Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

> Return address PO Box 43006 3540 AA Utrecht

To the Minister of Agriculture, Nature and Food Quality

To the Inspector-General of the Netherlands Food and Consumer Product Safety Authority

With a copy to the Minister for Medical Care

From the Director of the Office for Risk Assessment & Research

Advice on animal and public health risks of insects reared on former foodstuffs as raw material for animal feed

Office for Risk Assessment & Research

Catharijnesingel 59
3511 GG Utrecht
PO Box 43006
3540 AA Utrecht
www.nvwa.nl

Contact

T +31 88 223 33 33
risicobeoordeling@nvwa.nl

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Background

In the coming decades, there is expected to be a sharply increased demand for dietary proteins for humans and animals. As a result, there is an increasing focus on reared insects as a new source of protein. According to the Ministry of Agriculture, Nature and Food Quality (LNV), the use of food chain residual flows such as former foodstuffs (FF)[1,2] as a rearing ground (substrate) for insects, which are subsequently used as raw material for animal feed for food-producing animals, is a sustainable and innovative development that must be further explored[3,4]. However, this development may entail risks to animal and public health.

The department Plant Supply Chain and Food Quality of the Ministry of LNV has asked the Office for Risk Assessment & Research (BuRO) of the Netherlands Food and Consumer Product Safety Authority (NVWA) the following questions:

1. What are the main risks to animal and public health of insects that are reared on FF and that are processed into animal feed for farm animals[5]?
2. How can these risks be adequately managed?

[1] Former foodstuffs (FF): 'foodstuffs, other than catering reflux, which were manufactured for human consumption in full compliance with the EU food law but which are no longer intended for human consumption for practical or logistical reasons or due to problems of manufacturing or packaging defects or other defects and which do not present any health risks when used as feed' (Commission Regulation (EU) No. 68/2013 of 16 January 2013 on the Catalogue of Feed Materials). This definition includes FF of animal origin, FF containing products of animal origin, and FF of fully plant origin.
[2] Food or foodstuff: relates to 'any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans' (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).
[3] Letter to Parliament 13058527. Status with regard to the use of insects in animal feed and aquaculture. 19 April 2013.
[4] Letter to Parliament 15079841. Scope for innovation through future-proof legislation and regulations. 20 July 2015.
[5] The term 'farm animal' as referred to in this advice means farm animals other than fur animals. The issued advice relates to food-producing animals, i.e. animals that or whose products enter the human food chain. This also includes aquaculture animals.

Approach

To answer these questions, BuRO has studied the reviews prepared by the European Food Safety Authority (EFSA) and the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) on the health risks of using insects and insect products as food and animal feed. The opinions drawn up based on these reviews (ANSES, 2015; EFSA SC, 2015) are extremely wide in scope: they cover more than 2000 edible insect species (Jongema, 2017), different life stages, rearing methods (substrates and production environment) and processing methods. These opinions also noted that there are many gaps in our existing knowledge. As a result, it was not possible to assess the specific health risks for each of the possible variants or to arrive at a generic assessment of the risks.

BuRO has carried out an additional literature review to make a specific assessment of the risks to animal and public health arising from the use of insects reared on FF that are processed into animal feed for farm animals. This risk assessment was performed based on the four risk assessment steps as defined by the Codex Alimentarius Commission (FAO & WHO, 2015). In its assessment, BuRO has limited itself to insect species that are suitable for large-scale production and that can be reared on FF. It has examined the animal and public health hazards associated with the use of FF as a substrate for these insects when these insects are used as (raw material for) animal feed and how these hazards change during the production process. Subsequently, control measures have been formulated for the risks that occur.

The research questions relate not only to the risk assessment and formulation of control measures, but also take into account legal aspects. BuRO has examined the current legislation and regulations relating to (feed for) farm animals that also apply to the rearing of insects on FF, the use of insects as animal feed raw material, and the underlying considerations. These are summarised in the present advice.

Scope and definition

The issued advice only assesses the chemical and microbiological animal and public health risks associated with the use of feed for food-producing farm animals produced from insects reared on FF. This concerns the rearing of larvae of the black soldier fly (*Hermetia illucens*), larvae of the housefly (*Musca domestica*), yellow mealworms (larvae of *Tenebrio molitor,* the yellow mealworm beetle) and lesser mealworms (larvae of *Alphitobius diaperinus,* the lesser mealworm beetle) that are reared on FF of both plant and animal origin. The chemical animal and public health risks of substances that may be present in FF packaging material residues in the substrate have not been taken into consideration here. It is not possible to make a risk assessment of this because of the lack of good exposure data and sufficient knowledge of the toxicity and kinetics of the undesirable substances in the specific feed matrices and the effect of particle size and shape (BuRO, 2006; EFSA SC, 2015; RIVM-RIKILT, 2006). However, a risk identification study carried out by the RIKILT Institute of Food Safety in 2011 on packaging residues in FF (van Raamsdonk et al., 2011) could provide an initial basis for such a risk assessment.

A number of other animal and public health risks have not been taken into consideration in this advice. Animal and public health risks that may arise due to antimicrobial resistance have not been taken into account. Animal health risks due to physical hazards such as glass or metal are also not included. Allergy-related animal health risks that may arise from feeding animals with feed originating from insects are not part of the risk assessment. Health risks to workers in insect farms, relating to occupational contact and inhalation allergy, also fall outside the scope of this risk assessment. After all, the risk of allergens is not specific to the use of FF as a rearing substrate, but to the use and rearing of insects in general. Earlier in 2014, BuRO had issued an advice regarding this to the Minister of Health, Welfare and Sport and the State Secretary for Economic Affairs (BuRO, 2014). Furthermore, the present advice does not address the risks associated with requirements or aspects concerning animal feed hygiene during the production of insects as animal feed raw material as laid down in the EU legislation[6,7] for the production of all animal feed, which are the preconditions for the rearing of insects.

Findings

The chemical and microbiological composition of insects is mainly determined by their diet. Hence, the possible chemical and microbiological risks to animal and public health due to the use of insects reared on FF as feed for farm animals are mainly determined by the chemical and microbiological agents that may be present in the substrate composed of FF. But these hazards may change during the production process.

The definition of FF implies that former foodstuffs have the same level of safety as regular food. However, food that is safe for humans may not be safe for animals.

Chemical risks

Concentrations of chemical contaminants in food, and therefore also in FF, are low and only occasionally exceed food safety norms.

Black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms may become contaminated to a greater or lesser extent during the rearing process with chemical agents present in the substrate on which they are reared. These chemical agents include mycotoxins, plant protection products, veterinary medical products, and environmental contaminants such as heavy metals, dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and flame retardants.

Accumulation[8] of harmful chemical contaminants in the larvae due to contaminated substrates can, to the best of our knowledge, only occur in case of

[6] 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 (General Food Law Regulation, GFLR).
[7] Regulation (EC) No. 183/2005 of the European Parliament and of the Council of 15 November 2005 laying down requirements for feed hygiene.
[8] Accumulation or bioaccumulation means that the concentration of a substance in insect larvae is higher than the concentration of the same substance in the substrate on which the insects are reared.

arsenic, heavy metals[9] such as cadmium, mercury and lead, dioxins and dioxin-like PCBs.

Although the larvae have the potential to accumulate contaminants from the substrate, the literature review has not shown that this leads to concentrations of these contaminants in insect larvae that are harmful to health. The contaminant concentrations in insect larvae are comparable to or lower than the concentrations of these substances measured in (former) foodstuffs. In view of the short lifespan of insect larvae and the limited extent to which FF exceed the maximum levels for these agents, the use of insects reared on FF as animal feed raw material will only lead to undesirable effects, i.e. a potential health risk, in very exceptional cases. Only in case of prolonged exposure of farm animals to exactly those insect products that exceed the maximum levels, there is a risk of undesirable effects. However, the burden of disease caused by chemical substances is very small and epidemiologically not demonstrable. The chemical safety of insects reared on FF is comparable to that of the FF on which they are reared and also comparable to that of other foodstuffs.

The presence of chemical contaminants in insects is controlled to a sufficient extent via the current control procedures for the presence of contaminants in (former) foodstuffs used in the substrate. However, attention also needs to be paid to the chemical agents that are known to accumulate in the insect larvae.

Microbiological risks
Food, and therefore also FF, may contain microbiological agents that are harmful to humans and/or animals, i.e. microorganisms[10] and prions[11]. Foods that are safe for humans may contain microbiological agents that are pathogenic to animals. This particularly applies to foods containing products of animal origin.

Insects are reared at high temperatures (26-32°C), which can lead to pathogenic microbiological agents in the FF substrate growing to concentrations that no longer meet safety standards for animal feed and food. However, this only applies to bacteria, yeasts and moulds, and not to viruses, parasites and prions.

Research shows that harmful microorganisms that may be present in the substrate can be transmitted to black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms. In case of prions, transmission from contaminated substrate to larvae of a different fly species - *Sarcophaga carnaria* - has been demonstrated. It is therefore likely that prions can also be transmitted via contaminated substrates to the larvae that are the subject of this study. It has been demonstrated that microorganisms and prions in the larvae maintain their biological activity and are able to colonise or infect larvae-fed animals.

These risks can be controlled for microorganisms by using an adequate germicidal treatment when making the end product (whole insect larvae, insect meal or

[9] Heavy metals are metals with a density of more than 5 g/cm^3.
[10] Microorganisms: 'bacteria, viruses, yeasts, moulds, algae, parasitic protozoa, microscopic parasitic helminths, and their toxins and metabolites' (Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for food). Helminths are worms.
[11] Prions are infectious proteins (Prusiner, 1998).

protein), by which the number of microorganisms is reduced to an acceptable level.

However, there are no suitable methods for inactivating prions in animal feed or food. Hence, the only way to control the chance of prion diseases, in order to ensure animal and public health, is to prevent the presence of prions in the FF on which insect larvae are reared.

Till now, prion diseases or TSEs[12] have only been detected under natural conditions in higher vertebrates (mammals). Among food-producing farm animals, this mainly concerns ruminants: cattle (BSE[13] or mad cow disease), sheep and goats (scrapie), cervids (chronic wasting disease, CWD). Prion diseases are (not yet) curable and invariably lead to a fatal outcome. However, prion diseases are almost never transmissible between different animal species, with the exception of BSE which can lead to the variant Creutzfeldt-Jakob disease (vCJD) in humans through the consumption of contaminated beef products. Naturally-occurring prion diseases have not yet been detected in non-ruminant farm animals or in fish, crustaceans and shellfish. Prions do not occur in insects.

The main reservoir of prions can be found in the brain, spinal cord, eyes, tonsils and parts of the intestines of ruminants. This specified risk material (SRM) is therefore removed from the feed and food chain. The reduction of TSE infectivity by removing SRM has been estimated to three orders of magnitude. In case of a low incidence of BSE in the bovine population, this residual low concentration of prions in ruminant meat or products thereof constitutes a negligible health risk for humans due to the species barrier[14]. The species barrier protects humans against infection. If this species barrier is absent, as in the case of materials derived from animals being used as feed for animals of the same species - a practice known as 'intra-species recycling' - this may lead to the accumulation of prions within the species and ultimately to an increased chance of disease. In other words, FF of animal origin considered safe for humans are not always suitable for feeding to every animal species, due to the hazard of prions.

The chance of the occurrence of prion diseases as a result of intra-species recycling is greatest in ruminant farm animals, but intra-species recycling in non-ruminant animal species could also, theoretically, lead to the accumulation of hitherto unknown prions in an animal population. However, there is uncertainty about the chance of this occurring. This depends on the lifespan of the susceptible animal species, infectivity of the prions and the dose of infectious material fed back to an animal population. Prion diseases have a long incubation period, as a result of which an infection of the population could go unnoticed until late in the process. For BSE, it has been calculated that, with the current level of surveillance[15], it will take at least 16 years before the reintroduction of BSE can be

[12] TSE: transmissible spongiform encephalopathy.
[13] BSE: bovine spongiform encephalopathy.
[14] The species barrier is a transmission barrier that limits the spread of prions between different species of animals; the size of the species barrier between cattle and humans is estimated to be 4000 (EFSA BIOHAZ Panel, 2006).
[15] In countries or regions with a negligible BSE risk, surveillance procedures are set up such that, with an assumed prevalence of at least 1 case per 50,000, the population of adult bovine animals in the country or region in question can be identified with a 95% reliability (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 (extended feed ban or the TSE Regulation); OIE, 2018).

detected. This allows the disease to spread easily unnoticed. Given the severity of prion diseases and the possibility of them being discovered too late after the prions have already spread within the susceptible animal species population, it is important to avoid intra-species recycling not only among ruminants but also among all farm animals.
Due to the species barrier between ruminants and non-ruminants, it is highly unlikely, but not impossible, that prions can be transmitted to non-ruminant farm animals via the consumption of ruminant materials. Since it is not possible to rule out the above risk, it is important to continue enforcing current EU legislation on the feeding of ruminants and the use of ruminant materials as animal feed.

Since the prions present in the substrate are transmitted to larvae that are cultivated on this substrate, animal feed consisting of larvae reared on substrate containing prions will contain prions. Therefore, there is a chance of the presence of prions if material from an animal species is recycled as feed, via passage in insects, within the same animal species. Whether this can lead to an accumulation of prions in a susceptible animal population is dependent on the lifespan of the animal species, the infectivity of the prions and the dose of infectious material fed back to an animal population.

Feeding farm animals with feed produced from larvae reared on substrates with FF derived from ruminant meat constitutes a risk for animal and public health due to the presence of prions. The magnitude of this risk is unknown. Feeding ruminants with feed produced from larvae reared on substrates with FF derived from ruminant meat creates the highest chance of infection. The chance of transmitting prions by feeding non-ruminants with insect larvae reared on FF containing ruminant meat is very small, although this cannot be excluded, due to the species barrier between ruminants and non-ruminants.

However, the use of substrates with FF derived from meat from non-ruminant animal species and from fish, crustaceans and shellfish poses a negligible risk of prions in relation to animal and public health, provided that the species being fed with the feed produced from insects is not a ruminant species and does not match the species in the FF. It is currently not possible to control this because there are no validated methods available for the detection and identification of DNA from non-ruminant farm animals in animal feed. Moreover, this requires the separation of FF flows by animal species.

Feeding farm animals with feed from larvae reared on substrates with FF of fully plant origin, FF from dairy, eggs, honey and rendered fat, and FF containing collagen of gelatine from non-ruminants does not pose a risk to animal and public health, because these substrates do not contain any prions.

The above risk assessment is independent of what is laid down in legislation. However, the EU legislation applies as follows to the rearing of insects on FF containing components of animal origin and to the use of insects as animal feed raw material for farm animals (regardless of the substrate on which the insects are reared):

- The Animal By-Products (ABP) Regulation[16] only allows the direct[17] (without prior processing) feeding of FF containing animal components to farm animals if these animal components are derived from dairy, eggs, honey, rendered fat, collagen and gelatine (see Regulation (EU) No. 142/2011[18]). The TSE Regulation[19] only allows the use of collagen and gelatine from non-ruminant farm animals.
- Insects are farm animals according to the ABP Regulation and, if used as animal feed, may only be fed with the above-mentioned FF.
- The ABP Regulation sets out conditions for the handling of certain animal by-products, including insects, before they can be used in/as animal feed, for example, that these by-products must first be processed using high-pressure sterilisation (see Regulation (EU) No. 142/2011).
- The TSE Regulation currently only allows a limited number of insect species[20] to be processed as animal proteins to be fed to aquaculture animals.

Just like all other farm animals, insects may be fed with FF of fully plant origin.

Answers to the research questions

1. *What are the main risks to animal and public health of insects that are reared on FF and that are processed into animal feed for farm animals?*

The chemical risks to animal and public health arising from the feeding of farm animals with feed produced from black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms that have been reared on a substrate composed of FF of plant and/or animal origin are comparable to the chemical risks of (former) foodstuffs, and therefore negligible.
However, feeding farm animals with this type of animal feed does pose microbiological risks to animal and public health. These risks relate to specific pathogenic microorganisms and prions that may be introduced into the insects via the FF.

Pathogenic microorganisms that may occur in FF present a potential risk when using FF of both plant and animal origin.

There is no risk of prions in insects fed with FF of fully plant origin, FF containing animal components derived from dairy, eggs, honey and rendered fat, and FF with

[16] 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 or the ABP Regulation).
[17] 'Direct feeding of FF' means that the FF have not been processed prior to being used as feed in the manner prescribed by Regulation (EU) No. 142/2011 for all Category 3 animal by-products intended as feed material for farm animals.
[18] 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 concerning certain samples and products exempt from veterinary checks at the border under this Directive.
[19] 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 (extended feed ban or the TSE Regulation).
[20] This is limited to the following insect species: black soldier fly (*Hermetia illucens*), housefly (*Musca domestica*), common mealworm beetle (*Tenebrio molitor*), lesser mealworm beetle (*Alphitobius diaperinus*), home cricket (*Acheta domesticus*), tropical house cricket (*Gryllodes sigillatus*) and Jamaican field cricket (*Glyllus assimilis*).

collagen and gelatine from non-ruminant animal species. These products are free of prions. When using FF containing products derived from animals other than those mentioned above (i.e. meat from non-ruminants, and fish, crustaceans and shellfish), the risk of prions is determined based on the animal species used in the FF on which the insects are reared, combined with the species to which these insects are fed. The risk of prions is highest if intra-species recycling takes place via passage in the larvae.

2. *How can these risks be adequately managed?*

Animal and public health risks arising from exposure to chemical agents are adequately controlled via the current control of contaminants in (former) foodstuffs that are used in the substrate. However, attention must be paid to harmful chemical agents, such as arsenic, cadmium, mercury, lead, dioxins and dioxin-like PCBs, that are known to accumulate in insect larvae.

The risks of pathogenic microorganisms in insects reared on a substrate composed of FF of plant and/or animal origin can be adequately controlled through an effective germicidal treatment of the end product. 'Adequate control' means that the end products must meet the microbiological safety standards applicable to (the currently permitted) processed animal proteins and other feed materials derived from animal by-products.

When using substrates with animal components other than those derived from FF containing dairy, eggs, honey or rendered fat, or FF containing collagen and gelatine from non-ruminant species (i.e. FF with meat from non-ruminants, and fish, crustaceans and shellfish), the risk of prions can be controlled by ensuring that the FF, which is used for feeding the insect larvae, does not contain any ruminant products; that the species fed with the feed produced from insects is not a ruminant; and that the non-ruminant animal species being fed is not the same as the non-ruminant animal species in the FF on which the insects are reared.

Advice of BuRO

To the Minister of Agriculture, Nature and Food Quality

- Based on this risk assessment, propose to the European Commission (EC) to make a further adjustment in the applicable legislation to allow for the following:
  - In addition to the permitted use of insects as processed animal proteins (within the meaning of the ABP Regulation) as raw material for animal feed for aquaculture animals, black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms may be fed to all non-ruminant farm animals, and adequate germicidal treatments other than those currently prescribed in the ABP Regulation may also be used.
  - These insect larvae may be reared on substrates containing FF with meat from non-ruminant animals, and fish, crustaceans and shellfish, on the condition that the proteins in the FF are not derived from the same animal species that is fed with the animal feed consisting of insects.

Office for Risk Assessment & Research

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To the Inspector-General of the NVWA

- Ensure specifically that insect-based products used as raw material for animal feed comply with the microbiological safety standards applicable to processed animal proteins and other feed materials derived from animal by-products under the current legislation applicable to the rearing of insects on FF and the use of insects as a feed raw material for farm animals.
- Ensure the traceability of the animal species in FF derived from meat from non-ruminant animals, and fish, crustaceans and shellfish for the rearing of insect larvae as animal feed, as soon as the legislation allows this.
- Monitor developments in the insect sector to maintain a good overview of potential new risks arising from the introduction of multiple types of insects and production methods, such as substrates, resources and veterinary medicines.

Yours sincerely,

Prof. Antoon Opperhuizen
Director of the Office for Risk Assessment & Research

SUBSTANTIATION

Insects in the animal feed chain

There are more than 2000 edible insect species worldwide (Jongema, 2017). Of these, crickets (house cricket, banded cricket, African migratory locust and American desert locust), mealworms (yellow mealworm, lesser mealworm and morio worm), fly larvae (larvae of the black soldier fly and housefly) and caterpillars (silkworm, wax moth caterpillars and lesser wax moth) have the greatest potential to be used as food or feed material (ANSES, 2015; BuRO, 2014; EFSA SC, 2015; FASFC, 2014).

Insects are a source of high-quality proteins, fats, vitamins, minerals and trace elements (Rumpold & Schlüter, 2013a; Rumpold & Schlüter 2013b). Insects reared on organic residual and waste streams have a lower ecological footprint in terms of greenhouse gases and water and land use compared to traditional sources of protein (Gaffigan, 2017; Oonincx & de Boer, 2012). Farmers, agriculture sector stakeholders and consumers are positive about the use of insects in animal feed (Verbeke et al., 2015).

The black soldier fly (*Hermetia illucens*), housefly (*Musca domestica*), yellow mealworm beetle (*Tenebrio molitor*) and lesser mealworm beetle (*Alphitobius diaperinus)* have a short reproductive cycle and their larvae can be reared successfully on low-grade organic residual and waste streams (Čičková et al., 2015; Diener et al., 2009; Diener et al., 2011; Nguyen et al., 2015; Oonincx et al., 2015; van Broekhoven et al., 2015; van Huis, 2016; Wynants et al., 2018b) to serve as high-quality sources of proteins and fats (Table 1). It also appears that the larvae of these insects are suitable for large-scale industrial production (Ferri et al., 2019; van Huis, 2013; van Huis et al., 2013; Veldkamp et al., 2012). This makes these insect species particularly suitable for being reared by feeding on FF so that they can be used as a raw material for the animal feed industry. The risk assessment will therefore be limited to the larvae of these insects.

Table 1.
Protein and fat content (based on dry matter) of larvae of four insect species that are highly suitable for large-scale industrial production.

Insect species	% Protein	% Fat	Reference
Alphitobius diaperinus	45	42	Siemianowska et al., 2013
Hermetia illucens	42	35	Müller et al., 2017
Musca domestica	60-64	20-24	Hussein et al., 2017; Rumpold & Schlüter, 2013a
Tenebrio molitor	47-49	36-43	Rumpold & Schlüter, 2013a

Former foodstuffs

The EU Catalogue of Feed Materials[21] defines former foodstuffs (FF) as: 'foodstuffs, other than catering reflux[22], which were manufactured for human consumption in full compliance with the EU food law but which are no longer intended for human consumption for practical or logistical reasons or due to problems of manufacturing or packaging defects or other defects and which do not

[21] Commission Regulation (EU) No. 68/2013 of 16 January 2013 on the Catalogue of Feed Materials.
[22] Kitchen and food waste.

present any health risks when used as feed'. This definition includes FF of animal origin, FF containing products of animal origin, and FF of fully plant origin.

Examples of FF are incorrectly packed, labelled, shaped or flavoured foods, surpluses of seasonal foods, surpluses due to the discontinuation of a food production line or defects in the food (BuRO, 2011; EFFPA, 2014; RIVM-RIKILT, 2010; Pinotti et al., 2019).

The definition of FF implies that the health risk of food downgraded to FF is the same as that of regular food because, just like regular food, FF must also meet the requirements of EU food law. Hence, FF offer the same level of safety as regular food. A number of products of animal origin may be safe for human consumption but not safe for animal health, for example, because they may contain pathogens that affect animals and not humans. In particular, this applies to foods that (partly) consist of products of animal origin.

The use of FF of animal origin or containing products of animal origin as (raw material for) animal feed is strictly regulated in the EU (see: current legislation and regulations for the use of FF as animal feed raw material). As a result, at present, mainly FF originating from energy-rich foods such as cake, bread, biscuits, chocolate bars, breakfast cereals, pasta, savoury snacks and sweets (Bouxin, 2012; EFFPA, 2014; Pinotti et al., 2019; Schripsema et al., 2015; Tretola et al., 2017a; Tretola et al., 2017b) are used as animal feed raw material. These FF are processed by waste processing companies and animal feed producers. Depending on the type of FF (i.e. packaged *versus* bulk or dry *versus* moist *versus* liquid), upgrading of FF to animal feed raw material is done through a combination of different processes such as collecting, unpacking, mixing, grinding, and drying (Bouxin, 2012; Tretola et al., 2017a; van Raamsdonk et al., 2011). These process steps also have an effect on the safety of FF (Tretola et al., 2017a). The processed product is delivered either as-is or mixed with other products directly to farmers.

Currently, the status of FF is clearly indicated as 'non-waste'. As part of the EU strategy to reduce food waste (part of the EU action plan for a circular economy), the European Commission (EC) will take measures together with the Member States to clarify EU legislation on waste, food and feed materials and facilitate the use of FF in the production of feed without endangering the safety of the feed materials (EC, 2015). Recently the EC has published a document[23] with guidelines for the use, as animal feed, of food that is no longer intended for human consumption, which includes not only FF but also residual and by-products from the food industry.

Current legislation and regulations for the use of FF as animal feed raw material

The use of FF of animal origin or containing products of animal origin as animal feed is legally laid down in the ABP Regulation (Regulation (EC) No. 1069/2009)[24]

[23] 2018/c 133/02. Communication from the Commission. Guidelines for the use of food no longer intended for human consumption as feed material.

[24] 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).

and the Regulation implementing the ABP Regulation (Regulation (EU) No. 142/2011)[25].

Former foodstuffs of animal origin or containing products of animal origin fall under Category 3 material animal by-products according to the ABP Regulation. This means that FF containing components of animal origin may only be fed directly[26] to animals that are not part of a food chain, except in the case of animal-origin components derived from dairy, eggs, honey, rendered fat, collagen and gelatine which must be processed as defined in the regulation on the hygiene of foodstuffs (Regulation (EC) No. 852/2004, Article 2(1)(m)[27]. The TSE[28] Regulation (Regulation (EC) 999/2001[29]) only allows the use of collagen and gelatine from non-ruminant farm animals (Appendix IV, Chapters I and II).

Current legislation and regulations for the use of insects as animal feed raw material

EU legislation is applicable to the rearing of insects on FF of animal origin and the use of insects as animal feed raw material for farm animals[30] (regardless of the substrate on which the insects are reared):

- Insects are farm animals according to the ABP Regulation and may only be fed directly with FF containing animal components if these are derived from dairy, eggs, honey, rendered fat, collagen and gelatine (see Regulation (EU) No. 142/2011, Annex X, Chapter II, Section 10). The TSE Regulation only allows the use of collagen and gelatine from non-ruminant farm animals (Appendix IV, Chapters I and II)
- The ABP Regulation sets out conditions for the handling of certain animal by-products, including insects, before they can be used in/as animal feed, for example, through high-pressure sterilisation (Regulation (EU) No. 142/2011, Annex IV, Chapter III and Annex X, Chapter II).
- The TSE Regulation currently only allows a limited number of insect species[31] to be processed as animal proteins to be fed to aquaculture animals.

[25] 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 concerning certain samples and products exempt from veterinary checks at the border under this Directive.

[26] 'Direct feeding of FF' is understood to mean that the FF have not been processed prior to being used as feed in the manner prescribed by Regulation (EU) No. 142/2011 for all Category 3 animal by-products intended as feed material for farm animals.

[27] Processing: 'any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes' (Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs).

[28] TSE: transmissible spongiform encephalopathy.

[29] 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 (extended feed ban or the TSE Regulation).

[30] The term 'farm animal' as referred to in this advice means farm animals other than furred animals. The issued advice relates to food-producing animals, i.e. animals that enter the human food chain either via direct consumption or via products made from these animals. This also includes aquaculture animals.

[31] This is limited to the following insect species: black soldier fly (*Hermetia illucens*), housefly (*Musca domestica*), common mealworm beetle (*Tenebrio molitor*), lesser mealworm beetle (*Alphitobius diaperinus*), home cricket (*Acheta domesticus*), tropical house cricket (*Gryllodes sigillatus*) and Jamaican field cricket (*Glyllus assimilis*).

The NVWA website includes an overview summarising which farm animal species may be fed with processed insect proteins, which animal products may be fed to insects, as well as the conditions under which this is permitted (NVWA, 2019).

The legislation and regulations described above (the ABP Regulation and TSE Regulation) aim to prevent or minimise the potential risks to animal and human health.

The ABP Regulation came into being after the outbreaks of foot-and-mouth disease, the BSE and dioxin crises, all of which arose due to the use of animal by-products (whether or not FF of animal origin) as animal feed.

The legislation on TSEs originated after the outbreaks of BSE or the mad cow disease in the 1990s. BSE is a fatal prion disease[32] in cattle caused by feeding them meat-and-bone meal derived from cattle infected with BSE. The feeding of animals with feed material derived from their own species (known as 'intra-species recycling'*),* in this case of bovine proteins, created a so-called 'feedback loop' (Figure 1). As a result, cattle were constantly exposed to BSE prions, causing accumulation in bovine populations, which in turn increased the transmission of BSE and eventually led to an epidemic.

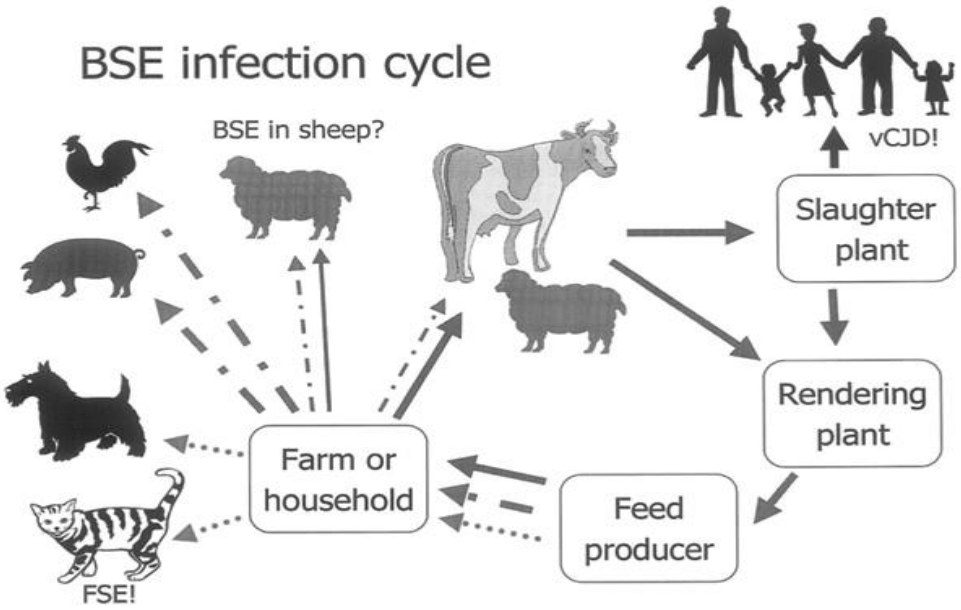

Figure 1.
BSE infection cycle and exposure of other species to products of bovine origin. Solid arrows (-) indicate direct exposure to products derived from cattle (food, animal feed), dashed arrows (-.-.) indicate exposure to animal feed for pigs or poultry, and dotted arrows (.....) indicate exposure to animal feed produced for pet animals (dogs and cats). Figure from Doherr, 2003.

[32] A prion is an infectious protein encoded by the Protease resistant Prion (PrP) gene. PrP^{c} is the cellular prion, PrP^{Sc} is the pathological isoform (Prusiner, 1998). The name 'prion' comes from ***pro****teinaceous* ***in****fectious particle*, actually 'proin', but renamed 'prion' by discoverer Stanley B. Prusiner because this sounds more melodic.

BSE also turned out to be a fatal zoonosis[33] that causes the variant Creutzfeldt-Jakob disease (vCJD) in humans (Bruce et al., 1997; Prusiner, 1998; Scott et al., 1999).

To control BSE and other TSEs, a ban on the use of animal protein derived from mammalian tissues for feeding ruminants such as cattle, sheep and goats has been in effect in the EU since 1994 (feed ban, Decision 94/381/EC[34]). In 2001, this ban was extended with a total ban on the feeding of processed animal proteins to all farm animals (extended feed ban or the TSE Regulation, Regulation (EC) No. 999/2001), with a few exceptions. This was because the permitted use of animal proteins in the feed of other animal species (for example, pigs and poultry) could lead to the cross-contamination of feed intended for ruminants (Simmons et al., 2018). This cross-contamination was a persistent problem in animal feed factories.
It has also been determined that SRM[35], i.e. tissues from cattle, sheep and goats with the highest TSE infectivity, must be removed and disposed of after slaughter, so that they do not enter the feed and food chain. SRM includes the spinal cord, brain, eyes, tonsils and parts of the intestines. The TSE Regulation not only prevents prions from accumulating in the animal populations and causing a burden of disease in these populations, but it is also effective at greatly reducing the spread of TSEs to humans.

When this legislation was adopted, insects were not yet in the picture as a protein source or as a farm animal. This has led to a situation where the feeding of insects as processed protein is limited by legislation, but there is no legislation for the feeding of live insects to farm animals.

Just like all other farm animals, insects may also be fed with FF of fully plant origin.

Production process of insect larvae
Insects produced in insect farms are reared in a closed and standardised environment. Here, exposure to harmful chemical and biological agents can be controlled better than in their natural environment (Belluco et al., 2013).

In general, the production process of insect larvae that are reared on FF to be used as animal feed raw material, consists of processing the FF into substrate for insect farming, rearing the larvae on the substrate, and then processing the larvae to form the end product. This end product may consist of the whole larvae, a larval preparation (insect meal) or a larval fraction (protein or fat). The end product is then further processed into animal feed, placing it at the start of the feed and food chains (Figure 2).

[33] A zoonosis is any disease or infection that is naturally transmissible between vertebrates and humans (WHO, 2019).
[34] Decision 94/381/EC. Commission Decision of 27 June 1994 on certain protective measures with regard to bovine spongiform encephalopathy and the feeding of protein derived from mammalian tissues (feed ban).
[35] SRM: specified risk material.

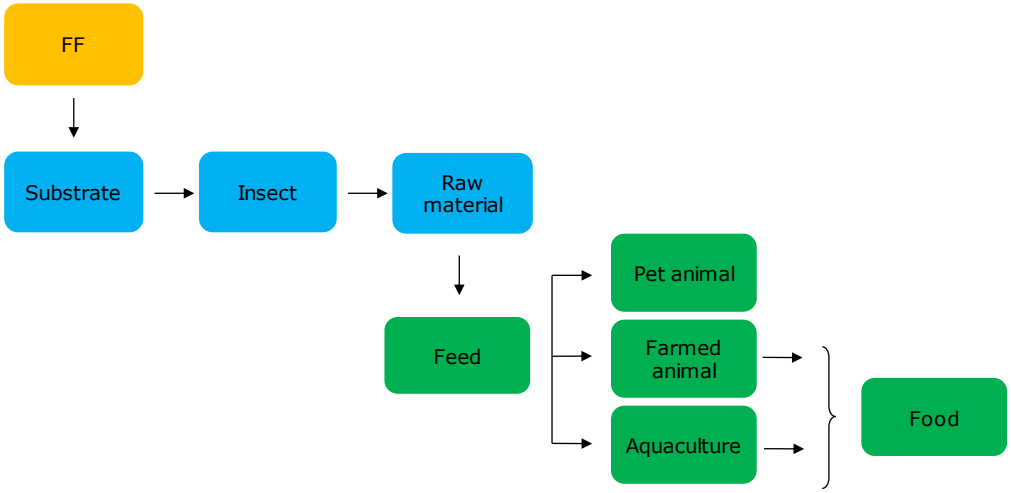

Figure 2.
Schematic representation of the production process of insects reared on FF to be converted into animal feed raw material and the further route within the feed and food chains. Fur animals and pet animals fall outside the scope of this risk assessment.

The various steps or techniques in the production process described below are not an exhaustive list of all the possible methods that can be used.

Processing of FF into substrate
The processing of FF into substrate for the rearing of insects is done in the same way as in the case of FF that is used as feed material for other farm animals. This is a combination of different processes such as collecting, unpacking, mixing, grinding, and drying, depending on the type of FF, i.e. packaged *versus* bulk or dry *versus* moist *versus* liquid (Bouxin, 2012; Tretola et al., 2017a; van Raamsdonk et al., 2011).

Rearing of insect larvae on substrate
Rearing conditions
Black soldier fly larvae are reared at a temperature of 26-32ºC, at a relative humidity (RH) of 65-70% in 2-6 weeks (Diener et al., 2009; Hilkens & de Klerk, 2016; Oonincx et al., 2015; Sheppard et al., 2002; Tomberlin et al., 2002). Housefly larvae are reared in 1-2 weeks at a temperature of 26-30ºC and an RH of 60-70% (Hogsette, 1992a; Hogsette, 1992b; Hussein et al., 2017; Keiding & Arevad, 1964). Yellow mealworms and lesser mealworms are reared at a temperature of 28-30ºC and 50-70% RH. Yellow mealworm rearing takes 5-10 weeks and lesser mealworms can be reared in 3 weeks (BuRO, 2014; Hilkens & de Klerk, 2016; Oonincx et al., 2015; Osimani et al., 2018; van Broekhoven et al., 2015; Wynants et al., 2018a).

Fly larvae are usually reared on moist substrates, while mealworms are usually reared on dry substrates (Ferri et al., 2019; IPIFF, 2019).

Most insect producers rear insects using batch farming. Here, the insects are monitored at the batch level during the entire rearing process. They are hatched in a single batch in a container, reared in the same container, and eventually also processed as a single batch into the end product. For the sake of continuity,

several batches of insects are reared side by side, but the batches remain separate from each other throughout the entire rearing period. The advantage of batch farming (compared to continuous farming) is that pathogens remain localised and any bad batches can be removed from the production process, so that the entire batch does not get contaminated.

Processing insect larvae into animal feed raw material

Harvest

The processing of insect larvae into animal feed raw material starts with the harvesting of the last larval stage, where the larvae are separated from the rearing residues (substrate, dead larvae, exuviae, pupae, faeces). In the case of the black soldier fly and the housefly, this can be done based on the migration behaviour of the last larval stage, when the larvae crawl from the wet substrate to a dry environment in a collection container (Diener et al., 2011; Hogsette, 1992a; IPIFF, 2019). Housefly larvae can also be separated from the substrate by lowering the oxygen concentration in a closed container (Hogsette, 1992a; IPIFF, 2019). After separation from the substrate, the larvae can be easily harvested by sieving manually or mechanically. Larvae can also be harvested directly from the substrate via sieving, as in the case of a number of yellow mealworm and lesser mealworm farms (BuRO, 2014; Wynants et al., 2018a). It is also possible to harvest them mechanically on vibratory plates, where the larvae are separated from the rearing residues, after which they are sieved via an air stream.

Pre-treatment

Insect larvae are processed in their entirety, without first removing the gut. Larvae can be rinsed with water after harvesting, if necessary after removal of the gut contents. For example, yellow mealworms and lesser mealworms are kept without food for a few hours to a few days after harvesting, so that they empty their guts (ANSES, 2015; BuRO, 2014; Wynants et al., 2017; Wynants et al., 2018a).

Killing

The method of killing most commonly used by insect farmers is freezing or freeze-drying (Erens et al., 2012; Ferri et al., 2019). Other suitable methods are cooking, steaming, blanching and grinding (ANSES, 2015; Erens et al., 2012; Hakman et al., 2013). Before grinding, larvae can be inactivated by cooling or incubating them in nitrogen, as in the case of yellow mealworms (Stoops et al., 2016; Wynants et al., 2017).

Processing

Freeze-drying is used to extract water from insects before they are ground. Depending on the method of killing, a drying step by, for example, heating them in an oven may be required during the further processing of the larvae in order to remove water and prevent microbial growth (ANSES, 2015; Charlton et al., 2015; IPIFF, 2019; Rumpold & Schlüter 2013b).

The insects are usually ground into insect meal (IPIFF, 2019).

Protein, fat/oil and chitin fractions can be obtained by using physical (e.g. centrifugation), chemical and biochemical extraction methods. Mechanical separation (pressing), heat treatment or organic solvents can be used to obtain fat/oil fractions. The dry residue that remains is fully defatted insect meal. Chitin extraction requires chemical and/or enzymatic processing (IPIFF, 2019).

Risk assessment

This risk assessment examines the chemical and microbiological risks to both animal and public health originating from chemical and microbiological agents present in animal feed produced from insects reared on substrates composed of FF. Specific control measures have been formulated for these risks.

In the production chain from insect larvae to animal feed raw material (Figure 2), insects can serve as a potential source of harmful chemical and microbiological agents transmitted from the substrate, as a result of which these hazards may possibly be passed on further in the animal feed and food chain (ANSES, 2015; Schlüter et al., 2017). These agents can enter the insects via the FF substrate.

The risk assessment follows the route taken by potentially harmful agents present in FF through the various steps of the production chain.
Feed hygiene requirements/aspects during the production of insect products as animal feed raw material, as laid down in the legislation[36,37] for the production of all feed materials, are preconditions for the rearing of insects as animal feed raw material. Hence, the risks associated with this are not included in this risk assessment.

Methodology
The assessment of the chemical and microbiological risks is carried out based on a hazard identification, hazard characterisation, exposure assessment and risk characterisation as defined by the Codex Alimentarius Commission (FAO & WHO, 2015).

To assess whether the use of insects reared on FF poses chemical or microbiological risks to animal and human health if these insects are used as raw material for animal feed, the following data are collected for the first three steps of the risk assessment:
- Hazard identification: data for identifying chemical and microbiological agents that are harmful to animals and/or humans that may occur in FF
- Hazard characterisation: data on how these hazards change while rearing insects on substrates composed of FF, i.e. whether there is an increase or decrease in the agents in the substrate, whether the agents can be passed on to the insects via the substrate, the extent of increase or decrease of the hazards in the insects, whether the microbiological hazards remain infectious and whether the insects can act as vectors of microbiological agents
- Exposure assessment: data on how often and to what extent the hazards occur in the reared insects

The above data forms the basis for the last step of the risk assessment:
- Risk characterisation: an assessment of the chemical and microbiological risks posed to animal and public health, including uncertainties, based on the probability (chance) of the occurrence of the hazards and the nature and severity of known or potential adverse health effects.

[36] 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 (General Food Law Regulation).
[37] Regulation (EC) No. 183/2005 of the European Parliament and of the Council of 15 November 2005 laying down requirements for feed hygiene.

Chemical risks

Chemical agents in (former) foodstuffs
(Hazard identification)

Foods that are downgraded as FF do not (according to the definition) present an increased chemical risk to public health compared to other foodstuffs, since they are required to comply with the maximum limits defined in EU food legislation. These include limits for mycotoxins[38], other chemical contaminants, and residues of veterinary medical products and plant protection products[39,40,41,42]. Therefore, the extent to which harmful chemical agents occur in FF is the same as that in other foods.

The most important categories of potentially harmful chemicals and the (former) foodstuffs of animal or plant origin in which they can be found are shown in Table 2.

Table 2.
Categories of potentially harmful chemical agents for animals and/or humans in (former) foodstuffs and their sources.

Category	Type of substance	Source[a]
Natural toxins	Mycotoxins (incl. aflatoxins, deoxynivalenol (DON), fumonisins, ochratoxin A (OTA), zearalenone (ZEA))	Plants, mainly grains (wheat, corn, rice), nuts, figs
Plant protection products	Fungicides, insecticides, herbicides	Residues on crops
Veterinary medical products	Medicines (including antibiotics), growth promoters	Residues in meat
Environmental contaminants	Heavy metals[b] (including cadmium, mercury, lead) and arsenic[c]	Fish, crustaceans and shellfish, organ meat, field-grown vegetable products (vegetables, legumes, grains)
	Dioxins and polychlorinated biphenyls (PCBs)	Animal oils and fats
	Polycyclic aromatic hydrocarbons (PAHs)	Vegetable oils
	Brominated flame retardants	Animal products

[a] van Kreijl & Knaap (2004) and the Netherlands Nutrition Centre (2019).
[b] Heavy metals are metals with a density of more than 5 g/cm^3.
[c] Arsenic is a metalloid that is often classified as a heavy metal.

[38] Mycotoxins are toxins that are produced by certain moulds (fungi). The most harmful mycotoxin is the carcinogenic aflatoxin formed by *Aspergillus flavus* and *Aspergillus parasiticus*.
[39] Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
[40] Commission Regulation (EU) No. 37/2010 of 22 December 2009 on pharmacologically active substances and their classification based on maximum residue limits in foodstuffs of animal origin.
[41] Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EC.
[42] EU Pesticides Database (EC, 2019).

Mycotoxins
NVWA's mycotoxin monitoring programme, in which various plant-origin products or plant-based foods for the presence of various mycotoxins were samples and examined, found exceedances of around 3% from defined EU limits in the period 2013-2017 (NVWA, 2014; NVWA, 2015a; NVWA, 2015b; NVWA, 2016a; NVWA, 2017a, NVWA, 2018c). These exceedances were found in nuts, seeds, dried subtropical fruits, herbs and spices. In the other product groups studied, including grains and grain products (including cake residues), no deviations or very low percentages of deviations were found.

Plant protection products
The number of exceedances of the maximum permitted levels in the EU for residues of plant protection products in food (mostly of plant origin) originating from the Netherlands and the rest of the EU is very small, i.e. between 2 and 3%. Exceedances are mainly found in products from third countries, with non-compliance percentages between 5 and 8% (EFSA, 2017a; NVWA, 2015c; NVWA 2015f).

Veterinary medical products and environmental contaminants
In the Netherlands, veterinary medical products and environmental contaminants are found only occasionally in food products of animal origin, usually in low concentrations. In the period 2014-2017, the results of 0.2% of the analyses were not in compliance with the set EU limits (NVWA, 2015d; NVWA, 2016b; NVWA, 2017a, NVWA 2018c). In most cases, these involved illegal growth-promoting hormones and antibiotics and in some cases, environmental contaminants (including lead and cadmium). The total number of non-compliant results has remained stable in recent years. This trend is similar to the findings at a European level. In the EU, the percentage of samples with non-compliant results was 0.4% in 2014 (EFSA, 2016a). In 2015 and 2016, this was 0.3% (EFSA, 2017c; EFSA, 2018a).

Environmental contaminants (dioxins and PCBs)
In the period 2001-2011, the fraction of samples in the Netherlands that did not meet the European maximum levels for dioxins and dioxin-like polychlorinated biphenyls (PCBs) was below 1% for most food products of animal origin, with the exception of lamb (Adamse et al., 2017b). In the EU in the period 1995-2010, the levels of dioxin and dioxin-like PCBs and those of non-dioxin-like PCBs in food of both animal and plant origin were, respectively, 10 and 3% above the maximum permitted levels. However, a decrease in the levels of contamination by dioxins and PCBs has been observed over the years (EFSA, 2012). More recent data could not be found.

The results of the above studies indicate low concentrations of mycotoxins, plant protection products, veterinary medical products, and environmental contaminants in food, and therefore also in FF. The limits are only occasionally exceeded.

Chemical agents in substrate and in insects during the rearing process (Hazard characterisation)
The concentrations of chemical agents in the substrate do not increase during the rearing of the insects. However, chemical agents may be transmitted to the insects via the substrate and as a result their level of concentration may increase in the insects (ANSES, 2015). This is known as (bio)accumulation[43].

The fact that the chemical composition of the insect feed determines the chemical composition of the insects themselves has also been demonstrated for black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms (Barlow, 1966; Latney et al., 2017; Oonincx et al., 2015; Tschirner & Simon, 2015; van Broekhoven et al., 2015). It is possible that, similar to other animal products, chemical agents from the feed accumulate in insect larvae (BuRO, 2014; Poma et al., 2017).

The black soldier fly, housefly, lesser mealworm beetle and yellow mealworm beetle have a short life cycle compared to other farm animals. Their larvae can be reared within a few weeks to few months. For insects with such a short life cycle, and therefore with limited repeated feeding, the accumulation of substances from the feed in these insects (bioaccumulation factor[44], BAF>1) is less likely to occur than in insects that are reared over a longer period of time (EFSA SC, 2015; Ferri et al., 2019) or in other farm animals.

Several studies are described below involving black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms which have examined possible accumulation in insects when they were reared on substrates that were (in almost all cases, experimentally) contaminated with chemical agents, including mycotoxins, plant protection products, veterinary medical products, and environmental contaminants (including heavy metals). In most studies, experimentally contaminated substrates were used or substrates that were known to be contaminated, such as manure or seaweed.

<u>Mycotoxins</u>
In black soldier fly larvae that were reared on a substrate to which the mycotoxins aflatoxin B1/B2/G2 (AfB1/B2/G2), deoxynivalenol (DON), ochratoxin A (OTA) or zearalenone (ZEA) were added, none of the mycotoxins tested in the study were found in the insect larvae (Purschke et al., 2017).

Camenzuli et al. (2018) also showed that there was no bioaccumulation of AfB1, DON, OTA or ZEA in black soldier fly larvae or in lesser mealworms reared on experimentally contaminated substrate. However, DON, OTA and ZEA were detected in the larvae.

In a study in which black soldier fly larvae and yellow mealworms were reared on a substrate of chicken feed to which AfB1 had been added, the mycotoxin did not accumulate in the larvae (Bosch et al., 2017). AfB1 could be detected in the mealworms, but not in the larvae of the black soldier fly.

[43] Accumulation or bioaccumulation means that the concentration of a substance in insect larvae is higher than the concentration of the same substance in the substrate on which the insects are reared.
[44] The bioaccumulation factor (BAF) or bioconcentration factor (BCF) is the ratio between the concentration of a substance in an organism and the concentration of this substance in the food/ingested water (Diener et al., 2015).

In yellow mealworms reared on a wheat substrate that had been experimentally or naturally contaminated with a high DON concentration, this mycotoxin could only be detected in the mealworm faeces and not in the larvae (van Broekhoven, 2015; van Broekhoven et al., 2017). In another study where yellow mealworms were reared on grain contaminated with DON (2 to 12 mg/kg), lower concentrations of DON ranging from 0.1 to 0.2 mg/kg were found in the mealworms after 24 hours of fasting (Ochoa Sanabria et al., 2017).

A study by Guo et al. (2014) in which yellow mealworms were fed with grains containing high concentrations of beauvericin, DON, enniatin A/A1/B/B1 (ENNA, ENNA1, ENNB, ENNB1), fumonisin B1 (FB1) or ZEA, the mycotoxin concentrations were low in the larvae compared to the concentrations in the grains, with the exception of ENNA (30 µg/kg) in the substrate *versus* 10 µg/kg in the larvae (BAF 0.33). DON was not detected in the larvae.

Abado-Becognee et al. (1998) demonstrated the presence of FB1 in yellow mealworms reared on corn flour experimentally contaminated with FB1, with approximately 40% of the ingested FB1 being excreted via the faeces.

Yellow mealworms reared on wheat flour contaminated with ZEN did not show the presence of any ZEN or ZEN metabolites. However, ZEN metabolites were found in the substrate residue, indicating intensive metabolism of ZEN in the larvae (Niermans et al., 2019).

Plant protection products

In a study involving black soldier fly larvae, where the insecticides chlorpyrifos, chlorpyrifos-methyl and pirimiphos-methyl were added to the substrate, it was found that the concentrations of these insecticides in the larvae were at least 50 times lower than the initial concentrations of these agents in the substrate (BAF<0.02) (Purschke et al., 2017). In black soldier fly larvae reared on a substrate experimentally contaminated with the fungicides azoxystrobin and propiconazole, no bioaccumulation of these fungicides was demonstrated. Neither fungicide was detected in the larvae (Lalander et al., 2016).
Chlorpyrifos was also found in yellow mealworms that had been pinned down on trees in a citrus orchard treated with chlorpyrifos, but here the concentration in the mealworms remained well below the initial concentration (BAF<1) (Brewer et al., 2003).

There is no bioaccumulation of the chiral[45] fungicides benalaxyl (BAF 0.01-0.05), diniconazole (BAF 0.015-0.1), epoxiconazole (BAF 0.01-0.09), furalaxyl (BAF 0.02-0.06), metalaxyl (BAF 0.015 -0.02) and myclobutanil (BAF 0.08-0.09) in yellow mealworms (Gao et al., 2013; Gao et al., 2014; Liu et al., 2013, Lv et al., 2013; Lv et al., 2014; Yin et al., 2017).

In yellow mealworms reared on carrots experimentally contaminated with a mixture of 12 plant protection products, four of these agents (2,4-D, bentazone, bifenthrin and clopyralid) were not found in detectable concentrations and the

[45] Chirality is a property of molecules that exhibit stereoisomerism. These molecules have the same structural formula but a different spatial structure.

remaining eight (diflufenican, fenpropimorph, isoproturon, linuron, mefenoxam (R-enantiomer of metalaxyl), pendimethalin, pyrimethanil and tebuconazole) were well below the initial concentrations in the substrate (Houbraken et al., 2016). This study also showed that starving the larvae (24 hours) reduces the amount of plant protection products in the larvae, which indicates the presence of these agents in the gut contents of the larvae.

Veterinary medical products

Although transmission of the antibiotics doxycycline, lincomycin and sulfadiazine from the substrate (manure) to black soldier fly larvae was observed, there was no accumulation of these antibiotics in black soldier fly larvae (Van Linden et al., 2017). Lalander et al. (2016) observed no transmission of the antibiotic roxythromycin from the substrate to these larvae, but did see the transmission of trimethoprim, with no bioaccumulation. The drug carbamazepine was also not detected in the larvae (Lalander et al., 2016).

Environmental contaminants

Heavy metals and other chemical elements

In black soldier fly larvae, housefly larvae and yellow mealworms reared on substrates experimentally or naturally contaminated with the heavy metals cadmium, chromium, cobalt, copper, iron, lead, mercury, nickel and zinc, or with arsenic and selenium, the presence of all of these chemical elements was demonstrated in the larvae (Bulak et al., 2018; Bednarska & Świątek, 2016; Biancarosa et al., 2018; Diener et al., 2015; Gao et al., 2017; Inouye et al., 2007; Lindqvist & Block, 1995; Purschke et al., 2017; Tschirner & Simon, 2015; van der Fels-Klerx et al., 2016; Vijver et al., 2003; Wang et al., 2017). Of the chemical elements mentioned above, arsenic, cadmium, lead and mercury are the most toxic. The other elements are trace elements that are toxic only in very high concentrations.

In black soldier fly larvae, accumulation of cadmium (BAF 2.3-9.5) and lead (BAF 1.1-2.6) was observed (Bulak et al., 2018; Biancarosa et al., 2018; Diener et. al., 2015; Gao et al., 2017; Purschke et al., 2017; Tschirner & Simon, 2015; van der Fels-Klerx et al., 2016). For mercury, the bioaccumulation factor was between 0.5 and 1.1 (Biancorosa et al., 2018; Purschke et al., 2017). The bioaccumulation factor for copper varied between 0.7 and 1.8 and that of zinc between 0.4 and 3.2 (Bulak et al., 2018; Diener et al., 2016; Tschirner & Simon, 2015).

Bioaccumulation has only been demonstrated for cadmium in three and four-day old housefly larvae (BAF 1.1 and 1.2), but not in five-day old larvae (BAF <1) or for selenium (BAF 1.2) -1.3) (Wang et al., 2017).

In yellow mealworms, only arsenic accumulation (BAF 1.4-2.6) was observed (van der Fels-Klerx et al., 2016).

Dioxins and polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) (Aroclor 1254) were ingested by housefly larvae reared on an experimentally contaminated substrate (*Chemical Specialties Manufacturers Association (CSMA) fly larval medium*), but no accumulation could be demonstrated (BAF 0.98) (Bryant & Cowles, 2000). Nordentoft et al. (2014) found that housefly larvae had a four times higher concentration of dioxins and

dioxin-like PCBs than the chicken manure on which they were reared (0.37 *versus* 0.09 pg WHO-TEQ/g dry weight).

Till now, only studies involving contaminated mealworms show that harmful chemical substances can be transmitted to mammals via insect larvae. Feeding bats with mealworms contaminated with the PCBs Aroclor 1254 and 1260 and the organochlorine pesticide (OCP) dichlorodiphenyldichloroethylene (p,p'-DDE, a derivative of dichlorodiphenyltrichloroethane (DDT)), can lead to their death (Clark 1978; Clark & Stafford, 1981; Clark & Prouty 1977; Reinhold et al., 1999). However, it is very probable that the effects of chemical contaminants in feed containing insects are the same as the effects of chemical contaminants in regular feed products.

The above studies show that black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms may become contaminated, to a greater or lesser extent, during the rearing process with the chemical agents present in the substrate on which they are reared. These agents include mycotoxins, plant protection products, veterinary medical products, and environmental contaminants such as heavy metals, dioxins, PCBs, PAHs and flame retardants. Accumulation of harmful chemical substances transmitted via the substrate to black soldier fly larvae, housefly larvae, mealworms and lesser mealworms occurs, as far is known, only for arsenic, cadmium, lead, mercury, dioxins and dioxin-like PCBs. In case of plant protection products, veterinary medical products, PAHs and non-dioxin-like PCBs, there are too few studies available to form an opinion regarding this.
The degree of contamination of the larvae is determined by the degree of contamination of the substrate and the extent of bioaccumulation (BAF).

Chemical safety of larvae for the feed and food industry
(Exposure assessment)
Mycotoxins
A study of the chemical safety of reared insects for the animal feed industry examined the presence of 69 mycotoxins in black soldier fly larvae reared on a mixture of brewery spent grain[46], fish feed and yeast and in housefly larvae reared on poultry or pig manure. Mycotoxins were only found in two of the five housefly larvae samples, where both had been reared on poultry manure. Beauvericin (6.9 µg/kg) was found in one sample and ENNA (12.5 µg/kg) and ENNA1 (7.3 µg/kg) was found in the other sample (0.7% of the analyses). However, these mycotoxins were not present in concentrations that could pose a safety risk and these did not include mycotoxins such as the carcinogenic aflatoxin for which the EC has stipulated a maximum concentration[47] limit for animal feed (Charlton et al., 2015). They also did not include the type of mycotoxins for which only indicative values for feed and feed materials are available (DON, ZEA, OTA, FB1, FB2, T-2 and HT-2) [48,49].

[46] Brewery spent grain is a residual product from beer breweries. It mainly consists of the chaff and protein parts of the malting barley from which the brewer has extracted the carbohydrates.
[47] Regulation (EC) No. 2002/32 of the European Parliament and of the Council on undesirable substances in animal feed.
[48] Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxins in cereals and cereal products.

A study which examined black soldier fly larvae reared on a semolina substrate for the presence of DON, AfB1/B2/G2, OTA and ZEA was unable to demonstrate any of these mycotoxins (Purschke et al., 2017).

Neither could the mycotoxins AfB1, DON, OTA and ZEA be demonstrated in black soldier fly larvae and lesser mealworms reared on a wheat substrate (Camenzuli et al., 2018).

Plant protection products

The concentrations of 393 pesticides were measured in black soldier fly larvae reared on a mixture of brewery spent grain, fish feed and yeast and in housefly larvae reared on poultry or pig manure (Charlton et al., 2015). The only residue detected was chlorpyrifos (800 µg/kg) in one of the five samples of housefly larvae reared on poultry manure (0.04% of the analyses). The concentration of this compound in animal feed is not regulated via EU legislation. The Codex Alimentarius recommends that the concentration of chlorpyrifos must remain below 5000 µg/kg in alfalfa that is used as feed (FAO & WHO, 2019).

In a study by Purschke et al. (2017), black soldier fly larvae reared on corn semolina were examined for the presence of chlorpyrifos, chlorpyrifos-methyl and pirimiphos-methyl. These compounds were not detected in the larvae.

Lalander et al. (2016) reared black soldier fly larvae on a substrate of dog food, but did not detect any azoxystrobin and propiconazole in the larvae.

Poma et al. (2017) examined commercially available yellow mealworms and lesser mealworms (rearing substrate unknown for both) for the presence of nine OCPs. The only two measurable OCPs were hexachlorobenzene (HCB) in yellow mealworms and lesser mealworms and p,p'-DDE in yellow mealworms, which were well below the lowest limit for animal feed. A qualitative pesticide suspect screening yielded nine possible identifications for yellow mealworms and seven for lesser mealworms. However, the structure of the compounds could not be confirmed.

In yellow mealworm that were reared on a substrate with carrots, residues of none of the 12 plant protection products tested, were found in measurable concentrations (Houbraken et al., 2016). These plant protection products were 2,4-D, bentazon, bifenthrin, clopyralid, diflufenican, fenpropimorph, isoproturon, linuron, mefenoxam, pendimethalin, pyrimethanil and tebuconazole.
In commercially available yellow mealworms (substrate unknown), no quantifiable amounts of these plant protection product residues were found, except for clopyralid (Houbraken et al., 2016).

In 2017, the NVWA has had an analysis performed on commercially available mealworms to detect the presence of 206 plant protection products, where one of the eight samples showed a low concentration of the insecticide tetramethrin (0.015 mg/kg) (not permitted in the EU) (NVWA, 2018a).

[49] Commission Recommendation (EC) No. 2006/576 of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 toxin and fumonisins in products intended for use as animal feed.

Veterinary medical products

In black soldier fly larvae and housefly larvae (five samples) reared on a substrate of brewery spent grain, fish feed and yeast, a veterinary medicine screening produced quantitative data for 68 agents and qualitative data for 492 agents, respectively. The larvae were also analysed for the presence of the antibiotic drug chloramphenicol which is banned in animal husbandry. Nicarbazin, a veterinary drug added to the feed of fattening chickens to prevent coccidiosis, was found in a sample of housefly larvae reared on poultry manure (0.04% of the analyses). The other tested agents were either not present or present in concentrations below the detection limit of the relevant agents (Charlton et al., 2015).

It is unlikely that substrates of plant origin such as wheat flour or brewery spent grain will contain residues of veterinary medical products as opposed to a substrate such as manure, which has been demonstrated in the study by Charlton et al. (2015) involving housefly larvae reared on poultry manure.

Environmental contaminants

Heavy metals and arsenic

The concentrations of 48 heavy metals and trace elements were studied in black soldier fly larvae reared on a substrate composed of brewery spent grain, fish feed and yeast (Charlton et al., 2015). The concentrations of arsenic (0.142 mg/kg), cadmium (0.12 mg/kg) and mercury (0.007 mg/kg) were below the lowest EU limits for these elements in animal feed (2 mg/kg for arsenic, 0.5 mg/kg for cadmium and 0.1 mg/kg for mercury). The concentration of lead was below the detection limit.

The presence of cadmium has also been demonstrated in other studies involving black soldier fly larvae reared on chicken feed (Diener et al., 2015; van der Fels-Klerx et al., 2016) and corn semolina (Purschke et al., 2017). The cadmium concentrations were between 0.05 and 0.40 mg/kg. Purschke et al. (2017) and van der Fels-Klerx et al. (2016) also carried out studies for the presence of arsenic and lead. Arsenic was not detectable in either study. Lead (0.03 mg/kg) was only detected in larvae reared on a corn semolina substrate (Purschke et al., 2017), where the concentration was well below the limit applicable to animal feed (5 mg/kg). Mercury was not detected in the larvae reared on corn semolina; chromium and nickel, which are much less toxic than cadmium, mercury and lead, were present in low concentrations (0.06 and 0.05 mg/kg respectively). Moniello et al. (2019) were, however, able to detect arsenic and mercury in black soldier fly larvae. But the concentrations of arsenic (0.23 mg/kg) and mercury (0.01 mg/kg) remained below the lowest EU limits for feed. In addition, cadmium (0.06 mg/kg) and lead (0.03 mg/kg) were also detected in this study and these concentrations were lower than the EU limits for feed.

Cadmium was also detected in all five analysed samples of housefly larvae reared on poultry or pig manure, where three of the five samples exceeded the lowest limit for cadmium in animal feed (complete feed) (Charlton et al., 2015); however, even these concentrations were below the lowest limit for feed materials (1 mg/kg). In addition, arsenic (0.1-0.4 mg/kg), cadmium (0.3-0.7 mg/kg), mercury (0.002-0.04 mg/kg) and lead (0.06-1.2) mg/kg) were detected in all samples, but the concentrations were below EU limits for these elements.

In mealworms reared on wheat bran (Adámková et al., 2017), a grain mix (van der Fels-Klerx et al., 2016) or an unspecified substrate (commercially available) (NVWA, 2017b; NVWA, 2018a; Poma et al., 2017), no arsenic, cadmium and lead were demonstrated in relevant concentrations (below the detection limit and/or below the lowest legal limit for animal feed).

Arsenic, cadmium and lead were also not demonstrated in lesser mealworms reared on a unspecified substrate (Poma et al., 2017).

Dioxins and PCBs
The presence of dioxins and PCBs was analysed in black soldier fly larvae reared on a substrate of brewery spent grain, fish feed and yeast, in housefly larvae (five samples) reared on a substrate of poultry or pig manure (Charlton et al. al., 2015), in commercially obtained mealworms (nine samples) (NVWA, 2018a; Poma et al., 2017), and in lesser mealworms (Poma et al., 2017). None of the samples exceeded the lowest legal maximum limits for dioxins and dioxin-like PCBs and indicator PCBs in animal feed. Even in housefly larvae reared on chicken manure, there was no exceedance of this limit, despite a quadruple accumulation of dioxins and dioxin-like PCBs.

Polycyclic aromatic hydrocarbons (PAHs)
The EU has established maximum concentrations for PAHs in foods that include all the four PAHs (PAH4, i.e. benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) and a separate maximum limit for benzo(a)pyrene (BaP). The maximum limits for PAH4 vary from 1 µg/kg in baby food to 35 µg/kg in mussels, and for BaP, between 1 and 6 µg/kg. No maximum limits have been set for animal feed. Charlton et al. (2015) reported that, in black soldier fly larvae reared on a substrate of brewery spent grain, fish feed and yeast and housefly larvae reared on poultry or pig manure, the concentration of PAH4 varied between 0.3 and 10 µg/kg and the concentration of BaP between <0.05-2.2 µg/kg.

Flame retardants
Commercially available yellow mealworm and lesser mealworm larvae were tested by Poma et al. (2017) for the presence of 18 different halogenated and phosphorus-based flame retardants. Four phosphorus-based flame retardants (PFRs) were detected in the yellow mealworms, three of which were also detected in the lesser mealworms. The concentrations varied between 8 and 24 µg/kg. The presence of PFRs arises due to the use of such PFRs during the processing and packaging of the insects, but possibly also of the substrate.

In cases where insect larvae were reared on substrates containing residues and by-products from the food industry and/or regular feed, and insofar as legal maximum limits are defined for animal feed, the above studies show that these larvae meet the lowest limits for mycotoxins, veterinary medical products, and environmental contaminants. The only quantifiable plant protection product detected was tetramethrin (banned in the EU), low concentrations of which were found in the mealworm sample. Just like FF, by-products or residual flows from the food industry are no longer considered as food intended for human consumption. They are comparable in origin to FF.

The concentrations of contaminants in the analysed insect larvae were found to be comparable to or lower than the concentrations of these substances that are usually measured in traditional feed materials (Adamse et al., 2015; Adamse et al., 2017a; NVWA, 2015e; NVWA, 2016c; NVWA, 2017b) or in products of animal origin such as meat, fish and eggs (NVWA, 2015d; NVWA, 2016b; Poma et al., 2017).

What are the risks? (Risk characterisation)
The chemical risks posed to animal and public health, due to the use of black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms reared on FF as animal feed raw material for farm animals, are mainly determined by the chemical composition of the substrate and the extent to which these agents are passed on to the insect larvae via the substrate. This risk may be intensified if the chemical contaminants present in the substrate are able to accumulate in the larvae.

The aforementioned larvae can ingest various harmful chemical agents and some of these agents have been shown to accumulate in the larvae. For the remaining chemical agents, there is no data or too little data to be able to conclude that there is no accumulation of these agents. However, it appears that the concentrations of these agents in fully grown larvae are comparable to those in (former) foodstuffs, including those of chemical agents that have been shown to accumulate in the larvae.

Given the short lifespan of the larvae and the occasional low rate of exceedance in (former) foodstuffs of the limits of these chemical agents, the use of insects reared on FF as animal feed is expected to pose a potential risk to animal and public health only in exceptional cases. Only in case of long-term exposure of farm animals to insect-based feed that exceeds the limits, there is a chance of undesirable effects. However, the burden of disease caused by chemical substances is very small and epidemiologically not demonstrable.

<u>Conclusion</u>
The chemical risks posed to animal and public health, by feeding farm animals with feed produced from black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms that have been reared on FF of plant and/or animal origin, are not estimated as being higher than those of (former) foodstuffs. This implies that larvae reared on FF represent a negligible risk to animal and public health.

How can the risks be managed?
Removing the gut contents of the larvae can drastically reduce concentrations of chemicals in the larvae, as has been demonstrated in the case of a number of plant protection products in mealworms. Moreover, this could also be an effective risk-reducing control measure for other larvae and other chemicals.

The presence of chemical contaminants in insects is mainly controlled by controlling the chemical risks in the substrate (BuRO, 2014; EFSA SC, 2015). When FF is used as a substrate, the current process of controlling the occurrence of chemical contaminants in foods provides a sufficient level of control. Over the years, a system of regulations has been developed in the EU which has ensured that these substances no longer occur in food or are present in such low quantities

that health risks are absent or can be considered negligible (van Kreijl & Knaap, 2004). This means that, in cases where low concentrations of these contaminants exceed the EU limits for food, large quantities of these products with increased concentrations of chemical substances must be consumed for a long period time before there is actually a chance of undesirable effects (NVWA, 2015c). When EU limits were exceeded or new potential risks identified, there was no real impact on health (van Kreijl & Knaap, 2004). The same logic applies in case of insects that are fed to farm animals. Control must be exercised by monitoring the substances that are known to accumulate.

Microbiological risks

Microbiological agents in (former) foodstuffs (Hazard identification)

Just like food, foodstuffs downgraded to the status of FF must be safe and not contain any microbiological agents in quantities that are harmful to health. They must meet the microbiological criteria defined in the EU (Regulation (EC) No. 2073/2005[50]). However, this does not mean - for example, in the case of perishable goods - that pathogens are never present. This is evident by the fact that, despite ensuring food safety through numerous measures, monitoring and adequate behaviour of food companies and consumers, it is estimated that 650,000 people in the Netherlands fell ill in 2018 by eating food contaminated with human pathogenic microorganisms[51] (Pijnacker et al., 2019). As stated earlier, foods may contain both human as well as animal pathogens. That is why (former) foodstuffs that are safe for humans are not always safe for animals.

Animal pathogens

The most relevant animal pathogens that may occur in (former) foodstuffs are identified based on the defined list of infectious animal diseases, outbreaks of which must be reported by the EU Member States to the EC via the Animal Disease Notification System (ADNS)[52] (EC ADNS, 2019). From this list, the animal diseases whose causative microbiological agents may be present in former or other foodstuffs and which can be transmitted to farm animals through feeding were selected (Table 3).

These microbiological agents are the causes of contagious and serious bacterial diseases such as bovine brucellosis, brucellosis in sheep and goat and bovine tuberculosis; viral diseases such as African swine fever (ASF), classical swine fever (CSF) and foot-and-mouth disease (FMD); and prion diseases/TSEs such as BSE, scrapie and chronic wasting disease (CWD) (Table 3).

[50] Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for food.

[51] Microorganisms: 'bacteria, viruses, yeasts, moulds, algae, parasitic protozoa, microscopic parasitic helminths, and their toxins and metabolites' (Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for food). Helminths are worms

[52] The legal basis for the ADNS is Directive 82/894/EEC on the notification of animal diseases in the Community. Annexes I and II have been amended by the Implementing Decree 2012/737/EU.

Table 3.
Relevant animal pathogens in Europe that can be transmitted by feeding farm animals with (former) foodstuffs, and their sources of contamination.

Organism	Source of contamination[a]
Bacteria	
Brucella abortus	Dairy (cattle)
Brucella melitensis	Dairy (sheep and goat)
Mycobacterium bovis	Dairy (cattle)
Viruses	
African swine fever (ASF) virus	Pigs
Classical swine fever (CSF) virus	Pigs
Foot-and-mouth disease (FMD) virus	Cattle, pig, sheep, goat, dairy
Prions	
BSE (mad cow disease)	Cattle
Chronic wasting disease (CWD)[b]	Deer, roe
Scrapie[b]	Sheep, goat

[a] GD Animal Health (2019)
[b] Not required to be reported in the ADNS (EC ADNS, 2019).

Human pathogens

The most relevant human pathogens that may occur in (former) foodstuffs in Europe are identified based on the lists of food-borne microorganisms maintained by the Enteric Disease Task Force and the Parasitic Disease Task Force of the Foodborne Disease Burden Reference Group (FERG) of the World Health Organization (WHO) (Havelaar et al., 2015; Kirk et al., 2015; Torgerson et al., 2015; WHO, 2015). From the microorganisms included in these studies, the ones relevant to this risk assessment have been selected: disease-causing zoonotic microorganisms occurring in Europe which may be present in (former) foodstuffs fed to farm animals and which can be transmitted to humans through the consumption of products from these animals.

The selected microorganisms include the infectious bacteria from the genera *Brucella*, *Campylobacter* and *Salmonella*; pathogenic *Escherichia coli*, *Listeria monocytogenes* and *Mycobacterium bovis*; protozoans (single-celled parasites) *Cryptosporidium parvum* and *Toxoplasma gondii*; and the helminth (parasitic worm) *Trichinella spiralis* (Table 4). The most important *Cryptosporidium* spp. that cause disease in humans (90% of cases of cryptosporidiosis) are *C. hominis* and *C. parvum* (RIVM-LCI, 2012)). *Cryptosporidium hominis* is mainly transmitted from person to person; *C. parvum* can be transmitted zoonotically, although not all *C. parvum* strains are zoonotic (Dawson, 2005; Smith et al., 2007).

Other relevant microorganisms are the Hepatitis E virus (HEV) and *Yersinia* spp. The WHO pathogen lists do not include these because of the lack of data on worldwide disease burden estimates. However, it is known that HEV gives rise to a relevant disease burden in Europe, with a high rate of incidence that has increased tenfold since 2005 (Aspinall et al., 2017; ECDC, 2017). Yersiniosis is most frequently reported in the EU, after campylobacteriosis and salmonellosis (EFSA & ECDC, 2018). Despite the current low human disease burden of vCJD - a TSE caused by the consumption of meat from cattle infected with the BSE prion (Bruce et al., 1997; Scott et al., 1999) - the BSE prion is a relevant human pathogen for this advice.

Table 4.
Relevant zoonotic microorganisms and prions in Europe that can be transmitted via (former) foodstuffs and their sources of contamination.

Organism	Source of contamination[a] Animal origin	Plant origin
Bacteria		
Brucella abortus/ melitensis	Cattle, sheep, goat, pig, dairy	
Campylobacter spp.	Cattle, sheep, goat, pig, chicken, dairy	Fruits, vegetables
Pathogenic *Escherichia coli*	Cattle, sheep, goat, pig, dairy	Fruits, vegetables
Listeria monocytogenes	Meat products, smoked fish, dairy	Ready-made raw vegetables (salads)
Mycobacterium bovis	Dairy	
Salmonella spp. (non-typhoid)	Cattle, sheep, goat, pig, chicken, fish, crustaceans, shellfish, dairy, egg	Vegetables, fruit, nuts, grains
Yersinia spp.	Pigs	
Viruses		
Hepatitis E virus (HEV)	Pigs	
Protozoans		
Cryptosporidium spp.[b]	Dairy	Fruits, vegetables
Toxoplasma gondii	Cattle, sheep, goat, pig, chicken, dairy, egg	Fruits, vegetables
Helminths		
Trichinella spp.	Pigs	
Prions		
BSE (vCJD)	Cattle	

[a] Hoffmann et al., (2017), van Kreijl & Knaap (2004) and the Netherlands Nutrition Centre (2019).
[b] Only the zoonotic *Cryptosporidium parvum* is relevant to the risk assessment.

Prevalence in food

Microbiological sampling conducted in the years 2014/2015-2016 showed that 0.6% to 0.7% of all food batches (of animal and plant origin) tested did not meet a food safety criterion. In 3.4% to 9.2% of the batch analyses, the batch did not meet a process hygiene criterion. A remaining group of analyses focused on non-statutory criteria. They relate to pathogens present in lower quantities than those known to cause illness. The analysis was positive in 6.5% to 17.2% of these batch analyses. The organisms that were found included *Campylobacter* spp., Shigatoxin-forming *E. coli* (STEC), *Salmonella* spp. and *Listeria monocytogenes* (NVWA, 2018b). The prevalence of these microorganisms in food is comparable with that in the EU (EFSA & ECDC, 2018).

Prevalence in FF

From the limited number of studies on the microbiological safety of FF it can be concluded that bakery products and cooked materials that mainly represent FF can, in general, be considered microbiologically stable. A microbiological analysis of FF starting from different food products (e.g. broken biscuits and chocolates, confectionery such as croissants and chocolate, surplus bread, rice cake and breakfast cereals) (n=6) showed a high microbiological quality for all samples

tested (less than 6 log cfu[53]/g aerobic bacteria). These results were expected for this type of FF which has a low moisture content and reaches high temperatures during the production process. The FF did not contain any *Salmonella* and the concentrations of *E. coli* were below the detection limit of log 2 cfu/g (Tretola et al., 2017b).

A study of FF of animal origin showed that *Salmonella* was present in FF meat (poultry, beef and pork, sampled from the cold storage warehouses of a wholesaler), which had been downgraded to pet food. From the poultry samples, 11.5% tested positive for *S.* Derby and *S.* Typhimurium (n=52), and from the pork samples, 13.3% tested positive for *S*. Typhimurium (n=30). None of the tested beef samples (n=30) contained *Salmonella*. A high prevalence of *E. coli* was found in FF of poultry (100%), pork (100%) and beef (93.3%) (Bacci et al., 2019).

Microbiological agents in substrate and insects during the rearing process (Hazard characterisation)
Biological activity of microbiological agents in substrate
As soon as FF are used as a substrate for insect rearing, there is a risk that any relevant pathogenic microbiological agents present in the substrate composed of FF will no longer remain limited to an acceptable level but will develop further to concentrations that are infectious to animals and/or humans. However, this only applies to bacteria and not to viruses, parasites (protozoans, helminths) and prions, which cannot multiply outside their natural hosts (Dijk, 2014). This means that there will be no increase in these agents in the substrate.

The above-mentioned biological agents can, however, 'survive' in the environment and on food and remain infectious (Dawson, 2005; Pirtle & Beran, 1991; Rzeżutka & Cook, 2004; Worley et al., 1985). This is also indirectly evident from the fact that consumers can fall ill by consuming food containing these pathogens (van Kreijl & Knaap, 2004).

Bacteria
The most important factors for the growth of bacteria in food are the availability of free water (water activity or a_w), acidity (pH), temperature and amount of oxygen required by these organisms (Dijk, 2014; US FDA, 2012; US FDA, 2019). Although each of these factors plays an important role, it is the interaction between these factors that determines whether or not a bacterium will grow in a particular food. Hence, the form (for example, dried, acidified or unchanged) in which the FF are included in the substrate for insects has an influence on how suitable this substrate is for serving as a culture medium for bacteria.

The range of temperatures at which insect larvae are reared is favourable for the growth of most bacteria (Dijk, 2014; US FDA, 2012). This is confirmed by studies on the dynamics of aerobic bacteria in substrates, substrate residues and black soldier fly larvae, yellow mealworms and lesser mealworms during the rearing of these larvae (see: Bacterial dynamics in substrate and larvae during the rearing process).

[53] Cfu = colony forming unit.

Viruses
The animal pathogenic FMD, ASF and CSF viruses are very stable in protein-rich environments such as meat and can survive for a few months in raw meat and meat products (Brown & Bevins, 2018; McKercher et al., 1978; Mebus et al., 1993; Mebus et al., 1997). The ASF and CSF viruses can survive for a number of months to a number of years in frozen meats (Blome et al., 2017; Mebus et al., 1993; Mebus et al., 1997; Ribbens et al., 2004). The ASF virus can also survive for 30 days in soybean meal (Dee et al., 2018). In the environment, the CSF virus is inactivated at high temperatures. Despite this, the virus can remain active for 3 days at 50ºC and 7 to 15 days at 37ºC (Ribbens et al., 2004). The FMD virus also survives in milk, even after HTST (high temperature/short time) pasteurisation (at least 15 seconds at 71.7ºC) and in cheese (Blackwell, 1976; Tomasula & Konstance, 2004). Hepatitis E virus can be detected in liver cell suspensions even after 43 days at 37ºC and remains infectious in faecal suspensions of HEV patients and in pig liver homogenates after heating for at 56ºC for one hour (Emerson et al., 2005; Feagins et al., 2008).

Parasites
The protozoans *C. parvum* and *T. gondii* can survive for a few months in and on moist foods (Dawson et al., 2005). The oocysts of *T. gondii* can survive in the environment for longer than a year and best in humid conditions at moderate temperatures (RIVM-LCI, 2009). Oocysts remain infectious for a long time: 200 days at 10°C, 15°C, 20°C and 25°C, 107 days at 30°C and 32 days at 37°C. Even after storage at -10ºC, the oocysts remain infectious for several months (Dubey, 1998).

Fifty to sixty percent of the helminth *T. spiralis* larvae in grizzly bear meat remain alive after storage for 27 months at -6.5°C to -20°C. Even after 34 months, this was as high as 30% to 50%. However, viable larvae were not found in meat products such as pepperoni, salami and sausage (Worley et al., 1986).

Prions
Prions are very stable in the environment (Wiggins, 2009), as a result of which a rearing temperature of 26-32°C has no influence on infectivity.

The above shows that the viruses, parasites and prions relevant to this advice can retain their biological activity in food and therefore also in a substrate composed of FF. This also applies to bacteria, which can also multiply in food and for which the relatively high temperature during rearing is favourable.

Bacterial dynamics in substrate and larvae during the rearing process
In a study by Wynants et al. (2018a), the concentration of aerobic bacteria in the substrate, during the five-week rearing period (one week at room temperature and four weeks at 30°C) of lesser mealworms on a dry and moist feed mixture, was about 4 log higher than the initial concentration in the dry feed and 2 log higher than the initial concentration in the moist feed. It was remarkable that the substrate residue, consisting of the remnants of dry and moist feed, including the faeces and moulting skins of the larvae, had a low water activity (a_w 0.5-0.6) and a low acidity (pH 5.5-6), which is not favourable for bacterial growth. The concentration of aerobic bacteria in the substrate, measured after two, three, four and five weeks, remained more or less the same during the rearing period. The concentration of aerobic bacteria of the mealworms was comparable to 1 log lower

than in the substrate residue during rearing, where the concentration was 3 log higher than the initial concentration in the dry substrate and 1 log higher than the initial concentration in the moist substrate.

Moreover, in another study by Wynants et al. (2018b) where black soldier fly larvae were reared for three weeks on different substrates with high water activity (a_w>0.95) at temperatures between 25°C and 29°C, the concentration of aerobic bacteria in the residue at the time of harvesting (substrate residues, skin and faeces) for all substrates was higher (2.5 log on average) than the initial concentration in the substrate. However, no correlation was found between the total viable aerobic counts of the substrates and their residues. The concentration of aerobic bacteria in the larvae was equal to or lower (on average, 0.6 log lower) than in the substrate residue when the larvae were harvested. The concentration of aerobic bacteria in the larvae was, on average, 2 log higher than the initial concentration in the substrate

In yet another study by Wynants et al. (2019), where yellow mealworms were reared at 28°C during a seven-day period, the concentration of aerobic bacteria in the substrate (wheat bran supplemented with carrot or chicory) (measured on days 1, 3 and 7) remained constant during the rearing period. In this study, the initial concentration of aerobic bacteria in the substrate was unknown. The concentration of aerobic bacteria in externally disinfected larvae was (1 to 3 log) higher than in the substrate residue during rearing. This is in contrast to the previous two studies, where the concentration of aerobic bacteria in the larvae was the same or lower than in the substrate residue. This study took measurements for up to seven days, so it is possible that after an increase in the first week, the concentration in the larvae remained approximately the same as that in the substrate residue.

When a mixture of *Salmonella* spp. (*S*. Enteritidis, *S*. Infantis and *S*. Typhimurium) was also added to the substrate, the *Salmonella* concentration remained constant in the substrate residue, but it was about 1 log lower than the initial concentration *Salmonella* in the substrate. The concentration of *Salmonella* in externally disinfected larvae was lower than that in the residue.

In the above study, the concentration of aerobic bacteria in the insects themselves was higher than the initial concentration in the substrate, but the concentration of *Salmonella* was lower. This could be an indication that the dynamics of pathogens are different from that of the total aerobic bacteria population.

The above studies do not provide a clear picture of the dynamics of bacterial populations in the substrate, substrate residue and larvae. No conclusions can be drawn about the degree of transmission of (pathogenic) bacteria from the substrate to the larvae.

Microbiota and vector potential of larvae
The gut microbiota[54] of adult insects consists mainly of bacteria (Cazemier et al., 1997; Steinhaus, 1941). Microorganisms that are present on the cuticle (exoskeleton) of the insects (van Huis et al., 2013) can contribute to the microbiota of insects. For building up the microbiota, reared insects depend on the microbiological composition of the culture medium (Bruno et al., 2019; De Smet et al., 2018; Dillon & Charnley, 2002; Engel & Moran, 2013; Jeon et al., 2011; Rinke et al., 2011; Wynants et al., 2018b; Yun et al., 2014). In case of insect larvae reared on a substrate composed of FF, the microbiota will therefore be a reflection of the microorganisms present in the FF.

Many insect species can be vectors of a whole range of pathogenic microorganisms (Belluco et al., 2013; Schlüter et al., 2017), including plant pathogens (Harris & Maramorosch, 1980). Also, black soldier fly larvae, housefly larvae, common mealworms and lesser mealworms can be temporarily colonised with pathogenic microorganisms.

Below is an overview of the information available on the combinations of microbiological agents and insect larvae relevant to this advice and the vector potential of these larvae with respect to farm animals. For this overview, studies were searched that demonstrate the transmission of the relevant microbiological agents to the larvae from experimental or naturally contaminated substrates. If this specific information was not available, information on other microbiological agents, other insect larvae and/or adults was searched.

Bacteria
Brucella spp.
No studies have been published on the presence of *Brucella* spp. in the insect larvae or their adults that are relevant to this advice. However, it is known that *B. melitensis* and *B. suis* are able to survive in larvae of the wax moth *Galleria mellonella,* which is used as an animal model to study virulence factors of pathogens (Sprynski et al., 2014).
Adults of the autumn housefly (*Musca autumnalis*), which is related to the housefly, are also capable to take up *B. abortus* (Cheville et al., 1989). Flies which were first fed on substrates contaminated with *B. abortus*, *B. melitensis* or *B. suis* and then on guinea pigs were able to transmit these bacteria to the guinea pigs, demonstrating that the bacteria remain infectious in the flies (Krinsky, 1976).

Campylobacter jejuni
In housefly larvae reared on manure to which *C. jejuni* had been added, the presence of *C. jejuni* could be demonstrated up to seven days after exposure (Nordentoft et al., 2017). *C. jejuni* could also be demonstrated in housefly larvae that had been kept for four hours on agar plates inoculated with *C. jejuni* (9 log cfu/ml). The concentration of bacteria decreased from 6.5 log cfu/ml to 3.6 log cfu/ml within eight hours following exposure (Bahrndorf et al., 2104).

Campylobacter jejuni can be transmitted to lesser mealworms via an experimentally or a naturally contaminated substrate (Hazeleger et al., 2008;

[54] Microbiota are ecological communities of commensal, symbiotic and pathogenic microorganisms in a given environment.

Strother et al., 2005; Templeton et al., 2006) and this pathogen can be transmitted to chickens by feeding them these mealworms (Hazeleger et al., 2008; Strother et al., 2005).

The experiments conducted by Strother et al. (2005) and Templeton et al. (2006) showed that it was still possible to isolate *C. jejuni* from the larvae up to three days after ingestion/exposure, but this was usually no longer possible after four days. Templeton et al. (2006) were able to isolate *C. jejuni* from the larvae even five days after exposure, but only after enrichment. Hazeleger et al. (2008) showed that, one week after ingestion/exposure, *C. jejuni* could no longer be demonstrated in larvae inoculated with a suspension of 8 log cfu/ml, resulting in initial infection levels of 5 log cfu/larva.

Escherichia coli
Escherichia coli (including STEC O157:H7) can be transmitted via the substrate to black soldier fly larvae (Erickson et al., 2004; Kashiri et al., 2018), housefly larvae (Nordentoft et al., 2017; Rochon, 2003; Rochon et al., 2004) and lesser mealworms (McAllister et al., 1996). In the study described in Rochon (2003) and Rochon et al. (2004), where housefly larvae were administered a single dose of 8.8 log cfu/ml of *E. coli* for 20 minutes, the *E. coli* concentration decreased from 5.2 log cfu/larva to 3.8 log cfu/larva 48 hours after ingestion. It has also been demonstrated that chickens that were fed with *E. coli*-positive lesser mealworms became infected with this pathogen (McAllister et al., 1996).

Listeria spp.
In a study in which yellow mealworms were reared over a one-week period on a substrate to which *L. monocytogenes* had been added, the bacterium could be detected in the larvae during the entire rearing period (Mancini et al., 2019). Several studies have shown that lesser mealworms can be infected with *Listeria* spp. (Garofalo et al., 2017, Grabowski & Klein, 2017, Wynants et al., 2017). The same applies to adults of the black soldier fly (Pava-Ripoll et al., 2015a; Pava-Ripoll et al., 2015b).

Listeria monocytogenes can infect wax moth larvae (Fedhila et al., 2010; Sprynski et al., 2014).

In adult horseflies fed with substrate (saline solution or blood) contaminated with *L. monocytogenes*, growth of the bacteria could be demonstrated up to 24 hours after ingestion (Krinsky, 1976).

Mycobacterium spp.
Mycobacterium spp. can be transmitted to yellow mealworms from an experimentally contaminated substrate (Fischer et al., 2004). In a source-tracing study for mycobacterial infections in pigs, *Mycobacterium* spp. was detected in *Musca* spp. larvae that were present in the stables (Matlova et al., 2003).

Salmonella spp.
Black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms are able to take up *Salmonella* (including *S*. Enteritidis and *S*. Typhimurium) (Crippen et al., 2012, Erickson et al., 2004, Gabler, 2014, Hazeleger et al., 2008, McAllister et al., 1994, Leffer et al., 2010, Nordentoft et al. 2017, Roche et al., 2009, Wynants et al., 2019, Zheng et al., 2012). A study by Hazeleger et al.

(2008) showed that one week after ingestion, *S.* Java was still detectable in larvae inoculated with a 7 log cfu/ml suspension, resulting in infection levels of 4 log cfu/larva.

It has been demonstrated that lesser mealworms are able to transmit this bacterium to chickens fed on the infected larvae (Hazeleger et al., 2008; Leffer et al., 2010; McAllister et al., 1994; Roche et al., 2009).

Yersinia spp.
The presence of *Y. pseudotuberculosis* has been demonstrated in housefly larvae contaminated via turkey bedding (Zurek et al., 2000). *Yersinia pseudotuberculosis* can survive in wax moth larvae (Sprynski et al., 2014).

Viruses
No studies have been published on the presence or the vector potential of the viruses relevant to this advice in the relevant insect larvae or their adults. However, it is known that lesser mealworms can get infected by viruses from vertebrates. The infectious laryngotracheitis virus can 'survive' in and on lesser mealworms and the virus can be re-isolated from homogenised mealworms inoculated on chicken embryos (Ou et al., 2012). When lesser mealworms that ingested turkey enterovirus and rotavirus from manure from turkeys with enteritis were fed to healthy turkey chicks, this caused enteritis in these animals (Despins et al., 1994).

In addition, it has been shown that larvae from other insects, i.e. the flesh flies *Lucilia sericata* (common green bottle fly) and *Calliphora vicina* (red-cheeked blue bottle fly), reared on ASF-infected liver can carry this virus (detected by a PCR[55]test, infectious virus could not be detected) (Forth et al., 2018). The CSF and FMD viruses could be detected in the larvae of the screw-worm fly (*Cochliomyia hominivorax*) reared on medium to which these viruses had been added (Chaudhury et al., 2008).

The *Stomoxys calcitrans* (stable fly) which, just like the housefly, belongs to the Muscidae family of flies, is capable to take up the ASF virus (Guinat et al., 2016; Mellor et al., 1987). It has been shown that pigs fed with *Stomoxys* flies, which have fed on blood contaminated with the ASF virus, can become infected with ASF (Olesen et al., 2018).

Parasites
Cryptosporidium spp.
Oocysts of the protozoa *C. parvum* were present in the intestinal tracts of housefly larvae that were reared on a contaminated substrate (Graczyk et al., 1999).

Toxoplasma gondii
Housefly larvae were able to acquire *T. gondii* oocysts from a substrate contaminated with *T. gondii* oocysts. Mice fed with these larvae were found to have a *Toxoplasma* infection (Wallace, 1971).

[55] PCR: polymerase chain reaction, a method for specifically multiplying (amplifying) one or more segments from very small amounts of genetic material.

The *Toxoplasm*a and *Cryptosporidium*-related protozoans of the genus *Gregarina* (eugregarine) and *Farinocystis tribolii* (neogregarine) can occur in lesser mealworms (Apuya et al., 1994). These are the frequently occurring parasites of this insect species. The bird parasite *Histomonas meleagridis* can be found in lesser mealworms from farms where a histomonas outbreak has occurred and in experimentally infected lesser mealworms (Huber et al., 2007).

Trichinella spp.
No studies have been published on the presence of *Trichinella* in the insect larvae or their adults that are relevant to this advice. However, larvae of *Sarcophaga argyrostoma* (type of flesh fly) reared on meat infected with *T. spiralis* were able to infect mice (Maroli & Pozio, 2000).

Prions
No studies have been published on the presence of the TSEs relevant to this advice in the relevant insect larvae or their adults. However, the scrapie prion was detected in the larvae of a parasitic fly species (*Oestris ovis*) isolated from the nasal cavity of sheep with scrapie (Corona et al., 2006).

In larvae of another parasitic fly species (*S. carnaria*) reared on brain tissue from scrapie-infected hamsters, not only could these prions be detected in the larvae but it was also demonstrated that ingestion of the internal organs of these larvae could cause clinical symptoms of scrapie in hamsters (Post et al., 1999).
Mites - which are arthropods just like insects - originating from farms with a history of scrapie, can also transmit scrapie to mice (Rubenstein et al., 1998; Wisniewski et al., 1996) and thus cause scrapie in mice (Carp et al., 2000).
These results suggest that insect larvae and mites may play a role in the spread of TSEs (Corona et al., 2006; Lupi, 2003; Lupi, 2005; Lupi, 2006). It cannot therefore be excluded that black soldier fly larvae, housefly larvae, mealworms and lesser mealworms may also be vectors of TSEs.
Replication of prions in an insect is considered impossible because PrP molecules, the building blocks for prions, are not present in insects (Forrest, 2003) and because PrP-coding genes (PRNP) have not been identified in any insect or other invertebrate animal (Málaga-Trillo et al., 2011; Post et al., 1999).

The above shows that a number of microorganisms relevant to this advice can be transmitted via the substrate to black soldier fly larvae, housefly larvae, mealworms and lesser mealworms and they can also remain biologically active. For prions, this transmission has only been demonstrated for larvae of a different fly species.

Although scientific literature only provides evidence of the survival of *Campylobacter spp.*, *E. coli*, *Salmonella* spp. and *T. gondii* and preservation of their capacity to infect black soldier fly larvae, housefly larvae, mealworms and lesser mealworms and does not provide a definitive answer on the potential survival of the other microbiological agents relevant to this advice, it is possible that these agents may be transmitted passively to animals fed with these insect larvae and form a risk to animal and public health.
For *C. jejuni*, *S.* Java and *E. coli*, it has been shown that they not only survive in larvae during exposure to substrates contaminated with these bacteria, but that they can also survive in the larvae for a few days after exposure of larvae to substrates contaminated with these bacteria. It seems that they are not actively

replicating in the larvae and are only colonising the larvae temporarily. This could explain the decrease in *Salmonella* in larvae observed by Wynants et al. (2019) with respect to the substrate and substrate residue.
Prions cannot replicate in insect larvae either. Insect larvae can, however, act as vectors of prions. It cannot therefore be excluded that black soldier fly larvae, housefly larvae, mealworms and lesser mealworms may also be vectors of TSEs.

There is uncertainty about the extent to which the microbiological agents can be transmitted to the insect larvae (and also ultimately about the extent to which the microbiological agents can be transmitted to farm animals fed with these insects). During the processing of the insect larvae into animal feed raw material, the larvae are used in their entirety, as a result of which all microorganisms and prions present in the digestive tract and on the cuticle of the larvae may end up in the animal feed.
As indicated earlier, it is not clear whether the concentration of bacteria in the insect larvae will be higher than, lower than or equal to that in the substrate. Nothing is known about the dynamics of the relevant viruses and parasites in the insect larvae. Prions cannot multiply in insect larvae. It is assumed that the degree of transmission of viruses, parasites and prions via passage in insects is at most equal to the degree of direct transmission of these pathogens via the direct feeding of FF to animals.
The relative share of the pathogens in the animal feed depends on the relative share of the larvae in the feed after further processing of the larvae into feed (from whole insects to the processing of ground insects or insect protein into feed materials).

Microbiological safety of larvae for the feed or food industry (Exposure assessment)
Fresh and freeze-dried black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms obtained from insect farms contain large numbers of bacteria. Values for the aerobic bacteria count lie between 7 and 9 log cfu/g, and for *Enterobacteriaceae,* this is 7 to 8 log cfu/g. The larvae contain 2 to 6 log cfu/g of endospores and up to 3 to 7 log cfu/g of moulds and yeasts (Agabou & Alloui, 2010; BuRO, 2014; Caparros Megido et al., 2017; Caparros Megido et al., 2018; Greenberg, 1959; Kashiri et al., 2018; Klunder et al., 2012; Osimani et al., 2018; Stoops et al., 2016; Stoops et al., 2017; Vandeweyer et al., 2017a; Vandeweyer et al., 2017b; Wynants et al., 2017; Wynants et al., 2018a; Wynants et al., 2018b). These analyses involved larvae that were reared on plant-based substrates consisting of residual and by-products from the food industry, which are similar to FF in terms of origin. Just like FF, they are no longer considered to be food intended for human consumption.

Black soldier fly larvae reared in a laboratory experiment on vegetable and fruit waste, supermarket waste and restaurant waste of both plant and animal origin, chicken blood and chicken manure contained, per gram, 9 to 10 log cfu aerobic bacteria, 8 to 10 log cfu *Enterobacteriaceae*, 6 to 8 log cfu endospores and 4 to 7 log cfu fungi (Wynants et al., 2018b). Three batches of commercially obtained black soldier fly larvae from this study had 1 to 2 log lower bacterial concentrations than larvae reared on waste substrates: approx. 8 log cfu/g aerobic bacteria, approx. 7.5 log cfu/g *Enterobacteriaceae*, 4 to 6 log cfu/g endospores and less than 3 to 5 log cfu/g fungi.

The concentration of *Enterobacteriaceae* in all the insect larvae analysed in all studies were well above legal microbial limits for *Enterobacteriaceae* in feed materials derived from animal by-products (300 cfu/g) (Table 5).

Until now, no *Salmonella* spp., *L. monocytogenes* (BuRO, 2014; Garofalo et al., 2017; Grabowski & Klein, 2017; Klunder et al., 2012; Osimani et al., 2018; Vandeweyer et al., 2015; Vandeweyer et al., 2017b), *E. coli* (Grabowski & Klein, 2017) and STEC (Osimani et al., 2018) had been found in fresh or freeze-dried housefly larvae, yellow mealworms and lesser mealworms reared for the feed or food industry. A number of studies were based on DNA analyses.

In dried larvae of the black soldier fly reared on barley chaff, average levels detected per gram were 5.9 log cfu of *E. coli* and 6.1 log cfu/g of *Salmonella* spp. With these levels, these larvae did not meet the legal microbiological limits for either *Enterobacteriaceae* or *Salmonella spp.*(Table 5). *Listeria* spp. were not detected (Kashiri et al., 2018). In the study by Grabowski and Klein (2017), no *E. coli*, *L. monocytogenes* and *Salmonella* spp. were found in commercially available black soldier fly larvae. Wynants et al. (2018b) also did not detect any *L. monocytogenes* and *Salmonella* spp. in seven batches of black soldier fly larvae. However, the rearing residue of one of the three batches of commercially available larvae did contain *S.* Agona (Wynants et al., 2108b). This suggests that this pathogen was either present in the initial substrate or had been excreted by the larvae. In black soldier fly larvae reared on food waste, calve forage or cooked rice, DNA of *E. coli* was detected (Jeon et al., 2011).

Table 5.
Microbiological standards for feed materials from derived products (Source: Regulation (EC) No. 142/2001, Annex X, Chapter I)

Microorganism	Standard	Additional requirements[a]
Enterobacteriaceae	300 cfu/g	n = 5, c = 2, m = 10, M = 300
***Salmonella* spp.**	Absent in 25 g	n = 5, c = 0, m = 0, M = 0

[a] n = number of samples to be tested; m = threshold value for the number of bacteria; the result is considered satisfactory if the number of bacteria in all samples does not exceed; m; M = maximum value for the number of bacteria; the result is considered unsatisfactory if the number of bacteria in one or more samples is M or more; and c = number of samples the bacterial count of which may be between m and M, the sample still being considered acceptable if the bacterial count of the other samples is m or less.

The studies described above show that fresh or freeze-dried larvae do not meet the microbiological standards applicable to *Enterobacteriaceae* in feed materials derived from animal by-products.
With the exception of *Salmonella,* no pathogens have been found so far. However, there are too few studies to conclude that the other pathogens cannot occur in animal feed made from these insects.
Larvae reared on waste substrates of animal and/or plant origin had 1 to 2 log higher bacterial counts than those reared on residual and by-products from the food industry. The latter are comparable in origin to FF. Just like FF, they are no longer considered to be food intended for human consumption.

What are the risks?
(Risk characterisation)

The microbiological risks to animal and public health, due to the use of larvae as animal feed raw material for farm animals, are mainly determined by the microbiological composition of the substrate (FF) and the vector competence of the larvae: i.e. the extent to which the microbiological contaminants that are present in the substrate can be passed on to farm animals by feeding them the larvae.

This risk can be increased if the animal species that is fed with the insect larvae (including the gut) is the same animal species as that processed in the FF. In such cases, there is a question of intra-species recycling via passage in insects (see Box 1: Insects as animal feed and intra-species recycling). When infected animals are fed back (feedback loop) as FF to insects that are subsequently used again as animal feed raw material, animal pathogens can spread easily in animal populations.

Box 1: Insects as animal feed and intra-species recycling

Intra-species recycling or cannibalism refers to the feeding of material from a particular animal species to other animals within its own species. This allows the accumulation and/or amplification of known or as-yet-unknown species-specific infectious agents through the continuous recycling within a species sensitive to the agent in question (EC SSC, 1999a).

A striking example of how intra-species recycling can lead to self-perpetuating epidemics is the aforementioned prion disease in BSE in cattle. Outbreaks of other animal diseases, such as swine fever, were also caused by intra-species recycling via feed from contaminated slaughter and food waste (swill made from e.g. meat product leftovers). It is theoretically possible that as-yet-unknown infectious agents can cause epidemics through intra-species recycling.
When feeding animals with feed originating from insect larvae (including the gut) reared on a substrate in which material from their own species is present, there is - strictly speaking - no question of intra-species recycling or cannibalism. However, the recycling of species-specific infectious agents within a species may occur indirectly, via a so-called 'two-species feedback loop'.

This is a feedback loop where infectious material from an animal species is consumed by a second animal species, the tissue of which is again consumed by the first animal species. Insects are consumed in their entirety, so microbiological agents present in their digestive tracts are transmitted to animals that are fed with insect products. However, this does not necessarily imply that the second species will get infected. A BSE modelling study shows that, for a feedback loop (both one-species and two-species), there are critical combinations of the life span of the susceptible animal species, the extent of infectivity and the dose of infectious material that is fed back into the loop determine below which the number of infections in a population may decrease over time and eventually disappear and above which the infection may spread epidemically (as in the case of BSE) (Barnes & Lehman, 2013). The model shows that it is mathematically possible to spread BSE in a population via a two-species feedback loop.

Since insect larvae can be vectors of microorganisms and prions, feeding animals with insects reared on a substrate containing FF derived from animals of the same animal species can therefore, via a two-species feedback loop, lead to an epidemic of a currently unknown pathogen as well as another BSE epidemic. As soon as the incidence among cattle reaches a certain threshold, the consumption of beef and products thereof also poses a health risk to humans.

However, there is uncertainty about the chance of this occurring. This depends on the lifespan of the susceptible animal species, the infectivity of the agents and the dose of infectious material that is fed back to an animal population. Moreover, the chance of the transmission and spread of infectious diseases is also uncertain.

With respect to the inventory of the available information on the occurrence of microbiological agents in and on black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms and the vector potential of these insect larvae, insufficient information is available for many combinations of pathogenic agents/insect larvae relevant to this advice to be able to make a proper assessment of the risk in terms of the vector potential of these insect larvae for the types of microbiological agents in question (bacteria, viruses, parasites and prions).
However, based on the inventory, it is evident that different types of pathogenic microorganisms and prions can be transmitted from their growth substrate to the insect larvae that are reared on them and remain biologically active in the larvae. The vector potential of insect larvae to farm animals has also been shown for all these types of agents.

Conclusion
Feeding farm animals with feed produced from black soldier fly larvae, housefly larvae, yellow mealworms and lesser mealworms that have been reared on FF of plant and/or animal origin may entail microbiological risks to animal and public health. These risks may arise due to pathogenic microorganisms and prions that could be introduced into the insects via the FF.

Microorganisms
Pathogenic microorganisms may also pose a risk when FF of either plant or animal origin are used.

Prions
There is no risk of prions in insects fed with FF of completely plant origin and FF derived from dairy, eggs, honey, rendered fat, collagen and gelatine from non-ruminant animal species. These products are free of prions. When using FF that contains products derived from animals other than those mentioned above (i.e. meat from non-ruminants, and fish, crustaceans and shellfish), the risk of prions is determined by the species of animal processed into the FF on which the insects are reared as well as the animal species fed with these insects.

How can the risks be managed?
Microorganisms
As indicated earlier, whole insects are used as animal feed raw material, including their guts. An option for reducing the microbial contamination of the larvae could be to empty the gut contents before harvesting. This is a common step in the process for producing animal feed raw material from insect larvae. Starvation of yellow mealworms and lesser mealworms has no effect (Wynants et al., 2017; Wynants et al., 2018a).
Washing mealworms and black soldier fly larvae is also not effective for reducing the amount of microbiological contamination (Van Linden et al., 2017; Wynants et al., 2017).

The microbiological risks of microorganisms can be limited by ensuring that the end product undergoes a germicidal treatment.

Freezing and freeze-drying has hardly any effect on the microbial quality of insect larvae (mealworms) (FASFC, 2014).

A number of studies on the effects of different methods of heating of yellow mealworms and lesser mealworms have been described. In these studies, the initial level of infection of these larvae was around 7-8 log cfu/g for the aerobic bacterial count and 6-7 log cfu/g for the *Enterobacteriaceae* count.

Oven-drying (110 minutes at 90ºC) of yellow mealworms reduced the aerobic bacterial count by 2 log and the *Enterobactericeae* count by 3 log (FASFC, 2014) and roasting (10 minutes, temperature unknown) of yellow mealworms reduced these counts by 3 log and 4 log, respectively (Klunder et al., 2012). A wet heat treatment (boiling for 10 minutes) of these larvae was more effective, resulting in a reduction of the counts by 5 log and more than 7 log, respectively (Klunder et al., 2012*).* Blanching (40 seconds in boiling water) of yellow mealworms resulted in a reduction of the counts by more than 5 log and more than 6 log, respectively (Vandeweyer et al., 2017a). Blanching lesser mealworms in water at 90ºC (approximately 5 minutes) resulted in a reduction of the counts by 4 log and 6 log, respectively (Wynants et al., 2018a). To meet the microbiological standard for *Enterobacteriaceae* in feed materials derived from animal by-products (Table 5), a reduction of >5 log from the initial contamination level is required.

The bacterial pathogens relevant to this advice (Tables 3 and 4) are not spore formers, so a pasteurisation (which is a 5-6 log reduction of the most heat-resistant organism) should be sufficient to reduce these bacteria to an acceptable level (Dijk, 2014).

The ASF, CSF and FMD viruses, HEV, *C. parvum* and *T. gondii* and *T. spiralis* are inactivated at temperatures between 60 and 70ºC for one to five minutes (the higher the temperature, the shorter the time required for inactivation) (Anderson, 1985; Dubey, 1998; Dubey et al., 1990; Feagins et al., 2008; Gubbins et al., 2016; Kotula et al., 1983; Turner & Wiliams, 1999; Turner et al., 2000). An adequate germicidal treatment of the larvae can stop any indirect recycling of microorganisms from (former) foodstuffs via insect larvae to animal feed and the animals fed with this feed.

Conclusion
The microbiological risks arising from pathogenic microorganisms in insects reared on a substrate composed of FF of plant and/or animal origin can be adequately controlled via an effective germicidal treatment carried out while producing the end product. 'Adequate control' means that the end products meet the microbiological safety standards applicable to processed animal proteins and other feed materials derived from animal by-products.

Prions
Prions are extremely resistant to standard inactivation methods such as heat, radiation and chemicals (Sakudo et al., 2011). Even heating to 133°C for 20 minutes at a pressure of 3 bar, which is the most stringent processing method required under EU legislation for processed animal proteins (Regulation (EC) No.

142/2011, Annex IV, Chapter III), only inactivates 2 log (RIVM-LCI, 2007). No suitable sterilisation techniques are currently available for the inactivation of prions in feed materials and food.

Prions in insects can only be controlled by preventing their introduction into the insects via the substrate.
Among farm animals and under natural conditions, TSEs only occur in ruminant farm animals, and lead to a fatal disease in both these ruminants and humans. In cattle, the majority of the BSE cases that are currently occurring are atypical BSE cases (EFSA, 2016b; EFSA, 2017b; EFSA, 2018b). The epidemiology of atypical BSE is still unclear (Requena et al., 2016; Tranulis et al., 2011) and experts in the field of TSEs expect these to remain present in the bovine population (Requena et al., 2016).
Naturally-occurring prion diseases have not yet been detected in non-ruminant farm animals or in fish, crustaceans and shellfish.

TSEs are controlled by implementing the applicable legislation and regulations to combat TSEs. As a result, not only has the incidence of BSE in the bovine population decreased (from more than 2000 cases in 2001 to 1 case in 2018) (EC ADNS, 2019), but the human disease burden of vCJD has also declined sharply. This has been achieved through the feed ban which (with a few exceptions) prohibits farm animals from being fed with other farm animals, thus preventing the accumulation of prions in populations of farm animals that may be susceptible to this. In addition, this is the result of the mandatory obligation to remove specified risk material (SRM) from the feed and food chain. SRM includes the spinal cord, brain, eyes, tonsils and parts of the intestines from ruminant farm animals. Together, these tissues are responsible for 99.7% of infectivity in a BSE case (EC SSC, 1999b). Lucker et al. (2011) have estimated the reduction of TSE infectivity through the removal of SRM to three orders of magnitude. As a result, there is only a very small chance of BSE being present in the feed and food chain. Prions may also occur in the muscle spindles of muscle fibres (Okada et al., 2014). These are not homogeneously distributed in the muscle, but are concentrated around the tendon junctions, which form the least edible part of a muscle.

The risk to humans is further limited by the species barrier. The species barrier is a transmission barrier that limits the spread of prions between different species of animals. The species barrier between cattle and humans is estimated at 4000 (1=no species barrier) (EFSA BIOHAZ Panel, 2006). Inter-species transmissions of prions are very inefficient and have longer incubation times than intra-species transmissions (Béringue et al., 2008; Requena et al., 2016). Controlling human exposure to BSE is due to the low incidence of BSE in cattle, the removal of SRM from the feed and food chain and the species barrier. As a result, there is a negligible risk to humans for contracting vCJD after consuming beef and other ruminant meat. The EFSA BIOHAZ Panel considers the removal of SRM as the most important public health measure for controlling TSEs (EFSA BIOHAZ Panel, 2014).

However, there is no species barrier between ruminants, as a result of which intra-species recycling can also lead to TSEs in the bovine population via passage in insects.

Due to the species barrier between ruminants and non-ruminants, it is highly unlikely, although not impossible, that TSEs will be transmitted to non-ruminant farm animals via the consumption of ruminant meat and vice versa, and therefore also very unlikely , although not impossible, that this will occur via passage in insects. Since it is not possible to completely rule out this risk, current EU legislation concerning the feeding of ruminants and using ruminant products as feed must continue to be enforced. The chance of transmission of TSEs from non-ruminants to non-ruminants is negligible as long there is no intra-species recycling (EFSA BIOHAZ Panel 2007a; EFSA BIOHAZ Panel, 2007b); this should also not occur via a two-species feedback loop with an insect in between.

With both forms of intra-species recycling, there is uncertainty about the effect: the increase or decrease of prions in an animal population depends on the dose of infectious material that is reused as feed, the degree of infectivity of the agent and the lifespan of the susceptible animal species.

Prion diseases have a long incubation period, as a result of which an infection of the population could go unnoticed until late in the process. For BSE, it has been calculated that, with the current level of surveillance [56], it will take at least 16 years before a reintroduction of BSE can be detected. This allows the disease to spread easily unnoticed (EFSA BIOHAZ Panel, 2014). Given the severity of prion diseases and the possibility of them being discovered too late after the prions have already spread within a susceptible animal species population, it is important to avoid intra-species recycling not only among ruminants, but also among all farm animals.

Given the aforementioned conditions for the safe use of FF as feed for farm animals, it is necessary to separate FF based on the type of animal protein processed into the FF. This traceability requirement relating to the origin of the food waste streams is difficult to guarantee, because there is limited insight into the previous links in the chain (Schripsema et al., 2015).

Providing safeguards against the hazard also includes monitoring its occurrence. The EC therefore states that, the lifting of the ban on the use of processed animal proteins from non-ruminants in non-ruminant feed while maintaining the existing ban on reuse within the same species, would only be acceptable if validated analytical methods are available to identify the animal species from which the processed animal proteins in the feed is derived (EC, 2010). At present, validated methods are only available for the detection and identification of ruminant DNA in animal feed (Fumière et al., 2016).

Conclusion
When using substrates with animal components other than those originating from FF containing dairy, eggs, honey, rendered fat or FF containing collagen and gelatine from non-ruminant species (i.e. FF with meat from non-ruminants, and fish, crustaceans and shellfish), the risk of prions can be controlled by ensuring

[56] In countries or regions with a negligible BSE risk, surveillance procedures are set up such that, with an assumed prevalence of at least 1 case per 50,000, the population of adult bovine animals in the country or region in question can be identified with a 95% reliability (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 (extended feed ban or the TSE Regulation); OIE, 2019).

that the FF, which is used for feeding the insect larvae, does not contain any ruminant products; that the species fed with the feed produced from insects is not a ruminant; and that the non-ruminant animal species being fed is not the same as the non-ruminant animal species in the FF on which the insects are reared.

Uncertainties

The risk assessment of the chemical and microbiological risks of the use of FF-reared black soldier fly larvae, housefly larvae, mealworms and lesser mealworms as animal feed raw material involves the following uncertainties:

- There are no studies available on the accumulation potential with respect to all the relevant combinations of insect larvae/chemical agents.
- There are no studies available on the vector potential with respect to all the relevant combinations of insect larvae/microbiological agents.
- The extent of transmission of microbiological agents from the substrate to the insect larvae is unknown.
- Data on the occurrence of chemical and microbiological agents in the insect larvae is very limited: data is not available for all agent/pathogen combinations.
- There is no information about the possible formation of process contaminants during processing into animal feed raw material.
- There is uncertainty about the effect (increase or decrease of microbiological agents in the population) of feeding farm animals with insect larvae that have been reared on FF containing meat, fish, crustaceans and shellfish belonging to the same animal species. This depends on the dose of infectious material that is recycled, the infectivity of the agent and the lifespan of the susceptible species.
- There is no information about the extent and frequency of the use of the insect larvae as animal feed raw material. At present, only the use of the larvae as feed for aquaculture is permitted (and where the FF, with which larvae may be fed directly, must be of completely non-animal origin, with the exception of FF derived from dairy, eggs, honey, rendered fat, and FF derived from collagen or gelatine from non-ruminants).
- There are no epidemiological data on incidents and outbreaks of disease caused by the use of insect larvae as animal feed (raw material).

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