Research Article

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Acute and chronic toxicity of acetamiprid, carbaryl, cypermethrin and deltamethrin to *Apis mellifera* larvae reared *in vitro*

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Abstract

BACKGROUND: The effects of exposing *Apis mellifera* larvae to common insecticides were tested in the laboratory.

RESULTS: The acute toxicity values of the four insecticides that we tested ranged from high toxicity to low toxicity: deltamethrin > cypermethrin > carbaryl > acetamiprid. The NOAEC (no observed adverse effect concentration) values of the chronic toxicity tests for each compound are 5 mg L^{-1} for acetamiprid, 2 mg L^{-1} for carbaryl, 1 mg L^{-1} for cypermethrin, and 0.2 mg L^{-1} for deltamethrin.

CONCLUSION: According to the risk quotient (RQ) values of acute and chronic toxicity that we obtained, the risk is acceptable at exposure rates that have been identified in the field. Overall, our results are valuable for evaluating the acute and chronic toxicities of these insecticides to developing honey bees.

Supporting information may be found in the online version of this article.

Keywords: *Apis mellifera*; larvae; acute toxicity; chronic toxicity; risk quotients

1 INTRODUCTION

Apis mellifera, western honey bee, colony decline has already occurred in many countries during recent decades.[1–4] However, no single causative factor has been identified as being responsible for this phenomenon.[5] Several factors, in combination with other stressors, have been identified to explain this decline, including *Varroa destructor* infestations, deformed wing virus (DWV) infections and the overuse of insecticides.[6–10] Insecticides may not directly cause colony decline, but they can potentially affect honey bees and/or compromise the bees and increase vulnerability to a range of other stressors.[11] Previous studies revealed that exposure to insecticides affected the homing capacity of honey bees,[1] colony stability,[12,13] olfactory learning and memory,[14] neuronal inactivation in the mushroom body,[15] and bumble bee colony growth and queen production.[16]

With the generalized use of insecticides worldwide, the impact of insecticides on bee larvae has attracted increasing attention.[17,18] The healthy development of bee larvae is an important basis for the survival of the entire bee colony. Pollen and nectar contaminated with insecticides are collected during the foraging process of adult honey bees and then stored in the hive.[11,19,20] Bee larvae are fed by nurse bee secretions and pollen collected by adult bees,[21] so they may also be affected by insecticides, and they may be more sensitive than adults because they require complex food to complete development.[21–23] There is clear evidence that bee larvae reared in treatment combs containing insecticide residues experience delayed development.[24] Reduced survival and weight loss are the main indicators of the effects of insecticides on bee larvae in the literature.[25–28] Several studies have even confirmed that insecticide toxicity occurs at lower exposure rates to larvae compared to adult bees.[29]

Neonicotinoids are insecticides that interact with the nicotinic acetylcholine receptors (nAChRs) of the insect central nervous system, resulting in abnormal behavior, inactivity and death of insects.[30–32] Field-realistic experiments showed that neonicotinoids increased worker mortality and reduced honey bee health.[33] Acetamiprid belongs to the family of neonicotinoid insecticides and is widely used in various agricultural crops against different types of insect pests.[34] Acetamiprid was detected in 3.1% of 350

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pollen samples collected from North American honey bee colonies located in various cropping systems.[35] Insecticide residues from 343 live bee samples from 16 provinces in Poland found that chlorpyrifos together with thiacloprid (4.7%) and acetamiprid (4.1%) were the top three insecticides detected in live honeybees.[7]

Carbaryl (1-naphthyl N-methylcarbamate) is a commonly used broad-spectrum insecticide that disseminates *via* drift from spray during aerial application or through runoff from adjacent agricultural fields or gardens.[36,37] Carbaryl is a member of a family of carbamates and significantly inhibits the activity of neonicotinoid acetylcholine receptors and acetylcholinesterase.[38,39] Residues of carbaryl were detected in wax, pollen and bee samples from North American honey bee colonies.[35] In a study of fresh pollen collected from 14 monitoring apiaries in Taiwan, carbamate accounted for 11% of the insecticides detected.[40]

Pyrethroids are also commonly used in crop protection against various insects considered as pests.[41] According to related reports, single-crop pollination requires 1.6 million bee colonies to provide services, while pyrethroids are used in nearly 1 million acres of orchard (including apples, apricots, sweet and tart cherries, peaches, plums, prunes, pears, and nectarines) in the USA.[42] Cypermethrin and deltamethrin are the two most common pyrethroid insecticides.[43] A study found that deltamethrin did not alter certain genes related to immune function, while cypermethrin had negative effects on the transcription of certain immune system genes in bees.[44,45]

Numerous examples of lethal and sublethal effects of insecticides on adult honey bees can be found in the literature.[46–49] Honey bee larvae, as an important part of maintaining the stability of bee colonies, should also receive sufficient attention. In this study, we determined the 72 h acute lethal concentration (LC_{50}) of four insecticides (acetamiprid, carbaryl, cypermethrin, deltamethrin) for honey bee larvae in a single 24 h exposure event and the sublethal effects of chronic exposure on honey bee larvae during a 4-day larval exposure event. Based on the acute toxicity and the chronic toxicity of the four insecticides, we conducted a preliminary risk assessment for *A. mellifera* larvae.

2 MATERIALS AND METHODS

2.1 Breeding and collection of bee larvae

A. mellifera larvae were reared *in vitro* using the methodology described by Dai *et al.*[26] Honey bee larvae and queens were obtained from experimental apiaries kept by the Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, in Beijing (N39°59′35.33″, E116°11′59.74″). Sufficient and healthy colonies were prepared for experimental determination. To provide a healthy bee colony, the queen was caged in the controller for 30 days and the colony did not contact insecticides before the experiment. The test queens in each bee colony originated from the same sister queens. At least five honey bee queens were caged on empty combs from their respective beehives for 24 h during the experiment. The successfully hatched combs were transported to the laboratory for grafting. Sterile 48-well cell culture plates (STCPs) were filled with 20 μL of diet A in each cell well for larval transfer. The STCPs were placed horizontally in a larval growth chamber that was maintained at 94% RH and 35 °C. The experiment was conducted from August 1 to September 16, 2018.

2.2 Feeding

Three *in vitro* larval diet compositions (A, B, C) were used in the study and administered on different days (D). Diet A (D1 – D2): royal jelly 44.25%, glucose 5.3%, fructose 5.3%, yeast extract 0.9% and water 44.25%; diet B (D3): royal jelly 42.95%, glucose 6.4%, fructose 6.4%, yeast extract 1.3% and water 42.95%; diet C (D4 – D6): royal jelly 50%, glucose 9%, fructose 9%, yeast extract 2% and water 30%.[50] Larvae were fed 20 μL of diet A within hours of coming into the lab and then consume that diet within 48 h. On day 3, each larva was fed 20 μL of diet B. On D4, 5 and 6, each larva was fed 30, 40, and 50 μL of diet C, respectively.

2.3 Acute toxicity

Five insecticides, namely, acetamiprid: purity 99.7%; carbaryl: purity 98.9%; cypermethrin: purity 98.2%; deltamethrin: purity 99.5% and dimethoate: purity 99.3%, were purchased from Dr. Ehrenstorfer GmbH Corporation, Germany. Each of the tested chemicals was dissolved in acetone to prepare a stock solution, and the volume of the tested solution in the diet was 2% of the final volume in the experiment. A number of preliminary experiments have been conducted to obtain a reasonable concentration range. Based on this, the following concentrations were designed to determine LC_{50}: acetamiprid: 25, 50, 100, 200 and 400 μg mL^{-1}; carbaryl: 10, 20, 40, 80 and 160 μg mL^{-1}; cypermethrin: 0.75, 1.5, 3, 6 and 12 μg mL^{-1}; and deltamethrin: 0.25, 0.5, 1, 2 and 4 μg mL^{-1}. We obtained the following doses based on the concentrations after the 30 μL diets were fully consumed: acetamiprid: 0.75, 1.5, 3, 6 and 12 μg $larva^{-1}$; carbaryl: 0.3, 0.6, 1.2, 2.4 and 4.8 μg $larva^{-1}$; cypermethrin: 0.0225, 0.045, 0.09, 0.18 and 0.36 μg $larva^{-1}$; and deltamethrin: 0.0075, 0.015, 0.03, 0.06 and 0.12 μg $larva^{-1}$.

On D4 post-grafting (larvae = 84 ± 12 h), 48 larvae per treatment (16 larvae × 3 colonies = 48 larvae) were selected, and each larva was fed 30 μL of diet C containing the treatments for each test substance.[50] Following the chemical exposure on D4, mortalities were checked and recorded at the time of feeding on D5, D6 and D7 (project termination). The 72 h LC_{50} was calculated for larvae (the cumulative mortality at D7).

2.4 Chronic toxicity

Six concentrations of each test substance were selected based on field-relevant doses (Table 1). The following treatments were conducted for each test solution: six concentrations of the test solution, a negative control, a solvent control, and 45 mg L^{-1} dimethoate (positive control). Three replicates were conducted for each treatment. Larvae tested within each replicate were sourced from a different colony. On D3, a minimum of 12 robust larvae per replicate were randomly selected for each treatment group and fed 20 μL of diet B containing the test solution appropriate to the group's assigned treatment. On D4, 5, and 6, the larvae were fed 30, 40, and 50 μL, respectively, of diet C containing the appropriate test solution. Larvae were transferred individually from the larval STCPs to the pupal STCPs when all available diet had been consumed (as early as day 7). Pupal STCPs were maintained horizontally in a pupal growth chamber maintained at 75% RH and 35 °C. Adult worker bees began to emerge as soon as 18 days after grafting. Mortality was determined daily by viewing larval movement and spiracle activity under a microscope. Immobile larvae or larvae failing to respire (no spiracle movement) were considered dead. The survival rate of the larvae was statistically determined as follows[52]:

$$\text{Larval survival} = (\#\text{larvae that reached D10}/\#\text{larvae D3}) \times 100.$$

The comparisons of larval weight are only for individuals that survived to day 7. Larval weight was measured on the seventh day

Table 1. The concentrations of each test substance relative to the residues reported in pollen/beebread or nectar/honey

Insecticides	Con. (mg L^{-1})	Pollen/beebread[35]	Nectar/honey[51]
Acetamiprid	2.5	42 × mean residue	188× maximum residue
	5	37 × maximum residue	376× maximum residue
	10	75 × maximum residue	752× maximum residue
	20	149 × maximum residue	1504× maximum residue
	40	298 × maximum residue	3007× maximum residue
	80	597 × maximum residue	6015× maximum residue
Carbaryl	2	17 × mean residue	48× maximum residue
	4	4 × maximum residue	95× maximum residue
	8	8 × maximum residue	190× maximum residue
	16	16 × maximum residue	381× maximum residue
	32	32 × maximum residue	762× maximum residue
	64	63 × maximum residue	1524× maximum residue
Cypermethrin	0.25	23× mean residue	3× maximum residue
	0.5	10 × maximum residue	5× maximum residue
	1	20 × maximum residue	11× maximum residue
	2	41 × maximum residue	22× maximum residue
	4	82 × maximum residue	43× maximum residue
	8	163 × maximum residue	87× maximum residue
Deltamethrin	0.025	0.4× mean residue	4× maximum residue
	0.05	0.5 × maximum residue	7× maximum residue
	0.1	maximum residue	15× maximum residue
	0.2	2 × maximum residue	30× maximum residue
	0.4	4 × maximum residue	60× maximum residue
	0.8	9 × maximum residue	120× maximum residue

by the following methods[52]:

Larval weight at D7 = weight of larval cellcup with larva-weight of empty larval cell cup.

2.5 Risk quotients

Risk assessment includes the integration of exposure and impact information to assess the likelihood of exposure adversely affecting eco-receptors. Based on the method described by the US EPA, RQ values are compared to levels of concern (LOC). The LOCs for acute and chronic exposure are 0.4 and 1.0, respectively. If the result is less than the LOC, then the risk is considered acceptable. If an RQ exceeds its LOC, then the chemical use being assessed poses a potential risk to insect pollinators, and the insecticide may require higher-tiered testing or label mitigation.[53]

Larval NOAEL was calculated as follows:

Larval NOAEL = NOAEC (no observed adverse effect concentration) × cumulative consumption of diet.

We used the US Environmental Protection Agency's BeeREX model to calculate risk quotients (RQs) for all test compounds based on larval LD_{50} and no observed adverse effect concentration (NOAEC). (https://www.epa.gov/ sites/production/files/2015-11/beerexv1.0.xlsx).

2.6 Statistical analysis

Acute toxicity data were analyzed by using the Microsoft Excel Data Analysis package. Mortality was corrected using a solvent to control mortality *via* the Abbott formula.[18] The SAS 9.2 software program was used for chronic toxicity data analysis. Comparison of survival curves for different treatments was based on Kaplan–Meier survival analysis. Differences in larval survival and weight were analyzed by ANOVA and Tukey's HSD tests.[52]

3 RESULTS

3.1 Acute toxicity

Mortality in the solvent control treatment was lower than 15% at 72 h (D7) after feeding diet C on D4. The toxicities of the compounds tested are reported in Table 2. The LC_{50} values of acetamiprid, carbaryl, cypermethrin and deltamethrin for honey bee larvae were 188.49, 44.24, 4.36 and 1.79 mg L^{-1}, respectively. The toxicities of the tested insecticides to honey bee larvae from most to least toxic were deltamethrin > cypermethrin > carbaryl > acetamiprid.

3.2 Chronic toxicity

3.2.1 Acetamiprid

A one-way ANOVA indicated differences in larval survival among the treatment groups in the acetamiprid study ($F_8 = 29.24$, $P < 0.0001$). The survival of larvae fed 10, 20, 40 and 80 mg L^{-1} acetamiprid was significantly lower than that of larvae fed the negative and solvent control diets. However, the survival of larvae fed 2.5 or 5 mg L^{-1} acetamiprid was not significantly different from that of larvae fed the solvent control and the negative control diets (Table S1, Fig. 1(A)). The NOAEC of acetamiprid was 5 mg L^{-1}.

Chronic exposure to acetamiprid ($F_8 = 5.53$, $P < 0.0001$, Fig. 2(A)) significantly affected larval weight on D7. Larvae fed a diet containing 20 or 80 mg L^{-1} acetamiprid had significantly lower weight than those fed a negative control (Fig. 2(A)).

Table 2. Acute oral toxicities of acetamiprid, carbaryl, cypermethrin and deltamethrin to *A. mellifera* larvae

Insecticides	n[a]	Intercept	Slope	R^2	LC_{50}[b] (mg L^{-1})	LD_{50}[c] (μg $larva^{-1}$)
Acetamiprid	336	0.93	1.79	0.95	188.49 (146.82–241.99)	5.65 (4.40–7.26)
Carbaryl	336	2.67	1.42	0.97	44.24 (35.18–55.62)	1.33 (1.06–1.67)
Cypermethrin	336	3.14	2.91	0.99	4.36 (3.65–5.21)	0.13 (0.11–0.16)
Deltamethrin	336	4.44	2.21	0.93	1.79 (1.48–2.16)	0.05 (0.04–0.06)

[a] Number of tested honey bees per treatment.
[b] Lethal concentration, with 95% confidence intervals in parentheses, causing 50% honey bee larval mortality at 72 h.
[c] Lethal dose, with 95% confidence intervals in parentheses, causing 50% honey bee larval mortality at 72 h.

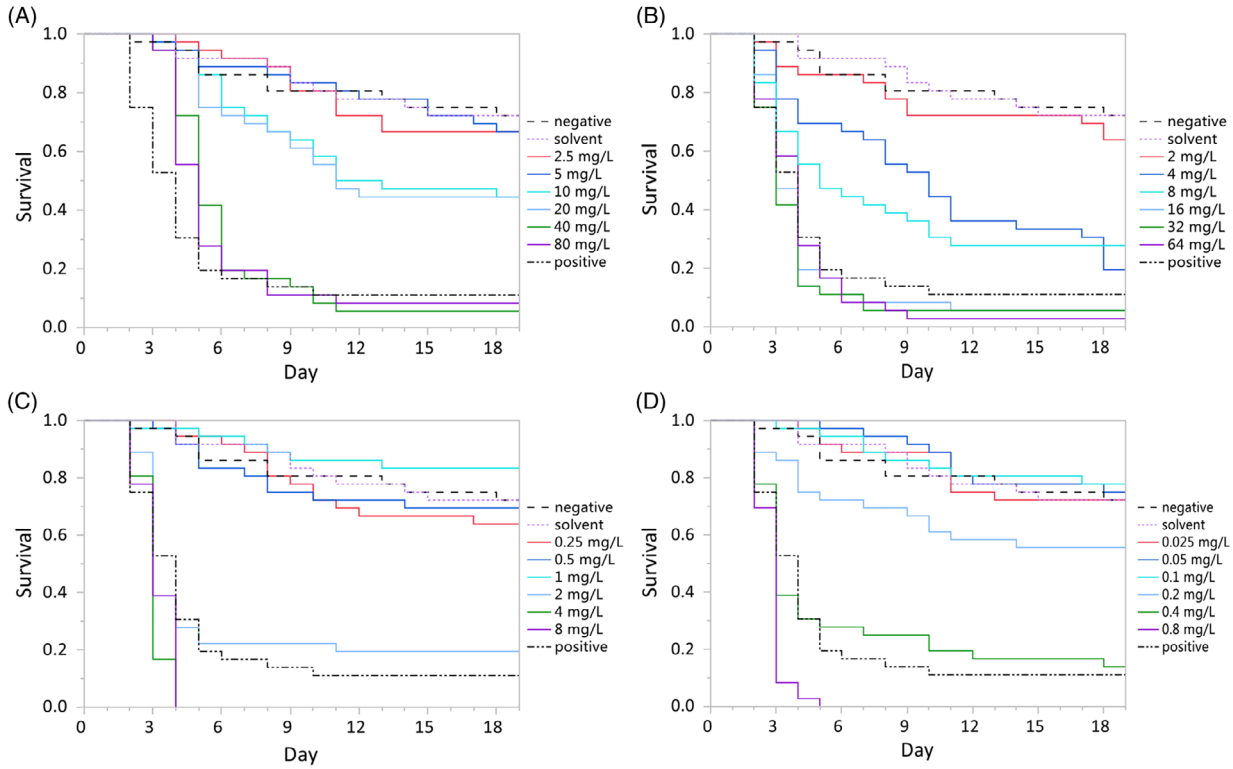

Figure 1. Overall survival of *Apis mellifera* exposed to sublethal concentrations of acetamiprid (A), carbaryl (B), cypermethrin (C) or deltamethrin (D) during larval development on D3 through D6 after grafting (n = 3 replicates of 12 larvae/replicate, or 36 larvae, per test substance). Larvae were fed a dimethoate-contaminated diet (45 mg L^{-1}) as a positive control, an acetone-contaminated diet as a solvent control, and a noncontaminated diet as a negative control. D18 in the figures corresponds to D21 from egg laying to adult emergence for the honey bee. Data analysis corresponds to Table S1–S4.

3.2.2 *Carbaryl*

A one-way ANOVA indicated differences in larval survival among the treatment groups in the carbaryl study ($F_8 = 35.11$, $P < 0.0001$). Overall survival was lower for larvae fed diets with 4, 8, 16, 32 or 64 mg L^{-1} carbaryl than for larvae fed negative and solvent control diets. The survival of larvae fed 2 mg L^{-1} carbaryl was not significantly different from that of larvae fed the negative and solvent control diets but was significantly higher than those fed 4, 8, 16, 32 or 64 mg L^{-1} carbaryl (Table S2, Fig. 1(B)). The NOAEC of carbaryl was 2 mg L^{-1}. Another one-way ANOVA showed differences in larval weight among the treatment groups in the carbaryl study ($F_8 = 2.75$, $P = 0.0075$, Fig. 2(B)). Despite this result, larval weight was not affected by carbaryl compared to the negative control and solvent control treatment groups.

3.2.3 *Cypermethrin*

A one-way ANOVA indicated differences in larval survival among the treatment groups in the cypermethrin study ($F_8 = 57.77$, $P < 0.0001$). Larvae fed 0.25, 0.5, and 1 mg L^{-1} cypermethrin diets did not have different overall survival curves compared to larvae fed the negative or solvent control diets. However, the overall survival of larvae fed diets containing 2, 4 or 8 mg L^{-1} cypermethrin was significantly lower than that of larvae fed the negative and solvent control diets (Table S3, Fig. 1(C)). The NOAEC of cypermethrin was 1 mg L^{-1}. Another one-way ANOVA showed differences in larval weight among the treatment groups in the cypermethrin study ($F_6 = 4.10$, $P = 0.0007$, Fig. 2(C)). Despite this result, larval weight was not affected by cypermethrin compared to the negative control and solvent control treatment groups.

3.2.4 *Deltamethrin*

A one-way ANOVA indicated differences in larval survival among the treatment groups in the deltamethrin study ($F_8 = 44.14$, $P < 0.0001$). Overall survival was lower for larvae fed diets with 0.4 or 0.8 mg L^{-1} deltamethrin than for larvae fed negative and solvent control diets. The survival of larvae fed 0.025, 0.05, 0.1 or

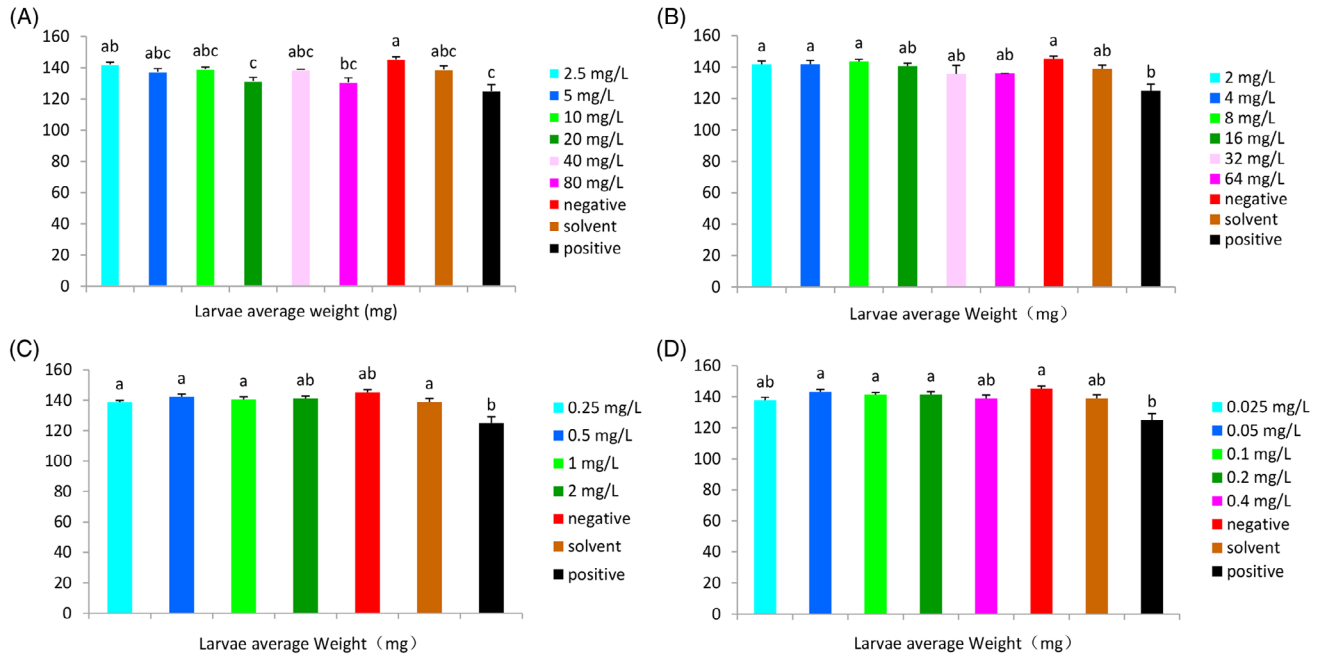

Figure 2. Body weight on D6 of honey bee larvae reared *in vitro* and exposed to acetamiprid (A), carbaryl (B), cypermethrin (C) or deltamethrin (D) in the diet on D3 through D6 after grafting. Larvae were fed a dimethoate-contaminated diet (45 mg L^{-1}) as a positive control or a noncontaminated diet as a negative control. Bars with the same letter are not significantly different at $\alpha \leq 0.05$.

0.2 mg L^{-1} deltamethrin was not significantly different from that of larvae fed diets containing the negative and solvent controls (Table S4, Fig. 1(D)). The NOAEC of deltamethrin was 0.2 mg L^{-1}. Another one-way ANOVA showed differences in larval weight among the treatment groups in the carbaryl study ($F_7 = 3.95$, $P < 0.0005$, Fig. 2(D)). Despite this result, no meaningful patterns are discernable. Larval weight was not affected by deltamethrin compared to the negative control and solvent control treatment groups.

3.3 Risk quotients

The NOAEL (no observed adverse effect level) was obtained for each of the four insecticides based on the NOAEC that we determined for the honey bee larvae. Using our data, the values of both acute and chronic RQ are less than 1 (Table 3).

4 DISCUSSION

Adult honey bees will bring pollen and nectar contaminated with insecticides back into the comb during their foraging activities, and acaricides used by beekeepers to control varroosis by beekeepers are also disseminated in-hive.[19,24,27] Honey bee larvae may be exposed directly or indirectly to these insecticides. Here, we focused on the acute and chronic toxicities of acetamiprid, carbaryl, cypermethrin and deltamethrin to honey bee larvae reared *in vitro*. The acute toxicity values of the four insecticides we tested ranged from high toxicity to low toxicity: deltamethrin > cypermethrin > carbaryl > acetamiprid. Larval survival was affected relative to the solvent control and the negative control, while larval weight was not affected except for acetamiprid. According to the risk quotient (RQ) values for acute and chronic toxicity that we obtained, the risk is acceptable at exposure rates that have been identified in the field.

A study of the oral acute toxicity of five neonicotinoids (acetamiprid, dinotefuran, nitenpyram, imidacloprid, thiamethoxam) against *Apis mellifera* and *Apis cerana* found that acetamiprid was toxic to adult bees.[54] Similarly, in another study, acetamiprid exhibited toxicity to adult bees, consistent with imidacloprid, nitenpyram, dinotefuran and thiamethoxam.[55] In our study, the LD_{50} of acetamiprid for larvae was 5.65 μg $larva^{-1}$, which was lower than the LD_{50} value (7.1 μg bee^{-1}) for adult bees.[55]

The chronic toxicity results indicate that the no observed adverse effect concentration (NOAEC) of acetamiprid was 5 mg L^{-1} for larvae, which is 37 times the maximum residual value in pollen, and 376 times the maximum residual value in nectar or honey at field-relevant exposure levels.[35,51] Based on our results, acetamiprid have little effect on the survival of larvae in the field.

Some studies have demonstrated that carbaryl can generate deleterious neurological, reproductive and genetic effects, and investigations have shown that carbaryl can accumulate in soil and fruit trees in relatively high amounts,[56,57] which may cause the abovementioned adverse effects on pollinating insects such as honey bees. Mullin found that the levels of carbaryl residues were as high as 4.5 ppb in wax, and reached 1010 ppb in pollen.[35] The LD_{50} value of carbaryl to adult honey bees was estimated as 0.0428 μg bee^{-1},[58] while the LD_{50} value for larvae was 1.33 μg $larva^{-1}$ in this study. Carbaryl is more toxic to adult honey bees than to larvae. In our chronic toxicity study, we observed a decrease in survival for larvae fed diets with 4, 8, 16, 32 or 64 mg L^{-1} carbaryl, but none of those concentrations affected larval weight. The no observed adverse effect concentration (NOAEC) of carbaryl was 2 mg L^{-1} for larvae, which was 17 times the mean residual value in pollen and 48 times the maximum residual value in nectar or honey.[35,51] Therefore, our study suggests that this compound is unlikely to have an effect on the survival of developing bees at the reported exposure levels.

Cypermethrin and deltamethrin are the most frequently detected pyrethroids in soil, crops, surface waters, sediments and aquatic organisms worldwide.[43] By measuring insecticide residues, investigators found that cypermethrin concentrations can reach 49 ppb in pollen and 92 ppb in honey or nectar,

Table 3. Quotient (RQ) analysis. The RQs were calculated using BeeREX from the United States Environmental Protection Agency (https://www.epa.gov/sites/production/ files/ 2015-11/beerexv 1.0.xlsx)[a]. *Maximum* residues in pollen/bee bread or in nectar are derived from the literature.[b] Larval NOAED values are derived from our data

Pesticide	Maximum residue reported in pollen/ bee bread (mg a.i. kg^{-1})[c]	Maximum residue reported in nectar (mg a.i./kg)	Larval LD_{50} (μg a.i./ larva)	NOAEC[d] (μg mL^{-1})	Cumulative consumption of diet D3 – D6 (mL)	Larval NOAEL[e] (μg a.i./larva)	RQs (acute dietary)	RQs (chronic dietary)
Acetamiprid	0.134	0.0133	5.65	5	0.14	0.7	<0.01	<0.01
Carbaryl	1.01	0.042	1.33	2	0.14	0.28	<0.01	0.05
Cypermethrin	0.049	0.092	0.13	1	0.14	0.14	0.04	0.12
Deltamithrin	0.091	0.0067	0.05	0.2	0.14	0.028	0.01	0.06

[a] The BeeREX model was modified in order to calculate the RQ based on cumulative dose. The RQs were calculated based on the cumulative amounts of nectar and pollen that are consumed during the worker's larval phase of development according to BeeREX.
[b] Mullin *et al.*[35]; Sanchez-Bayo and Goka·[51]
[c] a.i., active ingredient.
[d] No observed adverse effect concentration.
[e] No observed adverse effect dose.

while those values were 91 ppb and 6.7 ppb for deltamethrin, respectively.[35,51] Previous studies found that the LD_{50} value of cypermethrin for adult bees was 0.06 μg bee^{-1}, while the LD_{50} value of deltamethrin for adult bees was 0.7 μg bee^{-1}.[51,59] In our study, the data suggested that the LD_{50} value of deltamethrin for honey bee larvae was 0.05 μg $larva^{-1}$ and cypermethrin was 0.13 μg $larva^{-1}$. The acute toxicity of cypermethrin towards larvae is less than that towards adult honey bees while the acute toxicity of deltamethrin to larvae is higher than that of adult honey bees. Deltamethrin is more toxic to larvae than cypermethrin, whereas the opposite is true for adult honey bees.

In the chronic toxicity test of cypermethrin, larval survival was significantly decreased at a concentration of 2 mg L^{-1}. However, the indicator can be achieved at 0.4 mg L^{-1} deltamethrin when compared to those of the negative and solvent controls. We observed that deltamethrin appeared to be more toxic than cypermethrin to honey bee larvae that were chronically exposed to the compound for 4 days (D3–D6) *in vitro*. Both the acute and chronic toxicity results indicate that deltamethrin is more toxic than cypermethrin to larvae. The no observed adverse effect concentration (NOAEC) of cypermethrin was 1 mg L^{-1} for larvae, which was 20 times the maximum residual value in pollen and 11 times the maximum residual value in nectar or honey, while the corresponding values for deltamethrin were 0.2 mg L^{-1}, two times, and 30 times.[35,51] Thus, these two pyrethroids were unlikely to have an effect on larval survival at the reported residue values.

The no observed adverse effect concentrations (NOAECs) of the four insecticides that we determined in the chronic toxicity test were higher than those in the field and were unlikely to have detrimental effects on the survival of the *A. mellifera* larvae in our study. Further, for both acute and chronic toxicity, the RQ values that we obtained were lower than the level of concern (LOC) values, indicating that the risk of these four insecticides to bee larvae are acceptable. However, we did not consider the long-term cumulative effects of insecticide residues in pollen and nectar.[60,61] A previous study found that the sublethal effects of insecticides are substantially altered by the interaction of specific environmental landscapes and exposure time.[62] The mechanisms of interactions and diverse modes of action of most insecticides are still unknown.[29] Larvae are fed royal jelly and worker jelly in the hive, and there is a good possibility that the residue levels in those worker bee secretions are different from those observed in pollen/bee bread/nectar/honey; thus, the actual field-relevant doses of pesticides to honey bee larvae are still unknown. All of these factors may influence the true level of exposure of bee larvae to insecticides under near-field conditions. Another nonnegligible problem currently identified by risk assessment schemes is whether the level of sublethal insecticide residues can cause adverse effects at the colony level.[62]

Sanchez-Bayo and Goka believe that toxicity is of the utmost importance when considering the three factors of residue loads, prevalence and toxicity.[51] In recent years, some studies of the transcriptome and metabolome based on the chronic toxicity of insecticides have been carried out in adult bees, and observed effects have included altering detoxification gene expression pathways, affecting components of the cellular response in the immune system,[63] downregulating genes involved in learning performance in the brain,[64] increasing the abundance of metabolites associated with oxidative stress and detoxification,[65] damaging DNA, reducing antioxidant enzyme activity,[66] and inducing transcriptional effects in the brains of bees.[45] Future research is needed to analyze the effects of insecticides on the transcriptome and metabolome of honey bee larvae based on chronic toxicity.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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