

# Intoxication of honeybees on chemical treated winter rape: problem of its verification

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## Abstract

The reported experiments were to clear the dynamics of the decomposition of two potential toxic active substances chlorpyrifos (Nurelle) and fipronil (Regent) on plants and bees. The analyses were made using GC/MS method, the determination of chlorpyrifos was done by GC/NPD.

The content of active substances in stored plant samples was not rapidly changed. The content of active substances in dead bees stored to 4 days at room temperature was rapidly lower, but remained useful because of the applied methods.

Intoxication of bees after application of Regent and Nurelle are demonstrable if samples arrive at the laboratory in 4 days after plant treatment.

**Key words:** honeybee, rape, intoxication, fipronil, chlorpyrifos, GC/MS, GC/NPD.

## Introduction

The honeybee takes irreplaceable position in the winter rape pollination. High yields may be achieved by the use of adequate chemical protection.

In the last 5 years were found more cases of intoxication of bee colonies after the treatment of winter rape against weevils. The treatment of this crop plant was based on application of two preparations toxic to bee, Regent 800 WG and Nurelle D.

If the grower does not keep all details of treatment methods, or under some special circumstances, the bees are poisoned.

The beekeeper wants compensation, but the grower does not accept it without evidence of coherence in plant protection and intoxication of bees.

Although the visual symptoms of dying of bees are typical for intoxication, the routine laboratory analyses is very often negative. The beekeeper does not get compensation and the grower has no interest to improve methods of plant protection.

We need better methods of analyses of pesticides residues on bees and plants.

The topic of the reported experiments was to study the dynamics of the decomposition of fipronil (Regent 800 WG) and chlorpyrifos (Nurelle D) on plants and bees.

## Materials and methods

The experiments were established on the plots and at the laboratory of the Bee Research Institute in Dol near Prague.

Plant and bee samples were processed in the Institute of Chemical Technology, Prague.

### Residues of active substances on treated plants

#### Experimental plots

Three insulators "Nurelle", "Regent" and "Control" were placed on a flowering winter rape plots 5 m<sup>2</sup> large.

We treated the rape stand in the insulator by spraying Nurelle D solution adequate to common dose (0.6 liter in 500 liter water per hectare) and Regent 800 WG (25 g in 500 liter water per hectare) respectively. The "Control" insulator was without treatment.

#### Trial 1 Sampling:

We took samples of leaves on treated plants each day in the period of 9 days after treatment. The samples were put immediately into the freezing box.

#### Trial 2 Sampling:

From plants treated as described in "Experimental plots" we took samples of leaves 24 hours after the treatment:

- One 1/3 of samples was put immediately into the freezing box.
- The second 1/3 was left 48 hours at room temperature (20 - 24°C) and then placed into the freezing box.
- The third 1/3 was left 96 hours at room temperature (20 - 24°C) and then placed into the freezing box.

#### Quantification of active substances

Matrix was extracted by acetonitril, determination of fipronil and their degradation products by MB45950 (photodegradation) and MB46136 (oxidation) and the confirmations of results were carried out by GC/MS method. The determination of chlorpyrifos was done by GC/NPD.

#### Simulated intoxication of bees and its possible evidence

##### Preparation of bees

Each bee from the group of 60 bees was individually treated by preparations as given in the following scheme:

The dose of active substances applied on individual bees in the model experiment starts from common doses

described in “Experimental plots”. Active substances were applied topically on individual worker bees by plastic pipette tip in acetone solution, volume 1.5 µl per a bee (table 1).

The samples of bees with the same level of active substance were sampled and stored:

- One 1/3 of samples was put immediately into the freezing box.
- The second 1/3 was left 48 hours at room temperature (20 - 24°C) and then placed into the freezing box.
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## Results

### Residues of active substances on treated plants

The data are reported in tables 2 and 3.

### Simulated intoxication of bees and its possible evidence

The data are reported in tables 4 and 5.

**Table 1.** Basic dose adequate to 0.5 cm<sup>2</sup> treated area.

Samples	Dose	number of bees	individual dose
Regent	1	20+20+20	fipronil 0.1 µg/bee
Nurelle	1	20+20+20	chlorpyrifos 1.5 µg/bee
Control	0	20	0

Remark: LD<sub>50</sub> of fipronil: 5 ng/bee; LD<sub>50</sub> of chlorpyrifos: 60 ng/bee.

**Table 2.** Trial 1: content of active substance fipronil and chlorpyrifos on rape leaves after treatment; sampling as described in “Experimental plots”. Trial 1 (content of active substance in %, 100% = content of active substance after spraying).

Time after spraying	fipronil (%)	chlorpyrifos (%)
1 <sup>st</sup> day	100	100
2 <sup>nd</sup> day	51	46
3 <sup>rd</sup> day	49	39
4 <sup>th</sup> day	39	48
5 <sup>th</sup> day	22	26
6 <sup>th</sup> day	6	26
7 <sup>th</sup> day	5	16
8 <sup>th</sup> day	1	8
9 <sup>th</sup> day	1	15

**Table 3.** Trial 2: content of fipronil and chlorpyrifos on rape leaves after treatment; storage and sampling as described in “Experimental plots”. Trial 2.

Free storage time	fipronil %	chlorpyrifos %
0	100	100
2 days	97	93
4 days	102	92

**Table 4.** Trial 3: content of fipronil and chlorpyrifos on bees after treatment; storage and sampling as described in “Preparation of bees”.

Free storage time	Fipronil %	chlorpyrifos %
0	100	100
2 days	63	24
4 days	38	24

**Table 5.** Decomposition of active substances fipronil and chlorpyrifos on bees after treatment; sampling as described in “Preparation of bees”.

Free storage time	Fipronil ( $\mu\text{g}/20$ bees)	MB45950 ( $\mu\text{g}/20$ bees)	MB46136 ( $\mu\text{g}/20$ bees)	chlorpyrifos ( $\mu\text{g}/20$ bees)
	Total dose 2,000	-	-	Total dose 30,00
0	1,703	0,019	0,159	20,55
2 days	1,077	0,011	0,945	4,89
4 days	0,645	0,005	0,707	4,89

## Conclusions

### Monitoring of the decomposition of active substances on plants

At Regent 800 WG the collected leaves indicated toxicity up to the 5th day after the treatment, at Nurelle D up to 8th day after the treatment. Content of fipronil on plants sinks considerably the 6th day after the treatment. Content of chlorpyrifos was reduced more slowly.

### Simulated intoxication of bees and its possible evidence

The content of active substances in plant samples collected for the treatment and successively stored in the freezing box was not changed. The content of active substances in dead bees stored to 4 days at room temperature at lower doses than  $LD_{50}$  was rapidly lower, but remained useful because of the applied methods. Intoxication of bees after application of Re-

gent 800 WG and Nurelle D are on the basis of applied chemical methods demonstrable if samples are analyzed in the term no more than 4 days after the treatment.

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## References

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