps of washing, cutting and sieving.
- Livestock manure can be heat treated or naturally fermented (composted). Non-pathogenic strains of microorganisms may be used to reduce the load of potential pathogens before heating or fermentation.

3. What are the harvesting practices?

Different harvesting methods are used to isolate or collect target instar insects from the substrates. For example, mealworm and black soldier fly larvae can be collected by sieving; grasshopper adults are catched by insect collecting nets; housefly larvae can be isolated from the substrates by lowering oxygen concentration in a close container.

Dipteran fly larvae can be separated from substrate by allowing the larvae to pupae which stimulate them to leave the substrate. The pre-pupae or pupae stages have higher chitin content and earlier harvesting by separating larvae from substrate is practiced for better nutritional composition.

4. Which are the products (whole insects and processed products) available already on the market and which are foreseen to be available in the future?

Products include whole insects and products thereof such as protein, hydrolysed protein, purified fat (i.e. free of proteins), chitin and its derivatives, such as chitosan and glucosamine. These products could be used as food, feed and pet food or in the case of chitin in pharmaceuticals, medical purposes (e.g. biocompatible polymers, surgery wires, medicine capsules), cosmetics, industrial and agricultural coatings, but also nutrition (complementary diets). Insect farming waste may be used as fertilizer. Breeding stock (as adult life insects or as eggs) is another product of farming.

In the case of whole locust and grasshoppers as food, labeling may suggest the removal of wings and legs prior to consumption.

5. What are the present and anticipated volumes produced inside and outside Europe?

Volumes are limited at the moment since this is a new area and there are legislative blockages on the production and use of insects as food and feed. However, production is expected to rise due to knowledge that is gathered and investments in new production plans. In addition, the increase in price of fishmeal and soymeal makes insect protein a potential alternative solution for the feed industry. Hearing experts anticipate that within 10 years the production may be raised up to 500 000 tonnes of insect protein per year.

Some concrete figures follow:

- According to Venik, the current annual production of insects as food in The Netherlands is approximately 15 tonnes live insect weight.
- IPIFF member Protix is now scaling up to industrial scale to supply permitted markets with insect protein meal and insect oil (1 000 tonnes per annum by the second half of 2015).
- IPIFF member Ynsect is currently producing around 1 000 Kg a month of insect protein meal (and in addition oil and chitin byproducts), and is currently building a full automated facility to increase the production to more than 30 tonnes a day of insect protein meal.
- In South Africa, Agriprotein produced 200–300 Kg insect products a day in 2014, whereas in the second half of 2015, due to a new investment, they will be able to process 100 tonnes of waste a day to produce seven tonnes of insect products.
- Thailand produces an estimate of 7 500 tonnes of crickets per year and the production is expected to increase.

6. How do you assess safety aspects (e.g. use of antibiotics and pesticides in the chain, presence of pathogens) of (i) the substrates; (ii) the insects and (iii) the products?

Several EU producers have been analyzing proteins derived from insects fed on purely plant based material for undesirable chemical substances and biological hazards. Laboratory results have been provided to this working group.

7. Are there safety data of edible insects (e.g. chemical residues, occurrence data on pathogenic microorganisms, antinutrients, chitin)?

Producers use Hazard Analysis Critical Control Points (HACCP) approach in order to eliminate the hazards (biological and chemicals) or reduce them to acceptable levels. These approaches are company specific based on the respective farming and production techniques followed.

Producers comply with existing regulations of food and feed safety, as well as with quality schemes such as the GMP+[3].

[3] www.gmpplus.org/

Before the producers can place their products on the market as food and feed, they are obliged to test for pathogens and against the maximum legislative limits of antibiotics, pesticides and other contaminants.

Farm hygiene is important, therefore, clean water is used throughout the production and equipment is disinfected with approved products between batches of insects.

8. Which are the most common production processes (e.g. killing practices, heat treatment, time intervals, solvents for fat extraction)?

The processes that are used depend on the species and the producer. Some examples follow:

- Mealworms, grasshoppers and crickets may go through a specific diet for intestine cleaning before harvesting. Mealworms could be left 12–24 hours after separation from substrate to empty the intestine before killing by freezing. Insects may also be killed with hot water or vapour. In case heat treatment is not sufficient to kill all the pathogens, labelling may inform the consumer to cook them before consumption.
- For the black soldier fly, the larvae are harvested with mechanical separation before the stage of pupae; washing, grinding and heating up to 95 °C for 5–10 min follow. This heat treatment process shall be sufficient in order to achieve the microbiological criteria set by the Processing method 7 of Commission Regulation (EU) No 142/2011[4], Annex IV, Chapter II, Point G.
- Cricket production for food production in Thailand includes shifting to feed vegetable, most common pumpkin, the last week of production, cooling on ice after harvesting, followed by boiling for 5–10 minutes, cooling by washing in cold water and packing in plastic bags.

9. Which are the storage and distribution practices?

Storage and distribution of insect products depend on the producer and the characteristics of the product. As other foodstuff and feed material, they can be stored and distributed in room temperature, chilled or frozen. They can be pasteurized, dried or sterilized.

Researchers from the University of Khon Kaen (Thailand) reported that microbiological tests showed that the shelf life of crickets boiled for 10 minutes and then packed with air and kept at 0 °C was 30 days. The shelf life was extended beyond 30 days when the crickets were packed under vacuum (at 0 °C).

10. Are there Good Manufacturing Process manuals for insect production and processing?

There are no published GMP manuals specific to insect production and processing, but breeders work according to the HACCP and GMP+, as well as to national food and feed safety legislation.

11. Are there data on consumption (as food and feed, including aquaculture)?

According to the hearing experts at this meeting, there are no data on consumption. Insects and their products are not included in the production and trade commodity nomenclatures used by international organisations such as FAOSTAT and Eurostat.

12. Are there data on allergenicity?

- Arginine kinase isolated from *Locusta migratoria manilensis* was reported to cause allergic reactions in some sensitive persons.

[4] Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. OJ L 54, 26.2.2011, p. 1–254.

- Individuals sensitive to crustaceans may be allergic to insects or products thereof. Due to cross reactivity with tropomyosin and arginine kinase in mites and crustaceans, allergic reactions may occur after consumption of proteins derived from certain insects in sensitive individuals.
- A report on allergenicity in humans will be published in the Netherlands, whereas there are no reports for allergenic reactions to animals.
- Some products are labelled with allergen advice.

13. What is the fate of the waste from insect farming (e.g. is it used on arable land)?

The waste from insect farming can be used as fertilizer and soil conditioner. It could also be used as a substrate for rearing other insects, earthworms, nematodes, and other species.

14. How do you consider environmental consequences of production and processing e.g. utilization of residual material and by-products?

In general terms, production and processing of insects is environmentally friendly, as it has the potential of transforming the waste or side streams of agricultural, horticulture, forestry, food/feed distribution and animal/fish processing production systems into food and feed, while the waste of insect farming can be used as fertilizer. In addition, insect farming produces less greenhouse gases comparing to other production systems (see PhD thesis of Onnincx, 2015 "Measurement of GHG emissions of different insects"). Smells from the production process are managed such that they can be minimized.