[redacted]

“Risk assessment regarding pollinating bees and arable crops seed-treated with imidacloprid”

Executive Summary

The neonicotinoid insecticide imidacloprid (Trade names: Gaucho® and Chinook®) reveals a good plant systemicity and is used as a seed dressing agent in various agricultural crops. Due to its high intrinsic toxicity to honeybees and strong allegations of some French beekeepers numerous special residue and toxicity assays were conducted to evaluate any potential risk posed to honeybees by the use of this systemic seed dressing.

More than 15 studies with imidacloprid have been carried out concerning uptake, translocation and metabolism in various plant species mainly after foliar, soil or seed treatment. The uptake after soil or seed treatment is about 5% of the applied dose (20% in maize). Less than 1.4% of the adsorbed amount is translocated into reproductive organs of crop plants. In pollen and nectar of oilseed crops (sunflower) residues consisted of the parent compound only. Field residue trials with imidacloprid after seed-dressing of sunflower, maize and oilseed rape revealed no residues higher than 5 ppb imidacloprid in either nectar or pollen for the currently registered European seed dressing rates. In nectar and pollen of succeeding (untreated) crop plants no residues higher than 2 ppb imidacloprid were detected.

As in pest insects, imidacloprid acts as a partial agonist of the nACh receptors in honeybees. In acute toxicity assays following EPPO 170, imidacloprid revealed a high intrinsic toxicity to honeybees with oral LD_{50} values of ≥ 3.7 ng/bee. In these acute laboratory assays, a no-observed effect dose of 1.2 ng as/bee could be determined which translates into a dietary concentration of 46 ppb. Additionlly, a high number of (sub-)chronic feeding studies were conducted in the laboratory and under semi-field and field conditions to determine longer-term safe dietary concentrations of imidacloprid for honeybees. Based on studies which showed reproducible results and based on endpoints which were considered as relevant for honeybee performance in the field, a no-observed-adverse dietary effect concentration (NOAEC) of 20 ppb was concluded.

When the chronic dietary NOAEC of 20 ppb to honeybees is compared with the reported residue data of ≤ 5 ppb, it becomes evident that seed-dressings with imidacloprid will pose only a negligible risk to honey bees. This conclusion is strongly supported by the findings of more than 26 semi-field and field studies which had been conducted under various climatic and soil conditions in all main target crops. No treatment-related adverse effects on honeybees were observed in any of these studies. In particular, symptoms as reported by French beekeepers were never recorded during any of these tunnel or field studies.

From reviewed toxicity data on bee species other than *Apis mellifera*, a higher susceptibility of wild bee species can not be concluded.

Introduction

The neonicotinoid insecticide imidacloprid reveals a highly specific affinity to the insect acetylcholine receptor ([redacted] 1991, [redacted] 1992, [redacted] 1993, [redacted] 1993, [redacted] 2001). Due to its excellent systemic properties, imidacloprid effectively controls foliar pests even when applied as a seed dressing and, therefore, qualifies best for virus vector control. As a seed dressing agent imidacloprid was first launched in the early 1990ies in sugar beets and cereal crops. Starting in 1994, imidacloprid was also marketed for use in sunflower crops in France under the brand name Gaucho®. Due to its excellent pest control performance [redacted] 1991, [redacted] 1992, [redacted]. 1994), the treated sunflower area increased strongly during the consecutive years. In 1996 (one suspicion already was made in 1994) a potentially novel bee malady was reported from Central and Western France which aggravated in the following years. The characteristic symptoms of this bee malady are given in Appendix I (as summarized in [redacted] 1999). Due to the temporal co-incidence of the Gaucho® market launch and the appearance of this bee problem some beekeepers assumed that this bee malady could be linked to imidacloprid or metabolites of this compound.

Introduction (cont.**)**

This allegation initiated extensive research activities in many countries worldwide. The present paper intends to (1) summarize the outcomes of all relevant research activities, (2) evaluate all these findings and (3) come finally to a scienctifically robust conclusion about the risks posed by imidacloprid seed dressings to honeybees.
This review paper is divided into 4 parts. The first paragraph deals with the nature and the quantitity of residues in pollen and nectar of crop plants which were raised from Gaucho®-treated seeds. The second paragraph examines the acute and chronic toxicity of imidacloprid and imidacloprid plant metabolites to honeybees and other pollinating hymenopterans. This is followed by a risk assessment in the third paragraph where all findings of the exposure and toxicity investigations are comparatively evaluated. The final paragraph summarizes all findings of field-based research where the response of bee colonies to seed-treated crops had been followed.

1.0 Nature and Quantity of Residues in Seed-Treated Crop Plants

When foraging on arable crops seed-treated with imidacloprid honeybees may be exposed to residual traces of the parent compound or its metabolites. Existing plant metabolism studies were reviewed to identify the nature of plant residues to which bees are potentially exposed during foraging.

1.1 Uptake and Distribution of Imidacloprid in Seed-Treated Crop Plants

Systemic properties of a molecule are mainly determined by its water solubility, its octanol/water-partition coefficient (log P_{OW}) and its acid constant (pK_a). Following the simulation model of [redacted] 1982 a good membrane penetration and a high xylem mobility can be predicted for imidacloprid (P_{OW} = 0.51). The high pK_a of 14 indicates that imidacloprid remains within the plants in a non-ionized state and, therefore, diffuses freely within the plant transportation system despite varying pH environments (pH value phloem = 8.0 and xylem = 5.0). However, bulk flow in the xylem system is faster by a factor of 50 to 100 than in the phloem tubes ([redacted] 1989). Accordingly, once imidacloprid has entered the leaves it remains trapped by the counter-current principle within the leaf and only minute amounts can be re-distributed to the plant stem. These theoretical considerations fit well with findings of plant metabolism studies [redacted] 2003). Following seed dressing or granular applications of imidacloprid on cotton, eggplant, potato and rice, uptake rates ranged from 1.6 to 4.9% of the applied radioactivity ([redacted]. 1989, [redacted] 1991, [redacted] 1993, [redacted] 1991, [redacted] 1989). After seed treatment of maize an uptake rate of 20% was recorded (Vogeler and Dräger 1989). The ratio of the radioactivity in the reproductive organs (i.e. seeds and fruits) compared to the radioactivity of the whole plant was very low in maize, cotton, eggplant and rice ranging from 0.7 to 1.4% (Tab. 1). The mobility and distribution of imidacloprid within the vascular system and the tissues of plants was further examined in translocation experiments where the as was applied to apple and tomato leaves while the fruits were covered with plastic sheets to prevent their contamination. At harvest, 14 days later, the amount of radioactivity in fruits compared to the activity applied was 0.1% at maximum [redacted] 1992, [redacted] 1989). Based on residue findings and translocation experiments it can be concluded that imidacloprid reveals a good acropetal translocation from the root system to shoots and leaves (excellent xylem mobility) but only a poor basipetal translocation to sinks, i.e. storage organs, roots and fruits (negligible phloem mobility). Consequently, highest residues are expected to occur in the older leaf parts of the plants.

1.1 Uptake and Distribution of Imidacloprid in Seed-Treated Crop Plants (cont.**)**

Conclusion: Crop plants adsorb a maximum of 20% of the applied seed dressing material. Leaves act as a sink of the seed dressing material. Less than 0.25% of the applied seed dressing material enters the reproductive organs.

1.2 Metabolism of Imidacloprid in Seed-Treated Crop Plants

Despite the wide variety of crops and application types having been investigated a rather uniform picture of the metabolic behavior of imidacloprid in plants was found consisting of three principal pathways (Fig. 1-3). In Fig. 1 the ethylene-bridge hydroxylation of the imidazolidine (dihydroimidazole) ring of the as leads to the formation of the monohydroxy-metabolite, which mainly undergoes a subsequent elimination of a water molecule to form the olefine-metabolite. Secondly, as depicted in Fig. 2, a nitro-group reduction takes place to form the nitrosimine compound. In a further step, after loss of the NO-group the guanidine and urea metabolites are formed. Starting with nitrosimine a cyclisation with endogeneous pyruvic acid (created in respiration during glycolysis) to the triazinone metabolite via a supposed but not found aminoguanidine intermediate occurs. However, only minute amounts of this compound were found and only in potato leaves. Thirdly, imidacloprid is oxidized to 6-chloropicolyl alcohol and in turn to 6-chloronicotinic acid. In addition, some alcohol conjugates with carbohydrates are also formed (Fig. 3). In seeds or fruits of seed-treated crop plants, only very low amounts of imidacloprid metabolites were detected. Based on the quantitative aspect the monohydroxy-, olefine-, dihydroxy-, urea- and 6-CNA-metabolites were most commonly found in these part of seed-dressed crop plants.

Conclusion: Plant metabolites of imidacloprid which may enter reproductive organs in relevant amounts include the monohydroxy-, olefine-, dihydroxy-, urea- and 6-CNA-metabolites.

1.3 Nature and Quantity of Imidacloprid Residues in Pollen and Nectar of Seed-Treated Sunflower Plants

The nature and quantity of imidacloprid residues in pollen and nectar of seed –treated crop plants was sprecifically investigated in sunflowers. This sunflower metabolism study was performed in a greenhouse with potted plants [redacted] 1999). The sunflower seeds were dressed with 0.7 mg radiolabelled imidacloprid/seed which is the commercial seed dressing rate in France. During flowering, the nectar was collected after sampling the female florets from the inflorescence by tweezers and extracting the nectar using glass capillaries. Pollen samples were collected in plastic boxes fixed below the inflorescences before flowering. The total radioactive residue (TRR) in pollen and nectar was analysed for chemical composition using ^{14}C-chromatographic techniques, e.g. TLC and HPLC. In total, 3.4 g nectar and and 9.6 g pollen were harvested from the sunflower plants. The TRR was equivalent to 0.0019 mg/kg and 0.0039 mg/kg for nectar and pollen, respectively. From pollen samples, 85.8% of the TRR could be extracted. The TRR in nectar and in the pollen extract consisted completely of imidacloprid. No known or unknown imidacloprid metabolites were identified in these bee-relevant sunflower matrices. The limit of detection in these studies was 0.1 ppb which makes it very unlikely that any relevant metabolite remained undetected.

Conclusion: Based on a greenhouse study with radiolabelled imidacloprid, only the parent compound is expected as residue in nectar and pollen of seed-treated crop plants.

1.4 Imidacloprid Residue Levels in Pollen and Nectar of Seed-Treated Crop Plants

Field-residue trials with non-labelled imidacloprid on sunflower, maize and rape were carried out in various countries to examine the imidacloprid residue levels which honeybees may encounter under realistic field conditions ([redacted] 1999, [redacted] 1999a-c, [redacted] *l.* 1999a-f, [redacted] 2000, [redacted] 2001, [redacted] 2001, [redacted] 2002, [redacted] 2002). Most samples of these studies were not only analysed for imidacloprid but also for the monohydroxy- and the olefine-metabolites, as these metabolites revealed a relevant toxicity to honeybees (see paragraph 2.3). The results of the reviewed field residue trials are compiled in Tab. 2. In all but two samples no quantifiable residues of imidacloprid and the two toxicologically relevant metabolites were recorded in pollen or nectar samples of maize, sunflower and rape crops.

In two pollen samples from spring canola and summer rape, maximum residue levels of 7.8 ppb ([redacted] 2001) and 10 ppb imidacloprid [redacted], personal communication) were detected. However, these residue levels were detected in rape crops seed-treated with 10 g imidacloprid / kg seeds which is 5 times higher than the currently registered European seed dressing rate in this crop. At the currently registered seed dressing rate of 2 g imidacloprid / kg rape seeds, no residues were detected in honey samples from 13 different locations ([redacted], personal communication).

A pollen sample from sunflower crops (0.7 mg as/seed) was reported to contain 15 ppb imidacloprid (Pham-[redacted] 1999). However, this value is considered invalid since the value was not compatible with the analytical result of a second laboratory which analysed the same sample and found residue levels < LOQ (i.e. < 10 ppb). Of the examined imidacloprid metabolites, the monohydroxy-metabolite was detected qualitatively (i.e. below 10 ppb) in 12 of 31 sunflower pollen samples ([redacted] 1999). These detects are, however, difficult to interpret since (1) detects were not confirmed by independent validation analyses (< LOD) and (2) no monohydroxy-metabolite could be detected in the greenhouse study at much lower detection limits (radiolabelled compound). Also honeybees were analysed but revealed no detectable residues except in one study ([redacted] 1999). In this analysis, 2 out of 44 honeybee samples showed a total residue level (i.e. imidacloprid and all metabolites with the 6-CNA moiety) of 5 and 8 ppb, respectively.

Conclusion: At the currently registered European seed dressing rates of Gaucho® honeybees will not encounter imidacloprid residue levels higher than 5 ppb in nectar or pollen of seed-treated crop plants.

1.5 Imidacloprid Residue Levels in Pollen and Nectar of Succeeding Crop Plants

No soil metabolites > 2% were detected in 4 aerobic soil metabolism studies with radiolabelled imidacloprid ([redacted] 1990 a & b; [redacted] 1990 a & b). Therefore, it can be assumed that soil residues left from previous imidacloprid treatments consist predominantely of parent compound. As shown in the sunflower metabolism study, only residues of the parent compound must be anticipated in nectar and pollen of seed-treated crop plants. Accordingly, it is reasonable to assume that nectar and pollen of succeeding crop plants growing in imidacloprid-contaminated soil will also contain, if any, only residues of the parent molecule. Nevertheless, the analytical method employed in the field residue studies allowed to identify also traces of the structurally related and toxicologically relevant monohydroxy- and olefine-metabolites.

Specifically designed succeeding crop studies were conducted on different locations with significantly different soil characteristics, imidacloprid soil residue levels and climate ([redacted]. 1999 a-e, [redacted] 2001, [redacted] 2002). Residue levels of imidacloprid were found in soils of all treated fields. In contrast, no residues of imidacloprid and the imidacloprid metabolites monohydroxy- and olefine- were detected in nectar, pollen or honey from rape, clover or maize planted as succeeding crops (Table 3). In sunflower crops, [redacted] (2001) reported detectable residues in 1 of 4 nectar (1.6 ppb) and in 1 of 14 pollen (1.5 – 2 ppb) samples but it is unclear from the study report whether the positive detects were obtained from seed-treated or untreated crop plants. From a comparative measurement in sunflower seedlings, [redacted] (2001) recorded a 40-fold higher imidacloprid adsorption rate in seed-treated sunflower crops compared to sunflower plants grown as succeeding crops.

Conclusion: Succeeding crop plants do not exhibit residue levels of imidacloprid (including the monohydroxy- and olefine-metabolites) higher than 2 ppb in nectar or pollen.

2. Toxicity of Imidacloprid and Imidacloprid Metabolites to Honeybees

The toxic mechanism of imidacloprid and its insecticidally active metabolites to honeybees have been investigated and reported by [redacted] (2001). In principle, the toxicity of imidacloprid and it insecticidally active metabolites to honeybees is based on an interference of these molecules with the bee nicotinic aceytlcholine receptors resulting in an overstimulation and, consequently, discoordination of the neuronal signal transmission system.

2.1 Acute Toxicity of Imidacloprid to Honeybees

2.1.1 Lethal Effect Thresholds

Six different laboratories from the United Kingdom, the Netherlands and Germany examined the acute oral and contact toxicity of technical or formulated imidacloprid to honeybees (Tab. 4) with the majority of tests performed according to EPPO 170. In these studies, the oral LD_{50} ranged between 3.7 and > 70.3 ng and between 5.3 and 53.0 ng a.s per honeybee for the technical and formulated material, respectively. The lowest no-observed effect dose (NOED) was 1.2 ng/bee (Table 1) with mortalities recorded at doses of ≥ 1.5 ng as/bee. The contact LD_{50} was between 42.9 and 104 and between 42.2 and 245 ng imidacloprid per honeybee for the technical and formulated material, respectively, with a good fit of the resulting dose-response curves. The lowest no-observed effect dose (NOED) was < 2.5 ng/bee (Table 1) with mortalities recorded at doses of ≥ 2.5 ng as/bee.

2.1.1 Lethal Effect Thresholds (cont.)

Similar toxicity data are reported from other apiaries. Contact LD_{50}-values of 44, 30, and 12-24 ng as/bee were reported by [redacted] *l* (1991), [redacted].(1999), and [redacted] (2000), respectively. For the oral toxicity LD_{50}-values of 151 [redacted]. 1999) and approximately 5 ng as/bee were reported ([redacted] 2000).

Whereas the contact LD_{50} values were rather comparable between laboratories (factor of < 3 between lowest and highest value for technical grade material), the oral LD_{50} values showed a considerable variability between laboratories (factor of 19). The contact LD_{50} values are considered more reliable than the oral LD_{50} values since the topical application of a definite volume of the test compound is relatively easy to standardize while the dose applied to an individual bee in the oral toxicity assay is difficult to determine as precisely. Oral dosing is typically done by offering compound-containing sucrose solutions to groups of bees which share this food by trophallaxis. The following factors could influence the doses which are ingested by the individual bees:

(1) Trophallaxis may be reduced if a compound is toxic to bees. Since during the test many bees do not directly feed on feeding tubes a suppressed trophallaxis will have a significant influence on the doses ingested by the individual bees,

(2) A homogeneous solvation of test compounds might be more difficult to obtain in the large volume of aqueous sucrose solution than in the small volume of acetone needed for the contact test. This might result in unequal doses for the replicate cages and cause some variability of mortality figures between replicates as frequently observed in oral toxicity tests with toxic compounds.

(3) The nutritive status of the honeybees at the time of application may be important as well since toxicity is likely correlated with the rate of digestion.

(4) There is an age-related difference in the acceptance of imidacloprid-containing sucrose solutions (antifeedant response) and the age of the tested bees is not well standardized in these routine toxicity tests ([redacted] 1999).

In summary, the larger differences in the oral toxicity values between different research facilities may be mainly attributed to the application technique rather than reflecting real differences in the sensitivity of tested bees. The poor fit of the dose-response curves frequently observed in oral toxicity tests underlines the assumption that factors other than differences in sensitivity/susceptibility can bias the oral LD_{50}-values.

In the oral toxicity tests, imidacloprid was diluted in 20 µL sucrose solution per honeybee. Accordingly, the oral doses can be converted into lethal food concentrations using the formula $a = [b / (20\ \mu L \times 1.3\ mg/\mu L)] \times 1000$ with a = sugar concentration in mg/kg and b = oral doses in µg per bee. At concentrations of 1 mg kg^{-1} diet or higher honeybees rejected in a dose dependent pattern the ingestion of the sucrose solution which may also explain the poor fit in the dose response curve. The food concentrations corresponding with the no-observed effect dose for imidacloprid of 1.2 ng/bee would convert into a food residue concentration of **46 ppb**. Theoretically, lethal concentrations could be calculated as well but these values would be biased by the antifeedant effect which is observed above the NOED.

Conclusion: In acute dietary toxicity tests, no adverse effects to honeybees were observed at sucrose concentrations of 46 ppb or less.

2.1.2 Sublethal Effect Thresholds

In addition to lethality, also sublethal effects of imidacloprid were investigated, e.g. on the learning capacity of the honeybee. The most simple modes of learning are non-associative learning features such as habituation and sensitization. The next higher mode represents the conditioning where an association is made between an event and a response [redacted] 1989, [redacted] 1995).

[redacted]. (2001) examined the effects of a single thoracal application of imidacloprid on habituation of incubator-bred honeybees to a repeated feeding stimulus. They found significant treatment-related alterations on the habituation response at sublethal doses of imidacloprid between 0.1 and 10 ng/bee. However, although response patterns changed significantly in relation to the length of the post-treatment period no dose relation was found for the observed effects (except 1 hr post-treatment in 7-day old bees). The opposing response patterns between 7- and 8-day old honeybes were explained by distinct sub-types of the nAChR which differ in their affinity to imidacloprid and its metabolites and which are expressed to different levels in the two age classes. A kinetic consideration, however, seems to be not in support of this hypothesis since responses in 7-day old honeybees did not differ between 15 minutes and 4 hours after application which would be expected in case that imidacloprid and its metabolites evoke different response patterns. Nevertheless, imidacloprid doses between 0.1 and 10 ng/bee clearly affected the habituation responses in honeybees. The key question concerns the biological significance of this finding. As discussed by the authors bees reared in an incubator differ significantly in their learning performance from conspecifics reared in a full hive colony [redacted], 1997). Also, handling procedures of honeybees alter significantly behavioral features of honeybees. In the study [redacted] (2001), bees were subject to an ice narcotisation before testing. [redacted]. (1980) investigated the effects of carbon dioxide and low temperature narcotisation on the behavior of honeybee workers and found strong impacts of these handling procedures on behavioral performances. Finally, whether or not the reported effects may have a relevant impact on honeybees under realistic exposure conditions can be examined by studies of increased complexity as those performed by e.g. [redacted] (1998) [redacted] (1999) and reported below.
Olfactory conditioning testing of the Pavlov type (conditioned proboscis extension reflex tests) were conducted by [redacted] (1998) and [redacted] (1999) using 12-15 days old *Apis mellifera*. In these tests a conditioned proboscis (bee mouthpart) extension reflex was triggered by stimulating the antennae with a 50% sucrose solution (stimulation of chemoreceptors) while simultaneously an olfactory stimulus (conditioned stimulus) was directed over the antenna via an air-stream (50 ml/s). When bees extended their proboscis they received a droplet of sucrose solution as reward. When repeating this procedure several times then ultimately the proboscis is extended when the olfactory stimulus alone is presented (conditioned reflex), i.e. due to the Pavlov-type conditioning ([redacted]). During the test, bees were placed in glass tubes and fixed by two adhesive tapes. Only the antennae and the mouth parts were freely moveable. The bees were subjected to a 4 h starvation period before testing. Three to 5 conditioning cycles, i.e. olfactory stimulus associated by a reward, were conducted at intervals of 15 to 20 minutes. Then, the olfactory stimulus was presented alone during five occasions at intervals of 15 to 20 minutes and the number of bees responding with a proboscis extension were counted. In the acute test, the compound was applied either with the reward feeding solution (oral exposure) or the honeybees were exposed to filter paper soaked with the test solution (contact exposure) before conditioning. No significant differences in the associate learning performance of honeybees was recorded [redacted] (1998) between the control and the treated bees up to the highest test concentration of 300 ppb for both routes of exposure. After oral exposure to imidacloprid no effect on the conditioned proboscis extension reflex were reported by [redacted] (2000) for a concentration of **50 ppb** while a significant impact was reported at 100 ppb (LOEC).

Conclusion: At the acute dietary NOEC of 46 ppb as found in EPPO 170 toxicity assays also no acute sublethal effects were observed, e.g. on the learning performance of honeybees.

2.2 Chronic Toxicity of Imidacloprid to Honeybees

2.2.1 Lethal Effect Thresholds

Imidacloprid and its insecticidally active metabolites are rapidly metabolized in the honeybee ([redacted] 2001). Accordingly, no striking differences were expected between the acute and the chronic toxicity of this compound to honeybees. However, [redacted]. (2001) reported an unexpectedly high chronic dietary toxicity of imidacloprid to honeybees with an approximative LC_{50} of 0.0001 ppb in 50% sucrose solution. A comparably high dietary toxicity was reported in the same paper for six imidacloprid plant metabolites (monohydroxy-imidacloprid, olefine-imidacloprid, 4,5-dihydroxy-imidacloprid, desnitro-imidacloprid, urea metabolite, 6-CNA) of which some had already lost the toxophor. [redacted] (2001) related the similarity of the observed dietary toxicity between metabolites with and without the toxophor to the chloropyridine structure which all these plant metabolites had in common. However, data from binding and electrophysiological studies ([redacted] 2001) were not in line with this hypothesis. Accordingly, the results presented by [redacted] (2001) concerning the chronic dietary toxicity of imidacloprid and its plant metabolites to honeybees were corroborated by two approaches. Firstly, a survey of all available data was made to examine whether other researchers had found a comparably high chronic dietary toxicity of either the parent or its plant metabolites to honeybees. Secondly, dietary toxicity tests were performed at four different research institutes with the urea metabolite (urea NTN) and the 6-chloro-nicotinic acid (6-CNA). These two metabolites were chosen since the toxophor (= N-nitril-increment) is already cleaved off in these metabolites and [redacted] (2001) had postulated a pharmacological mechanism related to the chloropyridine structure of the molecules. Any chloropyridine related toxicity should be most clearly expressed in these two metabolites since an interference with the primary toxophor can be excluded for these plant metabolites.

The review of chronic dietary toxicity data of imidacloprid to honeybees from various publications revealed reproducible NOLEC values of 10 ppb and higher (Tab. 5). This chronic NOLEC value is only slightly lower than the acute dietary NOLEC value of 46 ppb as concluded above. Such a relation between acute and chronic values is to be anticipated given the rapid metabolism of imidacloprid and its insecticidally active plant metabolites in the honeybee ([redacted] 2001). Accordingly, the very high chronic dietary toxicity of imidacloprid reported by [redacted] (2001) could not be confirmed based on a survey of literature information from published and unpublished sources.

In addition to the literature search, no-choice chronic dietary tests were conducted with the urea NTN and the 6-CNA metabolite at four different independent laboratories to test the hypotheses of [redacted]. (2001) of a secondary toxicity mechanism of imidacloprid and its metabolites which is related to the chloropyridine part of the molecule (Tab. 6). Tests starting with older workerbees (22–45 days) do not appear to give reliable results as shown by the high mortality in the controls (30-44%). In the tests with older honeybees mortality did not follow a dose-response relation and fluctuated randomly between replicates (controls: 10-60%) and dose groups (34-77%). During the test, older bees were occasionally classified as being knocked down or stumbling but in these tests the same number of affected specimens were recorded in the control as in the treatment groups. It appears that in tests with older bees it may be very difficult to distinguish between natural mortality at the end of the life cycle of the honeybee and compound-related lethal effects. In contrast, younger bees remained vital over the entire test period and tests with these bees ended in more reliable results as revealed by reasonably low mortality rates in the controls ($\leq$ 10%). No statistically increased mortality rates relative to the control were noted for young bees, except of the laboratory Germany II (Tab. 6).

2.2.1 Lethal Effect Thresholds (cont.)

A more detailed analysis of this test ([redacted] 2000 b and 2000c) revealed that it was not conducted according to standard experimental practices (e.g. no randomization procedure) and the recorded sucrose ingestion rates of bees indicated a strong starvation stress. Honeybees in these tests showed an overall lower sucrose intake rate than in other test runs (c. 5 g total consumption in the controls vs. 8 g in other test runs) with a strong treatment-related antifeeding response (consumption rates of 19-33% relative to the control). In the test with the urea metabolite a significant variability in the mortality figures was observed between the three replicates of the highest treatment group (nominal dose = 3.57 ng as/bee) and no dose relation was found. In this treatment group, mortalities of 90, 80 and 20% were recorded for actually ingested doses of 2.3, 4.1 and 4.3 ng as/bee, respectively. In these tests, honeybees and/or test boxes were not randomly assigned to treatments as required for good experimental practice. If the recorded mortality rates are arranged in the order of the test box loading, the following picture is obtained:

Loading Sequence	Test Variant	Mortality [%]
1	Control	10
2	0.1 ppb urea-metabolite	37
3	1.0 ppb urea-metabolite	3
4	10.0 ppb urea-metabolite	63
5	0.1 ppb 6-CNA-metabolite	67
6	1.0 ppb 6-CNA-metabolite	77
7	10.0 ppb 6-CNA-metabolite	97

This obvious correlation between loading sequence and mortality rates indicates a sampling artefact (the bees sampled latest were the least vital) rather than a treatment-related effect. After amending these experimental deficiencies, a repeat test at the same laboratory [redacted] 2000) resulted in no mortality and data were consistent with those of the other three independent research institutes.
Several factors may have caused false positive results in the study of [redacted]. (2001) and during the first test run of laboratory Germany II ([redacted]y 2000b and 2000c). These include

(1) *Age of tested bees*: As shown by all repeat tests, foraging honeybees sampled from the flight board do not qualify for chronic dietary toxicity tests due to their limited life span. A batch of honeybees randomly sampled from the flight board has an average life span of only 10 days [redacted]r, pers. communication). Ten days was the test period of the feeding test conducted by [redacted]. (2001).

(2) *Randomization procedure for allocating bees to treatment groups*: As shown for various experiments, non-randomization can strongly bias the test results. This could be demonstrated in the first test run of the laboratory Germany II where mortality rates were strongly correlated with the sequence of the test box loading.

(3) *Sucrose ingestion rates*: In the first test run of the laboratory Germany II, the reported sucrose ingestion rate in the control groups were lower than in all other laboratories. Treatment groups had an even greater reduction in ingestion rates (consumption rates were 19-33% relative to the control). Sucrose ingestion rates as reported by [redacted] (2001) are also at least 50% lower than rates obtained by other researchers under comparable rearing conditions (Tab. 7).

2.2.1 Lethal Effect Thresholds (cont.)

Certainly, it cannot be finally concluded from this analysis which factor(s) might have influenced the results reported by [redacted] (2001). However, the data reported by [redacted]. (2001) have at least to be considered with care since (1) they are not consistent with unpublished and published data generated by other researchers, (2) they could not be reproduced by four different independent research facilities which all had a minimum testing experience of three years (Germany II had only 1 year testing experience), (3) the sucrose ingestion rates reported by [redacted] (2001) indicate some starvation stress of these honeybees and (4) the experimental methodology was not fully described in the paper of [redacted]. (2001). No information on the age of bees and the randomization procedure applied in this test are included from the paper.

Conclusion: Under laboratory conditions, no increased mortality rates of honeybees after chronic dietary exposure to imidacloprid is anticipated for concentrations of 24 ppb and lower. A chronic risk posed by plant metabolites of imidacloprid to honeybees as reported by [redacted]. (2001) could not be confirmed (see also paragraph 2.3). Under semi-field and field conditions, for imidacloprid a chronic dietary NOLEC of 20 ppb was found.

2.2.2 Sublethal Effect Thresholds

Under laboratory conditions [redacted]e (1998) reported from proboscis extension reflex (PER) tests NOEC values of 200 and < 4 ppb, respectively, after a repeated contact and a continous 10-day dietary exposure of honeybees to imidacloprid. However, a more rigid review of the oral exposure test raised some concerns over the validity of these study results since higher doses did not cause a poorer performance of treated bees (no dose-response relationship) and a subsequently conducted analysis of the raw data showed that even during the conditioning phases (= reward feeding) bees of all three treatment groups responded to the direct feeding stimulus with a significantly lower frequency than control bees [redacted]1999). If, however, treated bees already had a lower motivation to extend the proboscis prior to testing, then it is not possible to conclude anything from the response pattern to the conditioned stimulus during the test. Kirchner (2000) re-evaluated the study of [redacted] (1998) on the impact of a chronic dietary exposure to imidacloprid on the learning performance of foraging honeybees. In his tests, Kirchner could not find an impact of imidacloprid on the learning performance of honeybees at a test concentration of **10 ppb** (testing limited to this concentration). Also [redacted] (2003) repeated his PER test and found NOEC values between 6 and 48 ppb depending on the physiological state of the tested bees (summer and winter bees, respectively). However, it has to be mentioned that the 6 ppb NOEC value is based on an one-point measurement recorded immediately after conditioning and lasting less than 20 minutes. It is considered highly unlikely that such a very transient short-term effect will have a biological significant effect under field conditions.

From a cage study, [redacted] (2000) reported treatment-related effects on foraging and feeding behavior at residue levels of as low as 1.6 ppb. This finding, however, is in contradiction to reports from other researchers ([redacted]1998, [redacted]l. 1998, [redacted] 1999, [redacted] 2000, [redacted]. 2003) and could not be verified in chronic dietary studies under semi-field or field conditions ([redacted] 1999d and 1999e, [redacted] 2003). In the studies of [redacted]g (1999d and 1999e), small bee colonies which consisted of about 500 workerbees of different ages and one sister-queen were fed exclusively with either nectar or pollen fortified with technical imidacloprid (groups with fortified nectar received untreated pollen and vice versa). The concentrations in the fortified matrix was verified by analysis before use. The development of the small bee colonies which were confined with 50 m² gauze tunnels on oat-cropped plots, was followed over 39 days. Regular records were made on activity pattern at the honey and pollen feeders, nectar and pollen consumption, wax production (comb increase), production of food stores, hive weight increase, egg laying performance of the bee queen, the breeding success of the colony (nursing activity) and the development of the colony strength. Both long-term studies showed that imidacloprid residues up to at least **20 ppb** (highest test concentration) in either nectar or pollen do not adversely affect any of the testing endpoints. [redacted]. (2003) fed colonies with syrup containing imidacloprid at various concentrations over a full year. Groups of eight hives each were fed syrup alone or imidacloprid at 0.5 or 5 ppb; a fourth, negative control group was unfed. The colonies were fed on 13 occasions (July-August, 3x/week, 1 L/hive) and their summer development and winter development followed. Assessments included mortality, colony weight, capped brood area and incidence of diseases. Population development and capped brood area showed a similar development in all colonies with no statistical differences between the colonies even at the higher dose of **5 ppb** (highest test concentration). Other parameters (e.g. mortality colony weight, diseases) also did not show any significant differences between the treatments.

2.2.2 Sublethal Effect Thresholds (cont.)

In a field feeding experiment [redacted] (1999) investigated potential effects of different residue levels of imidacloprid in nectar on foraging intensity, orientation and longevity of honeybees. He found no indications that imidacopрrid at concentration of < 20 ppb reduced the foraging activity on a feeding bottle which was positioned in a distance of 500 m from the bee hive. He also found that the precision of information transfer regarding direction and distance of the food source was not different between control bees and those bees fed with a **10 ppb** imidacloprid sucrose solution for up to 10 days. Furthermore Kirchner stated in his report that no delayed effect could be found for any of the considered endpoints and any effect observed at higher residue concentrations (50-100 ppb) was fully reversible within 24 hr. In particular, up to the highest tested residue levels of 100 ppb no increased mortality was observed for these bees which were marked and observed individually over up to 10 consecutive days.

Conclusion: From the results of the reviewed studies, it is concluded that under field conditions no adverse effects are expected from a dietary exposure to imidacloprid at levels of 20 ppb or less. The definition of this field-relevant NOAEC is based on the following findings:

- No chronic mortality occurred at concentrations of ≤ 20 ppb (reported lower NOEC values from laboratory trials were found to be not consistent with findings from other researchers and are not supported by findings from higher Tier studies).
- No antifeedant effects occurred at concentrations of ≤ 20 ppb (reported lower NOEC values were not supported by a weight-of-evidence approach)
- Short-term behavioral effects reported in one study for concentrations < 20 ppb were very transitory (< 1 hr, Decourtye et al., 2003)
- Hive development was not adversely affected at concentrations of ≤ 20 ppb over 39 days
- No loss of foraging bees was observed at concentrations of ≤ 100 ppb

2.3 Toxicity of Imidacloprid Plant Metabolites

2.3.1 Acute Toxicity of Imidacloprid Plant Metabolites

All imidacloprid plant metabolites which were recorded in reproductive organs of crop plants during various plant metabolism studies (see review of [redacted] 2003) were examined for their acute toxicity to honeybees. All metabolite toxicity tests were conducted in the same research laboratory ([redacted] 1999b-g, [redacted] 2000). The parent compound was tested in this facility in parallel to faciliate a comparative assessment of the metabolites. The reported approximated LD_{50} value for the parent compound was 40.9 ng/bee [redacted]). As shown in summary Table 8 four out of the seven identified plant metabolites revealed some effects to honeybees under laboratory conditions whereas the guanidine-, the 6-CNA- and the 6-CPA-metabolite proved to be non-toxic to honeybees. The 4,5-dihydroxy-metabolite caused a very strong antifeedant response at doses higher than 49 ng/bees which did not allow to determine a LD_{50} value. However, since (1) no lethal or sublethal effect was observed for this metabolite at the highest ingested dose of 49 ng/bees, (2) this metabolite could not be detected in nectar and honey during the sunflower metabolism study of [redacted] (1999) and (3) the strong antifeedant response would act as a protective measure in case of residues this metabolite is considered as not relevant to honeybees. Also the nitrosimine-metabolite is considered as not relevant since it has only been detected in trace amounts in plant reproductive organs but was not detected in nectar and honey during the sunflower metabolism study of [redacted] (1999). Accordingly, of the 7 imidacloprid plant metabolites only the olefine- and the monohydroxy-metabolites are considered as relevant for a risk evaluation to honeybees. These metabolites were considered in the majority of (semi-)field studies with residual analyses of pollen, nectar, honey or honeybees.

Conclusion: Of the 7 imidacloprid plant metabolites only the olefine- and the monohydroxy-metabolites are considered relevant for evaluating the risk to honeybees from a crop seed treatment with imidacloprid.

2.3.2 Subacute Toxicity of Imidacloprid Plant Metabolites

The data of [redacted] (2001) on the chronic toxicity of imidacloprid plant metabolites are considered invalid (see paragraph 2.1.1) and are, therefore, not further discussed in this section.
[redacted] (2000) investigated the sublethal effects of imidacloprid and two of its metabolites, olefine-imidacloprid and dihydroxy-imidacloprid, on the behavior of honeybees in laboratory as well as in field experiments. In the field, sucrose solutions containing olefine-imidacloprid were fed to honeybee foragers and possible effects on foraging activity and communication behavior were analyzed. The behavioral effects of olefine-imidacloprid were found to resemble those of the parent compound. However, the effects were much less pronounced. The only effect, which was significant in the range of concentrations tested, was an increase in the frequency of tremble dances at 100 ppb. No significant disorientation could be found in the dances of bees fed with the olefine-metabolite and no significant effect was found on the foraging activity up to 100 ppb. The effects of imidacloprid, the olefine-metabolite and the dihydroxy-metabolite on learning and memory of honeybees were studied using the proboscis extension reflex paradigm. Imidacloprid fed to honeybees through the rewarding sucrose solution was found to reduce the learning performance at 100ppb, but not at 50ppb, 20 ppb or 10 ppb. Both of the metabolites, olefine- and dihydroxy-, did not significantly affect the learning performance at 100ppb. However, with the olefine-metabolite effects were found at 500ppb, with the dihydroxy-metabolite at 2 ppm.

Conclusion: The imidacloprid plant metabolites olefine- and dihydroxy- revealed a significantly lower subacute toxicity than the parent compound. A no-adverse-effect concentration of ≥ 50 ppb can be concluded for the olefine-metabolite. No significant subacute toxicity could be found for the dihydroxy-metabolite

2.4 Toxicity of Imidacloprid to Other Pollinating Hymenopterans

Based on NOED values from acute toxicity tests [redacted] (1999a-d) found a somewhat lower species susceptibility to imidacloprid for bumblebees compared to honeybees (see Tab. 4 and 9). The observed difference in sensitivity can, however, at least partly be explained by weight differences between honey bees and bumblebees. Average body masses of 100 and 150 mg are reported for worker honeybees and bumblebees, respectively ([redacted] 1993, [redacted]. 1999, [redacted]. 2000, [redacted] 2002). When this average body weights are accounted in the calculation, the relative NOED values for oral/contact exposure are 44/< 420 and 67/347 µg as per g body mass for honey bees and bumblebees, respectively, indicating no significant difference in species susceptibility.

[redacted]. (1994) examined the relative susceptibility of 4 bees species (*Apis mellifera* L. avg weight 126 mg, *Megachile rotundata* F. avg weight 31 mg, *Nomia melanderi* Cockerell avg weight 87 mg, *Bombus occidentalis* Greene avg weight 168 mg) to foliar residues of imidacloprid (contact toxicity). Some 40 m² alfalfa plots were sprayed with a FS 240 formulation and a rate of 0.168 kg as/ha suspended in a water volume of 234 litre per hectare. In the subsequent residual tests, bees were exposed in wire cages to field-weathered foliar residues on 500 cm² chopped leaf samples taken from the upper 15 cm portion of treated plants (4 replicates each). Groups of 50 (*A. mellifera*), 20 (*Megachile rotundata*), 20 (*Nomia melanderi*) and 12 (*Bombus occidentalis*) individuals were exposed to the foliar residues for 24 hours.
Recorded mortalities were as follows:

Time from spray to harvest	Mortality after 24 hours [%]			
[hr]	*Apis mellifera*	*Nomia melanderi*	*Megachile rotundata*	*Bombus occidentalis*
2	14	28	66	56
8	19	3	71	24

In this study, no clear correlation was found between average body weight and susceptibility. From these data it appears that leafcutting and bumble bees are more susceptible to foliar residues than alkali and honeybees. However, these differences may be attributed to the species-specific activity pattern and, hence, to the received dose rather than to intrinsic susceptibility as evidenced by standardized laboratory toxicity assays where bumblebees revealed no higher susceptibility to imidacloprid than honeybees ([redacted] (1999a-d). In addition, at application rates of 112 g as/ha or less as proposed in EU countries mortality rates did not differ significantly between honeybees, alkali and leafcutter bees ([redacted] 1997) and were all below 50% after foliar residues were field-weathered for 2 and 8 hr, respectively.

Conclusion: Based on reviewed data it can not be concluded that a Gaucho® seed treatment poses a higher risk to wild than to domestic bees.

3. HQ and TER-Based Evaluation of Risks Posed by Imidacloprid Seed Dressings to Honeybees

If a hazard quotient approach according to the EU directive 91/414 (CEU 1991) is followed using the proposed per hectare rates of imidacloprid with LD_{50} values from laboratory assays (Tab. 4), a high hazard to honeybees has to be concluded (HQ >> 50).
In a refined risk assessment, residue concentrations in nectar and pollen of seed-treated crop plants can be compared with lethal, sublethal and no-observed-effect concentrations.

The following TER values can be derived when using dietary NOEC values for various endpoints:

Endpoint	NOEC (ppb)	Residue level (ppb) in nectar and/or pollen	TER value
Acute lethal effects (see paragraph 2.1.1)	46	< 5	> 9.2
Acute behavioral impacts (see paragraph 2.1.2)	50	< 5	> 10
Chronic lethal effects (see paragraph 2.1.2)	24	< 5	> 4.8
Chronic behavioral impacts, including hive development (see paragraph 2.1.2)	20	< 5	> 4.0

These TER values support the conclusion that imidacloprid seed treatments at the European use rates do not pose an unacceptable risk to honeybees. This conclusion of safety to honeybees of imidacloprid crop seed treatments is finally demonstrated by the results of numerous semi-field and field studies as reported in paragraph 4.

4. Results of Tunnel and Field Studies

4.1 Sunflower Crops

In response to the allegations of the bee-keepers in Western and Central France, 2 tunnel and 2 field studies on seed-treated sunflower crops were conducted in 1995 and 1997 ([redacted] 1999) to examine potential effects of Gaucho® on honeybees under field-relevant exposure conditions. The tunnel tests were performed according to the official French testing guideline CEB 129 (adapted for a seed dressing product) with dressing rates between 0.35 and 1.05 mg as/plant (recommended dressing rate for sunflowers is 0.7 mg as/plant). The field studies were conducted on 1.5 to 8 ha large sunflower plots with 4-6 bee-hives per plot (dressing rates 0.7 mg as/seed = 49 g as/ha). In the field studies, the minimum distance between the control and the treatment fields was 2.5 km to exclude an exchange of honeybees and food between the control and the treated plots. Both, the tunnel and field tests were biased towards worst case exposure conditions by late sowing of a fast growing sunflower variety and a high planting density. Both, the tunnel and the field tests revealed no indications that honeybees reduced their foraging activity on Gaucho® treated sunflower plants. No treatment-related increases of mortality or behaviorally affected bees were recorded in any of the four studies.

4.1 Sunflower Crops (cont.)

In the field studies, no enhanced losses of foraging honeybees were noted which would indicate a potential impact on the navigation capacity of the exposed honeybees. The hive weight development which directly reflects the amount of sampled nectar was not adversely affected by the seed treatment. Likewise, fertilisation of sunflowers was good in both years on both sites.

Comparable findings are reported from the 1998 field research program of the French Ministry of Agriculture ([redacted] 1999). Four field studies were conducted on 16-51 ha large sunflower fields in the French departments [redacted]. In all four studies bee-hives on treated fields achieved the same honey yield as bee-hives on control fields. The same held true for the vitality of the bee-hives. The losses of foraging bees from the bee-hives on the treated plots were not higher than those experienced in the controls. The principal investigators of all four departments came to the same conclusion, i.e. there were no indications for a massive depopulation of bee-hives exposed to blooming sunflowers which had been seed-treated with Gaucho®. At two test sites ([redacted] bumble bees and honey bees were marked while feeding on sunflower heads and observed during the return flight. No impaired navigation performance was noted.

A further field study in sunflower crops (0.7 mg as/seed = 49 g as/ha) was conducted in 1998 in Germany ([redacted] 1998). At full blossom, 75 days after drilling, 4 bee hives each were moved to 1.25 ha plots (control and treatment site) and monitored during the following 14 days. No increased mortality or reduced foraging intensity was recorded at the treatment site compared with the control site. At both sites, sunflower pollen was intensively collected by the honeybees. There was no increase of hive weights during the observation period indicating a low nectar flow which was traced back to insufficient precipitations before blooming. Some 100 honeybees were sampled while foarging on sunflower inflorescences. The honeybulbs were dissected and analysed for residues of imidacloprid, the monohydroxy- and the olefine-metabolites. No residues were detected in these samples. Accordingly, during bloom no residues are apparently present in the sunflower inflorescences and, therefore, no adverse effects are to be expected.

A field study on Gaucho®-treated sunflowers was also performed by the Federal Educational and Experimental Institute for Agriculture, Viticulture and Horticulture [redacted] ([redacted] 2000). Shortly before flowering there were large precipitations which strongly favoured the nectar production of the sunflower plants. The distance between the control (about 5 ha) and the treated field (about 4 ha) was more than 10 km. Nine commercially managed bee hives were placed on each field partially equipped with either pollen traps (2 hives) or bee scan counters (3 hives). No anomalies were observed on either honeybees or simultaneously foraging bumblebees on the treated field. No differences were recorded for the return frequency of honeybees either. Numerous aphid colonies on the treated sunflowers demonstrated that Gaucho® is no longer active during flowering. The honey yield on both fields corresponded well with the foraging activity and consisted to a large extent of sunflower honey. An analytical evaluation of pollen, nectar and honey samples revealed no quantifiable residues of imidacloprid during the flowering period.

[redacted] (1999) performed a field study with Gaucho® seed-treated sunflowers (0.7 mg as/seed = 49 g as/ha) in 1999 in Hungary. Fifteen beehives each were moved to the treatment (45 ha) and to the control site (35 ha). When compared with the control field no reduced foraging or pollen collection intensity was recorded for the treatment field. Foraging bees in the treated field displayed a normal behavior and so did honeybees around their hives. Except for 2 days at the control hives, bee mortality was always in the normal range of < 100 dead bees per day and colony. The size of the brood area increased in the colonies at the treated field but decreased in the colonies at the control site. The honey production of the bees was generally poor in 1999 in Hungary.

4.1 Sunflower Crops (cont.)

Under the conditions of the experiment the weight gain of bee hives at the treatment site was lower than at the control site. The author related this difference primarily to a higher energy demand of the bee colonies at the treated field which produced substantially more brood compared to control colonies. In summary, [redacted] (1999) concluded that a Gaucho® seed treatment of sunflowers had no adverse effect on the forager bees, the queens or the brood.

In Argentinia, [redacted] (2000a) examined the effects of a Gaucho® seed treatment in sunflower. Due to a different target pest spectrum, the seed dressing rate in Argentinia is 0.25 mg as/seed compared to 0.7 mg as/seed in Europe. The seed dressing favoured the health and, subsequently, the attractivity of sunflowers to honeybees. Accordingly, bee foraging activity was higher in the treated than in the untreated crop. Mortality measured in front of the bee hives was statistically not significantly different between sites. There were also no significant differences in the number of bees returning to hives with pollen. By the end of the exposure period increases were recorded at both sites for the average hive weight, the quantity of honey and nectar in the top supers and the quantity of pollen and brood in bottom supers. Increases were higher at the treated site. No residues of imidacloprid or the monohydroxy- and olefine-metabolites were found in samples of either pollen or honey samples.

The author extended the hive monitoring through the post-hibernation period to highlight potential long-term effects of honeybees exposure to Gaucho® seed-treated sunflowers ([redacted] 2000b). The comparison between the hives from the treated and the control plots revealed that the hives from the treated field could maintain stronger populations through the hibernation period and, consequently, had a better start into the next season as evidenced by a better brood index.

Conclusion: In total, 12 tent/field studies were conducted to investigate potential adverse effects of a Gaucho® seed treatment of sunflower crops to honeybees. Adverse effects were not recorded in any of these studies.

4.2 Rape and Phacelia Crops

Honeybee nuclei were used for sampling nectar and pollen from the rape crops (10.5 g imidacloprid and 2 g beta-Cyfluthrin per kg rape seeds) in all residue studies of [redacted] (1999a-c) and [redacted] (1999e and 1999f). During all sampling days honeybees never displayed treatment-related behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or an increased mortality.

The risk posed by a seed treatment with Gaucho® to honeybees was further examined by Wallner (2001) in tent and field tests using seed-treated *Phacelia tanacetifolia* as honey forage crop. In the tent, the bees (2 colonies with 5 combs) on the treated area (120 m², 0.005 g as/m²) showed no symptoms of intoxication or disorientation. Flight activity (10 observations: 5 min./5 days), honey crop, and the daily mortality (7 days) was not different from control colonies. Residue analysis of the honey bulb contents (40 bees pooled) showed traces of imidacloprid in the nectar of *Phacelia* plants and in the bee bread (< LOQ; LOQ = 10 ppb). In the field studies, the attractiveness of seed treated *Phacelia* to honeybees was not different from untreated *Phacelia* plants. A negative effect on honeybees could not be observed.

4.2 Rape and Phacelia Crops (*cont.*)

[redacted] (1999) investigated the impact of Gaucho® seed-treated canola (8 g as/kg) in a tunnel study with 63 m² study plots. In his study, [redacted] (1999) followed the larval development within the colonies from a series of eggs that were monitored until the cells were sealed. In addition, cohorts of 100 worker bees were individually marked with paint and their survival recorded every seven days. Foraging intensity was not different between study plots. Worker bee survival was similar in the control and the treatment plot. Dead worker bees were not observed in front of the colony at levels that would indicate that bee poisoning had occurred. No difference in brood survival was recorded between the control and the treatment plot.

A combined large-scale tunnel/field study with imidacloprid seed-treated summer rape was conducted by the Federal German Agency for Agriculture and Forestry in Braunschweig ([redacted] 1999). The colza seed was dressed with 10.5 g/kg imidacloprid and 2 g/kg ß-cyfluthrin (control seed untreated) and drilled with 6 kg/ha on a 1,250 m² plot on April 19, 1999. Rape plants started flowering on June 9, 1999. On June 14, 1999 (=51 days after drilling), bee hives with 5 combs were placed within open tunnel constructions which were placed on 60 m² of either control or treatment plots. During the next 3 days honeybees were allowed to move freely between control and treatment plot and get accustomed to the study site. On June 17, 1999, the bee hives were then confined to the respective plot (control or treated) by covering the tunnel constructions with fine gauze. On 5 occasions during the study, a total of 2,500 foraging honeybees were collected from each hive for residue analysis. In spite of this sampling, both bee hives (control and treated) developed such strong colonies that they had to be transferred in larger hives with 11 combs. Pollen sampling activity was very high with up to 80% of the returning bees which demonstrates the strong breeding activity of both hives. The home apiary was only 100 m apart from the study field and these bees had free access to both, the treatment and the control part outside the tunnels. Counts of foraging activity revealed that honeybees did not discriminate between the control and the treated part of the field. No behavioral abnormalities were observed. Residue analyses of the honey bulb contents showed no quantifiable residues of imidacloprid and of the monohydroxy- and olefine-metabolites. Pollen samples contained imidacloprid residues up to 10 ppb.

Two field studies were conducted in southern Ontario, Canada and Minnesota, USA by expert scientists of the local universities to determine whether canola seed treated with 10 g imidacloprid per kg rape seed, had any effect on the honey producing ability, or on foraging and hive behavior of honeybees ([redacted] 2000). At each testing site a 1 ha planting of spring canola, *Brassica napus*, was established for the control and the treatment plot. When 20% of the canola plants were flowering, 4 two-super colonies of honeybees containing sister queens of approximately the same age were placed at each of the test sites. Prior to placement at each site, colonies were equalized for strength, food stores, sealed brood and adult bees (covering at least 10 frames). To determine whether changes in colony strength occurred over the course of the experiment, the total amount of sealed brood and frames of adult bees were again estimated prior to colony removal at the end of the canola bloom period. Over the course of the experiment all hives were weighed weekly. The number of dead bees found on white sheets surrounding each colony was recorded and removed every other day. To determine honey bee foraging activity, 6 x 1 m² collection plots were defined in each of the test fields. Honey bees were also monitored for abnormal behavior (aggressiveness, convulsiveness, or other erratic behavior) while foraging in the field and as they returned to the hive. For each hive, behavior of foragers (returning to the hive) was monitored and recorded every other day for 2 minutes intervals. Although colony strength varied between the US and the Canada sites, the results of both studies clearly showed that there was no significant difference in the amount of sealed honey bee brood in colonies exposed to the canola treatment with imidacloprid. There were also no significant differences in the nectar and pollen foraging activity of these colonies.

4.2 Rape and Phacelia Crops (*cont.*)

The results indicated that bee mortality was not significantly increased or honey yields decreased of colonies exposed to canola treated with imidacloprid by the seed treatment. No aggressive, convulsive, erratic or any other kind of abnormal bee behavior was observed at any of the test sites in southern Ontario and Minnesota throughout the entire duration of the study.

A field study winter rape seed-treated with 10.5 g imidacloprid and 2 g Beta-Cyfluthrin per kg rape seed was conducted in Lower Saxony (Germany) to examine the effects of this seed treatment to honeybee colonies. The size of the treated and the control plot was approximately 2.5 ha and six beehives were placed next to each plot. No adverse effects of the treatment were noted on the foraging activities of the bees, the colony weight and development or mortality. Also, no behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) were observed on the honey bees collecting rape nectar and pollen on the test substance field. The development of bee brood was not affected by the test substance and was comparable between hives placed at the test substance field and at the control field. No residues of imidacloprid or the monohydroxy- or olefine-metabolites were detected in pollen or honey. Only in nectar samples taken from the hive combs residues of imidacloprid (< LOQ) were detected.

Conclusion: In total, 12 tent/field studies were conducted to investigate potential adverse effects of a Gaucho® seed treatment of rape crops to honeybees. Although the seed dressing rate in all studies was 5 times higher than the typical use rate in Europe, no adverse effects were recorded in any of these studies.

4.3 Maize Crops

Honeybees occasionally collect pollen from maize crops. Standard field studies in maize fields, however, are technically not feasible since maize does not provide nectar to honeybees. For this reason, pollen from Gaucho® seed-treated maize crops (1 mg as/seed = 50 g as/ha) were harvested and fed to honeybees. In this feeding study, small bee colonies which consisted of about 500-1,000 worker bees of different ages and one sister-queen were exclusively provided with maize pollen harvested from Gaucho® seed-treated maize plants for protein supply ([redacted] 2001). A residue analysis of the fed pollen revealed no detectable residues of imidacloprid or its metabolites. Uncontaminated sunflower honey was fed to cover the energy demand of these honeybees. The development of the small bee colonies which were confined within 50 m² gauze tunnels on oat-cropped plots, was followed over 39 days. Regular records were made on activity pattern at the honey and pollen feeders, nectar and pollen consumption, wax production (comb increase), production of food stores, hive weight increase, egg laying performance of the bee queen, the breeding success of the colony (nursing activity) and the development of the colony strength. This long-term feeding study clearly demonstrated that maize pollen from Gaucho® seed-treated maize crops do not adversely affect any of the measured endpoints.

A second study using a replicated test design was performed with pollen also harvested from Gaucho® seed-treated maize crops (1 mg as/seed = 49 g as/ha; [redacted] 2001). A residue analysis of the fed maize pollen revealed imidacloprid residues below the limit of quantification (Table 2). As in the previous study, no treatment-related effects on any testing endpoint (foraging activity, honey and pollen consumption, comb cell production, honey storage, hive weight increase, population development, mortality, breeding activity, and breeding success) were observed.

<u>Conclusion</u>: In two long-term feeding studies using maize pollen from Gaucho® seed-treated maize crops no adverse effects were recorded to the development of expanding honeybee colonies.

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Tab. 1: **Plant uptake rates, compartimentalization and main metabolites of imidacloprid after soil application/seed treatment**
(TRR: total radioactive residue, 6-CNA: 6-chloronicotinic acid, 6-CPA: 6-chloropicolyl alcohol)

Crop	Time to harvest [days after treatment]	Uptake of applied activity [%]	Activity in reprod. organs rel to whole plant [%]	Nature of the residue (Compounds >1% of TRR)								
				Imi	OH-5	Ole	OH-Di	Gua	Guar-op	Nit*	CNA-6	CPA-6
Maize	134	20	1.2	x	x	x	x	x	x	x	x	x
Cotton	211	4.9	1.4	x		x		x		x	x	x
Egg-plant	69	1.6	1.0	x	x	x		x			x	x
Potato	129	2.5	n.a.	x	x	x		x		x	x	x
Rice	79	4.5	1.1	x	x			x		x	x	
Rice	124	4.4	0.7	x	x	x		x			x	

* Nitrosimine <<1% of TRR in reproductive organs

Imi = Imidacloprid, OH-5 = 5-Hydroxy-, Ole = Olefine, OH-Di = Dihydroxy-, Gua = Guanidine, Gua r-op = ring-open Guanidine, Nit = Nitrosimine, CNA-6 = 6-Chloronicotinyl, CPA-6 = 6-Chloropicolyl

n.a. = not applicable due to subsoil fruit setting

Tab. 2: Residue Levels of Imidacloprid and Relevant Imidacloprid Metabolites in Nectar and Pollen of Seed-Dressed Crop Plants under Field Conditions.

Study Location [Dressing rate]	Limit of Quantification[2]			Residue Concentration [mg/kg]				Reference
				Imidacloprid		Imidacl Metabolites[1]		
	Parent	5-OH	Olefine	Nectar or Honey	Pollen	Nectar or Honey	Pollen	
Sunflower Crops								
Deux-Sèvres, France [105 g as/U]	A	A	X	< LOQ (1/3)	< LOQ (10/10)	n.d.	< LOQ[a] (4/10)	[redacted] 1999
Indre, France [105 g as/U]	A	A	X	< LOQ (2/4)	< LOQ (5/5)	n.d.	n.d.	[redacted]999
Vendèe marais, France [105 g as/U]	A	A	D	< LOQ (1/12)	< LOQ (8/8)	n.d.	n.d.	[redacted] 1999
Vendèe plaine, France [105 g as/U]	A	A	D	< LOQ (1/4)	< LOQ (8/8)	n.d.	< LOQ[a] (8/8)	[redacted] 1999
Monheim, Germany [90 g as/U]	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 1999a
Burscheid, Germany [90 g as/U]	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 1999b
Gross-Winterheim, Germany [90 g as/U]	B	B	A	< LOQ	< LOQ	n.d.	n.d.	[redacted] 2000 und [redacted] 2003
Estancia, Brazil [37.5 g as/U]	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 2000

[a] monohydroxy-metabolites

Tab. 2: Residue Levels of Imidacloprid and Relevant Imidacloprid Metabolites in Nectar and Pollen of Seed-Dressed Crop Plants under Field Conditions (cont.)

Study Location [Dressing rate]	Limit of Quantification[2]			Residue Concentration [mg/kg]				Reference
				Imidacloprid		Imidacl Metabolites[1]		
	Parent	5-OH	Olefine	Nectar or Honey	Pollen	Nectar or Honey	Pollen	
Maize Crops								
Monheim, Germany [49 g as/U]	B	B	A	--	< LOQ	n.d.	n.d.	[redacted] 1999c
Burscheid, Germany [49 g as/U]	B	B	A	--	n.d.	n.d.	n.d.	[redacted] 1999d
Louans, France [49 g as/U]	B	B	A	--	< LOQ (2/2)	n.d.	n.d.	[redacted] 2001
Sales Oliveira, Brasil [60 g as/U]	B	B	A	--	n.d.	n.d.	n.d.	[redacted] 2002

n.a. = not analyzed; n.d. = below limit of detection (=LOQ/3); LOQ = limit of quantification

[1] Olefine- and monohydroxy-metabolites;

[2] Limit of quantification: A = 0.01 mg/kg; B = 0.005 mg/kg; C = 0.001 mg/kg, X = 0.1 mg/kg

Tab. 2: Residue Levels of Imidacloprid and Relevant Imidacloprid Metabolites in Nectar and Pollen of Seed-Dressed Crop Plants under Field Conditions (cont.)

Study Location [Dressing rate]	Limit of Quantification[2]			Residue Concentration [mg/kg]				Reference
				Imidacloprid		Imidacl Metabolites[1]		
	Parent	5-OH	Olefine	Nectar or Honey	Pollen	Nectar or Honey	Pollen	
Rape Crops								
Conches, France [10 g as/kg]	A	A	A	< LOQ	< LOQ	n.d.	n.d.	[redacted] 1999a
Borlunda, Sweden [10 g as/kg]	A	A	A	< LOQ	n.a.	n.d.	n.a.	[redacted] 1999b
Bury St.Ed-munds, UK [10 g as/kg]	A	A	A	< LOQ	< LOQ	n.d.	n.d.	[redacted] 1999c
Monheim, Germany [10 g as/kg]	B	B	A	< LOQ	n.d.	n.d.	n.d.	[redacted] 1999e
Burscheid, Germany [10 g as/kg]	B	B	A	< LOQ	< LOQ	n.d.	n.d.	[redacted] 1999f
Ontario, Canada [10 g as/kg]	C	C	C	n.d.	n.d.	n.d.	n.d.	[redacted] 2001
Minnesota, USA [10 g as/kg]	C	C	C	< LOQ (2/2)	4.4 - 7.6	n.d.	n.d.	[redacted] 2001
Celle, Germany	B	B	A	< LOQ	n.d.	n.d.	n.d.	[redacted] 2002

n.a. = not analyzed; n.d. = below limit of detection (=typically 1/3 of LOQ); LOQ = limit of quantification

[1] Olefine- and monohydroxy-metabolites;

[2] Limit of quantification: A = 0.01 mg/kg; B = 0.005 mg/kg; C = 0.001 mg/kg, X = 0.1 mg/kg

Tab. 3: Residue Levels of Imidacloprid and Relevant Imidacloprid Metabolites in Nectar and Pollen of Succeeding Crop Plants under Field Conditions

Soil Residue Level [mg/kg]	Limit of Quantification[2]			Residue Concentration [mg/kg]				Reference
				Imidacloprid		Imidacl Metab[1]		
	Parent	5-OH	Olefine	Nectar	Pollen	Nectar	Pollen	
Sunflower Crops								
0.0157	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted]. 1999a
0.0127	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 1999a
0.0143	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 1999a
0.0178	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 1999b
< 0.006	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 1999b
not reported	C[a]/D[b]	C[a]/D[b]	C[a]/D[b]	1.6 (1/4)	< LOQ (1/14)	n.d.	n.d.	[redacted] 2001
[a] Pollen; [b] Nectar								
Maize Crops								
0.0157	B	B	A	--	n.d.	n.d.	n.d.	[redacted] 1999c
0.0127	B	B	A	--	n.d.	n.d.	n.d.	[redacted] 1999c
0.0143	B	B	A	--	n.d.	n.d.	n.d.	[redacted] 1999c
0.0178	B	B	A	--	n.d.	n.d.	n.d.	[redacted] 1999d
< 0.006	B	B	A	--	n.d.	n.d.	n.d.	[redacted]. 1999d
Rape Crops								
0.0157	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 1999c
0.0127	B	B	A	n.d.	< LOQ	n.d.	n.d.	[redacted] 1999c
0.0143	B	B	A	n.d.	< LOQ	n.d.	n.d.	[redacted] 1999c
0.0178	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 1999d
< 0.006	B	B	A	n.d.	n.d.	n.d.	n.d.	[redacted] 1999d
Clover Crops (soil residue ageing period: approx 28 month)								
0.025	C	C	C	n.d.	n.d.	n.d.	n.d.	[redacted] 2002
0.014	C	C	C	n.d.	n.d.	n.d.	n.d.	[redacted] 2002
0.024	C	C	C	n.d.	n.d.	n.d.	n.d.	[redacted] 2002
0.017	C	C	C	n.d.	n.d.	n.d.	n.d.	[redacted] 2002

n.a. = not analyzed; n.d. = below limit of detection (= typically 1/3 of LOQ); LOQ = limit of quantification

[1] Olefine- and monohydroxy-metabolites;

[2] Limit of quantification: A = 0.01 mg/kg; B = 0.005 mg/kg; C = 0.002 mg/kg; D = 0.001 mg/kg

Tab. 4: **Acute Toxicity of Imidacloprid to Honeybees as Deteremined According to EPPO Guideline No. 170**

Test Material	Oral Toxicity [ng a.i./bee] LD50	Oral Toxicity [ng a.i./bee] NOEL[1]	Contact Toxicity [ng a.i./bee] LD50	Contact Toxicity [ng a.i./bee] NOEL[1]	Literature
techn. grade	3.7	< 1.5[2]	81.0	< 2.5[2]	[redacted] 1990
techn. grade	> 40.9 [50%]	1.5	not determined	not determined	[redacted] 1999a
techn. grade	> 20.4 [0%]	4.4	104.0[4] (83.0 – 130.0	< 42	[redacted] 999a, b
techn. grade	> 45.0 [13%]	2.8	50.0 (9.1 – 71.0)	< 40	[redacted] 2000a, 2000b
techn. grade	> 34.7 [17%]	7.0	42.9 (34.6 – 53.2)	< 40	[redacted] 2000a
techn. grade	> 70.3 [47%]	9.0	74.9 (61.8 – 90.9)	< 40	[redacted] 2000a
SC 200	20.6	3.5	58.0	< 10.0[3]	[redacted] 1995a
WG 70	11.7	1.2	245.0	35.0	[redacted] 1995b
SL 200	5.3[5] (3.4 – 8.4)	1.2	42.2 (20.9 – 85.0)	< 50	[redacted] 2001
SL 200	53.0[5] (38.0 – 74.0)	12.8	45.0[5] (34.0 – 60.0)	5.7	[redacted] 2001

[1] Mortality of less than 10% was considered as natural mortality and as not treatment-related.

[2] Lowest dose caused 20% mortality

[3] Lowest dose caused 10% mortality

[4] LD50 (72 h)

[5] LD50 (96 h)

Table 5: **Results of the Literature Survey on the Chronic Dietary Toxicity of Imidacloprid to Honeybees**

Laboratory Studies

Location / Season	Age of bees[1] / strain	Feeding duration [n=number of repetitions]	Syrup ingestion rate [µL/bee/day]	Mortality rates [%, corrected for control mortality]	Reference
Bures, France / March-April 1998	4 days [Apis mellifera ligustica]	11 days (n=2)	25–46 [50% sucrose, 33°C, dark]	4 µg/L: 3 8 µg/L: 20 40 µg/L: 36 [control mort.: 7] ***[NOLEC: 4 µg/L]***	[redacted] 1998 ***Invalidated study result***
Bures, F Nov-Feb, 1999/2000	4 days [A. mellifera ligustica]	11 days (n=3)	c. 28.8–33.7 [50% sucrose]	1.5 µg/L: 1 3 µg/L: 0 6 µg/L: 0 12 µg/L: 0 24 µg/L: 5 48 µg/L: 10 [control mort.: 12] NOLEC: 24 µg/L	[redacted] 2003 (repetition of 1998 study)
Bures, F Jun-Jul, 2000	4 days [A. mellifera ligustica]	11 days (n=3)	c. 28.8–33.7 [50% sucrose]	1.5 µg/L: 5 3 µg/L: 5 6 µg/L: 2 12 µg/L: 4 24 µg/L: 5 48 µg/L: 6 96 µg/L: 15 [control mort.: 3] NOLEC: 48 µg/L	[redacted] 2003 (repetition of 1998 study)
Avignon, F May-June, 1998	Not specified	15 days (n=1)	not specified [32°C]	No difference in mortality compared to control at 10 µg/L NOLEC: 10 µg/L[2]	[redacted] 1998
Konstanz, Ger May-June, 2000				No difference in mortality compared to control at 10 µg/L NOLEC: 10 µg/L[2]	[redacted] 2000
Avignon, F not specified	Not specified [Apis mellifera L.; strain not specified]		c. 12 [50% sucrose]	0.1 µg/L: ca. 35 1 µg/L: ca. 65 10 µg/L: ca. 70 [control mort.: < 15] NOLEC: < 0.1 µg/L[3]	[redacted] ***2001***

[1] at study initiation
[2] highest test concentration
[3] lowest test concentration

Table 5: Results of the Literature Survey on the Chronic Dietary Toxicity of Imidacloprid to Honeybees (*cont.*)

Cage and Field Feeding Studies

Location / Season	Initial no. of bees / strain	Exposure duration	Syrup ingestion rate [µL/bee/day]	Mortality rates [%, corrected for control mortality]	Reference
Avignon, France April, 1998	5,000 [A. mellifera Buckfast]	10 days Individually marked bees	not specified [50% sucrose]	No difference in mortality compared to control reported for 100 µg/L, reduced foraging at 1,000 µg/L NOLEC: 100 µg/L	[redacted] 1998
Konstanz, D May-June, 1999	5,000 [A. mellifera carnica]	4-10 days Individually marked bees	not specified [50% sucrose, 21°C, semi-dark]	No difference in mortality compared to control reported for 10, 20, 50 and 100 µg/L, reduced foraging at 50 and 100 µg/L NOLEC: 100 µg/L[2]	[redacted] 1999
Euskirchen, D June-July, 1999	500[1] [A. mellifera carnica]	39 days	c. 25d [90% sucrose]	No difference in mortality compared to control reported for 2, 5, 10, and 20 µg/L, NOLEC: 20 µg/kg[2]	[redacted] 2001

[1] at study initiation
[2] highest test concentration

Table 6: Dose-related mortality of honeybees during the repeat chronic feeding tests compared to values reported [redacted]. (2001)

Urea-Metabolite

Test Facility [Testing Period]	Young Honeybees [1-17 days]		Old Honeybees [22-45 days]	
	Dose [ng a.i./bee/day]	Mortality [%]	Dose [ng a.i./bee/day]	Mortality [%]
Germany [redacted] (2000a and 2000b)	0	0	0	20[a]
	0.004	8	0.003	34
	0.045	6	0.029	20
	0.432	0	0.288	16
Germany [redacted] (2000b) *Test run I*	0	10	0	30
	0.005	37	0.004	60
	0.039	3	0.048	50
	0.357	63	0.478	60
Germany III, [redacted] (2000b)	0	4	0	44
	0.008	10	0.008	26
	0.073	8	0.071	36
	0.727	12	0.730	36
	Dose [ng a.i./bee/day]		Mortality [%]	
United Kingdom, [redacted] (2000c)	0		44	
	0.007		34	
	0.066		46	
	0.640		50	
[redacted] (2001)	0		< 15	
	0.001		c. 50	
	0.012		c. 60	
	0.120		c. 75	

[a] test had to be prematurely terminated on day 4 due to the increased control mortality

Table 6: Dose-related mortality of honeybees during the repeat chronic feeding tests compared to values reported by [redacted] et al. (2001)

6-Chloro-Nicotinic Acid

Test Facility [Testing Period]	Young Honeybees [1-17 days]		Old Honeybees [14-45 days]	
	Dose [ng a.i./bee/day]	Mortality [%]	Dose [ng a.i./bee/day]	Mortality [%]
Germany [redacted] (2000c and 2000d)	0	0	0	20[a]
	0.005	2	0.003	8
	0.046	4	0.033	10
	0.468	0	0.273	6
Germany II, [redacted] (2000c) Test run I	0	10	0	30
	0.004	67	0.003	77
	0.038	77	0.040	70
	0.388	97	0.404	73
Germany II, [redacted] (2000) Repeat Test	0	7		
	0.006	10	n.d.	n.d.
	0.055	7		
	0.580	7		
Germany III, [redacted] (2000c)	0	4	0	44
	0.007	6	0.008	32
	0.073	4	0.082	40
	0.724	10	0.806	30
United Kingdom, [redacted] (2000d)	0		54	
	0.007		58	
	0.065		58	
	0.667		52	
[redacted] (2001)	0		< 15	
	0.001		c. 50	
	0.012		c. 55	
	0.120		c. 70	

[a] test had to be prematurely terminated on day 4 due to the increased control mortality

Table 7: **Sucrose (50% w/v) ingestion rates of honeybees in the repeat chronic feeding tests compared to rates reported by Suchail [redacted] (2001)**

Test Facility	Average Temp [°C]	Control [µl/bee/day]	Young Bees [µl/bee/day]	Old Bees [µl/bee/day]
Germany I, [redacted] (2000a, 2000b, 2000c and 2000d)	24–28	39.1–47.1	43.2–50.4	27.0–33.3
Germany II [redacted] (2000b and 2000c)	23–27	38.9–40.2	28.6–37.1	27.1–38.6
Germany III [redacted] (2000b and 2000c)	24-26	58.4–63.5	57.5–60.3	56.6–66.3
United Kingdom I	24–26	55.8–69.9	56.5–72.0	--
[redacted] **(2001)**	25	12		

Tab. 8: **Acute Oral Toxicity of Imidacloprid Plant Metabolites to Honeybees as Determined According to EPPO Guideline No. 170**

Plant Metabolite	Oral Toxicity [ng a.i./bee] LD50	NOED[1]	References
5-Hydroxy- (M01)	159	1.2	[redacted]
Olefine- (M06)	> 36	2.4	[redacted]
4,5-Dihydroxy- (M03)	> 49	49.0	[redacted]
Guanidine- (M09)	ca. 93,200	1,200.0	[redacted]
Nitrosimine- (M07)	80	< 80.0	[redacted]
6-Chloro- nicotinic acid (M14)	> 121,500	121,500.0	[redacted]
6-Chloro- picolylamin	> 119,800	119,800.0	[redacted]

[1] based on significant mortality or behavioral impacts compared to control bees

Tab. 9: Acute Toxicity of Imidacloprid to Bees Other Than Honeybees

Tested bee species	Test material	Oral Toxicity [ng as/bee]		Contact Toxicity [ng as/bee]		Literature
		LD50 (48 hr)	NOED[1]	LD50	NOED[1]	
Bombus terrestris L.	techn. material	220	10[2]	--	--	[redacted]
Bombus terrestris L.	techn. material	--	--	c. 100	52[2]	[redacted]

[1] based on significant mortality or behavioral impacts compared to control bees

[2] based on results of range finding test

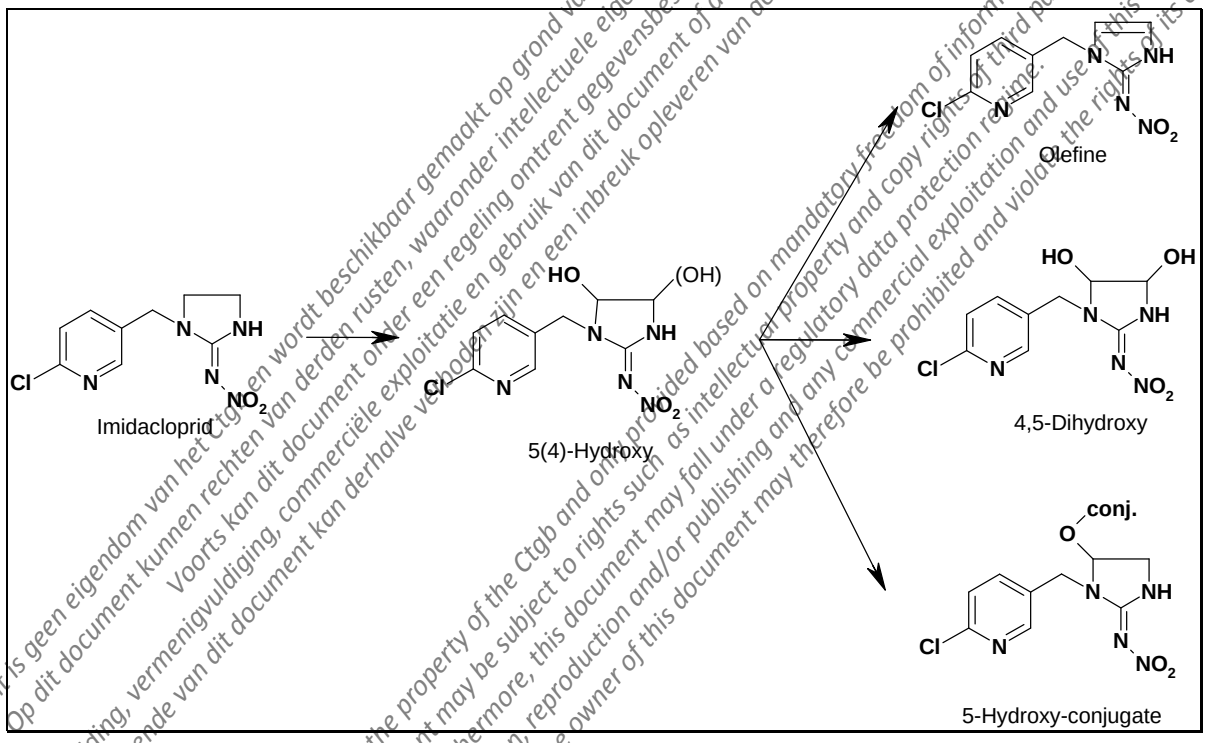

Fig. 1: Metabolism of imidacloprid (I): ethylene-bridge hydroxylation of the imidazolidine ring and elimination of water

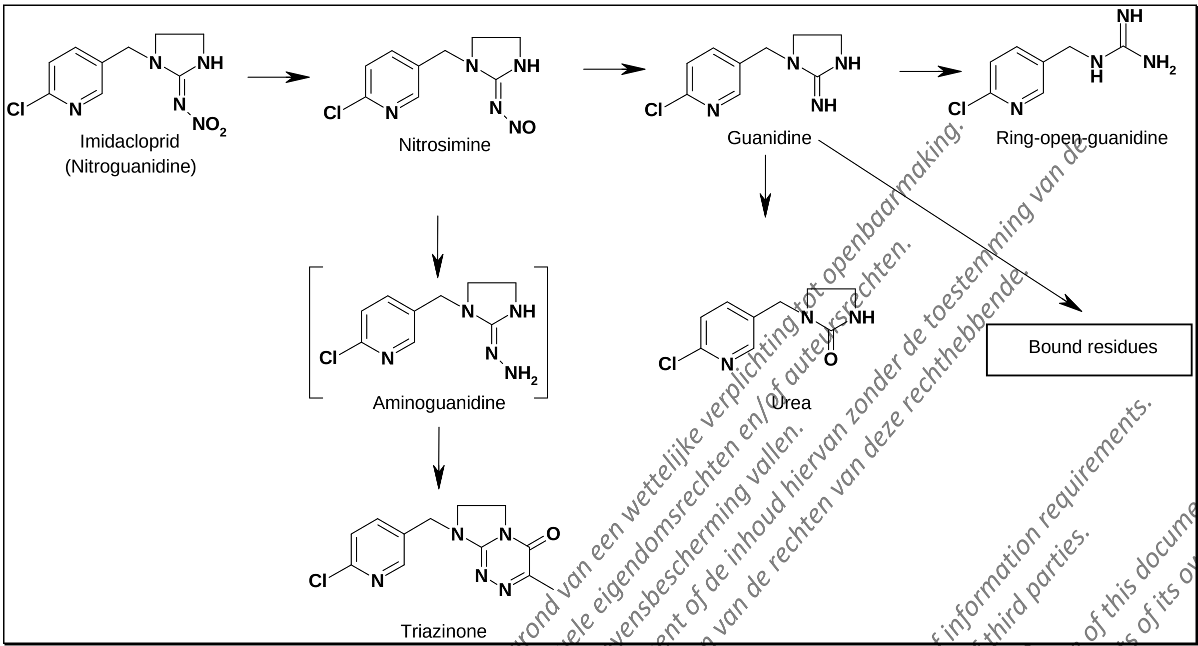

Fig. 2: Metabolism of imidacloprid (II): nitro-group reduction to nitrosimine and further loss of NO to form guanidine

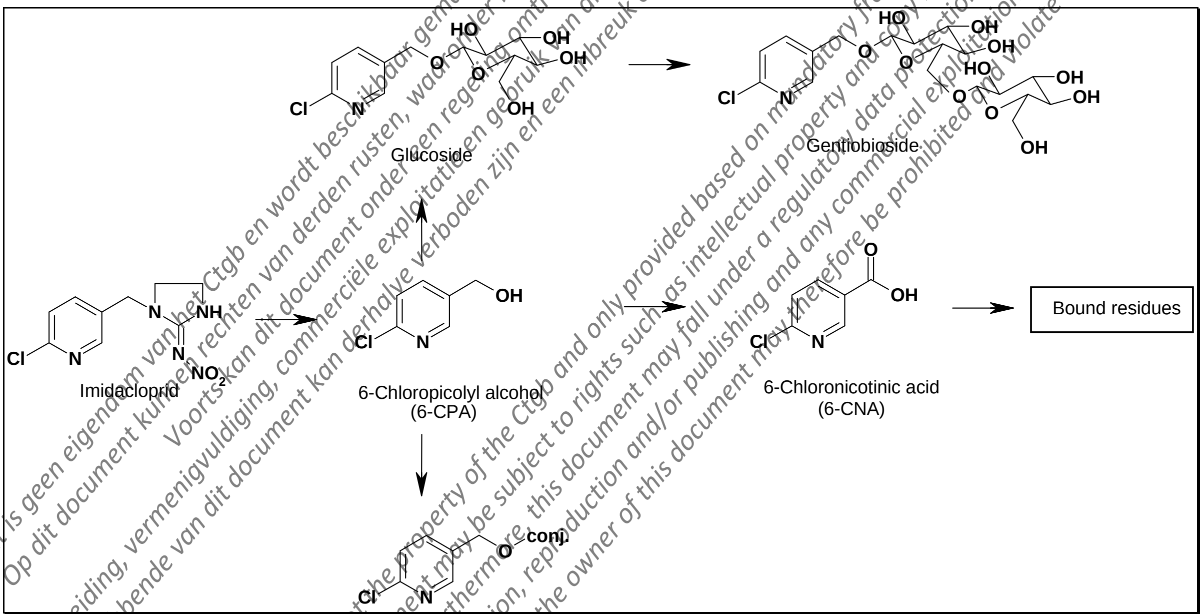

Fig. 3: Metabolism of imidacloprid (III): Oxidative cleavage of the methylene bridge to form 6-chloropicolyl alcohol and subsequent oxidation to 6-chloronicotinic acid

Appendix I: Characteristic Symptoms of the „French Bee Syndrome“

According to information from concerned commercial bee-keepers in Western and Central France and to written reports ([redacted] 1998) the French bee problem is characterized by the following symptoms:

Symptoms of individual bees

- impaired sense of balance, bees missed the board when they return to the hive.
- bees are incapable of flying, honeybees crawl on the ground in front of the hives and form clusters.
- bees appear to be weak, they are unable to climb up hive posts or grass stems.
- bees are of a black shiny appearance (abdomen) and show loss of fur.
- hive guards prevent trembling and apparently hectic foraging bees from entering the hive.
- some honeybees show sudden cleaning attacks (up to 5 minutes) while foraging on sunflower heads.
- honeybees remain sometimes apathetic at the outer edge of the sunflower heads.
- honeybees respond with trembling when touched while resting on the sunflowers.
- disorientation, honeybees fail to return to the hive.

Symptoms of affected bee-hives

- about one third of the honeybees of affected hives exhibit the above described symptoms.
- affected hives have no or only minor increases in population strength.
- there is a massive loss of foraging bees.
- nectar is stored in an unusual pattern within the honey combs.
- honey yield is substantially reduced.

The concerned beekeepers reported that the above symptoms became apparent about 3 to 5 days after the peak blossom of the sunflower fields. They also stated that the situation had worsened drastically over the last two years. The affected bee-hives were reported to be weakened up to some complete hive losses. Honey yields from the surviving hives were said to be unusually low. However, not all bee-hives of an apiary were affected. On average, one of four bee-hives was reported to be free from symptoms. This observation was explained by healthy colonies foraging on weed plants and crops other than sunflowers.

According to published statistics (Agreste, 1994-97) the honey yields in France were higher in 1994 and 1995, similar in 1996 and significantly lower in 1997 than the 10-year-average yield.

Honey yield data of a professional French bee-keeper were in 1994 and 1995, i.e. after Gaucho® was launched on the market, ranged between 73 and 76 kg per bee-hive. In 1996, honey yields decreased to 45 kg/bee-hive. However, it was not clear from the reported data sheet whether these data reflect the annual honey yield or only the amount harvested from sunflowers.