

Food Safety Issues Related to Uses of Insects for Feeds and Foods

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Abstract: Edible insects are expected to become an important nutrient source for animals and humans in the Western world in the near future. However, before insects can be put on the market, the safety of their use for feed and food is warranted. This literature study was prepared to provide an overview of the actual knowledge of possible food safety hazards, including chemical, microbiological, and allergenic agents and prions, to human and animal health upon the use of insects for food and feed, and to highlight data gaps and suggest the way forward. From the data available, heavy metals of concern are cadmium in black soldier fly and arsenic in yellow mealworm larvae. Investigated mycotoxins do not seem to accumulate. Residues of pesticides, veterinary drugs, and hormones, as well as dioxins and PCBs, are sometimes found in insects. Contamination of insects with pathogens to human health is a consequence of a combination of the substrates used and the farming and processing steps applied. Insects harbor a wide variety of microorganisms, and some human pathogenic bacteria may be present. In addition, insects may harbor and transmit parasites. There is no evidence so far insects may harbor pathogenic viruses or prions, but they may act as vectors. Insects and insect-derived products may have allergenic potential. In this review, evidence on some safety aspects is displayed, and data gaps are identified. Recommendations are given for future research to fill the most relevant data gaps.

Keywords: edible insects, feed, food, review, safety

Introduction

Insects are seen as one of the possible solutions to face several challenges approaching in the near future: population growth and increasing demand for animal protein, food and feed insecurity, and environmental pressures (Van Huis et al., 2013). Although insects are widely consumed in parts of Africa and Asia as traditional foods, insects are not yet included in regular diets of consumers and in animal feeds in Europe and North America. However, the momentum regarding increased use of insects for feed and food in Western countries has been getting stronger and more visible in the past few years. The longstanding ban on feeding processed insect proteins to aquaculture animals has recently been lifted (for example, Regulation EU No. 2017/893 amending Regulations EC 999/2001 and EU No. 142/2011). Before insects can be used as a regular part of feed and food in Western countries, possible food safety concerns should be investigated. To this end, some literature reviews are available (Belluco et al., 2013; van der Spiegel, Noordam, & van der Fels-Klerx, 2013) and some opinions have

been published (ANSES, 2015; FASFC, 2014; Netherlands Food and Consumer Product Safety Authority [NVWA], 2014). Next, EFSA investigated all available information on the safety of insects for use in feed and food in Europe and published its risk profile in 2015 (EFSA, 2015). This opinion clearly identified data gaps, and as a follow-up, recent studies have focused on specific food safety hazards in insects reared under controlled conditions, leading to new insights.

The objective of this study was to provide an overview of the possible chemical, microbiological, and allergenic hazards and prions that may pose a risk to human and animal health upon the use of insects for feed or food, through a review of published studies and to highlight data gaps and illuminate the ways forward. The focus of this study was on the insect species currently considered for use in feed and/or food in Europe, as reported in NVWA (2014) and Charlton et al. (2015). These species include the following.

Chemical Hazards

Chemical contaminants may be present in food and feed originally derived from insects, and they occur as a result of the presence of the particular contaminant in the environment and/or the substrate. Such chemicals include, among others, heavy metals, veterinary drug residues, organohalogen compounds, and pesticide residues. The main chemical exposure route is through the substrate on which the insects are grown and, therefore, the chemical(s) of concern will vary accordingly. For instance, insects reared on agricultural waste may be exposed to plant protection products

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and mycotoxins, while insects reared on manure may be exposed predominantly to veterinary drug residues.

Experimental research on the chemical safety of insects reared for food and feed is scarce. More data are available for insects harvested in the wild. Some information is also available from the monitoring of insects on the market. In the latter two types of studies, however, there is generally no information available on the substrate on which the insects have fed. Below is a brief overview of the current knowledge of chemical safety for insects reared for food and feed.

Heavy metals and arsenic

The concentration of heavy metals and arsenic in insects depends on the specific metal (including arsenic), the metal concentration in the substrate, the insect species, and the growth phase of the insect (EFSA 2015; van der Fels-Klerx, Camenzuli, Lee, & Oonincx, 2016). Although information on the safety of insects for food and feed with respect to heavy metals is limited, there are a number of studies that have focused on the bioaccumulation of metals in insects harvested in the wild, or recently also under controlled conditions.

The metabolism of essential metals, such as copper and zinc, is efficiently regulated in insects, such that no correlation is generally observed between the substrate concentration and the insect internal concentration (Crawford, Lepp, & Hodgkinson, 1996; Maryanski, Kramarz, Laskowski, & Niklinska, 2002; Vijver, Jager, Posthuma, & Peijnenburg, 2003). For nonessential metals like cadmium, lead, mercury, and arsenic, a positive correlation between the substrate concentration and the insect internal concentration has been observed in many studies (Diener, Zurbrugg, & Tockner, 2015; Jamil & Hussain, 1992; Lindqvist & Block, 1995; Maryanski et al., 2002; van der Fels-Klerx, Camenzuli, van der Lee, & Oonincx, 2016; Vijver et al., 2003; Zhang, Lu, Wang, & Zheng, 2009). However, the extent of accumulation varies with the metal, the insect species, and the growth phase of the insect.

In a study by Charlton et al. (2015), the larvae of 4 different fly species (*Musca domestica*, *Calliphora vomitoria*, *Chrysomya* spp., and *Hermetia illucens*) using diverse rearing methods and a range of waste substrates from the United Kingdom, China, Mali, and Ghana (nine fly samples in total) were analyzed for the presence of 48 heavy metals and trace elements. Cadmium (Cd) concentration was found to be above the detection limit (LOD of 10 to 50 $\mu\text{g/kg}$) in every sample analyzed. In 3 of the 5 *M. domestica* larvae, the levels of Cd exceeded the EU maximum limit for complete feed (0.5 mg/kg at 88% dry matter), however, the maximum allowable content in feed materials was not exceeded. In this study, the concentration of metals in the substrate was not measured and, therefore, no conclusions can be made on the accumulation pattern of Cd in the larvae of the fly species included in their study. In the work by Diener et al. (2015) and van der Fels-Klerx et al. (2016), Cd bioaccumulation in *H. illucens* larvae was confirmed through analysis of both the larvae and substrate. In both studies, larvae Cd concentration increased as substrate concentration increased. Since both studies spiked the substrate with Cd, the metal may be less bound than under natural contamination conditions, so results have to be interpreted with care. Diener et al. (2015) investigated the accumulation, expressed by the bioaccumulation factor (BAF), namely the ratio of the concentration of a contaminant in the organism to the concentration of that contaminant in the diet, at different life stages, and they observed Cd accumulation in both larvae and prepupae (BAF of

2.3 to 2.9). Comparable levels of Cd were also measured in the larval and pupal exuviae. However, heavy metal concentration in adults (BAF < 1) was significantly lower than in prepupae. The work by van der Fels-Klerx et al. (2016) concluded that Cd accumulation in *H. illucens* larvae should be considered carefully, since in their work, when the substrate contained Cd at the current EC maximum limit in complete feed, the Cd concentration in the larvae exceeded the maximum limit for Cd in feed materials (Directive 2002/32/EC). These authors also investigated the uptake of Cd by the larvae of the mealworm *Tenebrio molitor*. Similar to the fly larvae, the concentration of Cd in the *T. molitor* larvae increased with increasing substrate Cd concentration. However, the Cd BAF was consistently below 1, and, unlike for the fly larvae, Cd concentration in the *T. molitor* larvae never exceeded the maximum Cd limit for feed material. Similar bioaccumulation results were obtained by Vijver et al. (2003) for *T. molitor* larvae fed on soil and sediment. Furthermore, the concentration of Cd decreases during molting and metamorphosis, resulting in lower adult concentrations (Lindqvist & Block 1995).

Purschke, Scheibelberger, Axmann, Adler, and Jäger (2017) investigated the accumulation of several heavy metals (2 mg/kg As, 1 mg/kg Cd, 10 mg/kg Cr, 0.1 mg/kg Hg, 10 mg/kg Ni, and 10 mg/kg Pb on a wet basis) in *H. illucens* larvae. The substrates were spiked to “common contamination levels found in animal feed,” but it is unclear which data were used to determine those values. They found BAF values of 9.1 ± 1.4 for Cd and 2.3 ± 0.3 for Pb, which is relatively comparable to the results of Tschirner and Simon (2015) (BAF for Cd: 7.4 ± 2 , and for Pb: 2.6 ± 0.6) and van der Fels-Klerx et al. (2016), as discussed above.

In the field study by Zhang et al. (2009), Cd bioaccumulated in all 4 herbivorous insects studied (*Eligma naricissus*, *Holotrichia*, *Acrida chinensis*, and *Locusta migratoria manilensis*). However, the BAF for *L. migratoria* was 1.07 only, indicating slight accumulation. Crawford et al. (1996) observed increasing Cd concentrations in *L. migratoria* with increasing substrate Cd concentrations, however, the concentration in the locust was consistently lower than in the host plant and feces.

In the study by Charlton et al. (2015), lead (Pb) was above the LOD (of 0.1 mg/kg_{dw}) in the *M. domestica* larvae (4 samples), but below the LOD for the other three fly species (5 samples in total). However, no information was available on the concentration of lead in the substrate. Diener et al. (2015) showed the Pb concentration in *H. illucens* larvae increased with increasing substrate concentration, however, the majority of the Pb accumulated in the exuviae. van der Fels-Klerx et al. (2016) observed that larvae provided feed at the current EC maximum limit for Pb in complete feed; the Pb concentration in the larvae was below the maximum limit for Pb in feed materials and complete feed (Directive 2002/32/EC).

Vijver et al. (2003) observed a positive correlation between the concentration of Pb in *T. molitor* larvae and in soil, however the BAF was consistently below 1. High concentrations of Pb were measured in the feces of the *T. molitor* larvae (van der Fels-Klerx et al., 2016), such that when exposed to feed at the EC maximum limit for Pb in complete feed, the concentrations of Pb in larvae were below the LOD (0.1 mg/kg). Pb bioaccumulation was not observed in the locust *L. migratoria* (Zhang et al., 2009).

The concentration of mercury (Hg) was above the LOD in 7 of the 9 larvae samples analyzed by Charlton et al. (2015), however, none exceeded the EC maximum limit for mercury in feed materials. Hg was not observed in the locust *L. migratoria* (0.62 ± 0.60 mg/kg) (Zhang et al., 2009).

Arsenic (As) was measured below LOD (0.1 to 0.5 mg/kg) in every sample of fly larvae analyzed by Charlton et al. (2015). However, van der Fels-Klerx et al. (2016) showed that, while the larvae of *H. illucens* accumulated As, the BAF were consistently below 1. Additionally, the larvae did not exceed the maximum limit for As in feed materials (Directive 2002/32/EC). However, in the same study, As did bioaccumulate in the larvae of the mealworm *T. molitor*: if reared on a substrate containing the current EC limits for As in complete feed (2 mg/kg), this would result in larvae exceeding the EC limit in feed materials (BAF 2.6 ± 0.23). As has also been shown to accumulate in the Bogong moths (*Agotis infusa*), however, the extent of bioaccumulation is unknown (Green, Broome, Heinze, & Johnston, 2001).

Toxins produced by or accumulated in insects

The presence of toxins in insects is either a result of the insect itself synthesizing a natural toxin, or of accumulation through the substrate on which they are reared. From the insects being considered for use as food and feed within the EU (Table 1), there are no immediate indications that they produce reactive, irritating, or toxic substances in the life stages being considered for consumption (FASFC 2014). Some studies have been performed on possible contamination of natural toxins in insects from the substrate, focusing on mycotoxins, but not (yet) on plant toxins.

Charlton et al. (2015) analyzed the presence of 69 mycotoxins in the fly species included in their study. Three mycotoxins were detected above the LOD; namely enniatin A ($12.5 \mu\text{g/kg}$) and A_1 ($7.3 \mu\text{g/kg}$) in 1 sample of larvae of *M. domestica*, and beauvericin in another sample of larvae of *M. domestica*. This was not believed to be a safety concern however by the authors because there are currently no maximum limits set for these mycotoxins by the European Commission and the authors believed the levels of these mycotoxins do not pose a safety risk.

In the study by Guo et al. (2014), larvae of the mealworm *T. molitor* were reared on wheat kernels infected with four *Fusarium* species. Several mycotoxins were measured in the kernels and larvae, however, the concentrations in the larvae were generally much lower than in the kernels. The mycotoxins detected in the larvae include fumonisin B1, deoxynivalenol (DON), zearalenone, beauvericin, and enniatins A, A_1 , B, and B_1 . In the work by Van Broekhoven, Gutierrez, De Rijk, De Nijs, and Van Loon (2017), larvae of *T. molitor* were reared on wheat flour spiked with DON ($8000 \mu\text{g/kg}$ pure DON) and no detectable levels of the DON and two derivatives analyses as well (DON3-G and 15-AC-DON) were measured in the larvae after 14 days (LOD 0.1 mg/kg). Up to 41% of the DON present in the feed was excreted, however, the remaining fraction could not be accounted for, suggesting that the mycotoxin may have been metabolized by the mealworm larvae. The authors suggested that enzymes are possibly involved in detoxifying DON, and that further research should be done to find out which enzymes are responsible for DON metabolism and which metabolites are formed.

Bosch, van der Fels-Klerx, De Rijk, and Oonincx (2017) investigated the aflatoxin B_1 (AFB $_1$) tolerance and accumulation in larvae of *H. illucens* and *T. molitor*. They found that the AFB $_1$ concentrations tested for (at levels of 0.01, 0.025, 0.05, 0.10, 0.25, and up to 0.5 mg/kg dry feed) did not affect survival and weight gains in both species. In addition, the concentration of AFB $_1$ in larvae of both insect species was very low: it was below the LOD (of $0.1 \mu\text{g/kg}$) for *H. illucens*, and detected in *T. molitor* larvae only when they were provided with feed containing 0.025 mg/kg

AFB $_1$ or more. It was concluded that both species have a high AFB $_1$ tolerance and do not accumulate AFB $_1$.

Camenzuli et al. (2018) investigated the possible accumulation of 4 different mycotoxins in larvae of both *H. illucens* and *Alphitobius diaperinus* (lesser mealworms). Mycotoxins (AFB $_1$, DON, zearalenone, and ochratoxin A) were added to the substrate, both individually and as a mixture, at three different concentrations. Besides the mycotoxins, also some metabolites were analyzed in the larvae and the excreta. Similar patterns were seen for the mixture as with the individual mycotoxin treatments. The mycotoxins did not accumulate in the larvae, and they were only found in very limited concentrations or below the LODs of the respective mycotoxins. The analyzed metabolites could not explain the differences, except for zearalenone given to the *H. illucens* larvae. In that case, the parent compound (zearalenone) was metabolized by the larvae, to a large extent, into the two analyzed metabolites α - and β -zearalenol.

Purschke et al. (2017) also investigated the accumulation of DON ($4600 \pm 1400 \mu\text{g/kg}$; naturally contaminated), AFB $_1$ ($88 \mu\text{g/kg}$), aflatoxin B_2 ($17 \mu\text{g/kg}$), aflatoxin G_2 ($46 \mu\text{g/kg}$), ochratoxin A ($260 \mu\text{g/kg}$), and zearalenone ($860 \pm 260 \mu\text{g/kg}$; naturally contaminated) in *H. illucens* larvae (concentrations on a wet basis). All the tested mycotoxins were not detected in the larvae after the feeding trials.

Veterinary drugs and hormones

Insects grown on manure may be exposed to a variety of veterinary drugs (residues) that were poorly absorbed in the animal to which they were administered. However, very little information is available on the presence or possible accumulation of veterinary drug residues in insects.

Lalander et al. (2016) investigated the uptake of two antibiotics (roxithromycin and trimethoprim) and an antiepileptic drug (carbamazepine) in larvae of *H. illucens* grown in a composting system, in which organic waste was converted into animal feed protein and treatment residue for use as organic fertilizer. No bioaccumulation of these pharmaceuticals was observed in the larvae. In another study, the veterinary drug nicarbazin was detected in 1 sample of *M. domestica* grown on poultry manure in the United Kingdom (Charlton et al., 2015).

Veterinary drugs may also be sprayed or mixed with the substrate during rearing of insects to combat infections from a variety of insect pathogens and reduce the probability of mortality from infections (Inglis & Sikorowski 2009; Roeder, Kuriachan, Vinson, & Behmer, 2010). According to Roeder et al. (2010), two broad classes to control microbial agents are used, being antibacterial and antifungal agents. Since many of these agents are also toxic to the insects themselves, finding the right combination to combat microbial growth while at the same time limiting negative effects on growth and survival of the insects is challenging.

Pesticide residues

Pesticide residues may be present in the substrate used for insect rearing. Pesticides used in agriculture may be present in plant materials and agricultural production wastes, which can be used as a substrate for insect production.

Lalander et al. (2016) observed no accumulation of the 2 fungicides azoxystrobin and propiconazole in the larvae of *H. illucens* grown in a composting system. In fact, they observed that the degradation half-life of these two fungicides was reduced from 78 and 214 d in soil, respectively, to less than 1 d in the composting system with the larvae. Charlton et al. (2015) also investigated

Table 1—Overview of insect species considered for use in feed and/or food in Europe (Charlton et al., 2015; NVWA 2014).

Insect species (English name)	Insect species (Latin name)	Envisioned for food/feed
Housefly	<i>Musca domestica</i>	Feed, food
Black soldier fly (BSF)	<i>Hermetia illucens</i>	Feed, food
Blue bottle fly	<i>Calliphora vomitoria</i>	Feed
Blow fly	<i>Chrysomya</i> spp.	Feed
Yellow mealworm	<i>Tenebrio molitor</i>	Food
Lesser mealworm	<i>Alphitobius diaperinus</i>	Food
Migratory locust	<i>Locusta migratoria</i>	Food

the presence of pesticides in the fly larvae: from the 393 pesticide residues analyzed, chlorpyrifos was detected in one sample of *M. domestica* reared on milk powder and sugar from China, and piperonyl butoxide was detected in 1 sample of *C. vomitoria* farmed in the United Kingdom.

In a study by Houben et al. (2016), larvae of *T. molitor* were exposed to carrots spiked with 12 pesticides with a log octanol–water partition coefficient (K_{ow}) ranging from -2.63 to 6.4 . They indicated that pesticides with the higher log K_{ow} appeared to bioaccumulate in the mealworm, while pesticides with a lower log K_{ow} appeared to be readily excreted by the insects. However, this reported correlation could not be readily identified from the data presented in that publication. Several other studies on chiral fungicides showed that *T. molitor* larvae metabolized and degraded the fungicides epoxiconazole (log K_{ow} 3.58) (Lv et al., 2014), metalaxyl (log K_{ow} 1.65) (Gao et al., 2014), benalaxyl (log K_{ow} 3.67) (Gao et al., 2013), and mycolobutanil (log K_{ow} 2.94) (Lv et al., 2013). These experimental results all are consistent with the K_{ow} pattern observed by Houben et al. (2016) and no bioaccumulation was observed for all four fungicides. Purschke et al. (2017) analyzed the accumulation of the chlorinated insecticides chlorpyrifos, chlorpyrifos-methyl, and pirimiphos-methyl (corn spiked with the pesticide to 2.5 mg/kg on a wet basis) in *H. illucens* larvae. For chlorpyrifos and chlorpyrifos-methyl, the spiked level was slightly below the EC allowable maximum residue level (MRL) for maize/corn (of 3 mg/kg, Regulation (EU) No. 396/2005), but was above the EU-MRL for pirimiphos-methyl in maize/corn (0.5 mg/kg). At these spiked levels, the generation of larval biomass was not affected compared to the control, and no absorption or accumulation of insecticides in larval tissue was observed at relevant levels.

Dioxins, dioxin-like polychlorinated biphenyls, and polyaromatic hydrocarbons

Dioxins (and furans—PCDD/F) and dioxin-like PCBs (dl-PCBs) are chemicals currently banned under the Stockholm Convention. However, a significant contamination of these chemicals still exists in the environment, predominantly in soils and sediment. PCDD/Fs and dl-PCBs were detected in all the fly larvae in the work by Charlton et al. (2015). Concentrations in the larvae of PCDD/Fs ranged from 0.14 to 0.44 ng_{TEQ}/kg dry weight (WHO-TEF), which are all below the EC maximum limit for feed materials of animal origin of 0.75 ng_{TEQ}/kg dry weight (Regulation (EU) No. 277/2012).

Similarly, PCBs were also detected in all of the larvae samples analyzed by Charlton et al. (2015). The sum of the indicator PCBs (iPCBs) for all samples was below the EC maximum limit for feed materials of animal origin of 10 µg/kg (Regulation (EU) No. 277/2012). From the work by Nordentoft, Cederberg, Fischer, and Bjerrum (2014), it was concluded that the larvae of *M. domestica* accumulated PCBs when reared on chicken

manure. The concentration in the larvae was up to 4 times the concentration in the manure.

Charlton et al. (2015) also analyzed the presence of 28 PAHs in the fly larvae. The concentrations of the PAH4 (benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, and chrysene) in all samples analyzed were between 0.28 and 9.82 µg/kg and the concentration of BaP (benzo[a]pyrene) ranged from <0.05 to 2.2 µg/kg. There are currently no maximum limits for PAHs in animal feed set by the EC, however, limits for PAH4 in food range from 1 to 35 µg/kg (Regulation (EU) No. 835/2011).

Conclusion on chemical hazards

The main route of exposure of insects to chemical hazards is through the substrate on which the insects are grown and, therefore, the chemical(s) of concern will vary accordingly. Potential accumulation also often depends on the insect type and growth phase at which the insect is harvested. Essential heavy metals do not accumulate in insects, whereas nonessential heavy metals and arsenic may accumulate, depending on the element, insect type, and growth stage. Elements of most concern, due to their accumulation potential in insects that are of current interest for use in feed and food, include cadmium in the black soldier fly and arsenic in yellow mealworm larvae. Other heavy metal–insect species combinations might be of concern as well, but they need to be further investigated in controlled experiments. There are no indications that insects being considered for use for food or feed in the Europe produce reactive, irritating, or toxic substances in the life stages being considered for consumption. Black soldier fly and lesser mealworms do not seem to accumulate some investigated EU-regulated mycotoxins at significant levels, rather it seems that larvae of these species may be able to metabolize these mycotoxins, but some other mycotoxins may accumulate. To date, few studies have been done on the accumulation of residues of pesticides, veterinary drugs, and hormones in insects, but the results so far indicate potential uptake of some of them. Also, dioxins and dl-PCBs may be taken up by some insect species.

Microbiological Hazards

In the last few years, several reviews have tried to summarize available evidence on the microbiological safety of edible insects, mainly highlighting important data gaps (Belluco et al., 2013, 2015; Mlcek, Rop, Borkovcova, & Bednarova, 2014; Rumpold & Schlüter 2013; van der Spiegel, Noordam, & van der Fels-Klerx, 2013). Data available in the literature have been obtained from studies driven by very different purposes and mainly focused on the understanding of insects as vectors of diseases in animal farms (Wales et al., 2010). These studies investigated the potential for insect to be mechanical (Agabou & Alloui, 2010; Ahmad, Nagaraja, & Zurek, 2007; Goodwin & Waltman, 1996; Nelson & Harris, 2006) or biological (Hazeleger, Bolder, Beumer, & Jacobs-Reitsma, 2008; Kobayashi et al., 1999; Templeton, De Jong, Blackall, & Mifflin, 2006) vectors of pathogenic microorganisms,

but did not give certainties about the potential role of insects as reservoir for such microorganisms. In the last years, the situation has changed and an increasing number of studies investigated the microbiological contamination of insects as feed and food.

Two types of microbiota should be considered when assessing the microbial community of insects: those that are intrinsically associated with insects as part of their life style and those that are introduced during farming and processing and carried forward (EFSA, 2015). In both cases, the farming environment plays an important role, because it can both modulate intrinsic microorganisms but also be the source for new ones. Hence, the results of published studies investigating the microbiota of wild insects should be interpreted cautiously, but they can give important information on how to target new investigations. Another important aspect in the modulation of microbiota is the species considered, thus it is inappropriate to consider the insect class as a whole or to extend limited results to a long list of different species. Despite these limitations, the available evidence and the data gaps are presented below.

Viruses

Insects have a specific plethora of viral pathogens that are considered safe for humans and are even, in some cases, approved as biocontrol agents in agriculture. Conversely, human viruses taxonomically related to insect ones are known to be unable to replicate in insects and would therefore not pose health concerns (Eilenberg, Vlak, Nielsen-Leroux, Cappellozza, & Jensen, 2015). However, studies are needed for particular groups of viruses, since the arthropod-borne viruses (arboviruses) are able to cause human diseases (such as dengue, West Nile disease, rift valley fever, hemorrhagic fever, chikungunya) and are able to replicate in their vectors (King, Adamse, Carstens, & Lefkowitz, 2012). As of now, the vector competence of insects used for food and feed has never been demonstrated (Eilenberg et al., 2015). Another important issue is related to the possibility that some viruses (norovirus, rotavirus, hepatitis E, and hepatitis A) could be introduced with substrate in insect production units and transferred beyond primary production. In this case, processing and cooking practices play an important role (Eilenberg et al., 2015). The primary (financial) burden caused by viruses is carried by insect farms because most viruses associated with insects are only pathogenic to the insects themselves (EFSA, 2015). A recent review (Maciel-Vergara & Ros, 2017) lists a broad array of viruses that have been found in insects most commonly reared for food and feed, some of which form a serious threat to insect mass rearing.

Bacteria

Entomopathogenic pathogens can spread easily in crowded production units and cause serious production losses due to the death of insects. The number of studies designed to provide specific evidence about the bacterial community of insects farmed for food purposes is increasing. Klunder, Wolkers-Rooijackers, Korpela, and Nout (2012) were the 1st, to our knowledge, to address edible insects from a food safety perspective, with an exploratory study. Mealworm (*T. molitor*) (farmed) and cricket, *Acheta domesticus* (farmed) and *Brachytrupes* spp. (harvested from the wild) from Lao PDR were analyzed in different conditions: fresh, boiled, roasted, and fresh and stored, and also boiled and stored, with 1 sample per condition (total of 8 samples). High counts of total viable bacteria, Enterobacteriaceae and spore-forming bacteria were isolated from the fresh insects, with an increase after crushing, attributed to the release of intestinal microbiota. Boiling insects for 5 min showed

to be an efficient process for eliminating Enterobacteriaceae, but not spore-forming bacteria. The correct management of insects with a potential for spore content requires storage at refrigeration temperature (5 to 7 °C). This temperature was also suitable to prevent the spoilage of boiled insects (stable for more than 2 weeks), but it was not efficient against spoilage of fresh insects. Roasting alone did not kill all Enterobacteriaceae; therefore, a few minutes of boiling were suggested before roasting (Klunder et al., 2012).

In a small-scale survey performed, NVWA (2014) on the microbiological status of 55 freeze-dried insect products (locusts, lesser mealworms, mealworms, and a mealworm snack) found that 59% of the investigated insect products exceeded the process hygiene criterion for aerobic bacteria in raw materials used in meat preparation (10^6 colony forming units [CFU]/g) as laid down in the Hygiene Code for Butchers (The Royal Dutch Butcher's Assn., 2010). Enterobacteriaceae exceeded the criterion for raw materials used in meat preparations (10^3 CFU/g) in 65% of the samples. *Clostridium perfringens*, *Salmonella*, and *Vibrio* were never isolated and *Bacillus cereus* was present at levels of less than 100 CFU/g in 93% of samples (NVWA, 2014).

The microbial community of fresh *T. molitor* larvae and grasshopper (*L. migratoria migratorioides*) fit for human consumption has been described by Stoops et al. (2016). Results highlighted a very high total viable aerobic counts and Enterobacteriaceae counts in all analyzed batches, whereas bacterial endospore counts were highly variables among batches. Proteobacteria, Firmicutes, and Actinobacteria dominated the bacterial composition in mealworm larvae, and Firmicutes and Proteobacteria in grasshoppers. *T. molitor* larvae were found to contain abundant operational taxonomic units such as *Haemophilus*, *Staphylococcus*, and *Clostridium*, which can contain pathogenic species. (Stoops et al., 2016).

Another study carried out on samples from crickets (*A. domesticus* and *Gryllobates sigillatus*) and *T. molitor* freshly reared and living larvae from seven different insect-rearing companies suggested the importance of pulverization before analyses to obtain better estimates of microbial counts (Vandeweyer, Crauwels, Lievens, & Van Campenhout, 2017a). These authors reported results from 21 different batches showing large counts of Enterobacteriaceae ranging from 6.8 to 8.3 log CFU/g in *T. molitor* and from 7.3 to 8.3 log CFU/g in crickets. Crickets and mealworm larvae contained on average 4.5 log CFU/g and 6.6 log CFU/g of psychrotrophic organisms, respectively, raising potential concerns for product storage. An important variation of microbial load within and between companies was also highlighted, despite the similarities of rearing protocols and of intrinsic parameters of different batches. Results suggest the existence of other factors influencing counts, such as feed and/or other rearing practices. *Salmonella* spp. and *Listeria* spp. were not isolated, whereas the presence of a notable amount of psychrotrophic bacteria suggests the potential for spoilage during low-temperature storage (Vandeweyer et al., 2017a). Analyzing samples from the same study, a variation of bacterial composition was observed through a metagenetic approach in larvae of *T. molitor* within and between investigated companies. *Spiroplasma* spp. was the most dominant operational taxonomic units (out). On the other hand, investigated crickets had a more similar microbial composition among companies with a high degree of similarities also between *A. domesticus* and *G. sigillatus*, and less dominant OTUs, if compared with mealworms (Vanderweyer, Crauwels, Lievens, & Van Campenhout, 2017b).

Osimani et al. (2018) analyzed different batches of fresh *T. molitor* larvae, their feeding substrate (wheatmeal) and frass. The microbial contamination of the wheat fed to the larvae was low, but the

larvae and frass contained high amounts of Enterobacteriaceae, lactic acid bacteria, and some mesophilic aerobes species. Spore-forming bacteria were detected to a lesser extent. *Enterobacter* spp., *Erwinia* spp., *Enterococcus* spp., and *Lactococcus* spp. were found to be the dominant genera in both the larvae and frass, whereas *Klebsiella* spp., *Pantoea* spp., and *Xenorhabdus* spp. were found to be in the minority. STEC was found to be absent. The authors hypothesized two routes of microbiological contamination, being transmission via feed and via the vertical route.

Garofalo et al. (2017) studied the microbial composition of marketed processed edible insects, including powdered small crickets (*A. domesticus*), and whole dried small crickets, whole dried locusts (*L. migratoria*), and whole dried mealworm (*T. molitor*) larvae. The samples were purchased from a company and shipped in plastic bags by international transport. The authors observed a strong variation in the microbial composition of the different species. Counts of total mesophilic aerobes, Enterobacteriaceae, lactic acid bacteria, *Clostridium perfringens* spores, yeasts, and molds were relatively low. *Salmonella* spp. and *Listeria* spp. were not isolated, but *Spiroplasma*, a bacterium linked to neurodegenerative diseases in humans and animals, was described in *T. molitor* (Garofalo et al., 2017).

A preliminary microbiological survey of insect-based products (38 samples) from a wide plethora of species (*A. domesticus*, *L. migratoria*, *T. molitor*, *Omphisca fuscidentalis*, *Oxya yezoensis*, *Vespula aviceps*, *Bombyx mori*, *A. diaperinus*, *M. domestica*, *H. illucens*) was carried out between 2014 and 2015. Products were obtained from Dutch and German market channels (locally produced or from Asia) and were treated with different processes (drying, deep-frying, heat-treatments, ...). *Salmonella* and *L. monocytogenes* were never isolated. Counts of total aerobic mesophilic bacteria, Enterobacteriaceae, yeasts and molds, staphylococci, *Bacillus cereus*, *E. coli* varied widely and were significantly different between heat-treated and not heat-treated products (Grabowski & Klein, 2017).

Caparros Megido et al. (2017) explored the microbial load of edible insects bought in Belgium. In particular, they analyzed fresh and treated (blanched and sterilized) and freeze-dried *T. molitor* and *A. domesticus* samples from two Belgian companies and other smoked insect species (Termites, Bingula caterpillar and Mikwati caterpillar) from a Belgian African market that obtained insect products from Congo (harvested insects). Results, also in this case, highlighted high total counts, and yeast and molds contamination in fresh and smoked specimen but consistent reductions after blanching (1 to 5 min), or in freeze-dried products (Caparros Megido, 2017).

The effectiveness of blanching was confirmed by Vandeweyer, Lenaerts, Callens, and Van Campenhout (2017) on *T. molitor* larvae. Larvae were blanched with different times and chilled or microwave-dried. Regardless of treatment times, blanching alone or blanching plus microwave drying was effective in killing vegetative cells but not or only hardly spores, thus spores needing special attention in this kind of products. Blanched mealworms can be stored in refrigerated conditions for (at least) 6 days without substantial microbial growth.

Heat treatment was shown to be effective in a study by Stoops et al. (2017) on minced meat-like products obtained with *A. diaperinus* and *T. molitor*. The authors observed high counts of total aerobic bacteria, Enterobacteriaceae and lactic acid bacteria in fresh living insects with a significant reduction after each heat treatment applied (steaming and frying for *T. molitor*, stir and pan frying for *A. diaperinus*). Reduction was not observed for bacterial spores whose counts in some cases increased. The use of modified

atmosphere (40% CO₂ and 60% N₂) reduced microbial growth if compared with in air packaging (Stoops et al., 2017).

Insects processing in the period immediately before and after killing was investigated by Wynants et al. (2017). The authors studied the effect of 48 hr starvation (at 10 and 30 °C) with and without contact with feces as well as the effect of rinsing on Total viable aerobic bacteria, Enterobacteriaceae, aerobic endospores, yeasts, and molds. No significant differences were observed among groups, suggesting that starvation and rinsing do not seem to increase the microbiological quality of products. Processing is a critical stage in determining microbial quality of edible insects, but the management of farming conditions should not be neglected. The microbiological contamination (total viable count, Enterobacteriaceae, lactic acid bacteria, aerobic endospores, and fungi) of *A. diaperinus* during large-scale farming was investigated in the Netherlands through microbiological qualitative and quantitative analyses of pulverized insects, insect feed, and remaining substrate (feces, feed, exuviae) before, during, and after farming (Wynants et al., 2018). Moist feed showed higher counts as compared to dry feed, with the exception of Enterobacteriaceae that were present at similar levels in both feeds, and showed an increase toward the end of the farming period in moist feed. The bacterial counts of larvae did not show a significant increase or decrease during the farming cycle (35 days, five sampling moments) as well as after starvation (24 hr) with similar levels between substrate and larvae. A significant reduction of the presence of all bacteria was observed after blanching with a higher reduction for Enterobacteriaceae, LAB and fungi, whereas endospores were the main survivors. *Salmonella* spp. and *Listeria monocytogenes* were not detected and *B. cereus* and *Staphylococcus aureus* were present in levels below the LOD (<100 CFU/g). According to metagenetic analyses, diversity indexes showed a reduction in the variability of microbial flora in larvae toward the end of life cycle if compared to substrate suggesting a selection of bacteria within the insect gut. Proteobacteria and Firmicutes were the dominating bacterial phyla (Wynants et al., 2018).

The reduction of bacteria diversity in insect midgut was confirmed in another study aimed at comparing the microbiota of insects reared in a Controlled Environment Agriculture Ecosystem with an open environment (Li, Xie, Dong, Wang, & Liu, 2016). In this metagenomic study, the number of OTU as well as Shannon and Chao1 index of diversity were higher in feed than in midgut and frass suggesting a selection due to midgut conditions.

In general, high counts of microorganisms are recovered from fresh insects, as described above, with heat treatment representing an important step to reduce contamination. In case of fresh insects marketing, the consumer phase is of great importance as is the case with household cooking of all non-RTE (ready-to-eat) products. The effect of different household heat treatments on fresh insects (*T. molitor*) was recently evaluated together with the impact on nutritional composition. Heat treatments were effective for reducing total microbial counts with levels of reduction ranging from 2 log CFU after oven cooking to 4/5 log CFU after vacuum cooking and frying, until 7 log CFU reduction after boiling (Caparros Megido et al., 2018). The intestinal mycobiota of *H. illucens* fed on chicken feed and/or vegetable waste was studied to highlight differences between diets. Results showed differences in the fungal communities between the group fed 17 days on vegetable waste plus 4 days on chicken feed, compared with the groups fed only on chicken feed as vegetable waste feeding was associated with a greater diversity of fungal species. This suggests that the type of

substrate selects diverse yeast and mold genera (Varotto Boccazzi et al., 2016).

As reviewed by Zurek and Ghosh (2014), insects represent a reservoir for antibiotic-resistant bacteria. However, data are very scarce with regard to the prevalence of ARGs in edible insects; recently Osimani et al. (2017) assessed the occurrence of transferable antibiotic resistance genes in commercialized edible grasshoppers, revealing a high occurrence of genes responsible for resistance to tetracycline (tet(M), tet(S) and tet(K)), lower occurrence of erythromycin and aminoglycosides resistance genes. Neither *vanA* nor *vanB* (genes conferring vancomycin resistance) were detected in this study. Similar results were reported by Milanović et al. (2016) in 11 species of marketed edible insects (small crickets powder, small crickets, locusts, mealworm larvae, giant waterbugs, black ants, winged termite alates, rhino beetles, mole crickets, silkworm pupae, and black scorpions).

Parasites

Another aspect of the microbiological safety of insects is the potential for harboring and transmitting parasites through the oral route as suggested by some scientifically published studies. Data from human autopsies and insect analysis, in areas where insect consumption is traditionally practiced, suggested the possible foodborne transmission of parasites (trematodes) belonging to the family Lecithodendridae and Plagiorchiidae (Chai, Shin, Lee, & Rim, 2009).

Several cases of infestation with *Gongylonema pulchrum* have been described worldwide and were occasionally associated with raw insect ingestion (Allen & Esquela-Kerscher, 2013; Molavi, Massoud, & Gutierrez, 2006). In addition, the potential oral transmission of *Trypanosoma cruzi* should not be neglected. This parasite causes the Chagas disease and affects about 10 million people in the Americas, with more than 10000 people dying each year. Poor housing conditions promote contact with infected vectors, and even though the oral route is not the main way of transmission cases have been reported in the literature linking infection with the accidental ingestion of insects (Pereira et al., 2010) or consumption of contaminated food (Trotta Barroso Ferreira, Melandre, Cabral, Branquinho, & Cardarelli-leite, 2016).

Dicrocoelium dendriticum is the causative agent of a rare foodborne zoonosis of the human biliary tract. Dicrocoeliosis can be transmitted to humans via the oral route through the ingestion of infested liver of ruminants (pseudodicrocoeliosis) or through the ingestion of infested ants that act as intermediate hosts in the parasite life cycle (Jeandron et al., 2011). Some insects' species (*Blatella germanica* and *Periplaneta Americana*) have been demonstrated to harbor pathogenic protozoa like *Entamoeba histolytica*, *Giardia lamblia*, *Toxoplasma* spp., and *Sarcocystis* spp.

Conclusion

In conclusion, the potential presence of microbiological hazards for human health in insects is affected by a combination of the substrates used and the processing steps applied between farming and consumption. The substrate used and the farming environment strongly influence the insects' microflora and, therefore, possible contamination with microbiological hazards is influenced by the nature and the hygienic conditions of the substrate and the farming environment. In general, insects harbor a wide variety of microorganisms, and some pathogenic bacteria may be present. In particular, insects are characterized by high counts of total viable bacteria and Enterobacteriaceae with higher counts when insects are analyzed after pulverization suggesting

that an important proportion of contamination is due to gut content. In addition, insects may harbor and transmit parasites upon consumption. There is no evidence yet they may harbor pathogenic viruses, though insects may act as vectors. The insect harvesting process may be of critical importance, since it will impact the probability of transfer of hazards from the substrates to the insects and further on to the derived products (EFSA, 2015). Proper heat treatment before consumption may eliminate most microbiological hazards and has been shown to be effective in particular against Enterobacteriaceae, whereas bacterial spores are not affected. Particular attention should be paid in the storage of processed products and in the household treatment of fresh insects.

Prions

No specific prionic diseases have been observed in insects due to the lack of PrP-encoding gene. However, they could act as mechanical vectors of prions derived from at-risk substrates of ruminant origin with potential concerns for humans or susceptible animals according to the destination of insect-based products (EFSA, 2015). The current knowledge on the risk of prionic diseases posed by insects was recently reviewed by Van Raamsdonk, van der Fels-Klerx, and De Jong (2017). In short, although prions cannot be expressed in insect genomes (Thackray et al., 2012), insects do appear to be able to act as vectors if reared on a contaminated substrate (Lupi, 2005, 2006; Post, Riesner, Walldorf, & Mehlhorn, 1999).

Allergens

Most studies on allergic reactions to insects report allergy caused by occupational exposure, via inhalation, or by direct contact handling the insects, or even by insect bites or stings (Van Broekhoven, Bastiaan-Net, De Jong, & Wichers, 2016). One study reported an allergic reaction in response to consumption of edible insects, in this case yellow *T. molitor* (yellow mealworm) and *Zophobas morio* (giant mealworm) (Freye, 1996). The symptoms of allergic reactions caused by insect consumption range from oral allergy to itching or swellings to asthma to anaphylactic shock (death) (Ji et al., 2009; Van Huis et al., 2013). Food allergy develops in 2 phases; in the 1st phase, a human becomes sensitized to specific food allergens. In the 2nd phase, when sensitized patients are again exposed to the respective allergen(s), an allergic reaction may be elicited (Verhoeckx, Broekman, Knulst, & Houben, 2016). An allergic reaction to food proteins is characterized by the presence of antigen-specific serum IgE antibodies, leading to the release of histamine among others.

Allergies can also be developed through cross-reactivity between related proteins in other food products (Van Huis, 2016). For example, people with seafood allergy can experience allergic reactions after consumption of edible insects (Van Huis et al., 2013). The potential of allergies to insects as a source of food may be based on the existence of common allergens (pan-allergens) of arthropods. Insect consumption by persons allergic to, for example, dust mites or shrimps, can induce allergic reactions associated with cross-reactivity (Verhoeckx et al., 2014). It is, however, important to distinguish whether the effects are actually from food allergy or from possible chemical contamination of the insects (Pener, 2014; Srinroch, Srisomsap, Chokchaichamnankit, Punyarit, & Phiriyangkul, 2015).

Cross-reactivity of insect allergens

Tropomyosin is identified as the major protein in arthropods to cross-react with IgE from crustacean-allergic patients (Van

Broekhoven et al., 2016). Tropomyosin is an actin-binding protein that regulates muscle contractions and is a well-known pan-allergen in arthropods. Allergenic tropomyosins are found in invertebrates such as crustaceans, arachnids, insects, and molluscs. Tropomyosin identified in mealworm (three species) showed a 60% to 90% identity to tropomyosin from other insect species and crustaceans (Van Broekhoven et al., 2016). Tropomyosin was identified in patients allergic to house dust mites (HDM) (Verhoeckx et al., 2014) and to crustaceans (Van Broekhoven et al., 2016), confirming the importance of this protein in cross-reactivity. Pender (2014) has reviewed allergy to locusts and acridid grasshoppers and described cross-reactivity to tropomyosin from shrimp, lobster, HDM, and cockroach (Pender, 2014).

Recently, Verhoeckx et al. (2014) demonstrated *in vitro* cross-reactivity between HDM and crustaceans and *T. molitor*. The allergic potential of *T. molitor* was determined, and cross-reactivity of mealworm proteins with IgE from crustaceans and HDM was assessed. Although no sequence information is available for all proteins involved (that is, tropomyosin and arginine kinase), several proteins were identified as potentially allergenic, such as α -amylase, which may indicate that HDM and crustacean allergic patients may react to food containing yellow mealworm proteins (Verhoeckx et al., 2014). Van Broekhoven et al. (2016) showed that IgE from patients allergic to HDM or crustaceans is also able to bind to proteins from giant mealworm and lesser mealworm *in vitro*, meaning that patients allergic to HDM or crustaceans might experience an allergic reaction when consuming these mealworm species. In a double-blind placebo-controlled food challenge test, 87% of a shrimp-allergic patient population showed an allergic reaction upon consuming *T. molitor* (Broekman et al., 2016). Most of the shrimp-allergic patients were sensitized to either mealworm tropomyosin and/or arginine kinase, although IgE-binding was also found for other unidentified proteins.

Srinroch et al. (2015) investigated the cross-reactivity of allergens in a small freshwater prawn (*Macrobrachium lanchesteri*) and the field cricket *Gryllus bimaculatus* (Srinroch et al., 2015). Arginine kinase was found in both prawn and field crickets, suggesting it as a cross-reactive, invertebrate pan-allergen, hereby assuming that prawn-allergic patients might also be allergic to edible insects (Srinroch et al., 2015). In addition, these authors identified a novel allergen in field crickets, HEX1B, and found structural similarity with an allergen in prawn. In contrast to Van Broekhoven et al. (2016) and Verhoeckx et al. (2016), they did not determine tropomyosin as an allergen (Srinroch et al., 2015). Verhoeckx et al. (2014) stated that, because *T. molitor* or other insects are not commonly eaten yet in Europe, sera from allergic patients are not readily available and that they could not yet determine the probability of sensitization to mealworm proteins (Verhoeckx et al., 2014). Besides tropomyosin and arginine kinase, various allergens are mentioned in the literature as possible proteins responsible for cross-reactivity with edible insects. Paramyosin and heavy chain myosin were recently identified as shrimp allergens. These proteins, as well as actin and larval cuticle proteins, could therefore be novel allergens in mealworm species, but this remains to be proven (Van Broekhoven et al., 2016; Verhoeckx et al., 2016).

It is unclear whether processing such as boiling will destroy potential allergenic components. Van Broekhoven et al. (2016) showed that heat-processing might decrease, but not eliminate, the risk for allergic responses when consuming mealworm species (Van Broekhoven et al., 2016). Tropomyosin allergenicity appeared to be decreased in fried samples and disappeared in fried, *in vitro* digested samples (Van Broekhoven et al., 2016). Additionally,

Broekman et al. (2015) found that heat-processing did not lower the allergenicity of mealworm proteins, but clearly changed the solubility of these proteins (Broekman et al., 2015). With the skin prick test, 13 of 15 patients did not react differently to the processed extracts (Verhoeckx et al., 2016). Other processing techniques can be explored for their ability to reduce the allergic potential of insect proteins, making them a more sustainable source of dietary proteins for human consumption.

The allergenic potential of chitin

Chitin is a naturally occurring cellulose-like polysaccharide of glucosamine (N-acetyl-D-glucosamine). It can be found in cell walls of fungi and arthropod exoskeletons (for example, shrimps and insects). In nature, chitin occurs in 3 polymorphic forms of ordered crystalline microfibrils. The most common form in nature (as found in insect exoskeletons), alpha-chitin, is arranged in antiparallel strands. These strands are grouped together in the aforementioned microfibrils, which are wrapped in proteins and at the next hierarchical level are clustered together into chitin-protein fibers. This proteinaceous matrix forms the insect cuticle, together with other materials, depending on species. The rigid outer layer of the procuticle (exocuticle) contains more cross-linked proteins than the relatively more flexible inner layer (endocuticle). Depending on species, life stage, and body part, the percentages of chitin/proteins, the respective matrix organization, and other molecules of the cuticle may differ (Chandran, Williams, Hung, Nowlin, & LaJeunesse, 2016; Hamed, Özogul, & Regenstein, 2016; Muzzarelli, 2011; Vincent & Wegst 2004).

Chitinases and chitinase-like proteins (C/CLP) are enzymes that cleave a chitin polymer down to its associated dimers and monomers (Lee et al., 2011; Muzzarelli et al., 2012). The most important human enzyme for chitin digestion appears to be acidic mammalian chitinase, which is synthesized by gastric tissues (Boot et al., 2005) and is expressed in gastric epithelia, in lung macrophages, and in the pulmonary epithelia during asthmatic inflammation (Zhu et al., 2004). After enzymatic proteolysis, the resulting product N-acetyl-glucosamine (a monosaccharide derivative of glucose) is easily metabolized by the human body, although certain particularly strong chitin matrices (for example, in fly wings) may be more resilient to enzymatic digestion (Muzzarelli et al., 2012).

Previous reviews and opinions focusing on the food and feed safety of insects (EFSA, 2015; FASFC, 2014; Van Huis et al., 2013) that specifically discussed the allergenic potential of chitin primarily cite Lee, Da Silva, Lee, Hartl, and Elias (2008), Muzzarelli (2010), and/or Brinchmann et al. (2011). These publications and subsequent research on this topic focused primarily on the association between inhalation of chitin from fungi and HDM, and lung diseases such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease (Lee et al., 2011; Mack et al., 2015; Ober & Chupp, 2009; Vega & Kalkum, 2012). This research has made clear that chitin has complex and size-dependent effects on innate and adaptive immune responses, and that chitin has been found to have properties that could improve the immune response of specific groups of people. The response to chitin appears to be dependent on the particle size; medium-sized chitin particles induce allergic inflammation, while small-sized particles may reduce inflammatory effects. Lee et al. (2008) hypothesized that C/CLP degrades the biologically inert large polymers into medium-sized particles which interact with macrophages, producing a large variety of cytokines and mediators, resulting in an allergic reaction, and still more C/CLP that will degrade the chitin polymers into

smaller particles (“small chitin”). The particles of that size, in turn, induce the production of anti-inflammatory molecules (for example, IL-10) that will lead to resolution of the immune response.

There are still several questions unanswered in this area and it is unclear whether and how the conclusions from the research on intake via inhalation can be translated to intake via consumption. Moreover, research specifically on the association between consumption of insects and allergenic effects of chitin is lacking.

Conclusion

The allergenic potential of insects, in principle, seems clear, although the specifics warrant additional research. Most importantly, the pan-allergen tropomyosin, which is common in arthropods (such as prawns, shrimp, HDM), was recently identified in mealworm species. Several other proteins, such as arginine kinase, α -amylase, and some newly identified allergens, may also play a role in the cross-reactivity with crustaceans and some insects. The role of chitin from insect cuticles as an allergen is poorly understood, but its allergenicity appears to be related to its particle size with medium-size particles causing an inflammatory response while smaller particles lead to resolution of the immune response. Some types of heat-processing may lower the allergenicity of proteins, but results so far are conflicting and additional research is therefore needed.

Conclusions and Recommendations

This study was aimed to provide an overview of the available knowledge on potential food safety hazards, including chemical, microbiological, and allergenic hazards and prions, in insects for use in feed and food. It showed that, in the last years, more and more research is being published on the safety of insects reared under controlled conditions. From these studies, it is clear that cadmium and arsenic may accumulate from the substrate in, respectively, black soldier fly and yellow mealworm larvae. Some EU-regulated mycotoxins do not accumulate in these two species. Insects harbor a wide variety of microorganisms, and some pathogenic bacteria may be present, in particular they are characterized by high counts of total viable bacteria and Enterobacteriaceae. Furthermore, insects do have allergenic potential, as was demonstrated for several mealworm species.

Possible accumulation of chemical hazards depends on the insect species, growth stage, and substrate use. Possible contamination with microbiological hazards depends mostly on the insect species, the substrate, and the farming and processing conditions.

Much more research is needed to obtain a full overview of the potential food safety hazards for insects for feed and food use. Such research should be done on a case-by-case basis, and the best can be done for a selected set of insect species that are promising for use as feed or food, farmed with an appropriate substrate in a hygienic environment under controlled conditions. Conclusions on obtained results should be limited to the species under investigation and to the specific farming and processing conditions. Future studies should focus on the research/data gaps identified in this literature review:

- Further evaluate the bioaccumulation of heavy metals (including elements), residues of pesticides, veterinary drugs and hormones, and dioxins and PCBs in different insect species.
- Evaluate substrates, on a case-by-case basis, for microbiological hazards, taking into account also the kind of harvesting applied.

- Evaluate heat treatment and other processing methods to reduce the presence of microorganisms in insect-derived products.
- Confirm the presence of paramyosin, heavy chain myosin, actin, and larval cuticle proteins as novel allergens in mealworm species and/or insects in general.
- Investigate the association between consumption of insects and allergenic effects of chitin.
- *In vivo* confirmation of allergenicity to insect proteins by oral food challenges, and.
- Effects of heat treatment on reducing allergenicity of insect products.

From the current results, in addition to offer a promising alternative protein source for food and feed, insects may offer other opportunities as well. Interesting developments identified in the literature are: the potential of insects to metabolize certain mycotoxins (specifically DON and AFB₁) in food or feedstuffs; the immunomodulatory properties of chitin; and the possibility of insects reducing bacterial counts in substrates. These potential applications should be further investigated as well.

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Author Contributions

H. J. van der Fels-Klerx performed the literature review on chemical safety and wrote the main part of the manuscript; L. Camenzuli performed the literature review on chemical safety and wrote the related section; S. Belluco and A. Ricci performed the literature review on microbiological safety and wrote the related sections; N. Meijer performed the literature review on allergenic hazards and wrote the related section. All authors commented on all sections.

Conflict of Interest

The authors declare no conflict of interest.

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