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Low acute toxicity of Siltac EC to the honey bee (*Apis mellifera*)

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SUMMARY

Various types of pesticides are commonly used in modern agriculture. Honey bees (*Apis mellifera*) are sensitive indicators of environmental contamination with these substances. Exposure of honey bees to pesticides can lead to changes in their behaviour and increase mortality, so it is important to develop alternative formulations to common ('chemical') pesticides. The recently developed preparation Siltac EC (patent no. WO 2016/061259) shows promise as an effective substitute. It is based on a physical interaction with the pest and does not contain chemicals classified as pesticides. The aim of the current study was to evaluate the toxicity of Siltac EC to adult honey bee workers. The experiments showed that both contact and oral acute toxicity were very low, and that the preparation can preliminarily be considered safe for honey bees.

KEY WORDS: pesticide, contamination, novel preparation, pollinator

INTRODUCTION

Modern agriculture relies on the application of pesticides on a massive scale as a measure to protect crops. Commonly used pesticides ('chemical pesticides') contain active ingredients specified in the Regulation of the European Parliament and of the Council (EC) 1107/2009. Chemical pesticides are widely known to be capable of exerting toxic side effects on non-target species, such as fish (Devia et al., 2013; Ullah and Zorriehzahra, 2015; Bojarski et al., 2018), frogs (Albert et al., 2007; Gilbertson et al., 2009), crocodiles (Khan, 2005), chickens (Garga et al., 2004), dogs, or cats (Xavier et al., 2007). Moreover, the effects of exposure to agrochemicals on human health have become an urgent issue. The presence of pesticides has been shown to produce adverse

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outcomes in people, including impaired fertility and neurotoxicity (Bjorling-Poulsen et al., 2008; Roeleveld and Bretveld, 2008; Jokanovic and Kosamivic, 2010). Research conducted by Koutros et al. (2013) revealed a significant correlation between exposure to insecticides and the risk of prostate cancer. Furthermore, pesticides have been shown to be toxic to numerous invertebrate species (e.g. Peterson et al., 2001; Hartnik et al., 2008). Costa et al. (2013) established that insecticides used in melon farming exhibit high toxicity towards honey bees (*Apis mellifera*). Improper pesticide application may lead to changes in honey bee behaviour or even death (Porrini et al., 2003; Thompson, 2003). Moreover, pesticide exposure may increase the risk of *Nosema* infection (Pettis et al., 2013). In recent years, honey bee colonies have been vanishing at an alarming rate (Riscu (Jivan) and Bura, 2013; Sanchez-Bayo and Goka, 2014). There are many factors that may contribute to the decline in honey bee populations, and pesticide pollution is only one of them (Pettis et al., 2012; Sanchez-Bayo and Goka, 2014). Pesticides and their interactions contribute to stress-induced colony losses (Sanchez-Bayo et al., 2016). Chemical pesticides may potentially be replaced with preparations containing microorganisms (Roh et al., 2007) or based on a physical interaction with the target pest (Vincent et al., 2003). Siltac EC is a preparation that affects the surface of the pests. The formula was developed by ICB Pharma (Jaworzno) and is patented (patent no. WO 2016/061259). Siltac EC is a mixture of organomodified trisiloxane and tetraethoxysilane. The trisiloxane derivative and tetraethoxysilane were selected on the basis of numerous chemical reactions carried out in order to determine the reactive structures capable of rapid unidirectional addition reactions in an aqueous environment (Patrzałek et al., 2020). Previous research has shown this preparation to be highly effective in controlling mites and aphids feeding on fruit trees and berries (Patrzałek et al., 2020).

Data on the toxicity of pesticides acting through a physical interaction with the pest remain insufficient. There is a need to evaluate the potential environmental risks associated with the commercialization and widespread application of these products. This study was aimed at assessing acute oral and contact toxicity of Siltac EC to adult honey bee workers (*A. mellifera* L.).

MATERIALS AND METHODS

The research was conducted following OECD 213 and OECD 214 guidelines in a GLP certified laboratory of the Institute of Industrial Organic Chemistry – Division in Pszczyna.

Tested preparation

Siltac EC is a transparent liquid with a distinctive smell. According to the certificate of analysis of 21 May 2013, the surface tension of a 0,1% emulsion of the material in hard water is 23 mN/m, and its density is 1,012 g/cm^3. The preparation was stored at room temperature, in a dry place without exposure to light. The material was handled according to the procedures used at the Department of Test materials and the Laboratory of Apitoxicology.

Reference material

Research reliability and bee condition were tested using the insecticide Bi 58 Nowy 400 EC, whose active ingredient is dimethoate, a substance recommended in the OECD 214 guidelines. This insecticide is a clear liquid with a distinctive organic smell. It is used to prepare a water-based emulsion (EC form). According to the product label (manufacturer: BASF AG, Germany; batch no.: 0001039224), the reference material contains 400 g/L of the active substance dimethoate.

Experimental organism

Acute toxicity testing of the preparation was performed on worker bees of the subspecies *Apis mellifera carnica*, Beskidka line, bred in the experimental apiary of the Institute of Industrial Organic Chemistry, Division in Pszczyna. Bees were obtained from healthy colonies of known origin, with queens that normally laid eggs. Bees of approximately 21 days of age were selected from the colonies, which had not been exposed to any chemical substances, including antibiotics and varroacides, for at least 4 weeks before being taken from the apiary. Each group of 10 bees (placed in one cage) was taken from 5 different source colonies (2 bees from each colony).

Assessment of acute contact toxicity

The preparation was dissolved in distilled water. The resulting solution was applied to individual bees using an Arnold Automatic Microapplicator 240 V (Burkard Manufacturing Co. Ltd). The bees were placed in glass tubes (15 cm long, diameter 2,5 cm), which were closed at the top with a mesh plastic cap. A1 µL volume of the preparation was applied to the thorax (25,0; 50,0; 100,0 or 200,0 µg preparation/bee), and then the bees were transferred in cages (10 bees per cage). Three control tests (10 bees per test) were run simultaneously with the toxicity tests. Distilled water (1 µL) was applied directly onto the control insects. The cage size (5 x 7 x 4,5 cm) provided adequate space for the bees. A hole in the cage wall allowed them to collect food consisting of a pure sugar solution from a 5 mL syringe. After the bees were exposed to the preparation, they were given access to a 50% sugar solution. Fresh food was restocked when necessary. Throughout the experiment the bee cages were kept in a shaded room under controlled environmental conditions:

- air temperature: 23–27°C (required 25 ± 2°C)
- relative humidity: 54–69% (required 50–70%)

The first observations, to evaluate insect mortality (or other possible toxic effects), were made 4 hours after the bees were exposed to the preparation. Subsequent tests were carried out every 24 hours after application of the preparation and were concluded 96 hours after exposure. The amount of sugar solution ingested was checked every 24 hours. Research reliability and bee condition were tested using the insecticide Bi 58 Nowy 400 EC, whose active ingredient is dimethoate, a substance of known toxicity to bees. Bi 58 Nowy 400 EC was applied in 3 doses containing 0,1, 0,2 or 0,4 µg of the active substance in 1 µL volume. Control bees were treated with 1 µL distilled water. The reference material was applied to bees in a similar manner as the tested compound.

Assessment of acute oral toxicity

The preparation was dissolved in a 50% (w/v) sugar solution. The resulting solution was administered to each bee group using a micro-pipette. The bees (10 insects per cage) were placed in plastic cages (5 x 7 x 4,5 cm) with a hole in the wall enabling free access to the contaminated sugar solution. Acute oral testing involved applying the same doses of the preparation (25,0, 50,0, 100,0 or 200,0 µg preparation/bee) as in the contact toxicity testing.

Toxicity was evaluated only in the groups of bees that had consumed the entire volume of the contaminated mixture within 6 hours. Each insect ingested approximately 10 µL of the solution contaminated with the preparation. The insects from the control group received a pure sugar solution, 10 µL/bee on average. The bees in each group consumed contaminated or control sugar solution from a shared micro-pipette. After the bees had consumed the solution, the pipettes with contaminated sugar syrup were replaced with 5 mL syringes with a pure sugar solution. Throughout the experiment the bee cages were kept in a shaded room under controlled environmental conditions. The values for

environmental parameters reached in the acute oral toxicity test were the same as in the acute contact toxicity test.

After consuming contaminated food, the insects were provided with unrestricted access to a 50% sugar solution. Fresh food was restocked when necessary. The first observations, to evaluate insect mortality (or other possible toxic effects), were made 4 hours after the bees were exposed to the preparation. Subsequent tests were carried out every 24 hours after application of the preparation and concluded 96 hours after exposure. The amount of sugar solution consumed was checked every 24 hours.

Research reliability and bee condition were tested using the reference material Bi 58 Nowy 400 EC, an insecticide whose active ingredient is dimethoate, as in the case of the contact toxicity tests. Bi 58 Nowy 400 EC was administered orally in 3 doses. Geometric progression (ratio 2) differed between doses. In 10 µL of a 50% sugar solution the bees consumed quantities of reference material containing 0,03, 0,06 and 0,12 µg of the active substance. The bees in the control group were fed a pure sugar solution. One bee received on average 10 µL of the solution. The reference material was administered in a similar manner as the test preparation.

The log-probit method was to determine LD_{50} values.

RESULTS AND DISCUSSION

The results show that all OECD criteria for experiments were met. There were no dead bees after 24 and 48 hours in the control groups, in either the contact toxicity or the oral toxicity test (maximum acceptable mortality 10%). Evaluation of bee condition involved application of dimethoate. The LD_{50} values fell within the threshold values set forth in the guidelines, that is 0,11 µg per bee in the oral test (limit values 0,10–0,35) and 0,26 µg per bee in the contact test (limit values 0,10–0,30). The results indicate that the condition of the bees used in the study was satisfactory.

The mortality rates for bees exposed to Siltac EC evaluated using contact testing are shown in Tables 1 and 2. The LD_{50} values estimated after 24 and 48 hours for Siltac EC were 161,31 µg per bee (confidence interval: 91,35–284,87) and 114,59 µg per bee (confidence interval: 77,53–169,36), respectively. Mortality was not observed in the control group. The LD_{50} value for dimethoate evaluated after 24 hours was 0,26 µg per bee (confidence interval: 0,193–0,363 µg/bee) (Table 3).

The mortality rates for bees exposed to Siltac EC established through oral toxicity testing are presented in Tables 4 and 5. The lethal dose in the oral test (LD_{50} per os) estimated after 24 and 48 hours was 165,72 µg per bee (confidence interval: 68,89–398,64) and 117,92 µg per bee (confidence interval: 66,78–208,22), respectively. Mortality was not observed in the control group. The LD_{50} value for dimethoate evaluated after 24 hours was 0,11 µg/bee (confidence interval: 0,064–0,183) (Table 6).

Table 1

Mortality of honey bees 24 hours after exposure – contact toxicity tests using Siltac EC

Dose [µg/bee]	Mortality [n] Replicates			Overall mortality [n]	Overall mortality [%]	LD_{50} [µg/bee]
	1	2	3			
0,0 (control)	0	0	0	0	0,00	161,31
25,0	0	1	2	3	10,00	
50,0	2	2	0	4	13,33	
100,0	3	5	4	12	40,00	
200,0	6	6	5	17	56,67	

n – number of bees

Table 2

Mortality of honey bees 48 hours after exposure – contact toxicity tests using Siltac EC

Dose [µg/ bee]	Mortality [n] Replicates			Overall mortality [n]	Overall mortality [%]	LD_{50} [µg/bee]
	1	2	3			
0,0 (control)	0	0	0	0	0,00	114,59
25,0	1	2	2	5	16,67	
50,0	2	3	2	7	23,33	
100,0	3	6	5	14	46,67	
200,0	6	6	8	20	66,67	

n – number of bees

Table 3

Mortality of honey bees 24 hours after exposure – contact toxicity tests using dimethoate

Dose [µg/bee]	Mortality [n] Replicates			Overall mortality [n]	Overall mortality [%]	LD_{50} [µg/bee]
	1	2	3			
0,0 (control)	0	0	0	0	0,00	0,26
0,10	2	2	1	5	16,67	
0,20	4	4	5	12	43,33	
0,40	6	6	7	19	63,33	

n – number of bees

Table 4

Mortality of honey bees 24 hours after exposure – oral toxicity tests using Siltac EC

Dose [µg/ bee]	Mortality [n] Replicates			Overall mortality [n]	Overall mortality [%]	LD_{50} [µg/bee]
	1	2	3			
0,0 (control)	0	0	0	0	0,00	
25,0	1	1	2	4	13,33	
50,0	2	2	0	4	13,33	165,72
100,0	3	5	4	12	40,00	
200,0	6	6	5	17	56,67	

n – number of bees

Table 5

Mortality of honey bees 48 hours after exposure – oral toxicity tests using Siltac EC

Dose [µg/ bee]	Mortality [n] replicates			Overall mortality [n]	Overall mortality [%]	LD_{50} [µg/bee]
	1	2	3			
0,0 (control)	0	0	0	0	0,00	
25,0	1	2	2	5	16,67	
50,0	2	3	1	6	20,00	117,92
100,0	3	6	5	14	46,67	
200,0	6	6	8	20	66,67	

n – number of bees

Table 6

Mortality of honey bees 24 hours after exposure – oral toxicity tests using dimethoate

Dose [µg/ bee]	Mortality [n] Replicates			Overall mortality [n]	Overall mortality [%]	LD_{50} [µg/bee]
	1	2	3			
0,0 (control)	0	0	0	0	0,00	
0,03	0	1	0	1	3,33	0,11
0,06	1	1	1	3	10,00	
0,12	7	6	6	19	63,33	

n – number of bees

The results of the experiment using Siltac EC indicate that the preparation is safe for bees. At the recommended doses (0,5–1,0 L per hectare), we can assume that the risk of toxicity to bees is low. The results are promising, especially as the preparation is highly effective in controlling mites and aphids (Patrzałek et al., 2020).

The effect of pesticides on bees depends on the intrinsic sensitivity of a given bee species as well as on their specific life cycle, nesting activity, and foraging behaviour (Arena and Sgolastra, 2014). Nevertheless, studies by many researchers have revealed that a number of substances commonly used as chemical pesticides exhibit high toxicity to bees. Badawy et al. (2014) tested the toxicity of four chemical pesticides, i.e. acetamiprid, dinotefuran, pymetrozine and pyridalyl, to *A. mellifera.* Dinotefuran was found to be the most toxic to *A. mellifera* (contact LD_{50} = 0,0006 μg/bee), while pyridalyl was the least toxic (LD_{50} 6,16 μg/bee), and pymetrozine and acetamiprid exhibited medium to low toxicity (LD_{50} of 0,16 and 1,69 μg/bee, respectively) compared to dinotefuran. Research carried out by Spurgeon et al. (2016) established pesticide toxicity in the following order: clothianidin > dimethoate > tau-fluvalinate > propiconazole > 2,4-D. Iwasa et al. (2004) evaluated contact toxicity of neonicotinoids to honey bees (*A. mellifera*). They showed that nitric compounds are the most toxic to bees, with LD_{50} values reaching 18 ng/bee for imidacloprid, 22 ng for clothianidin, 30 ng for thiamethoxam, 75 ng for dinotefuran and 138 ng for nitenpyram. Cyano-substituted neonicotinoids showed significantly lower toxicity, with LD_{50} values of 7,1 and 14,6 μg per bee for acetamiprid and thiacloprid, respectively. Laurino et al. (2011) investigated the toxicity of four neonicotinoids to honey bees. LD_{50} values were assessed using oral and contact tests at 24, 48 and 78 hours from the start of the experiment. LD_{50} values were 2,844, 2,689 and 2,608 ng/bee for clothianidin, and 4,679, 4,411 and 4,316 ng/bee for thiamethoxam, while LC_{50} values reached 4,485, 2,967 and 2,667 ng/μL for clothianidin and 5,200, 3,313 and 2,462 ng/μL for thiamethoxam. Acetamiprid and thiacloprid caused higher mortality as compared with the control group only in oral toxicity tests. Pesticide exposure may also cause pathophysiological alterations in honey bees. Badiou-Beneteau et al. (2012) demonstrated that thiamethoxam treatment modified the activity of acetylcholinesterase (AChE), carboxylesterases (CaE1, CaE2 and CaE3), glutathione-S-transferase (GST), alkaline phosphatase (ALP), and catalase (CAT). Similarly, prochloraz and propiconazole strongly inhibited cytochrome P450 enzymes CYP9Q2 and CYP9Q3 (Haas and Nauen, 2021). Paleolog et al. (2020) reported that imidacloprid can affect the activity of haemolymph aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in honey bees. In addition, pesticides may affect the immunity of bees. Thiacloprid (200 μg/L or 2000 μg/L) and imidacloprid (1 μg/L or 10 μg/L) have been shown to reduce haemocyte density, encapsulation response, and antimicrobial activity in the insects (Brandt et al., 2016).

CONCLUSIONS

The high toxicity of chemical pesticides has prompted a search for more environmentally-friendly solutions. This study revealed that the toxicity of Siltac EC to honey bees is potentially low. It appears that Siltac EC is fairly safe for honey bees and natural biocoenoses. However, there is a need for further studies on the potential effects of Siltac EC on physiological parameters.

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