Neonicotinoid Insecticides and Honey Bees: Technical Answers to FAQs

Prepared by
David L. Fischer, Ph.D and Alison Chalmers, Ph.D
Ecotoxicology Section, Bayer CropScience LP
May 16, 2007

What are neonicotinoid insecticides?

Neonicotinoids are a class of insecticides with the same mode of action as nicotine. Examples include imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid and dinotefuran. These compounds interfere with the transmission of impulses in the nerve system of insects. Similar to the naturally occurring signal-transmitting acetylcholine, neonicotinoids stimulate certain nerve cells by acting on a receptor protein. Neonicotinoids bind tightly to the nicotinic acetylcholine receptor (nAChR) and are not broken down by the enzymes which normally and rapidly remove acetylcholine.[1] They therefore disrupt normal nerve transmission and produce toxic effects. Unlike nicotine, these chemicals have a much higher affinity for the insect receptor than for the mammalian receptor, resulting in a much more favorable toxicological profile than nicotine.[2] They have both contact and ingestion activity to insects.

How long have neonicotinoid insecticides been used in the USA?

Imidacloprid was the first commercially successful neonicotinoid insecticide developed. It was first synthesized by Bayer researchers in Japan in 1985 and was commercially introduced into US markets in 1994. Imidacloprid-based products in the US include *Gaucho* (seed treatment), *Admire*® (soil application), *Provado*® (foliar application), *Merit*® (turf and ornamental use), and *Premise*® (termite control). Other neonicotinoid insecticides, namely thiamethoxam (2001), acetamiprid (2002), clothianidin (2003), thiacloprid (2003) and dinotefuran (2005) have entered the US market more recently.

Are neonicotinoid insecticides toxic to honey bees?

Like many insecticides, imidacloprid, thiamethoxam, clothianidin and dinotefuran are intrinsically toxic to honey bees if they are exposed to a sufficient dose. These compounds belong to the subclass of neonicotinoids known as nitroguanidines. Members of this subclass are essentially equally toxic to honey bees with contact LD_{50} values (dose that kills 50% of the test population) approximately, 0.05 micrograms per bee and acute oral LD_{50} values approximately, 0.005 micrograms per bee.[3]

Acetamiprid and thiacloprid belong to the chemical subclass known as cyanoamidines. These two compounds are relatively non-toxic to honey bees.[4] These two neonicotinoids could be applied to blooming crops as a spray application with minimal risk to honey bee colonies. However, as an additional precaution, both product labels caution against or prohibit direct exposures to foraging bees.

To aid in understanding potential exposure doses, it is useful to convert lethal dose (LD_{50}) values which are expressed in units of micrograms per bee to an equivalent food concentration (LC_{50}) expressed in parts per billion (ppb). This provides a benchmark for comparison to concentrations that might be analytically measured in bee foods (pollen, nectar). To make this conversion, one simply divides by the amount of sucrose solution ingested by a bee in an LD_{50} test (26 mg) and then multiplies by 1 million (to convert the answer from parts per thousand to parts per billion).

$LC_{50} = (y / 26) \cdot 1{,}000{,}000$

Where y is the LD_{50} in micrograms per bee.

Example: The acute oral LD_{50} of 0.005 μg/bee equals a concentration of 192 ppb.

Are honey bees exposed to neonicotinoids?

Honey bees could be exposed to neonicotinoids in two general ways:

(1) to residues deposited on flowers or foliage by foliar sprays; and

(2) to residues taken up systemically by plants and translocated into nectar and pollen in the flowers.

Exposure via the first route should be minimal to non-existent for products toxic to bees because the product labels generally restrict foliar applications to plants that are not in bloom. With respect to exposure to systemic residues, research has shown that imidacloprid enters the xylem (water transport system in the plant) and moves very rapidly upwards into the leaves to provide protection against insect damage. Transport back to other parts of the plant, including the flowers, can only take place via the phloem, and studies show that very little imidacloprid is transported this way. Studies with imidacloprid have reported generally less than 5 parts per billion in the pollen and nectar of treated crops.[5] Bottom-line: honey bees can be exposed to neonicotinoids via translocation, but at levels in the low ppb range.

Will the low levels of neonicotinoids that may occur in pollen and nectar of treated crops kill bees?

No.

As explained above, the median lethal dose of the most potent neonicotinoids is equivalent to a food concentration of 192 ppb. So there is a large margin of safety between the maximum concentration expected in pollen and nectar (5 ppb) and concentrations that could kill bees.[8]

What about toxic effects on bees from long-term exposure?

Long-term (chronic) exposures do NOT represent a significantly greater risk than acute exposures because neonicotinoids are rapidly metabolized and do not bio-accumulate.[6] A published study that claimed chronic toxic effects in honey bees at very low exposure levels of imidacloprid was found to be unsubstantiated when other laboratories attempted to replicate the findings.[7] The chronic, No-Adverse-Effect Concentration (NOAEC) for the nitroguanidine compounds (derived from extensive field and semi-field testing) is in the range of 20 parts per billion.[8]

What about sublethal effects such as disorientation and interference with memory function?

Laboratory and field studies have been conducted on the effects of neonicotinoids (mainly imidacloprid) on the bee nervous system, and in particular on learning, memory and foraging behavior. These effects are referred to as 'sublethal', although it is interesting to note that many of the studies that showed effects actually used concentrations of imidacloprid which could be predicted to cause lethal effects.[9]

In controlled field studies in which bees were trained to use artificial feeders located up to 500 m from the hive, the orientation behavior of bees was unaffected when the concentration was 10 ppb and 20 ppb, slightly affected with no overall damage to the colony when the concentration was 50 or 100 ppb, and severely affected (most bees not returning to the colony) when the concentration was 500 or 1000 ppb.[10] Studies examining feeding behavior have generally reported the threshold for effects (reduced feeding rate, increase in trembling dances, etc.) to be at concentrations ranging from 20 to 100 ppb.[11] Studies examining impairment of memory via the Proboscis Extension Reflex assay have yielded variable results. These studies report thresholds for impairment of learning performance to be in the range of 12 to 100 ppb, essentially the same as for feeding behavior.[12] In studies that were conducted under realistic field conditions and that looked at the performance and fate of entire bee colonies, no adverse effects have been observed at concentrations < 20 ppb.[8]

What about effects on the queen and brood development?

These endpoints have been assessed in simulated and actual field studies of imidacloprid and clothianidin and no adverse effects have been found.

Is the toxicity of neonicotinoid insecticides increased when they are mixed with fungicides?

The toxicity of the nitroguanidine compounds (imidacloprid, thiamethoxam, clothianidin and dinotefuran) which are the ones that are highly toxic to honey bees, is NOT increased when they are mixed with certain fungicides.

There is some interesting research that showed that certain azole fungicides synergize the toxicity of the cyanoamidine subclass of neonicotinoid compounds (acetamiprid and thiacloprid). These compounds are relatively non-toxic to honey bees because the bees have enzymes that break them down quickly to non-toxic byproducts. However, the presence of azole fungicides blocks the activity of these detoxifying enzymes. The result is these compounds become much more toxic (but not more toxic than nitroguanidines) when the bee has also been exposed to the fungicide. This synergistic effect has only been demonstrated to occur under highly artificial laboratory conditions. In field trials in which azole fungicides and cyanoamidine compounds were mixed and applied, there was no evidence of synergistic toxicity to honeybees.[13]

Did imidacloprid cause widespread losses of honey bees in France?

This has been a matter of much controversy. However, we believe it is now clear that the answer to this question is an emphatic “No”.

The imidacloprid-based product *Gaucho* was introduced as a seed treatment for sunflowers in France in 1994. Beginning in 1995, French beekeepers reported mysterious losses of colonies in the late summer and fall and blamed *Gaucho* as the causative agent. The hive depopulations were referred to in the press as, “Mad Bee Disease” and “French Bee Malady”. In 1999, the French government responded to political pressure from the French beekeeping union and suspended the use of imidacloprid in sunflowers. In 2004 - in the wake of an ongoing, very emotional public discussion in France - the suspension was expanded to include use as a seed treatment in corn (and also to include another chemical, fipronil). In the media a risk assessment prepared by a Scientific and Technical Committee (CST) in 2003 is usually mentioned to support the decision. This paper claimed that imidacloprid use as a seed treatment posed a potentially high level of risk to honey bee colonies. However, the validity of this assessment was challenged on the basis of use of questionable laboratory studies, failure to consider results of field studies, and use of inappropriate risk assessment methods.[14] One French study that showed very low concentrations of imidacloprid caused chronic toxicity was repeated at four independent laboratories elsewhere in Europe and all four failed to show a similar effect.[7] The product suspension was also controversial because numerous field studies, including one conducted by the French Government’s Bee Pathology Lab, found no adverse effects.[15] Finally, the general lack of similar problems by beekeepers in other parts of Europe where imidacloprid was also in widespread use as a seed treatment suggested that the cause of the French Bee Malady was something other than imidacloprid. No other European country followed the French lead. Official statements were written by the governments of Germany and the United Kingdom that they were confident that use of imidacloprid as directed would not harm honey bee colonies.

It has now been almost 8 years since imidacloprid use in sunflowers, and 3 years since use in corn was suspended in France, and there has been no major recovery of the general health status of bee colonies in France.[16] This strongly suggests that imidacloprid did not cause the French Bee Malady. Another fact is that there is no difference in the losses beekeepers are suffering between regions of France where *Gaucho* had been used and those where it had never been used.[17]

A multifactorial study of the problems affecting bees in France was carried out from 2002-2005 by the AFSSA (French Food Safety Agency). A final report of the complete study has not yet been released officially but preliminary findings have been reported in the national press.[18] According to an article January 25, 2007 in the major French newspaper "Le Figaro", the conclusion reached by AFSSA is there are multiple factors involved including *Varroa* mite infestations and accompanying pathogens, inappropriate chemical treatments for *Varroa*, poor bee management practices, and other stresses such as weather and climate change, and decline in preferred bee forage (e.g. wildflowers) due to agricultural intensification.[19]

Did imidacloprid cause widespread losses of honey bees in Canada?

No.

Some beekeepers in the Maritime provinces, particularly PEI and NB, reported high over-wintering losses of honey bees in the spring of 2001 and questioned whether imidacloprid might be the cause. Imidacloprid was and is used widely in potatoes in Prince Edward Island and New Brunswick and the hypothesis was put forward that after these fields were rotated to clover in subsequent years, residues of imidacloprid remaining in the soil were being taken up by the clover plants and bees were being exposed by foraging on clover flowers. A study in 2001 found no detectable residues of imidacloprid in the pollen and nectar that bees harvested from clover fields on Prince Edward Island. All hives placed on fields where imidacloprid had been used performed as well or better than those on fields where imidacloprid had never been used. Another study spanning 2002-2003 found many problems affecting the hives of Maritime beekeepers, including high populations of a parasitic mite, diseases, nutritional deficiencies, inappropriate management practices, and poor quality bee genetics. The conclusion drawn from all of these studies was that the problems experienced by beekeepers in Maritime Canada were multifactorial in nature with agricultural pesticide use being a minor factor.[20]

Has any research been performed in North America to evaluate the risks neonicotinoids pose to bees?

While most studies have been conducted in Europe, several studies have been conducted in North America.

Dr. Jim Kemp (University of Prince Edward Island) and Mr. Richard Rogers (Wildwood Labs) conducted investigations in the maritime provinces in Canada from 2001-2004.[20]

Dr. Cynthia Scott-Dupree (University of Guelph, Ontario) and Dr. Marla Spivak (University of Minnesota) conducted a field study in 2000 that evaluated the safety to honey bees of imidacloprid and clothianidin use in test plots of canola grown from treated seeds. Endpoints evaluated included brood measurements, foraging activity, bee mortality, honey production and bee behavior. No adverse effects were found.[21]

Dr. Patty Elzen and colleagues at the USDA Bee Research Lab, Weslaco, TX conducted a field trial of effects of soil-applied imidacloprid on pollinators of cantaloupes. No adverse effects on the honey bee colonies that were used in the study were noted.[22]

Dr. Chris Cutler and Dr. Cynthia Scott-Dupree (University of Guelph) conducted a study of clothianidin used as a canola seed treatment following a protocol approved by the US EPA and conducted in compliance with Good Laboratory Practices (GLP). This study followed the fate of colonies for nearly an entire year and found no adverse effects.[23]

Is exposure to neonicotinoids a potential cause of CCD?

Neonicotinoids are unlikely an important factor because:

1) There is no correlation between incidence of CCD and use of neonicotinoid insecticides. CCD has either just recently appeared or increased in frequency whereas neonicotinoids have been in widespread use in the US for many years;
2) Based on laboratory toxicity tests and field measurements of exposure levels, there is a large margin of safety between concentrations bees are likely to be exposed to and those that cause adverse effects, including sublethal effects such as disorientation and impaired foraging behavior;
3) Controlled field studies have demonstrated over and over again that use of neonicotinoid insecticides per label directions does not harm bee colonies.

Questions, comments? Please contact:

Dr. Alison Chalmers
Ecotoxicology
Bayer CropScience LP
2 TW Alexander Drive
Research Triangle Park, NC 27709
USA
919-549-2491
alison.chalmers@bayercropscience.com

Footnotes:

[1] For more details on mode of action, see Bai et al. (1991), and Nauen et al. (2001)
(*Back to text*)

[2] Tomizawa and Casida (2003)
(*Back to text*)

[3] Over 10 studies of acute contact and acute oral toxicity to honey bees following EPPO test guideline No. 170 have been performed with imidacloprid. The reported oral LD_{50} values range from 0.0037 to >0.070 µg/bee, with all but one study reporting a value $\geq$ 0.005 µg/bee. The reported contact LD_{50} values are less variable, and range from 0.042 to 0.104 µg/bee. Fewer tests have been performed with thiamethoxam and clothianidin; however, the results indicate approximately equivalent toxicity to imidacloprid.
(*Back to text*)

[4] Chemicals are classified by the US EPA as highly toxic, moderately toxic or practically non-toxic to honey bees if the LD_{50} is <2, 2-11, or >11 µg/bee, respectively. According to EPA Office of Pesticide Programs' one-liner database, acetamiprid and thiacloprid have oral LD_{50} values of 14.5 and 17.3 µg/bee, respectively, which puts them in the "practically non-toxic" category.
(*Back to text*)

[5] Residues levels of imidacloprid found in pollen and nectar of crops (e.g., sunflower, oil-seed rape or canola, corn) grown from treated seeds were reviewed for the French Ministry of Agriculture by the Scientific and Technical Committee (CST, 2003 - Halm et al., and Maus et al. 2003). Both papers concluded that 5 ppb represents the maximum expected concentration. A recent paper by Chauzat et al. (2006) reported detections of imidacloprid residues in pollen of between 1.1 and 5.7 µg/Kg.
(*Back to text*)

[6] Suchail et al. (2003) reported the metabolism half-life of imidacloprid in honey bees was 4.5 to 5.0 h, with no detectable residues present 24 h post-dosing.
(*Back to text*)

[7] Suchail et al. (2001) conducted acute and chronic (10 day) oral toxicity studies with imidacloprid and 6 metabolites in honey bees and reported unexpectedly high chronic toxicity. Significant mortality was seen for each of the 7 chemicals tested at dietary concentrations of 0.1, 1 and 10 ppb. There was little difference in chronic toxicity between dose levels or between compounds. The latter finding was very surprising given that 4 of the compounds tested were non-toxic to honey bees in the acute test. The temporal pattern of mortalities observed was largely independent of the compound or dose being tested, suggesting an extraneous factor and not the test compounds might have been responsible for the mortality observed in this study. Schmuck (2004) had four independent laboratories repeat Suchail et al's chronic test with imidacloprid. Three contract labs in Germany and one in the UK again exposed honey bees via the diet for 10 days to spiked sucrose solution containing 0.1, 1 and 10 ppb. In all four labs, no compound-related mortalities were observed at any of these test concentrations. Kirchner (Unpublished report, 2003) conducted an additional test of the chronic toxicity of imidacloprid to honey bees and evaluated the effects of 10 days dietary exposure to 0.1, 1, 10, 20, 50 and 100 ppb. Only the two highest concentrations showed significantly greater mortality than in controls. The no-observed effect concentration was 20 ppb.
(*Back to text – 7.1*) (*Back to text – 7.2*)

[8] An overall no-adverse-effect concentration of 20 ppb for nitroguanidines is based on semi-field and field studies with imidacloprid (reviewed by Maus et al 2003) and clothianidin (Maus and R. Schöning, 2001). Unpublished report reviewed by US EPA, MRID 45422440) that evaluated mortality, behavior and colony development.
(*Back to text – 8.1*) (*Back to text – 8.2*) (*Back to text – 8.3*)

[9] For example, Decourtye et al (2004) reported that imidacloprid impaired medium-term olfactory memory (while not affecting short-term and long-term memory) at an oral dose level of 0.012 *µ*g/bee. This is more than two-fold greater than the oral LD_{50} benchmark value of 0.005 *µ*g/bee, previously discussed. (*Back to text*)

[10] Bortolotti et al. (2003) quantified the proportion of honey bees returning to the colony after visiting a feeder 500 m from the hive. Three concentrations of imidacloprid-spiked sucrose solution were tested: 100, 500 and 1000 ppb. At the two higher levels, the majority of the individuals never returned to the hive. At 100 ppb, the numbers of bees returning to the hive was similar to controls, but the time required for this to occur was longer than in controls. Kirchner (unpublished report, 1999) conducted experiments on the effect of imidacloprid on foraging behavior and communication ability of honey bees that used a feeder 500 m from the hive. Kirchner found no effects on bees exposed to 10 ppb. At 20 ppb, there was a slight increase in the frequency of tremble dances by the returning worker bees, but no impairment of orientation ability as determined by the ability to communicate distance or direction to the food source via waggle dances. At 50 and 100 ppb, precision of information communicated via waggle dances decreased and a transient reduction in the visitation rate at the feeder was noted. (*Back to text*)

[11] Guez et al. (2001) noted altered foraging behavior when honey bees were exposed to 1000 ppb but not 100 ppb imidacloprid in the field. Kirchner (1998, 1999) found a dose-related increase in tremble dances in worker bees returning from feeding on sucrose solutions spiked with 20-100 ppb imidacloprid. Recruitment of new foragers and visitation to the feeder was reduced when the concentration was 50 and 100 ppb, but not at 10 or 20 ppb. (*Back to text*)

[12] Decourtye et al. (2003) reported the threshold effect level for associative learning using the Proboscis Extension Reflex paradigm was chronic exposure (12-13 days of dietary exposure prior to testing) to 12 ppb for honey bees in summer and 48 ppb for bees in winter. There was a poor dose-response relationship for the summer bees, with learning performance of bees exposed to higher concentrations (24, 48 and 96 ppb) being equal or better than for bees at 12 ppb. Kirchner (2003) conducted similar tests of associative learning ability of honey bees chronically exposed to 10 ppb imidacloprid and found no effects at this concentration. Kirchner also reported that imidacloprid fed to honey bees through the rewarding sucrose solution was found to reduce the learning performance at 100 ppb, but not at 50, 20 or 10 ppb. Decourtye et al. (2004) reported that acute exposure to oral doses of 12 ng/bee affected medium-term, but not short-term or long-term olfactory memory. Similar oral doses of 0.12 ng/bee had no affect. Lambin et al. (2001) reported that topical application of a dose of 1.25 ng/bee improved learning performance of honey bees, whereas topical doses of $\geq$2.5 ng/bee had a negative effect on learning performance. (*Back to text*)

[13] For further details, see Iwasa et al. (2004) and Schmuck et al. (2003). (*Back to text*)

[14] An abbreviated version of the CST assessment is contained in Halm et al. (2006). Helen Thompson (former leader of the Bee Research Unit, and current leader of the Ecological Risk Assessment Unit, Central Science Laboratory, UK Department of Environment, Food and Rural Affairs) and Wolfgang Kirchner (Professor, Ruhr-University Bochum, Germany) prepared critical reviews of the CST report which were submitted by Bayer CropScience to the French Ministry of Agriculture. Both reviewers faulted the CST for using safety factors that are designed to protect entire ecosystems rather than individual species. In the case of assessing risk of imidacloprid to honey bees, tests have been performed with the actual species of concern, so the 10 to 100 fold-safety factors used for extrapolation from the species tested in the lab to the species of concern in the field is inappropriate. Kirchner pointed out that if the same methods were applied to assess risk of human consumption of red wine, one would conclude ingestion of more than 5 ml constitutes a high risk. Thompson pointed out that the existing regulatory directive in the EU for pesticides adequately addresses the case of systemic

pesticides by triggering field studies to directly address risk concerns and that there was no need to invent a new approach for these products as the CST did. Both Kirchner and Thompson pointed out that a multitude of field study data was available that contradicted the conclusions of the CST assessment.
(*Back to text*)

[15] Faucon et al. (2005) exposed bee colonies to 0.5 or 5 ppb imidacloprid in sucrose solution during summer for a period simulating the flowering period of sunflower cultivations and monitored the development of these colonies through the following spring. No adverse effects were noted. The development of imidacloprid-treated colonies did not differ from untreated control colonies.
(*Back to text*)

[16] According to a recent press release from the National Assembly of France, Jacques Remiller and 31 other deputies filed a motion on 2 February, 2007 to create a board of inquiry to determine the causes of severe losses of honey bees that continue to plague beekeepers in France. According to the press release, new scientific evidence (the AFFSA multifactor study, not yet published) has cleared *Gaucho* (imidacloprid) and Regent (fipronil) as being responsible for the severe losses of honey bees in France. In the press release, Dominique Bussereau, Minister of Agriculture is quoted as stating, "in spite of the prohibition of the plant health products suspected in the mass casualty of the bees, these most recent mortalities are completely abnormal" (*La Vendée agricole,* September 8, 2006).
(*Back to text*)

[17] Hervé Gaymard, who was Minister of Agriculture in France in 2004 when the decision was made to suspend *Gaucho* and Regent, stated in an interview with VSD (a weekly news magazine) on November 18, 2004, "In the business of the Régent and *Gaucho*, there was a media frenzy which was undoubtedly similar to the regional elections of 2004. But the problem is that scientific studies say today that this mortality of the bees was not due only to the accused pesticides, and there, not a word in any newspaper in the last 20 hours. There are well two weights, two measurements... Why would these new studies be stronger than the others which led me to prohibit the two pesticides? And I can reveal to you that they show the high mortality of the bees is also noted in areas where these products were not used."
(*Back to text*)

[18] In May /June 2006 Paul Faucon, a researcher at AFSSA gave clear statements that French hives suffer from bad and inappropriate *Varroa* control, not from pesticides. In August 2006 Michel Aubert, director of insect pathology at AFSSA clearly stated that his studies could not confirm any connection of imidacloprid to the bee problems.
(*Back to text*)

[19] The article in Le Figaro and a link to the unpublished AFFSA draft multifactor study report (both in French) can be accessed at:
http://www.lefigaro.fr/sciences/20070125.FIG000000023_mortalite_des_abeilles_pas_de_cause_unique_selon_l_afssa.html
(*Back to text*)

[20] For additional details on the initial studies, see Hivelights, 15(5): 24-27, 2002; Bulletin of Insectology 56(1):83–88, 2003. In a follow-up study, Rogers and Kemp (unpublished report to the PEI ADAPT Council, October 15, 2004) conducted a general survey of residues in hive products (honey, pollen, wax) in all three Maritime provinces did not detect imidacloprid in any samples. There was only one sample where a plant protection product (phosmet) was found, and most samples of beeswax contained fluvalinate (a beekeeper applied product for controlling parasitic mites). In New Brunswick, there were low levels of residues of neonicotinoid insecticides found in pollen and nectar that bees harvested from canola grown from treated seeds, but no adverse effects on the bee colonies at these sites were observed.
(*Back to text – 20.1*) (*Back to text – 20.2*)

[21] The summary of the study report (Scott-Dupree C.; Spivak, M.; Bruns, G.; Blenkinsop, C.; & Nelson, S. The Impact of *GAUCHO*® and TI-435 Seed Treated Canola on Honey Bees, *Apis mellifera* L. Bayer Corporation Report No. 110403, EPA MRID No. 45422435) stated:

"SUMMARY

Honey Bee Information

It is evident from the results of the large scale commercial study conducted in southern Ontario and Minnesota that none of the canola seed treatments tested, VITAVAX RS FLOWABLE®, *GAUCHO*® or TI-435, had statistically significant impacts on brood, foraging activity, bee mortality, honey yield or bee behavior.

Residue Analysis Information

Although some samples of pollen and nectar collected from canola seed-treated with TI-435 and *GAUCHO* (imidicloprid + metabolites = olefin and hydroxy) contained residues of these products, all levels detected were substantially below the NOAEC of 20 ppb. The fact that the residue levels were below the NOAEC supports our results that indicate no negative impact on bee behaviour and hive variables (ie. sealed brood, honey yield) for any of the colonies exposed to canola seed-treated with the test products."

(*Back to text*)

[22] Applications to cucurbits may represent the worst-case scenario for exposure of honey bees to systemic insecticides because these crops have relatively high application rates and short intervals between application and flowering. Elzen, et al. (2004) studied this scenario and noted no adverse effects.

(*Back to text*)

[23] A publication from this study is in press in the April 2007 issue of the Journal of Economic Entomology. The abstract is reproduced below.

"ABSTRACT: We conducted a long-term investigation to ascertain effects on honey bee, *Apis mellifera* L., colonies during and after exposure to flowering canola, *Brassica napus* variety Hyola 420, grown from clothianidin-treated seed. Colonies were placed in the middle of 1-ha clothianidin seed-treated or control canola fields for 3 wk during bloom, and thereafter they were moved to a fall apiary. There were four treated and four control fields, and four colonies per field, giving 32 colonies total. Bee mortality, worker longevity, and brood development were regularly assessed in each colony for 130 d from initial exposure to canola. Samples of honey, beeswax, pollen, and nectar were regularly collected for 130 d, and the samples were analyzed for clothianidin residues by using high-performance liquid chromatography with tandem mass spectrometry detection. Overall, no differences in bee mortality, worker longevity, or brood development occurred between control and treatment groups throughout the study. Weight gains of and honey yields from colonies in treated fields were not significantly different from those in control fields. Although clothianidin residues were detected in honey, nectar, and pollen from colonies in clothianidin-treated fields, maximum concentrations detected were 8- to 22-fold below the reported no observable adverse effects concentration. Clothianidin residues were not detected in any beeswax sample. Assessment of overwintered colonies in spring found no differences in those originally exposed to treated or control canola. The results show that honey bee colonies will, in the long-term, be unaffected by exposure to clothianidin seed-treated canola."

(*Back to text*)

Bibliography of Key References

(Bold type indicates article referenced in preceding document)

Bai D., Lummis S.C.R., Leicht, W., Breer, H., and Sattelle, D.B. (1991): Actions of imidacloprid and a related nitromethylene on cholinergic receptors of an identified insect motor neurone. Pestic Sci 33:197-204.

Bitterman, M. E.; Menzel, R.; Fietz, A.; Schäfer, S. (1983): Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). Journal of Comparative Psychology; 97 (2), 107-119.

Bortolotti, L., Montanari, R., Marcelino, J., Medrzycki, P., Maini, S., Porrini, C. (2003): Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees, Bulletin of Insectology 56 (1): 63-68.

Chauzat, M-P, Faucon, J-P, Martel, A-C, Lachaize, J, Cougoule, N and Aubert, M. (2006) J. Econ. Entomol. 99(2) 253 – 262

CST Report:
Comite Scientifique et Technique de l'Etude Multifactorielle des Troubles des Abeilles- Imidaclopride utilisee en enrobage de semences (*Gaucho*®) et troubles des abeilles (Sept 2003).
http://www.agriculture.gouv.fr/spip/IMG/pdf/rapportfin.pdf

Cutler, G.C. and Scott-Dupree, C.D. (2007): Exposure to Clothianidin Seed-Treated Canola Has No Long-Term Impact on Honey Bees. J. Econ. Ent. (in press).

Decourtye, A.; Le Metayer, M.; Pottiau, H.; Tisseur, M.; Odoux, J. F.; Pham-Delegue, M. H. (2001): Impairment of olfactory learning performances in the honey bee after long term ingestion of imidacloprid. Hazards of Pesticides to Bees; 98, 113-117.

Decourtye, A.; Lacassie, E.; Pham-Delegue, M.H. (2003): Learning performances of honeybees (*Apis mellifera* L.) are differenially affected by imidacloprid according to the season. Pesticide Management Science; 59 (3), 269-278.

Decourtye, A., Armengaud, C., Renou, M., Devillers, J., Cluzeau, S., Gauthier, M., Pham-Delegue, M. H. (2004): Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L). Pestic. Biochem. Physiol. 78: 83-92.

Elzen, P. J., Elzen, G. W., and Lester, G. E. (2004): Compatibility of an Organically Based Insect Control Program with Honey Bee (Hymenoptera: Apidae) Pollination in Cantaloupes. J. Econ Entomol 97(5) 1513-1516.

Faucon, J.-P.; Clément, A.; Drajnudel, P.; Mathieu, L.; Ribière, M.; Martel, A.-C.; Zeggane, S.; Chauzat, M.-P.; Aubert, M. F. (2005): Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. Pest Management Science; 61 (2), 111-125.

Guez, D.; Suchail, S.; Maleszka, R.; Gauthier, M.; Belzunces, L.P. (2000):
Sublethal effects of imidacloprid on learning and memory in honeybees. ; 7th Bee Protection Symposium, Avignon: Hazards of pesticide to bees 98 (abstract).

Guez, D., Suchail, S., Gauthier, M., Maleszka, R., and Belzunces, L.P. (2001):
Contrasting effects of Imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). Neurobiology of Learning and Memory; 76, 183-191.

Halm, M.P., Rortais, A., Arnold, G., Tasei, J.N. and Rault, S. (2006): New Risk Assessment Approach for Systemic Insecticides: The Case of Honey Bees and Imidacloprid (*Gaucho*). Environ. Sci. Technol.**2006**, 40, 2448-2454.

Iwasa T., Motoyama,N., Ambrose,J.T., Roe, R.M. (2004): Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Protection 23 (2004) 371–378.

Kirchner, W. H. (1998): The effects of sublethal doses of imidacloprid on the foraging behaviour and orientation ability of honeybees. Unpublished Study Report. University of Konstanz, Germany, 13pp.

Kirchner, W. H. (1999): Mad-bee-disease? Sublethal effects of imidacloprid (*Gaucho*) on the behaviour of honeybees. Apidologie 30: 421-422.

Kirchner, W. H. (2003): The effects of sublethal doses of imidacloprid, dihydroxy-imidacloprid and olefine-imidacloprid on the behaviour of honeybees Unpublished Study Report, Ruhr-Universitat Bochum,

Kirschner, W.H. (2004): Evaluation of the possible effects of imidacloprid used as a seed dressing on honeybees as reported by the Comite Scientifique and Technique de l'Etude Multifactorielle des Troubles des Abeilles. Expert Report, Ruhr-Universitat Bochum (unpublished report).

Lambin, M.; Armengaud, C.; Raymond, S.; Gauthier, M. (2001): Imidacloprid-Induced Facilitation of the Proboscis Extension Reflex Habituation in the Honeybee.
Archives of Insect Biochemistry and Physiology; 48, 129-134.

Maus, C., Curé, G and Schmuck, R. (2003): Safety of imidacloprid seed dressings to honey bees: a comprehensive overview and compilation of the current state of knowledge, Bulletin of Insectology 56 (1): 51-58.

Maus, C. and Schoning, R. (2001): Effects of TI-435 Technical Residues in Pollen on the Development of Small Bee Colonies and on Behavior and Mortality of Honey Bees: Lab Project Number: E319 1833-8: 110059. Unpublished study prepared by Bayer AG. 60 p.

Nauen, R., Ebbinghaus-Kintscher, U., Schmuck, R. (2001): Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). Pest Manag Sci 57:577-586.

Pflüger, W., and Schmuck, R. (1991): Ecotoxicological profile of imidacloprid. Pflanzenschutz-Nachrichten Bayer, 44: 145-148.

Rogers, R.E.L. and Kemp, J.R. (2003): Imidacloprid, potatoes and honeybees in Atlantic Canada: is there a connection? Bulletin of Insectology 56 (1): 83-88.

Schmuck, R. (1999): No causal relationship between *Gaucho* seed dressing in sunflowers and the French bee syndrome. - Pflanzenschutz-Nachrichten Bayer; 52 (3), 267-309.

Schmuck, R. (2004): Effects of a Chronic Dietary Exposure of the Honeybee *Apis mellifera* (Hymenoptera: Apidae) to Imidacloprid. Archives of Environmental Contamination and Toxicology; 47 (4), 471-478.

Schmuck, R., Nauen, R., Ebbinghaus-Kintscher, U. (2003): Effects of imidacloprid and common plant metabolites of imidacloprid in the honeybee: toxicological and biochemical considerations, Bulletin of Insectology 56 (1): 27-34.

Schmuck, R., Schöning, R., Stork, A. and Schramel, O. (2001): Risk posed to honeybees (*Apis mellifera* L, Hymenoptera) by an imidacloprid seed dressing of sunflowers, Pest Manag Sci 57:225-238.

Schmuck, R., Stadler, T., and Schmidt, H-W. (2003): Field relevance of a synergistic effect observed in the laboratory between an EBI fungicide and a chloronicotinyl insecticide in the honeybee (*Apis mellifera* L, Hymenoptera). Pest Manag Sci 59:279–286.

Scott-Dupree C., Spivak, M., Bruns, G., Blenkinsop, C. and Nelson, S. (2001): (The Impact of *GAUCHO*® and TI-435 Seed Treated Canola on Honey Bees, *Apis mellifera* L. Bayer Corporation Report No. 110403, EPA MRID No. 45422435

Suchail S. Guez D. and Belzunces LP., (2000): Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. Environ Tox Chem, 19: 1901-1905.

Suchail, S., Guez, D., Belzunces, L. P. (2001): Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. Environmental Toxicology and Chemistry; 20 (11), 2482-2486.

Suchail, S., Debrauer, L. and Belzunces, L. P. (2003): Metabolism of Imidacloprid in *Apis mellifera*. Pest Manag. Sci.; 60, 291-296

Sur R. and Stork A., (2003): Uptake, translocation and metabolism of imidacloprid in plants. Bulletin of Insectology 56 (1): 1-7.

Thomson, H. (2004): Expert Opinion: "Imidacloprid used as a seed dressing (*Gaucho*) and disorders in bees" Final Report by The Scientific and Technical Committee responsible for the multi-factorial study of disorders in bees (CST) (unpublished report)

Tomizawa, M and Casida, J.E. (2003): Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu. Rev. Entomol. 2003. 48:339–364

EPA/OPP - Label Review Manual Chapter 8: Environmental Hazards - See Section E, Category 4: http://www.epa.gov/oppfead1/labeling/lrm/chap-08.htm