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Chemical food safety hazards of insects reared for food and feed

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REVIEW ARTICLE

Abstract

Insects are a promising future source of sustainable proteins within a circular economy. Proving the safety of insects for food and feed is necessary prior to supplying them to the market. This literature review provides a state-of-the-art overview of the chemical food safety hazards for insects reared for food and feed, focusing mainly on transfer of contaminants from the substrate. Contaminants covered are: heavy metals, dioxins and polychlorinated biphenyls, polyaromatic hydrocarbons, pesticides, veterinary drugs, mycotoxins, and plant toxins. The twelve insect species reported as having the largest potential as feed and food in the EU are included. Transfer and bioaccumulation of contaminants depend on the chemical, insect species, life stage, and source of contaminant (spiked vs natural), as well as the particular substrate and rearing conditions. The heavy metals lead, arsenic, mercury, and cadmium can accumulate, whereas mycotoxins and polycyclic aromatic hydrocarbons (PAHs) seem not to accumulate. Mycotoxins and veterinary drugs could be degraded by insects; their metabolic routes need to be further investigated. Data are generally limited, but in particular for PAHs, plant toxins, and dioxins and dioxin-like polychlorinated biphenyls. Further research on chemical safety of different edible insects is therefore warranted.

Keywords: contaminants, edible insects, exposure, literature review

1. Introduction

Before insects are put on the European market as an ingredient for feed and food, they should be proven to be safe for livestock, pets, and humans. Insects can be efficiently reared with a minimal amount of resources on a wide range of substrates, such as organic side streams, at a high conversion rate (Van Huis *et al.*, 2013; Varelas, 2019). Insects emit lower greenhouse gasses and ammonia in comparison to conventional production animals (Oonincx *et al.*, 2011). Furthermore, insects contain high quality protein, amino acids, and vitamins for animal and human health (Rumpold and Schlüter, 2015). Insects can contain high fat fractions, including omega-3 fatty acids, that are essential for fish and human nutrition (Van Huis *et al.*, 2013). In Europe, insects are seen as a novel source of protein for feed and food production, that could help in producing enough food and feed for the growing European population (Bordiean *et al.*, 2020). The use of insect protein can partly replace the heavy import of protein sources from non-European areas.

In the European Union (EU), under Regulation (EC) No 142/2011, seven species of insects are currently legally allowed to be fed to aquaculture animals (EC, 2011a). In principle, there is no restriction on which insect species are allowed to be used in pet food (Regulation (EC) No 1069/2009) and feed for fur animals (Regulation (EC) No 999/2001) (EC, 2001, 2009). To produce and market insects for food, the producer should submit a dossier to the European Commission requesting for approval since insects were not consumed in Europe before the 15th of May 1997 (Regulation (EU) No 2015/2283) (EC, 2015a). Currently, several of the dossiers are under evaluation of EFSA (EC, 2015b). As a part of the dossier, evidence of the safety of insects for human consumption should be included. In particular, the possible presence of food safety hazards, including physical, microbiological, and chemical hazards, should not cause any short- or long-term human health problems. Information on the safety of insects for feed and food have been collected and brought together initially by EFSA (2015) and later in an extensive literature review (Van der Fels-Klerx *et al.*, 2018). Since then, additional experiments have been performed to fill the identified data gaps on the chemical safety of edible insects.

The current review aims to present an updated overview of the potential chemical food safety hazards that could be present in insects reared for feed and food. It is focused on contamination of insects by their exposure to chemicals in the substrates in the rearing phase, because of: (1) the relative importance of this contamination route for the presence of chemicals in harvested insects; (2) limited available data on effects of processing on chemicals; and (3) the relevance of food safety control at the upstream stages of the supply chain. Chemicals are generally stable and difficult to remove or reduce by processing steps, like cooking, further downstream the chain. Therefore, if contaminants do accumulate in insects, this could potentially pose a problem, either within the fat or protein fractions of the insects or the insect products themselves, through the processing, distribution, and consumption stages (Mutungi *et al.*, 2019).

2. Materials and methods

The comprehensive review by Van der Fels-Klerx *et al.* (2018) was completed in the beginning of 2017. Therefore, additional recent published studies were searched for in the current study. The review used the bibliographic databases CAB Abstracts, Web of Science, and Scopus to collect peer-reviewed papers written in the English language and published in the time period 1 January 2017 to 1 September 2020. In the case of limited data available on a particular contaminant group, earlier published papers were also included to provide a complete overview.

The literature search focused on chemical hazards in insects reared for food and/or feed, thus excluding insects harvested from the wild. Species covered included those considered by EFSA as having the largest potential to be used as food and feed in the EU, being: black soldier fly (*Hermetia illucens*) and common housefly (*Musca domestica*); yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), and giant mealworm (*Zophobas atratus*); house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*); greater wax moth (*Galleria mellonella*), lesser wax moth (*Achroia grisella*), silkworm (*Bombyx mori*), African migratory locust (*Locusta migratora migratorioides*), and American grasshopper (*Schistocerca americana*) (EFSA, 2015). Particular attention was given to bioaccumulation of chemicals in the larvae and/or cricket species from the substrate. All types of substrates were considered, also including the ones not currently legally allowed in the EU. Results are presented in the next sections for each of the main group of chemical contaminants separately, including: heavy metals, dioxins and polychlorinated biphenyls, polyaromatic hydrocarbons, pesticides, veterinary drugs, mycotoxins, and plant toxins.

3. Heavy metals

Possible bioaccumulation of several heavy metals has been investigated in various insect exposure studies held under controlled conditions. The focus of these studies was on the heavy metals currently regulated in feed and food in the EU, being; cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) (Regulation (EC) No 1881/2006) (EC, 2006a). Table 1 summarises the results from the recent exposure studies involving a substrate contaminated by the four mentioned heavy metals with *H. illucens* and *T. molitor* larvae. Results show that the heavy metal accumulation in insects depends on the type of heavy metal, the insect species, the substrate and, possibly the packaging material of the substrate (Van der Fels-Klerx *et al.*, 2020). For example, bioaccumulation factor (BAF) of Pb in *T. molitor* fed 100% organic wheat flour was 34, whereas it was 6.1 when fed 75% organic wheat meal and 25% organic olive-pomace (Truzzi *et al.*, 2019). The authors speculated that this high accumulation may have been due to elevated levels of Pb in the carrots that had been provided as a water source to all treatments; but this had not been verified by (Truzzi *et al.*, 2019). In addition, *H. illucens* fed vegetable-based substrate in a carton had a BAF of 20.4 for Cd, but BAF for this metal was 7 when *H. illucens* were fed the same vegetable-based substrate but in a plastic container (Van der Fels-Klerx *et al.*, 2020). Furthermore, *H. illucens* fed on seaweed-enriched media accumulated Cd, Pb, Hg, and As (Biancarosa *et al.*, 2018). The two recent studies that investigated As in *H. illucens* reported BAF <1 (Table 1). Possible As accumulation in *T. molitor* was studied in only one recent study reporting a BAF of 1.1, confirming results from (Van der Fels-Klerx *et al.*, 2016) who reported a BAF of 1.4-2.6 in *T. molitor* larvae.

Gao and co-authors (Gao *et al.*, 2019a) fed *M. domestica* with food waste including different percentages of dish waste, and reported a lower concentration of Cd in the larvae compared to other elements tested, which is congruous with previous reports (Jiang *et al.*, 2017). Currently, not all combinations of the four regulated heavy metals and the insect species considered in this review have been investigated. Therefore, species not investigated yet, such as *A. diaperinus* and *A. domesticus* among others, should be considered in further studies.

4. Dioxins/PCBs/PAHs

Dioxins (and furans – PCDD/F) and dioxin-like polychlorinated biphenyls (dl-PCBs) are chemicals currently banned under the Stockholm Convention due to well documented toxic effects throughout the food chain. However, local contamination of these chemicals still exists in the environment, predominantly in soils, sediment, and air, which may result in contamination of crops used for feed or food (Pius *et al.*, 2019). As insects are being reared on a variety of waste streams, these lipophilic pollutants are

Table 1. Bioaccumulation of heavy metals in insect larvae.

Heavy metal	Bioaccumulation factor	Species	Reference
Arsenic	0.8±0.1	*Hermetia illucens*	Proc *et al.* (2020)
	0.5-1.1	*H. illucens*	Schmitt *et al.* (2019)
	1.1±0.1	*Tenebrio molitor*	Truzzi *et al.* (2019)
	0.88-0.99	*H. illucens*	Truzzi *et al.* (2020)
Cadmium	9.1±1.4	*H. illucens*	Purschke *et al.* (2017)
	3.9±0.6	*H. illucens*	Proc *et al.* (2020)
	2.5±0.1	*H. illucens*	Schmitt *et al.* (2019)
	7-20.4	*H. illucens*	Van der Fels-Klerx *et al.* (2020)
	0.8-1.7	*T. molitor*	Truzzi *et al.* (2019)
	1.8-2.5	*T. molitor*	Mlček *et al.* (2017)
	4.2-6.9	*H. illucens*	Truzzi *et al.* (2020)
Lead	2.3±0.3	*H. illucens*	Purschke *et al.* (2017)
	0.03±0.03	*H. illucens*	Proc *et al.* (2020)
	0.8±0.1	*H. illucens*	Schmitt *et al.* (2019)
	2.1-3.1	*H. illucens*	Van der Fels-Klerx *et al.* (2020)
	5.2-34	*T. molitor*	Truzzi *et al.* (2019)
	1.6-2.3	*H. illucens*	Truzzi *et al.* (2020)
Mercury	0.5	*H. illucens*	Purschke *et al.* (2017)
	1.5±0.1	*H. illucens*	Proc *et al.* (2020)
	1.6±0.1	*H. illucens*	Schmitt *et al.* (2019)
	1.5-6.2	*T. molitor*	Truzzi *et al.* (2019)
	1.4-4.5	*H. illucens*	Truzzi *et al.* (2020)

being investigated for safe insect rearing. The concentration of dioxins and dl-PCBs may substantially concentrate in fat extracts of insects for food/feed. Dioxins and dl-PCBs concentrations were analysed in reared insects for feed, and reported to range from 0.23 to 0.63 ng toxic equivalency factor (TEQ)/kg dry weight (Charlton *et al.*, 2015). The authors noted that these figures are below the EC maximum allowed content in feed materials of animal origin (1.25 ng WHO-PCDD/ F-PCB-TEQ/kg, considering 88% dry matter) (EC, 2006a). In addition, dioxins and dioxin-like PCBs were analysed in insects and insect products for food and feed. Concentrations ranged from 0.05-0.28 pg WHO-TEQ/g ww in the edible insects, which was two times higher than in the insect derived products, probably, due to dilution of other ingredients within the insect product (Poma *et al.*, 2017). In a recent study in which *H. illucens* was fed on supermarket waste, larvae were analysed for dioxins and dl-PCBs (Van der Fels-Klerx *et al.*, 2020). These treatments included substrates packaged in either plastic or carton and with or without meat. Concentrations of dioxins and dl-PCBs (WHO-2005-PCDD/F-PCB-TEQ upper bound) were 0.2-0.3 ng TEQ/kg dw for the substrates, and 0.3-0.4 ng TEQ/kg dw for both the larvae and the residual materials. Concentrations of both the substrates and reared *H. illucens* larvae did not exceed current EC limits for these contaminant groups in feed materials. In the same study, concentrations of polycyclic aromatic hydrocarbons (PAHs) were also analysed. Reported PAH16 (upper bound) concentrations were low, with levels in the substrates of 1.8-6.6 μg/kg dw, in the larvae of 1.9-2.1 μg/kg dw, and in the residues of 2.1-5.2 μg/kg dw (Van der Fels-Klerx *et al.*, 2020). PAHs are chemically stable compounds in the environment which can be formed after burning. Currently, there are no legal limits for the presence of PAHs in feed materials in Europe. However, since these compounds can be carcinogenic and DNA damaging, the concentration of PAHs in insects should be as low as possible. PAHs are increasingly found in vegetable based aquafeed materials and potentially in vegetable based waste streams (Berntssen *et al.*, 2015).

Table 2 shows that dioxins and dl-PCBs as well as PAHs did not accumulate in *H. illucens* larvae, or only accumulated to very little extent, up to BAF of 2 for dioxins and dl-PCBs and up to 1.2 for PAHs in the various treatments (Van der Fels-Klerx *et al.*, 2020).

Controlled experiments on possible accumulation of dioxins, dl-PCBs, and PAHs have not been identified for the other insect species under consideration of this review. Since these compounds are known to accumulate in fatty tissues of production animals, they should be investigated in future studies (Fries, 1995). Based on the results for *H. illucens* larvae, accumulation of dioxins and dl-PCBs is expected in other insect species used for food and feed.

Table 2. Bioaccumulation of dioxins, dioxin-like polychlorinated biphenyls, and polycyclic aromatic hydrocarbons in *Hermetia illucens* larvae from supermarket returns (Van der Fels-Klerx *et al.*, 2020).

Contaminant	Bioaccumulation factor
Dioxins (WHO2005-PCDD/F-TEQ (ub))[1,2]	1.0-2.0
Sum of dioxins and dioxin-like PCBs (WHO2005-PCDD/F-PCB-TEQ (ub))[1,2]	1.0-2.0
PAH16 (ub)[2]	0.3-1.2

[1] World Health Organization (WHO) toxic equivalency factor (TEQ).
[2] ub = upper bound value (limits of detection used per congener).

5. Pesticides

In literature on the effects of reared insects discussed in a previous review (Van der Fels-Klerx *et al.*, 2018), it was largely concluded that tested pesticides did not accumulate in the considered species. These included azoxystrobin and propiconazole (Lalander *et al.*, 2016), chlorpyrifos and chlorpyrifos-methyl, and pirimiphos-methyl (Purschke *et al.*, 2017) in *H. illucens* larvae, and epoxiconazole (Lv *et al.*, 2014), metalaxyl (Gao *et al.*, 2014), benalaxyl (Gao *et al.*, 2013), and mycolobutanil (Lv *et al.*, 2013) in *A. diaperinus* larvae. In addition, Poma and co-authors (Poma *et al.*, 2017) confirmed the presence of pirimiphos-methyl in *Locusta migratoria* and in some insect products including a Buggie burger. Recent literature on the effects of pesticides on insect species reared specifically for food or feed purposes is scarce. Available data suggest that accumulation is most likely not a food safety concern. However, for some pesticides, the presence of low concentrations of pesticides in the substrate, below the EC legal limit, may affect insect growth and survival (unpublished data). More research is needed to determine effects of a larger variety of pesticides that are commonly found in residues of feed materials used for rearing insects.

6. Veterinary drugs

There is only limited data available on the possible presence or accumulation of veterinary drug residues in edible insects. When insects are reared on manure (though currently not allowed in Europe), they could be exposed to residues of veterinary drugs. Furthermore, veterinary drugs may also be used during the rearing of insects to prevent infections. The use of antibiotics may, however, also affect the development of edible insects and the spread of antibiotic-resistant pathogens, which could outweigh the possible benefits of using antibiotics (Grau *et al.*, 2017). Therefore, veterinary drugs could be used during insect rearing, however it is a challenge to find the optimal balance between limiting microbial growth and optimal growth and survival of the insects (Roeder *et al.*, 2010).

Some studies investigated the ability of *H. illucens* larvae to reduce pharmaceuticals in the environment. Cai and co-authors (Cai *et al.*, 2018) studied the mechanisms of tetracycline degradation by the intestinal microflora of *H. illucens*. Non-sterile substrates of moistened wheat bran were spiked with tetracycline in concentrations of 20, 40, and 80 mg/kg dw. It was shown that *H. illucens* could rapidly degrade tetracycline, which was due to the intestinal microbiota of the larvae. More than 75% of tetracycline was degraded at day 8, while at day 12 the reduction was between 95-96%. Several possible biodegradation products were identified in larval intestinal isolates (Cai *et al.*, 2018). Another study (Lalander *et al.*, 2016) showed that the half-life of the pharmaceuticals carbamazepine, roxithromycin, and trimethoprim, as spiked in the substrate, was shorter with *H. illucens* feeding off of the compost substrate and no bioaccumulation was detected in the larvae. Composting of organic waste by *H. illucens* larvae could, therefore, reduce the pharmaceuticals in the environment (Lalander *et al.*, 2016). Biodegradation of antibiotics present in swine manure and chicken manure was also observed with *M. domestica* larvae. Concentrations of nine antibiotics, including tetracyclines, sulfonamides, and fluoroquinolones were clearly decreased during a six-day larvae manure vermicomposting process. The cumulative removal of oxytetracycline, chlortetracycline, and sulfadiazine was around 70% (Zhang *et al.*, 2014). The veterinary antibiotic monensin, a feed additive according to EU regulation (EC, 2020), which is widely used in broiler feed was also reduced in a 12-day vermicomposting experiment with *M. domestica* larvae. After four days, the concentration of monensin in the chicken manure was reduced by 95%, while this reduction took twelve days for the control group. It was concluded that the reduction was due to monensin degrading bacteria in the gut of the larvae (Li *et al.*, 2019).

In other studies, some residues of veterinary drugs were detected in edible insects (Table 3). Several insects intended for human or animal consumption including *A. diaperinus*, *H. illucens*, and *A. domesticus* were screened for the possible presence of 75 (veterinary) drugs, pesticides, and mycotoxins. The veterinary drugs salicylic acid and metoprolol were detected in the three insect species (1-3 μg/kg; De Paepe *et al.*, 2019). Furthermore, it was shown that the antibiotic sulfonamide could affect the growth of *H. illucens*. Sulfonamide was spiked at concentrations of 0, 0.1, 1, and 10 mg/kg in the substrate. Only the highest concentration of sulfonamide affected the survival of the larvae, resulting in 30% survival. The body weight and development of the larvae were also affected by sulfonamide. Sulfamonomethoxine, sulfamethoxazole, and sulfamethazine were not detected in the prepupae, while only sulfadiazine was detected with a treatment of 1 and

10 mg/kg (0.5-0.8 mg/100 prepupae). It was concluded that *H. illucens* larvae can be used to partly remove veterinary drugs from manure to protect the environment (Gao *et al.*, 2019b).

From the limited data available, it can be concluded that residues of some veterinary drugs could be found in edible insects and could affect the larval growth, however, it has also been shown that veterinary drugs can be degraded by insects, possibly due to the gut microbiota. More research is needed on the effects of residues of specific veterinary drugs as present in potential substrates for insect rearing, in combination with the particular insect species reared on that substrate.

7. Other environmental contaminants

Poma and co-authors (Poma *et al.*, 2019) investigated the contamination levels of a large variety of insect food products derived from different insect species, purchased from five European countries (Austria, France, UK, Belgium, and the Netherlands) and three Asian countries (P.R. China, Japan, and R. Korea). A variety of species belonging to six orders, being Orthoptera, Coleoptera, Lepidoptera, Hemiptera, Odonata, and Hymenoptera, were analysed. The list of targeted compounds consisted of 31 persistent organic pollutants (POPs), including: 20 polychlorinated biphenyls (PCBs) and 11 organochlorine compounds (OCPs); 11 halogenated flame retardants (HFRs), including 9 polybrominated diphenyl ethers (PBDEs) and 2 dechlorane plus (DPs); 18 plasticisers, including 7 legacy plasticisers (LPs), and 11 alternative plasticisers (APs); 17 phosphorous flame retardants (PFRs), including 12 legacy PFR, and 5 emerging PFRs (ePFRs); 8 LP biotransformation products (LPs-BT), 11 AP biotransformation products (APs-BT), and 12 PFR biotransformation products (PFRs-BT). The authors concluded that contamination varied between insect species and products, nevertheless levels were generally low. They speculated that industrial post-harvesting handling and other ingredients may have contributed more to elevated contaminant levels in the insect products rather than the insects themselves. Bioaccumulation of contaminants was not investigated in this study.

8. Mycotoxins

Mycotoxins are secondary metabolites produced by certain fungal species, mostly from the genus *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp., that are toxic to animals and humans. Certain mycotoxins, such as aflatoxin B_1 (AFB1), deoxynivalenol (DON), zearalenone (ZEN), ochratoxin A (OTA), and fumonisins (FB1, FB2, FG1, FG2), are known to pose threats to livestock and human health (Gashaw, 2016). Therefore, the presence of these mycotoxins in feed and/or food products has been regulated or guidance levels have been established in Europe (EC, 2006a,b, 2011b).

Possible accumulation of mycotoxins in insects has been investigated in several studies with spiked and naturally contaminated substrates from different feed materials and waste streams, and with different insect species. Results from these studies lead to a better understanding of mycotoxin uptake, transformation, and excretion by insects for feed and food (Leni *et al.*, 2019). Results of recent studies showed that tolerance to mycotoxins varies, depending on insect species and mycotoxins. For example, *H. illucens* fed poultry feed spiked with AFB1 did not accumulate this toxin, and levels in the larvae were below the detection

Table 3. Possible accumulation of veterinary drugs in insect larvae.

Veterinary drugs	Results / accumulation	Species	References
Tetracycline	Degradation in the substrate by microbiota of *H. illucens*	*Hermetia illucens*	Cai *et al.* (2018)
Roxithromycin, trimethoprim, and carbamazepine	Degradation in the substrate; no accumulation in the larvae	*H. illucens*	Lalander *et al.* (2016)
Tetracycline, oxytetracycline, chlortetracycline, doxycycline, sulfadiazine, norfloxacin, ofloxacin, ciprofloxacin, and enrofloxacin	Degradation in the substrate	*Musca domestica*	Zhang *et al.* (2014)
Monensin[1]	Degradation in the substrate by the microbiota	*M. domestica*	Li *et al.* (2019)
Salicylic acid, metoprolol	Low levels found in insects intended for human or animal consumption	*H. illucens, Alphitobius diaperinus*	De Paepe *et al.* (2019)
Sulfonamide	Sulfamonomethoxine, sulfamethoxazole, and sulfamethazine were not detected, while only sulfadiazine was detected in the prepupae	*H. illucens*	Gao *et al.* (2019b)

[1] According to EU regulation monensin is a feed additive (EC, 2020).

Table 4. Possible accumulation of mycotoxins in insect larvae.

Mycotoxins	Concentration in substrate (µg/kg)	Concentration in larvae (µg/kg)[1]	Species	References
Aflatoxin B_1	415	<LOD[2], 10% legal limit[3]	*Hermetia illucens, Tenebrio molitor*	Bosch *et al.* (2017)
	390	<LOQ[2,4]	*H. illucens, Alphitobius diaperinus*	Camenzuli *et al.* (2018)
	13	<LOD	*H. illucens*	Purschke *et al.* (2017)
Zearalenone	14.9-79.9	<LOQ	*T. molitor*	Niermans *et al.* (2019)
		<LOD[2],60[3], <LOD[5]	*H. illucens, T. molitor, Acheta domesticus*	De Paepe *et al.* (2019)
	13,000	>LOQ[2], <LOQ[4]	*H. illucens, A. diaperinus*	Camenzuli *et al.* (2018)
	39	<LOD	*H. illucens*	Purschke *et al.* (2017)
	173	<LOD	*T. molitor*	Leni *et al.* (2019)
Deoxynivalenol	12,000	<131	*T. molitor*	Ochoa Sanabria *et al.* (2019)
	112,000	>LOQ[2], <LOQ[4]	*H. illucens, A. diaperinus*	Camenzuli *et al.* (2018)
	698	<LOD	*H. illucens*	Purschke *et al.* (2017)
	779[2], 1,207[3]	<LOD[2], 726[3]	*H. illucens, T. molitor*	Leni *et al.* (2019)
Ochratoxin A	1,700	>LOQ[2], <LOQ[4]	*H. illucens, A. diaperinus*	Camenzuli *et al.* (2018)
	130	<LOD	*H. illucens*	Purschke *et al.* (2017)
Fumonisin B_1 and B_2	573[2], 727[3], 441[2], 294[3]	<LOD[2], <LOD[3], <LOD[2], <LOD[3]	*H. illucens, T. molitor*	Leni *et al.* (2019)

[1] LL = legal limit; LOQ = limit of quantification; LOD = limit of detection.
[2] *H. illucens*: initial/larval concentration.
[3] *T. molitor*: initial/larval concentration.
[4] *A. diaperinus*: initial/larval concentration.
[5] *A. domesticus:* initial/larval concentration.

limit (0.10 µg/kg) of the analytical methods used (Table 4). However, *T. molitor* larvae fed with the same spiked substrate contained up to 1.44 µg/kg of AFB1, which is about 10% of the EU's legal limit for feed materials (Bosch *et al.*, 2017).

None of the considered mycotoxins, as presented in Table 4, accumulated in the insect species considered. *H. illucens, T. molitor,* and *A. diaperinus* were fed different substrates at varying concentrations of mycotoxins, with several being well above the EC legal limit for the maximum presence of the particular toxin in feed materials (EC, 2003). For example, Camenzuli and co-authors (Camenzuli *et al.*, 2018) fed *A. diaperinus* and *H. illucens* a wheat-based substrate contaminated with 390 µg/kg AFB1, which is 20 times the EC legal limit for AFB1 in feed materials. Not all combinations of mycotoxins and different insect species have been investigated. Based on results of studies performed so far, accumulation of mycotoxins is not expected in insects. However, this needs to be confirmed in future studies involving the species that have not been investigated so far, such as *A. domesticus, L. migratoria,* and *M. domestica*.

Even though mycotoxins were seldom found in the insect body, they were regularly found in the residues from excretion throughout these studies. Signifying, biotransformation of the mycotoxins was reported, resulting in varying levels of metabolites. For example, in a study in which *T. molitor* were fed with 79.9 µg/kg ZEN, the residual materials was found to contain 26.2 µg/kg ZEN, 6.8 µg/kg α-ZEL, and 17.3 µg/kg β-ZEL (Niermans *et al.*, 2019). In addition, when *H. illucens* were fed with 698 µg/kg DON, it resulted in 1,136 µg/kg DON found in the residual materials after excretion. This could be due to masked mycotoxins, which means that the mycotoxins could be bound to a carbohydrate or protein matrix (Purschke *et al.*, 2017). Recently, several studies on the conversion of mycotoxins with phase I and II enzymes have been performed (Meijer *et al.*, 2019). Additional studies of processes of different phase I and II enzymes regarding mycotoxin conversion are recommended to further unravel transformation of mycotoxins by insects.

9. Plant toxins

Plant toxins is referred to as the collective group of secondary metabolites in plants which have toxicological properties. The most known plant toxins include cyanogenic glycosides and alkaloids. Cyanogenic glycosides can release hydrogen cyanide when chewed or digested. Alkaloids in plants are nitrogen storage compounds that are involved to protect them against predators, function as growth regulators, and substitutes for minerals like potassium and calcium. Plants naturally synthesise alkaloid compounds based on their needs. Some of the synthesised alkaloids like pyrrolizidine, indolizidine, piperidine, and tropane alkaloids have been documented to cause toxic effects on animals and humans (Schramm *et al.*, 2019). Macel (2011) cites older studies that report that several insect species sequester Pyrrolizidine Alkaloids for their own defence, and that concentrations of these compounds in insects can exceed concentrations in the plant, from which they feed. The insect species currently being reared for food or feed are not known to exhibit this behaviour in the live stage in which they are harvested (EFSA, 2015). Bioaccumulation of plant toxins from substrates in insects for feed and food has, however, not been investigated so far.

10. Conclusions and recommendations

This review provides recent information on the chemical safety of insects reared for feed and food use, primarily focusing on accumulation of contaminants from substrates into reared insects. Generally, available data are fragmented over a wide range of contaminants and insect species, with most data collected for *H. illucens* and *T. molitor*. Factors related to the rearing phase, such as insect species, life stage, and source of the contaminant (spiked or naturally contaminated) were confirmed to affect the accumulation of contaminants in insects. Our results showed that, in addition, aspects related to the experimental setting may play a role, as well as the substrate type.

For most contaminants for which experimental data has been collected, an effect on growth and survival of insects was not observed, except for veterinary drugs and pesticides, which may lead to undesirable production effects. Bioaccumulation of some contaminants including the heavy metals, cadmium and lead can occur, and differences between species in accumulation have been observed. Dioxins and dl-PCBs and plant toxins could also potentially accumulate in insects but limited or no data, depending on the insect species, are available for these contaminants. Accumulation of mycotoxins and PAHs has not been observed so far, though, data on the later compound group are very limited. Further research is, therefore, recommended on possible accumulation of plant toxins, dioxins and dl-PCBs, and PAHs from substrates into insects. Furthermore, research on metabolic pathways of mycotoxins and veterinary drugs in insects, regarding possible detoxification/bioactivation pathways is recommended to unravel underlying mechanisms.

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Conflicts of interest

The authors declare no conflict of interest.

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