

Maisons-Alfort, 1st December 2009

OPINION

of the French Food Safety Agency on the results of the monitoring programme for marketing authorisation for the insecticide Cruiser

The French Food Safety Agency (Afssa) received a request on 11 September 2009 from the Directorate-General for Food (DGAI) for an Opinion on the results of a monitoring programme for marketing authorisation for the insecticide, Cruiser.

After consulting the Scientific Panel on "Plant protection products: chemical substances and preparations" which met on 25 November 2009, the French Food Safety Agency has reached the following conclusions.

BACKGROUND

Since 21 November 2007, several Opinions have been issued following the assessment of Cruiser, a thiamethoxam-based insecticide treatment for seed. Afssa issued a favourable Opinion, subject to recommendations for specific management measures for this insecticide.

At the request of the minister responsible for agriculture, the DGAL implemented a programme to follow-up the application of these management measures. The results of the 2008 monitoring programme are now available, as well as the initial data collected for the 2009 monitoring programme.

The DGAL has asked Afssa for an Opinion as to whether the data collected for the field monitoring programme might call into question its previous Opinions on Cruiser insecticide.

DOCUMENTS EXAMINED

For the 2008 monitoring programme:

- the memorandum BBMLE/2008-04-004 sent by the DGAL to the authorities responsible for implementing the monitoring programme (DDSV, DRAF/SRPV¹);
- the report entitled 'Cruiser monitoring programme 2008', including the summary document on insect monitoring (MNHN, 2009);
- the reports on analysis of the glycerol water, seed, bees, pollen and their amendments updates to these reports when additional analysis were carried out at Afssa's request;
- an annex concerning pollen analyses (at the Landes site²);
- an annex concerning the health of the apiaries, for all three regions.

For the 2009 programme:

- the memorandum 090311_PS-CRUISER_2009_vdef-fo sent by the DGAL to the authorities responsible for implementing the monitoring programme (DDSV, DRAF/SRPV).

Aquitaine region:

- the preliminary report for the Aquitaine region drawn up in November 2009 and the previous notes, including a summarised review of hive health;

¹ the devolved French government bodies responsible for food and environmental safety and plant protection at local level

² south-west France

- the reports on analysis of the glycerol water, seed, bees, pollen and updates to these reports when additional analysis were carried out at Afssa's request;
- maps of the focus zones.

Centre region:

- a description of how the monitoring programme was implemented at the pilot sites;
- the hive inspection report;
- the reports on analysis of the glycerol water, seed, bees, pollen and updates to these reports when additional analysis were carried out at Afssa's request;
- maps of the focus zones.

GENERAL OBJECTIVES OF THE MONITORING PROGRAMME

The objective of the programme is to verify that there are no unintended effects on pollinators, particularly honey bees, due to the marketing and use by farmers of seed coated with the plant protection product Cruiser, containing thiamethoxam.

1. OVERVIEW OF THE PROCEDURE FOR 2008

A general procedure was laid down in the memorandum BBMLE/2008-04-004. The principal aspects of the procedure can be summarised as follows.

- **Choice of the 'Cruiser-treated' and 'non-Cruiser-treated' sites.** Each of the three regions involved (Midi-Pyrénées, Rhône Alpes and Aquitaine) must set up 20 sites (10 'Cruiser-treated' and 10 'non-Cruiser-treated', for a total of 30 sites for each treatment case. The 'Cruiser-treated' and 'non-Cruiser-treated' sites must be in small agricultural regions³ with similar landscapes and environmental conditions. These sites will consist of a 'focus zone' with a radius of 1 km, surrounded by a 'safety' zone with a radius of 3 km, within which certain land use criteria must be satisfied:
 - at least 50% of the 'focus zone' must be planted with maize, 50% of which are Cruiser-treated, giving at least 150 ha of treated maize (for 'Cruiser-treated' sites), or 'non-Cruiser-treated' but with at least 150 ha of untreated maize (for 'non-Cruiser-treated' sites).
 - the 'safety zone' covers a surface area of between 2500 ha and 3000 ha, in which, for 'non-Cruiser-treated' sites, no more than 10% of the area may be sown with Cruiser-treated maize.

The 'non-Cruiser-treated' sites must be at a minimum distance of 5 km from 'Cruiser-treated' sites.

The characteristics of each site must be recorded carefully.

- **Collection of landscape data** for all observation areas (with specific data for 'focus zones' and more general data for 'safety zones').
- **Collection of biological data** (on pollinators and diurnal butterflies in areas sown with maize and in the flora on edges of fields) for all 'focus zones'.
- **Experimental installation of apiaries at some of these sites ('pilot sites')**. The purpose of these experimental installations is to compare two populations of bees: on the one hand, those whose hives are placed at 'Cruiser-treated' sites and on the other, those whose hives are placed at 'non-Cruiser-treated' sites. Four sites are designated 'pilot sites' in each of the 3 regions, with each receiving an apiary of 7 hives. This gives a total of 12 apiaries (6 at 'Cruiser-treated' sites and 6 at 'non-Cruiser-treated' sites), made up of 84 hives for observation.

It is imperative that the apiaries installed at 'non-Cruiser-treated' sites be located at a minimum distance of 10 km from sites with 'Cruiser-treated' maize and that apiaries installed at 'Cruiser-

³ In French, one of these is called a *petite région agricole* and has a strict definition in terms of the local cropping, soil and climate

treated' sites be located at a minimum distance of 10 km from sites with 'non-Cruiser-treated' maize.

At the pilot zones, health inspections of the bee populations will be carried out as well as toxicological monitoring of samples from the different compartments of the hive (bees, bee bread, honey and larvae). The hives will then be relocated together on a single site at the beginning of September for a health inspection before overwintering.

- **Collection of data on agricultural practices** in the 'focus zones' of the 30 'pilot sites', particularly information on the use of insecticide plant-protection products over the previous two years or of seeds coated with fungicides.
- **Undertaking of a 'dust' test**, to assess the amount of active substance (thiamethoxam) in dust emitted during seed-drilling operations at 'Cruiser-treated' pilot sites.
- **Sampling and analyses to be carried out.**
 - On purchase and installation of hives
Sampling live bees to search for pathogenic agents, and analysis of thiamethoxam and clothianidin content;
 - On installing hives
Sampling live bees to search for pathogenic agents, and live bees and pollen to be analysed for thiamethoxam and clothianidin content;
 - During seed-drilling
 - At pilot sites, sampling treated and untreated seed to measure the dose of thiamethoxam and of its major metabolite, clothianidin;
 - At pilot sites, collecting dust in Petri dishes placed at various distances from the seed drill, to be analysed for thiamethoxam and clothianidin, and also for imidacloprid. It is also planned to collect dust using a high-flow sampling device (from the ORAMIP air-quality observatory⁴) placed at the edge of the field at one 'Cruiser-treated' site and one 'non-Cruiser-treated' site in the Midi-Pyrénées region, for analysis for the same active substances;
 - Sampling live bees and larvae to be analysed for pathogenic agents, and live bees, larvae and pollen to be analysed for thiamethoxam and clothianidin;
 - During flowering
 - On one field of 'Cruiser-treated' maize and one field of 'non-Cruiser-treated' maize at each of the sites in each region, two sets of pollen samples will be taken, from 10 maize panicles, i.e. a total of 30 samples in duplicate for each type of crop (i.e. treated and untreated); the pollen from treated fields to be analysed for thiamethoxam and clothianidin while the pollen from untreated fields will undergo multi-residue analyses;
 - Before and during flowering, sampling live bees to search for pathogenic agents, and live bees, larvae, bee bread and pollen to be analysed for thiamethoxam and clothianidin;
 - At the end of flowering, sampling live bees and larvae to be analysed for pathogenic agents, and live bees, larvae, bee bread and pollen to be analysed for thiamethoxam and clothianidin content;
 - Before overwintering
Sampling live bees to be analysed for pathogenic agents and live bees and bee bread for multi-residue analysis.

The field monitoring will therefore last from the spring of 2008 until the health inspection at the end of overwintering, in the spring of 2009.

⁴ <http://www.oramip.org/content/oramip/index.php>

2. OVERVIEW OF THE PROCEDURE FOR 2009 UP UNTIL MAIZE SEED-DRILLING

A general procedure was described in the memorandum 090311_PS-CRUISER_2009_vdef-fo. It has the same structure as the 2008 monitoring programme, but was extended to a total of 6 regions. It should be remembered that, at the DGAL's request, Afssa issued an Opinion concerning this monitoring plan⁵. The principal aspects of the procedure can be summarised as follows.

- **Choice of the 'Cruiser-treated' and 'non-Cruiser-treated' sites.** Each of the six regions must set up 16 sites (eight 'Cruiser-treated' and eight 'non-Cruiser-treated'), for a total of 48 sites for each treatment procedure. The 'Cruiser-treated' and 'non-Cruiser-treated' sites must be in small agricultural regions with similar landscape and environmental conditions. These sites will consist of a 'focus zone' with a radius of 1 km, surrounded by a 'safety' zone with a radius of 3 km, within which certain land use criteria must be satisfied:
 - At least 50% of the 'focus zone' is occupied by maize, 50% of which are Cruiser-treated, giving at least 150 ha of treated maize (for 'Cruiser-treated' sites), or maize not treated with Cruiser and a minimum of 150 ha of untreated maize crops (for 'non-Cruiser-treated' sites).
 - the 'safety zone' covers an area of between 2500 ha and 3000 ha, in which, for 'untreated' sites, no more than 10% of the area may be sown with Cruiser-treated maize.

The 'non-Cruiser-treated' sites must be at a minimum distance of 5 km from 'Cruiser-treated' sites.

The characteristics of each site must be recorded carefully.

- **Collection of landscape data** for all observation areas (specific data for 'focus zones' and more general data for 'safety zones').
- **Collection of biological data** (on pollinators and diurnal butterflies in areas sown with maize and in the flora on edges of fields) for all 'focus zones'.
- **Experimental installation of apiaries at some of these sites ('pilot sites').** The purpose of these experimental installations is to compare two populations of bees: on the one hand, those whose hives are placed at 'Cruiser-treated' sites and on the other, those whose hives are placed at 'non-Cruiser-treated' sites. Four to six sites are designated 'pilot sites' in each of the six regions, with each receiving an apiary of seven hives. This gives a total of 30 apiaries (15 at 'Cruiser-treated' sites and 15 at 'non-Cruiser-treated' sites), made up of 210 hives for observation. Twelve of these apiaries were already involved in the 2008 monitoring programme and will be placed at sites in 2009 subject to the same procedure as the previous year, for the second year running.

At the pilot zones, the bee populations will be inspected together with toxicological monitoring of samples from the different compartments of the hive (bees, bee bread, honey and larvae). The hives will then be relocated together at a single site at the beginning of September for a health inspection before overwintering.

- **Collection of data on agricultural practices** in the 'focus zones' of the 30 'pilot sites', particularly including information on the use of plant-protection insecticides or seeds coated with fungicides over the last two years.
- **A 'dust' test is to be carried out,** to analyse the quantity of active substance (thiamethoxam) in the dust emitted during seed-drilling operations at 'Cruiser-treated' pilot sites.
- **Sampling and analyses to be made.**
 - On purchasing and installing hives
Sampling live bees to be analysed for pathogenic agents, and analysis for thiamethoxam and clothianidin content;
 - On installing hives

⁵ Opinion 2009-SA-0071 of 30 March 2009.

Sampling live bees to search for pathogenic agents, and live bees and pollen to be analysed for thiamethoxam and clothianidin content;

- During seed-drilling
 - At pilot sites, sampling treated and untreated seed to measure the dose of thiamethoxam and of its major metabolite, clothianidin;
 - At pilot sites, collecting dust in Petri dishes placed at various distances from the seed drill, to be analysed for thiamethoxam and clothianidin, and also for imidacloprid. It is also planned to collect dust using a high-flow sampling device (from the ORAMIP air-quality observatory) placed at the edge of the field at one 'Cruiser-treated' site and one 'non-Cruiser-treated' site, for analysis for the same active substances;
 - Sampling live bees and larvae to search for pathogenic agents, and live bees, larvae and pollen to be analysed for thiamethoxam and clothianidin content.

3. ANALYSIS OF THE RESULTS FOR THE 2008 MONITORING PROGRAMME

3.1. Implementing the monitoring

The programme was carried out in all 3 regions. For each region, the sites were chosen by comparing data on useable agricultural areas (UAA) in each *commune*⁶ and the doses of maize sold per *commune* for the 2008 maize season for the principal distributors. The number of sites installed per region is given in the following table:

Region	Number of Cruiser-treated sites	Number of control sites
Aquitaine	8 including 2 pilot sites*	8 including 2 pilot sites
Midi-Pyrénées	7 including 2 pilot sites	5 including 3 pilot sites
Rhône-Alpes	2 pilot sites	2 pilot sites

*plus two sites at which dust samples were taken.

3.2. Characteristics of sites chosen and exposure of apiaries with regard to the procedure

The total exposure of the apiaries to treated maize as specified by the procedure includes proximity to a field during seed-drilling (with the apiary being installed before sowing) and an environment with a substantial area of treated maize during flowering (150 ha of 'Cruiser-treated' maize in the focus zone). In order for the programme to be capable of showing any possible effects on bee colonies, the colonies installed at the chosen sites must first be inspected to ensure that they are in good health when purchased or when they are installed at the site.

The descriptions provided for the three regions were analysed with regard to the requirements specified in the procedure, but with the following interpretation applied concerning the area sown with maize. The procedure requires that each site include 150 ha of maize. A deviation from the procedure of 20% as regards the target area of 150 ha was not deemed to cause a major reduction in the extent to which the hives were exposed to flowering maize grown from treated seed. Sites whose focus zone contained more than 120 ha of 'Cruiser-treated' maize were therefore included.

Surface areas of maize at chosen sites

Based on the records of land use in the focus zones of the 'Cruiser-treated' and 'non-Cruiser-treated' pilot sites, the following table gives the areas of 'Cruiser-treated' and 'non-Cruiser-treated' maize:

Region	Number of 'Cruiser-treated' sites with more than 120 ha	Number of control sites with more than 120 ha under maize
Aquitaine	2 pilot sites	2 pilot sites
Midi-Pyrénées	-	2 sites
Rhône-Alpes	-	2 sites

⁶ A *commune* is the smallest administrative division in France

⁷ For locations, see administrative map at <http://www.adminet.com/tow/reg.html>

Situation in terms of bee exposure

The following table gives the situation concerning sowing dates and installation of the apiaries at the treated pilot sites:

Region	Pilot site	Date apiary was installed	Date of sowing
Rhône-Alpes	Ambronay	27-28/04/08	02/05/08
	Lagnieu	27-28/04/08	05/05/08
Midi-Pyrénées	Cintegabelle	2 hives on 01/05/08 7 hives on 06/05/08	02/05/08
	Segos	06/05/08	07/05/08
Aquitaine	Samadet	Hives unavailable	15/05/08
	Mant		15/05/08

The number of sites chosen as representative of an environment exercising long-term pressure on populations of bees present due to the high density of maize, as specified in the requirements for areas sown with maize **was therefore lower than the total number of sites planned for all regions.**

However, the impact on exposed hives during seed-drilling operations is less a function of the area under maize than of the proximity of a field to the apiary and the manner in which any dust generated is dispersed. For this reason, a distinction has been made between sites where it is possible to measure the effects on hives at seed-drilling time and sites with a sufficiently large area of maize to be taken into account when measuring the long-term effects on the bees.

On this basis, only the pilot sites in the Aquitaine region have sufficient areas under maize to be taken into account for an assessment of the long-term effects on bees with respect to the flowering of maize.

As regards the installation of the hives at the sites relative to the seed-drilling dates, the hives were installed a few days before sowing in Rhône-Alpes and at one treated site in Midi-Pyrénées. At the second Midi-Pyrénées site, only two hives were in place before seed-drilling. In Aquitaine, the hives were not available before seed-drilling.

On this basis, only the apiaries at the treated pilot sites in the Rhône-Alpes and Midi-Pyrénées regions can be taken into account to assess the effect on the bees of dust generated during seed-drilling.

Situation in terms of health inspections of the apiaries

Lastly, the procedure requires health inspections (and taking samples) of the hives used for the experiment, at the time of purchase and for every region.

No report is available for the health inspections at the time of purchase and installation for hives at the sites in the Rhône-Alpes region. In Midi-Pyrénées, the hives were not inspected for health on purchase at the 'Cruiser-treated' sites, although the report does mention samples being taken at the time of installation.

There is therefore no guarantee that the colonies installed at these sites were in good health and free from pathogens. Taking these into account in a detailed analysis of the monitoring data from subsequent visits, with respect to exposure data, must therefore be treated with caution.

A general analysis of the way the colonies evolved was nonetheless carried out, based on the hypothesis that if the apiaries installed at the control and treated sites were homogenous in terms of their health, a comparison would reveal the most significant effects on the apiaries of an environment made up of 'Cruiser-treated' maize.

3.3. Overall analysis of the evolution of colonies exposed to dust generated during seed-drilling

Exposure during seed-drilling

The **results of the analyses of the seed** indicate concentrations of thiamethoxam of between 1397 and 2037 mg a.s.⁸/kg of seed for the Midi-Pyrénées sites and between 1384 and 1952 mg a.s./kg of seed for the Rhône-Alpes sites. The mean thousand grain weight (TGW) is estimated to be about 300 g (330 g according to FAO data and 297 g [220 – 390 g]⁹ according to information provided by Arvalis¹⁰). The application rate of thiamethoxam contained in the seed coating used at these sites therefore equates to between 0.42 and 0.61 g a.s./1000 grains for the Midi-Pyrénées sites and between 0.42 and 0.59 g a.s./1000 grains for the Rhône-Alpes sites. These results should be compared to a mean dose of 0.63 g a.s./1000 grains corresponding to an application of 69.3 g a.s./ha for a seed rate of 110,000 grains/ha¹¹. The small number of samples taken, however, is deemed insufficient for estimating the doses of thiamethoxam actually applied per hectare. Precise estimation would only be possible with more extensive sampling.

The results of the analyses of dust generated during seed-drilling are available for the pilot sites of both regions (see Annex 1).

For the Rhône-Alpes region:

At Ambronay, the results show the presence of thiamethoxam in the Petri dishes placed downwind, decreasing as the distance from the drilling row increases. In all cases, the concentration of clothianidin is lower than the limit of quantification. The presence of thiamethoxam in the control dishes is due to contamination related to the re-use of funnels used for recovering the contents of the dishes.

At Lagnieu, the results show the presence of thiamethoxam in the Petri dishes, decreasing as the distance from the drilling row increases. In all cases, the concentration of clothianidin is lower than the limit of quantification. There are no quantifiable residues in dishes placed laterally, or in the control dishes.

For the Midi-Pyrénées region:

At Cintegabelle, the results show the presence of thiamethoxam in the Petri dishes placed downwind, decreasing as the distance from the drilling row increases. Higher quantities were found than at the other sites. Weather conditions, the direction of airflow from the seed drill and the way sacks were handled before loading the drill are singled out as factors likely to affect the quantity of dust emitted. No quantifiable residues were found in the control dishes. A quantifiable amount of thiamethoxam was found in one of the dishes placed laterally.

At Ségos, the results show the presence of thiamethoxam in the Petri dishes placed on both sides of the drilling row, with a tendency to decrease as the distance from the drilling row increases. No quantifiable residues were found in the control dishes. A quantifiable amount of thiamethoxam was found in the dishes placed laterally.

Some uncertainty remains about expressing the results in terms of equivalent active substance per hectare, as the procedure recommends that dust be collected in 50 mL of glycerol water plus rinsing twice with 25 mL of glycerol water, but it would seem that the rinsing operation was not carried out at all the sites because of constraints related to conditions in the field. Since the operating procedure actually followed at each site is not given in the report, both calculations have been performed in order to estimate the equivalent dose of active substance per hectare. They show that dust generated by seed-drilling could be detected at some distance from the field being sown, with dispersal being fairly consistent with wind direction. The doses per hectare are in all cases lower than the NOAEL¹² dose of 1 g/ha as defined for honeybees¹³. Placing kit-type

⁸ a.s.: active substance.

⁹ Depending particularly on the variety of maize.

¹⁰ ARVALIS - *Institut du végétal*, a French agricultural applied research organisation financed and managed by producers

¹¹ Maximum authorised seed rate when using Cruiser insecticide (seed treatment for maize).

¹² NOAEL: No observed adverse effect level.

weather stations at the fields and the Petri dish locations would allow for more refined analysis of the way the particles are dispersed.

Monitoring the hives

In Rhône-Alpes, the observations made during visits subsequent to the installation (sowing, intermediary pre-flowering visit, flowering, beginning and end of winter) reveal:

- at treated sites, scattered symptoms of chronic paralysis, a few mortalities in front of hives or feeders (the mortalities observed in front of one hive during one visit were not observed on later visits), a reduction in the density of brood in one hive, a suspicion of wax moth and weakness attributed to ageing lineages. Two colonies died before overwintering at one of the sites, while the five others were described as strong and were still living at the end of winter.
- at control sites, scattered symptoms of chronic paralysis, a reduction in the density of brood in some colonies with periods in which they were temporarily described as weak. One colony died during overwintering at one of the sites, while the six others were described as strong and were still alive at the end of winter.

These observations indicate that the hives installed in the Rhône-Alpes region had a generally homogenous state of health. The results concerning the presence of chronic bee paralysis virus are not yet available, however. These observations do not reveal any signs that the colonies located at 'Cruiser-treated' sites underwent any greater decline than those located at control sites.

In Midi-Pyrénées, the visits following installation of the apiaries (seed drilling, intermediary pre-flowering visit, flowering, beginning and end of winter) revealed different anomalies in terms of health:

- at treated sites, varying bee mortality in front of the hives, symptoms of chronic bee paralysis (CBPV) at the hive entrance, symptoms of American foulbrood, European foulbrood, sacbrood and fungal infections of brood were observed. Reduced reserves of honey and pollen were also observed. The presence of American foulbrood, European foulbrood, sacbrood and fungal infections were confirmed after analysis. Despite these observations, all the colonies were still alive at the end of winter;
- at the control sites, varying bee mortality in front of the hives and (slight) symptoms of chronic bee paralysis (CBPV) at the hive entrance were observed. It was also found that 3 out of 5 hives were orphaned and were raising a new queen. The presence of CBPV was confirmed by analysis. During the final visit of the experiment in October, symptoms of varroa infestation were observed in all the hives at both sites (phoretic varroa mites, bees with deformed wings and brood cannibalism). At the end of winter, all the colonies were alive at one site while 3 out of 7 colonies were dead at the other site (Calmont).

The anomalies listed above therefore do not allow us to assume that the hives installed in the Midi-Pyrénées region were in a particularly good state of health. However, these observations do not reveal any signs that the colonies located at 'Cruiser-treated' sites underwent any greater decline than those located at control sites.

3.4. Analysis of long-term effects related to flowering

The hives at pilot sites in the Aquitaine region were installed after seed-drilling had been completed, but in an environment where it was possible to study the long-term effects on colonies of exposure to 'Cruiser-treated' maize during the flowering period.

Descriptions of sites

The areas sown with maize varied from one site to the next. At 'Cruiser' pilot sites, the remaining areas were given over to crops (cereals) and woodland, meadows and set-aside/fallow land, in varying proportions. At the 'non-Cruiser' pilot sites, the remaining areas were under crops of cereals, oilseed rape or grapevines, as well as woodland, meadows and set-aside/fallow land, in varying proportions.

¹³ NOAEL estimated for application of a preparation in the form of dispersible granules containing thiamethoxam, on phacelia in flower

The insecticides applied in the focus zones over the two years preceding implementation of the study were recorded. These were carbamates, applied directly to the soil along the drilling row or, more rarely, applied to foliage, sometimes replaced by a treatment based on organophosphates and foliar treatments using a pyrethroid (on oilseed rape or maize).

An exact description of the safety zones was not available when the report was finalised.

The focus zones of the 12 other sites (observation sites) were also described. Maize covered 32 to 81% of the UAA of the control focus zones, compared with 51 to 86% of the UAA of the 'Cruiser-treated' focus zones. The percentage of Cruiser-treated maize in the area under maize was between 0 and 13% in the control focus zones compared with 50 to 89% in the 'Cruiser-treated' focus zones.

The remaining area of both the control and 'Cruiser' focus zones was occupied by crops (mainly cereals, oilseed rape and sunflower), woodland, meadows and set-aside land, in varying proportions.

Installation and monitoring of the hives

The hives were not installed before seed-drilling but one week before flowering. The results from these sites can therefore only be exploited for observations at the time of flowering. The first health inspection took place two days after the installation of the hives. The following table lists the observations made during visits to the apiaries from flowering to the end of winter:

Site	Type	State of hives before flowering	State of hives at flowering	State of hives at end of flowering	State of hives at end of winter
Serreslous	Control	Strong hives	4 strong hives and 3 medium	Strong hives	Medium hives
Cazères-sur-Adour	Control	6 strong hives and 1 weak	5 strong hives and 2 weak	5 strong hives, 1 weak and 1 dead	4 medium hives and 2 weak
Samadet	Cruiser	Strong hives	3 strong hives and 4 medium	5 strong hives, 1 medium and 1 weak	5 medium hives and 2 weak
Mant	Cruiser	Hives stolen and replaced	3 strong hives and 4 medium	4 strong hives, 2 medium and 1 weak	5 medium hives and 2 weak

At the Serreslous apiary (control), some symptoms of chronic paralysis were identified after transhumance.

At the Cazères-sur-Adour apiary (control), a case of tubular brood¹⁴ [caused by the lesser wax moth, *Achroea grisella*] was observed during flowering. The presence of greater wax moth¹⁵ [*Galleria mellonella*] was also found in a colony described as "weak" at the end of flowering¹⁶.

At the Samadet apiary (Cruiser-treated), tubular brood was observed in 5 hives at purchase, and then at different times, i.e. in 2 of the 7 hives during flowering and 2 different hives of the 7 at the end of flowering. Varroa mites were observed in one of these hives. No abnormal mortality was noted before overwintering.

Lastly, at the Mant apiary (Cruiser-treated), the observations concern a hive introduced during the experiment (15 July) to replace those stolen between 2 and 8 July. One case of chalk brood¹⁷ was observed before and during flowering, with remission. One colony showed atrophied wing symptoms during flowering, with remission. Trembling bees were observed in a colony before flowering, with remission. Greater wax moths were seen in two colonies during flowering. Finally, mortality was found among larvae in two colonies during flowering. The results of the pathological

¹⁴ *Couvain tubulaire* and *petite fausse teigne des abeilles* in French;

¹⁵ *fausse teigne des abeilles* in French

¹⁶ Translation sources used for this section: Haensch/Haberkamp, *Dictionary of Agriculture*. Munich: BLV, 1975; <http://ressources.ciheam.org/om/pdf/b25/99600245.pdf>

¹⁷ *Couvain plâtré* in French

analyses mentioned in a preliminary report sent by the laboratory responsible for these analyses found chronic paralysis virus in living bees showing various disorders at the hive entrance, with a viral count compatible with a manifest case of disease. No abnormal mortality was observed before overwintering.

Analyses of pollen from maize panicles sampled during flowering reveal concentrations of thiamethoxam or clothianidin at levels too low to be quantified. The results of the analyses of bees mentioned in the intermediary report sent by Afssa's laboratory in Sophia-Antipolis indicates the absence of any residue of thiamethoxam and clothianidin (concentrations too low to be quantified) in bees sampled between 25 June and 17 September.

The hives from this region were brought together on a single site (Pissos) at the beginning of September.

Reports for the end of winter are available. Symptoms such as uneven brood patterns (showing brood disease) were observed in the colonies from the apiaries at the Samadet and Mant sites. No lost colonies were recorded.

Lastly, a report was compiled on the health of bees in the *départements* concerned. Three cases of hive mortality had been reported to the DDSV 64 in the *communes* of Peyrehorade (Landes), Hastingues (Landes) and Sames (Pyrénées-Atlantiques), which were the subject of the Opinion issued by Afssa on 6 March 2009¹⁸. Based on the information provided, the hive mortalities observed cannot be attributed to exposure to any residue of thiamethoxam (thiamethoxam or clothianidin) or to the use of Cruiser on fields close to the hives concerned.

Observations concerning the flora and fauna

Observations were made of the populations of pollinators and butterflies and of flora populations.

According to the report by the French National Museum of Natural History (MNHN), the analyses, based on more than 3000 insects counted and more than 38 000 insects trapped, did not reveal any significant impact of Cruiser, except in the case of homoptera and heteroptera, which are significantly less numerous at the 'Cruiser-treated' sites. However, the heterogeneity of the results for the other groups, and in particular the differences in relative abundance between the 'Cruiser' and control sites from region to region, mean that these results should be interpreted carefully: environmental factors not taken into account in the analyses may influence the abundances observed and could make their interpretation more complex. The MNHN recommends the monitoring network be extended to other regions and other environmental and agricultural variables be taken into account so that the results can be refined.

3.5. Conclusions for the 2008 monitoring programme

The purpose of the monitoring programme set up in three regions (Aquitaine, Midi-Pyrénées and Rhône-Alpes) was to evaluate the unintended effects of thiamethoxam on pollinators, particularly honey bees, related to the use of Cruiser, which contains thiamethoxam, applied to seed as a pesticide.

The reports available enable us to assess the manner in which the monitoring was carried out. A delay in drawing up and distributing a procedure made it difficult to find sites with the characteristics detailed in the procedure and where maize had not already been sown.

Consequently, the apiaries were not established in the Aquitaine region in time to study the effects of the seed-drilling process on the colonies.

For the Rhône-Alpes and Midi-Pyrénées regions, apiaries were installed before the treated fields were sown but some of them in conditions that precluded the possibility of health inspections. It was nonetheless possible to conduct an overall analysis of the evolution of the colonies exposed to seed-drilling on the pilot sites of these regions.

¹⁸ Opinion by the French Food Safety Agency relative to the results of the analyses for the mortality of bees in the Pyrénées-Atlantiques in response to Request No. 2009-SA-0040.

Furthermore, the areas under maize were insufficient for the focus zones in the Rhône-Alpes and Midi-Pyrénées regions, so the long-term effects related to the flowering period of maize were only studied for the sites in the Aquitaine region.

Since the sites were located widely across *départements*¹⁹, it was necessary to perform multiple visits and sampling almost simultaneously, leading to certain deviations from the procedure, which reduced the volume of exploitable data.

It should be noted, however, that optimum monitoring conditions also require a correspondingly complete review of the health of the hives, so that any effects can be detected that may potentially be associated with exposure to the active ingredient, whether at the time of seed-drilling or related to the flowering of crops grown from treated seed. In this case, a satisfactory state of health of the hives during this monitoring programme could not be guaranteed, as it was not possible to perform all the visits, particularly when the apiaries were installed, and there was no analysis for pathogenic agents when the hives were purchased. In the Aquitaine region, in which monitoring was carried out after exposure to flowering, pathologies were found in all the apiaries, at both control and treated sites.

However, the exploitable results under the monitoring conditions described above offer no evidence, of losses of colonies that could be attributed to the use of maize seed treated with Cruiser.

4. ANALYSIS OF THE RESULTS OF THE 2009 MONITORING PROGRAMME UP TO SOWING OF THE MAIZE

4.1. Setting up the monitoring programme

The monitoring programme was undertaken in all six regions. The following table gives the number of sites per region:

Region	Number of Cruiser-treated sites	Number of control sites
Aquitaine	7 including 3 pilot sites	5 including 3 pilot sites
Midi-Pyrénées	8 including 3 pilot sites	5 including 3 pilot sites
Rhône-Alpes	5 including 2 pilot sites	2, both pilot sites
Alsace	3, all pilot sites	5 including 2 pilot sites
Poitou-Charente	3 including 2 pilot sites	2, both pilot sites
Centre	2, both pilot sites	2, both pilot sites

Although the minimum number of pilot sites was established in each region, the planned total of 16 sites per region (eight 'Cruiser-treated' sites and eight control sites) was not reached, because of the difficulty of finding sites which satisfied all the conditions for the area sown with maize in the zones to be monitored.

4.2. Characteristics of the sites chosen and exposure of the apiaries with regard to the procedure

Afssa has not yet received all the data relative to the areas sown with Cruiser-treated maize, which were intended to serve as the basis for verifying the installation conditions with regard to the procedure. The available data can be analysed as follows:

Region	Number of Cruiser-treated sites exceeding 120 ha	Number of control sites with 120 ha under maize
Aquitaine	2 pilot sites	1 site
Midi-Pyrénées	3 pilot sites	6 sites
Rhône-Alpes	1 pilot site	1 site
Alsace	2 pilot sites	1 site
Poitou-Charentes	1 pilot site	Area under maize < 120 ha
Centre	Area drilled with maize < 120 ha	Area under maize < 120 ha

¹⁹ The French administrative division subordinate to a region; a district. See also the administrative map referred to in the footnote on p 5 above.

The same criteria were used as for the 2008 monitoring programme to verify that sites satisfied the requirements of the procedure.

The number of sites chosen to represent an environment exercising long-term pressure on populations of bees present due to the high density of maize, was lower than the number of sites planned. As before, a distinction was made between sites where it is possible to measure the effects on hives at seed-drilling time and sites with a sufficiently large area under maize to be taken into account when measuring the long-term effects on the bees.

All the results of analyses of samples taken at seed-drilling time, together with a description of the health of the apiaries at purchase and at seed-drilling time, are available for the Aquitaine region and the Centre region. For the other regions, a summary of the health of the apiaries is currently being written in a consolidated form. Therefore, only the data for the Aquitaine and Centre regions have so far been examined. They deal mainly with the pilot sites, at which apiaries were installed, as data for the observation sites (without hives) are still being collected.

Lastly, for these two regions, the hives installed were examined at purchase (Centre region) and/or at the end of winter when hives monitored in 2008 at the same sites were concerned (Aquitaine region). The visit reports are available and indicate that the hives used in both these regions were in a good state of health.

4.3. Results for the Aquitaine region

Description of sites

The locations of sites likely to satisfy the requirements of the procedure were identified after analysis of information supplied by the industry about the sales of treated maize seed.

A detailed plan of land use in the focus zones of pilot sites was acquired. Maize covers the largest area, followed by meadows and straw cereals (wheat and triticale). When the information from the land use plan was compared with the requirements of the procedure, it was found that two 'Cruiser-treated' pilot sites (Cazères sur Adour and St Jean de Marsacq) and two control pilot sites (Bascons and Poyanne) satisfied all the conditions of the monitoring programme, including the flowering period. Regarding the effect on the hives of dust from seed-drilling operations, all the treated pilot sites were taken into consideration, as the total area of Cruiser-treated maize in the focus zone was not in itself relevant.

Exposure during seed-drilling

During seed-drilling, dust was collected in Petri dishes at two of the three 'Cruiser-treated' sites. It was not possible to take air samples (as planned with the ORAMIP air-quality observatory) at the 'Cruiser' sites, but samples were taken at a control site. Seeds were also sampled to measure the dose of thiamethoxam contained in the coating.

The results of analysis of the seed show concentrations of thiamethoxam between 1523 and 1551 mg a.s./kg of seed (results for the two sites). On the basis of an average weight of about 300 g for 1000 grains (see above), the dose of thiamethoxam contained in the seed coating used at the two sites is between 0.46 and 0.47 g a.s./1000 grains, compared with an average dose of 0.63 g a.s./1000 grains corresponding to a use of 69.3 g a.s./ha. Here again only limited sampling was carried out and is not deemed sufficient for estimating the real doses of thiamethoxam applied per hectare. Larger-scale sampling would be necessary to produce conclusive results on this point.

The results of the analyses of the Petri dishes are given in the tables in Annex 2. They show very low concentrations of thiamethoxam in the samples of glycerol water, i.e. between 0.098 and 0.24 µg/L, with only two dishes containing concentrations greater than the limit of quantification at the Cazères sur Adour site and two dishes at the Souprosse site. These concentrations correspond to doses per hectare of between 0.006 and 0.016 g of active substance²⁰, well below the NOAEL

²⁰ On the basis of resuspension in 50 mL followed by two rinses, corresponding to the highest calculated dose per hectare

of 1 g a.s./ha defined for honey bees²¹. No trace of clothianidin or imidacloprid was found²². On both sites, seed-drilling was performed with drills fitted with deflectors and the wind was between 0 and 1 on the Beaufort scale. These results, compared with the data collected in 2008, indicate that the precautions taken when seed-drilling at the two sites, such as the use of effective deflectors and the choice of favourable weather conditions, can significantly reduce the dispersal of dust from the seed.

Installation and monitoring of the apiaries

The apiaries were installed on 15 and 16 April 2009. Two apiaries monitored in 2008 were used again (1 Cruiser apiary and 1 control apiary) with four new apiaries being added. Visits took place 24 hours after installation and the apiaries were visited 12 times until the beginning of winter.

The observations recorded during visits to the apiaries from installation to flowering are given in the following table (figures in brackets give the number of frames of brood):

Site	Type	State of hives after installation	State of hives just after seed-drilling	State of hives at the intermediary visit	State of hives before flowering
Bascons	Control	4 strong hives (6-7), 1 medium (6)	5 strong hives (7-8 frames), 2 hives split into 2	3 strong hives, 2 medium hives (4-5 frames), 1 laying worker colony	4 strong hives (7 frames) out of the 5 visited
Poyanne	Control	2 strong hives (7), 3 medium (4-6)	3 strong hives (7), 2 medium, 1 hive without pollen and 1 hive with an unfertilised queen	3 strong hives (7-8), 3 medium (3-7), 1 weak	2 strong (6-7) and 1 with a change of queens out of the 3 visited
Biarrotte	Control (2008 apiary)	1 strong hive (7), 2 medium and swarming, 1 weak (6) and 1 very weak (0.5) out of the 6 visited	5 medium hives (swarming) and 1 very weak	4 strong hives (7-8), 1 medium hive (6), 1 orphan and 1 laying worker colony	3 strong hives (5-8) et 2 weak out of the 5 visited
Saint Jean de Marsacq	Cruiser (2008 apiary)	State not recorded	4 strong hives (7-8), 1 medium (7)	2 strong hives (7-8), 4 medium (3-6)	1 strong hive, 2 medium out of the 3 visited
Cazères sur Adour	Cruiser	State not recorded	4 strong hives (7-8), 1 medium, 2 without pollen	1 strong hive (8), 2 medium (6), 3 weak (no queen observed)	No visit
Souprosse	Cruiser	State not recorded	2 strong hives (8), 2 medium (6-7)	6 strong hives (8), 1 drone hive	No visit

Samples of pollen were taken from traps installed on 2 of the 7 hives at each site, when the apiaries were installed and during seed-drilling. The results of the analysis (which tested for residues of thiamethoxam, clothianidin and, for some samples, multiple residues) are not available.

When the apiaries were installed, the colonies were inspected to assess their health and the results are available for the control sites. They show that the colonies were in good condition except for one (a hive described as very weak which later became a 'laying worker colony'). At the time of seed-drilling and subsequently, it was observed that some weak hives at the control sites became laying worker colonies, as the measures taken by the bee-keeper (feeding the queen, introducing brood) had met with varying success.

In the case of the treated sites, the results of the visits carried out at installation are not described in the preliminary report. The visit reports mention no mortalities among colonies exposed at

²¹ Estimated NOAEL for application of a preparation of dispersible granules containing thiamethoxam on phacelia in flower

²² All the results are below the limits of quantitation

seed-drilling time. As with the control sites, one laying worker colony was found during the intermediary visit, and three hives described as weak, apparently queenless during the intermediary visit at the Cazères sur Adour site. In all cases of colonies described as weak, bees were taken as samples to be analysed for residues of thiamethoxam, the results of which are not yet available.

Conclusions for the Aquitaine region

The available, incomplete results so far are insufficient to allow any interpretation in terms of impact on the colonies.

However, the concentrations of active substance in the Petri dishes were lower than those found during the 2008 programme, which indicates that the precautions taken in 2009 during seed-drilling, such as installing effective deflectors and choosing favourable weather conditions can significantly reduce the dispersal of dust from the seed.

The colonies continue to be monitored and once the toxicological analyses and the search for pathogens are complete the results will allow a better understanding of the colonies described as “weak” in apiaries at both control and ‘Cruiser-treated’ pilot sites.

4.4. Results for the Centre region

Description of sites

The locations of sites likely to satisfy the requirements of the procedure were identified after a survey of specific agricultural regions with similar growing conditions and a map of the area under maize, followed by an analysis of sales data for treated maize seed, provided by the industry.

Maps were available showing the details of crop distribution in the focus zones for the pilot sites. There is a good amount of maize but its proportion is insufficient to satisfy the requirements of the procedure (the two treated sites had areas of 72 and 82 ha of maize in their focus zones, while the control zones had 30 and 78 ha). As in the previous case, these sites did not satisfy the conditions enabling them to be taken into account for the full monitoring programme including the flowering period, but could be taken into consideration for the effect of dust on the hives from seed-drilling operations.

Exposure during seed-drilling

During seed-drilling, dust was collected in Petri dishes at the two ‘Cruiser-treated’ sites. There were no plans to take air samples using the ORAMIP system. Seed samples were taken for analysis of the dose of thiamethoxam contained in the coating.

The available results of the analyses performed on the seeds (from the Lutz en Dunois site) show a concentration of thiamethoxam equal to 3312 mg a.s./kg of seed. On the basis of an estimated average weight of 300 g for 1000 grains, the dose of thiamethoxam contained in the seed-coating used at the site is in the region of 1 g a.s./1000 grains, compared with an average of 0.63 g a.s./1000 grains estimated for a sowing operation applying 69.3 g a.s./ha with a density of 110,000 grains/ha. As mentioned above, this degree of sampling is not deemed sufficient for estimating the real doses of thiamethoxam applied per hectare.

During seed-drilling, the observers noted clouds of dust, attributed to the soil being particularly dry at the time of seed-drilling and also the practice of rolling the fields after seed-drilling.

The deflectors are fitted with nozzles that open at heights (about 50 cm above the ground at Villamblain and 1 metre above the ground at Lutz-en-Dunois) which seem too high to allow the dust generated by the drill to be deflected significantly.

In addition, at the two ‘Cruiser-treated’ pilot sites, the strong wind conditions reported on the days when seed-drilling occurred are confirmed by the observations made at nearby weather stations (winds of up to 46.8 km/h recorded at Orléans, near the Villamblain site, and up to 43.2 km/h recorded at Ouzouer, near the Lutz-en-Dunois site).

The results of the analyses of the Petri dishes for the Villamblain site show concentrations of thiamethoxam in the samples of glycerol water between 0.24 and 3.6 µg/L, corresponding to doses per hectare of between 0.017 and 0.25 g a.s.²³, lower than the NOAEL (1 g a.s./ha). No traces of clothianidin or imidacloprid were found²⁴.

At Lutz-en-Dunois, the results of the analyses of the Petri dishes show concentrations of thiamethoxam in the samples of glycerol water between 0.07 and 5.46 µg/L, corresponding to doses per hectare of between 0.005 and 0.38 g a.s.²⁵, lower than the NOAEL (1 g a.s./ha). No traces of clothianidin were found²⁶.

Installation and monitoring of apiaries

The apiaries were installed between 5 and 14 April 2009. The hives used to make up the apiaries came from different places. The apiaries were installed before seed-drilling at the Lutz-en-Dunois site (a treated site) and on the day of seed-drilling at the Péronville site (a control site) and the Villamblain site (a treated site). For the fourth site (Villeneuve-sur-Conie), the apiary was installed after seed-drilling. This site will therefore not be taken into account when evaluating the effects of seed-drilling on colonies.

A detailed examination of the choices made when installing the apiaries at the two 'Cruiser-treated' pilot sites (installation on the sown field itself, on the ground, downwind and next to a wall, at some distance from vegetation that could provide food (pollen and nectar) and, at the Villamblain site, on the very morning of the seed-drilling operation) casts doubt on whether monitoring conditions at these sites were truly representative of field conditions.

The reports are available for the four health inspections to the four pilot sites, which took place on 9, 15, 22 and 29 April 2009. The 9 April visit corresponds to the day of purchase.

The following table summarises the observations recorded during these visits:

Site	Type	State of hives on 09/04/09	State of hives on 15/04/09	State of hives on 22/04/09	State of hives on 29/04/09
Villeneuve-sur-Conie	Control	Strong colonies	1 strong colony, 6 medium	7 strong colonies	1 strong colony, 5 medium colonies, 1 weak colony
Péronville	Control	Strong colonies	6 strong colonies, 1 medium	7 medium to strong colonies	3 strong colonies and 4 medium colonies
Villamblain	Treated	Strong colonies	No visit (heavy rain)	7 strong colonies	3 strong colonies et 4 medium colonies
Lutz-en-Dunois	Treated	Strong colonies	7 medium colonies	1 strong colony, 6 medium	2 strong colonies, 1 medium and 4 weak

The observations recorded for the purchase visits describe strong, active colonies.

The observations recorded on 15 April 2009 describe the presence of tubular brood in all the hives at the Péronville site and of the ropy stage of American foulbrood in one of the hives at this site. Tubular brood was also found in 3 of the 7 hives at Villeneuve-sur-Conie.

At Villamblain, the hives were installed on 10 April 2009, on a field drill-sown on the same day. Bee mortalities described as 'abnormal' were noted the next day. On 15 April, the health inspector noticed some dead bees but considered this mortality to be non-significant²⁷. At Lutz-en-Dunois, mortalities were observed on the same day as seed-drilling, i.e. 15 April 2009, before

²³ On the basis of resuspension in 50 mL followed by two rinses, corresponding to the highest calculated dose per hectare

²⁴ All the results are inferior to the limits of quantitation

²⁵ On the basis of resuspension in 50 mL followed by two rinses, corresponding to the highest calculated dose per hectare

²⁶ All the results are inferior to the limits of quantitation

²⁷ These mortalities are not recorded on the inspection file.

the hives were opened and the frames examined. Deaths were estimated at 150 to 200 bees per hive.

Samples were taken for pathological analysis and to screen for residues of thiamethoxam in the bees and, in the case of Lutz-en-Dunois, in bee bread taken from the hives. Samples of bee bread were also taken from the hives at the control pilot sites.

The visits on 22 April 2009 describe strong colonies at three of the four sites. At Lutz-en-Dunois, the colonies were found to be in medium condition, except for one that was strong. Activity was described as strong throughout the apiary. Supers were added as a precaution on Saturday 18 and Wednesday 22 April.

The visits on 29 April 2009 describe medium colonies at one of the control sites and strong to medium at the other control site, with some mortalities observed in front of the hives, though no indication is given of the numbers concerned. At Villamblain, the colonies are described as strong to medium, with some mortalities observed in front of the hives but with no indication of quantities. At Lutz-en-Dunois, the colonies are described as strong to weak, also with some non-quantified mortalities, in front of the hives.

Interpretation of the mortalities observed at Villamblain

The results of the toxicological and health analyses conducted on the samples of dead bees and bees with symptoms reveal:

- the presence of residues of thiamethoxam and clothianidin at concentrations of 2.68 µg/kg²⁸ and 1.76 µg/kg²⁹ respectively, i.e. 0.268 and 0.176 ng/bee, respectively.
- a manifest case of chronic paralysis (3.98×10^{11} copies/bee).

The doses of thiamethoxam and clothianidin measured are lower, not only than the acute toxicity reference values³⁰, but also than the 10-day NOEL³¹ (2 ng/bee), even when considering the accumulated residues as being equivalent to thiamethoxam³², and cannot explain a significant and visible mortality among bees.

The results of the analyses of the bee bread are not available.

The viral load per bee for the Chronic Paralysis Bee Virus indicates a strongly positive diagnosis and corresponds to a manifest case of disease^{33, 34}. The mortalities reported (whose extent is not given) can therefore be explained by chronic paralysis, as detected in the dead bees.

Interpretation of the mortalities observed at Lutz-en-Dunois

The results of the toxicological and sanitary analyses practised on the samples of dead bees and bees showing symptoms reveal:

- among the dead bees:
 - the presence of residues of thiamethoxam and clothianidin at respective concentrations of 2.9 µg/kg and 1.1 µg/kg (double analysis)³⁵ and 3.2 µg/kg and 3.2 µg/kg (double analysis)³⁶, i.e. an average of 2.0 and 3.2 µg/kg respectively, or 0.2 and 0.32 ng/bee;
 - declared chronic paralysis (1.07×10^{11} copies/bee);

²⁸ Limit of quantitation: 0.5 µg/kg

²⁹ Limit of quantitation: 1 µg/kg

³⁰ DL50 by ingestion and by contact: respectively 5 ng/bee and 24 ng/bee for thiamethoxam, and 4.79 ng/bee and 44 ng/bee for clothianidin

³¹ NOEL: No observed effect level

³² It should be noted that the thiamethoxam could have begun to degrade into clothianidin in dust falling on to the bodies of the bees, then in the bees, and finally during their decomposition on the ground. The residues of thiamethoxam and clothianidin should be considered together as equivalent thiamethoxam, which leads to an estimate of the dose of thiamethoxam for the initial exposure of 0.474 ng/bee (with a molar ratio for thiamethoxam/clothianidin of 1.168).

³³ Detection of Chronic bee paralysis virus (CBPV) genome and its replicative RNA form in various hosts and possible ways of spread. Olivier Celle, Ph. Blanchard, V. Olivier, F. Schurr, N. Cougoule, J. P. Faucon, M. Ribière. *Virus Research* 133 (2008) 280–284.

³⁴ Evaluation of a real-time two-step RT-PCR assay for quantitation of Chronic bee paralysis virus (CBPV) genome in experimentally-infected bee tissues and in life stages of a symptomatic colony. Ph. Blanchard, M. Ribière, O. Celle, P. Lallemand, F. Schurr, V. Olivier, A.L. Iscache, J.P. Faucon. *Journal of Virological Methods* 141 (2007) 7–13.

³⁵ Limit of quantitation: 0.5 µg/kg

³⁶ Limit of quantitation: 1 µg/kg

- among bees showing symptoms collected in front of the hives, infestation by *Nosema* sp. was observed (6.54×10^6 spores/bee) and chronic paralysis bee virus was also found (1.15×10^6 copies/bee).

The bee bread collected was analysed and contained 8.16 µg/kg of thiamethoxam. In order to ingest the equivalent of LD₅₀ of this source of food, a bee would need to consume 0.6 g of bee bread. Bee bread – whose thiamethoxam residues are contributed solely by the pollen - is used principally to feed larvae and nurse bees, and for the sake of comparison, the daily needs in pollen of larvae and nurses are estimated to be 5.1 mg and 6.5 mg, respectively³⁷. It should be noted that bee bread from the control pilot sites contained 0.54 µg a.s./kg of thiamethoxam at the Villeneuve site and 1.21 µg a.s./kg at Péronville. No explanation for the presence of these residues found in non-exposed hives during the monitoring programme has been suggested.

As before, the doses of thiamethoxam and clothianidin are not only lower than the acute toxicity reference values, but also below the 10-day NOEL (2 ng/bee), even when considering the accumulated residues as being equivalent to thiamethoxam, and cannot explain any significant and visible mortality of bees.

The mortalities observed could, however, correspond with the chronic bee paralysis found in the dead bees.

Conclusions for the Centre region

The conditions under which the pilot sites in the Centre region were monitored are disappointing. The health condition of the colonies and the circumstances under which the hives were installed on the 'Cruiser-treated' pilot sites at Villamblain and Lutz-en-Dunois, combined with inappropriate seed-drilling conditions, cannot be considered as representing field conditions as intended by the monitoring procedure, which had been discussed with the profession. It is therefore very difficult to interpret the data collected.

On both these sites, the abnormal nature of the mortalities observed in the apiaries remains to be established precisely. The results of the analyses of the samples of dead bees show that despite the conditions of extreme exposure to dust from seed-drilling, the levels of residues of thiamethoxam and clothianidin measured cannot explain any significant mortality in the hives. On the other hand, the mortalities recorded could well be related to the chronic paralysis found in the dead bees.

4.5. Conclusions for the 2009 monitoring programme

The monitoring programme was undertaken in six French regions: Aquitaine, Midi-Pyrénées, Rhône-Alpes, Centre, Poitou-Charentes and Alsace. The results received to date, for only two regions out of the six involved in the monitoring plan, are very incomplete and, for some of them, difficult to interpret. However, the levels of exposure, as measured by contamination of Petri dishes, are lower than the NOAEL for the active substance for bees, even in the extreme installation conditions in the Centre region.

The initial results do not show any loss of colonies attributable to the seed-drilling of maize using seed coated with Cruiser.

A comparison of the conditions under which seed-drilling was carried out in the two regions with conditions applying in 2008 provides evidence in support of the precautions that can be taken during seed-drilling, with a view to reducing the exposure of pollinators.

³⁷ Rortais A, Arnold G, Halm MP and Touffet-Briens F, Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36: 71-83 (2005).

5. CONCLUSIONS

The purpose of the monitoring system deployed in 2008 and 2009 was to evaluate the unintended effects of thiamethoxam on pollinators, in particular honey bees, resulting from the use of Cruiser, which contains thiamethoxam, applied as a seed treatment. Monitoring took place in three regions in 2008 and was extended to six in 2009.

Initial data from the surveillance plan have been made available. Afssa has examined these results, but is disappointed to note the following:

- in 2008, a much lower number of sites and pilot sites were installed than the number planned, and in 2009, only a very incomplete amount of the data collected are so far available and is limited to the seed-drilling period (for two regions out of six);
- there were deviations from the procedure concerning the conditions under which the hives were installed (location, number and timetable) and the monitoring itself;
- the health condition of the hives was often mediocre, with several pathogens found to be present, making it very difficult to analyse the causes of bee mortalities and assess the respective impact of their state of health and the possible presence of pesticide residues.

The results of the 2008 programme procedure nonetheless confirmed that dust is created during seed-drilling, which had been taken into account in the risk evaluation study made by Afssa in 2007³⁸. This observation led to the recommendation, published in October 2008³⁹, to rapidly adopt additional technical measures to reduce the emission and dispersal of dust during seed-drilling.

In addition, it has been possible to examine the results from the seed-drilling period in two of the regions involved in the 2009 procedure:

- The analyses carried out confirm the effectiveness of fitting deflectors to seed drills to reduce the dispersal of dusts containing the active substance.
- In the Aquitaine region the procedure was implemented under good conditions and led to the conclusion that there were no abnormal mortalities in the colonies exposed to seed-drilling with maize seed coated with Cruiser.
- In the Centre region, the pilot sites were installed under disappointing conditions (poor health of the hives, circumstances of installation at the 'Cruiser-treated' sites and of seed-drilling operations) that do not conform to the monitoring procedure, making it very difficult to interpret the data collected. Abnormal mortalities were observed in the apiaries installed in the fields drilled with treated maize. These mortalities are considered to correspond principally to the chronic bee paralysis disease detected in dead bees, and not to be due to exposure to dust containing thiamethoxam.

Pathologies were frequently observed in the colonies monitored under the programme, which illustrates the difficulty of clearly identifying the origin of bee mortalities reported in France and shows the advantages of multifactor studies intended to improve understanding of these complex phenomena.

In light of the data received so far, the French Food Safety Agency considers that the conclusions and recommendations expressed in the previous Opinion⁴⁰ are not called into question.

While waiting for the final report on the 2009 monitoring programme, the technical measures recommended previously should be taken to reduce the generation and dispersal of dust during seed-drilling:

- equipping seed drills with anti-drift equipment that has been demonstrated by a manufacturer's tests to guide dust back towards the ground;

³⁸ Opinion 2007-3845 dated 21 November 2007.

³⁹ Opinion 2008-SA-0307 dated 23 October 2008.

⁴⁰ Opinion dated 20 December 2007 concerning the conclusions of the evaluation of Cruiser with regard to the long-term risk for bee colonies.

- verifying the effectiveness of this type of equipment for guiding dust from seed-coating back towards the ground in windy conditions (between 20 and 30 km/h);
- implementing supplementary precautions such as restricting seed-drilling to slight wind conditions (2 on the Beaufort scale) or restricting the practice of rolling after seed-drilling, which can compromise the effectiveness of efforts to reduce the dispersal of dust.

Marc MORTUREUX

**Annex 1: results of measurements of the dispersal of dust during seed-drilling
as estimated by the Petri dish method
2008 monitoring programme**

The Petri dishes were placed at varying distances (5, 10 and 50 metres) from the drilling row. The dose/ha is calculated on the basis of 50 or 100 mL of glycerol water in dishes 13.5 cm in diameter.

For 2008, the procedure recommended collecting dust in 50 mL of glycerol water, then two rinses with 25 mL of glycerol water. Given the constraints involved in performing the rinses in the field, the 2009 procedure was amended to remove the rinses.

Since the exact method followed at each site is not recorded in the report, both calculations were carried out to estimate the equivalent dose (application rate) of active substance per hectare.

The results for the sites in the Rhône-Alpes region are given in the following tables. (If the wind was blowing at 90° to the line of drilling, the dishes were placed upwind or downwind. If the wind was blowing parallel to the line of drilling, the dishes were placed to the left or right of the line of drilling.)

Site	Ambronay							
	Upstream			Downstream (downwind)			Outside the drilling line	Control
Distance from the drilling line (m)	5 m	10 m	50 m	5 m	10 m	50 m		
Concentration in µg/L of glycerol water	0.05	< LQ	< LQ	1.2	1.05	0.24	< LQ and 1.1	0.07
Equivalent in g/ha if diluted in 50 mL	0.002	< LQ	< LQ	0.042	0.038	0.08	< LQ and 0.038	0.002
Equivalent in g/ha if diluted in 100 mL*	0.004	< LQ	< LQ	0.084	0.076	0.016	< LQ and 0.076	0.004

Site	Lagnieu							
	To the left of the drilling line			To the right of the drilling line			Outside the drilling line	Control
Distance from the drilling line (m)	5 m	10 m	50 m	5 m	10 m	50 m		
Concentration in µg/L of glycerol water	< LQ	< LQ	0.11	1.6	0.9	< LQ	< LQ	< LQ
Equivalent in g/ha if diluted in 50 mL	< LQ	< LQ	0.004	0.056	0.031	< LQ	< LQ	< LQ
Equivalent in g/ha if diluted in 100 mL*	< LQ	< LQ	0.008	0.112	0.062	< LQ	< LQ	< LQ

* As indicated in the procedure and mentioned in a memo from the DGAL to the authorities responsible for implementing the monitoring programme (DDSV, DRAF/SRPV).

Results for the pilot sites in the Midi-Pyrénées region are listed in the following tables:

Site	Cintegabelle							
	Upstream			Downstream (downwind)			Outside the drilling line	Control
Distance from the drilling line (m)	5 m	10 m	50 m	5 m	10 m	50 m		
Concentration in µg/L of glycerol water	< LQ	< LQ	< LQ	16	6	2.14	< LQ and 0.46	< LQ
Equivalent in g/ha if diluted in 50 mL	< LQ	< LQ	< LQ	0.58	0,24	0.075	< LQ and 0.016	< LQ
Equivalent in g/ha if diluted in 100 mL*	< LQ	< LQ	< LQ	1.16	0.42	0.15	< LQ and 0.032	< LQ

Site	Ségos							
	To the left of the drilling line			To the right of the drilling line			Outside the drilling line	Control
Distance from the drilling line (m)	5 m	10 m	50 m	5 m	10 m	50 m		
Concentration in µg/L of glycerol water	0.37	0.46	0.21	1.4	0.76	0.16	0.11 et 0.78	< LQ
Equivalent in g/ha if diluted in 50 mL	0.013	0.016	0.007	0.049	0.027	0.0056	0.0038 et 0.027	< LQ
Equivalent in g/ha if diluted in 100 mL*	0.026	0.032	0.014	0.098	0.054	0.0112	0.0076 et 0.054	< LQ

* As indicated in the procedure and mentioned in a memo from the DGAL to the authorities responsible for implementing the monitoring programme (DDSV, DRAF/SRPV).

**Annex 2: results of measurements of the dispersal of dust during seed-drilling
as estimated by the Petri dish method
2009 monitoring programme**

The memorandum issued for the 2009 monitoring programme recommended collecting dust in a volume of 50 mL of glycerol water, without further rinsing of the Petri dishes. It seems, however, that rinsing was carried out in some regions. Both calculations were therefore performed in order to estimate the equivalent dose of active substance per hectare.

The results for the Aquitaine region are given in the following tables:

Site	Cazères sur Adour							
	To the left of the drilling line			To the right of the drilling line			Outside the drilling line	Control
Distance from the drilling line (m)	5 m	10 m	50 m	5 m	10 m	50 m		
Concentration in µg/L of glycerol water	< LQ	< LQ	< LQ	< LQ	0.098	0.24	< LQ	< LQ
Equivalent in g/ha if diluted in 50 mL*	< LQ	< LQ	< LQ	< LQ	0.0035	0.0085	< LQ	< LQ
Equivalent in g/ha if diluted in 100 mL	< LQ	< LQ	< LQ	< LQ	0.007	0.017	< LQ	< LQ

Site	Souprosse							
	To the left of the drilling line			To the right of the drilling line			Outside the drilling line	Control
Distance from the drilling line (m)	5 m	10 m	50 m	5 m	10 m	50 m		
Concentration in µg/L of glycerol water	< LQ	0.1	0.17	< LQ	< LQ	< LQ	< LQ	< LQ
Equivalent in g/ha if diluted in 50 mL*	< LQ	0.0035	0.0056	< LQ	< LQ	< LQ	< LQ	< LQ
Equivalent in g/ha if diluted in 100 mL	< LQ	0.007	0.0112	< LQ	< LQ	< LQ	< LQ	< LQ

* As indicated in the procedure and mentioned in a memo from the DGAL to the authorities responsible for implementing the monitoring programme (DDSV, DRAF/SRPV).