THE DIRECTOR GENERAL

Maisons-Alfort, 31 May 2012

# OPINION
## of the French Agency for Food, Environmental and Occupational Health & Safety

### in response to a request for scientific and technical support following publication of the article entitled *"A common pesticide decreases foraging success and survival in honey bees"*



On 23 March 2012, ANSES received a formal request from the Directorate General for Food (DGAL) for scientific and technical support, following publication of the article entitled *"A common pesticide decreases foraging success and survival in honey bees"* written by Mickaël Henry, Maxime Béguin, Fabrice Requier, Orianne Rollin, Jean-François Odoux, Pierrick Aupinel, Jean Aptel, Sylvie Tchamitchian and Axel Decourtye.

This article, which was initially published in *Sciencexpress* on 29 March 2012, appeared in the journal *Science* 336 p.348-350 on 20 April 2012.

It should be noted that on 3 April 2012, EFSA[1] had received a request from the European Commission for scientific and technical support related to two articles that had been published in the journal *Science*, including the above-mentioned article by Henry *et al.*. In this context, ANSES and EFSA initiated a collaborative process to exchange data.

## 1. BACKGROUND AND PURPOSE OF THE REQUEST

The referenced article suggests that exposure of bees to sub-lethal doses of the active substance thiamethoxam causes a number of behavioural impairments in bees and, by altering their homing skills, may contribute to bee-colony weakening at a level likely to place the hive in a critical situation.

ANSES has been requested to determine whether the dose administered in the experiment reported in the article corresponds to situations that represent natural conditions of bee exposure and whether these studies are likely to call into question the conclusions of the previous risk

[1] EFSA: European Food Safety Authority

assessments undertaken on the active substance thiamethoxam and the various products that contain it.

## 2. Organisation of the expert appraisal

The expert appraisal was carried out in accordance with the French standard NFX50-110 "Quality in Expert Appraisal Activities – General Requirements of Competence for Expert Appraisals (May 2003)".

In the context of this request, the DGAL requested CETIOM[2] to take nectar samples from oilseed rape flowers in order to determine the sugar concentrations of nectar from various varieties of oilseed rape treated with CRUISER OSR[3] [4] and the corresponding thiamethoxam and clothianidin concentrations. The protocol summary and the results of the investigation are presented in this Opinion.

On 16 May 2012, the Agency and two experts from the “Expert Committee on Plant protection products: chemical substances and preparations” held a hearing of the authors of the referenced article, CETIOM, which implemented the protocol for sampling nectar from oilseed rape, and at the request of a beekeeper association, two experts in the fields of apiology and beekeeping techniques.

Following these hearings, and after consulting with the “Expert Committee on Plant protection products: chemical substances and preparations”, which met on 30 May 2012, the French Agency for Food, Environmental and Occupational Health & Safety is issuing the following Opinion.

## 3. The Agency’s conclusions and recommendations

### Summary of the protocol and results according to the authors

This section describes the study’s methodology, experiments and results as reported by the authors in the available documents. Some points were clarified during the hearing with the authors on 16 May 2012.

The aim of the study was to test the assumption according to which exposure to a sub-lethal dose of a neonicotinoid substance indirectly increases bee colony mortality by altering forager bees' ability to return to the hive. The effects on homing of a single administration of a sub-lethal dose of thiamethoxam (1.34 ng/bee) were measured using RFID (Radio-Frequency Identification)[5] microchips glued to the thorax of forager bees and recording detectors installed at the entrance to the hives. The impact of the observed effects on colony dynamics was then evaluated using a model of bee population dynamics described in the literature[6].

Each group of bees treated with 1.34 ng of thiamethoxam/bee was compared to a control group.

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[2] French technical centre for research and development of production procedures for oilseeds and industrial hemp

[3] CRUISER OSR contains 280 g/L of thiamethoxam (minimum purity 98%) [insecticide] 8 g/L of fludioxonil (minimum purity 95%) [fungicide] and 32.3 g/L of metalaxyl-M (minimum purity 91%) [fungicide], in the form of a flowable concentrate for seed treatment (FS)

[4] ANSES's Opinion of 15 October 2010 on an application for marketing authorisation (MA) for the CRUISER OSR formulation containing thiamethoxam, fludioxonil and metalaxyl-M by the company SYNGENTA AGRO SAS. Marketing authorisation no. 2100180.

[5] mic3®-TAG 64-bit RO, iID2000, 13.56 MHz system, 1.0×1.6×0.5mm; microsensys GmbH, Erfurt, Germany

[6] S. Khoury, M. R. Myerscough, A. B. Barron, A quantitative model of honey bee colony population dynamics. *PLoS ONE* **6**, e18491 (2011). doi:10.1371/journal.pone.0018491 Medline

<u>Study experiments</u>

Four experiments, each comprising a treated group and a control group, were undertaken in different conditions with respect to the pathway back to the hive. Experiments 1 and 3 used bees that were considered familiar with the pathway back to the hive. They were released at a distance of 1 km (experiment 1) and 70 m (experiment 3) from their colony. Experiments 2 and 4 used foragers considered unfamiliar with the pathway back to the hive. They were released from an intensive farming environment (experiment 2) and a suburban environment (experiment 4) at a distance of 1 km from their colony.

The foragers were captured in the morning upon returning to the hive at the hive entrance and then transported to the laboratory. Their nutritional status was synchronised through the *ad libitum* provision of a professional beekeeping candy for 60 minutes followed by a 90-minute fasting period. Each bee was then individually fed 20 µL of sucrose syrup (50% weight/weight) that either did or did not contain thiamethoxam. Only those bees that consumed the full 20 µL were used for the experiment. Each bee was fitted with an RFID microchip and was kept in the laboratory for 40 additional minutes to allow the administered dose to be fully assimilated before being released on the chosen site. RFID readers positioned between the body of the hive and its entrance recorded the time at which each bee first returned to the hive after release. Recording continued for five to seven days after release to cover all returning bees. In all of the experiments, the control and treated bees were released simultaneously at each point, and for each experiment, the bees were released over two or three consecutive days. The collected data were transferred to a computer and saved as .txt files.

<u>Administered dose of thiamethoxam</u>

The dose of 1 ng thiamethoxam/bee was used, since:

- it is lower than the oral $LD_{50}$[7] for bees for this active substance[8];
- the article's authors had verified that it does not induce mortality;
- a preliminary experiment had determined that it had an effect on the homing rates and times of treated bees released at a distance of 1 km.

The analysis undertaken in the glucose syrup showed that the actual administered dose was 1.34 ng/bee.

<u>Pathways back to the hive and choice of sites</u>

The bees considered familiar with the pathway back to the hive ('familiar' bees, experiments 1 and 3) were selected after they had been captured carrying *Phacelia* pollen, a blue pollen that is easily recognisable, from a single field of this crop located in the experimental zone. In the experiment, they were released from this field.

The bees that had not returned with *Phacelia* pollen were released at six sites selected for the experiment, not including the *Phacelia* field, that were located 1 km from the hive. The bees' prior knowledge of the site from which they were released, and the pathway back to the hive, was random (experiments 2 and 4). These bees were therefore considered 'unfamiliar' with the return to the hive (but not with foraging, which they had already practised the day before the experiment).

The experiments were conducted in two zones:

- the 450 $km^2$ 'Zone Atelier Plaine et Val de Sèvre' farming zone (Deux-Sèvres *département*[9]), whose landscape, farming practices and domestic and pollinating bee populations are subject to georeferencing, for experiments 1 to 3;
- the suburban area of the Avignon INRA station (Vaucluse *département*), for experiment 4.

<u>Processing of homing data</u>

For each experiment, the group of treated bees was compared to a control group.

Cumulative homing probability as a function of time (calculated based on the time taken for the bees to first return to the hive after being released) was presented in the article in the form of a

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7 $LD_{50}$ (Lethal Dose 50%) is a statistical value expressing a single dose of a substance/preparation that, when administered orally, kills 50% of the treated animals.

8 For acute oral exposure in adult bees, the $LD_{50}$ is equal to 0.005 µg of thiamethoxam/bee.

9 administrative division

curve, with the x-axis representing the time after release, from 0 to 3000 minutes, and the y-axis representing cumulative homing probability, from 0 to 1.

The statistical analysis that was undertaken compared the treated group and the control group, using an exact binomial test comparing the cumulative probabilities obtained four hours after release and at the end of the experiment. When the difference was statistically significant ($P<0.05$), mortality due to homing failure, $m_{hf}$, was calculated using the following formula:

$$m_{hf} = \frac{\text{homing probability for control bees} - \text{homing probability for treated bees}}{\text{homing probability for control bees}}$$

$m_{hf}$ was therefore an estimate of the proportion of foragers not returning due to the treatment, excluding other causes of homing failure such as natural mortality, predation and handling stress.

The $m_{hf}$ values obtained in experiment 1 ('familiar' bees) and experiment 2 ('unfamiliar' bees) were considered representative of the lower and upper bounds, respectively, of the proportion of treated foragers not returning to the hive.

Population dynamics model

Population dynamics were simulated by introducing the measured $m_{hf}$ values (lower and upper bounds) into a model described in the literature[10] in order to describe best- and worst-case scenarios for population dynamics. The first three months of a beekeeping season after the wintering period were taken into account so as to include oilseed rape's flowering period, with the following parameters:

- the queen bee's daily egg-laying rate (2000, 1800 or 1600 eggs/day),
- the proportion of forager bees exposed each day (50 or 90%),
- the size of the colony at the start of exposure (15 000 or 18 000 bees),
- the proportion of forager bees: 25% of the total population,
- the natural mortality of forager bees: 0.154 individuals/day based on an average life span of 6.5 days, or a mortality rate of 15.4%,
- the other parameters were those defined in the original model.

The following assumptions were used for the simulations:

- for unexposed forager bees, a constant mortality rate,
- for exposed forager bees, a 30-day exposure period, with the mortality rate increasing by the proportion of bees not returning to the hive (natural rate of mortality + $m_{hf}$) each day.

Results

On the curves showing cumulative homing probability as a function of time, the treated groups in all four experiments appeared to have homing rates that were lower than those of the control groups.

Comparing the cumulative probabilities obtained four hours after release and at the end of the experiment gives the following results:

10 S. Khoury *et al*, *op. cit*.

**Table 1**

| | **Experiment 1:** 'Familiar' bees released 1 km from the hive – intensive farming area - ***(treated-control)*** | **Experiment 2:** 'Unfamiliar' bees released 1 km from the hive – intensive farming area - ***(treated-control)*** | **Experiment 3**: 'Familiar' bees released 70m from the hive – intensive farming area - ***(treated-control)*** | **Experiment 4:** 'Unfamiliar' bees released 1 km from the hive – suburban area - ***(treated-control)*** |
|---|---|---|---|---|
| Number of released forager bees | 72-74 | 118-118 | 67-68 | 82-54 |
| Homing probability 4 hours after release | 68.1%-81.1% | 33.9%-57.6% | 67.2%-82.4% | 68.3%-81.5% |
| (Exact binomial test for homing proportions) | ($P$=0.005) | ($P$<0.001) | ($P$=0.002) | ($P$=0.003) |
| Homing probability at the end of the experiment | 76.4%-85.1% | 56.8%-83.1% | 92.5%-98.5% | 76.8%-85.2% |
| (Exact binomial test for homing proportions) | ($P$=0.036) | ($P$<0.001) | ($P$=0.003) | ($P$=0.029) |
| Homing failure mortality induced by treatment ($m_{hf}$) | 0.102 | 0.316 | 0.061 | 0.098 |

The percentages of bees that returned to the hive differed significantly between the treated group and the control group, in all of the experiments, four hours after the foragers were released and at the end of the experiment.

Six demographic scenarios were simulated with the population dynamics model, taking into account homing failure induced by bee exposure to thiamethoxam with the measured $m_{hf}$ values of 0.102 and 0.316 from experiments 1 and 2. Graphs illustrating time (0 to 90 days) on the x-axis and total population size on the y-axis showed the corresponding changes in the bee population, for the control and treated groups, based on the various input parameters indicated above.

Simulations A and D were performed with a daily egg-laying rate of 2000 eggs (normal development), simulations B and E with a daily egg-laying rate of 1800 eggs (equilibrium dynamic) and simulations C and F with a daily egg-laying rate of 1600 eggs (deficient development). The period of exposure to thiamethoxam was 30 days. Scenarios A, B and C were computed when 90% of forager bees were exposed and scenarios D, E and F when 50% of forager bees were exposed each day during the exposure period.

In all cases, the size of the exposed colonies decreased during the exposure period and then increased after exposure but did not reach the size of the unexposed colonies after three months. In the worst-case simulations (A, B and C, 90% of foragers exposed, maximum $m_{hf}$), colonies initially containing 15 000 bees could drop to or below 5000 bees after one month of exposure. In the event of longer exposure (90 days instead of 30), the plots show a continuous decline until death of the colony.

The authors conclude that this study clearly demonstrates that exposure of foragers to non-lethal but commonly encountered concentrations of thiamethoxam can impact forager survival, with potential contribution to the risk of colony collapse. The extent to which exposure affects forager survival appears dependent on the landscape context and the foragers' prior knowledge of this landscape. Higher risks are observed when the homing task is more challenging. As a result, impact studies are likely to underestimate sub-lethal pesticide effects when they are conducted on bee colonies placed in the immediate vicinity of treated crops by not showing possible effects on homing flights.

**GENERAL COMMENTS ABOUT THE ARTICLE**

The experimental study described above presents an original approach to the behavioural assessment of forager bees exposed to a plant protection substance. The RFID technique tracks the homing behaviour of individual bees. The administered dose of thiamethoxam is precisely known. The protocol uses an elegant method for taking into account several levels of difficulty in the homing flights of forager bees. The results clearly show that the administered dose of thiamethoxam has an impact on homing.

However, from a methodological standpoint, a few points should be clarified.

Composition of the groups of 'unfamiliar' forager bees
The bees considered familiar with the pathway back to the hive ('familiar' bees) were selected after having returned to the colony, before the experiment, with *Phacelia* pollen from a single field of this crop, from which they were then released.

The bees that had not returned with *Phacelia* pollen ('unfamiliar' bees) were released on six sites selected for the experiment, not including the *Phacelia* field, that were located 1 km from the hive. The bees' prior knowledge of the site from which they were released and of the pathway back to the hive was random. In these experiments 2 and 4, the proportion of forager bees that were already familiar with the return pathway was therefore not known. It can be considered that this proportion was the same in the control and treated groups. Nevertheless, none of the available data can confirm this hypothesis.

It should be noted that a significantly different proportion of bees familiar with the return pathway between the control and treated groups would result in a bias that would overestimate or underestimate the effects, thus limiting the interpretation of these experiments' results.

Statistical analysis
The statistical analysis undertaken compared the treated group and the control group, using an exact binomial test comparing the cumulative probabilities obtained four hours after release and at the end of the experiment.

The exact binomial test is used to compare an observed percentage and a theoretical percentage. The comparisons made in the article were different, since the two percentages that were compared both came from field observations made in the treated group and control group. Fisher's exact test is an appropriate test for comparing two observed percentages. Considering the relatively large sizes of the various groups of bees in the study, a chi-squared test would also be relevant.

It should be pointed out that when Fisher's exact test is used to compare the cumulative probabilities obtained four hours after release and at the end of the experiment in the treated and control groups, based on the data presented in the article, it produces *P* values that are different from those in Table 1:

**Table 2**

| | **Experiment 1**: 'Familiar' bees released 1 km from the hive – intensive farming area - ***(treated-control)*** | **Experiment 2:** 'Unfamiliar' bees released 1 km from the hive – intensive farming area - ***(treated-control)*** | **Experiment 3:** 'Familiar' bees released 70m from the hive – intensive farming area - ***(treated-control)*** | **Experiment 4:** 'Unfamiliar' bees released 1 km from the hive – suburban area - ***(treated-control)*** |
|---|---|---|---|---|
| Number of released forager bees | 72-74 | 118-118 | 67-68 | 82-54 |
| Homing probability 4 hours after release | 68.1%-81.1% | 33.9%-57.6% | 67.2%-82.4% | 68.3%-81.5% |
| (Fisher's exact test for homing percentages) | ($P$=0.0874) | **($P$<0.001)** | **($P$=0.0489)** | ($P$=0.1126) |
| Homing probability at the end of the experiment | 76.4%-85.1% | 56.8%-83.1% | 92.5%-98.5% | 76.8%-85.2% |
| (Fisher's exact test for homing percentages) | ($P$=0.210) | **($P$<0.001)** | ($P$=0.115) | ($P$=0.276) |
| Homing failure mortality induced by treatment ($m_{hf}$) | 0.102 | 0.316 | 0.061 | 0.098 |

The *P* values are those obtained for a bilateral test.

According to the results of these tests, only the three comparisons that give a bold *P* value in the above table are considered as showing a significant difference between the control group and the treated group.

<u>Population dynamics model</u>
Population dynamics were simulated by introducing the measured $m_{hf}$ values into a mathematical model in order to assess the impact on the colony of mortality caused by homing failure in exposed bees.

The model used was described in a publication by Khoury *et al*. (2011). According to the authors, it is a very simple theoretical model developed solely to predict how forager death rates affect the recruitment of other workers in the hive into the foraging population. In fact, when there is a decline in the number of foragers, the development of young bees is accelerated and they begin foraging precociously to compensate. However, it has been shown that these young bees that are precociously recruited for foraging are less efficient and have a reduced life span (up to 2.8 days versus 6.5 days on average for a normal forager). The model by Khoury *et al*. was designed to highlight effects related to this phenomenon and deliberately ignores all other mechanisms that make hive population dynamics much more complex. This simplification can also be found in the modelled phenomenon. For example, the model considers that the queen's laying rate is constant over time whereas it varies depending on the season, resource availability, etc. There is no actual brood. Cannibalism of larvae is not taken into account whereas it is a significant phenomenon in regulating the bee population.

This extremely simple theoretical model cannot therefore be used to simulate the dynamics of a bee population *in situ*.

### REPRESENTATIVENESS OF THE ADMINISTERED DOSE WITH REGARD TO EXPOSURE IN FIELD CONDITIONS

Sub-lethal nature of the administered dose of thiamethoxam

The dose that was orally administered to each forager bee individually was a single dose of 1.34 ng/bee in 20 µL of sucrose syrup (50% w/w). The non-lethal nature of the dose administered selectively in the experiment by Henry *et al*. (2012) was verified in a laboratory.

This is consistent with the data from the application dossier for the active substance, which shows that the lethal dose killing 50% of bees in a 48-hr. period after oral ingestion (acute $LD_{50}$) is 5 ng/bee. The dose of 1.34 ng/bee is also lower than the sub-lethal dose of 2 ng/bee with acute administration but is higher than the daily no-effect level after ten days of repeated exposure of 0.2 ng/bee/day.

In a study submitted in the application dossier for the active substance, in which forager bees were released 500 m from their colony, bees fed with a feeder containing 10 µg of thiamethoxam/L (corresponding to average exposure of 1.1 ng/bee) all returned to the hive within the next 24 hours, whereas absences were recorded in bees fed 25 µg of thiamethoxam/L (corresponding to average exposure of 3.0 ng/bee). It should be noted that exposure to the active substance occurred in the context of daily consumption and not a single dose like in the experiment reported by Henry *et al*. (2012), which also used a more precise measurement methodology.

Principle for calculating the exposure of forager bees

Forager bees eat nectar and are therefore exposed to residues of the active substances it contains. The quantity of nectar eaten to fulfil energy requirements, which themselves are related to a bee's flight time, depends on its sugar content. The amount needed is higher when the sugar content is low.

The article by Rortais *et al*. (2005) indicates that forager bees consume 32 to 128.4 mg of sugar per day, for flight times ranging from 4 to 10.7 hours.

In the risk assessment undertaken by ANSES in the framework of the application for marketing authorisation (MA) of CRUISER OSR, a plant protection product containing thiamethoxam that is intended for the treatment of oilseed rape crops, maximum bee exposure was taken into account, to illustrate a 'worst-case' scenario, and 128.4 mg of sugar consumption/day was used in the calculation.

If X is the sugar concentration (%) and Y is the concentration of residues in the nectar (µg/kg), theoretical exposure corresponding to the 'worst-case' consumption scenario is obtained with the following formula:

$$\text{Exposure level (ng/bee/day)} = 128.4 \times Y \times 0.1/X$$

Calculation made by the authors

The authors claim that the dose of 1.34 ng/bee is representative of the exposure of bees that forage the nectar of winter oilseed rape treated with CRUISER OSR.

In this study, the residue level of the active substance in the nectar was the mean concentration measured in the nectars collected in the stomach of forager bees exposed in a tunnel during the flowering period of treated winter oilseed rape (1.85 µg/kg), as reported in the ANSES Opinion.

The sugar concentrations in oilseed rape nectar used by the authors came from a study by the INRA[11] research station at Rennes which was published in 1999[12] and that reported concentrations

[11] French national institute for agricultural research

ranging from 10.6 to 30.2% for the 'Samourai' oilseed rape variety and from 8.3 to 66.6% for other varieties.

Exposure levels in 'worst-case' sugar consumption scenarios are therefore 1.19 ng/bee/day for nectar containing 20% sugar and 2.38 ng/bee/day for nectar containing 10% sugar.

For a bee that flies for only four hours per day and eats only 48 mg of sugar, the theoretical level of exposure is 0.89 ng/bee/day. It is 0.20 ng/bee/day for a bee that eats 32 mg of sugar.

Calculation performed by ANSES (Opinion of 15 October 2010 on the CRUISER OSR formulation)
Considering consumption of 128.4 mg sugar/day, a residue level for the active substance of 1.85 µg/kg and a 40% sugar concentration in the nectar, the level of exposure in 'worst-case' consumption scenarios is 0.59 ng/bee/day.

This sugar concentration, which was initially extrapolated from the sugar content of a sunflower nectar described in the publication by Rortais *et al*. (2005), was also cited for oilseed rape nectar in a classic bee-research publication (Vaissière *et al.*, 2002)[13]: "Nectar secretion: 0.2 to 2 mg/flower/day with 40 to 60% sugars".

Discussion on sugar concentrations in oilseed rape nectar
The sugar content of oilseed rape nectar is an extremely important parameter when calculating exposure. Indeed, the lower the sugar content, the more nectar is consumed by forager bees to fulfil their energy requirements, and therefore at equal residue concentrations, the more foragers are theoretically exposed.

The sugar concentrations taken into account by the authors in their calculation came from the publication by Pierre *et al. (*1999), which describes quantitative and qualitative variability in nectar secretion among 71 genotypes of winter oilseed rape. The aim of the study was to examine the possibility of selecting genotypes, including male-sterile lines, that secrete sufficient amounts of nectar to attract bees to ensure pollination of male-sterile lines for hybrid seed production. The sugar concentrations used in the calculations were those measured for a 'Samourai' male-fertile line. The 'Samourai' male-fertile line was the reference for group 1, which included the 64 conventional male-fertile lines. Sugar concentrations were very different for the 'Fu 27-Hokkaido/Bienvenu' F1 male-sterile and male-fertile lines in group 3 in which they reached 66.6% on one of the collection dates. A 'Darmor' variety had extremely low concentrations (8.2%). The attractiveness of oilseed rape nectar to bees in these conditions is questionable.

These data indicate that sugar concentrations and nectar quantities are linked to oilseed rape varieties, the flowering stage and weather conditions.

Analyses undertaken in 2012 in oilseed rape treated with thiamethoxam
Cultivated varieties of winter oilseed rape are steadily and rapidly changing.

On 2 and 11 May 2012, using micropipettes, CETIOM took samples of floral nectar from winter oilseed rape in five fields of this crop treated with CRUISER OSR, in order to analyse sugar concentrations and residues of active substances (thiamethoxam and its metabolite, clothianidin). The five varieties of oilseed rape in which nectar was collected (DK EXSTORM, ADRIANA T, PAMELA, DK EXQUISITE and EXOCET) accounted for 30% of the surface area given over to winter oilseed rape in 2011-2012. The samples of 2 May corresponded to the 'full flower' stage and those of 11 May corresponded to the 'early end of flowering' stage.

Sugar concentrations were measured using a portable electronic refractometer immediately following field sampling.

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12 Pierre J., Mesquida J., Marilleau R., Pham‐Delègue M.H., Renard M. (1999). Nectar secretion in winter oilseed rape, *Brassica napus* ‐ quantitative and qualitative variability among 60 genotypes. Plant Breeding 118:360‐365.

13 Vaissière *et al*., 2006, Chapitre IV : Pollinisation, apiculture et environnement, in Le traité Rustica de l'apiculture, Rustica Editions.

The samples were sent to the ANSES laboratory in Sophia-Antipolis for residue analyses that were undertaken using an LC-MS/MS method (LoQ[14] = 0.3 ng/mL, LoD[15] = 0.1 ng/mL).

The available results, in addition to a theoretical calculation of exposure levels in 'worst-case' sugar consumption scenarios, are presented in the following table.

**Table 3**

| Variety | Date | Mean sugar concentration (%) | Thiamethoxam concentration (ng/mL) | Clothianidin concentration (ng/mL) | Volume of nectar per 128.4 mg sugar (mL) | Exposure level (ng/bee) |
|---|---|---|---|---|---|---|
| DK EXSTORM | 02/05/2012 | 32.6 | 0.4 | <LoD | 0.39 | 0.16 |
| ADRIANA T | 02/05/2012 | 28.7 | 0.4 | <LoD | 0.45 | 0.18 |
| PAMELA | 02/05/2012 | 33.3 | 0.7 | <LoD | 0.39 | 0.27 |
| DK EXQUISITE | 02/05/2012 | 34.7 | 0.4 | <LoD | 0.37 | 0.15 |
| EXOCET | 02/05/2012 | 25.8 | 0.4 | <LoD | 0.50 | 0.20 |
| DK EXSTORM | 11/05/2012 | 59.5 | 0.7 | <LoD | 0.22 | 0.15 |
| ADRIANA T | 11/05/2012 | 51.0 | 0.8 | <LoD | 0.25 | 0.20 |
| PAMELA | 11/05/2012 | 66.6 | 1.6 | <LoD | 0.19 | 0.31 |
| DK EXQUISITE | 11/05/2012 | 50.5 | 1.3 | <LoD | 0.25 | **0.33** |
| EXOCET | 11/05/2012 | 61.4 | 0.5 | <LoD | 0.21 | 0.10 |

Furthermore, nectar samples collected on 7 May 2012 from untreated oilseed rape used as a negative control showed sugar concentrations ranging from 13.7 to 21.2%.

The mean and maximum exposure levels were respectively 0.21 and 0.33 ng/bee and per day.

While concentrations of clothianidin, which were lower then the limit of detection in all the samples, were considered equal to 0.1 ng, the mean and maximum exposure levels were respectively 0.24 and 0.36 ng/bee and per day.

These values, calculated based on analyses undertaken in 2012, reflect daily exposure in situations of 'worst-case' sugar consumption, which is lower than the theoretical exposure of forager bees to nectar that was estimated at 0.59 ng/bee/day by ANSES and the value of 1.34 ng/bee administered as a single dose in the experiment by Henry *et al*. (2012).

<u>Conclusion regarding the administered dose</u>
The theoretical calculations made by the article's authors were based on high sugar consumption, low sugar concentrations in nectar and a residue level of thiamethoxam in nectar obtained in a worst-case exposure scenario involving bees in a tunnel.

In light of the observed variability in sugar concentrations in oilseed rape, such a field situation cannot be excluded.

However, recent analyses confirm that oilseed rape nectar can contain very high sugar concentrations, particularly in weather conditions in which bees engage in intense foraging activity. The exposure level calculated based on sugar concentrations and residue levels in 'worst-case' sugar consumption scenarios was, in all the samples, lower than the dose administered in the study.

[14] LoQ: Limit of Quantification.
[15] LoD: Limit of Detection.

The authors' interpretation according to which the dose of 1.34 ng of thiamethoxam/bee is commonly encountered in the field is therefore not considered to be confirmed by the available observations.

However, experiments in conditions directly exposing forager bees to the residues in the crop would be necessary to specify the effects on homing in field situations.

EFSA, whose Opinion was formally requested on the same issue, concluded based on the data collected from all of the Member States, that the concentration of thiamethoxam in the syrup administered to the bees in the study by Henry *et al*. (2012) was approximately ten times higher than the maximum concentration observed in a sample of nectar and that, although actual bee exposure may theoretically exceed the dose administered in the study, such exposure occurs in the field only gradually over a day of foraging.

### REPRESENTATIVENESS OF THE EXPERIMENTAL CONDITIONS WITH REGARD TO FIELD REALITY

Homing was measured in forager bees fitted with RFID microchips that returned to their hive after having been released on a site 1 km from the hive and that had not made the trip from the hive to this site immediately before. The experimental design used forager bees that were considered either familiar or unfamiliar with the pathway back to the hive.

The bees considered familiar with the pathway back to the hive (experiments 1 and 3) were selected after they had returned to the hive carrying *Phacelia* pollen, a blue pollen that is easily recognisable, from a single field of this crop located in the experimental zone. They were released on this field.

The bees that had not fed on *Phacelia* pollen were released on six sites selected for the experiment located 1 km from the hive, excluding the *Phacelia* plot. Their prior knowledge of the site from which they were released and the pathway back to the hive was random (experiments 2 and 4). In experiment 2, the bees were released in an intensive farming environment with few visual landmarks. In experiment 4, the bees were released in a suburban environment in which there were by definition more landmarks.

Whether or not these conditions were representative of reality is worth discussing. In fact, in normal situations, forager bees return to the hive after having left it, even after transhumance. Comparing the homing curves from experiments 1 and 2 shows that bees released on a site from which their knowledge of the pathway back to the hive is random experience significant stress, as some of the bees only returned to their colony the following day.

The field trials submitted with the MA application for the CRUISER OSR plant protection product also had limitations but they were not the same. In fact, in order to determine maximum exposure to residues of the active substance in bees, the field trials were performed by placing the hives as close as possible to the treated crops. Even though this situation may be encountered in the field, in the case of hives installed in front of a foraging area, since the bees do not have a sufficiently long distance to cover to return to the hive after foraging, these trials risk not showing that exposure to the active substance affects their motor or sensory capacities or their orientation skills.

In that sense, implementing field trial protocols that keep the hives at a certain distance, while ensuring realistic exposure of bees to the tested substance would make it possible to study a situation in which the locomotive, sensory and orientation skills of forager bees are put more severely to the test.

### CONSEQUENCES FOR PAST RISK ASSESSMENTS

The experimental study described in the article by Henry *et al*. (2012) presents an original approach to the behavioural assessment of forager bees exposed to a plant protection substance. The protocol and the RFID technique allow for the homing behaviour of individual bees to be tracked. In this way, the potential effects of compounds that may affect their locomotive, sensory or orientation skills can be understood.

However, the article's conclusion regarding the potential long-term effects on the colony relies on the use of a model. No experimental studies are currently available comparing effects on the homing of individual bees and actual effects on colonies. And yet, the conclusions of the risk assessment undertaken before the plant protection products were marketed were based on a series of trials that resulted in a multi-stage assessment. The final stage involved trials undertaken in a tunnel and in the field. In these trials, bee colonies were exposed to treated crops. The effects of bee exposure related to foraging activities and therefore the consumption of pollen and/or nectar potentially contaminated with the active substance and/or its metabolite were directly observed in the colonies. These trials enabled colony development and survival to be monitored.

Forager bees are primarily exposed to pesticide residues in the nectar they consume. In France, thiamethoxam is authorised as a seed treatment for crops of maize (CRUISER 350), beets (CRUISER 600 FS), peas (CRUISER FS) and crucifer oilseed crops (CRUISER OSR). Only crucifer oilseed crops (oilseed rape) are nectar-producing crops whose flowers are accessible to bees for foraging. For the other crops (maize, beets, peas), bees are only exposed to residues in the nectar-producing plants of succeeding crops, in which residue levels are low. A product (ACTARA [containing thiamethoxam]) is authorised for foliar application on various crops, but not during flowering or exudate-production periods or when bees are present. A few uses in greenhouses, on indoor plants and on fertiliser spikes are also available.

For this reason, the reported effects should be compared to the data that were evaluated as part of the risk assessment undertaken for the MA authorisation for CRUISER OSR , which is intended as a seed treatment for oilseed rape, a nectar-producing crop that is particularly attractive to bees.

To support the MA application for CRUISER OSR, the applicant submitted several tunnel and field studies undertaken in oilseed rape crops cultivated from treated seeds. These trials, some of which were performed with multi-year follow-up of the exposed hives, did not show that exposure to these crops had any effects on bee survival, foraging activity, the development of adult and larval populations or behaviour. However, as indicated above, it can be considered that they had limitations regarding their sensitivity for detecting effects such as impaired orientation skills.

The risk assessment for bees undertaken in the regulatory framework for plant protection products was based on the fate of bee colonies. The results obtained with the innovative methodology used in the article by Henry *et al*. (2012) were obtained for individual bees and cannot currently be reliably interpreted in terms of effects on the fate of colonies, in real conditions of exposure corresponding to agricultural and beekeeping practice, considering that an inappropriate model was used to predict the effects on population dynamics. This work should therefore be continued in order to verify whether effects on homing such as those observed in the study have a medium- or long-term impact on colony development and survival.

Furthermore, the effects of thiamethoxam that were reported in the article may be considered to explain phenomena associated with exposure that have been observed in the field. Approximately 790 000 ha of winter oilseed rape treated with CRUISER OSR were cultivated in France in 2011-2012. In the current state of available knowledge, this first year in which the product was used was not associated with any accidents affecting bees that were attributed to exposure to thiamethoxam residues in oilseed rape flowers. However, according to beekeeping professionals, given the particular weather conditions of spring 2012, bees seldom visited this flower crop. No conclusions, on which the results of Henry *et al*. (2012) could have shed additional light, can therefore be drawn from this field campaign.

In the current state of knowledge, the results presented in the article by Henry *et al*. (2012) are not considered as calling into question the conclusions of the risk assessment conducted according to the current regulatory criteria in the context of the Marketing Authorisation application for CRUISER OSR. However, they do emphasise the limitations of the models used in this context in terms of sensitivity. The bee toxicity properties taken into account for the approval of thiamethoxam under Regulation (EC) no. 1107/2009[16], which are summarised on page 8 of this Opinion, are not modified by this study's results.

Forager bees can also theoretically absorb residues found in guttation fluid. Guttation is the formation of small clear liquid droplets that can be observed at the apical end of maize leaves, among other plants[17]. According to results communicated in 2009[18], guttation fluid may contain residues of active plant protection substances and be a route of exposure for bees. Exposure of forager bees to a sub-lethal dose could thus affect bees located near fields of maize treated with the CRUISER 350formulation.

However, tests have shown that the guttation fluid of maize leaves is not an attractive source of water that is used by bees. Exposure of forager bees to the residues contained in the guttation droplets of maize treated with CRUISER 350 is therefore considered unlikely. Moreover, in the context of the assessment of the MA application for this product, a field trial was assessed by the Agency to evaluate the risk to bee colonies placed on the edges of fields, relating to the formation of guttation droplets on young plants of treated maize. During this trial, no significant mortality was observed nor any difference in the strength of the colonies and the surface area occupied by reserves and larval stages.

### General conclusion

The French Agency for Food, Environmental and Occupational Health & Safety considers that the results reported in the article by Henry *et al*. (2012) show the harmful effect of a sub-lethal dose of thiamethoxam on forager bees' ability to return to the hive. However, the consequences of these effects on the fate of colonies, which were examined using a mathematical model that has not been validated for this use, cannot be clearly established.

The single dose administered in the described experiments (1.34 ng/bee) is lower than the doses that induced bee mortality in an acute toxicity test. The available data regarding concentrations of the active substance and sugar concentrations in oilseed rape nectar, whether taken from application dossiers submitted in a regulatory framework or from analyses undertaken in 2012 in current farming practice conditions, indicate that bee exposure to thiamethoxam through oilseed rape nectar residues is lower than this dose, although exposure to this dose cannot be totally excluded in certain circumstances. This conclusion is consistent with that of EFSA, whose Opinion on the same issue was formally requested by the European Commission.

Moreover, trials undertaken with CRUISER OSR containing thiamethoxam, some of which have included multi-year follow-up of hives exposed to crops of treated oilseed rape, have not shown that exposure to these crops has significant effects on bee survival, foraging activity, the development of adult and larval populations or behaviour. Despite the fact that these trials have limitations regarding their sensitivity for detecting effects such as impaired orientation skills, their relevance for assessing risks to a colony cannot be called into question by a single experimental study that is based on exposure levels and conditions that are less representative of field reality.

16 Regulation (EC) no. 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
17 See details in the Agency's Opinion no. 2009-SA-0065 of 30 April 2009.
18 Gioro C. *et al*. (2009), How can guttation drops kill bees? The lethal effect of neonicotinoid insecticides, XXIII Congresso Nazionale della Società Chimica Italiana, Sorrento, 5-10 July 2009 (Abstract).

Nonetheless, the Agency considers that the trials emphasise limitations in the methodologies used in field studies, including their failure to take into account certain parameters such as effects on homing flights.

Based on this study's results, the Agency therefore recommends:

- Pursuing experiments based on RFID technology, using a range of exposure levels to more closely match the doses to which bees are commonly exposed, and delving more deeply into the consequences of the harmful effects observed in individual bees on the dynamics of the bee colony as a whole. This work could make it possible to validate a research protocol to more effectively describe the sub-lethal effects of exposure to neonicotinoids, and be taken into account as European-level regulations develop.

- Instigating a review at the European level of neonicotinoid active substances (thiamethoxam, clothianidin, etc.) based on new scientific data from recent studies, as was proposed by EFSA.

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**KEYWORDS**

Plant production products, thiamethoxam, bee

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