1998 NATIONAL PROGRAMME

EFFECTS OF CROP PROTECTION PRODUCTS ON BEES
Effects of GAUCHO seed dressing on losses of foraging bees

FINAL REPORT
March 1999

[redacted]

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FOREWORD

This study was carried out under the auspices of the French Ministry of Agriculture and Fisheries at the request of representatives of the beekeeping industry in order to answer the following question:

Is the seed dressing Gaucho (active ingredient = imidacloprid), a systemic product that makes plants resistant to aphid attack, responsible for losses in hive populations and the falls in sunflower nectar production that have been observed during the sunflower flowering period over the past few years?

A study programme involving field trials and laboratory experiments was devised and carried out by a number of organisations working together: scientific research bodies, technical institutions, representatives of the Ministry of Agriculture and Fisheries and the Ministry of Land Planning and the Environment, the farming industry, beekeepers, seed producers and BAYER.

The work was carried out under considerable pressure; the group had to set up the programme, carry out the experiments, process the data and draft the reports within a short time frame. We should like to take this opportunity to thank all those involved for their commitment to this programme which has great ecological and economic importance.

All the results obtained from the study and summarised in this report are undoubtedly worthy of more detailed investigation. They could form the basis of scientific or technical publications, allowing lessons to be learnt not only in the particular case of Gaucho as a dressing for sunflower seeds but also in more general terms to help develop new methods of assessing the effects of crop protection products on bees, taking account of both lethal and sub-lethal effects.

REASONS FOR THE PROGRAMME

INTRODUCTION

Between 1993 and 1997 beekeepers observed increasingly large falls in their sunflower honey yields; the central and western-central regions of France were particularly badly affected. The lower yield figures were attributed to losses of foraging bees at the time when the crop was in flower. The sharp fall in nectar production coincided with a rise in the area of land given over to sunflower cultivation using seed dressed with Gaucho (active ingredient: imidacloprid). Field surveys carried out by CNEVA and ACTA, field trials carried out by Bayer and the observations and questions raised by beekeepers highlighted the need for concerted action to discover whether sunflower seed dressing was affecting bee populations.

SIGNIFICANT DATES

24 October 1997
First joint meeting called by ACTA to discuss the causes of the fall in sunflower nectar production.

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Comparison of data submitted by BAYER and by beekeepers' representatives.
Annexe I-1: minutes of the meeting

November-December 1997
The Ministry of Agriculture and Fisheries (MAF) commissions two scientific experts ([redacted], INRA, Avignon; [redacted] INRA, Lusignan) to draw up a "Report on the effects of Gaucho (imidacloprid) used as a sunflower seed dressing: Impact on bee populations and nectar production".
Report submitted to the commission responsible for examining the toxicity of agricultural antiparasitic products and similar products (Toxicity Commission).
Annexe I-2: experts' report

26 January 1998
Creation of a Steering Committee at the request of the Crop Protection Unit (General Food Directorate, GFD) to monitor additional studies.
Start of work to set up a programme of multidisciplinary studies.
Annexe I-3: minutes of the meeting and press release from the Ministry of Agriculture and Fisheries

5 February 1998
Ban on the use of sunflower seed treated with Gaucho in Deux-Sèvres (already banned in Indre and Vendée). Work now organised in the following structures: Steering Committee, Operational Group, Local Committee (to cover the individual départements).
Presentation of a study programme involving field work, work in a controlled environment (tunnel) and in the laboratory costed at 6 million francs.
Programme to be submitted by the GFD to the PTD (Production and Trade Directorate) for European funding under Council Regulation no. 1221/97 containing implementation rules for action aimed at improving honey production.
Annexe I-4: minutes of the meeting

17 March 1998
The EU agrees to refund 50% of the cost of the studies.
Financial support from the Ministry of Land Planning and the Environment and from BAYER.
Local committees set up in the 3 départements.
Steering Committee approves experimental zones and study protocols.
Decision taken to ask the GFD to draw up specifications for the analyses.
The GFD draws up a plan for monitoring and inspecting seeds in the 3 départements.
Annexe I-5: minutes of the meeting

26 May 1998
Information on establishment of the experimental sites.
Report on progress made in reaching administrative agreements.
List of participating laboratories drawn up following the call for tenders for the analytical work.
Annexe I-6: minutes of the meeting

24 June 1998
Select committee: Analyses for individual matrices and laboratories distributed.

Annexe I-7: minutes of the meeting

17 July 1998
Steering Committee visits the field experiments.

31 July 1998
Technical meeting in Luçon.
Observation of problems in hives in the Vendée affecting areas treated with Gaucho and areas free from Gaucho.
Annexe I-8: minutes of the meeting

17 September 1998
Discussion on the need to keep the results confidential as not all experiments have been concluded.
Presentation of completed experiments and submission of preliminary reports.
Timetable for outstanding analytical work and for submission of final reports.
Annexe I-9: minutes of the meeting and MAF press release (dated 5/10/98) on the timetable.

15 October 1998
Final reports submitted by the groups involved.

6 November 1998
Coordinator presents a first draft of the summary report.
Comments of members of the Steering Committee incorporated into the summary report to be sent to the Ministry of Agriculture and Fisheries.

18 November 1998
First meeting of the Toxicity Commission.
Coordinator presents the summary report.
Discussion postponed for one month to collect additional information.

16 December 1998
Second meeting of the Toxicity Commission.
Additional information presented by the coordinator *(Annexe I-10)*.
Residue analyses presented by those responsible for conducting the tests in the two selected laboratories.
Decision taken to continue investigations and to maintain the ban on using Gaucho in the 3 départements in which the product was banned in 1998 (Vendée, Deux-Sèvres, Indre).
Annexe I-11: Press release by the Toxicity Commission

15 January 1999
Decision taken by Jean Glavany, Minister of Agriculture, to continue investigations, to introduce a temporary ban on the use of the product on sunflower seeds and to set up an additional epidemiological study.
Annexe I-12: MAF press release

Final report GAUCHO bees

06/04/99

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LIST OF ANNEXES

Annexes I

- Annexe I-1: minutes of the meeting held on 24 October 1997
- Annexe I-2: experts' report presented to the Toxicity Commission
- Annexe I-3: minutes of the meeting held on 26 January 1998 and press release from the Ministry of Agriculture and Fisheries
- Annexe I-4: minutes of the meeting held on 5 February 1998
- Annexe I-5: minutes of the meeting held on 17 March 1998
- Annexe I-6: minutes of the meeting held on 26 May 1998
- Annexe I-7: minutes of the meeting held on 24 June 1998
- Annexe I-8: minutes of the meeting held on 31 July 1998
- Annexe I-9: minutes of the meeting held on 17 September 1998 and MAF press release issued on 5 October 1998

(Annexes I-1 to I-9 were submitted with the reported published on 6 November)

- Annexe I-10: additional information submitted by the coordinator to the Toxicity Commission on 16 December 1998
- Annexe I-11: Press release by the Toxicity Commission issued on 16 December 1998
- Annexe I-12: MAF press release issued on 15 January 1999

***Annexe II**: Detailed experimental protocols presented by each group involved*
(submitted with the report published on 6 November)

Annexes III:

- Annexe III-1: Report by C. Fléché – CNEVA
- Annexe III-2 to 4: ACTA reports
- Annexe III-5: Report by M.-E. Colin – INRA Avignon
- Annexe III-6: Report by J.-N. Taséi – INRA Lusignan
- Annexe III-7A: Report by F. Faivre d'Arcier et al. *– INRA Avignon*
- Annexe III-7B: Report by J. Brun – INRA Avignon
- Annexe III-8: Report by M.-H. Pham-Delègue – INRA Bures-sur-Yvette
- Annexe III-9: Report by L. Belzunces – INRA Avignon
- Annexe III-10: ACTA report

(Annexes III-1 to III-10 were submitted with the report published on 6 November)

***Annexe IV**: Report on varietal tests – ACTA/CETIOM*
(submitted with the report published on 6 November)

***Annexe V**: Results of residue analyses*

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COMMITTEE MEMBERSHIP

National Steering Committee
- PTD-MAF [redacted]
- GFD-MAF [redacted]
- DPPR-MATE
- ACTA [redacted]
- CETIOM
- BAYER [redacted]
- Beekeeping organisations [redacted] SPMF, [redacted] UNAF, [redacted] SNA)
- INRA [redacted]
- CNEVA [redacted]
- AMSOL [redacted]
- SdPV [redacted]
- FOP [redacted]

Representatives of local committees and analytical laboratories attended some of the meetings.

Operational Group Coordinators
- [redacted] (ACTA)
- [redacted] (GFD-MAF)
- [redacted] (INRA)

Local Committees (for each département)
- farmers (Chamber of Agriculture)
- seed producers
- beekeepers
- government bodies (DDAF-DRAF-SRPV-DSV)
- BAYER
- technical institutions (ACTA-CETIOM)
- CNEVA (Deux-Sèvres)

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STUDY PROGRAMME

A summary of the experiments carried out as part of the programme is given below (*Annexe II: Detailed experimental protocols presented by each group involved).*

The results have been divided into two main groups:
- results of **field experiments** aimed at studying the effects observed by beekeepers on sunflowers treated with Gaucho and at dosing the product in a number of substrates;
- results of **experiments performed under controlled conditions** intended to characterise any symptoms under tunnel or laboratory conditions.

Additional varietal tests conducted without bees are presented separately. The purpose of these tests is to evaluate the kinetics of the product and the variability of nectar secretion across a range of cultivated sunflowers.

The reports were submitted by each team leader summarising the individual experiments for which he or she was responsible (the full reports are given in annexes III and IV).

The content of each report is summarised. Results supported by statistics are given priority, and the observations of the research staff are given as additional information. Each summary contains author's comments on compliance with the stated protocol or any difficulties in interpreting the results submitted.

Beekeepers' representatives and Bayer sent additional information to the authors. This information is summarised at the end of this report.

Participating teams
ACTA [redacted]
CETIOM [redacted]
CNEVA [redacted]
INRA Avignon [redacted]
INRA Avignon [redacted]
INRA Lusignan [redacted]
INRA Bures [redacted]
CNRS BIOTEC Orléans [redacted]
GIRPA Angers [redacted]

Verification
- Pesticide/honey monitoring programme (DSV).
- Seed treated with Gaucho monitored (dose) before and up to sowing (SPRV).
- Seed sown in Gaucho-free zones monitored (SPRV).

I – FIELD EXPERIMENTS

1 – Effect of Gaucho on the loss of foraging bees (ACTA-CNEVA)

1.1- Aim

To ascertain whether or not Gaucho is responsible for hive depopulation during the sunflower flowering period by comparing the behaviour of bee colonies on a site treated with Gaucho and an untreated site.

1.2- Protocol

4 experimental zones consisting of 2 sites each (treated / untreated) are set up in the 3 Gaucho-free départements (Vendée, Deux-Sèvres, Indre). An experimental plot is selected in each site.

The sites, plots and colonies observed are broadly similar.

- Observation of sunflowers (stages of growth, foraging activity)
- Observations of bee colonies (weight, mortality, activity / bee counters, state of health, population, volume of pollen and honey collected)
- Samples of seeds, leaves, flowers, soil, bees, honey and pollen taken for analysis.

Reminder of definitions

Zone: Area selected for the installation of two sites.

Site: Area within which a group of hives is set up for the experiment (two sites = one zone).

Experimental plot: sunflower field covering at least 8 ha, with the experimental hives on the edge of the plot (one plot per site).

D0 = day on which the hives are set up on the experimental plots.

1.3- Results

(see following page)

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*** Département of Deux-Sèvres** (CNEVA Report by C. Fléché – annexe III-1)

	Control site	**Gaucho site**
- Site survey	*Sites broadly homogeneous*	
- Initial hive visit	D-10	D-10
	Comparable on both sites	
- Phenology:		
Sowing date	4/05 (D-60)	23/04 (D-70)
	Difference between sowing dates: 12 days	
Start of flowering	8/07 (D+5)	4/07 (D+1)
	Difference between start of flowering dates: 4 days	
Peak flowering	18/07 (D+15)	14/07 (D+11)
Time from sowing / peak flowering	73 days	78 days
	Difference between peak flowering dates: 4 days	
- Treatments applied to experimental plots:		
Insecticides		
Seed / soil	Lindane 1/04 (D-90)	Gaucho 23/04 (D-70)
Plants	0	0
Fungicides		
Plants	0	0
Herbicides		
Trifluralin	1/04 (D-90)	1/04 (D-93)
Flurochloridone	4/05 (D-60)	25/04 (D-68)
Other treatments applied to the zone	*Nothing while the sunflowers were flowering in the zone*	
- Weather conditions	*Comparable on both sites*	
- Bees on the disk flowers	Presence at earlier stages	
	Comparable on both sites at peak flowering	
- Hive mortality	Min = 141 bees/day	Min = 98 bees/day
(for 10 hives)	Max = 810 bees/day	Max = 640 bees/day
	Mean = 410 bees/day	Mean = 340 bees/day
	Fluctuations comparable on both sites	
- Hive activity		
Visual observation	*Fluctuations comparable on both sites*	
Counters	No data submitted	
- State of colonies		
Population	Intercolony heterogeneity (drift?)	Homogeneous
	No depopulation over time	
Brood	*Identical loss of brood on both sites*	
- Honey yield (D+26)	Intercolony heterogeneity (edge)	Homogeneous
	Min = 0.6 kg	Min = 18.3 kg
	Max = 145.4 kg	Max = 70.7 kg
	Mean comparable yield: 41-42 kg/hive	
- Pollen yield	Min = 30 g	Min = 10 g
(3 traps/hive)	Max = 220 g	Max = 150 g
	Mean = 100 g	Mean = 90 g
	Comparable on both sites	
- Pollen analysis (estimate)	*Sunflower the major source only in peak flowering period*	

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Conclusions

The differences between the control site and the site treated with Gaucho were not significant overall in terms of the criteria under consideration.
The differences observed included the fact that bees were present on the disk flowers at different stages in the treated plot compared to the control plot. The colonies were also more heterogeneous in the control site as regard populations and honey yields (which were higher in the hives on the edge of the site).

Comments

The author pointed out some deviations from the planned protocol:
- Bad weather meant that the preliminary hive visit took place on D-10 instead of D-8, and observation of bee activity took place at a different time of day.
- The weight measurement systems could not be used correctly.
- The bee counters broke down towards the end of the experiment. The data were processed late and are not included in the report.
- There was no detailed survey on the other plots in the zone. Nevertheless, the author states that there was no Fipronil seed treatment in the control site and no air spraying of sunflower and maize in the whole of the zone.
- Very large areas were given over to broods in some hives.
- VBarroa was observed in the hives at the start and end of the experiment.
- The author states that aphids were present on the sunflowers, even in the treated plot.

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*** Département of Indre** *(ACTA report – annexe III-2)*

	Control site	**Gaucho site**
- Site survey	*Detailed survey as per protocol* *Sites broadly homogeneous*	
- Initial hive visit	D-14	D-14
	Comparable on both sites	
- Phenology:		
Sowing date	12-13/05 (D-64)	8-9/05 (D-68)
	Difference between sowing dates: 4 days	
Start of flowering	16/07 (D0)	16/07 (D0)
	No difference between start of flowering dates	
Peak flowering	24/07 (D+8)	24/07 (D+8)
Time from sowing / peak flowering	72 days	76 days
	No difference between peak flowering dates	
- Treatments applied to experimental plots:		
Insecticides		
Seed / soil	0	Gaucho 8-9/05 (D-68)
Plants	Mavrik Systo 6/06 (D-31)	Mavrik Systo 7/06 (D-30)
Fungicides		
Plants	Corvet Flo 1/07 (D-15)	Corvet Flo 4-5/07 (D-12)
Herbicides		
	Trifluralin 11/05 (D-65)	Pendimethalin 15/05 (D-61)
Aclonifen	6/06 (D-31)	15/05 (D-61)
Other treatments applied to the zone	*Nothing while the sunflowers were flowering in the zone*	
- Weather conditions	Heavy rainfall	
- Bees on the disk flowers	More intense at the start/end of flowering	
	Comparable on both sites at peak flowering	
- Hive mortality	Min = 110 bees/day	Min = 80 bees/day
(for 10 hives)	Max = 720 bees/day	Max = 320 bees/day
	Mean = 280 bees/day	Mean = 120 bees/day
	Low, varied according to hive opening and weather	
- Hive activity		
Visual observation	*Fluctuations comparable on both sites*	
Counters	Higher number of exits/day	
	Comparable shortfall between exits/entrances	
- State of colonies		
Population	Homogeneous	Fall at the end of flowering
	Comparable except at the end of flowering	
Brood		Larger fall
- Honey yield (D+32)	Min = 6.5 kg	Min = 6.5 kg
	Max = 19.3 kg	Max = 30.8 kg
	Mean = 13.5 kg/hive	Mean = 18.3 kg/hive
	Difference between mean figures not significant	
- Pollen yield	Min = 20 g	Min = 40 g
(3 traps/hive)	Max = 130 g	Max = 190 g
	Mean = 80 g	Mean = 130 g
		Larger yield
	Kinetics closely linked to flowering	
- Pollen analysis (estimate)	*Sunflower the major source throughout flowering period*	

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Conclusions

The differences between the control site and the site treated with Gaucho were not significant overall in terms of the criteria under consideration.
It was noted that the average honey yield was low on both sites, especially the treated site; however, the difference between the sites was not significant.

Comments

The protocol applied was as stated, except for the **addition of an isolated hive** (100 m from the experimental group of hives) fitted with a bee counter on each site. This hive was to be used as a reference to evaluate any problems with drift that might occur in the experimental groups of hives and would conceal depopulation. This change in the protocol was introduced at the request of beekeepers.
In Indre, the percentage of bee loss was higher in the control site than in the Gaucho site. No explanation was found for this. No marked differences were found between the isolated hive and the other hives fitted with counters on the same site.

With regard to the use of **bee counters**, a shortfall of around 3,000 bees a day was observed (total number of exits – total number of entrances). This seems to be a very high figure and is not in line with the general observations of the colonies which did not reveal any massive bee losses. The author puts forward the hypothesis that there may be a systematic error in counting entrances (underrating entrances compared to exits during periods of intense activity).

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*** Département of Vendée marais** *(ACTA report – annexe III-2)*

	Control site	**Gaucho site**
- Site survey	*Detailed survey as per protocol*	
Size of experimental plots	20 ha	50 ha
	Heterogeneous sites (crops, plot sizes)	
- Initial hive visit	D-11	D-11
	Comparable on both sites	
- Phenology:		
Sowing date	10/05 (D-71)	12-18/05 (D-63/69)
	Difference between sowing dates: 2-8 days	
Start of flowering	20/07 (D0)	20/07 (D0)
	No difference between start of flowering dates	
Peak flowering	24/07 (D+4)	28/07 (D+8)
Time from sowing / peak flowering	75 days	71-77 days
	Difference between peak flowering dates: 4 days	
- Treatments applied to experimental plots:		
Insecticides		
Seed / soil	Carburufan 10/05 (D-71)	Gaucho 12-18/05 (D-63/69)
Plants	0	0
Fungicides		
Plants	0	0
Herbicides		
	Flurochloridone 10/05 (D-71)	Trifluralin (pre-emergence)
	Paraquat	Linuron
Other treatments applied to the zone	*Nothing while the sunflowers were flowering in the zone*	
- Weather conditions	Heavy rainfall	
- Bees on the disk flowers		Later
	Low but comparable on both sites at peak flowering	
- Hive mortality	Min = 100 bees/day	Min = 100 bees/day
(for 10 hives)	Max = 880 bees/day	Max = 600 bees/day
	Mean = 300 bees/day	Mean = 215 bees/day
	Higher mortality at the end of the flowering period	
- Hive activity		
Visual observation	Higher activity at the end of the flowering period	
	Fairly stable during the flowering period	
Counters		Temporary rise in bee numbers (drift?)
	Comparable with the total number of exits	
- State of colonies		
Population	Slight fall at the start of the flowering period (drift?)	
	No depopulation over time	
Brood	Larger fall	
- Honey yield (D+32)	Min = 11.6 kg	Min = 22.7 kg
	Max = 77.4 kg	Max = 65.1 kg
	Mean comparable yield: 41 kg/hive	
	Sites heterogeneous (effect of hives at the edge of the site)	
- Pollen yield	Min = 5 g	Min = 8 g
(3 traps/hive)	Max = 115 g	Max = 75 g
	Mean = 40 g	Mean = 30 g
	Increase at the end of the flowering period – kinetics closely linked to flowering	
- Pollen analysis (estimate)	*Sunflower (and maize) the major sources in peak flowering period*	

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Conclusions

The criteria analysed were not found to be different on the Gaucho site when compared with the control site. Significant problems of drift were seen on both sites, which made the observations more heterogeneous.
Pollen analysis showed that significant levels of maize pollen were present on both sites. On the treated site, maize plots were located more than 3 km away from the experimental plot.

Comments

Problems of drift occurred as soon as the experiment had been set up on the control plot. The configuration of the experimental hives was changed to counter this problem; the hives were set up in an arc and wind-breaks were installed. On the control plot, a hive fitted with a counter was placed at the end of the line instead of a hive fitted with a pollen trap. Following this, some observations indicated that drift was no longer taking place in the two groups of hives, except for the period from 3 to 11/07 when the counters in the group of hives on the treated plot recorded a rise in bee numbers which could be due to further drift for which no explanation has been found.

Edge effects were observed in the groups of hives on both sites, with the hives at the ends of the lines producing more honey.

Looking at the results of the **pollen analyses** performed on samples taken from the pollen traps on both sites, sunflower was found to be the major source during the flowering period, but maize pollen was also present, sometimes in greater volumes. On the treated site, maize plots were more than 3 km away from the experimental plot. One possible explanation is that the bees travel long distances to find this pollen, especially at the end of the sunflower flowering period. When the experimental plots were in flower, sunflower was the preferred pollen.
The Steering Committee stresses the need for in-depth quantitative pollen analysis.

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*** Département of Vendée plaine** *(ACTA report – annexe III-4)*

	Control site	**Gaucho site**
- Site survey	*Detailed survey as per protocol* *Sites broadly homogeneous*	
- Initial hive visit	D-12	D-12
	Comparable on both sites	
- Phenology:		
Sowing date	5/05 (D-63)	6/05 (D-62)
	Difference between sowing dates: 1-5 days	
Start of flowering	9/07 (D+2)	9/07 (D+2)
	No difference between start of flowering dates	
Peak flowering	15/07 (D+8)	18/07 (D+11)
Time from sowing / peak flowering	71 days	73 days
	Difference between peak flowering dates: 3 days	
- Treatments applied to experimental plots:		
Insecticides		
Seed / soil	Fipronil 5/05 (D-63) 6 ha Lindane 10/05 (D-58) 14 ha	Gaucho 6/05 (D-62)
Plants	0	0
Fungicides		
Plants	0	0
Herbicides		
Trifluralin	25/03-10/05 (D-58)	19/03
Flurochloridone	10/05-13/05	7/05 (D-61)
Linuron	13/05	
Other treatments applied to the zone	*Some treatments (European corn borer)*	
	Comparable on both sites	
- Weather conditions		
- Bees on the disk flowers	Lower	Closely linked to flowering
- Hive mortality	Min = 180 bees/day	Min = 50 bees/day
(for 10 hives)	Max = 1100 bees/day	Max = 1200 bees/day
	Mean = 390 bees/day	Mean = 380 bees/day
	Fluctuations comparable on both sites	
- Hive activity		
Visual observation	*Comparable on both sites*	
Counters	Rise in bee numbers at the end of the flowering period	
	Daily activity comparable	
- State of colonies		
Population		Slightly larger fall
	No significant depopulation over time	
	Identical loss of brood on both sites	
Brood		
- Weight gain	Min = 25.2 kg	Min = 16.5 kg
between D0 and D34	Max = 144.4 kg	Max = 130.9 kg
	Mean = 61.9 kg/hive	Mean = 52.4 kg/hive
	Each group of hives was heterogeneous	
- Pollen yield	Min = 20 g	Min = 40 g
(3 traps/hive)	Max = 250 g	Max = 230 g
	Mean = 90 g	Mean = 110 g
	Increase at the end of the flowering period	
	Comparable on both sites	
- Pollen analysis (estimate)	*Sunflower the major source even after the flowering period on the plot*	

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Conclusions

There were no marked differences between the control site and the site treated with Gaucho in terms of the main criteria under consideration. However, some points should be emphasised:

- One of the two plots on the site not treated with Gaucho (6 ha) was given a Régent seed dressing (fipronil). This may reduce the value of part of this site as a control.

- There were wide variations in honey yields on both sites.

- A fall in population figures was recorded for both sites during hive inspection visits. The fall was more pronounced on the site treated with Gaucho (around 7%).

- The bee counters showed comparable daily activity, but with some increase in bee numbers on the treated site.

Comments

As one of the plots on the control site had been treated with Régent (fipronil), the results for this zone must be considered with caution, even though there were no significant differences between the two sites, as was the case with the other zones. The report indicates that some treatments had been applied to control European corn borers in the zone.

Mortality statistics highlight two unexplained peaks in mortality (not linked to prior opening of the hives or weather problems). This occurred on both sites a few days apart.

The counters on the treated site recorded increases in bee numbers. There were also temporary increases in bee numbers in the isolated hive on the treated site. However, it is hard to say for certain whether or not this is due to the use of Gaucho.

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2- Analyses of residues of imidacloprid and its main metabolites (GIRPA-CNRS)

2.1- Aim

To detect the product in a number of different matrices:
- soil samples
- sunflower disk flowers
- dead bees collected outside the hives
- pollen balls collected in the traps
- fresh honey (nectar substitute) collected in the combs
- old honey collected at harvest time

2.2- Protocol

The matrices were divided evenly between the GIRPA and CNRS (BIOTEC) laboratories. 10% of the samples were analysed twice.
The samples were kept for possible counter-analyses.

Methods used by the laboratories:
- CBRS BIOTEC performed analyses to detect imidacloprid alone and four of its derivatives in isolation: guanidine, urea, olefin, monohydroxy imidacloprid.
The results were presented on the basis of three levels of detection:
LQ = Limit of Quantification for all compounds (0.01 mg/kg) except olefin (0.1 mg/kg)
ILQ = Present but Inferior to LQ
ND = Not Detected

- GIRPA performed analyses to detect imidacloprid alone and total residues (imidacloprid and metabolites expressed as 6-chloronicotinic acid).

LQ for imidacloprid	=	0.02 mg/kg for the disk flowers (first set of samples), bees and harvested honey.
	=	0.08 mg/kg for the soil, leaves, pollen (second set of samples for the cross-analyses).
LQ for residues	=	0.005 mg/kg for the disk flowers and leaves, bees, pollen, soil.
	=	0.012 mg/kg for the harvested honey.
ILQ	=	Inferior to the LQ
ND	=	Not Detected

Tables showing the full results are presented in annexe V.

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Distribution of results of analyses by sample and level of detection

	Plots not treated with Gaucho					Plots treated with Gaucho				
Sample (laboratory)	Deux-Sèvres	Indre	Vendée marais	Vendée plaine	*All zones*	Deux-Sèvres	Indre	Vendée marais	Vendée plaine	*All zones*
1. Soil (Girpa)										
>0.005	0	0	0	0	*0*	0	0	0	0	*0*
<LQ	0	0	0	0	*0*	0	0	0	0	*0*
ND	1	1	1	2	*5*	1	1	1	1	*4*
Total	1	1	1	2	*5*	1	1	1	1	*4*
2. Leaves (Biotec)										
>0.01	0	0	0	0	*0*	0	0	0	0	*0*
ILQ	0	0	2	2	*4*	2	1	2	2	*7*
ND	2	1	0	0	*3*	0	0	0	0	*0*
Total	2	1	2	2	*7*	2	1	2	2	*7*
2.1 Leaves *(cross-analyses)* (Girpa)										
>0.005	0	0	0	0	*0*	2	1	0	0	*3*
<LQ	0	0	0	0	*0*	0	0	2	2	*4*
ND	2	1	2	2	*7*	0	0	0	0	*0*
Total	2	1	2	2	*7*	2	1	2	2	*7*
3. Pollen in trap (Biotec)										
>0.01	0	0	0	0	*0*	0	0	0	0	*0*
ILQ	2	0	1	7	*9*	10	5	8	8	*31*
ND	10	5	5	1	*21*	0	0	0	0	*0*
Total	12	5	6	8	*30*	10	5	8	8	*31*
3.1 Pollen in trap *(cross-analyses)* (Girpa)										
>0.05	1	-	0	1	*2*	0	-	0	1	*1*
<LQ	0	-	0	0	*0*	0	-	0	0	*0*
ND	0	-	1	4	*5*	1	-	1	0	*2*
Total	1	-	1	5	*7*	1	-	1	1	*3*
4. Dead bees (Girpa)										
>0.005	0	0	0	0	*0*	2	0	0	0	*2*
<LQ	0	0	0	2	*2*	0	0	0	0	*0*
ND	8	10	13	23	*54*	6	10	9	17	*42*
Total	8	10	13	25	*56*	8	10	9	17	*44*
5. Fresh honey (Biotec)										
>0.01	0	0	0	0	*0*	0	0	0	0	*0*
ILQ	1	0	0	0	*1*	1	2	1	1	*5*
ND	2	6	14	5	*27*	2	2	11	3	*18*
Total	3	6	14	5	*28*	3	4	12	4	*23*
6. Honey harvested (Girpa)										
>0.012	0	0	0	0	*0*	0	0	0	0	*0*
<LQ	0	0	0	0	*0*	0	0	0	0	*0*
ND	1	2	2	2	*7*	1	1	1	1	*4*
Total	1	2	2	2	*7*	1	1	1	1	*4*
6.1 Honey harvested *(cross analyses)* (Biotec)										
>0.01	-	-	-	0	*0*	0	0	0	0	*0*
ILQ	-	-	-	0	*0*	1	1	0	0	*2*
ND	-	-	-	1	*1*	0	0	1	1	*2*
Total	-	-	-	1	*1*	1	1	1	1	*4*

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1 – Soil

In each département one analysis was performed on the control plot (two analyses were performed in Vendée plaine) and one on the treated plot. Imidacloprid was not detected in any of the analyses.

2 – Leaves

In Deux-Sèvres two analyses were conducted by both laboratories on each site. Nothing was detected on the control plot. On the treated plot, imidacloprid and its residues were detected in both samples at levels of 0.015 and 0.022 mg/kg.

A sample was taken from each site in Indre and analyses were performed by both laboratories. Nothing was detected on the control plot. On the treated plot, imidacloprid (and its residues) were detected at a level of 0.007 mg/kg. Plant samples were taken at the BBCH 53 stage in this zone.

2 sets of samples were taken in Vendée marais: 50 days after sowing at the BBCH 51-53 stage and 60 days after sowing at the BBCH 55-57 stage. Both laboratories analysed the samples. The presence of imidacloprid was not detected by GIRPA and detected but not quantified by Biotec in the two samples from the control plot. On the treated plot, both laboratories detected the presence of imidacloprid but did not quantify it (lowest LQ = 0.005 mg/kg).
2 sets of samples were taken in Vendée plaine: 50 days after sowing at the BBCH 51 stage and 60 days after sowing at the BBCH 57-59 stage. The results were similar to those obtained in the Vendée marais.

3 – Pollen

In Deux-Sèvres 12 samples of pollen were taken from the control site between D+2 and D+41. Imidacloprid and its metabolites were not detected in 10 analyses. Imidacloprid was detected but not quantified (ILQ) in two samples. One of the two samples was analysed by the second laboratory: imidacloprid was detected at 0.008 mg/kg
Out of the 10 samples analysed from the treated site, imidacloprid was detected but not quantified (ILQ) in all the analyses and the monohydroxy derivative was detected but not quantified in 4 of the 10 samples. One of the samples was analysed by the second laboratory, which did not detect the product.

In Indre 5 samples were analysed for each site. Imidacloprid and its metabolites were not detected on the control site. On the treated site imidacloprid was found at ILQ levels (<0.01 mg/kg) in the 5 samples analysed. The other metabolites were not detected.

6 samples from the control site in Vendée marais were analysed: 1 contained imidacloprid and the monohydroxy derivative at ILQ levels, but the products were not detected in the other samples. The second laboratory analysed the “ILQ” sample and did not detect imidacloprid or its metabolites. Imidacloprid was found at ILQ levels in all of the 8 samples analysed from the treated sites. The other metabolites were not

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detected. Imidacloprid was not detected in the cross-analysis performed by GIRPA on one of these samples.

8 samples from the control site in Vendée plaine were analysed by Biotec: imidacloprid was present at levels below the limit of quantification (<0.01 mg/kg) in 7 of the samples. The other metabolites were not detected. Girpa analysed 5 of these samples and did not find imidacloprid. All the 8 samples from the treated site analysed by Biotec were found to contain imidacloprid and the monohydroxy derivative at concentrations below 0.01 mg/kg. Girpa analysed one of these samples and detected imidacloprid at 0.015 mg/kg.

4 – Dead bees

In Deux-Sèvres 8 samples of dead bees from the group of hives on the control site and 8 from the hives on the treated site were analysed. Imidacloprid was not detected on the control site. 2 of the 8 samples analysed from the treated site showed total residue levels above the limit of quantification (0.005 and 0.008 mg/kg).

10 samples from each site in Indre were analysed. Imidacloprid was not detected in any of the samples analysed.

In Vendée marais 13 samples were analysed from the control site and 9 from the treated site. Imidacloprid and its metabolites were not detected in any of the samples analysed.

A larger number of samples were analysed in Vendée plaine: one sample was taken from the site under quarantine before the hives were installed on the experimental plots, 24 were taken from the group of hives on the control site and 17 from the treated site. Imidacloprid was detected only in two samples from the control site; the levels of imidacloprid and total residues were below the limit of quantification (0.005 mg/kg).
Following the meeting held to discuss the fieldwork on 31 July, 9 bee samples were taken from "problematic" and "problem-free" hives in the Vendée plaine zone.
Nothing was found in 8 samples and one sample contained 0.015 mg/kg of imidacloprid and metabolites.

4 – Fresh honey

In Deux-Sèvres, 3 samples from the control site and 3 samples from the treated site were analysed. Imidacloprid was present at levels below the LQ (0.01 mg/kg) in one sample from the control site and one sample from the treated site.

In Indre, 6 samples from the control site were analysed; two samples were analysed by both laboratories. Imidacloprid was not detected in any of the samples. Four samples from the treated site were analysed, and two were found to contain levels of imidacloprid below the limit of quantification (0.01 mg/kg) while the other two samples contained no imidacloprid.

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In Vendée marais, 14 samples from the control site and 12 from the treated site were analysed. Only 1 sample from the treated site contained imidacloprid at levels below the limit of quantification (0.01 mg/kg).

Nothing was found in the 5 samples from the control site analysed for Vendée plaine. Out of the 5 samples analysed from the treated site, imidacloprid was found at levels below the limit of quantification (0.01 mg/kg) in one sample.

6 – Honey harvested

Honey samples were taken from the harvest of each group of hives in the four départements. In Indre, Vendée plaine and marais an additional sample was taken on D0 in the hives which remained in the quarantine site. 11 analyses were conducted: 2 in Deux-Sèvres and 3 in each of the other zones. Girpa did not detect imidacloprid or its metabolites in any of the 11 samples. Biotec re-analysed 5 samples: one sample from the treated sites in Deux-Sèvres and Indre. The results of these two analyses showed that imidacloprid was present at levels below the limit of quantification (0.01 mg/kg). Nothing was found in the sample from the treated site in Vendée marais, or in the samples from the control site and the treated site in Vendée plaine.

Comments
The results are difficult to interpret since the two laboratories did not adopt the same limit of quantification and did not use exactly the same method of analysis.

Conclusions
If we consider the results above the limits of quantification laid down, overall there are few "positive" results: 2 out of 148 samples analysed (all matrices) from the control sites and 6 out of 128 samples analysed from the treated sites. The product's presence is detected in a larger proportion of samples at levels "below the limit of quantification", even on the control sites: 16 out of 148 samples from the control sites and 49 out of 128 from the treated sites.

It should also be emphasised that the seed was inspected by the SRPV at the time of sowing. Additional verification may be necessary (some members of the Steering Committee mentioned the possibility of under-dosage during the seed dressing process), but information provided to the authors of the report indicates that the doses of imidacloprid administered to the seed used on the treated sites were in accordance with the approved dose.

3- Observation of symptoms in productive colonies *(INRA Avignon* Report by [redacted] *– annexe III-5)*

3.1- Aim
To record and quantify individual or collective symptoms.

3.2- Protocol
The experiments were carried out in the Vendée plaine district. Three colonies (cohorts of marked worker bees in each colony) were placed in each group of hives:

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one group on the site treated with Gaucho, one on the site not treated with Gaucho and one on the edge of the untreated site.
Daily counts of bees returning to the hive were performed throughout the flowering period and the bees were filmed as they entered the hive.

3.3- Results and conclusions

The results indicate that daily returns of marked bees to the hive were similar on the site treated with Gaucho and the untreated sites.
Observations of symptoms of intoxication were reported.

3.4- Comments

The author states that the control site was treated with Régent and questions the value of this site as a control. This means that non-significant results should be considered with caution.
The protocol stated that observations would be made of the third group of hives at the edge of the site not treated with Gaucho but they were not included in the report.
Film taken of bees entering the hives and on the disk flowers provides qualitative information here.

4- Effects of Gaucho used as a sunflower seed dressing on bumblebees (INRA Lusignan *Report by* [redacted] *annexe III-6)*

4.1- Aim

To ascertain the effect of the treatment on the return of bumblebees to the hive.

4.2- Protocol

Twenty colonies of individually marked bumblebees were distributed in groups of ten colonies on a control site and a site treated with Gaucho. The experiment was conducted in the Deux-Sèvres département on a site described in detail by the author.
Observations took place over 10 days from the beginning of the sunflower flowering period.

4.3- Results

The following parameters were recorded:
- Visits to sunflowers:
A comparison of the foraging bees visiting sunflowers showed that there was no difference in the percentages foraging for nectar and pollen on the disk flowers on the control plot and on the plot treated with Gaucho. Examination of the pollen carried on the bodies of the worker bees showed a similar percentage of sunflower pollen on the control plot and on the plot treated with Gaucho.
- Activity on entering the nest:
No difference was observed between the two sunflower plots.
- Loss of worker bees:
9 days of observation in the field showed identical losses in the treated plot and the control plot.
- Development of colonies:
Observation of the colonies 26 days after marking showed no difference between the plots in terms of the number of additional workers in the colony, the number of virgin queens and the breeding rate.

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4.4- Conclusions

No difference in the activity or development of bumblebee colonies was observed after field observations comparing sunflowers in the control plot and the plot treated with Gaucho.

5- Effect of Gaucho on beneficial entomofauna present on sunflowers (INRA Avignon *Report by* [redacted] *et al. – annexe III-7 A)*

5.1- Aim

Survey of the various species of beneficial insects present on sunflower disk flowers during the flowering period.

5.2- Protocol

Insect samples were taken from treated and untreated plots in order to identify and evaluate the populations of the different species.

5.3- Results

A comparison of sunflower biodiversity was carried out on a control plot and a plot treated with Gaucho in the Vendée plaine district. Samples of crop pests and beneficial fauna were taken on two dates by the beating method. The samples were sent to the laboratory for identification and counting. The laboratory work looked at a number of characteristics in the populations found, including the range of entomofauna and indices of diversity and balance.
The data obtained for the various types of insects found on each plot (phytophagous insects including aphids, beneficial fauna) were then processed to give overall results for each plot and each sampling date.

5.4- Conclusions

The use of Gaucho does not appear to cause any marked effect on the numbers of the various insects identified, nor to have any other dramatic effect.
However, the author noted the following points:
- The Gaucho plot had a broader range of insect life than the control plot as regards taxon and individual capture figures (for absolute population figures).
The author stressed that there was no link between the presence of individuals and their physiological condition.
- Detailed analysis of the total population, plot population, phytophagous population, apterous aphids and beneficial fauna found no significant difference between the Gaucho plot and the control plot. However, a significant date effect was found for these different population groups on the same plot. This indicates that the populations change over time; it is difficult to interpret this change as samples were taken on only two dates.

5.5- Comments

The report contains details of the methods used and the taxons identified.
The author believes that the population difference between the treated plot and the control plot could be due to external factors not taken into account in the study, to the fact that the control plot had been treated with Régent (fipronil seed dressing), or to the fact that the samples were taken once Gaucho residues were no longer present.

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II – EXPERIMENTS UNDER CONTROLLED CONDITIONS

These observations enabled the research teams to assess the risks caused to bees by making foraging bees visit artificial contaminated sources and to assess the toxicity of the product in laboratory tests. The experiments are presented in two sections, one for each of these two categories.

A – STUDY OF FORAGING BEHAVIOUR

1- Tunnel tests with bee micro-colonies (INRA Avignon *Report by* [redacted] – *annexe III-5)*

1.1- Aim

To ascertain whether the product affected the bees' behaviour when visiting a food source.

1.2- Protocol

Foraging bees were trained to visit a food source, were subjected to oral or contact contamination with Gaucho and the effects on their behaviour at the feeder were observed.

1.3- Results

A first test was performed in an insect-proof tunnel to ensure that bees that had received a topical injection of 1 ng of imidacloprid per bee were able to find their way back to the hive after being released 300 m away from the hive. Then small groups of bees kept under cloches were studied to investigate the following reactions:

- Visits to feeders

40% syrups containing imidacloprid at concentrations of 0.2, 0.1, 0.05 and 0.025 mg/kg (and one control syrup solution) were offered to colonies (one colony was tested for each concentration) for 4 days. Contaminated syrup and a control syrup were offered in alternation for 2 hours and the number of bees feeding was recorded. Visits to feeders were observed to stop before the end of the period during which the contaminated syrup was offered at concentrations above 0.025 mg/kg.

- Amount of syrup taken

40% syrups containing imidacloprid at concentrations of 0.05 – 0.025 mg/kg were offered to colonies (two colonies per concentration) for 3 days. The syrups were offered for 2 hours each day, alternating between the contaminated syrup and the control syrup. Then only the control syrup was offered for 2 hours a day for the next 3 days. The amount of syrup taken was recorded. Consumption fell significantly when the 2 imidacloprid concentrations were offered.

- Length of visits to feeders

Syrups containing imidacloprid at concentrations of 0.1, 0.025, 0.013 and 0.006 mg/kg (and a control syrup containing no imidacloprid) were offered to a micro-colony (one per concentration). On the first day the colonies fed from the control syrup, and for the next 4 days had unrestricted access to the contaminated syrup. The length of visits to feeders was observed and was found to be inversely proportional to the concentration of product in the syrup. This effect was noted even at the lowest concentration tested (0.006 mg/kg).

1.4- Conclusions

The results indicate that imidacloprid affects:

- The number of visits to feeders containing contaminated syrup at concentrations above 0.025 mg/kg

- The amount of syrup taken at concentrations of 0.025 mg/kg and above

- The length of visits to feeders containing contaminated syrup at concentrations of 0.006 mg/kg and above

2- Outdoor tests with bee colonies (INRA Avignon *Report by* [redacted] – *annexe III-9)*

2.1- Aim

To ascertain whether the product affects behaviour during visits to a food source.

2.2- Protocol

Hives containing Buckfast bees were set up outdoors with 2 food sources placed in diametrically opposite directions 150 m away from the hive. 30 individuals were marked at each source. One of the sources was contaminated with sublethal doses of imidacloprid of 1 – 0.1 mg/l (the LD50 for these bees is 15 – 30 ng/bee). The marked bees were observed as they returned to these sources for 12 days.

2.3- Results

At 1 mg/l, the number of visits to the contaminated feeder was seen to fall rapidly. As the marked individuals were still present on the control source, the author concluded that there was no contamination in the hive. Symptoms of intoxication were also reported (trembling, immobility on the contaminated source).

At 0.1 mg/l there was no difference between the treated source and the control source. Activity was intense and no symptoms of intoxication were observed.

2.4- Conclusions

The number of visits by foraging bees to a source of food contaminated with 1 mg/l of imidacloprid was observed to fall, and symptoms of intoxication from the source were noted. These phenomena were not observed for a concentration of 0.1 mg/l.

2.5- Comments

The 30 marked bees for each source represent a small proportion of the recruited population as a whole. It would be interesting to know how the rest of the population behaved on both sites.

The use of the Buckfast bee may explain the lack of symptoms at a concentration of 0.1 mg/l, as this bee could be less sensitive to the product than *Apis mellifera mellifera*.

3- Tests with a colony of bees in flight cages (INRA Bures *Report by* [redacted] – *annexe III-8)*

3.1- Aim

To ascertain whether the product affects behaviour during visits to a food source.

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3.2- Protocol

Observations were conducted in an outdoor flight cage containing a small hive (around 4,000 bees) fitted with a bee counter. The bees were offered a control food source or a food source contaminated with 0.05 mg/l of imidacloprid, both scented with a floral scent (linalool), for 1-2 hours a day. The bees visiting the source were marked and counted. Activity on entering the hive was recorded by the counter. The bees were offered a control syrup for 2 weeks followed by a contaminated syrup for 2 weeks, and then a control syrup again.

3.3- Results

The following observations were made:

- Recruitment

The number of new foraging bees visiting the food source increased regularly during the period when the control syrup was offered, remained stable while the contaminated syrup was offered, and then increased again when the control syrup was offered.

- Olfactory discrimination abilities

After being offered the control syrup for two weeks, the bees were given a choice between linalool, the same scent as the food source, and odourless sites. The foraging bees preferred the scent, showing that they remembered it.

The experiment was repeated after offering the contaminated syrup. The bees no longer recognised the scent.

After offering the control syrup again, the bees were once again able to identify the scent.

- Hive entrances and exits

The bees had a daily peak of activity closely related to the introduction of the control syrup. There was a fall in activity in the presence of the source when the contaminated syrup was offered. Activity rose again once the control syrup was offered.

3.4- Conclusions

Imidacloprid at a concentration of 0.05 mg/l leads to a significant reduction in the number of individuals recruited to a food source, a lowering of the ability of foraging bees to recognise a learnt scent, and a fall in overall activity in the presence of this contaminated source. However, these phenomena are reversible and activity returns to normal once an uncontaminated source is offered.

3.5- Comments

These experiments may have been skewed by the installation of the bee counter, which restricted the level of visits to the source offered. Nevertheless, observations of relative fluctuations in activity over time in the presence of contaminated and uncontaminated sources are still relevant.

4- Tunnel evaluation of the effects of Gaucho on bees *(Report by* [redacted] *ACTA – annexe III-10)*

4.1- Aim

To compare the effect of Gaucho seed dressing on bees during the flowering period in sites treated with fungicide and sites which had not been treated.

4.2- Protocol

The experiment was designed on the basis of the ANP CEB no. 129 test (1996), modified to suit the needs of the study.

6 scenarios were compared:

- Untreated tunnel
- Fungicide treatment in 2 out of 4 zones
- Gaucho treatment in 2 out of 4 zones
- Gaucho + fungicide treatment in 2 out of 4 zones
- Gaucho treatment in 4 out of 4 zones
- Gaucho + fungicide treatment in 4 out of 4 zones

One hive fitted with a bee counter was placed in each tunnel. Observations were conducted during the flowering period (7 days) to examine foraging activity on the flowers, mortality, development of the colonies and activity on entering the hive.

4.3- Results and conclusions

In tunnels, Gaucho seed dressing administered alone and in association with fungicide to control phomopsis affected neither foraging activity nor bee mortality in the short term.

4.4- Comments

This work contributes to the study of possible synergy between Gaucho and other crop protection products. Combined treatment does not seem to affect bee activity in tunnels when compared with control scenarios.

It should be borne in mind that the use of bee counters was not planned for this experiment. The author states that the rhythms of activity recorded by the counters was in line with visual observations (end-of-day flight for excretion).

5- Effects of Gaucho used as a sunflower seed dressing on bumblebees (INRA Lusignan *Report by* [redacted] *– annexe III-6)*

5.1- Aim

To evaluate the effect of treatment on the foraging activity of female worker bees and male bees.

5.2- Protocol

Foraging activity on the disk flowers of treated and untreated container-grown sunflowers was measured. Disk flower samples were taken for residue analysis.

5.3- Results

Flowering was identical in the treated sunflowers and the control flowers. 173 bumblebees visited the control flowers and 217 visited the treated flowers; this is not a significant difference. The length of the visits was the same in both groups of plants.

5.4- Conclusions

There was no difference in the number and length of visits to disk flowers of sunflowers treated with Gaucho and control plants.

5.5- Comments

Sunflower samples were taken for residue analysis. The results have been submitted to the person in charge of this study.

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B- TOXICITY TESTS

1- Acute toxicity and social behaviour (INRA Avignon *Report by* [redacted] *annexe III-5)*

1.1- Aim

To establish the dose-mortality ratio for product doses below LD50.
To evaluate the effects of imidacloprid on social behaviour.

1.2- Protocol

24-hour mortality for various product doses administered orally or by contact to caged bees.
The development of micro-colonies and recognition of related bees were monitored.

1.3 – Results

The purpose of these tests was to:

- Define the sublethal range

The protocol refers to the application of various ranges of concentration or doses of imidacloprid, imidazolidone or chloronicotinic acid administered orally or topically in order to define the sublethal range. The author considers that the sublethal range is below the dose of 1 ng/bee when administered topically, and that the acute toxicity of the metabolites tested is lower than that of imidacloprid.

- Understand the physiopathology of intoxication

The aim of this test was to investigate how long any **"knock down"** effect took to appear and the conditions under which it was reversible. The knock down effect is an indicator of acute intoxication and is defined by mobility problems affecting the bees, extending as far as complete paralysis and apparent death, although it is reversible.
The report states that high doses (doses of 0.2 and 1 mg/kg of imidacloprid were administered orally and doses of 1 and 2 ng/bee were administered topically) were followed by a knock down effect after a few hours, although in some cases symptoms did not appear until more than 24 hours after application.
Concentrations of 0.2, 0.1, 0.05 and 0.025 mg/kg of imidacloprid in a 40% sugar syrup were administered to caged bees in groups of 20-30 individuals. The results of the experiment obtained for the two strongest concentrations (0.2 and 0.1 mg/kg) showed that **food consumption** fell by half when compared with the control group; the fall was even greater in cages where the feeder had been placed in a less accessible position.

-To study the impact of the substance on certain aspects of social behaviour.

*** Maintenance and development of micro-colonies**

Micro-colonies made up of 120 newborn worker bees and one queen were fed on sunflower pollen harvested from control plots and plots that had been treated with Gaucho or with pollen from another untreated plant (rosemary). The pollen from the sunflowers treated with Gaucho was not found to affect mortality, consumption of crystallised sugar, the number of nectar storage cells or the number of brood cells. However, the consumption of pollen from sunflowers treated with Gaucho was much lower than the consumption of the other types of pollen and bees raised on Gaucho pollen produced much less wax.

*** Recognition of related bees**

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The purpose of this test was to examine the bees' ability to recognise their sisters or half-sisters from their colony. To find out whether imidacloprid affected this ability, groups of 3 newborn worker bees were placed in cages and a worker bee from the same colony or from another colony was placed in the cage after 5 days. In principle the bees should accept their fellow bees from the same hive and reject bees from other hives. The results show that, when compared with control bees, bees fed on a syrup containing imidacloprid at a concentration of 0.05 mg/kg were "passive" and less likely to reject outsiders. This may indicate a decline in their ability to recognise related individuals.

1.4- Conclusions

We can conclude that:

- **Consumption of syrup containing imidacloprid at concentrations of 0.2 – 0.1 mg/kg leads to a significant fall in food consumption in groups of caged bees.**
- **Consumption of pollen from sunflowers treated with Gaucho is lower than consumption of untreated pollen and causes a significant fall in wax production in micro-colonies.**
- **Consumption of syrup containing 0.05 mg/kg of imidacloprid leads to a decline in the ability of caged groups of 3 worker bees to recognise related worker bees.**

1.5- Comments

The report on the test to determine the sublethal range and the knock down effect does not contain all the figures.
As regards the test on recognition of related bees, the author states that the results reported for the 0.05 mg/kg concentration are currently being confirmed for lower doses (0.012 mg/kg).

2- Chronic toxicity and learning (INRA Bures *Report by* [redacted] – *annexe III-8)*

2.1- Aim

To evaluate the effects of ingesting various doses of imidacloprid on the long-term survival and olfactory learning ability of bees following long-term and short-term exposure.

2.2- Protocol

- Chronic toxicity in caged bees
- Study of the learning abilities of individual bees by means of a biological test examining the conditioned proboscis extension reflex of worker bees of a known age (15 days at the time of the test) that had been raised in cages. The doses were selected with reference to the LD50 or sublethal doses indicated by BAYER (as the residue doses were not yet known at that time).

2.3- Results

- Short-term effects on learning ability

In order to simulate brief exposure of a worker bee to the product which may be present in sunflower nectar, the conditioning procedure involved giving a brief reward to the bee consisting of a 30% sugar solution containing imidacloprid at

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concentrations of 0.3, 0.06 and 0.03 mg/l (2.5, 0.5 and 0.25 ng/bee respectively under our conditions). When compared with the control over the course of the experiment (about 2 hours) no mortality was observed and there was no difference between the bees according to whether they received a contaminated reward or a control reward.

- Long-term effects

Bees raised in cages received imidacloprid (and a control):

- By contact: 10, 5 and 2.5 ng/bee (0.5, 0.25 and 0.125 mg/l respectively under our conditions)
- By ingestion: 0.04, 0.008, 0.004, 0.002 and 0.0001 mg/l (1.3, 0.25, 0.13, 0.065 and 0.0325 ng/bee respectively under our conditions). The ingestion experiments were conducted in two separate phases, the first with the 3 strongest concentrations and the second with the 3 weakest concentrations (one concentration was administered in both phases).

*** Effects on mortality**

In the ingestion experiment, significant mortality compared with the control was observed for concentrations of 0.008 mg/l (0.25 ng/bee) and above after 11 days.
In the contact experiment, significant mortality was observed at doses of 10 ng/bee (0.5 mg/l) and above after 13 days.

*** Effects on learning**

- 11 days after ingesting the contaminated syrup, comparison with the control showed a significant decline in learning performance at all doses tested, even the dose of 0.004 mg/l (0.13 ng/bee). The contaminated solutions were seen to have no repellent effect at any dose since all the solutions offered were consumed. It was noted that administration of the product did not affect the motricity of the reflex itself, but only the process of memorisation.
- 13 days after topical application, comparison with the control showed no significant difference even at the highest dose tested (10 ng/bee or 0.5 mg/l).

2.4- Conclusions

No short-term effect, simulating possible exposure of a bee foraging on a contaminated flower, was shown at doses below 0.3 mg/l (2.5 ng/bee).
However, prolonged exposure to the product led to:

- **A significant rise in mortality after contact application of a dose of at least 10ng/bee (0.5 mg/l) and ingestion application of at least 0.008 mg/l (0.25 ng/bee).**
- **A significant decline in olfactory learning performance following the ingestion of imidacloprid at a concentration of at least 0.004 mg/l (0.13 ng/bee).**

Prolonged topical application of the product (up to 10 ng/bee) did not cause a decline in performance.
From this we can conclude that the product will significantly affect learning if bees are exposed to contaminated food over several days, even if the dose is low.
In contrast, contact exposure appears to have less significant effects.

2.5- Comments

It should be noted that the long-term study of mortality in the presence of the product was to have been conducted over two months but could not be completed before the deadline. The report contains results for mortality observed over a period of around ten days.

The results indicate that the dose of 0.13 ng/bee administered orally over a long period does not cause a significant increase in mortality compared to the control group, but on the other hand does have a significant detrimental effect on learning performance.

3- Metabolic study of imidacloprid in bees (INRA Avignon *Report by* [redacted] *– annexe III-9)*

3.1- Aim

To characterise the kinetics of imidacloprid breakdown in order to establish a solid basis for diagnosing intoxication by the product.
To determine which metabolites may be useful indicators for imidacloprid intoxication.
To study the toxicity of imidacloprid metabolites in bees.

3.2- Protocol

The first step involved redefining acute toxicity according to the CEB95 method. The research team then studied the metabolisation of imidacloprid and the appearance of its metabolites, olefin and hydroxy-imidacloprid, following oral administration of sublethal doses of imidacloprid (2 and 5 ng/bee).

3.3- Results

The following values were obtained:

Contact toxicity: LD50 (24h) = 23.8 ± 1.5 ng/bee
LD50 (48h) = 24.4 ± 2.7 ng/bee

Oral toxicity: LD50 (24h) = 4.7 ± 0.2 ng/bee
LD50 (48h) = 4.3 ± 0.4 ng/bee

The product metabolised rapidly (40% of imidacloprid left 20 minutes after ingestion). Lower doses metabolised more quickly. The metabolites also appeared rapidly, accounting for 5% of the parent product immediately on ingestion, peaking between 4 and 6 hours after ingestion.

3.4- Conclusions

When ingested at doses of 2 and 5 ng/bee imidacloprid was metabolised very rapidly, and its metabolites (olefin, OH-imidacloprid) also appeared very rapidly. From this the author concludes that it will be impossible (or very difficult) to detect the presence of imidacloprid in bees intoxicated with doses below 2 ng/bee.

3.5- Comments

The author mentions the fact that mortality induced by increasing doses of imidacloprid followed a "particular kinetic pattern". The dose-response curves showed unusual peaks.

4- Synergic effects in the presence of imidacloprid in bees (INRA Avignon *Report by* [redacted] *– annexe III-9)*

4.1- Aim

To ascertain whether the toxicity of imidacloprid is potentiated by the presence of other products used to treat sunflowers (azole fungicides, insecticides and acaricides).

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4.2- Protocol

Effects (mortality) of
- staggered and simultaneous treatments
- oral and contact intoxication
- various product doses

4.3- Results

A number of combinations of imidacloprid and other crop protection products were tested. The products were administered orally or topically at sublethal doses and included pyrethrinoids (deltamethrin, fluvalinate) and azole fungicides (prochloraz, difenoconazole). There was no significant difference in terms of mortality between the individuals treated with these combinations and the controls.

4.4- Conclusions

Synergy with products likely to be present during imidacloprid's period of action was not demonstrated.

5- Effects of Gaucho used as a sunflower seed dressing on bumblebees (INRA Lusignan *Report by* [redacted] *– annexe III-6)*

5.1- Aim

To observe the effects of ingesting feed contaminated with the product on the reproductive abilities of micro-colonies of orphaned female worker bees.

5.2- Protocol

Micro-colonies of 3 worker bees were fed on contaminated syrup; food consumption and reproduction of individuals were observed.

5.3- Results

Micro-colonies of 3 individuals were fed on feed contaminated with imidacloprid at concentrations of 0.01 and 0.025 mg/l. The experiment consisted of observing the effects of this feed on:

- Consumption

Over a 5-day period the control group and the groups treated with both doses of contaminated feed consumed equivalent amounts of pollen and syrup. The bumblebees consumed 2.15 and 4.8 ng of imidacloprid per worker bee per day.

- Mortality

Mortality increased significantly from the 7^{th} day for the 0.01 mg/l concentration. On the 14^{th} day longevity was affected by both treatments. The differences between the control group and the treated groups stabilised from the 30^{th} day.

- Time until the appearance of the first males

There was no difference between the control group and either of the treated groups.

- Production of males

Consumption of imidacloprid at a concentration of 0.01 mg/l (2.15 ng/worker bee/day) caused a significant fall in the number of males produced. However, the same number of micro-colonies produced males irrespective of the treatment received.

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- Ejection of larvae

The same number of micro-colonies ejected larvae in the control group and the treated groups, but the number of larvae ejected was significantly higher in the control group than in the treated groups.

- Brood size

The number of colonies with a brood did not vary according to the treatments, but the brood size of the bumblebees treated at a concentration of 0.01 mg/l was significantly lower than that of the control group.

5.4- Conclusions

Ingestion of 2.15 (0.01 mg/l or 10 ppb) and 4.8 (0.025 mg/l or 25 ppb) ng of imidacloprid/bee/day caused a significant reduction in bumblebee longevity. Treatment with 2.15 ng/bee/day caused a significant reduction in the number of male offspring. Both treatments caused a significant reduction in the ejection of larvae but did not affect the speed of development of the first brood.

5.5- Comments

Some effects appear at the lowest dose (2.15 ng/bee/day) but not at the higher dose. These apparently contradictory results need to be discussed.

The author notes that bumblebees are intrinsically less sensitive to imidacloprid than honeybees.

6- Effects of Gaucho used as a sunflower seed dressing on ladybirds (INRA Avignon *Report by* [redacted] *– annexe III-7B)*

6.1- Aim

To evaluate the effects of the product on ladybird development.

6.2- Protocol

10 pairs of ladybirds were fed on crushed pollen from sunflowers treated with Gaucho, 10 pairs were fed on untreated sunflower pollen and 10 control pairs were fed on their normal replacement food (*Ephestia kuehniella* eggs). Fecundity and fertility were measured in the adults and experiments were also conducted on larvae at different stages in order to assess the speed of larval development.

6.3- Results and conclusions

Pollen from sunflowers treated with Gaucho had no significant effects on the larval or adult stages of the ladybird *Harmonia axyridis*. However, only females fed on untreated sunflower pollen laid fertile eggs.

6.4- Comments

It is clear that the experiment came up against the difficulty of maintaining ladybirds on a diet of pollen, which seems to have an inhibitory effect irrespective of whether it is obtained from treated or untreated sunflowers.

The author also suggests that a more appropriate experiment would involve feeding ladybirds on aphids taken from untreated sunflowers and from sunflowers that had been treated with Gaucho.

III – VARIETAL TESTS *(ACTA-CETIOM Report – annexe IV)*

Aim

To investigate imidacloprid and its major metabolites at various stages of crop growth in order to assess the risks posed to foraging bees exposed to the product.
To ascertain whether seed treatment affects the nectar-producing capacity of sunflowers.
To assess the nectar-producing capacity of the main sunflower varieties currently grown in order to estimate their relative level of attraction for bees.

Protocol

The tests were carried out in 4 geographical areas (Surgères, St. Florent, Chonas, En Crambade) with different soils and climates. A varietal test was carried out on each site, comparing 5 varieties (3 new varieties, one old variety and one local variety).
An irrigation test was also performed on two sites, aimed at studying the effect of irrigation on one variety (treated and untreated).
A dose test was performed on the other two sites, examining the effects of three doses (N, 1.5N and 2N) on one variety.
A number of samples were taken:
Samples of leaves and disk flowers were taken at 4 phenological stages before, during and after flowering. Some of these samples from each site were sent for residue analysis.
Nectar samples were pipetted (at 3 sites) and sent for quantitative and qualitative analysis of the sugars secreted.

3- Results

(see overleaf)

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Breakdown of results of leaf analyses by level of detection (imidacloprid only (BIOTEC))

Varietal test	Untreated plots				Plots treated with Gaucho			
Stage / Site	39 BBCH	59 BBCH	61 BBCH	65 BBCH	39 BBCH	59 BBCH	61 BBCH	65 BBCH
C1 St-Florent								
>0.01	0	0	0	0	5	0	0	0
ILQ	0	0	1	0	0	5	5	5
ND	1	1	0	1	0	0	0	0
Total	1	1	1	1	5	5	5	5
C2 Surgères								
>0.01	0	0	0	0	0	0	0	0
ILQ	1	1	0	0	1	5	1	1
ND	0	0	1	1	0	0	0	0
Total	1	1	1	1	1	5	1	1
A1 En Crambade								
>0.01	0	0	0	/	0	0	0	/
ILQ	0	1	0	/	1*	4	1	/
ND	1*	0	1	/	0	1	0	/
Total	1*	1	1	/	1*	5	1	/
A2 Chonas								
>0.01	0	0	0	0	0	0	0	0
ILQ	0	1	1	1	1	5	1	1
ND	1	0	0	0	0	0	0	0
Total	1	1	1	1	1	5	1	1
Total								
>0.01	0	0	0	0	5	0	0	0
ILQ	1	3	2	1	3	19	8	7
ND	3	1	2	2	0	1	0	0
Total	4	4	4	3	8	20	8	7

* sample taken late: BBCH stage 54

Irrigation test	Untreated plots				Treated plots			
Stage / Site	39 BBCH	59 BBCH	61 BBCH	65 BBCH	39 BBCH	59 BBCH	61 BBCH	65 BBCH
C1 St-Florent								
>0.01	0	0	0	0	0	0	0	0
ILQ	0	1	1	1	2	1	1	2
ND	1	0	0	0	0	1	1	0
Total	1	1	1	1	2	2	2	2
C2 Surgères								
>0.01	0	0	0	0	1	0	0	0
ILQ	3	1	0	0	7	2	2	2
ND	1	0	1	1	0	0	0	0
Total	4	1	1	1	8	2	2	2
Total								
>0.01	0	0	0	0	1	0	0	0
ILQ	3	2	1	1	9	3	3	4
ND	2	0	1	1	0	1	1	0
Total	5	2	2	2	10	4	4	4

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Dose test	Untreated plots				Plots treated with Gaucho			
Stage / Site	39 BBCH	59 BBCH	61 BBCH	65 BBCH	39 BBCH	59 BBCH	61 BBCH	65 BBCH
A2 Chonas								
>0.01	0	0	0	0	0	0	0	0
ILQ	0	1	1	1	1	5	1	1
ND	1	0	0	0	0	0	0	0
Total	1	1	1	1	1	5	1	1

Limit of quantification LQ = 0.01 mg/kg for imidacloprid and its derivatives except for olefin (LQ = 0.1 mg/kg)

ILQ: below the limit of quantification ND = not detected

Breakdown of results of disk flower analyses by level of detection (imidacloprid and total residues (GIRPA))

Varietal test	Untreated plots				Plots treated with Gaucho			
Stage / Site	54 BBCH	59 BBCH	61 BBCH	65 BBCH	54 BBCH	59 BBCH	61 BBCH	65 BBCH
C1 St-Florent								
>0.005		0	0	0		2	2	3
<LQ		1	1	1		3	3	2
Total	/	1	1	1	/	5	5	5
C2 Surgères								
>0.005			0	0			0	0
<LQ			1	1			1	1
Total	/	/	1	1	/	/	1	1
A1 En Crambade								
>0.005	1	1	0		0	0	0	
<LQ	0	0	1		1	5	1	
Total	1	1	1	/	1	5	1	/
A2 Chonas								
>0.005		0	0	0		0	0	0
<LQ		3	1	1		3	1	1
Total	/	3	1	1	/	3	1	1
Total								
>0.005	1	1	0	0	0	2	2	3
<LQ	0	4	4	3	1	11	6	4
Total	1	5	4	3	1	13	8	7

<LQ: below the limit of quantification = 0.005 mg/kg

Irrigation test	Untreated plots			Treated plots		
Stage / Site	59 BBCH	61 BBCH	65 BBCH	59 BBCH	61 BBCH	65 BBCH
C1 St-Florent						
>0.005	1	1	0	1	0	1
<LQ	0	0	1	1	2	1
Total	1	1	1	2	2	2
C2 Surgères						
>0.005		0	0		0	0
<LQ		1	1		2	2
Total	/	1	1	/	2	2
Total						
>0.005	1	1	0	1	0	1
<LQ	0	1	2	1	4	3
Total	1	2	2	2	4	4

<LQ: below the limit of quantification = 0.005 mg/kg

Final report GAUCHO bees

06/04/99

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Dose test	Untreated plots			Treated plots		
Stage Site	59 BBCH	61 BBCH	65 BBCH	59 BBCH	61 BBCH	65 BBCH
A2 Chonas						
>0.005	0	0	0	0	0	0
<LQ	1	1	1	3	3	3
Total	1	1	1	3	3	3

<LQ: below the limit of quantification = 0.005 mg/kg

Kinetics of the product in the plant

- **Leaf analysis (LQ = 0.01 mg/kg):**

*** Varietal tests**

This test was conducted on 4 sites. 58 residue analyses were performed (43 samples from treated plots and 15 from untreated plots). Out of the 43 samples taken from treated plots, 5 showed levels above the limit of quantification (between 0.012 and 0.020 mg/kg) and 37 showed levels below the limit of quantification (ILQ) which were not quantified.

Imidacloprid was detected but not quantified in 7 of the 15 samples taken from untreated varieties (ILQ) and was not detected in the 8 others.

Comments

The 5 analyses showing values above the limit of quantification were performed on samples taken at BBCH stage 39 on a single site 49 days after sowing.

*** Irrigation tests**

This test was conducted on 2 sites. 33 samples were analysed (11 from treated irrigated plots, 11 from treated unirrigated plots and 11 from untreated irrigated plots). Out of the 22 samples from the treated plots, 1 sample from an irrigated plot contained 0.012 mg/kg of imidacloprid and in 19 samples imidacloprid was detected but not quantified (ILQ). In the untreated samples, the presence of imidacloprid was detected but not quantified (ILQ) in 7 samples and not detected in the other 4.

Comments

Of the 22 samples taken from the treated plots, the derivative guanidine was also found at levels above the limit of quantification (between 0.011 and 0.015 mg/kg) in 3 samples from irrigated plots and 1 sample from an unirrigated plot. These samples were taken from a single site at BBCH stage 39, 44 days after sowing.

*** Dose tests**

This test involved analysing 16 samples (12 from treated plots and 4 from control plots). The results obtained for the samples from the treated plots were as follows: 1 result above the limit of quantification (0.011 mg/kg) with imidacloprid being detected but not quantified (ILQ) in the other 11 samples. In the samples taken from untreated varieties, 3 results were in the ILQ range.

Comments

The sample containing a quantifiable level of imidacloprid was taken from a variety that had been treated with twice the approved dose that had been sampled late (BBCH 65).

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- Analyses conducted on disk flowers (LQ = 0.005 mg/kg for imidacloprid and total residues (GIRPA) and 0.01 mg/kg for imidacloprid only (BIOTEC))

*** Varietal tests**

GIRPA analysed 29 samples taken from treated plots. 7 samples showed levels of imidacloprid and metabolites above 0.005 mg/kg. 2 of the 13 samples taken from the control plots showed levels above the limit of quantification while the other samples were below the limit of quantification.

BIOTEC also analysed disk flowers at BBCH stage 59. All the 5 samples from treated plots were found to contain imidacloprid at unquantified levels (ILQ), as did the single control sample.

*** Irrigation tests**

10 samples from treated plots were analysed. 2 results were above the limit of quantification while the rest were below the limit. 5 samples from untreated plots were analysed and 2 results were above the limit of quantification.

BIOTEC analysed one treated sample and one control. Both results were ILQ.

*** Dose trials**

9 samples from treated plots and 3 samples from control plots were analysed. Imidacloprid and its metabolites were detected but not quantified in all the samples.

Comments

The GIRPA laboratory analysed 69 samples in total: 48 treated samples and 21 controls. 18% of the treated samples (9 samples) and 19% of the controls (4 samples) were found to contain imidacloprid and total residues at levels above the limit of quantification (0.005 mg/kg).

The CNRS BIOTEC laboratory used an LQ of 0.01 mg/kg and analysed 115 leaf samples in total: 83 treated samples and 32 controls. Most of the samples contained imidacloprid: 88% of the treated samples and 60% of the control samples. This raises problems of interpretation.

11 samples were cross-analysed by both laboratories. The results were similar: when imidacloprid was detected, both laboratories reported close findings.

<u>Effect of the use of Gaucho on nectar secretion</u>

- Varietal test

There was considerable variation in secretion according to variety, and when the varietal effect was significant the performance of individual varieties differed from one site to the next. The effect of treatment on the quantity of nectar produced was expressed in different ways on different sites: on one site, the 5 treated varieties produced more nectar than the untreated varieties; on another site this favourable effect was observed in 3 varieties out of 5. In the last site treatment had a detrimental effect on the least productive varieties.

- Dose test

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Although the differences were not significant, the test conducted to compare doses found that the product had a favourable effect when applied at the approved dose. The effect was less marked when larger doses of the product were applied.

- Irrigation test
Irrigation was found to have no overall effect on secretion.

Comments
Secretion measurements indicated strong variability, but this seems to be linked mainly to the site. The favourable effect observed when the product was administered at the approved dose may reflect the fact that the treated plants were in a better physiological condition.

Sugar composition of the nectar

The initial aim of this test was to investigate the overall sugar content and the level of sucrose, a sugar which bees find particularly attractive.

The results obtained are more difficult to interpret, but in general terms it was found that the sugar content of varieties fluctuated from one site to the next and that treatment and irrigation did not cause any significant differences.

Comments
As regards methodology, it should be noted that there were sometimes significant differences between the volume of samples taken as recorded in the field and the measurements undertaken in the laboratory as part of the sugar analyses. These distortions may be due to errors in reading associated with the presence of air bubbles in the capillaries or storage problems.
It is clear that a more in-depth analysis of this data is required before definite conclusions can be drawn.

Conclusions on the varietal tests
This experiment confirmed that at a threshold of 10-20 ppb the presence of imidacloprid residues in the upper part of the plant is quantified mainly at the earliest stages, but that the presence of the product is detected in the vast majority of the samples analysed, and even in some untreated plants which raises a problem of interpretation. Investigations revealed that in two out of four sites no Gaucho-dressed seeds had been planted since 1995, and not since 1994 in one case. It would have been preferable for the treated and untreated varieties to have been planted separately.
The effects of treatment and variety on the quantity and quality of the nectar produced varied from one site to the next. In general terms, approved doses of Gaucho were not found to affect sunflower nectar production.

CONCLUSIONS

- The **methodology** used in this study was highly innovative, involving
* the concerted implementation of large-scale experiments,
* the first ever use of bee counters,

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* modification of biological tests under controlled conditions to assess sublethal effects,
* individual analysis of imidacloprid metabolites and a (tenfold) reduction in the residue quantification threshold.

- Comparison between all the **fieldwork** experiments conducted on sites treated with Gaucho and control sites showed that **there was no marked difference between the sites as regards foraging activity, mortality, colony development and the honey yield**. No dramatic phenomena (depopulation, collapse in nectar production) were reported in the zones studied.
However, it should be noted that **drift phenomena** were observed in **three out of the four treated sites**.
It is also important to draw a distinction between what was observed in the context of the experiments themselves and **extra-experimental observations**; beekeepers have been reporting **wide variations in sunflower nectar production and continuing hive depopulation**.
Finally, **symptoms associated with intoxication** (trembling, immobility, abnormal appearance etc…) have been mentioned, especially in Vendée. However, these observations relate to the whole of the zone, not simply to sites treated with Gaucho.

- Experiments performed under **controlled conditions** (tunnel, laboratory) showed that even low doses of the product had **significant effects** on various aspects of bee biology and behaviour. In order to demonstrate the effects produced by the range of doses tested by the individual research teams we have drawn up a graph with a logarithmic scale of concentrations or doses tested on the X-axis and the various types of reactions studied by each team on the Y-axis.
Figure A: 1 = INRA Bures; 2 = INRA Avignon; 3 = INRA Avignon; 4 = INRA Lusignan (bumblebees). The concentrations are shown in mg/l or ng/bee on the X-axis.
Figure B: 1 = INRA Bures; 2 = INRA Avignon; 3 = INRA Lusignan (bumblebees). The concentrations are shown in mg/l or ng/bee on the X-axis.
The crosses indicated the doses tested. The thicker lines indicate a significant effect produced by imidacloprid. Dotted lines indicate that the threshold for which an effect is observed is located in this zone.
In figure A we indicate the lowest LQ stated in the specifications of the analytical laboratories (0.01 mg/kg) and in figure B the various known lethal or sublethal doses.
It was clearly demonstrated that significant effects were reported when imidacloprid was present at concentrations close to or even below the LQ (figure A). The following biological effects were observed at or below 0.01 mg/kg (= 10 ppb):
- In micro-colonies: reduction in the quantity of "Gaucho" pollen consumed and reduction in the number of cells built
- Under controlled conditions: inhibition of recognition of related bees (0.012 mg/kg)
- In insect-proof tunnels: bees stopped foraging at a source contaminated with syrup containing 0.006 mg/l of imidacloprid; this was followed by apathy among the adult bees and the queen inside the micro-colonies
- In cages: increase in long-term mortality (over more than 10 days) following ingestion of contaminated syrup (above 0.008 mg/l)
- Among individuals: decline in olfactory learning performance in bees raised (for around ten days) on contaminated syrup (at concentrations of 0.004 mg/l and above).

- The **residues** obtained in the various matrices were not often found in quantities above the LQ **between 0.005 and 0.02 mg/kg** (the figures varied according to the matrices, type of residues and methods of analysis). However, the presence of imidacloprid was detected (but not quantified) in most plant samples analysed, even in some of the untreated plants. These results raise the problem of persistence of the product in the soil or accidental contamination of samples.
In the light of the results of the laboratory experiments, it is noted that there is a negative effect on bees at doses corresponding to and below the LQ.

These data highlight an apparent contradiction: the laboratory tests indicated that bees are at risk when exposed to imidacloprid concentrations that they are likely to encounter in sunflowers under natural conditions as shown by the residue analyses. However, this apparent risk is not confirmed by field observations.
If we simply consider the initial question, we find that the dramatic phenomena (loss of foraging bees in the first few days of the flowering period followed by a fall in nectar production) were not observed on the sites treated with Gaucho whereas beekeepers continued to report these phenomena in areas outside the experimental sites. It is therefore difficult given the field data to reach any conclusion as to the role of the product in the phenomena described during the sunflower flowering period. It is possible that the potential effect of Gaucho on bees observed in laboratory tests was not expressed in the field. The effect may have been masked or countered by factors beyond our control, particularly the presence of the product in plants on the control sites.

The large volume of data obtained during this study is worthy of further investigation, and some aspects are still not complete. This would help us refine our interpretation of the initial results and develop working hypotheses for the future.

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4 Brood
Ejection of larvae
Production of males
Mortality
3 Time spent at feeders
Consumption (tunnel)
Visits to feeders (tunnel)
Recognition of related bees
Consumption of feed
2 Visits to feeders / symptoms of intoxication
1 Olfactory discrimination
Recruitment
Long-term consumption / reflex motricity
Long-term learning / contact
Long-term learning / ingestion
Long-term mortality / ingestion
Long-term mortality / contact
Short-term learning and acute toxicity / ingestion
mg/l (logarithmic scale)
Figure A

estimated sublethal D Belzunces Taséi
Oral LD50 Bayer
Oral LD50 Belzunces
Oral LD50 Buckfast
Contact LD50 Belzunces
Contact LD50 Bayer
3 Brood
Ejection of larvae
Production of males
Mortality
2 Sublethal range
1 Long-term consumption / reflex motricity
Long-term learning / contact
Long-term learning / ingestion
Long-term mortality / ingestion
Long-term mortality / contact
Short-term learning and acute toxicity / ingestion
ng/bee (logarithmic scale)
Figure B

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COMMENTS ON THE SUMMARY REPORT:
"EFFECTS OF CROP PROTECTION PRODUCTS ON BEES"
Effects of GAUCHO sunflower seed dressing on losses of foraging bees
[redacted] – November 1998"

1 - RESIDUE RESULTS

a – Residues in leaves (open field experiments) page 21:

On the control plots, no quantifiable residue (LOQ = 10 ppb) was found in the leaves in any of the 4 experimental zones. The C.N.R.S. reported that detectable but not quantifiable residues were found in the 2 Vendée zones (Marais and Plaine) and the explanation given pointed to residues from the previous crop; however, in the "Vendée marais" experimental crop the previous crop was a common wheat that had not been treated with Gaucho. These results should therefore be confirmed via an analysis performed with a limit of quantification of 5 ppb.

In any event, the results of analyses carried out in the treated plots do not show any quantifiable residue where the limit of quantification is 10 ppb. It would nevertheless be reasonable to expect levels to be higher in plants grown from seed treated with Gaucho in 1998 than in the control plants.

In addition, these levels cannot disturb bees to such an extent that the hive becomes depopulated over the course of a few days (see studies with treated solutions).

b – Residues in the flowers of plants grown from treated seed

Residues in the disks (stage 59: "Yellow ray flowers visible between the bracts") and in the disk flowers (stages 61 and 65: "First flowers open" and "Full flower") were examined in 4 sites (varietal tests) and on 5 varieties per site. Total residues were determined by the GIRPA laboratory with a limit of quantification of 5 ppb (see table 1).

No quantifiable residues were found on 2 sites (En Crambade/31 and Chonas/38). It should be noted that the analyses performed on the flowers produced by seed treated at 1.5 times (1.5N) and 2 times (2N) the approved dose at the Chonas site gave the same result: no quantifiable total residues (LQ = 5 ppb).

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Table 1: TOTAL RESIDUES in the disks of treated plants

LOCATION	Stage 59	Stage 61	Stage 65
En Crambade			
Chonas			
Chonas dose study			
Surgères			
Surgères irrigation			
RIGASOL irrigated			
RIGASOL unirrigated			
St. Florent			
St. Florent irrigation			
RIGASOL irrigated			
RIGASOL unirrigated			

LOQ = 5 ppb
*: imi.ILQ = imidacloprid < 10 ppb
*: guan.ILQ = guanidine < 10 ppb
*: metaND = metabolite not detected

On a third site (Surgères/17), the samples taken at stage 59 were mistakenly sent to the BIOTEC laboratory which uses a method that involves searching for individual residues with a limit of quantification of 10 ppb. There was no quantifiable residue for the 5 varieties (LOQ = 10 ppb). The samples taken from the irrigation study performed at the same site and analysed by the GIRPA laboratory found no quantifiable total residues (LOQ = 5 ppb) for the variety used (Rigasol) irrespective of whether it was grown under unirrigated or irrigated conditions. There was no quantifiable residue for the other stages (61 and 65) (LOQ = 5 ppb).

On the last site (Saint Florent/18) 21 flower samples were analysed (5 varieties at 3 stages plus 1 variety in an irrigation study (unirrigated, irrigated)); 12 were found to contain no quantifiable residues and 9 contained total residues at levels between 8 and 12 ppb. The results obtained on this site contradicted those obtained on the others, but it was also on this site that [redacted] detected in the leaves of the control varieties. The explanation given by CNRS scientists, i.e. that the residues were the remains of treatments applied in previous years, is highly unlikely since no crops were treated with Gaucho in 1997 or 1996 at Saint Florent. It should also be noted that on the Chonas site (38) no quantifiable residues were detected in the flowers although a wheat crop grown on this site in 1997 had been treated with Gaucho.

The conclusion is that on 3 out of 4 sites total residues in the disks and disk flowers are not quantifiable with a limit of quantification of 5 ppb, provided that the results obtained by BIOTEC (LOQ = 10 ppb) for Surgères are confirmed with a lower limit of quantification (LOQ = 5 ppb). No satisfactory explanation for the results obtained on the last site (St Florent) has yet been found.

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In any event, even the worst figures (under 5 ppb to 12 ppb in Saint Florent according to the variety and stage) cannot explain the bees' disorientation and failure to return to the hive, given the results of the sublethal tests. This view is confirmed by the fact that the total residues found include some metabolites that do not have an insecticidal action comparable to the parent compound (for example: guanidine).

2 - OBSERVATIONS UNDER CONTROLLED CONDITIONS

All the studies on the effects of offering contaminated syrup conclude that doses below 20 ppb have no effect, with the exception of 3 tests: "length of time spent at feeders" ([redacted] et al., INRA Avignon), "recruitment test" and "long-term toxicity test" [redacted] et al., INRA Bures-sur-Yvette).

a – In the tunnel test performed by [redacted] with microcolonies (page 25 of the summary report), the length of time the bees spend at the feeders starts to fall on the second day for all doses of imidacloprid contained in the syrup (100, 25, 13 and 6 ppb), although the higher doses did not have a stronger effect as is shown by the following findings:
- On the second day the 6 and 100 ppb doses produced the strongest effects and the 13 and 25 ppb doses produced the weakest effects.
- On the third day the most significant fall in time spent at the food source was caused by the 25 ppb dose.
- On the fourth day the strongest effect was produced by the 100 ppb dose and the weakest by the 13 ppb dose, with the 6 and 25 ppb doses lying between these extremes.
- Finally, on the fifth day the 13 ppb dose had the least effect on the bees, with the 6, 25 and 100 ppb doses all producing a similar effect.

Taking the average of the 5 days, the 6 ppb dose produced effects between those caused by the 25 and 100 ppb doses and above those caused by the 13 ppb dose; this indicates an erratic response curve.

Following this study, although the length of time spent at the feeders is a relevant and reproducible criterion it is worrying that we are unable to demonstrate a logical response curve for doses between 6 and 100 ppb given the results observed in other tests and the dynamics normally observed in toxicology.

b – The flight cage recruitment test (page 26 of the summary report) concluded that syrup contaminated with 10 ppb of imidacloprid had an effect. No tests performed on outdoor colonies have confirmed that the recruitment of worker bees is affected. In addition to all the tests conducted in France, Prof. Kirchner (Konstanz University, a graduate of the highly respected von Frisch college) has studied the effects of different doses of imidacloprid on the process by which foraging bees are recruited in the hive; he observed bee dances which are known to be the means by which these social insects communicate. At the end of this work, a concentration of 10 ppb did not interfere with the recruitment dances in any way.

c – In the laboratory experiments conducted by [redacted] et al., the minor effects on the motivation and learning performance of bees that had been observed elsewhere were confirmed only in long-term studies (ingestion of imidacloprid over 11 days) of isolated bees; no clear dose-response curve could be established. This result is not sufficient to explain

the dramatic losses of foraging bees observed by beekeepers (in the 2 to 3 days following the start of nectar production).
Moreover, although this test opens up an interesting opportunity for developing a way of investigating sublethal effects caused by chemical molecules, it needs to be validated, i.e. the practical significance of these results for the behaviour of bees in colonies and in the field need to be determined. A further problem is that at present there is no database covering a significant number of phytopharmaceutical active ingredients that could be used to draw up a parallel comparison between different insecticidal substances both by means of this test and in agriculture and apiculture (in fields and on bees in colonies).

In conclusion, all the studies performed in situations that closely reflect natural conditions (bees in colonies and test performed outdoors with food sources far from the hive) find that the effects are only produced at doses of 20 ppb of imidacloprid and above. These doses have never been found in the flowers, and the symptoms observed do not explain the dramatic losses of foraging bees (no effect on orientation and return to the hive).

3 - CONCLUSIONS (page 38 of the summary report)

a – Comment on the "phenomena of drift in three out of four treated sites":

These phenomena are well known to beekeepers who set their hives in a straight line. They do not undermine the results obtained in the field because they are unlikely to hide the dramatic and spectacular losses of population that the research work was attempting to investigate.

It should also be remembered that the hives in the Indre zone were set up in undergrowth and in a curved line in order to reduce drift. Under these conditions no significant difference was observed.

b – On the test performed under controlled conditions which indicated inhibition of recognition among related bees at a dose of 12 ppb: The report on this test is not yet available. Moreover, this symptom is not observed in the field as marked worker bees in the field study conducted by [redacted] were accepted by their siblings throughout the three-week experiment.

c – On residues: With regard to the residues detected in untreated plants and the suggestion that they could be due to the presence of residues in the earth, it should be noted that these residues were not quantified (LOQ = 10 ppb) and that on some sites no Gaucho-treated crop had been grown the previous year (e.g. common wheat not treated with Gaucho in 1997 in the Vendée marais zone; varietal tests in Saint Florent where no treated crop had been grown in 1997 or 1998).

d – If we concentrate just on the question that was asked, neither the field trials nor the studies conducted under controlled conditions permit us to conclude that there is a causal link between Gaucho used as a seed dressing and the dramatic fall in hive populations in the first few days following the start of sunflower nectar production. In fact, the symptoms observed following ingestion of syrup contaminated with doses of imidacloprid never indicate disorientation or failure to return to the hive.

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e – On the possibility of a potential effect that is hidden in the field: This is contradicted both by observations of hive populations in the treated sites (no dramatic and serious effect throughout the experiment) and by the fact that beekeepers again observed these losses of population in 1998 (outside the experimental site) even in micro-regions where sunflowers that are not treated with Gaucho were grown (see report of a meeting held at the Ministry of Agriculture on 31/07/98). It should also be noted that non-quantified residues (LOQ = 10 ppb) were not found in untreated plants at the Indre or Deux-Sèvres sites.

To conclude, in all ecotoxicological work, studies performed in the field under conditions closely reflecting reality are always considered to be more relevant in evaluating the true risk of a technique than studies performed under laboratory conditions. It is therefore important to stress that it has not been possible to find a causal link between Gaucho used as a seed dressing and the genuine problem posed by the dramatic loss of bee populations in the first few days after the start of sunflower nectar production.

[redacted] **(Bayer S.A.), 16 November 1998**

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EFFECTS OF PLANT PROTECTION PRODUCTS ON BEES
Effect of the treatment of sunflower seeds with GAUCHO on the disappearance of foraging bees

Supplementary comments to the summary report

Since the summary report was presented to the meeting of the Study Committee on the Toxicity of Plant Protection Products for Agricultural Use and Similar Products on November 18, 1998, we have received a number of results from supplementary analyses and comments by various members of the steering committee.

Supplementary analyses

- **relating to the results of the INRA** [National Agricultural Research Institute], **Bures experiments** conducted in the laboratory and flying cage. All used the same stock solution containing imidacloprid, but subsequent verification of the concentration of this solution in product (December 1998) carried out at our request by the GIRPA laboratory, indicates that the initial concentration was higher by a factor of 5 than the concentration which was initially stated. All the concentrations at which the biological effects were studied therefore need to be modified. Thus, **the lowest dose for which an effect on the learning behaviour of the caged bees was observed is of the order of 4 ppb (0.004 mg/l) and the effect on the foraging behaviour in the flying cage is seen on a contaminated source at 50 ppb** (see attached dossier).

- **relating to the analyses of residues:**

Soil analysis samples taken by the GIRPA from control and treated experimental plots and from reference plots (plots not cultivated) with a quantification limit of 8 ppb (0.008 mg/kg) for imidacloprid alone and 5 ppb (0.005 mg/kg) for total residues indicate that **the results are below the quantification limit in all the samples** (see attached document).

The cross-over analyses originally provided for in the protocol are in progress. At present we have the results of leaf analyses at the trial sites, initially carried out by BIOTEC (QL = 0.01 mg/kg for imidacloprid) and subsequently by GIRPA (QL = 0.008 mg/kg for imidacloprid and 0.005 mg/kg for total residues). It seems that the samples taken from **control sites** show that the values which according to BIOTEC are below the QL, correspond to non-detectable values (ND) according to GIRPA. In addition, in the case of samples from **treated sites**, imidacloprid, when quantifiable, was found by the two laboratories in similar amounts (with the exception of one sample, currently being checked) (see attached document).

Samples of dead bees from the INDRE control site, analysed by GIRPA, showed imidacloprid and residues of between 0.005 and 0.007 mg/kg (for 4 out of 5 samples). A check of these samples and analysis of 4 additional samples (taken on dates on either side of those of the first samples) by GIRPA with a QL of 0.02 mg/kg for imidacloprid and 0.005 mg/kg for total residues, give results below the QL (see attached document).

- **relating to pathological analyses:** following the site meeting on July 31, 1998, samples of live bees on their return to the hive were taken from 9 apiaries (with or without problems) not involved in the experiments. The analyses carried out by CNEVA [National Centre for

Veterinary and Food Research] gave negative results for acariasis, nosema disease and "black disease".

Comments by the association of bee-keepers

- In the case of studies based on the consumption of sources artificially contaminated by variable doses of the product (shown in Figs. A and B of the summary report), it would be preferable to specify how much of the syrup was actually ingested by the bees.
This information cannot always be monitored.

- Figs. A and B of the summary report, which represent the doses at which biological effects were noted by the various researchers, have been corrected in accordance with the comments.

- It is suggested that the experiments should be continued under controlled conditions in doses below those which were used (when an effect was noted at the lowest dose tested).

- It is noted that the protocol of the observations on foraging behaviour carried out by [redacted] (INRA, Avignon) was not submitted initially to the Pilot Committee.

- Analysis of residues on additional matrices are requested.
Some of these have been done (soil analyses), others are awaited (freshly harvested honey). In the case of nectar pipetted from the flower, the problem of the small quantities of samples which can be taken was discussed in the steering committee. As regards the sunflower seeds which were harvested, such analyses may be a possibility.

A request to lower the quantification thresholds is desired (to 5 ppb).
For the moment, priority has been given to cross-over analyses (see above).

- Comments are made regarding the presence of imidacloprid in the samples from control sites.
These comments should be reviewed on the basis of new information provided by the analytical laboratories.

- The validity of the controls was called into question because of the presence of Gaucho-treated maize on the control sites. Residue analyses of maize are requested.

- Regarding the field experiments, the activity counts by the counters, the visual observations of activity in the hive and on the sunflowers, the evaluation of the population in the hives and the figures for the honey harvest are all disputed.

- The conclusions of the summary report regarding the decrease in nectar flow in sunflowers at the experimental sites are disputed. Decreases over the last 3 - 5 years of 30% to 75% in Indre and of 40 - 60% in Deux-Sèvres and the Vendée are pointed out.

- The establishment of an **auxiliary insect and pollinating fauna protection service** is highly desirable.

Comments by Bayer

- The figure of 0.1 mg/bee presented as a sublethal dose in the Summary Report is rejected.

This is a misinterpretation of a figure mentioned in the expert report by [redacted]. This dose is in fact a sublethal dose estimated on the basis of the LD50.

- Additional experiments carried out by [redacted] in Germany indicate that the wagging dance of bees inside a colony visiting a syrup source contaminated with imidacloprid was affected above a dose of 20 ppb, but not at 10 ppb.

- Samples from bees with symptoms of tremors and dead bees in front of the hive in 6 hives in Vendée Plaine (3 of them at the treated site, 1 at the control site and 2 off-site) which were analysed by CNEVA show positive results for "black disease" for the six samples of live bees and 4/6 of the dead bees.

- The establishment of a monitoring service for adverse effects on bees is approved.

Comments by [redacted] and colleagues (INRA Avignon)

- The validity of the ACTA [Association of technical co-ordination in agriculture] tunnel trials is called into question because method CEB 129 was not strictly adhered to.
ACTA notes that the protocol was always presented as an adaptation of the CEB method.

- It is requested that the foraging behaviour experiment conducted by [redacted] (INRA Avignon) be validated.

- In the case of field experiments, the value of the control sites is disputed because of the history of Gaucho on the sites.

- The absence of imidacloprid in the dead bees, even those which had visited highly contaminated sources, is reported and is probably the result of the rapid metabolisation of the product in the bees.

- Video tapes showing bees with symptoms of poisoning are available.

Comment by GRAPPA

GRAPPA states that it in no way participated in the work of L. Belzunces.
The inclusion of the name INRA-GRAPPA in the summary report to designate [redacted], team was the responsibility of those who produced the Report.

[redacted]. Done at Bures, 15 December 1998

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