BRILL
WAGENINGEN
ACADEMIC

JOURNAL OF INSECTS AS FOOD AND FEED (2024) DOI:10.1163/23524588-00001167

Journal of Insects as Food and Feed
brill.com/jiff

RESEARCH ARTICLE

Toxicity, transfer and metabolization of the pyrethroid insecticides cypermethrin and deltamethrin by reared black soldier fly larvae

N. Meijer[1], L. Zoet[2], D. Rijkers[1], R. Nijssen[1], M. Willemsen[1], P. Zomer[1] and H.J. van der Fels-Klerx[1]*

[1]Wageningen Food Safety Research (WFSR), Wageningen University and Research, Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands; [2]Bestico B.V., Veilingweg 6, 2651 BE Berkel en Rodenrijs, The Netherlands; *nathan.meijer@wur.nl

Received 16 February 2024 | Accepted 8 May 2024 | Published online 7 June 2024

Abstract

Reared insects such as black soldier fly larvae (*Hermetia illucens*) are considered a potential alternative feed protein. However, dietary exposure to insecticide residues via the substrate could adversely affect performance indicators (yield/survival) and substance-transfer from substrate to larval biomass could result in non-compliance with low legal limits. Effects of pyrethroid insecticides cypermethrin and deltamethrin were tested at varying concentrations, with or without the synergist piperonyl butoxide (PBO). Concentration/response curves for yield were estimated and samples were analysed to determine concentrations of parent compounds and selected metabolites. Results suggest that deltamethrin is highly toxic to *H. illucens* larvae: the critical effect dose for 10% yield loss was estimated to be 0.04 mg/kg, compared to a legal limit in wheat of 2.0 mg/kg. Cypermethrin was comparatively less toxic, in line with prior studies, but may also cause significant adverse effects even for exposure levels below the legal limit – especially when combined with PBO. For both substances, transfer from substrate to larvae is a potential issue due to low limits, and transfer as well as toxicity are increased by presence of PBO. Some metabolites could be detected, but more research is needed to determine resistance mechanisms involved.

Keywords

Hermetia illucens – pesticides – residues

1 Introduction

Insects reared for food and feed purposes are an emerging market in the European Union (EU) (Sogari *et al.*, 2019; Van Huis, 2020). The insect species that are currently being reared for food and feed purposes can generally be considered safe if reared on conventional feed-grade materials included in the EU feed catalogue (Regulation (EC) No 68/2013), such as cereal grains and legumes (EFSA, 2015a). However, the presence of certain chemical contaminants in the substrate can affect the safety of the final product, which includes residues of insecticides from agricultural use in the field or storage (Lievens *et al.*, 2021; Van der Fels-Klerx *et al.*, 2020). Product-specific maximum residue levels (MRLs) for plant protection products (pesticides) have been laid down in Regulation (EC) No 396/2005, which are based on application according to Good Agricultural Practice (GAP) and toxicological limits to protect vulnerable consumers such as children (preamble 5). The potential issues associated with the presence of insecticide residues in insect rearing substrates are twofold.

Published with license by Koninklijke Brill BV | DOI: 10.1163/23524588-00001167
 | ISSN: 2352-4588 (online)

Firstly, significant reductions in survival and yield resulting from exposure to such substances could present a financial and insect welfare issue to organizations rearing BSFL. Secondly, the presence of elevated pesticide concentrations in the larval biomass presents a potential food safety risk (Meyer *et al.*, 2021). Such transfer could result in non-compliance of the insect product with the applicable MRLs as laid down in Regulation (EC) No 396/2005. For instance, while the MRLs for deltamethrin and cypermethrin in wheat are 2.0 and 1.0 mg/kg, respectively; they are much lower at 0.05* and 0.02* mg/kg for insects. The asterisk (*) signifies that the substance/matrix-specific MRL is equal to its limit of quantification (LOQ). These two pyrethroid insecticides are used on a large variety of (stored) crops, including grains, legumes such as soy, potatoes, and fruits and vegetables; and residues are stable for at least one year in storage of plant-based commodities (EFSA, 2015b, 2023).

Meijer *et al.* (2023) showed highly significant adverse effects of dietary exposure of the insecticides cypermethrin and deltamethrin on reared black soldier fly larvae (BSFL, *Hermetia illucens*, L. 1758; Diptera: Stratiomyidae), manifesting in significant reductions in yield, at concentrations at or below the applicable maximum residue limit (MRL). Calculations to determine a concentration/response curve for cypermethrin showed a critical effect dose (CED) of 10% less yield to be 0.40 mg/kg. Correcting for the moisture content of BSFL diets (~65% water), exposure to a concentration equal to the MRL of cypermethrin in wheat (2.0 mg/kg) was estimated to result in 20.5% yield reduction. Toxicity of deltamethrin was substantially higher: dietary exposure to 1.51 and 0.53 mg/kg resulted in 98.2 and 94.1% yield loss, compared to the control, respectively. Addition of the synergist piperonyl butoxide (PBO) to these pesticides reduced yields even further. As such, it was concluded that feed materials containing cypermethrin, deltamethrin, and/or PBO at concentrations that are legally permitted may significantly affect commercial yields. Further, comparatively high concentrations of deltamethrin were detected in the larval biomass (1.14 mg/kg) against the spiked dietary concentration (1.51 mg/kg, i.e. 75.5% transfer). Furthermore, elevated transfer from substrate to larvae was observed for cypermethrin (30.8%), deltamethrin (26.3%), and permethrin (26.4%) when a cocktail of these substances was used in conjunction with PBO than without it (12.8, 10.4, 8.3%, respectively). This suggested that elevated mortality and reduced yield were correlated with higher transfer of pesticides to the larval biomass, and that this was mediated by the presence of PBO (Meijer *et al.*, 2023). The latter would be in line with the intended effect of PBO to block the detoxifying capacity of cytochrome P450 enzymes (Snoeck *et al.*, 2017).

The primary objectives of this study were to determine which factors affect toxicity, transfer and metabolization of the pyrethroid insecticides cypermethrin and deltamethrin by BSFL. The respective compounds may be broken down into certain metabolic products, which may be more or less toxic for the insect, or for the subsequent consumer, than the parent compound. We hypothesised that deltamethrin was highly toxic for BSLF and that safe levels (less than 10% reduction in yield) were below the analytical LOQ. Further, we hypothesised that metabolic breakdown products of cypermethrin and deltamethrin, with or without PBO, could provide an indication of the relevant resistance mechanisms involved. This study partly expands on the prior work in Meijer *et al.* (2023) but focuses more on the individual toxicity of deltamethrin and its potential metabolites.

2 Methods and materials

For a period of seven days, BSFL were exposed to different concentrations of cypermethrin and deltamethrin, with and without PBO. A concentration/response curve was calculated for deltamethrin. Data for cypermethrin was compared to the curve calculated in Meijer *et al.* (2023). The substrate (pre-trial) and selected larvae and frass samples (post-trial) were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS) to determine concentrations of the primary compounds. In addition, selected samples were analysed using liquid chromatography-high resolution mass spectrometry (LC-HRMS) to determine potential metabolites. Data from both analytical methods was used to perform mass balance calculations.

Selection of treatments

The MRLs for cypermethrin (2.0 mg/kg) and deltamethrin (1.0 mg/kg) in wheat, as laid down in Regulation (EC) No 396/2005, were used as the basis of the intended concentrations in this study. Cypermethrin was tested at this concentration and 50%, and at these same concentrations in combination with PBO at a ratio of 1:10. Deltamethrin was tested in five treatments so as to facilitate the creation of a concentration/response curve: the highest tested concentration was equal to the MRL, and lower concentrations were conducted in

TABLE 1 Overview of experimental treatments with intended concentration (mg/kg) in wet and dry feed

Number	Treatment	Concentration (mg/kg)	
		Dry feed	Wet feed
1	Control	n/a	n/a
2	Solvent control	n/a	n/a
3	Piperonyl butoxide	20.0	5.77
4	Cypermethrin	1.0	0.29
5	Cypermethrin	2.0	0.58
6	Cypermethrin +	1.0	0.29
	PBO	10.0	2.88
7	Cypermethrin +	2.0	0.58
	PBO	20.0	5.77
8	Deltamethrin	0.06	0.02
9	Deltamethrin	0.13	0.04
10	Deltamethrin	0.25	0.07
11	Deltamethrin	0.50	0.14
12	Deltamethrin	1.00	0.29
13	Deltamethrin +	0.13	0.04
	PBO	1.25	0.36
14	Deltamethrin +	0.25	0.07
	PBO	2.50	0.72
15	Deltamethrin +	0.50	0.14
	PBO	5.00	1.44

n/a = not applicable.

a sequence of steps of 50% lower for every treatment. The three 'middle' treatments (i.e. excluding lowest and highest concentrations) containing deltamethrin were tested in combination with PBO at the same ratio of 1:10 as cypermethrin. Treatments were performed with 6 replicates each (Table 1).

Spiking of feed

Substrates were spiked with analytical reference standards of cypermethrin, deltamethrin, and piperonyl butoxide (PBO) (Sigma Aldrich, Zwijndrecht, the Netherlands). Firstly, five batches of feed were prepared by creating a slurry of dry feed and methanol (MeOH): using the same feed material (Meelfabriek de Jongh, Steenwijk, the Netherlands) and method as described in Meijer *et al.* (2023). For the blank control, 2.5 kg of dry feed was prepared in this manner; for the solvent control, this was 100 g; for the treatments containing active substances, this was 1.0 kg. Batches containing active substances were spiked to 4.0 mg/kg for cypermethrin, 2.0 mg/kg for deltamethrin, and 40.0 mg/kg for PBO. After the MeOH in the slurries had evaporated in a fume hood (~3 days), n = 3 aliquots were taken from each batch for LC-MS/MS analysis to verify the spiked concentrations. Secondly, based on the analysed concentrations of active substances, spiked feed from each of the batches was mixed with blank (unspiked) feed at the appropriate ratios for the intended concentration(s) for each treatment to a total of 17.5 g of dry feed, inside each of the replicate containers. Finally, 43.2 ml tap water was added to each replicate container, totalling 60.7 g wet feed, and these were stored at 4 °C for 1 day prior to the start of the experiment, to reduce fungal growth in the wet substrates.

Animal procedures

Animal procedures were identical to as described in Meijer *et al.* (2023). In short, n = 50 seven-day old larvae (provided by project partner Bestico B.V., Berkel en Rodenrijs, the Netherlands) were counted and added to each of the replicate containers. The containers were kept in stacked trays in a climate chamber at 28 °C and 60% RH for a period of seven days. After this period, all larvae were removed from the respective containers using metal tweezers and subsequently washed, dried, and weighed. All larvae and approximately 5.0 g of frass was placed in plastic tubes and finally stored frozen (–18 °C), which killed the larvae.

LC-MS/MS analyses for primary compounds

Samples of substrate, frass, and larvae were analysed with a liquid chromatography-mass spectrometry (LC-MS/MS) based analytical procedure, after a QuEChERS extraction. For the extraction of larvae, between 1.0 and 2.5 grams of the frozen sample material was weighed into a tube. This was diluted by adding MilliQ water and a solution of 1% acetic acid in acetonitrile, accordingly to the amount of sample so the ratio stayed the same. Then the sample was homogenised using a ultra-turrax machine. A mixture of sodium acetate and $MgSO_4$ (1:4) was subsequently added to the tube. After vortexing, the tubes were centrifuged. The extract was being diluted (1:1) with 0.1% acetic acid in MilliQ in a LC vial before being analysed. For extraction of the residual material, the same procedure was followed except that the homogenisation step using an ultra-turrax machine was replaced with 30 min of end-over-end mixing.

LC-HMRS screening analyses

The extracts of larvae and residue material as prepared for LC-MS/MS measurement were also used for the screening of metabolites. The measurements were performed using LC-Q-Orbitrap-MS. Positive and neg-

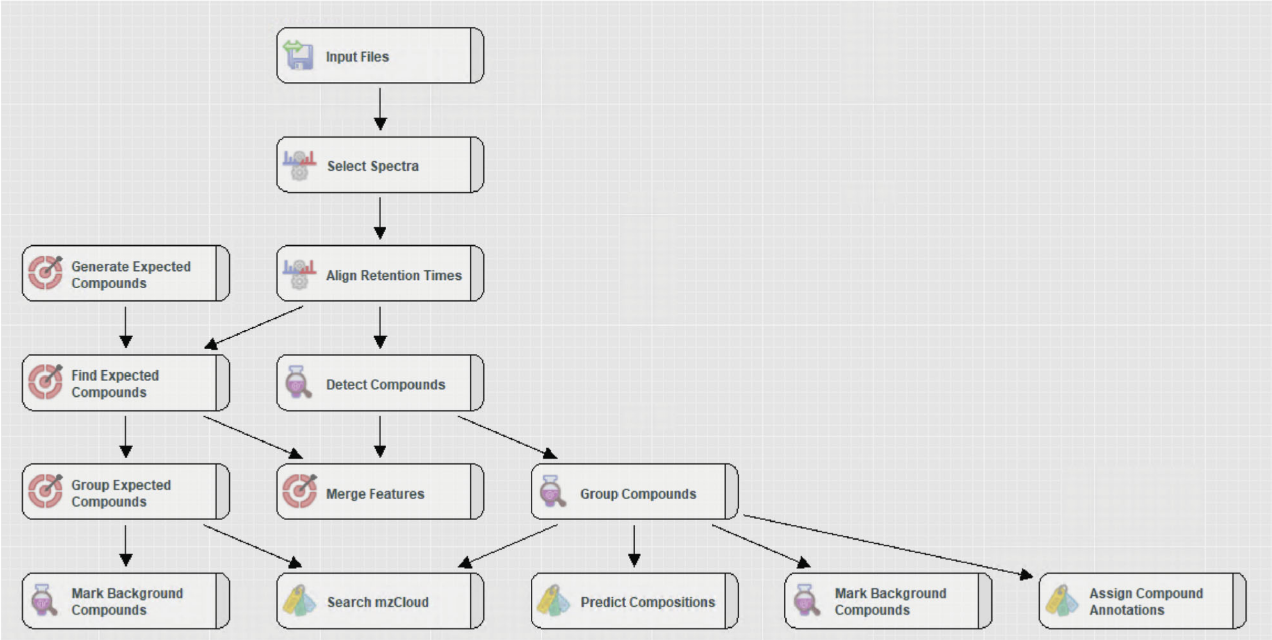

FIGURE 1 Workflow for processing HRMS data.

ative ionization modes were acquired separately (Supplementary Table S1). In positive mode eluents consisting of water (A) and MeOH (B) both containing 2 mM ammonium formate and 0.1% FA were used. In negative mode eluents were water (A) and 95% MeOH (B) both containing 10 mM ammonium carbonate. In both ionization modes the same gradient was used, which consisted of linear gradient from 0% B to 100% B in 15 minutes, followed by 6 minutes isocratic at 100% B, return to 0% B in 1 minute and 8 minutes of equilibration. The flow rate was 0.3 mL/min. Dedicated Waters UPLC BEH C18 columns (1.7 µm, 2.1 mm × 100 mm) were used for each ionization mode eluents. The column temperature was 50 °C, autosampler temperature 10 °C and the injection volume was 5 µL. Data acquisition was performed in full scan combined with data independent acquisition (vDIA) mode for two samples of each treatment, and full scan combined with top-5 data dependent acquisition (DDMS2) for one sample of each treatment. DDMS2 results in cleaner MS2 spectra as compared to the vDIA method, but MS2 spectra are only triggered for the top 5 highest signals measured in full scan in each scan cycle, whereas vDIA provides spectra for all signals, but of lower quality.

Data processing was performed using Compound Discoverer 3.3 (Thermo Scientific, Waltham, MA, USA). The workflow used for data processing is shown in Figure 1. The Compound Discoverer workflow consisted of an expected compounds search and an unknown search, as described in Meijer *et al.* (2022). Using this software, the samples were grouped by treatment. The reagent blanks were used to perform a background subtraction for data reduction. The signal intensity threshold for the detection of features was set to 1×10^5. Data in positive and negative ionization mode were processed separately, as well as insects and frass samples. In this study mzCloud was used to obtain tentative annotations, features not matched to mzCloud were evaluated based on their predicted elemental composition.

To select relevant features, differential analysis was performed comparing the control samples to the treatment samples. All up-regulated features (*P*-value: 0.05 and Log2 fold change: (1) were selected in both the unknown search and the expected compounds search. These selected features are not necessarily metabolites of the pesticides, as endogenous metabolites can also be up-regulated following pesticide exposure. Therefore, the selected upregulated features were manually evaluated based on their molecular formula and HRMS-spectra to identify pesticide metabolites.

The molecular identification confidence scoring was performed by using the method by Schymanski *et al.* (2014) in the same manner as described in Meijer *et al.* (2022).

Statistics and data analysis

A concentration/response curve was created using all data for treatments containing only deltamethrin. This was done using the PROAST software package version 70.5 (RIVM National Institute for Public Health and the Environment, Bilthoven, the Netherlands) for the R programming language version 4.2.0 (EFSA, 2017; Slob,

TABLE 2 Intended and analysed concentration (mg/kg) in dry feed used to prepare experimental substrates. Arithmetic mean and standard deviation for n = 3 samples per treatment

Substance	Concentration (mg/kg)		Recovery (%)
	Intended	Analysed	
Cypermethrin	4.0	3.97 ± 0.05	73%
Deltamethrin	2.0	2.01 ± 0.02	72%
Piperonyl butoxide	40.0	39.35 ± 1.99	93%

2018; RIVM, 2022). The data was plotted for the E-5 model, as described in Meijer *et al.* (2023). The treatments with either of the active substances cypermethrin or deltamethrin and PBO were compared against the treatments with equivalent concentrations of the active substance but without PBO, using a Mann-Whitney U test ($\alpha = 0.05$) in SPSS Statistics for Microsoft Windows 6 (version 25.0.0.2, IBM Corp., Armonk, NY, USA). Mass balance calculations were performed by expressing the molecular weight (g/mol) of the parent compound and/or tested metabolites in larvae and frass samples as a percentage of the molecular weight of the parent compound as present in the substrate. Molecular weights used to calculate were as follows: cypermethrin (416.3), trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylic acid (trans-DCCA, 209.07), and 3-PBA (214.22 g/mol). Since there were no metabolites detected in any samples associated with deltamethrin treatments, the mass balance was based on the mass pre- and post-experiment as expressed in mg; as was done for PBO. If the concentration of a metabolite exceeded the limit of detection (LOD), but not the limit of quantification (LOQ), a value equal to half of the LOQ was used for mass balance calculations.

3 Results

Quality control

Concentrations of spiked substances in the feed materials used for the rearing substrates were checked. Results of these analyses are shown in Table 2. Analysed concentrations were in accordance with intended concentrations and recovery percentages were within the acceptable range (70-130%). After this the results were corrected for the recovery.

An overview of the larval performance in response to each of the tested treatments is provided in Supplementary Table S2.

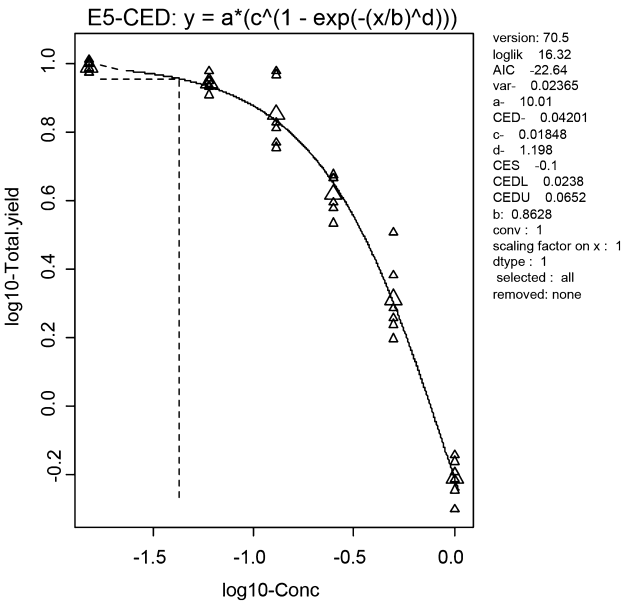

FIGURE 2 Concentration/response curve for the effect of the concentration (mg/kg) of deltamethrin on the total black soldier fly larvae (*Hermetia illucens*) biomass yield (g) on log10-scale axes.

Larval performance

Using the concentration/response curve based on the data for cypermethrin from Meijer *et al.* (2023), the effect sizes for exposure to 1.0 and 2.0 mg/kg in dry feed were estimated. The estimated effect sizes (90% confidence interval) for these concentrations would be reductions in yield of 4.3-9.4% for 1.0 mg/kg, and 12.1-20.4% for 2.0 mg/kg. For both tested concentrations, the actual measured effects compared to the estimated size effects were exceeded, for most replicates as well as the mean value for the treatment.

Figure 2 shows the concentration/response curve for deltamethrin. The estimated CED_{10} was 0.04 mg/kg in dry feed, with a 95% CI of 0.02-0.07 mg/kg. Corrected for added water (17.5 g dry feed + 43.2 g water), this CED_{10} is equivalent to 0.01 mg/kg in wet feed.

Figure 3 shows the synergizing effect of PBO on yield when combined with the active substances cypermethrin and deltamethrin at different concentrations, at a ratio of active substance to PBO of 1:10. At the highest

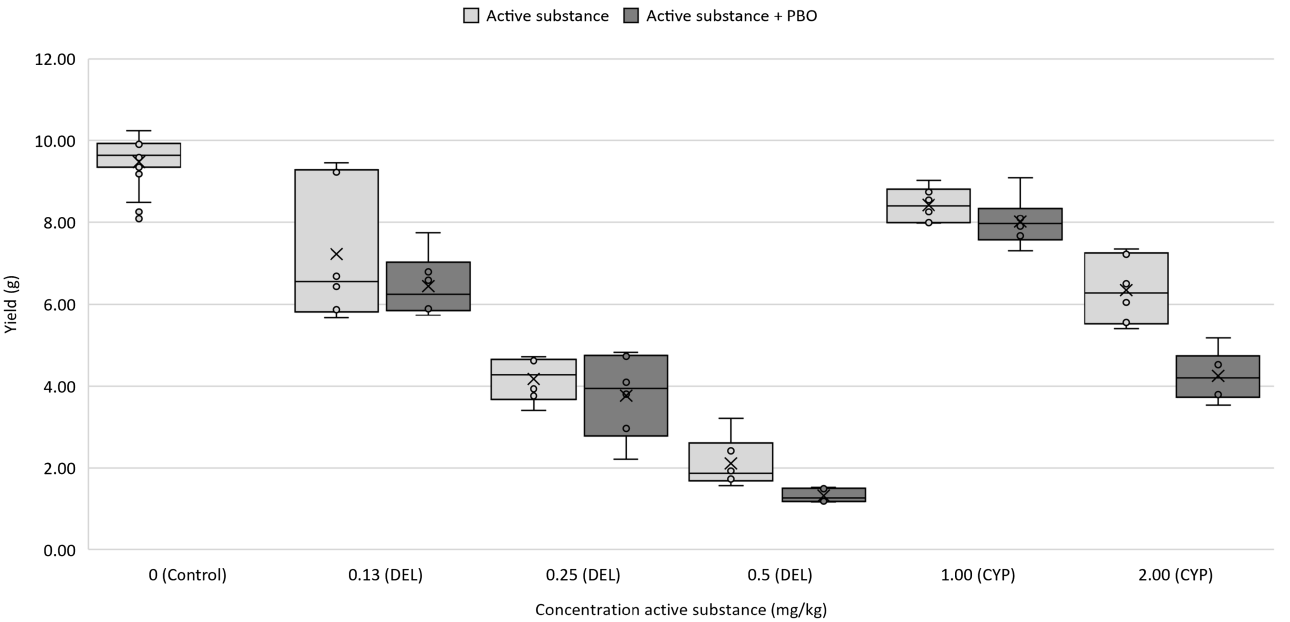

FIGURE 3 Effects of active substances cypermethrin (CYP) and deltamethrin (DEL) on yield of black soldier fly larvae (*Hermetia illucens*), with or without the synergist piperonyl butoxide (PBO), tested at different concentrations depending on the active substance. Treatments were tested with n = 6 replicates each, and n = 18 for the pooled control. Asterisks (*) denote significance of differences between treatments with and without PBO ($P < 0.05$).

tested concentrations of each active substance cypermethrin (2.0 mg/kg) and deltamethrin (0.50 mg/kg), the addition of PBO had a significant additive effect compared to the treatment without PBO ($P < 0.05$).

Concentrations and metabolites

Table 3 shows the analysed concentrations in mg/kg in samples of larvae and frass, against the intended concentrations in the wet substrate. The MRLs for insect products as laid down in Regulation (EC) No 396/2005 are 0.02* for deltamethrin and 0.05* for cypermethrin. These limits were exceeded for cypermethrin when tested at the highest concentration in the substrate (2.0 mg/kg in dry feed), both with and without PBO. For deltamethrin, this MRL was exceeded at the highest substrate exposure level (1.0 mg/kg). This means that it is possible for reared BSFL exposed to cypermethrin or deltamethrin to be non-compliant with MRLs, even if reared on feed materials that are compliant – due to major differences in tolerable levels between the two matrices.

For samples of frass and larvae from the analysed deltamethrin treatments, no metabolites were identified. For samples of cypermethrin treatments, the metabolite trans-DCCA was detected in the larvae (<5 µg/kg); and trans-DCCA and 3-PBA were found in the frass samples, as shown in Table 4. For both detected metabolites, a reference standard was measured. Using these reference standards, the identity of both metabolites was confirmed (Schymanski level 1), and semi-quantification was performed. Note that correction for recovery or matrix effect is not possible using solvent standards, therefore the concentrations should be considered indicative. The presence of PBO in addition to cypermethrin appeared to result in reduced concentrations of both metabolites.

Figure 4 shows the mass balance for the treatments containing cypermethrin at 1.0 mg/kg in the dry feed, with and without PBO at a ratio of 1:10; and for deltamethrin at 0.25 and 0.50 mg/kg, also with and without PBO at that ratio, respectively. Most of the spiked parent compounds in the substrate were recovered in the frass; the contribution of the larval matrix to this mass balance was comparatively minor. The same was true for the tested metabolites trans-DCCA and 3-PBA. Overall recovery was approximately 50% and did not appear to differ much between each of these treatments.

4 Discussion

The severe adverse effects of deltamethrin on this particular population of *Hermetia illucens* larvae, as observed in Meijer *et al.* (2023), were maintained in this study. When BSFL were exposed to deltamethrin at a

TABLE 3 Overview of analysed concentrations (mg/kg) in larvae and frass samples, against intended concentrations in (wet) substrate. Arithmetic mean and standard deviation for n = 3 samples per treatment. Carry-over defined as the concentration in the feed as a percentage of the mean concentration in the larvae

Treatment	Concentration (mg/kg)			Carry-over substrate to larvae (%)
	Substrate (wet)	Larvae	Frass	
Control	n/a	n/a	n/a	n/a
Solvent control	n/a	–	–	n/a
Piperonyl butoxide (PBO)	5.77	0.02 ± 0.00	2.69 ± 0.20	0.3 ± 0.1
Cypermethrin	0.29	0.03 ± 0.01	–	11.0 ± 2.6
Cypermethrin	0.58	0.10 ± 0.02	0.57 ± 0.03	17.1 ± 3.4
Cypermethrin +	0.29	0.04 ± 0.01	–	14.9 ± 3.0
PBO	2.88	0.03 ± 0.01		1.1 ± 0.2
Cypermethrin +	0.58	0.06 ± 0.02	0.56 ± 0.01	9.7 ± 2.7
PBO	5.77	0.07 ± 0.02	2.71 ± 0.40	1.3 ± 0.3
Deltamethrin	0.02	<LOQ (0.01)	–	–
Deltamethrin	0.04	<LOQ (0.01)	<LOQ (0.01)[2]	–
Deltamethrin	0.07	0.02 ± 0.00	0.07 ± 0.01	21.0 ± 1.8
Deltamethrin	0.14	0.02 ± 0.00	0.12 ± 0.02	13.3 ± 3.5
Deltamethrin	0.29	0.05 ± 0.01	–	16.1 ± 3.5
Deltamethrin +	0.04	<LOQ (0.01)[1]	0.04 ± 0.00	–
PBO	0.36	<LOQ (0.01)[1]	0.23 ± 0.05	
Deltamethrin +	0.07	0.02 ± 0.00	0.05 ± 0.01	27.5 ± 2.8
PBO	0.72	0.02 + 0.00	0.61 ± 0.09	2.5 ± 0.6
Deltamethrin +	0.14	0.02 ± 0.00	0.13 ± 0.01	11.8 ± 2.0
PBO	1.44	0.02 ± 0.01	1.10 ± 0.11	1.3 ± 0.4

n/a = not applicable; – = concentration not measured, or transfer ratio not calculated due to larval concentration being < LOQ.
[1]Two out of three samples < LOQ (0.01 mg/kg), one sample 0.01 mg/kg.
[2]Two out of three samples < LOQ (0.01 mg/kg), one sample 0.04 mg/kg.

TABLE 4 Analysed concentrations (mg/kg) of detected metabolites trans-DCCA and 3-PBA in samples of frass. Arithmetic mean and standard deviation for n = 3 analysed samples

Treatment	Concentration (mg/kg)	
	Trans-DCCA	3-PBA
Cypermethrin (2.0 mg/kg)	0.040 ± 0.018	0.005 ± 0.0
Cypermethrin (2.0 mg/kg) + PBO (20.0 mg/kg)	0.020 ± 0.007	<0.005

concentration that was equivalent to the MRL in dry wheat (1.0 mg/kg), the resulting yield was 6.5 ± 0.9% of the mean control value, i.e. a reduction of ~93%. The calculated CED_{10} for deltamethrin was 0.04 mg/kg in dry feed, which is only slightly above the deltamethrin LOQ in this study of 0.01 mg/kg. Caution is therefore advised for commercial BSFL rearing organizations when using feed materials that contain quantifiable residues of deltamethrin. Results of this study further highlight the drastic differences in toxicity between different pyrethroids for commercially reared BSFL. This is exemplified by the CED_{10} for cypermethrin being much higher at ~1.14 mg/kg in dry feed, as calculated in Meijer *et al.* (2023).

One of the primary motivations to conduct this study was to verify and further assess the comparatively high residue transfer from substrate to larval biomass for deltamethrin, as observed in Meijer *et al.* (2023). This was as high as 75.5% for exposure to 1.51 mg/kg of this substance in the (wet) substrate. Elevated residue transfer could present a food, feed, and/or compliance issue if legal limits are exceeded in larvae-based products. The highest mean carry-over from substrate to larvae observed in this study for deltamethrin was 21% at

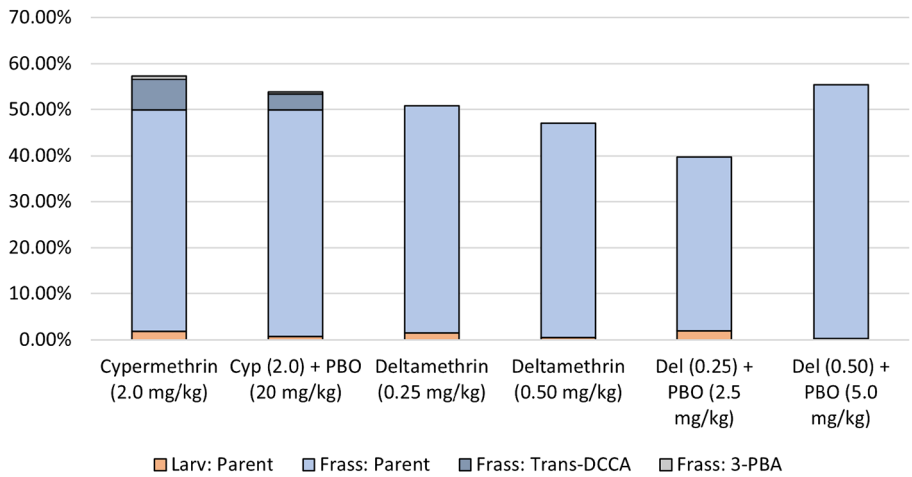

FIGURE 4 Mass balance for cypermethrin (Cyp) and deltamethrin (Del) treatments, with and without the synergist piperonyl butoxide in black soldier fly larvae (*Hermetia illucens*). Molecular mass of parent compound and/or detected metabolites 3-PBA and trans-DCCA in post-experiment samples larvae and frass, as a percentage of the molecular mass present in the substrate pre-experiment. Arithmetic mean for n = 3 analysed samples per treatment.

0.07 mg/kg (wet feed), and 27.5% when present at that concentration in conjunction with PBO. This suggest that exposure to comparatively lower concentrations, that cause lower reductions in biomass, are correlated to lower transfer from substrate to larvae – although results from this study are not conclusive in that regard. Two studies on reared *Tenebrio molitor* L. 1758 (Coleoptera: Tenebrionidae) larvae suggested that pesticide transfer was related to the substance-specific fat/water solubility (expressed as octanol/water partition coefficients (K_{ow})) (Houbraken *et al.*, 2016; Dreassi *et al.*, 2020). We speculate that this factor also plays a role for BSFL: more research is needed on this topic, as well as on the potential effects of insecticide exposure on BSFL nutrient composition (fat/protein/chitin).

It was hypothesised that the determination of metabolic breakdown products of tested insecticides in larvae and frass samples could provide an indication of the toxicity pathways involved. Mass balance calculations indicated that approximately 50% of both cypermethrin and deltamethrin could be recovered; largely in the frass. This suggests that metabolic resistance plays a major role. However, metabolites were not or only barely quantifiable by the used HRMS method – especially in the case of the parent compound deltamethrin. An alternative analytical approach, consisting of isolating potential enzymes involved in pyrethroid resistance, could provide a better view of these pathways but such research on *H. illucens* is lacking. In general, metabolic pyrethroid resistance in insects is most likely modulated both by microsomal hydrolytic esterases as well as microsomal and mitochondrial cytochrome P450 monooxygenases (Khambay and Jewess, 2004). One important P450 enzyme for pyrethroid resistance that has been isolated in house fly (HF, *Musca domestica*, L.; Diptera: Muscidae) is CYP6D1 (Tomita *et al.*, 1995; Liu and Scott, 1998), but other P450 enzymes may (also) play a role, depending on species (Khambay and Jewess, 2004). In *M. domestica*, CYP6D1 was only expressed in adults (Scott *et al.*, 1996), and possibly late pupae (Tomita and Scott, 1995). Scott (1996) investigated 21 potential inhibitors of CYP6D1 in house fly microsomes. In previous studies, that team had purified this enzyme (Wheelock and Scott, 1989) and developed specific assays (methoxyresorufin O-demethylation (MROD) and ethoxycoumarin Odeethylation (ECOD)) to evaluate potency and specificity of CYP6D1 inhibition of various substances (Wheelock and Scott, 1990; Scott, 1996; Wheelock and Scott, 1992). They found that CYP6D1 was most strongly inhibited by xanthotoxin, chlorpyrifos, β-naphthoflavone, PBO, and 5-methoxypsoralen. Greatest selectivity was observed for 5-methoxypsoralen, xanthotoxin, β-naphthoflavone, chlorpyrifos oxon, isosafrole, and psoralen (Scott, 1996). As observed for PBO in this study, these other substances may also synergise pyrethroids against BSLF.

5 Conclusion and recommendations

Deltamethrin was found to be highly toxic for this particular commercial population of *H. illucens*. The CED_{10} for this substance was 0.04 mg/kg, which is only slightly higher than the analytical LOQ. It is assumed that other populations than the one assayed are similarly vulnerable, which warrants the recommendation for BSFL-

rearing organizations to have their rearing substrate analysed especially for the presence of deltamethrin, as well as cypermethrin and PBO – and to reject any batch of feed that contains concentrations of these substances that exceed commercially safe limits. Transfer patterns for other substances may be different, and the presence of synergists and/or other pesticides have been shown to increase the transfer percentages compared to substances tested in isolation. The relatively low existing MRLs for insect products as laid down in Regulation (EC) No 396/2005 (0.02* mg/kg for deltamethrin) thus require continued vigilance to avoid non-compliance. Results suggest that pyrethroid resistance and substance transfer in BSFL are partly modulated by enzymatic processes, but more research is needed to elucidate the mechanisms involved.

Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.25808782

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 861976 (project SUSINCHAIN). Additional financing from the Netherlands Ministry of Agriculture, Nature and Food Quality (through project BO-57-102-005) is acknowledged. Co-author L.Z. is employed by Bestico B.V., which is a commercial entity producing black soldier fly larvae for animal feed purposes.

References

Dreassi, E., Mancini, A., Corbini, G., Botta, M., Tarchi, F. and Francardi, V., 2020. Bioaccumulation of deltamethrin, tebuconazole and chlormequat chloride in *T. molitor* larvae and risks associated with their human consumption. Journal of Insects as Food and Feed 6: 457-465. https://doi.org/10.3920/JIFF2020.0007

EFSA (European Food Safety Authority), 2015a. Risk profile related to production and consumption of insects as food and feed. EFSA Journal 13: 4257. https://doi.org/10.2903/j.efsa.2015.4257

EFSA (European Food Safety Authority), 2015b. Review of the existing maximum residue levels for deltamethrin according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 13: 4309. https://doi.org/10.2903/j.efsa.2015.4309

EFSA (European Food Safety Authority), 2017. Update: use of the benchmark dose approach in risk assessment. EFSA Journal 15: e04658. https://doi.org/10.2903/j.efsa.2017.4658

EFSA (European Food Safety Authority), 2023. Review of the existing maximum residue levels for cypermethrins according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 21: e07800. https://doi.org/10.2903/j.efsa.2023.7800

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. https://doi.org/10.1016/j.foodchem.2016.01.097

Khambay, B.P.S. and Jewess, P.J., 2004. Pyrethroids. In: Gilbert, L.I., Iatrou, K. and Gill, S.S. (eds.) Comprehensive molecular insect science. Pergamon Press, Oxford, UK.

Lievens, S., Poma, G., De Smet, J., Van Campenhout, L., Covaci, A. and Van Der Borght, M., 2021. Chemical safety of black soldier fly larvae (*Hermetia illucens*), knowledge gaps and recommendations for future research: a critical review. Journal of Insects as Food and Feed 7: 383-396. https://doi.org/10.3920/JIFF2020.0081

Liu, N. and Scott, J.G., 1998. Increased transcription of CYP6D1 causes cytochrome P450-mediated insecticide resistance in house fly. Insect Biochemistry and Molecular Biology 28: 531-535. https://doi.org/10.1016/S0965-1748(98)00039-3

Meijer, N., Nijssen, R., Bosch, M., Boers, E. and Van der Fels-Klerx, H.J., 2022. Aflatoxin B1 metabolism of reared *Alphitobius diaperinus* in different life-stages. Insects 13: 357. https://doi.org/10.3390/insects13040357

Meijer, N., Zoet, L., de Rijk, T., Zomer, P., Rijkers, D., van der Fels-Klerx, H.J. and van Loon, J.J., 2023. Effects of pyrethroid and organophosphate insecticides on reared black soldier fly larvae (*Hermetia illucens*). Insect Science. https://doi.org/10.1111/1744-7917.13269

Meyer, A.M., Meijer, N., Hoek-Van den Hil, E.F. and Van der Fels-Klerx, H.J., 2021. Chemical food safety hazards of insects reared for food and feed. Journal of Insects as Food and Feed 7: 823-831. https://doi.org/10.3920/JIFF2020.0085

RIVM, 2022. Proast manual: menu version, version 70.3. Available at: https://www.rivm.nl/documenten/proast-manual-menu-version

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P. and Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: Communicating con-

fidence. Environmental Science & Technology 48: 2097-2098. https://doi.org/10.1021/es5002105

Scott, J.G., 1996. Inhibitors of CYP6D1 in house fly microsomes. Insect Biochemistry and Molecular Biology 26: 645-649. https://doi.org/10.1016/S0965-1748(96)00008-2

Scott, J.G., Sridhar, P. and Liu, N., 1996. Adult specific expression and induction of cytochrome P450tpr in house flies. Archives of Insect Biochemistry and Physiology 31: 313-323. https://doi.org/10.1002/(SICI)1520-6327(1996)31:3<313::AID-ARCH6>3.0.CO;2-Y

Slob, W., 2018. Joint project on benchmark dose modelling with RIVM. EFSA Supporting Publications 15: 1497E. https://doi.org/10.2903/sp.efsa.2018.EN-1497

Snoeck, S., Greenhalgh, R., Tirry, L., Clark, R.M., Van Leeuwen, T. and Dermauw, W., 2017. The effect of insecticide synergist treatment on genome-wide gene expression in a polyphagous pest. Scientific Reports 7: 1-12. https://doi.org/10.1038/s41598-017-13397-x

Sogari, G., Amato, M., Biasato, I., Chiesa, S. and Gasco, L., 2019. The potential role of insects as feed: a multi-perspective review. Animals 9: 119. https://doi.org/10.3390/ani9040119

Tomita, T., Liu, N., Smith, F.F., Sridhar, P. and Scott, J.G., 1995. Molecular mechanisms involved in increased expression of a cytochrome P450 responsible for pyrethroid resistance in the housefly *Musca domestica*. Insect Biochemistry and Molecular Biology 4: 135-140. https://doi.org/10.1111/j.1365-2583.1995.tb00018.x

Tomita, T. and Scott, J.G., 1995. cDNA and deduced protein sequence of CYP6D1: the putative gene for a cytochrome P450 responsible for pyrethroid resistance in house fly. Insect Biochemistry and Molecular Biology 25: 275-283. https://doi.org/10.1016/0965-1748(94)00066-Q

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. https://doi.org/10.3920/JIFF2020.0024

Van Huis, A., 2020. Insects as food and feed, a new emerging agricultural sector: a review. Journal of Insects as Food and Feed 6: 27-44. https://doi.org/10.3920/JIFF2019.0017

Wheelock, G.D. and Scott, J.G., 1989. Simultaneous purification of a cytochrome P-450 and cytochrome b5 from the house fly *Musca domestica* L. Insect Biochemistry 19: 481-489 https://doi.org/10.1016/0020-1790(89)90030-9

Wheelock, G.D. and Scott, J.G., 1990. Immunological detection of cytochrome P450 from insecticide resistant and susceptible house flies (*Musca domestica*). Pesticide Biochemistry and Physiology 38: 130-139. https://doi.org/10.1016/0048-3575(90)90046-5

Wheelock, G.D. and Scott, J.G., 1992. Anti-P450$_{lpr}$ antiserum inhibits specific monooxygenase activities in LPR house fly microsomes. Journal of Experimental Zoology 264: 153-158. https://doi.org/10.1002/jez.1402640206