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DE SÉCURITÉ SANITAIRE
DES ALIMENTS

THE DIRECTOR GENERAL

Afssa – Request no. 2007-SA-0393
subject related to no. 2007-3845 – Cruiser

Maisons-Alfort, 20 December 2007

OPINION

of the French Food Safety Agency (Afssa) on the conclusions of the Cruiser assessment regarding the long-term risk to bee colonies

On 13 December 2007, the Directorate-General for Food (DGAI) requested that the French Food Safety Agency (Afssa) issue an Opinion relating to the conclusions of the Cruiser assessment.

On 20 September 2007 Afssa received a marketing authorisation application for a thiamethoxam-based product, submitted by the company Syngenta Agro SAS as part of a mutual recognition procedure, on which it issued an Opinion on 20 November 2007.

Following this Opinion, Afssa was asked to examine additional data provided by Syngenta Agro SAS on the assessment of the long-term risk to bee colonies.

After consulting the Scientific Panel "Plant protection products: chemical substances and preparations", which met on 19 December 2007, Afssa issues the following Opinion.

1 CONTEXT OF THE REQUEST

The Opinion issued on 20 November 2007 concerned the thiamethoxam-based insecticide, Cruiser, intended to treat maize and sweet corn seeds, and embodied the following conclusion:

"In conclusion, the short-term risks are considered to be acceptable for adult bees. No effect associated with exposure to the pollen or nectar of treated crops was detected in the plastic-tunnel or field tests. The long-term risks for a colony have not been fully assessed as the results of a field study conducted over the last three years are not yet available. Information on the exposure levels through pollen and nectar confirm a low exposure level in a scenario where barley is cultivated in the springtime followed by rape in the winter.

Pending the results of studies still under way, and to reduce exposure via the gathering of pollen or nectar from the treated crop or following crops which may contain residues of thiamethoxam, hives should be moved more than 3km away from the crops cultivated from treated seeds during the flowering period. In addition, plants that may be attractive to bees should not be introduced in the crop rotation, and measures should be applied to limit bee exposure."

Afssa also recommended monitoring pilot hives under realistic conditions to quantify the potential contamination level in hives and refine the incidence and nature of long-term risks.

This current Opinion is based on the examination of additional data concerning the preliminary reports of three field studies set up in 2006 to study the effects on bees, and the final reports of three plastic-tunnel studies conducted to analyse the residues in different materials for bee exposure to treated maize (the use requested for the Cruiser insecticide).

The studies on the long-term effects on bees exposed to treated rape (preliminary reports of two field studies initiated in 2005) and on the measurement of exposure levels (final reports of three plastic-tunnel tests) in the nectar and pollen of winter rape will be evaluated for a later Opinion.

As a result, this Opinion only concerns the request on maize crops treated with the insecticide Cruiser.

The additional data submitted by the applicant, plastic-tunnel studies and field studies, are summarised below.

2 PLASTIC-TUNNEL STUDIES TO DETERMINE THE POTENTIAL EXPOSURE OF BEES BY POLLEN FROM MAIZE SEEDS TREATED WITH THIAMETHOXAM

The plastic-tunnel studies were set up in 2005 and 2006 to estimate the potential exposure of bees to residues that can reach the maize pollen by systemic properties.

Pollen-gathering bees and colonies were exposed to treated maize in plastic tunnels in three French regions. The hives were exposed for two years in a row (2005 and 2006). Maize was sown for two years in a row on the same plot (28kg/ha) to measure the transfer of thiamethoxam residues and its metabolite CGA 322704 to the plant, pollen, hive and soil. On each site, three plastic tunnels of treated maize and one plastic tunnel of untreated maize were constructed. A plant sample was taken during the exposure stage. The samples of pollen harvested by the pollen-gathering bees were taken on three dates during the exposure stage. Samples of pollen and wax in the frames were taken just before the hives were put into the plastic tunnels and continued until around 50 days after exposure.

The limit of quantification (LOQ) of 0.001mg/kg was validated in the soil, plant and pollen for thiamethoxam and CGA 322704 and in the wax for CGA 322704. The limit of quantification of 0.0005mg/kg was validated in the wax for thiamethoxam.

In each test, only one colony examination was carried out at the start and end of exposure (number of frames with bees and surfaces filled with egg, larval and pupal stages).

In Alsace and Champagne-Ardenne, the maize varieties Delitop and Moncada were used in 2005 and 2006 respectively. In Midi-Pyrénées, the variety NK Terra was used. For all three regions, a formula containing thiamethoxam and two fungicides was used and the dose of thiamethoxam on the seeds was the same as the one claimed for the insecticide Cruiser (315 g s.a./quintal).

In Alsace, the hives were exposed to flowering maize for nine days in 2005 and three days in 2006.

Analysis showed the presence of residues at quantifiable levels in all plant samples. The residues were quantified in the plants while the bees were exposed (2005: 0.010-0.018 mg thiamethoxam/kg, 0.010 – 0.016 mg CGA322704/kg; 2006: 0.003 – 0.012 mg thiamethoxam/kg, 0.002 – 0.008 mg CGA322704/kg).

The residues were quantified in pollen gathered by bees in 2005 (0.05-0.015mg thiamethoxam/kg, 0.003-0.007mg CGA322704/kg) but were generally lower than the limits of quantification in 2006 (<0.001–0.002 mg thiamethoxam/kg, <0.001–0.002 mg CGA322704/kg).

In the frames' pollen, the residues were less than the limits of quantification, except in a third of the samples (maximum 0.004mg thiamethoxam/kg and 0.006mg CGA 322704/kg). In the wax, the residues were less than the limits of quantification. The residues in the soil before the sowing period in 2006 were below the limits of quantification.

In Champagne-Ardenne, hives were exposed to flowering maize for six days in 2005 and five days in 2006.

Analysis showed the presence of residues at quantifiable levels in all plant samples. The residues were quantified in the plants while the bees were exposed (2005: 0.003-0.006 mg thiamethoxam/kg, 0.002 – 0.005 mg CGA322704/kg; 2006: 0.003 – 0.004 mg thiamethoxam/kg, 0.002 – 0.004 mg CGA322704/kg).

The residues were quantified in pollen gathered by bees in 2005 (0.05-0.004 mg thiamethoxam/kg, 0.002-0.003 mg CGA322704/kg) but were generally lower than the limits of quantification in 2006 (<0.001–0.002 mg thiamethoxam/kg, <0.001–0.002 mg CGA322704/kg).

In the frames' pollen, the residues were below the limits of quantification. In the wax, the residues were less than the limits of quantification, except in a few samples with a maximum of 0.0014mg of thiamethoxam/kg. The residue concentrations of thiamethoxam and CGA 322704 in the soil before the sowing period in 2005 were very low (0.002mg/kg for each substance).

In Midi-Pyrénées, hives were exposed to flowering maize for eight days in 2005 and seven days in 2006.

Analysis showed the presence of residues at quantifiable levels in all plant samples. The residues were quantified in the plants while the bees were exposed (2005: 0.009-0.020 mg thiamethoxam/kg, 0.004 – 0.008 mg CGA322704/kg; 2006: 0.017 – 0.050 mg thiamethoxam/kg, 0.006 – 0.012 mg CGA322704/kg).

The thiamethoxam residues were quantified in the pollen gathered by bees (2005: 0.001-0.012 mg thiamethoxam/kg, 2006: 0.001-0.008 mg thiamethoxam/kg). Residues of CGA 322704 were found at lower concentrations in the pollen gathered by bees in 2005 (<0.001–0.002 mg CGA322704/kg; 2006: <0.001–0.003 mg CGA322704/kg).

In the frames' pollen, the residues were below the limits of quantification, except in a very limited number of samples (maximum 0.002 mg thiamethoxam/kg and 0.001 mg CGA 322704/kg). In the wax, the residues were below the limits of quantification, except in one case (0.0009 mg thiamethoxam/kg). The residue concentration of CGA 322704 in the soil before the sowing period in 2006 was very low (0.001 mg/kg) and thiamethoxam was below the limit of quantification.

Conclusions of the plastic-tunnel tests to determine the potential exposure of bees via pollen from maize seeds treated with thiamethoxam

In maize pollen harvested by the bees, the mean concentration levels of thiamethoxam residue were 4.81 µg/kg in 2005 (77 analyses) and 2.09 µg/kg in 2006 (53 analyses). The mean concentration levels of CGA 322704 residue were 2.65 µg/kg in 2005 (77 analyses) and 1.37 µg/kg in 2006 (53 analyses). The means of the results obtained over the two years were 3.45 µg thiamethoxam/kg and 2.01 µg CGA 322704/kg (106 analyses for each substance)[1].

In the pollen harvested in the frames, the concentrations of thiamethoxam and CGA 322704 were lower than in the pollen taken from the bees. The concentrations measured were not higher in 2006 than in 2005.

3 FULL-FIELD STUDIES WITH MULTI-YEAR FOLLOW-UP OF HIVES EXPOSED TO MAIZE POLLEN

Full-field studies were set up in 2006 for three years in a row by exposing hives during the flowering period of maize from seeds treated with the insecticide Cruiser. The follow-up of hives from one season to the next should make it possible to study the impact of exposure to residue in the maize pollen on the winter survival of the colony, the [spring] recovery of pollen-gathering activity and on the health of the colonies.

- ***Test conditions***
  These field tests were conducted in three French regions (Alsace, Lorraine and Aveyron). They got under way in 2006 and are expected to last until spring 2009 so as to assess the

[1] The mean concentrations were used in the risk assessment for nurse bees to avoid the overestimation of exposure that would occur if occasional peaks in concentration were taken into account..

long-term effects of annual exposure to the pollen of maize grown from seeds treated with thiamethoxam-based insecticide.

The same colonies are kept in the same test plots during the maize flowering period over the three successive years (exposure in 2006, 2007 and exposure intended in 2008). When the maize crop begins to flower (BBCH 59-61), six colonies are placed in front of each treated plot (one treated plot per site) and six others in front of each control plot (one control plot per site). Once the maize has finished flowering (BBCH 69), the colonies are moved to a maintenance site selected to minimise additional exposure to other pesticides. In this test, the short-term assessment of the exposure impact is completed with a health check-up of the colonies during the rest of the pollen-gathering season and the success of the end of over-wintering for colonies can be assessed the following spring.

Special attention has been paid to bee exposure to maize pollen. The plots are carefully selected so that they are well away from any attractive crop that may flower at the same time as the maize, to ensure that the bees gather pollen from the treated and control plots only. The plots planted were large, approximately 2 ha each, with the treated plots placed at about 2 km from the control plots so as to limit the movement of bees from one plot to the next.

The agronomic parameters in the plots and their environment are not available in the interim reports.

The health of the bee colonies and development of the brood are recorded during exposure and at regular intervals afterwards. Comments are made on deaths, pollen-gathering activity, colony numbers (estimation of the number of adult worker bees), the presence of healthy eggs laid by the queen, verified by the presence of freshly laid eggs (less than a day old), estimation of the surface area containing eggs, larvae and operculated cells (in % of surface area), estimation of the pollen and nectar storage surface area (in % of surface area), colony weight and signs of disease in the bees.

Every unusual event observed (bee death, hairless bees, crawling bees or that are unable to fly, abnormal brood), symptom of disease (chalk brood, sac brood, *Nosema*, American or European foul brood) or parasitisation (*Aethina tumida, Tropilaelaps* spp.) is recorded. The infestation of each colony by *Varroa* is monitored constantly and recorded by routine assessment. Bee samples from each colony are also collected and stored at around -30°C for viral analyses.

Samples were taken from plants and bees for analysing pesticide residue (plants, pollen on plants, pollen harvested by the bees, stomach nectar) and for the taxonomic identification of pollen types. The results of these analyses are not yet available. The two methods are distinct only by the positioning of the hives in front of an untreated maize plot (NT hive) or a treated maize plot (T hive).

The colonies have been divided up to prevent risks of swarming. For this, the old queen and some of the worker bees are transferred to a swarming hive with 1-2 frames of larvae and 1-2 spare frames. The original colony should normally raise a new queen. If it does not, the old queen is put back into the original hive. The divisions were carried out on 02/05/2007, 03/05/2007 and 05/04/2007 for the hives in Alsace, Lorraine and Aveyron respectively.

Records of apiculture measures taken, other than those stipulated in the protocol, are not available in the interim reports.

- ***Test results in Alsace***
  The maize varieties were Moncada in 2006 and PR39G12 in 2007. The seeds were treated with the insecticide Cruiser (315g thiamethoxam/100 kg), together with a fungicide. For the control plot, untreated maize was sown. The hives were exposed for five days in 2006 and eight days in 2007.

  During exposure, death rates of the adult bees in front of the hives were low and similar for both plots. Only one hive in front of the control plot showed a higher death rate, unrelated to

another observation. No deaths were found in the test plots. Pollen-gathering on the flowering maize was assessed and no significant difference was observed between the two plots. At the hive entrances, no abnormal behaviour among the bees was observed. Unprocessed records of these observations are not available in the interim reports.

The size of the colony, estimated in number of bees per hive according to the Liebefeld method, was observed during the colony activity periods. Growth of the colonies was similar for both plots, with, in particular, a healthy recovery of activity in all hives in spring at the end of the over-wintering period. The growth of only one T colony was disrupted by sac brood, detected in spring 2007. Development in colony weight was similar, except for the hive infected with sac brood.

The surfaces covered by larval stages in the frames evolved in similar ways between the hives of both plots. Some differences were noted over short-term periods. For one T hive, the queen was lost before the hive was divided up and the colony successfully raised a new one. The food reserves developed in a similar way for both plots.

Nosemosis was detected in almost all the hives at least once in 2006, and not at all in 2007. Varroasis was present in over half of the hives for both plots in at least one sample, and the infestation rate was lower in 2007 than in 2006. Amibiasis (2006,2007), acarapisosis (2006[2]) and American foul brood (2006, 2007) were not detected.

The viral analysis results are available for the 2006 samples and some of the 2007 samples (spring and July, before exposure to the maize). KBV (Kashmir Bee Virus), ABPV (Acute Bee Paralysis Virus) and CBPV (Chronic Bee Paralysis Virus) were not detected. SBV (Sac Brood Virus) was detected in 2006 in three NT hives and DWV (Deformed Wing Virus) was detected in one NT hive in 2006.

- ***Test results in Lorraine***

  The maize varieties were Moncada in 2006 and PR39G12 in 2007. The seeds were treated with the insecticide Cruiser (315g thiamethoxam/100 kg), together with a fungicide. For the control plot, untreated maize was sown. The hives were exposed for six days in 2006 and eight days in 2007.

  During exposure, death rates of the adult bees in front of the hives were low and similar for both plots. In July 2007, before the hives were exposed to the maize, a high death rate was recorded for one T hive for a short time, due directly to the reintroduction of the swarming colony in its original hive[3]. A single dead bee was found on the treated maize plot in 2007. Pollen-gathering on the flowering maize was assessed and no significant difference between the two plots was found. At the hive entrances, no abnormal behaviour among the bees was observed. Unprocessed records of these observations are not available in the interim reports.

  The size of the colony, estimated in number of bees per hive according to the Liebefeld method, was observed during the colony activity periods, with, in particular, a healthy recovery of activity in all hives in spring at the end of the over-wintering period. Colony growth was similar for both plots. Development in colony weight was similar.

  The surfaces covered by larval stages in the frames evolved in similar ways between the hives of both plots. In July, before the hives were exposed to the maize, disturbances in the surfaces covered by the larval stages were observed in four T hives. Male larvae were found in three of these hives. The reintroduction of swarming colonies into their original hive meant that larval growth could pick up again. For one NT hive, the larval stages were no longer observed in the frames at the end of August, because of the loss of a queen in mid-July 2007.

  Nosemosis was detected in all the NT hives and in two T hives at least once in 2006, and in two T hives and one NT hive in 2007. Varroasis was present in over half of the hives for both plots in at least one sample, and the infestation rate was lower in 2007 than in 2006.

2 The results for acaripisosis are not yet available for 2007.
3 This was carried out after no queen was found during the observations in mid-July.

Amibiasis (2006,2007) and acarapisosis (2006) were not detected. American foul brood was not detected, except for one sample in one NT hive in 2007.

The viral analysis results are available for the 2006 samples and some of the 2007 samples (spring and July, before exposure to the maize). KBV (Kashmir Bee Virus), ABPV (Acute Bee Paralysis Virus) CBPV (Chronic Bee Paralysis Virus) and DWV (Deformed Wing Virus) were not detected. SBV (Sac Brood Virus) was detected just the once in a sample from one T hive in 2007.

- ***Test results in Aveyron***
  The maize varieties were Dakovo in 2006 and Justina in 2007. The seeds were treated with the insecticide Cruiser (315g thiamethoxam/100 kg), together with a fungicide. For the control plot, untreated maize was sown. The hives were exposed for six days in 2006 and eight days in 2007.

  During exposure, death rates of the adult bees in front of the hives were low and similar for both plots. A single dead bee was found on the treated maize plot in 2007. On the untreated maize plot, three and two dead bees were found in 2006 and 2007 respectively. Pollen-gathering on the flowering maize was assessed and no significant difference was observed between the two plots. At the hive entrances, no abnormal behaviour among the bees was observed. Unprocessed records of these observations are not available in the interim reports.

  In this test, observation was more difficult due to a high infestation of American foul brood. Two T colonies highly infected with American foul brood were destroyed and replaced by a swarm from the same plot. At the end of summer 2007, a colony C was highly infected. Comparisons between apparently healthy hives were nevertheless carried out.

  The size of the colony, estimated in number of bees per hive according to the Liebefeld method, was observed during the colony activity periods, with, in particular, a healthy recovery of activity in all hives in spring at the end of the over-wintering period. In May-June 2007, one NT hive developed more than others. In June-July 2007, the NT hives grew slightly more on average than the T hives. Development in colony weight was similar. The NT hives had a better supply of nectar in May-June 2007.

  The surfaces covered by larval stages in the frames presented similar developments between hives on both plots, except in the three colonies highly infected by American foul brood. At the end of summer, one T colony had no larval stages left, but the link with the foul brood was not established.

  Nosemosis was detected in two T hives at least once in 2006 but not at all in the other hives. In 2007, nosemosis was detected at least once in almost all of the hives. Varroasis was present in half of the hives from both plots in at least one sample in 2006 and in over half of the hives in at least one sample in 2007. Amibiasis (2006, 2007) and acarapisosis (2006) were not detected. American foul brood was detected in one NT hive and four T hives in 2006. The analysis results of the 2007 samples are not available.

  The viral analysis results are available for the 2006 samples and some of the 2007 samples (spring and July, before exposure to the maize). KBV (Kashmir Bee Virus), ABPV (Acute Bee Paralysis Virus), CBPV (Chronic Bee Paralysis Virus) and SBV (Sac Brood Virus) were not detected. DWV (Deformed Wing Virus) was detected just the once in a sample from one T hive in 2007.

- ***Conclusion on the initial results of the field tests on flowering maize (2006-2007)***
  In the three full-field tests, pollen-gathering on male maize flowers was observed without it being possible to assess the scale because of a lack of reference data on this activity in maize. The analytical and palynological results in samples of harvested pollen on pollen-gathering bees are not yet available. The representativeness of hive exposure during a single period of maize flowering has not been discussed in the interim reports.

The results available do not show any abnormal behaviour among the bees or any excess or unexplained deaths. Instead, they show that development of the adult and larval populations and of hive weight is consistent with a good state of health and healthy recovery in colony growth in spring at the end of the 2006/2007 over-wintering period.

Specific observations on each test were undertaken and do not show any apparent link with exposure to pollen derived from treated seed, but a more likely link with beekeeping measures, health or environmental factors. The presence of nosemosis, varroasis and American foul brood in several hives was noted in particular.

4 GENERAL CONCLUSIONS ON THE ADDITIONAL DATA

Potential exposure of bees was determined in an enclosed environment. The analytical results show that residue can be brought back to the hive by pollen-gathering bees. That said, the residue concentrations are low, non-quantifiable even, in most of the samples taken from inside the hives exposed for two successive years to pollen from maize grown from treated seed.

The full-field tests show that exposure over five to eight days during two sessions in a row to plots of maize grown from treated seeds did not have a significant impact on colony survival or development. This impact was monitored over an observational period spanning two seasons and one over-wintering period in three different regions.

The applicant has not discussed how representative the hive exposure periods (from five to eight days depending on session and region) are in the reports, in terms of the reality of bee exposure periods under realistic maize cultivation conditions (staggering flowering spells depending on sowing dates and variety, for example). However, the exposure period is relevant for grain or forage[4] maize sown before 15 May and for female varieties of maize seed plants, irrespective of the sowing date. This reduces the flowering variability and therefore bee exposure to approximately a week (five to eight days). With regard to sweet corn, the exposure period is longer due to more spaced-out flowering spells. As a result, the exposure periods of these studies are not representative for sweet corn.

Exposure quantification out in the field is not available. No link has been made with the residue results obtained from the plastic-tunnel studies, during which pollen-gathering was not assessed.

Lastly, these are interim results. Other results are expected, which will allow the analysis of these trials to be completed, embodying three years of exposure and three over-wintering periods.

To conclude, the results of the studies examined allow reconsideration of the recommendation given in the Opinion of 20 November 2007, with a view to reducing bee exposure to treated crops.

The French Food Safety Agency therefore recommends:

- maintaining the 3km distance between the hives and plots sown with maize treated with the insecticide Cruiser for sweet corn and male maize seed plants only, due to a lack of representative exposure data. This precaution is no longer necessary for grain maize or forage maize for silage, or for female maize seed plant if the sowing period is limited according to the point below;

- limiting the sowing period for grain maize, forage maize and female maize seed plants treated with the insecticide Cruiser to a period ending on 15 May;

[4] [Specifically, maize for silage]

- that the company Syngenta Agro SAS continues and finalises the long-term tests already set up.

The corresponding precaution is as follows:

SPe8 : Hazardous for bees. Do not introduce plants that may attract bees in the crop rotation, or apply measures that may limit bee exposure (e.g. reaping before flowering). For sweet corn or male maize seed plant, move the hives more than 3 km away from the crops grown from the treated seeds during the flowering season.

Afssa also recommends creating an independent organisation in 2008 to monitor particularly-exposed apiaries[5] due to their location in relation to plots of treated maize.

[Signed by the Director-General]

[5] [also known as bee yards]