

## Acute contact toxicity of insecticidal baits on honeybees *Apis mellifera*: a laboratory study

T.F. Domatskaya, A.N. Domatskiy, M.A. Levchenko, E.A. Silivanova\*

All-Russian Scientific Research Institute of Veterinary Entomology and Arachnology

Branch of Federal State Institution Federal Research Centre

Tyumen Scientific Centre of Siberian Branch of the Russian Academy of Sciences

(ASRIVEA – Branch of Tyumen Scientific Centre SB RAS)

Mira St. 5 - 7, 625517 Malkovo, Tyumen oblast, Russian Federation

Corresponding author E-mail: [silivanovaea@mail.ru](mailto:silivanovaea@mail.ru)

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The honeybee *Apis mellifera* L. (Hymenoptera: Apidae) is an important pollinator, an object of human economic activity and a bioindicator of environmental pollution. Insecticides used in Agriculture for treatment of plants, bee colonies, and farm animals may be dangerous for bees. This paper reports the results of a comparative assessment of the acute contact toxicity of four toxic bait formulations designed for insect pest control on livestock farms to the honeybees. Toxic baits consisted acetamiprid, ivermectin, chlorfenapyr, and fipronil, with residues of which on the filter paper adult bees contacted on plastic containers. It was found that the bait formulation with ivermectin manifested toxicity slower than the other ones. We used probit analysis to calculate median lethal doses (LD<sub>50</sub>) of each active substance showed that their toxicity to bees decreased in the following order: fipronil – ivermectin – chlorfenapyr – acetamiprid. Considering the mass content of active substances in formulations, median lethal doses of toxic baits were 202.6 µg/bee (bait with fipronil), 229.3 µg/bee (bait with ivermectin), 1188.3 µg/bee (bait with chlorfenapyr), and 6552 µg/bee (bait with acetamiprid). Thus, bait formulations themselves may be considered practically non-toxic to bees, since their LD<sub>50</sub> exceeded the threshold value of 100 µg/bee. Assessment of hazard degree for bees by the hazard index showed that the insecticide bait containing acetamiprid were less dangerous than the other three bait formulations under laboratory conditions. The future semi-field and field studies are needed to evaluate the possible exposure and the hazard degree of insecticidal baits for bees under conditions close to practice use of these formulations.

**Keywords:** acetamiprid; ivermectin; fipronil; chlorfenapyr; honeybee

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### Introduction

Currently, in agriculture (livestock breeding and plant protection), insecticides are widely used to control the number of harmful insects (Simon-Delso et al., 2015). Intensive use of insecticides increases the likelihood of environmental contamination and increases the risk of exposure of non-target organisms (Gorbatov, 2008; Bonmatin et al., 2015), including insect pollinators.

Insect pollinators are a critical importance for wild growing and cultivated plants, to a stability of ecosystems (Potts et al., 2010). Bees are important pollinators: according to FAO, 90% of the world's food is provided by 100 crop species, 71 of which are pollinated by bees (<http://www.efsa.europa.eu/en/topics/topic/bee-health/>). The honeybee *Apis mellifera* L. (Hymenoptera: Apidae) is an object of human economic activity and an indicator of ecological well-being (Celli and Maccagnani, 2003; Sadeghi et al., 2012).

The greatest danger to bees is posed by pesticides used to protect plants. For example, in 2015 in Russia poisoning with such pesticides was a reason of the main causes of death of bees (Klochko and Blinov, 2016). There are cases of poisoning of bee colonies with various insecticides, for example, in Canada (Cutler, 2014). As a rule, the death of bees is noted during the period of mass pesticide treatment of agricultural crops, orchards, forests. Poisoning of bees can occur during insecticide treatment of non-nectar sources located next to blooming melliferous plants, hitting an apiary during an aircraft treatment, as well as transferring of insecticides from the treated areas to neighboring areas visited by bees. Adult bees can be exposed to pesticides not only through contact with treated plants but also through contaminated pollen, nectar, water and soil (Rortais et al., 2005; Botias et al., 2015; Kiljanek et al., 2016; Krupke et al., 2012). Pesticides get into hives transferred with pollen and nectar by adult foraging bees. Both wild and cultivated bees are being exposed to pesticides (Hladik et al., 2016; Kiljanek et al., 2017).

Surveys of live and dead bees identified insecticides which are used both for plant protection and veterinary medicine for treatment of bee colonies and farm animals (Kiljanek et al., 2017; Lambert et al., 2013). In our opinion, insecticides used in

livestock can also be dangerous for bees. Firstly, the residues of insecticides after treatment of animals and buildings on farms can get into the soil with rainwater and washings and spread to the territory of the growth of melliferous plants. Secondly, insecticide baits against, for example, flies or cockroaches, usually contain a food attractant (most often sugar), which can attract bees, especially during the absence of honey flow. There is a possibility of contact of bees with such insecticides on a cattle-breeding farm in violation of the rules of handling pesticides (storage, disposal). This study was carried out to assess the acute toxicity of insecticide baits designed for flies control on livestock farms to bees.

## Materials and methods

### Insects

Adult honeybees *Apis mellifera* were taken from a colony wintering under the snow (the colony was in a sixteen-frame beehive with normal quantity of honey and perga) at a temperature of -8 to -10°C. The bees were brought to the laboratory, placed in cages 50 bees each, and kept at a temperature of 23-24°C. The bees were fed with a 50% aqueous solution of sugar. A day later, the bees were inspected, the intact (healthy and viable) were used for the experiment.

### Insecticides

We used four insecticidal baits designed to flies control in livestock premises, developed and manufactured by the staff of the All-Russian Scientific Research Institute of Veterinary Entomology and Arachnology (Levchenko and Silivanova, 2015). The insecticidal baits include one of the active substances (fipronil 0.15%, or acetamiprid 1.5%, or ivermectin 0.6%, or chlorfenapyr 6%), as well as sex attractant (cis-9-tricosene), food attractant (sucrose or glucose), and excipients.

### Bioassays

The experiments were conducted under laboratory conditions at a temperature of 23-24°C. The toxicity of insecticidal baits for bees was studied in accordance with Titov et al. (1989) with minor changes by contacting the bees with the treated filter paper for 30 minutes in plastic containers. Pieces of filter paper with an area of 51 cm<sup>2</sup> were placed in plastic containers; then tested baits were applied to filter paper in a volume of 1 mL, evenly distributed over the surface, and dried in the air. After that, 10 bees were introduced into the containers contacting with the treated paper for 30 minutes. After contacting, the bees were placed in cages and their mortality rates were recorded after 1, 4 and 24 hours. Control bees were placed in containers with filter paper soaked in water in a volume of 1 mL. The baits tests were carried out in 5-7 dilutions (doses). For each studied dilution (dose), 10 bees were used. The experiments were repeated three times.

### Data analysis

The results of the dependence of the death of bees on the dilution (dose) of the insecticidal agent were analyzed by the probit analysis method. The median lethal dose (LD<sub>50</sub>) with a 95% confidence interval and a slope was calculated using the Free LD50/LC50 Calculator (July 7, 2016, posting by Dr. M. Alpha Raj by Calculating LD50/LC50 using Probit Analysis in Excel, Blog). LD<sub>50</sub> was expressed in µg per bee and per cm<sup>2</sup>.

## Results

During the period of contacting with the residues of insecticidal baits in plastic containers, the death of the bees was not observed. The most death of bees was recorded within an hour and four hours after contacting (Table 1). Contact with preparations containing acetamiprid and chlorfenapyr resulted in the death of most of the bees within the first hour after exposure. The most death of bees after the contact with the ivermectin containing preparation was noted after 4 hours. After contacting with the residues of the fipronil containing preparation, the death of the bees occurred both in the first hour of observation and in the following 24 hours. In the control group, the death of bees was not recorded during the entire observation period.

**Table 1.** Percent mortalities (%M) of bees at various times (h) after 24 h to ingest toxic baits in the laboratory.

Active substance	Bees number	Time after contacting			Total in 24 hours
		1 h	4 h	24 h	
Acetamiprid	180	36.7	1.70	0	38.3
Ivermectin	210	0	17.6	7.60	25.2
Fipronil	210	27.6	3.30	19.0	50.0
Chlorfenapyr	180	19.4	0	5.00	24.4

Fipronil had the highest toxicity for bees (Table 2). The slope value was the lowest; the slope of the regression line is less steep compared to other insecticides, indicating a lower rate of change in toxicity with an increase in the dose of the preparation. The LD<sub>50</sub> of ivermectin was 4.5 times higher than that of fipronil. Low toxicity for bees was found in chlorfenapyr and acetamiprid: their LD<sub>50</sub> was 234.5 and 323.3 times higher than in fipronil, respectively. LD<sub>50</sub> value of these insecticides was of the same order, but acetamiprid was less toxic. The slopes in the case of ivermectin, chlorfenapyr, and acetamiprid did not differ. Median lethal doses of active substances in tested baits are comparable with the literature data (Table 3).

Considering the mass content of the active substances, the LD<sub>50</sub> of each tested bait have the following values: 6552 µg/bee (bait with acetamiprid), 229.3 µg/bee (bait with ivermectin), 202.6 µg/bee (bait with fipronil), and 1188.3 µg/bee (bait with chlorfenapyr). It should be noted that the median lethal doses (LD<sub>50</sub>) of baits with fipronil and ivermectin differed in the active

substance, being 0.3 and 1.4 respectively, but their preparation LD<sub>50</sub> values were similar. The toxicity of the bait with acetamiprid (LD<sub>50</sub> of the preparation) was 5.5 times lower in comparison with the chlorfenapyr-containing preparation, whereas the LD<sub>50</sub> difference of these baits towards the active ingredient was 1.4 times.

**Table 2.** Acute contact toxicity and test results

Active substance	Number of bees	LD <sub>50</sub> (95% CI) µg a.i./bee	LD <sub>50</sub> (95% CI) µg a.i./cm <sup>2</sup>	Slope (standard error)
Acetamiprid	180	98.28 (61.73-156.48)	19.61 (12.37-31.01)	1.817 (0.102)
Ivermectin	210	1.376 (0.797-2.374)	0.270 (0.156-0.465)	1.611 (0.121)
Fipronil	210	0.304 (0.155-0.599)	0.0597 (0.0304-0.1174)	1.075 (0.150)
Chlorfenapyr	180	71.30 (38.21-133.05)	13.98 (7.49-26.07)	1.623 (0.138)

**Table 3.** Toxicity of studied insecticides to *Apis mellifera* (by literature data).

Insecticide	Preparation (Example)	LD <sub>50</sub> , topical application, µg a.i./bee	Source	LD <sub>50</sub> , contact with the surface, µg a.i./bee	Source
Acetamiprid	substance (technical grade)	8.09	EFSA, 2012	-	-
	Mospilan 20 SP	5.27 (3.56-7.80)	Derkach, 2009	118.86 (80.66-176.17)	Derkach, 2009
	Assail 30SG	64.6	Biddinger et al., 2013	-	-
Ivermectin					
Abamectin	substance (technical grade)	7.8 (6.5-8.9)	Del Sarto et al., 2014	1.54 (1.21-1.90)	Del Sarto et al., 2014
Fipronil	substance (technical grade)	0.00593	EFSA, 2006	-	-
		0.00106	Roat et al., 2012	-	-
	Regent 80 WDG	0.010 (0.006-0.016)	Derkach, 2009	0.282 (0.182-0.437)	Derkach, 2009
Chlorfenapyr	substance	0.12 (0.10-0.14)	US EPA, 1993	-	-

## Discussion

The tested insecticidal baits contain active substances from different chemical classes, differing in the mode of action and the degree of toxicity for insects. The rate of insecticidal action of acetamiprid, fipronil and chlorfenapyr in the composition of the tested baits after a 30-minute contact of the bees with the treated surface was similar. The bait with ivermectin was slower than the other ones.

Studying the toxicodynamics of neonicotinoids when contacting bees with treated surfaces, Illarionov and Derkach (2009) did not observe insect death in the first 3 hours after exposure, followed by a uniform mortality for every 3 hours of experiment, and maximum mortality after 24 hours. Perhaps, the reason for the difference in the rate of death of bees after contacting in our experiment is related to the characteristics of the formulation. Since baits contain sugar, there is a high probability of them being eaten by bees, and as a result of the contact-intestinal action of the insecticide, this killed the insects faster.

According to Costa et al. (2013), the median lethal time at the contact of bees with plant leaves treated with chlorfenapyr and acetamiprid-containing insecticides was 44.12 hours and 60.89 hours respectively; the abamectin insecticide was much faster (LT<sub>50</sub> 18.45 hours). When spraying the bees with insecticides directly, the median lethal time of acetamiprid and chlorfenapyr did not differ, and with abamectin it was two times less (Costa et al., 2013). Stanley et al. (2015), when studying the effect of pesticides on bees in doses recommended by manufacturers, found that after 30 minutes of contacting of *A. mellifera* bees with a surface treated with acetamiprid-containing preparation (Albis 20 SP, Atul Ltd., Gujarat), 20% of the bees died after 48 hours. As can be seen from the obtained results, acute contact toxicity for bees of the studied insecticidal baits decreased in the following order: fipronil – ivermectin – chlorfenapyr – acetamiprid. The results obtained by other authors (Table 3) confirm that fipronil is the most toxic for bees when applied topically, and when applied to the surface, and acetamiprid is the least toxic in experiments on contacting bees with a treated surface.

There are several classifications of the acute contact toxicity and the danger of pesticides for honeybees, which are given in Russian (Osintseva, 1999; Titov et al., 1989) and foreign literature (Felton et al., 1986; USEPA, 2014). According to these classifications and the data in Table 2, chlorfenapyr and acetamiprid in the tested baits are of low toxicity; ivermectin is of medium toxicity, and fipronil is a highly toxic pesticide. However, the baits themselves are practically non-toxic to bees, since the LD<sub>50</sub> of the preparation exceeds the threshold value of 100 µg/bee.

From a practical point of view, it is useful to assess the degree of danger of insecticides for bees. Such an assessment is mandatory for pesticides used in plant protection and is carried out using a risk factor (RF) (Osintseva, 1999). For contact exposure, the risk factor is calculated as the ratio of the preparation application rate (in grams of active ingredient per 10000 m<sup>2</sup>) to the median lethal dose at contact exposure (in µg of a.i./bee). In international practice, the risk is assessed with RQ (risk quotient), which is calculated using a special mathematical Bee-REX model (<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#terrestrial/>); or hazard factor HQ (hazard quotient), which is used to evaluate pesticides intended for spraying plants (Rortais et al., 2005). HQ is the ratio of the predicted pesticide concentration in the environment, to which the bees may be exposed, to the LD<sub>50</sub> value that reflects the acute toxicity of the pesticide for bees (Rortais et al., 2005).

According to another method, the hazard degree of plant protection products for bees is assessed using the insecticide hazard index (Derkach, 2009), which is calculated by the formula:

$$I = N \times 0.5 / 100 \times LD_{50},$$

where I is Hazard Index; N is application rate of the active ingredient of the insecticide, g/ha; LD<sub>50</sub> is median lethal dose, µg a.i./bee or cm<sup>2</sup>.

The baits tested should be used the following way. First, they should be mixed with water in a ratio 1:3 (for example, 10g of powder bait and 30g of cold water) under constant stirring, then, the resulting thick mass should be locally applied for the treatment of surfaces at the rate of 250 ml per 1 m<sup>2</sup>. Therefore, the following amount of active substance will be applied to 1 m<sup>2</sup> of the surface: 5g of chlorfenapyr, 1.25g of acetamiprid, 0.125g of fipronil, 0.5g of ivermectin. If we use this formula, the hazard index of bait with chlorfenapyr will be 3.51, with ivermectin - 18.17, with fipronil - 20.6, and with acetamiprid - 0.636. That means that while contacting the treated surfaces, the danger of insecticide bait containing acetamiprid for bees is negligible compared to the other three baits.

According to the literature data, residues of pesticides and their metabolites are often found in dead bees; it includes both insecticides used for plant protection as well as veterinary medical products (Kiljanek et al., 2017). Some authors reported about finding of acetamiprid, fipronil and its derivatives in the dead bees (Kiljanek et al., 2016). In our opinion, insecticides widely used in livestock breeding to control the number of insects (for example, flies in the premises) may be an additional source of risk for bees. After assessing the acute contact toxicity to honey bees of four insecticidal baits under laboratory conditions, we founded that these baits were practically non-toxic for bees, whereas the bait with acetamiprid was the least toxic. The future semi-field and field studies are needed to evaluate the possible exposure and the hazard degree of insecticidal baits for bees under conditions similar to practice use of baits.

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## Conflict of Interest

Levchenko M.A. and Silivanova E.A. are authors of patent "Method of controlling flies in livestock buildings", RU, Pat. 2540553.

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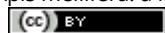
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