Feeding experiments with insects and assessment of feed-related risks from kitchen- and food waste and possibly other by-products

Advisory report from DCA – National Center for Food and Agriculture

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Data sheet

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1 Preface

Industrial production of insects for food and feed is associated with several challenges with regard to food and feed safety, including adjusting the current legislations to facilitate this new industry without risks to production animals and consumers. In 2019, the Danish Ministry of Food, Agriculture and Fisheries (MFVM) asked Aahus University (AU), University of Copenhagen (KU), and the Technical University of Denmark (DTU) to frame the research needed to implement a sustainable industrial production of insects in Denmark. The current legislation prohibits feeding with several biomasses, which obviosly would support a green transition in food and feed production Thus, it was pointed out that knowledge is needed on former foods and household waste, and on biomasses containing packaging material residuals. The intention is that the research based knowledge can support potential changes in EU regulations. The present report focuses on transfer of pesticide residues, perfluorakyl substances used on packaging material surfaces, and DNA from raw meat to larvae of the black soldier fly.

The topics of packaging materials and former food and household waste for insect production contain endless possibilities in setting up studies. The transition from the above mentionend overall aim to specific studies was a process involving key staff from the Danish Veterinary and Food Administration at MFVM, the authors of the current report representing the National Food Institute at DTU, and Department of Animal Science at AU. In collaboration it was specified which insect species and which feed components should be included in the studies to exemplify potential chemical and biological hazards. After aligning the expectation regarding the aim and objective of the studies, the university partners were responsible for choosing appropriate materials and methods to answer the objectives.

The present report presents the results of three sets of experiments performed to gain knowledge on the consequences for food/feed safety when growing black soldier fly larvae (BSFL) on feed spiked with chemical residues from packaging material, in this case perflourinated compounds. The larvae were housed at AU where the experiments were performed, and samples were shipped to DTU where chemical analyses were performed. Further, a literature study was performed to collect the available studies of potential risk parameters associated with insects as waste-to-feed converters, when produced for food or feed purposes.

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2 Dansk sammendrag

Der er stort potentiale i brugen af insekter til foderproduktion ved udnyttelsen af affaldsprodukter fra fødevareindustrien og generelt via genanvendelse af madaffald fra industrielle køkkener og restauranter. Genanvendelse af madaffald fra køkkener til insektproduktion som foderobjekt er dog under de nuværende regler ikke tilladt, da fødevaresikkerheden forbundet hermed ikke er grundigt undersøgt. Til beslutningsgrundlaget for lovgivning om brug af mad- og køkkenaffald i insektproduktion til foder er der derfor brug for at kunne vurdere under hvilke omstændigheder mad- og køkkenaffald kan og ikke kan anvendes i insektproduktion. Dette vil afhænge af, om skadelige eller potentielt skadelige stoffer overføres til insekterne, og dermed videregives til de husdyr, de fodres til. Vi undersøger her om forskellige substanser, der kan findes i køkken- og madaffald, vil overføres til og ophobes i larver af sorte soldaterfluer, der udvikles på medier indeholdende disse substanser.

Vi har her fokuseret på tre forskellige grupper af skadelige eller potentielt skadelige stoffer: pesticider brugt i frugtproduktion, perfluorerede stoffer brugt i mademballage, og animalsk DNA fra kød. Pesticider og perfluorerede stoffer kan ophobes i kroppen på insekterne og føres videre i fødekæden til de endelige fødevarer, hvor de kan have sundhedsskadelige konsekvenser hos forbrugerne. DNA kan hypotetisk overføres til insekter og videre til husdyr, hvor det principielt kan forårsage sygdom, og uforarbejdet kød er derfor indtil videre forbudt i insektproduktion. I alle undersøgelser har vi brugt larver af den sorte soldaterflue.

I de udførte eksperimenter har vi tilsat pesticider (boscalin, etofenprox og fluopyran), perfluorakylforbindelser (PFOA, PFNA, PFHxS og PFOS) eller frysetørret råt kød (kylling, gris og kvæg) til vækstsubstraterne for larver af sorte soldaterfluer. Hvert replikat (ni per behandling) bestod af 123 seks dage gamle larver, der ved forsøgets start blev tildelt 100 g vækstsubstrat i plastikbeholdere med en diameter på 7,9 cm. Efter otte dages vækst på de givne substrater målte vi larvernes vækst og overlevelse, og analyserede larverne for indhold af de tilsatte stoffer. Undersøgelserne foregik i et klimakontrolleret rum ved 27°C og 65% relativ luftfugtighed.

Undersøgelserne viser, at de tre pesticider alle bliver overført til og ophobes i soldaterfluelarverne når de vokser på medier, der indeholder disse pesticider. Tilsvarende fandt vi, at de fire perfluorakylforbindelser alle ophobes i insektlarverne. Til gengæld fandt vi ikke DNA fra hverken kylling, gris eller kvæg i larverne efter at de var opvokset på substrater indeholdende kød fra de tre arter, med undtagelse af et enkelt fund af DNA fra gris. Larvernes vækst var ikke påvirket af hverken pesticider eller perfluorakylforbindelser. Vi fandt en negativ påvirkning af larvernes vækst ved lave, men ikke høje, kødkoncentrationer i substraterne. Dette må skyldes en påvirkning af substratet ved lave kødkoncentrationer måske forårsaget af mykotoxiner, nærmere end en direkte effekt af kødet på larverne.

Vi kan ud fra undersøgelserne konkludere, at pesticidrester fra frugtproduktion og perfluorakylforbindelser fra emballagerester kan udgøre en risiko hvis de indgår i vækstsubstraterne for larver af sorte soldaterfluer, da de optages og ophobes i larverne og dermed indgår i fødekæden til forbrugerne. Modsat fandt vi ikke nogen begrundet risiko forbundet med at tilføje råt kød til substraterne, hvilket kunne åbne for muligheden for at tillade uforarbejdet kød i vækstsubstraterne til larver af sorte soldaterfluer.

3 Introduction

Background for the assignment

Insects as feed and food has in the last decade been internationally recognized as an alternative source of protein to a growing world population, and this has been articulated as a solution in the green transition of food production. To fully exploit the sustainable potential of insects in efficiently coverting biomasses into protein, it will be necessary to utilize a variety of low-value biomasses not already used for food or animal feed. However, the current regulation on feedstuffs for insect production results in use of feedstuffs which are already used for pigs and poultry. There is therefore a need to discuss if insects could be fed with alternative biomasses which are not currently utilized. Obvious feed substrates in an industrial production of insects would include former foods and household waste.

In recent years, the black soldier fly (*Hermetia illucens*) has become an insect of high interest for sustainable protein production. Black soldier fly larvae (BSFL) are ideal for converting former food and household wastes into protein-rich insect biomass, and BSFL production for animal feed is an emerging industry in Denmark with a large economic potential. However, innovation is required to exploit the insects to their full potential and make the production environmentally and economically sustainable. Former foods and household waste are challenging feed substrates because they may contain chemical contaminants from the food production or from packaing materials, or they may impose biological risks as mixtures of former vegetable and animal food items may be in decay.

Selection of parameters for investigation

Several studies have investigated risks that can occur when feeding BSFL with kitchen and food waste, such as contamination with salmonella and accumulation of mycotoxins. Adequately investigated risks as well as parameters relevant for the feed legislation are selected based on a literature review preceeding the experimental work presented in this report. In this literature review (Appendix 1), we identified which risk areas need more investigation based on the number of studies. A number of different chemical risks have been investigated and reported in the literature: mycotoxins, heavy metals, pesticides, dioxins, and PCBs. Especially mycotoxins and heavy metals are covered in many studies. Neither pesticides nor dioxins and PCBs are very well covered. In our search, we found only four articles on pesticides in BSFL (Charlton et al., 2015; Lalander et al., 2016; Meijer et al., 2021; Purschke et al., 2017), and one in mealworm larvae (Houbraken et al., 2016). We found only one study on chemical residues from emballage (van der Fels-Klerx *et al.*, 2020), such as perflourakyl substances. Of biological risks, *Campylobacter*, *Salmonella*, *Listeria*, *Escherichia coli* and *Staphylococcus areus* (MRSA) are covered in the literature with at least three and generally more than ten studies in black soldier flies, while only one study has covered detection of animal DNA in insects (Belghit et al., 2021).

We selected pesticides, perfluorakyl substances, and DNA from animal meat as parameters for further study, as limited information exists on their transfer from substate to insects due to no or only few studies on these factors in insects. The selected parameters are relevant for the legislation of 1) waste containing fruit and vegetables with pesticide residues, 2) waste containing packageing residues with coated surfaces, and 3) waste containing animal material.

Pesticides

Pesticide residue limits for animal feed, including insect feed, are regulated through EU Regulation 396/2005[1] and ammendments. Former foods and household wastes include fruit and vegetables, which may contain pesticide residues. The pesticides included in this study were selected based on a review of the latest data in the Danish pesticide control at the Danish Veterinary and Food Administration. From their list, three pestcides used in fruit, vegetable, and cereal production with high detection frequencies were seleted; boscalid (a fungicide), etofenprox (an incecticide) and fluopyram (a fungicide and nematicide). These pesticides are representative for residues that will be expected to occur in conventionally produced agricultural products that may end up as insect feed.

Perfluorakyl substances

In Denmark there is no tolerance for food packaging materials in feed for livestock, which appears from the list of prohibited feed materials, cf. Marketing Regulation 767/2009 Annex III. However, in practice it can be difficult to remove the last packaging material residues from former foods used for feed. The level of chemical contaminants in feed ingredients and animal feed are regulated through EU Directive 2002/32 and later amendments. Perfluoroalkyl substances (PFAS) are not included in the animal feed legislation, including for insects. Perfluoroalkyl substances can be used to make the surface of food contact materials of paper and cardboard surfaces impermeable to fat and water (e.g., cookie sheets, food paper and fastfood packaging). Denmark has, as of 1 July 2020, banned the use of PFAS in cardboard and paper which is to be used as food contact material (food packaging material). But perfluoroalkylated substances are still in use in other European countries. The four main PFAS accumulating in the human body were selected for this study. These are perfluorooctanic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS).

Animal DNA

Animal products not intended for human consumption are referred to as animal by-products in accordance with the EU Animal By-Products Regulation (1069/2009)[2]. This applies to e.g. former foods or household wastes containing meat. The use of kitchen and food waste as feed for animals, including insects, is currently prohibited[2] due to pathogen risk. We lack knowledge of the possible tranfer of animal DNA from substrate to BSFL. Animal meat from chicken, pork and beef was included in this study.

Overall objective

The objective is to assess risk factors of using former foods and household wastes as feed substrate in a future industrial production of BSFL. Since the ingredient composition of kitchen- and food waste varies enormously on a daily and seasonal basis, as well as in between waste provisioners (kitchens, restaurants, etc.), we addressed the objective through experiments in which larvae were not fed kitchen and food waste, but a standard substrate spiked with substances that are proxies for the relevant risks. The effect of chemical contaminants or animal DNA on the larval performance were assessed based on larval survival, growth, and emergence to flies. The accumulation of chemical contaminants and animal DNA in BSFL was recorded.

[1] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin

[2] Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption

Aims of the experiments

The aims of the experiments were to determine if:

1. Spiked pesticides are transferred from substrate to BSFL
2. Spiked perfluorakyl substances are transferred from substrate to BSFL
3. DNA from chicken, pork and beef is transferred from substrate to BSFL
4. BSFL growth and survival are affected by any of the above substrate additions

4 Materials and methods

General procedures

Larvae for all three experiments were collected from Enorm Biofactory A/S, Hedelundvej 15, Flemming, Denmark. On the day of collection, the larvae were 6 days old. All experiments were performed in 370 mL plastic containers with a bottom diameter of 7.9 cm. Nine replicates were set up per treatment. Six of these were frozen at the end of the experiment for use in chemical analyzes, and the remaining three were used to observe the emergence of flies from pupae. In all experiments, chicken feed (Paco Start, DLG, Fredericia, Denmark) was used as control and base substrate. For all experimental units, 100 g feed was weighed into the container at experiment start, and 123 larvae were added. After 8 days, larvae were separated from the frass, rinsed under running water and dried with napkins. They were then weighed, and the number of surviving larvae were counted. The larvae were then transferred to a clean container without feed for approximately 24 hours to empty their guts. The experiments took place in a climate chamber with a temperature of 27.4 ± 0.8°C and a relative humidity of 65.3 ± 0.9%. Samples of frass and feed were stored at -20°C and larvae at -80°C until analysis. Effects of substrate on larval survival, individual growth, biomass production and adult emergence were analysed using analysis of variance (ANOVA) tests followed by Student's t-tests when $P < 0.05$. Statistical analyses were performed using JMP Pro 15.0 (SAS Institute, Cary, NC, USA).

Experiments with pesticides

Substrates were spiked with pesticides selected based on a review of the latest data in the Danish pesticide control by the Danish Veterinary and Food Administration. This review focused on samples of fruits, vegetables and cereals, and a list of pesticides with the higest detection frequencies was compiled. From that list three pestcides were seleted; boscalid (a fungicide), etofenprox (an incecticide) and fluopyram (a fungicide and nematicide). The spike levels in the experiment was set a the MR (maximum residue level) for apricots (European Union regulation 396/2005 and amendments; Table 1). The substrates were spiked with boscalid at 5 mg/kg substrate, etofenprox at 0.6 mg/kg substrate, or fluopyram at 1.5 mg/kg substrate. The stock solution for all pesticides was prepared in toluene further diluted in methanol (MeOH) at a total volume of 10 mL (Table 1). The solution was mixed with the volume of water, which was added to the basis feed mix. The basic feed mix was the same for all treatments, consisting of 300 g chicken feed and 700 g water. Both MeOH and toluene are expected to have completely evaporated before addition of the larvae, which was facalitated by placing the feed mixes overnight in a fume cupboard. Two control treaments were included; one without addition (control) and one with added toluene and MeOH (MeOH control).

Table 1: Amounts of pesticides and stock solutions spiked to the substrates.

	Control	MeOH control	Boscalid	Etofenprox	Fluopyram
Spike level (mg/kg) (corresponding to MRL for apricot)	-	-	5	0.6	1.5
Concentration stock solution (mg/mL)	-	-	1.0	1.0	1.0
Volume stock solution (in toluene) to be added to the substrate (mL)	-	-	5.5	0.66	1.65
Volume MeOH (mL)	-	4.50	4.50	9.34	8.35
Volume toluen (mL)	-	5.50	-	-	-
Total volume (mL)	0	10	10	10	10

Analyzes of larvae and feed

Pesticide concentrations were determined using the QuEChers method with minor modifications, as BSFL are a new matrix. In brief, samples of BSFL were homogenised using an Ultra turrax and extracted in acetonitrile (ACN). After phase separation the extracts were cleaned up using PSA (primary and secondary amine exchange material). Samples of substrate and frass, were extracted with the same method using higher amount of sample. All samples were analysed by LC-MS/MS (liquid cromatography – triple mass spectrometry) and the pestcides were quantified using external calibration.

Experiments with perfluorakyl substances

The substrates were spiked with four equally mixed PFAS; perfluorooctanic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS). The compounds were spiked in a mix of the four compounds at two levels; 10 µg/kg substrate (Mix 10) and 100 µg/kg substrate (Mix 100) for each of the four PFAS (Table 1). The basic feed mix was the same for all treatments, consisting of 330 g chicken feed and 770 g water. The accumulation of PFAS has not been studied in insects, and spike concentrations were therefore based on experience from a study on zebrafish (Rainieri et al., 2018). The stock solutions were prepared and further diluted to 50 ml in ethanol (EtOH). The spike solution and chiken feed were gently mixed and left overnight in a fume cupboard to ensure that the EtOH had completely evaporated. Then the feed was mixed with water. Two control treatments were included, one with only water (control) and one with EtOH and water (EtOH control) (Table 2). The transfer rate of PFAS from substrate to larvae was calculated by dividing the concentration in the larvae at the end of the growth period with the mean substrate concentration at experiment start. Effects of individual PFAS and their concentration in the substrate on the transfer rate to the larvae was analysed using two-way ANOVA followed by a Student's t-test.

Table 2. Compositions of the four substrates. The PFAS mixes consisted of the four perflourinated compounds PFOS, PFOA, PFNA and PFHxS at two levels of concentration: 10 µg/kg substrate (Mix 10) or 100 µg/kg substrate (Mix 100) for each of the compounds.

	Control	EtOH control	Mix 10	Mix 100
Spike level (µg/kg substrate)	-	-	10	100
Substrate (kg)*	1.1	1.1	1.1	1.1
Amount of each PFAS (µg)	0.0	0.0	11	110
Concentration of stock solution (µg/ml)	-	-	200	200
Volume stock solution (in EtOH) of each of the four PFAS to be added to the substrate (µL)	-	-	55	550
Volume EtOH (mL)	-	-	49.8	47.8
Total volume (mL)	0	50.0	50.0	50.0

* Substrate is the mixture of chicken feed and water

Analyzes of larvae and feed

DTU Food has an accreditated method for the determination of PFAS in eggs. The method was slightly modified for the analysis of PFAS in BSFL, substrate and frass. In brief, PFAS were extracted using a QuEChERs method. Samples of BSFL were homogenised using an Ultra Turrax homogenizer and extracted in acetonitril (ACN). Samples of substrate and frass were extratced in ACN as is. All extracts were then analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The perfluorinated compounds were quantified using external calibration.

Experiments with meat

Minced chicken, pork and beef were purchased in a supermarket, and freeze-dried and ground to a powder in the laboratory. Four substrates were mixed to include different amounts of animal meat (3%, 15%, and 30%), as shown in Table 3. Three of these substrates contained chicken meat, pork and beef in equal proportion but differing in total amount, and one substrate contained 5% beef without other meat types. The basis chicken feed substrate and freeze-dried meat were first mixed and water was then added to obtain a dry matter content of 27 ± 1% and 73% water in all treatments. The control treatment consisted of only chicken feed at 27% dry matter content.

Table 3. Composition of the five substrates.

	Control	3% mixed meat	15% mixed meat	30% mixed meat	5% beef
Chicken feed	100%	97%	85%	70%	95%
Freeze-dried chicken	0%	1%	5%	10%	0%
Freeze-dried pork	0%	1%	5%	10%	0%
Freeze-dried beef	0%	1%	5%	10%	5%

Analyzes of larvae and feed

Larvae and feed samples were analysed at the Danish Veterinary and Food Administration's laboratory in Ringsted. Frozen samples were ground in liquid nitrogen. The samples were then analysed by real-time PCR based on SOPs from the EURL Animal protein (https://www.eurl.craw.eu/legal-sources-and-sops/method-of-reference-and-sops/). Separate analyses were applied for the detection of DNA from each type of meat.

5 Results

Pesticides

Growth and survival

Pesticide addition did not affect larval survival (mean = 90.95%, P = 0.24), individual end mass (mean = 153.42 mg, P = 0.68), or total larval biomass produced (mean = 17.17 g, P = 0.85). Success of emergence into adults was between 81% and 98%, with highest appearance in larvae exposed to etofenprox and lowest in larvae exposed to fluopyram (P = 0.044).

Detection of pesticides in substrate, larvae, and frass

The concentration of the pesticides in the spiked substrates aligned with the intended spiking level of the substrates (Table 4). For all substrates, only the spiked pesticide was detected in the substrates, and none of the three pesticides were detected in the control substrate (Table 4). Taken together, this confirms a successful spiking of the substrates.

The three pesticides were all found in larvae developing on substrate spiked with the given pesticide (Table 4). None of the pesticides were detected in larvae developing on the control substrate, nor in other substrates than those where they were spiked (Table 4). The results show that the three pesticides are transferred from substrate to the larvae.

The three pesticides were also detected in the frass (Table 4). For boscalid and fluopyram, the concentrations in frass were lower than in the substrate, further supporting the finding of a transfer for the two pesticides from substrate to larvae. For etofenprox, the concentrations were similar in substrate and frass.

Conclusions

Boscalid, etofenprox and fluopyram do not appear to have any influence on survival or growth of BSFL, but were found in the bodies of larvae developed on substrate containing pesticide. This shows that these pesticides are transferred from substrate to the BSFL during larval feeding and growth.

Table 4: Concentrations (mean ± SD) of pesticides in larvae (n = 3), substrate (n = 2) and frass (n = 3). Please note the different units for insects and for substrate and larvae. Undetected pesticide was described as not detected (n.d.).

	Treatment	Boscalid (ng/g)	Etofenprox (ng/g)	Fluopyram (ng/g)
Larvae	Control	n.d.	n.d.	n.d.
	MeOH control	n.d.	n.d.	n.d.
	Boscalid	7.30 ± 1.49	n.d.	n.d.
	Etofenprox	n.d.	13.90 ± 1.30	n.d.
	Fluopyram	n.d.	n.d.	0.56 ± 0.16
		Boscalid (mg/kg)	Etofenprox (mg/kg)	Fluopyram (mg/kg)
Substrate	Control	n.d.	n.d.	n.d.
	MeOH control	n.d.	n.d.	n.d.
	Boscalid	4.95 ± 0.09	n.d.	n.d.
	Etofenprox	n.d.	0.42 ± 0.05	n.d.
	Fluopyram	n.d.	n.d.	1.54 ± 0.36
Frass	Control	n.d.	n.d.	n.d.
	MeOH control	n.d.	n.d.	n.d.
	Boscalid	2.73 ± 0.02	n.d.	n.d.
	Etofenprox	n.d.	0.45 ± 0.02	n.d.
	Fluopyram	n.d.	n.d.	0.90 ± 0.12

Perfluorakyl substances

Growth and survival

We did not find differences between substrates on larval survival (mean = 97.02%, P = 0.49), individual mass (mean = 203.34 mg, P = 0.31), or biomass produced (mean = 24.26 g, P = 0.14). Similarly, emergence did not differ between substrates (mean = 97.38%, P = 0.89). Thus, addition of PFAS did not affect any of the investigated larval fitness and performance parameters.

Detection of PFAS in substrate, larvae, and frass

The concentrations of the PFAS in the 10 µg/kg mix ranged from 8,0 to 9,9 µg/kg substrate, and in the 100 µg/kg mix from 83 to 98 µg/kg substrate (Table 5). The measured concentrations are comparable to the theoretical concentrations, confirming a successful spiking of the substrates.

The four PFAS were all detected in BSFL fed on substrates spiked with the compounds in higher concentrations than in the substrates (Table 5), showing that all four PFAS accumulated in the larvae. The concentrations were higher in the BSFL fed on the substrate with the higher concentration of PFAS, showing that the accumulation of the four compounds depends on concentration in the substrate (Table 5).

The PFAS were detected in the frass in concentrations comparable to those in the substrates. The accumulation rate fram substrate to larvae depended both the exact substance ($P < 0.0001$) and the substance concentration ($P<0.0001$). PFOS was transferred at significantly higher rate to the larvae than the other perfluorakyl substances (Figure 1). PFOA, in contrast, was transferred at lower rate than the other substances, although not at significantly lower rate than PFNA when the two were at high concentration (Figure 1). All perfluorakyl substances were incorporated into larvae at a significantly higher rate when at the lower concentration in the substrate (Figure 1).

Conclusions

The four investigated PFAS (PFOA, PFNA, PFHxS and PFOS) do not influence growth or survival of BSFL within the concentrations tested. The four PFAS all accumulated in BSFL fed on substrates spiked with the compounds. Overall, the study shows that PFAS are transferred from substrate to BSFL and accumulate as the larvae eat and grow. More PFAS are accumulated at higher substrate concentration. It should be kept in mind that the used substrates were spiked, and that concentrations of the four PFAS in substrates containing packaging residues would likely be lower. However, substances at lower substrate concentration accumulate at higher efficiency in larvae.

Table 5. Concentrations (µg/kg sample, mean ± SD, n = 3) of perfluoroalkyl substances (PFAS) in larvae, substrate and frass. The four compounds were perfluorooctanic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS). The compounds were spiked in a mix of the four compounds at two levels of concentration: 10 µg/kg substrate (Mix 10) and 100 µg/kg substrate (Mix 100). Concentrations below 0.2 µg/kg sample were not detected (n.d.).

	Treatment	**PFOA**	**PFNA**	**PFHxS**	**PFOS**
Substrate	Control	n.d.	n.d.	n.d.	n.d.
	EtOH control	n.d.	n.d.	n.d.	n.d.
	Mix 10	9.6 ± 1.4	9.6 ± 1.6	9.9 ± 1.2	8.0 ± 1.4
	Mix 100	87 ± 8	92 ± 9	98 ± 10	83 ± 3
Larvae	Control	n.d.	n.d.	n.d.	n.d.
	EtOH control	n.d.	n.d.	n.d.	n.d.
	Mix 10	37 ± 3	48 ± 4	57 ± 1	59 ± 6
	Mix 100	213 ± 40	297 ± 54	369 ± 76	411 ± 76
Frass	Control	n.d.	n.d.	n.d.	n.d.
	EtOH control	n.d.	n.d.	n.d.	n.d.
	Mix 10	9.5 ± 0.6	7.2 ± 0.6	7.5 ± 1.0	3.1 ± 0.3
	Mix 100	114 ± 11	92 ± 9	93 ± 4	42 ± 4

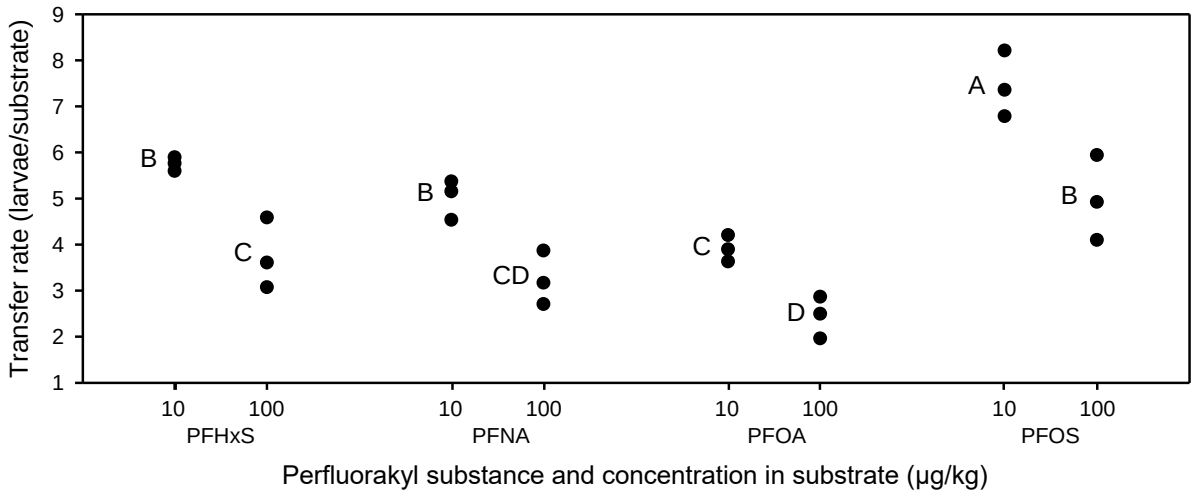

Figure 1: Transfer rate of perfluorakyl substances from substrate to larvae during the experimental growth period. Different letters indicate significant differences (Student's t-test at $\alpha = 0.05$).

DNA from meat

Growth and survival

We found significant effects of substrate on survival throughout the experiment, with highest survival of larvae on the control substrate (Figure 2A). Survival on substrates containing meat was positively associated with meat content irrespective of meat source (Figure 2A). In contrast, individual larval mass at the end of the experiment was significantly lower on the control substrate than on the substrates containing meat (Figure 2B). This led to highest larval biomass production on the substrate with highest proportion of meat and lowest biomass production on the diet with the lowest meat proportion, with intermediate biomass production on the control substrate (Figure 2C). Emergence success as adults from pupae was at 96-100% with no difference between substrates ($P = 0.87$).

Detection of animal DNA in substrates and larvae

Ruminant DNA, chicken and turkey DNA, and porcine DNA were all detected in substrates containing all three types of meat (Table 6). Ruminant DNA, and chicken and turkey DNA were not detected in the control substrate, while porcine DNA was detected in all three control samples (Table 6), indicating a contamination of the control substrate with porcine DNA. Ruminant DNA was detected in the substrates with added beef only, but chicken and turkey DNA and porcine DNA were also each detected in two of these samples (Table 6).

Porcine DNA was detected in one sample of BSFL (Table 6). However, ruminant DNA, chicken and turkey DNA, or porcine DNA were not detected in any other BSFL sample (Table 6). This indicates that DNA from meat is not transferred to BSFL, and that the single detection is likely a result of contamination during grinding.

Conclusions

Survival of BSFL was reduced in substrates with low additions of meat. This could be due to an increased fungal growth occuring at low meat addition. Individual larval growth was positively affected by meat, resulting in heavier larvae when meat was added. Emergence to adults was not affected by meat.

Ruminant DNA, chicken and turkey DNA, and porcine DNA were not detected in the BSFL in any of the treatments, with one exception. This indicates that animal DNA present in substrates is not transferred to the BSFL.

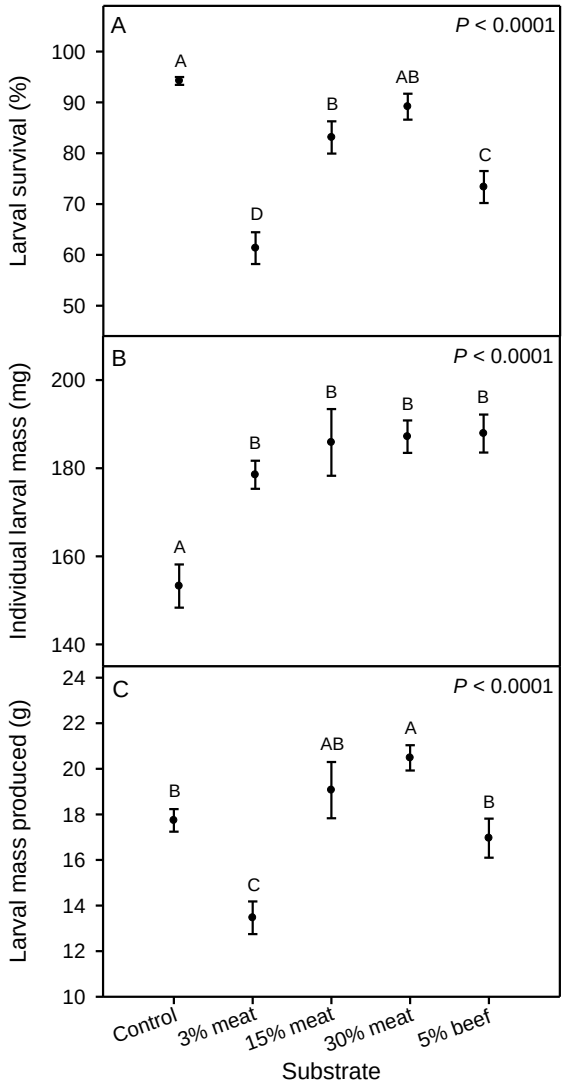

Figure 2: (A) Larval survival, (B) individual mass, and (C) biomass produced during the seven days on experimental substrates. Points show mean ± SE. The P-values are from ANOVA tests. Different letters indicate significant differences (Student's t-test at $\alpha = 0.05$).

Table 6: Detection of animal DNA in substrate and larvae (n = 3). A plus sign (+) indicates that DNA was detected and a minus sign (-) indicates that DNA was not detected.

	Treatment	Ruminant DNA	Chicken and turkey DNA	Porcine DNA
Substrate	Control	-	-	+
		-	-	+
		-	-	+
	3% mixed meat	+	+	+
		+	+	+
		+	+	+
	15% mixed meat	+	+	+
		+	+	+
		+	+	+
	30% mixed meat	+	+	+
		+	+	+
		+	+	+
	5% beef	+	+	-
		+	+	+
		+	-	+
Larvae	Control	-	-	-
		-	-	-
		-	-	-
	3% mixed meat	-	-	-
		-	-	-
		-	-	+
	15% mixed meat	-	-	-
		-	-	-
		-	-	-
	30% mixed meat	-	-	-
		-	-	-
		-	-	-
	5% beef	-	-	-
		-	-	-
		-	-	-

6 Discussion

We find that three pesticides used in fruit production, as well as four PFAS used on surfaces of food packageing material accumulate in BSFL when these are present in the feed substrate. In contrast, we did not find support for the hypothesis that animal DNA would be present in BSFL after developing on a feed substrate containing raw meat of three species.

None of the tested parameters affected larval growth and survival, except additions of meat in low levels. This shows that the larvae are robust, as they are able to handle the investigated chemical contaminants without consequences for their fitness and performance. Emergence to adults was also unaffected or only little affected by the studied chemicals, and larger sample sizes would be required to identify the exact effect of different pesticides relative to each other. The negative effect of a small meat content in the substrate could be caused by a higher growth of fungi and other microorganisms that possibly benefit more from this protein source than the larvae. Because the larvae were unaffected at high concentration of meat, we do not think the result is a direct negative effect of the meat on the larvae in these cases, but that the effect must have other causes, for example a derived effect of microorganisms such as higher mycotoxin levels.

The finding of porcine DNA in the basis substrate suggests that the substate may have been contaminated with pork meat when prepared, or that the samples were contaminated during the grinding process after freeze drying. It could also suggest that the commercial chicken feed used contains pork meat, although this is not the case according to the label. However, the findings of chicken and turkey DNA in the substrate with beef only, but not the controls, suggests contamination. Thus, substates may have been contaminated when prepared, or the samples may have been contaminated during grinding. However, this does not have a negative effect on the quality of the main result, concluding that animal DNA is not transferred to BSFL. The one finding of porcine DNA in BSFL is also likely a result of contamination.

We can, from the investigations, conclude that pesticides and PFAS will accumulate in the BSFL, if used in BSFL growth substrates. Pesticides and PFAS in the growth substrate thus constitute risk factors, as they have potential to accumulate in the food chain. Since PFAS are not any longer allowed for food packaging materials coating in Denmark, they should not impose a problem if waste providers follow the legislations. The addition of raw meat does not appear to involve a risk to the consumers of the larvae, which could potentially open for the possibility to permit organic household waste, slaughter waste and other raw meat sidestreams in BSFL production.

7 References

Belghit, I., Varunjikar, M., Lecrenier, M.C., Steinhilber, A.E., Niedzwiecka, A., Wang, Y.V., Dieu, M., Azzollini, D., Lie, K., Lock, E.J., Berntssen, M.H.G., Renard, P., Zagon, J., Fumière, O., van Loon, J.J.A., Larsen, T., Poetz, O., Braeuning, A., Palmblad, M. and Rasinger, J.D., 2021. Future feed control – tracing banned bovine material in insect meal. Food Control 128: 108183. 10.1016/j.foodcont.2021.108183

Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior, R. and Smith, R., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. Journal of Insects as Food and Feed 1: 7-16. 10.3920/jiff2014.0020

Houbraken, M., Spranghers, T., De Clercq, P., Cooreman-Algoed, M., Couchement, T., De Clercq, G., Verbeke, S. and Spanoghe, P., 2016. Pesticide contamination of *Tenebrio molitor* (Coleoptera: Tenebrionidae) for human consumption. Food Chemistry 201: 264-269. 10.1016/j.foodchem.2016.01.097

Lalander, C., Senecal, J., Calvo, M.G., Ahrens, L., Josefsson, S., Wiberg, K. and Vinneras, B., 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. Science of the Total Environment 565: 279-286. 10.1016/j.scitotenv.2016.04.147

Meijer, N., de Rijk, T., van Loon, J.J.A., Zoet, L. and van der Fels-Klerx, H.J., 2021. Effects of insecticides on mortality, growth and bioaccumulation in black soldier fly (*Hermetia illucens*) larvae. PLoS One 16: e0249362. 10.1371/journal.pone.0249362

Purschke, B., Scheibelberger, R., Axmann, S., Adler, A. and Jager, H., 2017. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain. Food Additives and Contaminants Part a - Chemistry Analysis Control Exposure & Risk Assessment 34: 1410-1420. 10.1080/19440049.2017.1299946

Rainieri, S., Conlledo, N., Larsen, B.K., Granby, K. and Barranco, A. (2018). Combined effects of microplastics and chemical contaminants on the organ toxicity of zebrafish (*Danio rerio*). Environmental Research 162, 135-143. 10.1016/j.envres.2017.12.019

van der Fels-Klerx, H.J., Meijer, N., Nijkamp, M.M., Schmitt, E. and van Loon, J.J.A., 2020. Chemical food safety of using former foodstuffs for rearing black soldier fly larvae (*Hermetia illucens*) for feed and food use. Journal of Insects as Food and Feed 6: 475-488. 10.3920/jiff2020.0024

8 Appendix 1 - Literature study

Overview of existing literature concerning risks when feeding kitchen waste and food packaging materials to larvae of the production insects *Hermetia illucens* and *Tenebrio molitor*

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9 Purpose

Several studies investigate risks that can occur when feeding BSFL kitchen and food waste or packaging residues. The purpose of this literature overview is to examine which relevant risks have been adequately investigated and to identify risks that require more examinations. We aim to give an overview of the number of investigations regarding certain risks, not the outcome of these investigations, to locate subject areas where further research is required.

10 Clarification of risks

In order to uncover relevant parameters within the feed legislation, a previous memorandum from the DTU Food Institute has been used (Fareprofil for substrater til insektopdræt; Danger profile for substrates for insect breeding, 2018). In the note from DTU (Danger profile for substrates for insect breeding, 2018), the danger profile of substrates for insect breeding is described within 10 groups of substrates. The first five groups consist of approved substrates: 1) dry food aimed at insects, 2) 'former foods' - e.g., bakery and cake residues, 3) vegetable by-products and residues, 4) mash/brewer's grain and 5) animal by-products - category 3 material. The next five groups are non-approved substrates: 6) kitchen and food waste, 7) animal by-products - category 3 material from slaughterhouses, 8) 'former foods' with animal content, 9) livestock manure and content from the digestive tract and 10) sludge.

11 Search for literature

Two independent literature searches were performed. Articles published between 2015 to May 2021 were included. This period partly overlaps with literature searches performed in the memo from DTU (2018).

12 Risks related to kitchen and food waste

The risk parameters that have been searched for are mainly selected according to group 6 and 8 in the note from DTU; (6) kitchen and food waste and 8) 'former foods' with animal content). The risk parameters can be divided in biological risks and chemical risks.

Biological risks

The biological risks include zoonotic infectious agents from especially unprocessed food waste (*Campylobacter*, *Salmonella*, *Listeria*, *Escherichia coli* and *Staphylococcus aureus* (MRSA)), as well as severe livestock diseases (foot-and-mouth disease, African and classical swine fever, Aujeszky's gastrointestinal disease). The mentioned livestock diseases typically do not occur in Danish livestock and are therefore not included in this overview. Zoonotic bacteria are typically found again on the surface of larvae after development in contaminated substrate, and therefore need heat treatment before use as food or feed.

Campylobacter

Campylobacter is responsible for a zoonotic disease, which is often transferred by fresh and undercooked meat, especially chicken meat. *Campylobacter* prevalence varies depending on production method and source. In slaughterhouse samples, *Campylobacter* occurs in 21.3% / 87.2% of the samples respectively (conventional / organic). In Danish retail, the prevalence is 12.8% / 71.0% and 37.9% / 78.8% in samples of imported meat (Anon 2017). There are only a few studies on the occurrence of *Campylobacter* in the black soldier fly or the common mealworm. To our knowledge, none of the studies have been carried out as spiking studies, but studies focusing on intestinal microbiology (*H. illucens* (Comm, 2015; Raimondi *et al.*, 2020; Tanga *et al.*, 2021).

Salmonella

The prevalence of *Salmonella* in Danish meat is generally very low (<1%). However, in imported meat from pork, chicken, and turkey, 7-12% of tested batches of *Salmonella* are positive. Danish eggs are almost entirely free from *Salmonella*, but the requirements for imported eggs are high incurring a *Salmonella* risk. Many studies examine the presence of *Salmonella* in larvae of both the black soldier fly and mealworms. The experiments have been done both on substrates where *Salmonella* occurs naturally (fertilizer), in spiking experiments and studies of the occurrence of *Salmonella* in larvae that are ready to use as feed or food. There are more than 10 studies for both *T. molitor* and *H. illucens*: Awasthi *et al.*, 2020; Borremans *et al.*, 2020; Comm, 2015; Garofalo *et al.*, 2017; Grabowski and Klein, 2017a; b; Ibanez-Peinado *et al.*, 2020; Jensen *et al.*, 2020; Kamau *et al.*, 2020; Kashiri *et al.*, 2018; Lalander *et al.*, 2013; Lalander *et al.*, 2015; Lee *et al.*, 2020a; b; Lopes *et al.*, 2020; Mancini *et al.*, 2019a; Marin *et al.*, 2019; Nyangena *et al.*, 2020; Raimondi *et al.*, 2020; Vandeweyer *et al.*, 2017; Wynants *et al.*, 2019a; Wynants *et al.*, 2019b.

Listeria

Several articles investigate how larvae of *T. molitor* and *H. illucens* cope with infections with *Listeria*. Many look at how specific genes in the larvae affect survival with *Listeria*, while some also investigate the *Listeria* occurrence in larvae and how it is reduced through various treatments. There are more than 20 articles in the field, some of the most relevant for *T. molitor* are: Belleggia *et al.*, 2020; Borremans *et al.*, 2020; Garofalo *et al.*, 2017; Grabowski and Klein, 2017a; Ibanez-Peinado *et al.*, 2020; Mancini *et al.*, 2019a; Mancini *et al.*, 2019b; Patnaik *et al.*, 2015; Vandeweyer *et al.*, 2017. For *H. illucens*: Awasthi *et al.*, 2020; Grabowski and Klein, 2017b; Kashiri *et al.*, 2018; Marin *et al.*, 2019; Raimondi *et al.*, 2020.

Escherichia coli

Escherichia coli have been very well investigated. Since 2017, more than 50 studies have been published on E. coli in the larvae of *H. illucens*, slightly fewer (about 40 studies) on *E. coli* in larvae of *T. molitor*.

Studies on *T. molitor*: Edosa *et al.*, 2020a; b; Grabowski and Klein, 2017a; Ibanez-Peinado *et al.*, 2020; Kavran *et al.*, 2018; Keshavarz *et al.*, 2020a; Keshavarz *et al.*, 2020b; Keshavarz *et al.*, 2020c; Mancini *et al.*, 2019a; Osimani *et al.*, 2018; Park *et al.*, 2019; Silva *et al.*, 2020; van der Fels-Klerx *et al.*, 2018.

Studies on *H. illucens*: Awasthi *et al.*, 2020; Bortolini *et al.*, 2020; Cifuentes *et al.*, 2020; Dobermann *et al.*, 2019; Grabowski and Klein, 2017b; Joosten *et al.*, 2020; Kashiri *et al.*, 2018; Lee *et al.*, 2020a; Liu *et al.*, 2019; Lopes *et al.*, 2020; Marin *et al.*, 2019; Miranda *et al.*, 2020; Shelomi *et al.*, 2020; Swinscoe *et al.*, 2020; Wynants *et al.*, 2019a; Zhang *et al.*, 2021.

Staphylococcus aureus (MRSA)

One of FVST's control reports "MRSA in Pork 2016" states that 122 (40%) of 305 meat samples were positive for methicillin-resistant *Staphylococcus aureus* (MRSA) (animal type) (FVST 2016). However, it does not appear to pose a significant food safety risk. Many studies examine the presence of *Staphylococcus aureus* in insects, including *T. molitor* and *H. illucens*.

Studies on *T. molitor*: Adamski *et al.*, 2019; Colasso *et al.*, 2020; Dorling *et al.*, 2015; Edosa *et al.*, 2020b; El Shazely *et al.*, 2019; Garofalo *et al.*, 2017; Grabowski and Klein, 2017a; Keshavarz *et al.*, 2020a; Keshavarz *et al.*, 2020b; Keshavarz *et al.*, 2020c; Makarova *et al.*, 2018; Mancini *et al.*, 2019a; McGonigle *et al.*, 2016; Nyangena *et al.*, 2020.

Studies on *H. illucens*: Awasthi *et al.*, 2020; Grabowski and Klein, 2017b; Lee *et al.*, 2020a; Nyangena *et al.*, 2020; Raimondi *et al.*, 2020.

Chemical hazards

Chemical hazards come from fungi as mycotoxins or from human pollutants, agricultural practices, and use on coated surfaces in food packaging.

Mycotoxins

There are various mycotoxins of which Aflatoxin B1 is the most frequently studied, but other mycotoxins have also been examined. Among others are: Deoxynivalenol, Zearalenone, Ochratoxin A, Fumonisin B1 + B2, Sum T-2 and HT-2 toxin. From 2017 to today, there are 12 articles on mycotoxins in larvae of *H. illucens* or *T. molitor*. All studies find that mycotoxins do not accumulate and often smaller amounts are found in the larvae than in the feed (Bosch *et al.*, 2017; Camenzuli *et al.*, 2018; Charlton *et al.*, 2015; Gulsunoglu *et al.*, 2019; Leni *et al.*, 2019; Meijer *et al.*, 2019; Niermans *et al.*, 2019; Purschke *et al.*, 2017; Sanabria *et al.*, 2019; Schrogel and Watjen, 2019; Van Broekhoven *et al.*, 2017; Wang and Shelomi, 2017).

Heavy metals and other elements

Many studies can be found on the accumulation of heavy metals in larvae. The elements most frequently investigated are cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), chromium (Cr), zinc (Zn), copper (Cu) and arsenic (As). Studies suggest that in particular cadmium accumulates in the larvae, but the results differ a little across studies (Biancarosa *et al.*, 2018; Cai *et al.*, 2018; Diener *et al.*, 2015; Gao *et al.*, 2019; Gao *et al.*, 2017; Purschke *et al.*, 2017; Schrogel and Watjen, 2019; Truzzi *et al.*, 2019; van der Fels-Klerx *et al.*, 2016; van der Fels-Klerx *et al.*, 2020; Wu *et al.*, 2020).

Pesticides

Only a few studies examine how pesticides in the substrate affect the content of pesticides in larvae of *H. illucens* and *T. molitor*. The results suggest that pesticides do not accumulate in larvae of *H. illucens* (Charlton *et al.*, 2015; Lalander *et al.*, 2016; Meijer *et al.*, 2021; Purschke *et al.*, 2017). The one study found on larvae of *T. molitor* indicates that some pesticides may accumulate in the larvae (Houbraken *et al.*, 2016).

Dioxins and PCBs

Only two studies could be found on the uptake of dioxins and PCBs from substrate to larvae of *H. illucens*. The levels of both dioxins and PCBs are lower in the larvae than in the substrate (van der Fels-Klerx et al., 2020), and both remain below the limit values for animal feed (Charlton et al., 2015).

13 Risks related to food packaging residues

This search has been based on packaging residues consisting of cardboard and paper. Some of the sources for problematic substances can be found in the treatment of the surfaces (e.g., fluorinated substances) and in the printing ink.

Problematic substances

Only one study has been found regarding the risk of feeding *H. illucens* larvae plastic packaging and cardboard / paper packaging with different mineral oils: n-decane D22, n-dodecane D26, n-eicosane D42 and n-hexatricontane D74 (van der Fels-Klerx *et al.*, 2020). The study found that mineral oils do not appear to accumulate in the larvae. Searches for other problematic substances such as bisphenol A, phthalates and fluorinated substances have not given results, underlining the lack of knowledge in this field.

14 Risks related to meat

The TSE Regulation (999/2001 and subsequent amendments) describes which processed animal proteins (PAP) can be used in feed for various livestock, including insects. For insects, it is permitted to use fishmeal and blood products from non-ruminants. It is not permitted to use former foods which contain meat and fish (Regulation 1069/2009). Only one study was found that investigated the transfer of PAP from substrate to *H. illucens* larvae (Belghit *et al.*, 2021). They found that animal DNA can be transferred from substrate to BSFL.

15 Summary

Except for *Campylobacter*, the primary biological risks such as *Salmonella*, *Listeria*, *Escherichia coli* and *Staphylococcus aureus* (MRSA) are well studied. In terms of chemical risks, mycotoxins and heavy metals are well studied while studies on pesticides / insecticides and dioxins and PCBs are lacking. Regarding the problematic substances that can occur in food packaging materials consistent of cardboard / paper, only one study on mineral oils exists. No studies could be found on bisphenol A, phthalates, and fluorinated substances. Finally, only one study has been found that investigates whether PAP in substrate is transferred to BSFL.

16 References

Adamski, Z., Bufo, S.A., Chowanski, S., Falabella, P., Lubawy, J., Marciniak, P., Pacholska-Bogalska, J., Salvia, R., Scrano, L., Slocinska, M., Spochacz, M., Szymczak, M., Urbanski, A., Walkowiak-Nowicka, K. and Rosinski, G., 2019. Beetles as model organisms in physiological, biomedical and environmental studies - a review. Frontiers in Physiology 10: 319. 10.3389/fphys.2019.00319

Awasthi, M.K., Liu, T., Awasthi, S.K., Duan, Y.M., Pandey, A. and Zhang, Z.Q., 2020. Manure pretreatments with black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae): a study to reduce pathogen content. Science of the Total Environment 737: 139842. 10.1016/j.scitotenv.2020.139842

Belghit, I., Varunjikar, M., Lecrenier, M.C., Steinhilber, A.E., Niedzwiecka, A., Wang, Y.V., Dieu, M., Azzollini, D., Lie, K., Lock, E.J., Berntssen, M.H.G., Renard, P., Zagon, J., Fumière, O., van Loon, J.J.A., Larsen, T., Poetz, O., Braeuning, A., Palmblad, M. and Rasinger, J.D., 2021. Future feed control – tracing

banned bovine material in insect meal. Food Control 128: 108183. 10.1016/j.foodcont.2021.108183

Belleggia, L., Milanovic, V., Cardinali, F., Garofalo, C., Pasquini, M., Tavoletti, S., Riolo, P., Ruschioni, S., Isidoro, N., Clementi, F., Ntoumos, A., Aquilanti, L. and Osimani, A., 2020. *Listeria* dynamics in a laboratory-scale food chain of mealworm larvae (*Tenebrio molitor*) intended for human consumption. Food Control 114: 107246. 10.1016/j.foodcont.2020.107246

Biancarosa, I., Liland, N.S., Biemans, D., Araujo, P., Bruckner, C.G., Waagbo, R., Torstensen, B.E., Lock, E.J. and Amlund, H., 2018. Uptake of heavy metals and arsenic in black soldier fly (*Hermetia illucens*) larvae grown on seaweed-enriched media. Journal of the Science of Food and Agriculture 98: 2176-2183. 10.1002/jsfa.8702

Borremans, A., Smets, R. and Van Campenhout, L., 2020. Fermentation versus meat preservatives to extend the shelf life of mealworm (*Tenebrio molitor*) paste for feed and food applications. Frontiers in Microbiology 11: 1510. 10.3389/fmicb.2020.01510

Bortolini, S., Macavei, L.I., Saadoun, J.H., Foca, G., Ulrici, A., Bernini, F., Malferrari, D., Setti, L., Ronga, D. and Maistrello, L., 2020. *Hermetia illucens* (L.) larvae as chicken manure management tool for circular economy. Journal of Cleaner Production 262: 121289. 10.1016/j.jclepro.2020.121289

Bosch, G., van der Fels-Klerx, H.J., de Rijk, T.C. and Oonincx, D., 2017. Aflatoxin B1 tolerance and accumulation in black soldier fly larvae (*Hermetia illucens*) and yellow mealworms (*Tenebrio molitor*). Toxins 9: 185. 10.3390/toxins9060185

Cai, M.M., Hu, R.Q., Zhang, K., Ma, S.T., Zheng, L.Y., Yu, Z.N. and Zhang, J.B., 2018. Resistance of black soldier fly (Diptera: Stratiomyidae) larvae to combined heavy metals and potential application in municipal sewage sludge treatment. Environmental Science and Pollution Research 25: 1559-1567. 10.1007/s11356-017-0541-x

Camenzuli, L., Van Dam, R., de Rijk, T., Andriessen, R., Van Schelt, J. and Van der Fels-Klerx, H.J., 2018. Tolerance and excretion of the mycotoxins aflatoxin B-1, zearalenone, deoxynivalenol, and ochratoxin A by *Alphitobius diaperinus* and *Hermetia illucens* from contaminated substrates. Toxins 10: 91. 10.3390/toxins10020091

Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior, R. and Smith, R., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. Journal of Insects as Food and Feed 1: 7-16. 10.3920/jiff2014.0020

Cifuentes, Y., Glaeser, S.P., Mvie, J., Bartz, J.O., Muller, A., Gutzeit, H.O., Vilcinskas, A. and Kampfer, P., 2020. The gut and feed residue microbiota changing during the rearing of *Hermetia illucens* larvae. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 113: 1323-1344. 10.1007/s10482-020-01443-0

Colasso, A.H.M., Barros, T.F., Figueiredo, I.F.D., Carvalho, A.R., Fernandes, E.S., Uchoa, M.R.B. and da Silva, L.C.N., 2020. The latex of *Euphorbia tirucalli* inhibits staphyloxanthin production and protects *Tenebrio molitor* larvae against *Staphylococcus aureus* infection. Natural Product Research 34: 3536-3539. 10.1080/14786419.2019.1582036

Comm, E.S., 2015. Risk profile related to production and consumption of insects as food and feed. EFSA Journal 13: 4257. 10.2903/j.efsa.2015.4257

Diener, S., Zurbrügg, C. and Tockner, K., 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. Journal of Insects as Food and Feed 1: 261-270. 10.3920/jiff2015.0030

Dobermann, D., Field, L.M. and Michaelson, L.V., 2019. Using *Hermetia illucens* to process Ugandan waragi waste. Journal of Cleaner Production 211: 303-308. 10.1016/j.jclepro.2018.11.176

Dorling, J., Moraes, C. and Rolff, J., 2015. Recognition, survival and persistence of *Staphylococcus aureus* in the model host *Tenebrio molitor*. Developmental and Comparative Immunology 48: 284-290. 10.1016/j.dci.2014.08.010

Edosa, T.T., Jo, Y.H., Keshavarz, M., Bae, Y.M., Kim, D.H., Lee, Y.S. and Han, Y.S., 2020a. TmSpz4 plays an important role in regulating the production of antimicrobial peptides in response to *Escherichia*

coli and *Candida albicans* infections. International Journal of Molecular Sciences 21: 1878. 10.3390/ijms21051878

Edosa, T.T., Jo, Y.H., Keshavarz, M., Bae, Y.M., Kim, D.H., Lee, Y.S. and Han, Y.S., 2020b. TmSpz6 is essential for regulating the immune response to *Escherichia coli* and *Staphylococcus aureus* infection in *Tenebrio molitor*. Insects 11: 105. 10.3390/insects11020105

El Shazely, B., Urabanski, A., Johnston, P.R. and Rolff, J., 2019. In vivo exposure of insect AMP resistant *Staphylococcus aureus* to an insect immune system. Insect Biochemistry and Molecular Biology 110: 60-68. 10.1016/j.ibmb.2019.04.017

Gao, M., Lin, Y., Shi, G.Z., Li, H.H., Yang, Z.B., Xu, X.X., Xian, J.R., Yang, Y.X. and Cheng, Z., 2019. Bioaccumulation and health risk assessments of trace elements in housefly (*Musca domestics* L.) larvae fed with food wastes. Science of the Total Environment 682: 485-493. 10.1016/j.scitotenv.2019.05.182

Gao, Q., Wang, X.Y., Wang, W.Q., Lei, C.L. and Zhu, F., 2017. Influences of chromium and cadmium on the development of black soldier fly larvae. Environmental Science and Pollution Research 24: 8637-8644. 10.1007/s11356-017-8550-3

Garofalo, C., Osimani, A., Milanovic, V., Taccari, M., Cardinali, F., Aquilanti, L., Riolo, P., Ruschioni, S., Isidoro, N. and Clementi, F., 2017. The microbiota of marketed processed edible insects as revealed by high-throughput sequencing. Food Microbiology 62: 15-22. 10.1016/j.fm.2016.09.012

Grabowski, N.T. and Klein, G., 2017a. Microbiological analysis of raw edible insects. Journal of Insects as Food and Feed 3: 7-14. 10.3920/jiff2016.0004

Grabowski, N.T. and Klein, G., 2017b. Microbiology of processed edible insect products - results of a preliminary survey. International Journal of Food Microbiology 243: 103-107. 10.1016/j.ijfoodmicro.2016.11.005

Gulsunoglu, Z., Aravind, S., Bai, Y., Wang, L., Kutcher, H.R. and Tanaka, T., 2019. Deoxynivalenol (DON) accumulation and nutrient recovery in black soldier fly larvae (*Hermetia illucens*) fed wheat infected with *Fusarium* spp. Fermentation 5: 83. 10.3390/fermentation5030083

Houbraken, M., Spranghers, T., De Clercq, P., Cooreman-Algoed, M., Couchement, T., De Clercq, G., Verbeke, S. and Spanoghe, P., 2016. Pesticide contamination of *Tenebrio molitor* (Coleoptera: Tenebrionidae) for human consumption. Food Chemistry 201: 264-269. 10.1016/j.foodchem.2016.01.097

Ibanez-Peinado, D., Ubeda-Manzanaro, M., Martinez, A. and Rodrigo, D., 2020. Antimicrobial effect of insect chitosan on *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* survival. PLoS One 15: e0244153. 10.1371/journal.pone.0244153

Jensen, A.N., Hansen, S.H. and Baggesen, D.L., 2020. *Salmonella* Typhimurium level in mealworms (*Tenebrio molitor*) after exposure to contaminated substrate. Frontiers in Microbiology 11: 1613. 10.3389/fmicb.2020.01613

Joosten, L., Lecocq, A., Jensen, A.B., Haenen, O., Schmitt, E. and Eilenberg, J., 2020. Review of insect pathogen risks for the black soldier fly (*Hermetia illucens*) and guidelines for reliable production. Entomologia Experimentalis et Applicata 168: 432-447. 10.1111/eea.12916

Kamau, E., Mutungi, C., Kinyuru, J., Imathiu, S., Affognon, H., Ekesi, S., Nakimbugwe, D. and Fiaboe, K.K.M., 2020. Changes in chemical and microbiological quality of semi-processed black soldier fly (*Hermetia illucens* L.) larval meal during storage. Journal of Insects as Food and Feed 6: 417-428. 10.3920/jiff2019.0043

Kashiri, M., Marin, C., Garzon, R., Rosell, C.M., Rodrigo, D. and Martinez, A., 2018. Use of high hydrostatic pressure to inactivate natural contaminating microorganisms and inoculated *E. coli* O157:H7 on *Hermetia illucens* larvae. PLoS One 13: e0194477. 10.1371/journal.pone.0194477

Kavran, M., Cupina, A.I., Zgomba, M. and Petric, D., 2018. Edible insects - safe food for humans and livestock. In: R. Petanovic (Ed.), Ecological and Economic Significance of Fauna of Serbia. Serbian Academy of Sciences and Arts Scientific Meetings, pp. 251-300.

Keshavarz, M., Jo, Y.H., Edosa, T.T., Bae, Y.M. and Han, Y.S., 2020a. TmPGRP-SA regulates antimicrobial response to bacteria and fungi in the fat body and gut of *Tenebrio molitor*. International Journal of Molecular Sciences 21: 2113. 10.3390/ijms21062113

Keshavarz, M., Jo, Y.H., Edosa, T.T. and Han, Y.S., 2020b. *Tenebrio molitor* PGRP-LE plays a critical role in gut antimicrobial peptide production in response to *Escherichia coli*. Frontiers in Physiology 11: 320. 10.3389/fphys.2020.00320

Keshavarz, M., Jo, Y.H., Patnaik, B.B., Park, K.B., Ko, H.J., Kim, C.E., Edosa, T.T., Lee, Y.S. and Han, Y.S., 2020c. TmRelish is required for regulating the antimicrobial responses to *Escherichia coli* and *Staphylococcus aureus* in *Tenebrio molitor*. Scientific Reports 10: 4258. 10.1038/s41598-020-61157-1

Lalander, C., Diener, S., Magri, M.E., Zurbrugg, C., Lindstrom, A. and Vinneras, B., 2013. Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) - from a hygiene aspect. Science of the Total Environment 458: 312-318. 10.1016/j.scitotenv.2013.04.033

Lalander, C., Senecal, J., Calvo, M.G., Ahrens, L., Josefsson, S., Wiberg, K. and Vinneras, B., 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. Science of the Total Environment 565: 279-286. 10.1016/j.scitotenv.2016.04.147

Lalander, C.H., Fidjeland, J., Diener, S., Eriksson, S. and Vinneras, B., 2015. High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling. Agronomy for Sustainable Development 35: 261-271. 10.1007/s13593-014-0235-4

Lee, K.S., Yun, E.Y. and Goo, T.W., 2020a. Antimicrobial activity of an extract of *Hermetia illucens* larvae immunized with *Lactobacillus casei* against *Salmonella* species. Insects 11: 704. 10.3390/insects11100704

Lee, K.S., Yun, E.Y. and Goo, T.W., 2020b. Evaluation of the antimicrobial activity of an extract of *Lactobacillus casei*-infected *Hermetia illucens* larvae produced using an automatic injection system. Animals 10: 2121. 10.3390/ani10112121

Leni, G., Cirlini, M., Jacobs, J., Depraetere, S., Gianotten, N., Sforza, S. and Dall'Asta, C., 2019. Impact of naturally contaminated substrates on *Alphitobius diaperinus* and *Hermetia illucens*: uptake and excretion of mycotoxins. Toxins 11: 476. 10.3390/toxins11080476

Liu, C., Wang, C. and Yao, H., 2019. Comprehensive resource utilization of waste using the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae). Animals 9: 349. 10.3390/ani9060349

Lopes, I.G., Lalander, C., Vidotti, R.M. and Vinneras, B., 2020. Reduction of bacteria in relation to feeding regimes when treating aquaculture waste in fly larvae composting. Frontiers in Microbiology 11: 1616. 10.3389/fmicb.2020.01616

Makarova, O., Johnston, P., Rodriguez-Rojas, A., El Shazely, B., Morales, J.M. and Rolff, J., 2018. Genomics of experimental adaptation of Staphylococcus aureus to a natural combination of insect antimicrobial peptides. Scientific Reports 8: 15359. 10.1038/s41598-018-33593-7

Mancini, S., Fratini, F., Tuccinardi, T., Turchi, B., Nuvoloni, R. and Paci, G., 2019a. Effects of different blanching treatments on microbiological profile and quality of the mealworm (*Tenebrio molitor*). Journal of Insects as Food and Feed 5: 225-234. 10.3920/jiff2018.0034

Mancini, S., Paci, G., Ciardelli, V., Turchi, B., Pedonese, F. and Fratini, F., 2019b. *Listeria monocytogenes* contamination of *Tenebrio molitor* larvae rearing substrate: preliminary evaluations. Food Microbiology 83: 104-108. 10.1016/j.fm.2019.05.006

Marin, C., Urbina, P., Salvatierra, P., Rodrigo, D., Fernandez, P. and Martinez, A., 2019. Effect of high hydrostatic pressure on natural contaminating microorganisms and inoculated *Salmonella* in *Hermetia illucens* larvae. Berliner Und Munchener Tierarztliche Wochenschrift 132: 257-263. 10.2376/0005-9366-18058

McGonigle, J.E., Purves, J. and Rolff, J., 2016. Intracellular survival of *Staphylococcus aureus* during persistent infection in the insect *Tenebrio molitor*. Developmental and Comparative Immunology 59: 34-38. 10.1016/j.dci.2016.01.002

Meijer, N., de Rijk, T., van Loon, J.J.A., Zoet, L. and van der Fels-Klerx, H.J., 2021. Effects of insecticides on mortality, growth and bioaccumulation in black soldier fly (*Hermetia illucens*) larvae. PLoS One 16: e0249362. 10.1371/journal.pone.0249362

Meijer, N., Stoopen, G., van der Fels-Klerx, H.J., van Loon, J.J.A., Carney, J. and Bosch, G., 2019. Aflatoxin B-1 conversion by black soldier fly (*Hermetia illucens*) larval enzyme extracts. Toxins 11: 532. 10.3390/toxins11090532

Miranda, C.D., Cammack, J.A. and Tomberlin, J.K., 2020. Mass production of the black soldier fly, *Hermetia illucens*(L.), (Diptera: Stratiomyidae) reared on three manure types. Animals 10: 1243. 10.3390/ani10071243

Niermans, K., Woyzichovski, J., Kroncke, N., Benning, R. and Maul, R., 2019. Feeding study for the mycotoxin zearalenone in yellow mealworm (*Tenebrio molitor*) larvae-investigation of biological impact and metabolic conversion. Mycotoxin Research 35: 231-242. 10.1007/s12550-019-00346-y

Nyangena, D.N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., Nakimbugwe, D. and Fiaboe, K.K.M., 2020. Effects of traditional processing techniques on the nutritional and microbiological quality of four edible insect species used for food and feed in East Africa. Foods 9: 574. 10.3390/foods9050574

Osimani, A., Milanovic, V., Cardinali, F., Garofalo, C., Clementi, F., Pasquini, M., Riolo, P., Ruschioni, S., Isidoro, N., Loreto, N., Franciosi, E., Tuohy, K., Petruzzelli, A., Foglini, M., Gabucci, C., Tonucci, F. and Aquilanti, L., 2018. The bacterial biota of laboratory-reared edible mealworms (*Tenebrio molitor* L.): From feed to frass. International Journal of Food Microbiology 272: 49-60. 10.1016/j.ijfoodmicro.2018.03.001

Park, S., Jo, Y.H., Park, K.B., Ko, H.J., Kim, C.E., Bae, Y.M., Kim, B., Jun, S.A., Bang, I.S., Lee, Y.S., Kim, Y.J. and Han, Y.S., 2019. TmToll-7 plays a crucial role in innate immune responses against gram-negative bacteria by regulating 5 AMP genes in *Tenebrio molitor*. Frontiers in Immunology 10: 310. 10.3389/fimmu.2019.00310

Patnaik, B.B., Patnaik, H.H., Park, K.B., Jo, Y.H., Lee, Y.S. and Han, Y.S., 2015. Silencing of apolipophorin-III causes abnormal adult morphological phenotype and susceptibility to *Listeria monocytogenes* infection in *Tenebrio molitor*. Entomological Research 45: 116-121. 10.1111/1748-5967.12099

Purschke, B., Scheibelberger, R., Axmann, S., Adler, A. and Jager, H., 2017. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain. Food Additives and Contaminants Part a - Chemistry Analysis Control Exposure & Risk Assessment 34: 1410-1420. 10.1080/19440049.2017.1299946

Raimondi, S., Spampinato, G., Macavei, L.I., Lugli, L., Candeliere, F., Rossi, M., Maistrello, L. and Amaretti, A., 2020. Effect of rearing temperature on growth and microbiota composition of *Hermetia illucens*. Microorganisms 8: 902. 10.3390/microorganisms8060902

Sanabria, C.O., Hogan, N., Madder, K., Gillott, C., Blakley, B., Reaney, M., Beattie, A. and Buchanan, F., 2019. Yellow mealworm larvae (*Tenebrio molitor*) fed mycotoxin-contaminated wheat - a possible safe, sustainable protein source for animal feed? Toxins 11: 282. 10.3390/toxins11050282

Schrogel, P. and Watjen, W., 2019. Insects for food and feed-safety aspects related to mycotoxins and metals. Foods 8: 288. 10.3390/foods8080288

Shelomi, M., Wu, M.K., Chen, S.M., Huang, J.J. and Burke, C.G., 2020. Microbes associated with black soldier fly (Diptera: Stratiomiidae) degradation of food waste. Environmental Entomology 49: 405-411. 10.1093/ee/nvz164

Silva, T.F., Cavalcanti, J.R.N., Fonseca, M., dos Santos, N.M., da Silva, A.C.B., Zagmignan, A., Abreu, A.G., da Silva, A.P.S., Lima, V.L.D., da Silva, N.H., Dutra, L.M., Almeida, J., da Silva, M.V., Correia, M.T.D. and da Silva, L.C.N., 2020. Products derived from *Buchenavia tetraphylla* leaves have in vitro antioxidant activity and protect *Tenebrio molitor* larvae against *Escherichia coli*-induced injury. Pharmaceuticals 13: 46. 10.3390/ph13030046

Swinscoe, I., Oliver, D.M., Ornsrud, R. and Quilliam, R.S., 2020. The microbial safety of seaweed as a feed component for black soldier fly (*Hermetia illucens*) larvae. Food Microbiology 91: 103535. 10.1016/j.fm.2020.103535

Tanga, C.M., Waweru, J.W., Tola, Y.H., Onyoni, A.A., Khamis, F.M., Ekesi, S. and Paredes, J.C., 2021. Organic waste substrates induce important shifts in gut microbiota of black soldier fly (*Hermetia illucens* L.): coexistence of conserved, variable, and potential pathogenic microbes. Frontiers in Microbiology 12: 635881. 10.3389/fmicb.2021.635881

Truzzi, C., Illuminati, S., Girolametti, F., Antonucci, M., Scarponi, G., Ruschioni, S., Riolo, P. and Annibaldi, A., 2019. Influence of feeding substrates on the presence of toxic metals (Cd, Pb, Ni, As, Hg) in larvae of *Tenebrio molitor*: risk assessment for human consumption. International Journal of Environmental Research and Public Health 16: 4815. 10.3390/ijerph16234815

Van Broekhoven, S., Gutierrez, J.M., De Rijk, T.C., De Nijs, W.C.M. and Van Loon, J.J.A., 2017. Degradation and excretion of the Fusarium toxin deoxynivalenol by an edible insect, the yellow mealworm (*Tenebrio molitor* L.). World Mycotoxin Journal 10: 163-169. 10.3920/wmj2016.2102

van der Fels-Klerx, H.J., Camenzuli, L., Belluco, S., Meijer, N. and Ricci, A., 2018. Food safety issues related to uses of insects for feeds and foods. Comprehensive Reviews in Food Science and Food Safety 17: 1172-1183. 10.1111/1541-4337.12385

van der Fels-Klerx, H.J., Camenzuli, L., van der Lee, M.K. and Oonincx, D., 2016. Uptake of cadmium, lead and arsenic by *Tenebrio molitor* and *Hermetia illucens* from contaminated substrates. PLoS One 11: e0166186. 10.1371/journal.pone.0166186

van der Fels-Klerx, H.J., Meijer, N., Nijkamp, M.M., Schmitt, E. and van Loon, J.J.A., 2020. Chemical food safety of using former foodstuffs for rearing black soldier fly larvae (*Hermetia illucens*) for feed and food use. Journal of Insects as Food and Feed 6: 475-488. 10.3920/jiff2020.0024

Vandeweyer, D., Crauwels, S., Lievens, B. and Van Campenhout, L., 2017. Microbial counts of mealworm larvae (*Tenebrio molitor*) and crickets (*Acheta domesticus* and *Gryllodes sigillatus*) from different rearing companies and different production batches. International Journal of Food Microbiology 242: 13-18. 10.1016/j.ijfoodmicro.2016.11.007

Wang, Y.S. and Shelomi, M., 2017. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. Foods 6: 91. 10.3390/foods6100091

Wu, N., Wang, X.B., Xu, X.Y., Cai, R.J. and Xie, S.Y., 2020. Effects of heavy metals on the bioaccumulation, excretion and gut microbiome of black soldier fly larvae (*Hermetia illucens*). Ecotoxicology and Environmental Safety 192: 110323. 10.1016/j.ecoenv.2020.110323

Wynants, E., Frooninckx, L., Crauwels, S., Verreth, C., De Smet, J., Sandrock, C., Wohlfahrt, J., Van Schelt, J., Depraetere, S., Lievens, B., Van Miert, S., Claes, J. and Van Campenhout, L., 2019a. Assessing the microbiota of black soldier fly larvae (*Hermetia illucens*) reared on organic wastes streams on four different locations at laboratory and large scale. Microbial Ecology 77: 913-930. 10.1007/s00248-018-1286-x

Wynants, E., Frooninckx, L., Van Miert, S., Geeraerd, A., Claes, J. and Van Campenhout, L., 2019b. Risks related to the presence of *Salmonella* sp. during rearing of mealworms (*Tenebrio molitor*) for food or feed: survival in the substrate and transmission to the larvae. Food Control 100: 227-234. 10.1016/j.foodcont.2019.01.026

Zhang, X.X., Zhang, J.Z., Jiang, L.L., Yu, X., Zhu, H.W., Zhang, J.L., Feng, Z.B., Zhang, X., Chen, G.Z. and Zhang, Z.J., 2021. Black soldier fly (*Hermetia illucens*) larvae significantly change the microbial community in chicken manure. Current Microbiology 78: 303-315. 10.1007/s00284-020-02276-w