AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES ALIMENTS

Maisons-Alfort, 2 March 2010

OPINION

of the French Food Safety Agency relative to the proposed protocol for the 2010 post-authorisation monitoring programme on the Cruiser 350 seed treatment product

On 10 February 2010, the French Food Safety Agency (AFSSA) issued a formal internal request to review the protocol proposed by the Directorate General for Food (DGAL) for the 2010 post-authorisation monitoring programme on the CRUISER 350 seed treatment product.

BACKGROUND

AFSSA received a marketing authorisation application dossier submitted by the company Syngenta Agro SAS for the thiamethoxam-based insecticide CRUISER 350, intended as a seed treatment for maize and sorghum.

Based on the examination of the dossier submitted for this product and the available data, AFSSA issued a favourable Opinion[1] on 1 December 2009 for the use of maize seeds treated with CRUISER 350, in addition to several recommendations with regard to the risk for bees.

Strictly identical to the CRUISER 350 seed treatment, the CRUISER seed treatment underwent post-authorisation monitoring in 2008 and 2009 to assess the unintended effects of thiamethoxam on pollinators, particularly honeybees. The results of this monitoring programme were examined by AFSSA and an Opinion was issued[2].

At the request of the Minister of Agriculture, the Directorate General for Food (DGAL) continued this monitoring and will implement a new protocol in 2010.

OBJECTIVE OF THE FORMAL INTERNAL REQUEST

Upon examination of the data provided from the two years of monitoring in 2008 and 2009, some results appeared to be difficult to use considering the protocol that had been implemented.

After consultation with the Scientific Panel on "Plant protection products: chemical substances and preparations" that met on 23 February 2010, the French Food Safety Agency issued the following guidelines to improve the monitoring protocol so that the results thereof can be used to their full potential.

- Systematic and daily monitoring of bee mortality and symptomatic bees should be carried out for all the hives in the treated and non-treated sites during the sowing and flowering periods. A device for collecting dead bees in front of the hive was described for this purpose.

- To avoid certain difficulties encountered during the analysis of results from the 2008 and 2009 monitoring programmes, apiaries should be installed at least 2 weeks before the sowing period for proper acclimation of the bees. A detailed calendar can be found in the annex of the proposed protocol. This calendar specifies the dates of the health inspections, as well as the samples to be taken during these inspections. As bee bread is a good indicator of potential

[1] Opinion no. 2009-1235 of 1 December 2009.
[2] Solicited request 2009-SA-0253 – result of the Cruiser monitoring programme of 1 December 2009.

contamination of the hive, sampling should thus be carried out during three key phases of this monitoring: at installation, before flowering and at the end of flowering.

- The proposed protocol mentions reinstalling hives that had been monitored in 2009. In order to avoid introducing new biases in the interpretation of results, especially to be able to compare agrosystem histories and plant protection practices, it would be preferable if the hives that had been monitored in 2009 not be reintroduced for 2010 monitoring purposes. Furthermore, some swarm homogeneity criteria are proposed for the selection of hives for monitoring in 2010, but these criteria may not be met by some of the hives that were monitored in 2009.

- The protocol calls for sampling flora in the event of excess mortality at the treated and untreated sites during the sowing period. This new monitoring parameter appears to be important.

  In order to be able to use these data, the sampling conditions (quantity of samples, whether or not they are grouped) and the storage of samples should be described in detail, in addition to the analyses to be performed (multi-residue, thiamethoxam, imidacloprid), and the name of the laboratory in charge of these analyses.

- The proposed protocol calls for monitoring insects in general. Monitoring should be limited to the populations of pollinators that include the honeybee and wild pollinators.

  In addition, the presence of wild pollinators, notably butterflies, depends directly on landscape features. Consequently, monitoring them only along the edges of [cultivated] fields does not seem relevant, and the monitoring zone should be delimited according to the objectives that the DGAL wishes to establish. It may be necessary to limit this monitoring to the monitoring sites chosen from zones that vary with respect to landscape features and to those chosen from zones that vary with respect to type of crop.

- The protocol mentions that “the beekeeper responsible for supervising the apiary during the study could also be in charge of the health inspections”. In order to avoid any conflict of interest, the beekeeper in charge of monitoring the hives as part of this monitoring programme should not also act as his own “health inspector”, regardless of his aptitude to do so.

The proposed protocol for the 2010 post-authorisation monitoring programme on Cruiser 350 is given in the Annex of this Opinion.

Marc MORTUREUX

Keywords: monitoring protocol, Cruiser 350, bee

ANNEX

PROPOSED PROTOCOL FOR THE 2010 POST-AUTHORISATION MONITORING PROGRAMME ON THE CRUISER 350 PRODUCT

AFSSA's Proposal as part of formel internal request no. 2010-SA-0029

Subject: National post-authorisation monitoring programme on the CRUISER 350 product

References: Rural code – Chapters III, IV, V and VII of Title V of Book II and texts taken for their application

Summary: The national post-authorisation monitoring programme on CRUISER 350 is based on the principle of comparing Cruiser-treated sites with control sites so as to identify the potential unintended effects of the CRUISER 350 seed treatment.

On the basis of experience from the two previous years of monitoring, this monitoring programme follows a simplified protocol, which is centred primarily on monitoring insects and apiary health. Monitoring is to take place during two key periods, i.e. during maize sowing and flowering periods, in the monitoring zones that have been well characterised from the viewpoint of cultivation practices and their landscape.

To select monitoring sites and to effectively conduct the surveys on agricultural practices (and particularly on the history of use of plant protection products) in the monitoring zones, the departmental public works and agriculture authorities (DDEA) of the *départements* concerned by this monitoring programme are to assist in the monitoring programme, especially in the survey of farmers with fields located in the monitoring zones, .

Keywords: biovigilance, maize, thiamethoxam, pollinators, monitoring

1. GENERAL OBJECTIVES

The national monitoring programme concerning CRUISER 350 is part a nationwide biological monitoring effort aimed at detecting unintended effects of agricultural practices, particularly of plant protection products, on the environment.

It is based on the principle of comparing monitoring data collected on biological indicators according to a harmonised protocol at monitoring sites that have been chosen as representative of a situation where seeds dressed with the preparation CRUISER 350 are predominantly used, with a situation where they are not or only rarely used. The sites where the monitoring programme is implemented must be characterised with regard to the agricultural practices implemented there, particularly in terms of the crops cultivated and the main landscape and environmental factors.

This monitoring programme has been enhanced with regard to monitoring unintended effects on pollinators, notably on honeybees.

The implementation of this monitoring must incorporate the various local authorities that carry out the inspections in the field (Cross-ministerial departmental authorities - DDI; Regional food service - SRAL).

2. SECTION OF THE MONITORING PROGRAMME RELATIVE TO THE CULTIVATED PLOTS

The monitoring programme is to be implemented in the five following regions: Midi-Pyrénées, Rhône-Alpes, Aquitaine, Centre and Poitou-Charentes. In each region, eight sites, identified by the SRAL, are to be partitioned as specified in the table below:

Region	CRUISER 350 sites	Non-treated sites
Midi-Pyrénées	4	4
Rhône Alpes	4	4
Aquitaine	4	4
Poitou-Charentes	4	4
Centre	4	4
Total	**20**	**20**

2.1 Definition and general data on the sites

2.1.1. Definition and criteria for site the choice of sites

The monitoring sites are composed of an apiary located in a sheltered spot, such as a hedge or grove following the usual good beekeeping practices. The sites include a group of maize fields, as a single, large plot or divided into many plots.

The monitoring focus zone (see diagram in Annex 2) consists of an area with a 1 km radius, i.e. approximately 300 ha. This focus zone does not cover the entire area over which the monitored bees gather nectar and pollen, but it represents a zone in which domestic and wild pollinators can be intensively monitored.

The SRAL is to select eight monitoring sites in each region according to the following criteria:

Cruiser-treated sites

- 2 sites in a zone of intensive crop cultivation and where the cultivation of maize predominates (150 ha of maize minimum);
- 1 site where the apiary can be installed in a zone that varies with respect to landscape features within the total surface (woods, hedges, brambles) and in which at least 30% of the surface area is cultivated with maize (90 ha of maize minimum);
- 1 site where the apiary can be installed in a zone that varies with respect to type of crop within the useful agricultural area and including around 30% of the surface cultivated with maize (90 ha of maize minimum).

For these sites, the surface area of maize grown from seeds dressed with CRUISER 350 must be around 80% of the area designated for maize cultivation, i.e. 120 ha, 72 ha and 72 ha, respectively.

Non-Cruiser-treated sites

- 2 sites in a zone of intensive crop cultivation and where the cultivation of maize predominates (150 ha of maize minimum);
- 1 site where the apiary can be installed in a zone that varies with respect to landscape features within the total surface (woods, hedges, brambles) and in which at least 30% of the surface is cultivated with maize (90 ha of maize minimum);
- 1 site where the apiary can be installed in a zone that varies with respect to type of crop within the useful agricultural area and including around 30% of the surface cultivated with maize (90 ha of maize minimum).

For these sites, the surface area of maize cultivation originating from seeds that have not been dressed with CRUISER 350 must be 100% of the area designated for maize cultivation.

The apiaries located between the two types of monitoring site ("Cruiser-treated" and "non-Cruiser treated") must ideally be separated by a minimum distance of 10 km, i.e. twice the maximum radius of bee activity. Nevertheless, if it proves to be difficult to find such locations, a minimum distance of 5 km can be allowed.

2.1.2 Recording the landscape and environmental factors of the sites

The different types of crop found in the sites are to be inventoried using graphic plot representations (Graphic plot registry – RPG) and field surveys, so as to perform accurate mapping. A description of the main landscape and environmental factors (edges of fields, woods, hedge, etc.) is to be provided for each site. Orthophotos from the National Geographic Institute (IGN, France) can also be used [to provide information] for site descriptions.

2.2 Collection of biological data (fauna and flora)

The collection of monitoring data from wild pollinators is to be carried out in all the monitoring zones during the sowing period and the maize flowering period.

Pollinators are the primary object of this part of the monitoring programme, carried out according to the trapping protocol that was instituted two years earlier. Butterflies can be good indicators of unintended effects, and dedicated monitoring is simple to implement. These monitoring activities will be coupled with the collection of flora. Vegetation along the edges of fields and to a greater extent from the environment of the monitored fields may indeed have a strong impact on the behaviour of the insects, particularly on pollinators. The objective is therefore to use monitoring data to detect the origins of unintended effects that could occur in the pollinators present in the environment of the cultivated field due to the presence of attractive flowers near them or any other unanticipated phenomena. Collecting information on the pre-determined biological indicators and cross-checking this information must help to both detect potential unintended effects and identify the factors that can explain and interpret them.

2.2.1 Landscape descriptors

Preparation in progress by the MNHN

2.2.2 Data collection on pollinators and other insects

The observational data on wild pollinators are collected through:

- recording the presence and abundance of butterflies during the maize sowing and flowering periods according to the method found in Annex 1A. These observations are reported on the tally sheet found in Annex 1B;
- trapping pollinators in the maize fields during flowering according to the method presented in Annex 2.

The tally sheets are to be sent electronically to the French National Museum of Natural History (MNHN) at the following address: fchiron@mnhn.fr, copy to brmmi.sdqpv.dgal@agriculture.gouv.fr, no later than 15 June 2010 for the observations made during sowing, and no later than 15 September for those made during flowering. All the sheets from the same region are to be sent on the same date.

The samples collected through trapping are to be stored and shipped according to the procedure indicated in Annex 2.

2.2.3 Data collection on flora along the edge of fields

The observational data on flora along the edge of fields are to be collected adjacent to the cultivated fields. These data are to be obtained according to the method described in Annex 3. As the proposed method is in the experimental stage, it is important to strictly follow the proposed protocol instructions so that the MNHN can validate this method. It is also imperative to note the presence of flowering trees and/or bushes (e.g. blackthorn, hawthorn) near the fields when maize is sown.

More thorough floristic data can also be communicated to the MNHN when local expertise is available, in addition to the method under validation by the MNHN.

The tally sheets are to be sent electronically to the MNHN at the following addresses: fchiron@mnhn.fr and machon@mnhn.fr, with a copy to brmmi.sdqpv.dgal@agriculture.gouv.fr, no later than 15 June 2010 for the observations made during sowing, and no later than 15 September for those made during flowering. All the sheets from the same region are sent on the same date.

2.3 Agricultural practices

Surveys are to be used to collect information on the agricultural practices over the last two years and the present year involving the use of plant protection products, particularly insecticides, for all the fields (even those without maize crops) included in the monitoring sites. It is also important to note the possible presence

of fields of flowering oilseed rape, because the period of maize sowing coincides with the flowering of oilseed rape.

2.4 Identification of the dressed seeds used in sowing

The batch numbers of the CRUISER 350-treated maize seeds, which are used for the cultivation of fields in the monitoring sites, are to be collected from the farmers and recorded by the agents from the corresponding regional authority on food, agriculture and forests (DRAAF) and/or the SRAL.

Information thus collected is to be completed by results from the tests carried out by the seed companies on each of the maize seed batches using the Ceres or Heubach test. This information is to be communicated by the French Official Inspection and Certification Service (SOC) for seeds and plants.

2.5 Sampling of flora in the event of excess mortality of bees

In the event of excess mortality of bees when treated and non-treated sites are seeded, flower samples (from herbaceous plants and/or shrubs) along the edges of fields and exposed to dominant winds are to be collected immediately in order to investigate the presence of plant protection product residues and identify their nature. The samples are to be kept in well-marked packages and immediately frozen after collection. The analyses to screen for these products may be based on the list of products used in the fields located in the zone in question.

3 SECTION OF THE MONITORING PROGRAMME RELATIVE TO APIARY HEALTH

This part of the programme only involves the veterinary aspect of the monitoring, the aim of which is to compare two populations of bees: those whose hives are placed in "Cruiser-treated" maize sites and those whose hives are placed in “non-Cruiser-treated” maize sites.

Glossary and the role of each Beekeeper
Health Inspector
DDI
Departmental health protection group for the honeybee (GDS)
Beekeeping technician from the Association for the development of beekeeping (ADA)
SRAL Agent

3.1 Installation of apiaries

Apiaries, each comprised of seven hives, are introduced in each of the monitoring sites (see paragraph 2.1.1). The purchase terms, their quality and their distribution over the monitoring sites are specified below.

3.1.1 Purchase of hives

The hives are to be purchased[3] by the DDI specifically for implementing this monitoring programme and are to be delivered by the seller to the monitoring site after passing a health inspection at the time of purchase to attest to satisfactory health of the hive (see paragraph 3.2.2).

The seven hives of a single apiary are to be furnished with a queen, which is marked so as to be recognised during the different samplings. Two hives per apiary are to be equipped with a pollen trap fitted to the hive body. These pollen traps are to be purchased from the beekeeper at the same time as the hives. Identical traps are used for the hives in a given *département*.

[3] The purchase includes the delivery of the hives to the monitoring sites by the beekeeper.

The purchased swarms must form a homogenous batch and be representative of locally used strains. The criteria of hive homogeneity are the following:
- the same number of frames colonised by bees when the hive is opened,
- the same number of brood combs (open and capped)
- a comparable agrosystem history and plant protection practices.

If these criteria cannot be met, homogenous and non-homogenous hives can be distributed equally across the monitoring sites.

3.1.2 – Layout on the sites

The hives must be installed at least two weeks before the date of the first sowing.

Each apiary is to be placed near a maize field and is introduced “at the heart” of the site so that the hives are suitably exposed with respect to the monitored maize fields.

The layout and installation of the hives is to take place following good beekeeping practices: the apiary is set up and sheltered by a hedge or grove at the centre of the monitoring site. During sowing it is prohibited to place the hives in the fields directly on the ground and/or on bare ground.

The health inspection report (see paragraph 3.2.2) should specifically describe under which conditions the apiaries are installed and, in particular, their immediate environment (photographed if possible and enclosed with the report).

3.1.3 Identification of the hives

Each apiary is to be clearly identified by a code or number (apiary no.; monitoring no., indicating the number of the *département* and the identifier of the monitoring site along with the letter C for Cruiser or T for Control). This code provides a reference throughout the duration of the study as to whether an apiary originates from a “Cruiser” site or “non-Cruiser” site, as well as its location.

Each hive is to also be clearly identified by an indelible identification code that must be clearly visible in order to avoid any error. This code must indicate throughout the duration of the study whether a hive originates from a “Cruiser” site or “non-Cruiser” site, as well as its location.

A correspondence table matching the hive’s code to the data pertaining to that hive is to be established and carefully kept by the DDI.

3.1.4 Number and distribution of the hives

The national monitoring system is comprised of a total of 40 apiaries (20 "Cruiser" apiaries and 20 "non-Cruiser" apiaries), i.e. a total of 280 monitored hives (140 "Cruiser" hives and 140 "non-Cruiser" hives).

Location and number of hives

Region	***Département***	**Number of apiary sites**	**Number of hives per apiary**
Midi-Pyrénées	Haute-Garonne (31) and Gers (32)	4 "Cruiser 350" apiaries	7*
		4 "non-Cruiser 350" apiaries	7*
Aquitaine	Landes (40) and Pyrénées-Atlantiques (64)	4 "Cruiser 350" apiaries	7*
		4 "non-Cruiser 350" apiaries	7*
Rhône-Alpes	Ain (01)	4 "Cruiser 350" apiaries	7*
		4 "non-Cruiser 350" apiaries	7*
Poitou-	Vienne (86)	4 "Cruiser 350" apiaries	7*

Region	*Département*	Number of apiary sites	Number of hives per apiary
Charentes		4 "non-Cruiser 350" apiaries	7*
Centre	Loiret (45)	4 "Cruiser 350" apiaries	7*
		4 "non-Cruiser 350" apiaries	7*
TOTAL hives			**280**

***7 hives per apiary, 2 of which are fitted with a pollen trap**

3.1.5 Recovery of hives before overwintering

At the end of the maize flowering period in the considered zone, the hives are relocated to a common site by the beekeeper in cooperation with the DDI and the health inspector, so that the preparation for overwintering is homogenous among the different categories of hives. The hives will thus form two batches (Cruiser and non-Cruiser) at the site of relocation and may be in close proximity but not mixed together.

3.2 Field monitoring and maintenance of the apiaries

The field monitoring is to be led by each DDI involved in the monitoring programme. The field monitoring protocol (visits and sampling) is to be carried out by a beekeeping sanitary assistant (beekeeping sanitary agent or beekeeping GDS agent or ADA beekeeping technician) who is accompanied by a DDI agent or if this is not possible, by an SRAL agent.

3.2.1 Monitoring of mortality

An account of bee mortality, due to natural causes or not, must be thoroughly performed during the two most critical periods for the bees relative to the monitoring programme. This monitoring must be carried out daily at the control sites (non-Cruiser-treated) and the Cruiser-treated sites throughout the entire sowing period of each monitoring zone, as well as during the maize flowering period. The number of dead bees (and/or the weight according to the number of dead bees) and the number of symptomatic bees per hive are to be reported on a specific form.

Dead bees should be recovered using the following device, which should be placed beneath the entry of all the hives used in the monitoring programme.

Cadaver recovery device

For each hive, a 0.60 x 1.00 m plywood board, fitted with an approximately 4 cm rim, is to be used. Several holes are to be drilled at one end of the board, to allow water to drain off; the board is placed beneath the entry of the hive on a wedge several centimetres thick and located under the edge opposite the drilled edge, to tilt the board and thus allow water to drain off. A wire frame is to be placed over this device to reduce predation.

3.2.2 Health inspections

A series of health inspections is to be conducted in each of the apiaries according to a pre-established inspection and sampling calendar, which is included in Annex 1.

At each health inspection, the health inspector is to fill out an inspection sheet (sheet established by AFSSA - Antipolis), and the collected samples are to be identified by an appropriate label and sent with an information sheet to the various laboratories involved.

The inspection upon purchase is to be performed by a DDI-trained agent in the presence of the beekeeper involved in the monitoring programme and responsible for the maintenance of the apiary. The health inspection at the time of purchase of the hives aims to verify the homogeneity of the proposed hives, the absence of disease and the quality of the colonies (see paragraph 3.1.1). If there is any doubt concerning the health status of the hives, the DDI will not purchase them.

The second inspection, performed within 24 hours following the installation of the apiary, is to be done by the health inspector in the presence of the beekeeper in charge of the apiary (and insofar as possible with a DDI agent). The goal of this collaborative inspection is to train the inspector in carrying out the inspection,

completing the inspection sheet, taking and packaging the samples, interacting with the DDI [agents], meeting with the beekeeper and installing the pollen traps (done by the beekeeper). It also makes it possible to establish a baseline. All of the samplings are then to be carried out (see paragraphs 3.2.3 and 3.2.4). The AFSSA Bee Pathology Unit is to participate in these inspections as much as possible.

The other inspections are to be done by the health inspector, accompanied if possible by the DDI agent, with prior notification of the beekeeper. During these health inspections, the development of the five colonies that do not have pollen traps should be monitored and reported on the "sample and inspection sheet for apiary health monitoring" based on the following criteria:
- the number of frames colonised by bees during the opening of the hive;
- the number of brood combs;
- the total weight of the hive.

3.2.3 Sampling and sample storage

The calendar of inspections and sampling found in Annex 1 is delivered by the DDI to the health inspector and the beekeeper.

For this purpose, the DDI is to provide sampling bags and adhesive labels to the health inspector. The health inspector uses one bag per type of sample and per recipient laboratory. The following is then indicated on each label affixed to the bag:
- type of sample (example: random sample of live bees within the hive / dead bees / symptomatic bees outside the hive)
- sample code
- apiary identification code
- sampling date
- recipient laboratory (AFSSA or GIRPA)
- type of analysis requested (toxicological or pathogen).

The samples intended for analyses (paragraph 3.2.4) must be collected under the conditions described below.

A copy of the inspection sheet is included with each sample for recordkeeping.

Bees

- *Symptomatic bees*: Sampling priority is given to live bees presenting abnormal clinical signs or behaviour.

- *Dead bees*: Only recently dead bees should be sampled and only at times other than rainy periods. If the bees are dried out or rotting, the analysis cannot be carried out. In the presence of abnormal behaviour, it is imperative that the "dead bee" sample be accompanied by a separate "symptomatic" bee sample from the same colony.

  Sampling must be as thorough as possible in number in order to have a sufficient quantity of bees so that the sample can be divided into two subsamples for the laboratory in charge of the analysis, with one subsample for analysis of pathogens and the other for toxicological analyses.

- *Live bees*: About one hundred live bees are to be sampled randomly in each of the five hives without pollen traps for a grand total of 500 live bees. In the presence of abnormal clinical signs or behaviour, it is imperative that this sample be accompanied by a sample of bees presenting these symptoms.

The bees must be placed in a brown paper envelope that has been labelled beforehand, one for the live bees (staple the corners of the envelope after sampling), one for each hive presenting dead bees and one for symptomatic bees. Each envelope is immediately placed in a plastic bag, then in a cool box containing frozen ice packs. These samples must then be frozen at –18° C at the end of the day before being sent frozen, without breaking the cold chain, to the laboratories in charge of the analyses (paragraph 3.2.4). The live bee samples will then be mixed and homogenised at the laboratory after freezing in a single sample representing the apiary.

Pollen

The pollen collected from the pollen traps of the two fitted hives must be combined and thoroughly homogenised before sampling for the laboratory analysis and then placed in paper bags or cardboard boxes, e.g. matchboxes, that have been labelled beforehand. A quantity of 50 g is necessary to be able to carry out the analyses. Each paper bag, put into a plastic bag, must be immediately placed in a cool box. These samples must then be frozen at– 18° C at the end of the day before being sent frozen, without breaking the cold chain, to the laboratories in charge of the analyses (paragraph 3.2.4).

Brood

Brood is to be sampled if the health inspection concludes that it is symptomatic. In this case, cut a 10x10 cm piece of brood that contains at least 15 larvae and/or pupae whose abnormal appearance prompted this analysis request. The samples must be placed in paper bags or cardboard boxes, e.g. matchboxes, which have been labelled beforehand. Each package must be immediately placed in a cool box containing frozen ice packs. These samples must then be frozen at –18° C at the end of the day before being sent frozen, without breaking the cold chain, to the laboratories in charge of the analyses (paragraph 3.2.4).

Bee bread

The bee bread is to be sampled from one single hive, with a different hive sampled at subsequent visits, in a number of alveoli containing bee bread (pollen stored in the cells), in sufficient quantity to obtain 30 g of bread. Be sure not to sample brood at the same time. The samples must be placed in paper bags or cardboard boxes, e.g. matchboxes, which have been labelled beforehand. Each package must be immediately placed in a cool box containing frozen ice packs. These samples must then be frozen at –18° C at the end of the day before being sent frozen, without breaking the cold chain, to the laboratories in charge of the analyses (paragraph 3.2.4).

3.2.4 Analyses

In this monitoring programme, analyses are requested for all the apiaries (see calendar of sampling and analyses in Annex 1). The analyses on the bees are to be carried out on the total quantity of bees from the same apiary. They include:

- **Detection of pathogens**
  Screening for pathogens, if necessary following the health inspection: this screening is to be carried out only if abnormalities are noted (abnormal behaviour in the bees, symptoms in front of the colonies, mortalities, etc.) in the samples of **live, symptomatic** and **dead** bees**.** The screening targets pathogenic agents that cause disease [acariosis, nosemosis, foulbrood, varroasis, chronic bee paralysis virus (CBPV), sacbrood virus (SBV), acute bee paralysis virus (ABPV)].

  The samples intended for bee pathogen detection are to be analysed by AFSSA - Sophia Antipolis – Bee Pathology Unit (105 route des Chappes – BP 111 – 06902 SOPHIA-ANTIPOLIS – Tel: 04 92 94 37 00 – Fax: 04 92 94 37 01).

- **Detection of plant protection product residues and the identification of observed potential toxic effects according to the calendar in the Annex:**
  - screening and quantification of thiamethoxam and its metabolite (clothianidin), as well as imidacloprid and its metabolite in the bees, bee bread and pollen pellets;
  - multi-residue analysis in the bee samples before maize flowering and a second analysis after maize flowering.

In case of abnormal mortality findings, complete samples (dead bees, symptomatic bees, pollen pellets and bee bread) are to be taken and analysed (pathogens and residues). The pathogen and residue screening will be conducted on two bee subsamples originating from the sample that showed abnormalities (symptoms and/or mortality). In the event of abnormal mortality, particular attention should be given to detailed description of the symptoms observed and to assessing of the quantity of dead bees or those presenting these symptoms, as well as the number of apiary colonies in which these phenomena are observed.

Important note: As the programme is based on the principle of comparison between the "Cruiser" sites and the "non-Cruiser-treated" control sites, samples (bees, bee bread, pollen) are to be taken from both sites ("Cruiser-treated" and corresponding 'non-treated"). The samples are to be stored in distinct packages and immediately frozen after collection.

The pollen and bee samples intended for investigation of plant protection product residues are to be analysed by GIRPA (8 rue Henri Becquerel – 49070 Beaucouze – Tel: 02.41.48.75.70 – Fax: 02.41.48.71.40).

The bee bread samples are to be analysed by AFSSA-Sophia Antipolis – Bee Pathology Unit (105 route des Chappes – BP 111 – 06902 SOPHIA-ANTIPOLIS – Tel: 04 92 94 37 00 – Fax: 04 92 94 37 01).

- **Identification of the origin of the pollen brought back to the hive**
  A fraction of the pollen collected in the pollen traps is to be taken to determine the origin of the pollen collected by the bees and to measure the proportion of maize pollen in these collections, expressed as the number of maize pollen grains over the total number of pollen grains and in volume percent of maize pollen in the sample.

  Beginning in September, the same palynologic analyses are to be carried out on a bee bread sample.

  The pollen samples taken to identify the plant species present are to be analysed in Aquitaine by the MICHAUD company (Domaine St Georges, chemin Berdoulou - 64290 GAN, Tel: 05 59 21 91 00, 2009 pollen analysis price – 28 €/analysis).

3.2.5 Maintenance of the apiaries

Apart from the programme/calendar of health inspections, the apiaries must undergo maintenance and supervision by a volunteer beekeeper.

The vendor who sells the hives intended for monitoring can also be the beekeeper responsible for supervising the apiary during the study. See *AFSSA's comments in its Opinion.*

The beekeeper agrees to inform the health inspector of his observations during the health inspection, and in the event of anomalies, to immediately and directly notify the DDI.

The beekeeping supervision of the apiaries, as well as any intervention, event or assessment are to be recorded by the beekeeper in his beekeeping record book. Likewise, the health inspector is to contact the beekeeper responsible for the supervision of the apiaries before each health inspection.

The apiaries that are the focus of the monitoring protocol are considered to be typical apiaries by the DDI. The apiary and the hives are subject to the usual notification measures. In the event of health problems, the DDI conducts the follow-up interventions prescribed by the regulations.

The beekeeper documents the quantities of products collected, e.g. honey, for each type of apiary (Cruiser and non-Cruiser). In order to compare production performances, harvesting from the Cruiser and non-Cruiser apiaries from the same site is done during the same week. As compensation for services rendered, the honey and other products from the hive may be offered to the beekeeper free of charge.

3.2.6 Shipment of samples and channels of information

According to the storage conditions described in 3.2.3, and without breaking the cold chain, the health inspector is to place all of the sample bags collected from an apiary during an inspection into a single bag that contains the original inspection sheet, and then gives it to the DDI in the *département* in which apiary has been set up.

Shipments must be avoided after Wednesday, as samples may be left unfrozen over the weekend, making the analysis unreliable. The samples must be sent to the laboratory in a rigid parcel in order to avoid any risk of crushing; a carrier must be used to ensure negative temperatures during all phases of the transport.

The DDI is to send a copy of each inspection sheet to the SRAL of the appropriate DRAAF.

The DDI is to send the collected samples (while verifying the compliance with the calendar of inspections and samples table attached in the Annex) to the relative laboratories with a copy of the inspection sheet, used for recordkeeping purposes, and dispatch note.

The DDI is to inform the laboratories beforehand of the sample shipment.

Each laboratory is to send the analysis results to the DDI that requested the analysis. The DDI is to send a copy of these results to the relevant SRAL and the DGAL. The DDI also communicates the results to the health inspector responsible for the samples, who in turn informs the beekeeper during a health inspection.

4. GOVERNANCE OF THE MONITORING

The duration of the field monitoring is to last from mid-April 2010 to Spring 2011. The intermediate and final results of the monitoring will be presented at each scientific and technical committee, as well as at each steering committee meeting. The governance organisation chart is to be used for overseeing the activities and for determining the information to be sent.

Please inform me of any potential difficulties encountered during the implementation of this memorandum.

Director General for Food

Pascale BRIAND

Annex 1

CALENDAR OF HEALTH INSPECTIONS AND SAMPLINGS TO BE DONE AS PART OF CRUISER 350 MONITORING

Inspection	Week*	Dates	Period	Inspection sheet	Samples (analyses to be done: in accordance with § 3.2.4)					
					Bees TOXICO (N = 500)	Bees PATHO (N = 100)	Brood PATHO If necessary	Bee bread TOXICO	Pollen TOXICO	Pollen qualitative
Preparations before monitoring	10		Inspection of potential sites							
	10		Inspection of apiary purchases	1						
	10-11		Installation of apiaries					AFSSA		
No. 1	10-11		Pre-sowing inspection within 24 hr after installation of apiaries on site	1	GIRPA	AFSSA	AFSSA			
	13 - 14		Sowing							
No. 2	13 - 14		As of 24 hr after sowing: - 3 health inspections in 3 weeks to be carried out once a week - Daily monitoring of deaths throughout the entire sowing period of a monitoring zone	1	GIRPA	AFSSA	AFSSA		GIRPA	
No. 3	15			1	GIRPA	AFSSA	AFSSA		GIRPA	
No. 4	16			1	GIRPA	AFSSA	AFSSA		GIRPA	
No. 5	20		Intermediate inspection (after sowing and before maize flowering)	1	GIRPA	AFSSA	AFSSA	AFSSA	GIRPA	
No. 6	25		Before flowering (3 to 6 days before flowering)	1	GIRPA	AFSSA	AFSSA			
No. 7	27		During flowering, 3 inspections over 3 weeks to be carried out once a week Daily monitoring of mortality throughout the entire sowing period of a monitoring zone	1	GIRPA	AFSSA	AFSSA		GIRPA	
No. 8	28			1	GIRPA	AFSSA	AFSSA		GIRPA	
No. 9	29			1	GIRPA	AFSSA	AFSSA		GIRPA	
No. 10	30 to 32		At the end of flowering	1	GIRPA	AFSSA	AFSSA	AFSSA	GIRPA	
No. 11	October		Beginning of winter 2010	1	GIRPA	AFSSA	AFSSA		GIRPA	
No. 12			End of winter (spring 2011)	1	GIRPA	AFSSA	AFSSA			

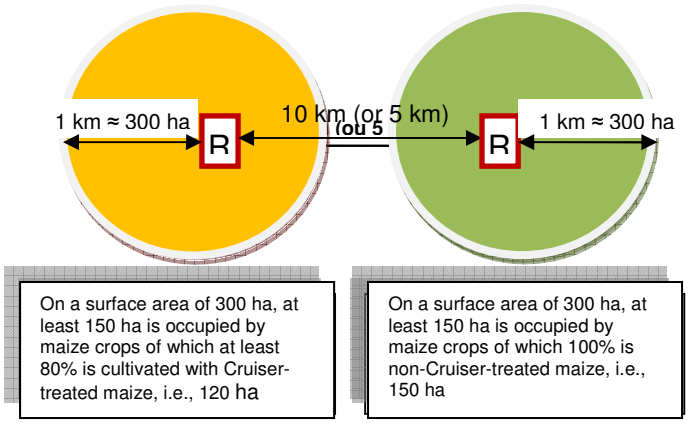
TOTAL			Per apiary	13	12	12	12	3	9	9

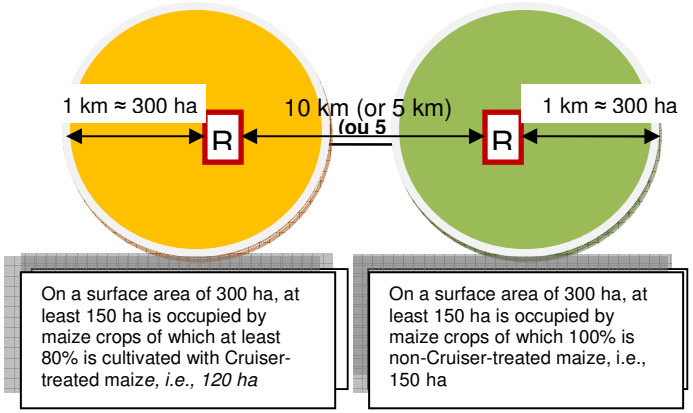
* The proposed calendar must be adjusted according to the sowing date in each region

Annexe 2

FOR EACH REGION

2 ZONES OF INTENSIVE MAIZE





: Site Traité CRUISER

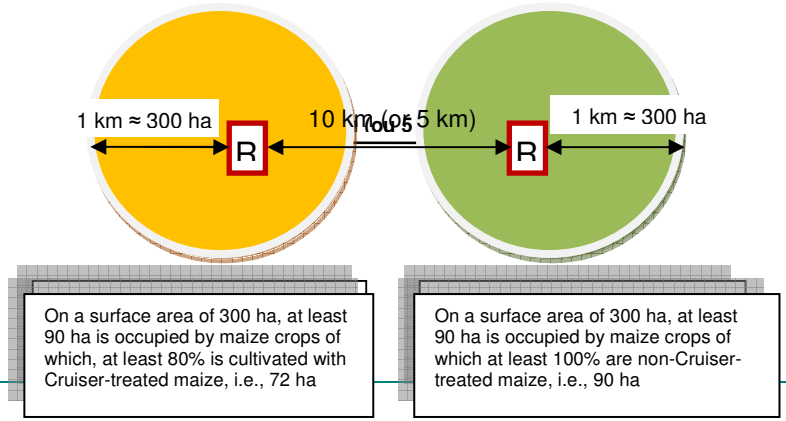
: Cruiser-treated

: Non-Cruiser-treated

R : Apiary

OTHER ZONES:

ZONE WITH VARIABLE LANDSCAPE FEATURES



ZONE WITH VARIABLE TYPES OF CROP