Poisoning of Apis mellifera bees with neonicotinoids and glyphosate

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Abstract. In the summer of 2019, in many regions of the Russian Federation, there was a high death rate of honey bee families in apiaries after treating rapeseed and other fields with pesticides during flowering. The purpose of our research: to study the effect of neonicotinoids and glyphosate contained in pesticides and herbicides on Apis mellifera honey bees in laboratory conditions, to study the clinical picture of poisoning. As a result of the study, it was revealed that imidacloprid and thiacloprid have a fumigant effect from the treated surfaces for 7 days. In addition, the accumulation and death of bees from small doses can be observed on the 9th day and later. The effect of glyphosate-containing preparations on bees in laboratory conditions revealed 100% death of bees when consumed with feed. The death of bees after consuming sugar syrup with the drug, even from small doses, occurred after 15 minutes, and in the fumigation test, no death was found with prolonged exposure of the drug near bees.

1. Introduction
In the summer of 2019, in many regions of the Russian Federation, there was a high death rate of honeybee colonies in apiaries after the treatment of rapeseed and other fields with pesticides during flowering.

The first telephone calls to the laboratory of bee diseases of the Federal State Budget Scientific Institution «Federal Scientific Centre VIEV» regarding the poisoning of bee colonies from the regions began to arrive in May 2019. There were appeals from veterinary specialists, heads of veterinary laboratories, beekeepers, heads of rural settlements and district administrations - everyone asked how to take samples, to which laboratory to send the collected material. They reported what the fields were treated with. The drugs have names such as: «Borei-Neo», «Biscaya, MD», «Roundup BP», «Uragan forte BP», «Monarch», etc., which include neonicotinoids, synthetic pyrethroids, both individually and in a mixture. The treatments were carried out in the middle of the day – in violation of the requirements of the Federal Law No. 109-FZ of 19.07.1997 «On the safe handling of pesticides and agrochemicals».

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2. Materials and methods
The research was conducted in the Laboratory of Bee Diseases of the Federal State Budget Scientific Institution «Federal Scientific Centre VIEV».

Of the pesticides containing neonicotinoids in their composition, the study used:
Sample No. 1. Preparation «Tanrek VRK» (active substance-imidacloprid 200 g / l), manufacturer-CJSC Firm "August", Russia; manufactured in 2018, shelf life 4 years. Hazard class for bees-1 (highly dangerous), it is recommended to spray in the early morning or late evening at a temperature below +15°C, with a wind speed of no more than 1-2 m / s; the border protection zone for bees is 4-5 km [1,2].

Sample No. 2. Preparation «Biscaya, MD» Bayer (active substance-thiacloprid 240 g / l), provided by an agronomist from the Borovsky district of the Kaluga region, the remains - after processing of flowering rapeseed. Baseline data for the drug were not provided. Hazard class for humans-2, for bees-3. Resistant to atmospheric precipitation, the period of protective action is not less than 14 days. The pesticide has intestinal and contact effects [1,2]. The consistency and color of the preparation correspond to the preparation «Biscaya, MD» Bayer.

Of the herbicides containing glyphosate in their composition, the following were used in the study:

Sample No. 3. Preparation «Roundup BP» (active substance - glyphosate acid in the form of isopropylamine salt 360 g / l), made in 2018, shelf life of 5 years. Manufacturer-CJSC Firm "August", Russia. For the treatment of plants, a solution of 40 ml per 10 liters of water is prepared, the consumption is 5 liters per 100 m²[1,2].

Sample No. 4. Preparation «Uragan forte BP» (active substance-glyphosate acid 500 g / l in the form of potassium salt), form HI TECH, manufacturer LLC «Syngenta» Russia, made in 2018, shelf life 3 years. Hazard class for humans-2, for bees-3. The drug is slightly toxic to birds and bees, but toxic to fish. It is recommended to process it in the morning or in the evening at a wind speed of 4-5 m / sec., the border zone is 2-3 km. The solution is prepared in 5 ml per 0.8 liters of water. Consumption 0.3 l / per 10 m²[1,2].

In the first series of experiments, all the preparations were tested by feeding bees with sugar syrup, or honey. For this purpose, an aqueous solution of the drug was prepared according to the manufacturer's recommendation for plant treatment. Then, in 10 ml of 50% sugar syrup, 0.1 ml of an aqueous solution of the drug was added, and in another case, 0.01 ml of an aqueous solution of the drug. Syrup or honey was fed from penicillin vials through 4 layers of gauze, placed in a special hole. After feeding the syrup (honey) with the drug, the consumption of feed by the bees, the behavior of the bees in the cage, and the time of death of the bees were monitored.

In the second series of experiments, a lemon leaf moistened with a prepared solution of the drug for spraying plants was placed at the bottom of the bee cage. The behavior of bees in cages and the time before their death were also monitored. In the control, a lemon leaf moistened with clean water was placed on the bottom of the cage (figure 1).

In the third series of experiments, the effect of all drugs on fumigant activity was tested. Cages with bees and pure sugar syrup were placed in a Plexiglass chamber, and a lemon leaf soaked in the preparation was placed on the bottom of the chamber away from the bees (excluding direct contact). The cell was loosely covered with a lid and left for the night. In the morning, the death of bees was taken into account (figure 2).
All experimental bees were placed in a fume hood at an ambient temperature of +25°C, and all drug concentrations in the experiments were given in accordance with the instructions prescribed by the manufacturers. Control groups of bees in similar conditions were in a different room.

3. Research results

When bees ate sugar syrup with imidacloprid, clinical signs of poisoning were observed after 45 minutes. The feed consumption was insignificant. In bees, excitement was noted. They beat the dorsal part of the chest and the head against the walls of the cage. Then the regurgitation of the contents of honey goiters was noted, and later the bees were oppressed. The bees lay on their sides and alternately moved their limbs, their death occurred after 1-2 hours.

In the cages, in which a moistened lemon leaf with imidacloprid was introduced, there was no contact of bees with the treated surface of the leaf, the bees rushed to the window in the cage, after 20 minutes, the excitement of the bees was observed, followed by depression, and after 1 hour, the death of 100% of the bees in the experimental group was observed.

Thus, imidacloprid and thiacloprid are highly toxic poisons for bees. Thiacloprid from the group of neonicotinoids cannot differ from other drugs with low toxicity. They have the same mechanism of action.

In the chamber where the fumigation of imidacloprid and thiacloprid took place, 100% death of bees was noted the next morning. In the control, the bees remained alive.

The lemon leaf, treated for the first time with neonicotinoids, remained in the chamber, and the bees were planted daily for 7 days in clean new cages with clean food. The chamber with the sheet was open during the day and was located in the fume hood. At the end of the working day, bees were placed there.

Accounting for the death of bees was noted in the morning of the next day. The death of bees in experimental cages was noted for 7 days.

After removing the dead bees from the cage in experiments with fumigation, the bottom of the cage was sticky due to regurgitation of the contents of honey goiters. The cage was washed with plenty of running cold water, wiped dry inside and out, and new bees were placed in these cages. Immediately noted the excitement, the bees began to beat the dorsal side of the chest against the walls of the cage. This phenomenon quickly ended within 5 minutes, the bees were calm, ate clean food, and on the 9th day they were depressed, regurgitating the contents of the honey goiter, the bees lay on their sides and moved their limbs.

Thus, imidacloprid and thiacloprid have a fumigant effect from the treated surfaces for 7 days. In addition, the accumulation and death of bees from small doses can be observed on the 9th day and later.

When removing the intestines from dead bees from poisoning, attention was paid to the color of the middle intestine, its thickness and the integrity of the rupture. With poisoning, the intestines of bees become thinner, become dark brown or black in color, and very easily tear when pulled out of the bee for the last segment of the abdomen. These studies should be carried out to confirm the diagnosis of chemical poisoning of bees (figure 3).
The intestines of bees: 1 – poisoning; 2,3,4,5 – normal.

The effect of «Roundup» and «Hurricane» preparations on bees: in laboratory conditions, 100% death of bees was detected when consumed with food. The death of bees after consuming sugar syrup with the drug, even from small doses, occurred after 15 minutes. In many dead bees, the wings were not pressed against the abdomen, they were not folded.

During the fumigation test, the death of bees was not established when the drug was found near bees for a long time.

Thus, roundup and hurricane are highly toxic drugs when ingested in the bee's intestines. The death of bees when using glyphosate acid occurs in 15 minutes.

4. Conclusion

Imidacloprid is a pesticide of the first class of danger for bees and the second class for mammals and humans. The mechanism of action consists in blocking postsynaptic acetylcholinesterase [3,4]. This mechanism of action is characteristic of the entire group of neonicotinoids. Our studies also confirm the high toxicity of thiacloprid for bees, despite the fact that the literature sources (including the State Catalog) describe the toxic effect only on insect pests of plants, and not on bees [1,2,5,6].

Simultaneously with the laboratory tests, we, with the help of beekeepers, monitored the clinical signs of poisoning, as well as the impact on bee families. So, in the Borovsky district of the Kaluga region, in one of the apiaries, the death of bee families occurred from the treatment of flowering rapeseed with the preparation of «Biscaya, MD». The bee families had printed honey in the second case. The bioassay in our laboratory did not reveal the presence of poison in the honey. The death in the families of the apiary in question lasted for 30-40 days. Although measures were taken to reduce the nest of bees, the dead bees were removed from the bottom of the hive.

The flight bees of the families died on the first day after processing, and their death continued for some time, since the bees were not closed before processing. Young, newly born bees (intra-bee bees) continued to die due to the fumigant action of thiacloprid, which evaporated from the bottom of the hive, moistened with the burp of dying bees and the accumulation of poison in the bee's body. These data obtained in laboratory experiments are confirmed in practice.

The following should be taken into account: pollen-collecting bees from processed rapeseed flowers bring poisoned pollen to the hive. They do not consume it, but only collect it and bring it to the hive, having time to put it in a cell. Young, newly born bees consume poisoned pollen. They die after that.

Thus, the death of bees will continue for some time, and the family of bees will not be able to survive under such conditions. Even after taking the frames of the printed brood from the hive to the insulator and leaving the cell of the honeycomb in the thermostat, the death of bees will continue, due to the consumption of poisoned pollen. As a result, the family of bees dies completely.
Honey from such families cannot be pumped out, it will be clogged with pollen grains with poison during centrifugation [3,4].

If the family of bees is closed before the treatment of crops with neonicotinoids, there will be no departure of bees, then when the poison is fumigated from the leaves of plants and soil for 7 days (which we noted in laboratory studies), the wind spread from the treated field can enter the hive through cracks and vents, and having a cumulative effect, will cause the death of bees. A similar thing was noted in an agronomist who treated a field of rapeseed with thiacloprid, closing the bees in the hives, however, after 3 days opened the bees-they died. The death of bees in the Kashirsky district of the Moscow region was noted in an apiary 12 km from a field with rapeseed. After processing, a moderate north-easterly wind (5-10 m/s) blew and vapors with neonicotinoids flew to the apiary. This fact confirms the results of our laboratory studies on the fumigant effect of neonicotinoids. It should be noted that when ingested through the bee's respiratory system, venom (drug) is required ten times less than with food to cause their death.

Thus, it is almost impossible to save bee families when treated with neonicotinoids. All restrictions on the removal of the apiary for 5-7 km from the cultivated field, prescribed in Federal Law of Russian Federation No. 109 of 19.07.1997, will not ensure its safety. The only way out is to stop using drugs from the group of neonicotinoids that have long-term fumigation and accumulation.

Based on this study, special attention should be paid to the use of imidacloprid-based drugs to control cockroaches and bedbugs in human habitation. Imidacloprid has a contact and systemic effect in the control of pests on plants. Imidacloprid acts by fumigating from the treated surface, according to our data given above, for 7 days and has a cumulative effect. We believe that this can also harm human health – this requires additional research in specialized medical research institutes.

Herbicides containing glyphosate acids – «Roundup BP», «Uragan forte BP», highly toxic drugs when ingested with food to bees. Death occurs after 15 minutes. The bee in such a short period of time will not be able to reach the hive and will die in the same field where it picked up the poison. This happens when the herbicide gets on a flowering plant from which bees collect nectar or pollen. When poisoning with roundup or another glyphosate-containing drug, there is no summer of bees in the apiary, there are no dead or crawling bees in front of the hives. It is not possible to take the dead bees for analysis. It is necessary to look for them in the field where the processing was carried out, or along the flight vector from the apiary to the flowering crops.

In the bee family, the weakening of the force in the streets is noted, if there is a large area of brood, the latter will not be incubated by bees. These preparations do not have fumigation, so closing the bees in a timely manner before processing can save them, waiting for the flower to wither and not be attractive to the bees, and the drug will completely evaporate and disperse in the atmosphere, open the hive.

These preparations are treated with hogweed, sunflower from the plane as a desiccant, so that the plant withers. The plume from the plane, can be blown away by the wind on flowering weeds on the outskirts of the field. All this threatens to poison the bees. The results of long-term use of both pesticides and herbicides for the life of bee colonies are still unknown. It is known that they persist in the soil for a long time, do not undergo hydrolysis and photolysis, and have a half-life of 120 days or more. This issue requires further study.

Thus, herbicides containing glyphosate acid, when ingested by bees with food, are highly toxic. Death in the apiary is difficult to identify. When making a diagnosis, it is necessary to exclude infectious diseases. But according to the complex of symptoms, we can conclude about bee poisoning: the mass weakening of families in the apiary or many apiaries in this area, the absence of bee summer in all apiary families (bees do not buzz in sunny weather and the presence of flowering honeybees).

5. Summary
Preparations from the group of neonicotinoids are highly toxic to bees [7,8,9], have a fumigant effect within 7 days after treatment and have a cumulative effect. The fumes of the poison are carried by the wind to considerable distances from the cultivated field, causing the mass death of bee families.
It is practically impossible to protect bees after treatment with insecticides of this group. The only way out is to abandon the use of drugs from the group of neonicotinoids, as the regulatory authorities from some EU countries have done.

It requires a thorough study of the preparations included in the Register of Pesticides and Agrochemicals in the Russian Federation, and an unambiguous ban on carrying out treatments during the flowering of entomophilic plants.

Herbicides based on glyphosate acid in violation of the requirements for use, namely: treatment of flowering plants with the drug, lead to the death of bees visiting them, and for an ordinary beekeeper, under certain conditions, the death may go unnoticed in the apiary due to what happens away from the hive.

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