



31 MAY 2011

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## **Explanatory Notes of Bayer CropScience (BCS) to the Ctgb evaluation of imidacloprid and clothianidin containing plant protection products and their associated uses authorised in The Netherlands**

### **1. PREFACE**

Imidacloprid is marketed by Bayer CropScience in The Netherlands in/on various crops including floriculture, flower bulbs, bulb flowers, tree nurseries, perennials (including orchards) as well as arable crops, either as a foliar application, soil application or as a seed-treatment; applications can be performed – depending on the crop and use – either outdoors or in greenhouses.

Clothianidin is marketed by Bayer CropScience in The Netherlands exclusively as a seed-treatment in sugar beets and in maize.

In February 2011, the Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) was asked by the House of Representatives in The Netherlands to review the existing uses of imidacloprid and clothianidin with regard to bee safety. In the meantime, Ctgb has elaborated a review document on which Bayer CropScience was given the opportunity to provide comments. This document summarizes the explanatory notes of Bayer CropScience regarding the questions and open points as identified by Ctgb; proposals of label adaptations are provided, where considered appropriate.

## 2. IMIDACLOPRID

### 2.1 APPROPRIATE WAITING PERIOD AFTER THE USE OF IMIDACLOPRID IN GREENHOUSES TO PROTECT HONEY BEES AND BUMBLE BEES

Imidacloprid is used in/on various crops grown under protection in The Netherlands since many years (>10). These uses include spray and soil applications in/on plants not depending on pollination by bees (e.g. floriculture) and crops where particularly bumble bees are used as pollinators to increase and stabilize fruit production (e.g. solanaceous crops). These uses include imidacloprid soil applications to cucurbits of up to and including 141 g a.s./ha and solanaceous crops with soil applications of up to and including 235 g a.s./ha.

In two crop pollination studies under confined conditions, considering imidacloprid soil drip/drench applications of up to 300 g a.s./ha (Doc. No.: M-030167-01-1; ) and 267 g a.s./ha (Doc. No.: M-304435-01-2), it was concluded that the use of imidacloprid does not impair the pollination efficacy of confined bumble bees (for details of the studies see chapter 2.5).

Moreover, Bayer CropScience is not aware of complaints or claims of damages by vegetable growers who use both, imidacloprid for aphid and whitefly control and bumble bees for crop pollination. As such, due to several years of coexistence between imidacloprid uses in greenhouses and pollination services provided mainly by bumble bees, Bayer CropScience does not see the imminent need to define on short notice waiting periods in greenhouses to protect pollinators. **Nonetheless, in light of the current discussions with Ctgb, Bayer CropScience will propose appropriate waiting periods for the entry of pollinators for those uses, where this is in line with common practice (i.e. tomato and bell pepper).**

### 2.2 RISK ASSESSMENT FOR HONEY BEES REFERRING TO THE FIELD USES IN FLORICULTURE, FLOWER BULBS, TREE NURSERIES AND PERENNIALS

All outdoor foliar uses of imidacloprid in The Netherlands in flowering plants exclude the flowering period. As such, honey bees are not exposed to residues of imidacloprid in blossoms of (potentially) bee attractive target plants. This conclusion is also valid for the post-flowering uses. With regard to pre-flowering applications, the question was asked whether there is a potential risk for foraging honey bees later on in the season when the (potentially) bee attractive flowering plant was sprayed before flowering. Bayer CropScience specifies the latest pre-flowering growth stage to be sprayed in floriculture for imidacloprid containing products – e.g. in recently submitted dossiers for Imidacloprid SC 350 – to be BBCH 49 (i.e. end of vegetative propagation, before inflorescence emergence [BBCH 50-59] and before beginning of flowering [BBCH 60]).

Bayer CropScience has investigated the potential impacts of pre-flowering applications in a highly bee attractive crop. i.e. in flowering apple orchards. In total, five independent studies have been conducted:

- One study in Germany, 1 × 105 g a.s./ha, application 24 days before exposure of honey bee colonies (Doc.-No.: M-084030-01-1)
- Four studies in Italy, 1 × 120 - 1 × 160 g a.s./ha, 15 - 20 days before exposure of honey bee colonies (Doc.-No.: M-355844-01-1 and M-064758-02-1), at which in study with the highest application rate (160 g a.s./ha) there was the shortest interval to honey bee exposure (15 days, Verona, Doc.-No.: M-355844-01-1)

In none of the studies any impact on foraging honey bees as well as on colony development has been recorded.

Moreover, the critical review of various translocation experiments after foliar application of imidacloprid (Doc.-No.: M-308631-01-1) revealed that when imidacloprid is applied on leaf surfaces there is a good translocation to shoots and leaves (xylem mobility) but a poor translocation to sinks, like e.g. storage organs, roots, fruits (negligible phloem mobility). The studies investigated in Doc.-No.: M-308631-01-1 revealed a consistent distribution pattern with predominant acropetal and only marginal basipetal transportation. The authors concluded that it is highly unlikely that a foliar application of imidacloprid will lead to any significant residues in nectar and pollen of plants treated in the pre-flowering stage. This conclusion is supported by the 5 studies conducted in highly bee attractive apple orchards. Moreover, it needs to be considered that the half life of total imidacloprid residues on plant surfaces is very low (< 1 up to max. 2.6 days; see DAR of imidacloprid).

The following pictures illustrate the predominant acropetal and the only marginal basipetal distribution of  $^{14}\text{C}$ -Imidacloprid by autoradiography; this predominant acropetal transport is also the reason of the empirical observations in commercial practice that new shoots are not protected from aphid infestations after imidacloprid spray applications (aphids are much more susceptible to imidacloprid than honey bees,  $\text{LD}_{50} = 0.54 \text{ pg/aphid}$ ; see Doc.-No.: M-110655-01-1)

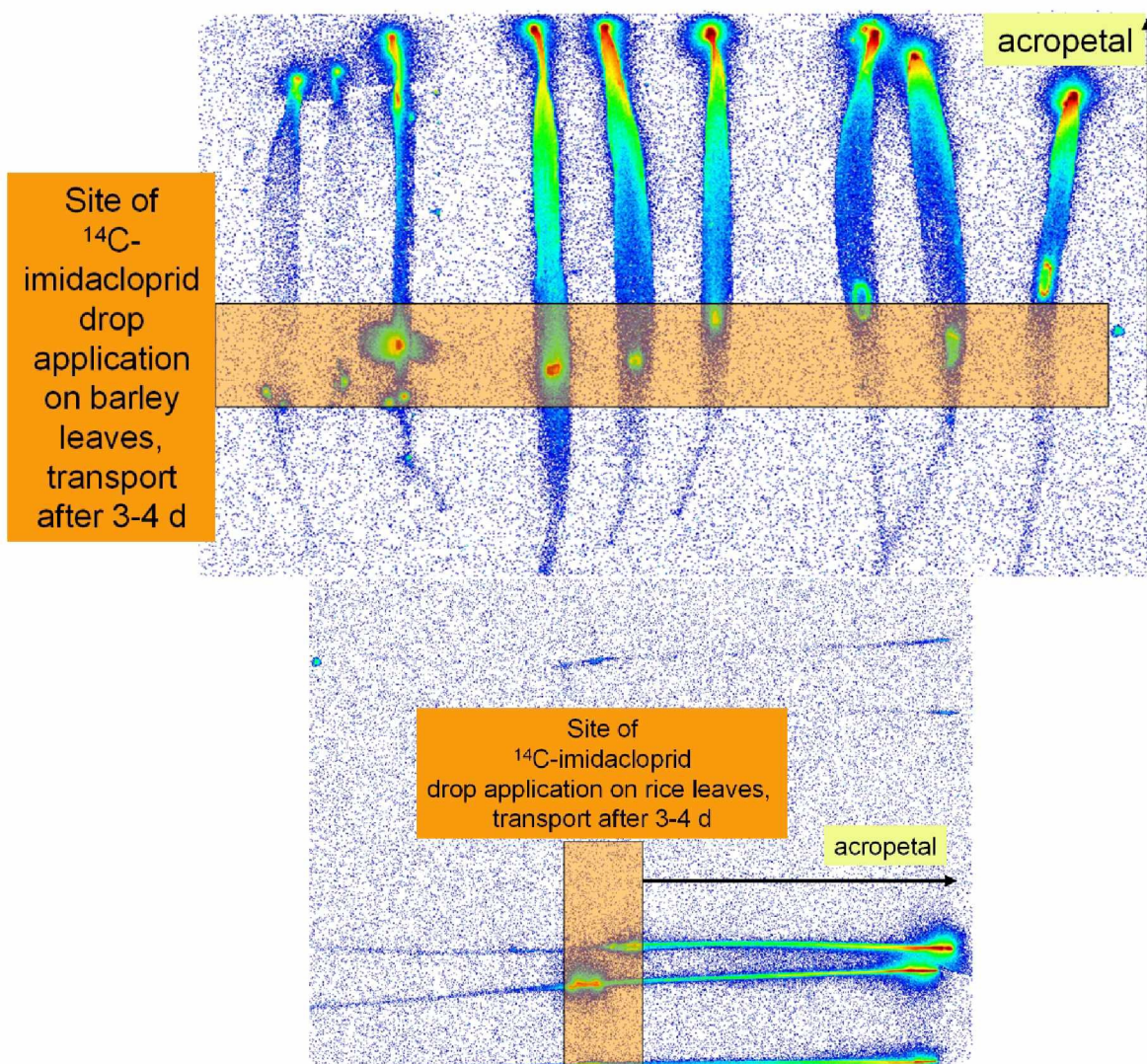


Figure 2.2.1: Illustration of the acropetal transport of imidacloprid after foliar application

Considering (i) the translocation behaviour of imidacloprid after foliar application, (ii) the short half-life of total imidacloprid residues of plant surfaces and (iii) no observable adverse effects on honey bees and honey bee colonies from a pre-flowering foliar application of up to 160 g a.s./ha, the weight of evidence suggests that pre-flowering applications of up to and including 70 g a.s./ha at the latest at BBCH 49 - still several days before onset of flowering - will not pose an unacceptable risk to honey bees. This conclusion is supported by the findings of Mayer and Lunden (1997; Doc.-No.: 110179-01-1) who found no impact on honey bee mortality from an imidacloprid spray application of 112 g a.s./ha in an apple orchard with 10% open bloom and with on average 6 flowering dandelions per m<sup>2</sup> understorey.

Regarding potential impacts of imidacloprid residues on hibernation, Faucon et al. (2005; Doc.-No.: 387723-01-1) fed honey bee colonies during summer repeatedly with sugar syrup, fortified with 0.5 and 5 µg/L imidacloprid. The authors have not observed elevated acute mortality, or sub-lethal or delayed effects, or effects on brood, colony development or finally overwintering mortality. A systematic investigation of Aubert et al. (2008; Doc.-No.: 400335-01-1), who investigated the effect of microbial and parasitic agents and pesticide residues on the evolution of domestic bee colonies under natural conditions revealed that the only parameters for which a statistically significant relationship to the mortality of the colonies could be found were the level of attention paid by the apiarist to preventive measures and the early detection and identification of Varroa disease. This conclusion is in line with the recent publication of the German Bee Monitoring (Genersch et al., 2010; Doc.-No.: M-408279-01-1) - which has been systemically scrutinizing impacts on up to 1200 honey bee colonies in Germany for many years - where it was concluded that no correlation between plant protection products and overwintering losses have been found and that the principal factor of overwintering losses is an insufficient or improper Varroa control.

**Considering all the available information and applying the weight-of-evidence principle, it can be concluded with reasonable certainty that honey bee colonies and bee keeping practices will not be impaired from pre-flowering foliar applications in apple orchards when sprayed at the latest at the mouse ear stage or from pre-flowering foliar applications in flowering ornamentals (for flowering ornamentals, Bayer CropScience fixed the timing of application to BBCH 10 – 49[end of vegetative propagation] and from BBCH 69 [end of flowering] onwards). However, for flowerbulbs and bulbflowers, spraying before the first flower buds are visible, is not a realistic option. As such, we propose to restrict the use in flowerbulbs and bulbflowers to post-flowering.**

**Regarding dip applications of imidacloprid on ornamental bulbs at the time of planting outdoors, Bayer CropScience proposes to restrict the use to those ornamentals which are not used for foraging by bees (e.g. *Amaryllis*), based on a list currently under development by PPO bollen and Bijen@WUR.**

### 2.3 RISK FOR HONEY BEES FROM POTENTIAL FLOWERING WEEDS IN THE ORCHARD UNDERSTOREY AFTER SPRAY APPLICATION

On the current label it is stated that application is not allowed when flowering weeds are present, i.e. eventual flowering understorey has to be removed before application. Ctgb has now raised a concern that weeds, which are not yet flowering in the understorey during application, may receive an imidacloprid dose from ground deposition during orchard spraying and that the systemic uptake of the spray residues may pose a risk to honey bees foraging later on in the orchard understorey. Whereas in principle the same facts as stated above for pre-flowering applications are considered to be also valid for the above mentioned scenario, it is acknowledged that the period between orchard spraying and flowering of potentially bee attractive weeds in the orchard understorey can be significantly shorter as investigated in the available studies. However, the findings of Mayer and Lunden (1997; Doc.-No.: 110179-01-1; see above) who applied imidacloprid at 112 g a.s./ha in an apple orchard with 10% open bloom and additionally with on average 6 flowering dandelions per m<sup>2</sup> understorey with no impact on honey bee mortality suggest that the relevance of emerging flowering and bee attractive weeds in the orchard understorey soon after an imidacloprid application in terms of associated risks for honey bees is acceptable. **Nonetheless, in light of the recent discussion with Ctgb on this subject, Bayer CropScience suggests to state on the label that the understorey has to be cut in any case before applying imidacloprid to orchards and tree nursery crops and that potential weeds in the understorey have to be managed to prevent them from flowering for two weeks after application (e.g. by frequent mowing which is common practice for most growers anyway).**

### 2.4 RISK FOR HONEY BEES FROM RESIDUES IN POTENTIALLY BEE ATTRACTIVE SUCCEEDING CROPS AFTER SPRAY AND SOIL APPLICATIONS

Carry-over of soil residues and subsequent uptake by succeeding, bee-attractive flowering crops has been investigated in a range of studies. The maximum residues in bee relevant matrices like nectar and pollen that has been found was 2 µg imidacloprid/kg, originating from a soil-borne imidacloprid residue levels ranging from 13 - 25 µg imidacloprid/kg soil. Ctgb has now calculated on the basis of the max. non-normalised field DT<sub>50</sub> of imidacloprid and the initial PEC<sub>SOIL</sub> after soil or foliar use of imidacloprid the time period until when the soil borne residue level has declined in the upper 20 cm to 25 µg imidacloprid/kg soil. The calculated time period for all imidacloprid used in The Netherlands is < 1 year (max. ≈10 months), i.e. whatever the field use of imidacloprid in The Netherlands, a bee attractive flowering crop can be sown on a field which received its last imidacloprid application about 1 year before. Studies on the time dependent sorption of imidacloprid in mineral soils with an organic carbon content of 0.9 and 1.8% showed a constant increase of the K<sub>OC</sub>-value of imidacloprid over time with increase factors of 3.2 and 3.8 after 100 days (Doc. No.: M-023945-01-1) which translates into a steadily decreasing bioavailability of soil borne imidacloprid.

However, **there are to date no studies with higher than 25 µg/kg soil residues available to experimentally prove that bee attractive crops can be planted with a shorter time interval than 10 months after the last imidacloprid application. Therefore, Bayer CropScience proposes to adjust the label with waiting periods before planting a bee attractive flowering crop that are in line with the suggestions made by Ctgb in their draft evaluation.**

## 2.5 RISK FOR BUMBLE BEES FROM RESIDUES IN FLOWERING POTATOES AFTER SOIL APPLICATION OF IMIDACLOPRID

Solanaceous crops are known to be a non-attractive feeding source for honey bees. Solanaceous crops do not provide nectar and release pollen only after the flowers are sonicated. Sonication is not performed by honey bees but bumble bees may obtain pollen from sonicated solanaceous crop-flowers. In The Netherlands, imidacloprid is used in potatoes after soil application of up to and including 175 g a.s./ha. No studies have been conducted specifically in potatoes after imidacloprid soil applications, but two studies are available where imidacloprid has been soil-applied in tomatoes – an other solanaceous crop. In one crop pollination study by the University of Cartagena in greenhouses in Spain (Doc. No.: M-030167-01-1), tomato plants received a soil drip/drench application corresponding to 10 mg a.s./plant (4 applications in a ten days interval, starting 4 days after the bumble bee colonies were introduced into the greenhouse). The plant density under investigation ranged from 1.5 - 3 plants/m<sup>2</sup> which corresponds to 15 - 30,000 plants/ha and in turn to 150 - 300 g a.s./ha. Bumble bees were used as crop pollinators. The authors concluded that there were no differences between control and the treatments and that the crop has been effectively pollinated by the foraging bumble bees. In a second study conducted by the department of plant protection of the University of Tuscia in Viterbo in Italy (Doc. No.: M-304435-01-2), tomato plants received a soil drip application of imidacloprid corresponding to 178 and 267 g a.s./ha. Bumble bee colonies were introduced into gauze netting enclosures 7 days after drip application. The author concluded that 7 days after drip application of imidacloprid to tomato plants bumble bee forage and pollinate imidacloprid treated tomato plants as effectively as untreated control plants.

Gels et al. 2002 (Doc. No.: M-210591-01-1) investigated the impact of imidacloprid soil applications in turf with 25 - 50% white clover coverage on bumble bees. The authors concluded that imidacloprid, when irrigated into the soil at rates corresponding to 336 g a.s./ha, cause no adverse effects on bumble bee colony vitality even when bumble bees are exposed to flowering white clover on the treated plots under confined conditions for up to 30 days. Moreover, available data on the sensitivity of bumble bees to imidacloprid as compared to honey bees show no distinct differences (DAR, imidacloprid).

Moreover, on the labels for Amigo and MonAmi, Bayer CropScience claims an efficacy against aphids for up to 8 -10 weeks. This is about the time potatoes need to develop flowers. Thus, when bumble bees are potentially exposed to potato pollen, imidacloprid levels in potato leaves are not longer sufficient to control aphids. Furthermore, considering the translocation information provided in chapter 2.2, it appears to be unlikely that the concentration in pollen will be higher than in the leaves. Moreover, it needs to be considered that there is a large difference in the imidacloprid-LD<sub>50</sub> between bees and aphids (0.00000054 µg/aphid vs. 0.0037 µg/bee).

**Considering (i) the findings of the two pollination studies with bumble bees in tomato plants, which received at least one soil application of imidacloprid >175 g a.s./ha with no impact on the pollination efficacy, which can be considered as an indirect parameter of the health status of the individual foraging bumble bees as well as the associated bumble bee colonies, (ii) the findings of Gels et al., where bumble bees were confined on plots with flowering white clover without adverse effects on colony vitality parameters after a soil application of 336 g a.s./ha, and (iii) the fact that aphids, which are much more sensitive than bees, are no longer controlled at the time of potato flowering, it is highly unlikely that an imidacloprid soil application to potatoes at the day of potato planting will pose an unacceptable risk to foraging bumble bees at the time of potato flowering, which occurs several weeks after planting.**

## 2.6 RESIDUES IN IMIDACLOPRID-SEED-TREATED SUCCEEDING CROPS, GROWN IN SOILS WHICH CONTAIN AN IMIDACLOPRID BACKGROUND CONCENTRATION

As indicated in chapter 2.4 above, studies on the time dependent sorption of imidacloprid in mineral soils with an organic carbon content of 0.9 and 1.8% showed a constant increase of the  $K_{oc}$ -value of imidacloprid over time with increase factors of 3.2 and 3.8 after 100 days (Doc. No.: M-023945-01-1) which translates into a steadily decreasing bioavailability of soil borne imidacloprid over time. A study with the neonicotinoid compound clothianidin in maize (Doc.-No.: M-256474-01-1) revealed no, or at best a non-significant marginal increase in the clothianidin residue level in maize pollen if seed-treated maize seeds are sown during springtime in clothianidin-treated soil compared to residue-free soil. In this study it needs to be considered that the soil was treated with clothianidin shortly before sowing, i.e. residues did not age in the soil matrix as typical for a "grown" soil background concentration. Therefore, the established soil background concentration of clothianidin in this study can be expected to be (nearly) fully bio-available due to the short aging period of only 55 and 42 days (see also chapter 3.3, below).

The findings and the conclusion made for the neonicotinoid compound clothianidin in maize (Doc.-No.: M-256474-01-1) are further supported by the findings in studies with imidacloprid, where either imidacloprid-seed-treated sunflowers, spring-OSR (oil seed rape) or maize was planted either in imidacloprid-treated (Doc. No.: M-016820-01-1, M-016827-01-1, M-016828-02-1, M-016830-01-1, M-016836-01-1 and M-016842-02-1) or in imidacloprid-free soil (e.g. Doc. No.: M-006815-01-1, M-006811-01-1, M-040023-01-1, M-018436-01-1, M-075630-01-1, M-052637-01-1 and M-052238-01-1; further reports can be found in the DAR of imidacloprid).

When comparing the results of these studies, it can be concluded that there is, if any, only a non-significant marginal increase in the imidacloprid residue level in pollen and nectar of imidacloprid-seed-treated crops when grown in imidacloprid-treated soil as compared to imidacloprid-seed-treated crops grown in imidacloprid-free soil.

Since (i) the comparison of a range of studies where imidacloprid-seed-treated crops were grown either in imidacloprid-treated or imidacloprid-free soil revealed, if any, only a non-significant marginal increase of the imidacloprid residue levels in bee relevant matrices, (ii) in none of all these studies any adverse effects on foraging honey bees have been observed when honey bee colonies were confined on flowering spring-OSR and sunflowers, grown from imidacloprid-treated seeds in imidacloprid-treated soil (Doc. No.: M-016820-01-1, M-016827-01-1, M-016828-02-1 and M-016842-02-1) or when honey bees were confined on flowering crops, grown from imidacloprid-treated seeds in imidacloprid-free soil, (iii) the measured imidacloprid residue levels in nectar and pollen were in any case and in every investigated crop - with and without an imidacloprid background concentration in soil - below the field-relevant NOEC of imidacloprid for honey bees (i.e. 20 µg imidacloprid/kg food matrix; Doc. No.: M-016832-01-1 and M-016845-01-1), (iv) also the study with clothianidin with fresh clothianidin residues showed no or only a marginal increase of residue levels in bee relevant matrices (max. 0.1 µg/kg; Doc.-No.: M-256474-01-1) and because (v) aged imidacloprid residues can be expected to be much less bio-available in terms of root uptake by succeeding crops than fresh imidacloprid soil residues (Doc. No.: M-023945-01-1), **it can be concluded that imidacloprid-seed-treated crops grown in soils with an imidacloprid background concentration as a succeeding crop will not pose an unacceptable risk to honey bees and bee keeping practices.**

## 2.7 NON-PROFESSIONAL USES OF IMIDACLOPRID IN HOME AND GARDEN AREAS

Provado® Garden is authorised in The Netherlands for uses in pome fruit, ornamentals and lawns. Concerns were raised by Ctgb whether the restriction to pre-flowering applications in pome fruits, as established for the agronomic uses (i.e. BBCH 10, mouse-ear stage), will reliably be respected by non-professionals. In order to address this question, Bayer CropScience has prepared a document (5.1.2.e 128; date: 04 MAR 2005), proposing a less and a more stringent wording as well as an illustration of the restriction to pre-flowering and post-flowering, i.e. when Provado® Garden can be used by non-professionals. Particularly the more stringent wording and illustration, as proposed in document 5.1.2.e 128, is considered to enable every non-professional to identify the crop stage where application of Provado® Garden is possible, considering honey bees foraging on flowering apple or pear trees. As such, Bayer CropScience is convinced that with an appropriate label in combination with an intuitive and illustrative user manual (e.g. illustration of growth stages as proposed in document 5.1.2.e 128 or illustration of situations where and when, respectively where and when not to apply), Povado® Garden can be used in pome fruit and ornamentals without adverse effects on honey bees. Moreover, it needs to be considered that potentially treated areas are small-scaled and as such deliver much less forage to bee colonies than e.g. commercial orchards, which require bee colonies to get hold of other pollen and nectar sources, which finally results in a dilution of potential residues at the hive level.

This holds also true for the question raised by Ctgb with regard to potentially flowering weeds around treated areas in house gardens.

Concerns were also raised with regard to the application of Provado® Garden to lawns. Bayer CropScience is convinced that also this use does not pose an unacceptable risk to bees, based on the risk assessment of Merit® Turf and the knowledge that the lawn use is commercialized as a specific product, which is mainly bought by consumers who will take proper care of their lawn. Furthermore, the use on private lawns is considered small scale in comparison to the Merit® Turf application.

When considering in addition the findings of Mayer and Lunden (1997; Doc.-No.: 110179-01-1) who applied imidacloprid at 112 g a.s./ha in an apple orchard with 10% open bloom and additionally with on average 6 flowering dandelions per m<sup>2</sup> understorey with no impact on honey bee mortality, in combination with the negligible phloem mobility of imidacloprid, **it can be concluded that risk for bees in house gardens from the use of Provado® Garden in pome fruit, ornamentals and lawns can be effectively mitigated by appropriated label instructions.**

## 2.8 PROFESSIONAL USES OF IMIDACLOPRID IN NON-AGRONOMIC AREAS

The label and the commercialisation of Merit® Turf will be adapted so that the product will be used in future only by professionals and only on intensively managed areas like golf courses, turf etc.. Also the label and the commercialisation of Maxforce® Quantum will be adjusted so that the outdoor applications are restricted to injection of the imidacloprid-treated sugar syrup directly into the ant nest entrances, which effectively mitigates exposure of bees.

### 3. CLOTHIANIDIN

#### 3.1 STATISTICAL HYPOTHESIS TESTING OF CLOTHIANIDIN LONG-TERM STUDIES

In France, on three locations, long-term exposure studies on honey bees are running (Hecht-Rost and others), by exposing the same honey colonies for three years in succession to flowering maize on isolated field plots, which have been grown from maize seeds, seed-treated with clothianidin at a rate of nominally 0.5 mg clothianidin a.s./kernel (nominal sowing rate: 100,000 seeds/ha). The treatment fields were matched by similar sized control fields. During summer/autumn 2011, the final reports for these studies are scheduled. On the basis of available data, no long-term adverse effects on honey bees and honey bee colonies can be concluded.

In a study in Austria, maize seeds seed-treated with clothianidin at a rate of nominally 1.25 mg clothianidin a.s./kernel were sown in 2010 in a cross-wise manner (nominal sowing rate: 100,000 seeds/ha) on cleared strips inside a full flowering OSR-field (Garrido et al.). During springtime 2011 the overwintering performance of the colonies has been assessed, during summer 2011 the final report of this study is scheduled. On the basis of available data, neither direct adverse effects on honey bees or honey bee colonies nor effects on the overwintering performance of the exposed colonies have been recorded.

In four studies on isolated field plots in France, honey bee colonies were exposed during sowing of maize seeds, seed-treated with clothianidin at a rate of nominally 0.5 mg clothianidin a.s./kernel (nominal sowing rate: 100,000 seeds/ha) and during the subsequent period when guttation was regularly observed on the maize seedlings (Liepold, 2010 a-d). The treatment fields were matched by similar sized control fields. In none of the studies adverse effects on honey bees and honey bee colonies have been observed, neither during the sowing operation of the clothianidin-treated maize seeds nor during the subsequent growing period of the maize seedlings when guttation occurred.

**Before finalisation of the long-term exposure studies in France and Austria, Bayer CropScience will contact Ctgb with regard to the request of the evaluator from *Bioresearch and Promotion* regarding the statistical hypothesis testing; in this discussion, also the already finalised Liepold-studies from 2010 will be included.**

#### 3.2 TEN DAYS ADULT CHRONIC FEEDING STUDY WITH CLOTHIANIDIN (LABORATORY); FURTHER HIGHER TIER STUDIES WITH CLOTHIANIDIN

Ctgb asked Bayer CropScience to submit their own data regarding the chronic laboratory NOEC in adult honey bees. Bayer CropScience can confirm that an own study is available, the endpoint currently used by Ctgb in their evaluations is identical (10 µg/kg; nominal) to the endpoint as determined in the previously submitted study Doc.-No.: M-255911-02-1.

In addition to this 10 day laboratory feeding study in adult honey bees, Bayer CropScience has in the interim also conducted an in-vitro study in honey bee larvae (Doc.-No.: M-359395-01-1) which revealed a NOEC of at least  $\geq 20$  µg/kg, verifying therefore the existing experience with imidacloprid and clothianidin, i.e. that these compounds control adult insects and do not have a specifically larvicidal activity. Neonicotinoids (as imidacloprid and clothianidin) act selectively on the insect central nervous system as agonists of the post-synaptic nicotinic acetylcholine receptors (Doc.-No.: M-347898-01-1).

Additional special investigations aimed at demonstrating whether clothianidin, brought into the bee hive via contaminated pollen, will be transferred to the next generation of honey bees (larvae) via larval food (royal jelly) provided by nurse bees (Doc.-No.: M-258354-01-1) are reported. In an additional assessment (Doc.-No.: M-259087-01-1), the effect of clothianidin in bee bread (mixture of pollen and honey consumed by nurse bees to produce royal jelly) on food gland development has been determined.

Colonies of approximately 10000 free-flying bees were deprived of their pollen stores and of the possibility of collecting pollen (pollen traps). While free to collect nectar, 10 hives were fed throughout the experiment with artificial bee bread (honey : pollen, 10 : 7.5), artificially contaminated at the target concentration of 10 µg clothianidin/kg; an other 8 hives received clothianidin-free artificial bee bread.

Of the 20 prepared hives, 2 were deserted by their bees (probably as a result of the death of the queens) and the study was therefore performed with 10 treated hives and 8 control hives. The food was distributed to the hives every 3 days and the amounts of food distributed were measured each time. On the 7<sup>th</sup> day of the experiment, the queens were removed from the hives to induce the bees to rear new queens and thus to encourage the production of royal jelly.

Artificial queen cells, grafted with larvae, were introduced into the hives on the 15<sup>th</sup> day of the experiment. From the 15<sup>th</sup> day of the experiment, all the young bees, old enough to be nurse bees and thus to produce royal jelly emerged during the experiment and their sole source of protein food was therefore the artificial bee bread with which the colony was provided. The royal jelly collected from that day onwards from the artificial queen cells was consequently produced by young bees whose sole pollen food source was the food provided in the experiment: either artificially contaminated in the case of the treated hives or uncontaminated in the case of the control hives. A total of 5 royal jelly samples were collected, in each case 3 days after the introduction of larvae into the queen cells.

The royal jelly samples were collected in accordance with strict measures to keep the samples separate so as to avoid contamination of the control samples and were immediately frozen.

The native honey and pollen batches used to prepare the artificial bee bread were analysed for random clothianidin contamination before spiking. No residues of clothianidin or of its metabolites TZNG and TZMU were detected in these samples (LOD 0.3 µg/kg). The artificial bee bread was spiked to achieve a nominal target concentration of 10 µg/kg. Analytical validation revealed a residue level of 9 µg/kg of clothianidin, the metabolites TZNG and TZMU being undetectable.

A stability test on residues in the artificial bee bread stored in the freezer was performed over a period of 4 months. The analysis showed no degradation of clothianidin in this matrix and under these storage conditions.

The residue analysis of all royal jelly samples revealed (Table 3.2.1) that there is no transfer of clothianidin from the food of nurse bees (bee bread contaminated with 10 µg/kg of clothianidin) to the royal jelly provided to the larvae when the nurse bees are exclusively fed with clothianidin-contaminated bee bread. Moreover, no impact on the development of the brood food glands of nurse bees was found when nurse bees exclusively fed on bee bread, contaminated with 10 µg/kg of clothianidin.

**Table 3.2.1: Clothianidin residues in royal jelly after consumption of clothianidin-treated (10 µg clothianidin/kg) and clothianidin-free artificial bee bread by nurse bees (Doc.-No.: M-258354-01-1)**

		Clothianidin residues (clothianidin, TZNG & TZMU, respectively) in royal jelly [µg/kg]				
Hive number	Treatment group	Date sampled				
		09 AUG 2005	11 AUG 2005	15 AUG 2005	19 AUG 2005	22 AUG 2005
1B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
2B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
3B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
4B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
5B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
6B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
7B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
8B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
9B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
10B	treated	<LOD	<LOD	<LOD	<LOD	<LOD
11A	control	Sample size too small	<LOD	<LOD	<LOD	<LOD
12A	control	<LOD	<LOD	<LOD	<LOD	<LOD
13A	control	absent	<LOD	<LOD	<LOD	<LOD
15A	control	<LOD	<LOD	<LOD	<LOD	<LOD
16A	control	<LOD	<LOD	<LOD	<LOD	<LOD
17A	control	<LOD	<LOD	<LOD	<LOD	<LOD
19A	control	<LOD	<LOD	<LOD	<LOD	Sample size too small
20A	control	<LOD	<LOD	<LOD	<LOD	<LOD

Limit of Quantitation (LOQ) = 1 µg/kg for all test items; Limit of Determination (LOD) = 0.3 µg/kg for all test items

Moreover, under confined conditions, potential long-term effects of clothianidin residues on honey bee populations were investigated on small honey bee colonies which consisted of about 500 worker bees of different ages and one sister-queen, which were exclusively fed with pollen respectively sunflower honey, artificially contaminated with clothianidin (Doc.-No.: M-031689-03-1, Doc.-No.: M-031695-01-1). The concentrations in the fortified matrix were verified by residue analysis before use. The development of the small bee colonies, which were confined within 50 m<sup>2</sup> gauze tunnels on oat-cropped plots, were examined for treatment-related impacts over a period of 41 days. The evaluated endpoints were mortality, comb cell production, food consumption, storage behaviour, hive weight increase, egg laying activity, breeding success, colony strength, foraging intensity and behavioural anomalies. Both long-term studies revealed that clothianidin residues of at least 0.02 mg/kg (= 20 µg/kg) in either nectar or pollen do not adversely affect any of the measured endpoints, including colony vitality and development. This field-relevant NOEC of 20 µg/kg has been verified in a further shorter-term field study (Doc.-No.: M-031717-01-1) where small honey bee colonies (approximately 5000 - 10000 honey bees) were exclusively fed over several days with a sucrose solution, containing 0, 10 or 20 µg clothianidin a.s./kg sucrose solution under non-caged conditions within an area with no alternative food supply.

As these studies were conducted on honey bee colonies and investigated potential impacts of continuous feeding on clothianidin-treated diets for two honey bee reproduction cycles, the NOEC of 20 µg/kg is considered to be ecologically more meaningful than a 10-day laboratory feeding study under laboratory conditions with individual bees. Accordingly, when assessing the risk of systemic clothianidin residues, the results of this higher-tier study should be used.

### 3.3 SUCCEEDING CROP STUDIES WITH CLOTHIANIDIN

Bayer CropScience was asked to conduct/submit studies to investigate the uptake of clothianidin by succeeding crops and to clarify whether the uptake of soil-borne residues may pose an unacceptable risk for honey bees. Bayer CropScience can confirm that those types of studies have been conducted. In the following, the studies are briefly summarised and discussed, followed by an evaluation of the results.

*Clothianidin-residues in bee relevant matrices from accumulated soil residues in a continuous maize mono-culture scenario (worst-case soil exposure situation)*

As a first step, the realistic worst-case long-term soil background concentration after successive use of clothianidin as maize seed treatment over several years has been calculated (Doc.-No.: M-257195-01-1). The calculation on the basis of 50 g a.s./ha per year resulted in 17.2 µg a.s./kg soil. To achieve the target background concentration, 90 g a.s. clothianidin / ha were applied via a spray treatment with Clothianidin FS 600 (Poncho®) and the spray deposit was incorporated into 20 cm depth on two fallow test plots, respectively, located at 5.1.2.e (Doc.-No.: M-256564-01-1) at 29 MAR 2005 and on 5.1.2.e (Doc.-No.: M-256474-01-1) at 21 MAR 2005. Another two fallow test plots remained without any clothianidin spray application, respectively.

Residue analysis of soil samples revealed, that the application and incorporation of clothianidin resulted on the location 5.1.2.e in a background concentration of 22.8 µg clothianidin/kg soil at the day of application and of 18.0 µg clothianidin/kg soil at the day of maize drilling and on the location 5.1.2.e in a background concentration of 19.7 µg clothianidin/kg soil at the day of application and of 19.2 µg clothianidin/kg soil at the day of maize drilling.

After an ageing period of 55 (location 5.1.2.e) and 42 days (location 5.1.2.e) respectively, following the spray application of Clothianidin FS 600, either “undressed” (= only fungicide treated) or clothianidin- (Poncho®)-dressed maize seeds were sown to the test plots on 23 MAY 2005 (location 5.1.2.e) and on 02 MAY 2005 (location 5.1.2.e), respectively, resulting in a study plot design per location as follows:

- Control plot (untreated soil, ‘undressed’ maize seeds)
- Treatment scenario 1 (untreated soil, clothianidin dressed maize seeds)
- Treatment scenario 2 (treated soil, clothianidin dressed maize seeds)
- Treatment scenario 3 (treated soil, ‘undressed’ maize seeds)

**Table 3.3.1: Clothianidin residues in maize pollen, investigating the up-take of accumulated soil residues (aging period simulating crop failure /continuous maize mono-culture situation) from repeated uses of clothianidin (Doc.-No.: M-256564-01-1 and M-256474-01-1)**

Residues in maize pollen [µg a.s./kg pollen]					
Location <sup>5.1.2.e</sup>					
Treatment	Clothianidin soil treatment	Clothianidin dressed seeds	Clothianidin	TZNG	TZMU
Scenario 1	-	x	1.2	< LOQ	< LOQ
Scenario 2	x	x	1.3	< LOQ	< LOQ
Scenario 3	x	-	< LOQ	< LOQ	< LOQ
Control	-	-	< LOQ	< LOQ	< LOQ
Location <sup>5.1.2.e</sup>					
Scenario 1	-	x	1.8	< LOQ	< LOQ
Scenario 2	x	x	1.9	< LOQ	< LOQ
Scenario 3	x	-	< LOQ	< LOQ	< LOQ
Control	-	-	< LOQ	< LOQ	< LOQ

LOQ = 1 µg/kg for all test items

The analytically verified soil concentration of 18.0 and 19.2 µg clothianidin/kg soil (day of drilling) did not result in a quantifiable up-take and translocation of clothianidin into pollen of undressed maize. Clothianidin residues in pollen from clothianidin-dressed maize in clothianidin-pre-treated soil were nearly identical (difference 0.1 µg a.s./kg) to the residues in maize pollen from clothianidin-dressed maize in native soil.

*Clothianidin-residues in bee relevant matrices from accumulated soil residues in a flowering succeeding crop scenario (worst-case soil exposure situation)*

Spring oil-seed rape (OSR) had been selected for the purpose of this study as it represents a bee-relevant crop with a short vegetation period. OSR-seeds only dressed with a standard fungicide were drilled on the test plot after the established background concentration (see above) aged for 23 days. Residue analysis of soil samples revealed that the application and incorporation of clothianidin resulted in a background concentration of 25.8 µg clothianidin/kg soil at the day of application and of 21.0 µg clothianidin/kg soil at the day of spring OSR drilling.

With begin of the flowering period (BBCH growing stage 62 - 63) a gauze tunnel (approx. 50 m<sup>2</sup>) was set up at each study plot. A bee colony of about 3000 bees (*Apis mellifera carnica*) was installed into each of the tunnels. During the flowering period of spring OSR, nectar and pollen sampling bees were manually collected in the tunnels and stored deep frozen. Afterwards, the frozen bees were worked up by separating pollen loads from the bees and by extracting sampled nectar by puncturing the honey bulbs of the bees with an ultra-fine needle. In the following, extracted pollen and nectar was analyzed to determine potential residues of clothianidin and its metabolites TZMU and TZNG.

**Table 3.3.2: Clothianidin residues in nectar & pollen of spring OSR, investigating the uptake of accumulated soil residues (aging period simulating crop failure situation) from repeated uses of clothianidin (Doc.-No.: M-256718-01-1)**

Residues in pollen or nectar [µg a.s./kg] [ppb]				
<b>Control (OSR grown in untreated soil; date of drilling: 22 APR 2005)</b>				
Sampling ID	Sampling date	Clothianidin	TZNG	TZMU
Pollen 1	2005-06-25 & 27	< LOQ	< LOQ	< LOQ
Pollen 2	2005-06-28 & 29	< LOQ	< LOQ	< LOQ
Pollen 3	2005-07-03/04 & 08	< LOQ	< LOQ	< LOQ
∅ Pollen	2005-06-25 – 2005-07-08	< LOQ	< LOQ	< LOQ
Nectar	2005-06-25 – 2005-07-08	< LOQ	< LOQ	< LOQ
<b>Treatment (OSR grown in clothianidin treated soil; date of drilling: 22 APR 2005)</b>				
Sampling ID	Sampling date	Clothianidin	TZNG	TZMU
Pollen 4	2005-06-25 & 27	3.6	< LOQ	< LOQ
Pollen 5	2005-06-28 & 29	3.6	< LOQ	< LOQ
Pollen 6	2005-07-03 & 04	4.0	< LOQ	< LOQ
Pollen 7	2005-07-08	2.8	< LOQ	< LOQ
∅ Pollen	2005-06-25 – 2005-07-08	3.5	< LOQ	< LOQ
Nectar	2005-06-25 – 2005-07-08	2.2	< LOQ	< LOQ

Limit of Quantitation (LOQ) = 1 µg/kg for all test items

An analytically verified soil concentration of 21.0 µg clothianidin/kg soil (day of drilling) resulted on average in a residue level of 3.5 µg clothianidin/kg pollen (max. 4.0 µg clothianidin/kg pollen). The induced residue level in nectar was found to be 2.2 µg clothianidin/kg.

*Clothianidin-residues in bee relevant matrices from accumulated soil residues in a flowering succeeding crop scenario (more realistic soil exposure situation)*

To achieve the target background concentration (17.2 µg a.s./kg soil, on the basis of 50 g clothianidin/ha per year; Doc.-No.: M-257195-01-1), 90 g a.s. clothianidin / ha were applied via Clothianidin FS 250 and incorporated into 20 cm depth on two fallow test plots, one located at

5.1.2.e

(Doc.-No.: M-291950-01-1), the other test plot was located at 5.1.2.e

5.1.2.e

(Doc.-No.: M-291947-01-1). A fallow test plot remained without any Clothianidin spray application and served as control plot on each study location, respectively. At the same day where the spray application and incorporation of Clothianidin FS 250 took place (establishment of soil background concentration), additionally clothianidin-dressed winter barley seeds were sown (0.5 g clothianidin/kg seeds, seed drilling rate: 160 kg seeds/ha, resulting in 80 g clothianidin/ ha). After harvesting of winter barley, clothianidin-untreated winter OSR-seeds were sown to the test plots on both study locations. No further crop was sown during the intervening period after harvesting of winter barley and sowing of winter OSR. A fallow test plot remained without any clothianidin treatment served as control plot on both locations, respectively. With the start of the OSR-flowering period (BBCH stage 60) a gauze tunnel (approximately 56 m<sup>2</sup>) was set up at each study plot. A bee colony of about 3000 bees (*Apis mellifera carnica*) was installed into each of the tunnels. During the flowering period of winter OSR, nectar and pollen foraging bees were manually collected in the tunnels and stored deep frozen. Then, the frozen bees were worked up by separating pollen loads from the bees and by extracting sampled nectar by puncturing the honey bulbs of the bees with an ultra-fine syringe. Afterwards, extracted pollen and nectar was analyzed to determine residue levels of clothianidin and its metabolites TZMU and TZNG.

Residue analysis of soil samples revealed, that the application and incorporation of Clothianidin FS 250 resulted on the location <sup>5.1.2.e</sup> in a background concentration of 33.6 µg clothianidin/kg and on the location <sup>5.1.2.e</sup> in a background concentration of 20.3 µg clothianidin/kg soil. After a period of nearly one year, one day before drilling of clothianidin-untreated winter OSR-seeds, the clothianidin concentration in soil decreased to 12.9 µg/kg soil on the location <sup>5.1.2.e</sup> and to 11.9 µg/kg soil on the location <sup>5.1.2.e</sup>. The control plots on both locations showed no residues of clothianidin. Residues of the metabolites clothianidin-MNG and clothianidin-TZNG in soil were always below limit of determination (LOD; 2 µg/kg soil).

**Table 3.3.3: Clothianidin residues in nectar and pollen of winter OSR, investigating the up-take of accumulated soil residues (more realistic, but still conservative soil residue aging) from repeated uses of clothianidin (Doc.-No.: M-291950-01-1 and M-291947-01-1 )**

Residues in pollen or nectar [µg a.s./kg]				
Location <sup>5.1.2.e</sup>				
<b>Control (OSR grown in untreated soil)</b>				
Sampling ID	Sampling date	clothianidin	TZNG	TZMU
Pooled Nectar Sample 002+004+005	2007-04-12 & 13 & 24	< LOD	< LOD	< LOD
Pollen Sample 001	2007-04-12	< LOD	< LOD	< LOD
Pollen Sample 003	2007-04-13	< LOD	< LOD	< LOD
Pollen Sample 006	2007-04-24	< LOD	< LOD	< LOD
<b>Treatment (OSR grown in clothianidin treated soil)</b>				
Sampling ID	Sampling date	clothianidin	TZNG	TZMU
Nectar Sample002	2007-04-12	< LOD	< LOD	< LOD
Nectar Sample 004	2007-04-13	< LOD	< LOD	< LOD
Nectar Sample 005	2007-04-24	< LOQ	< LOD	< LOD
Pollen Sample 001	2007-04-12	< LOQ	< LOD	< LOD
Pollen Sample 003	2007-04-13	< LOQ	< LOD	< LOD
Pollen Sample 006	2007-04-24	< LOQ	< LOD	< LOD
Location <sup>5.1.2.e</sup>				
<b>Control (OSR grown in untreated soil)</b>				
Sampling ID	Sampling date	clothianidin	TZNG	TZMU
Pooled Nectar Sample 001 + 004	2007-04-10 & 11	< LOD	< LOD	< LOD
Nectar Sample 006	2007-04-12	LOD	< LOD	< LOD
Nectar Sample 008	2007-04-13	< LOD	< LOD	< LOD
Pooled Pollen Sample 002 + 003	2007-04-10 & 11	< LOD	< LOD	< LOD
Pooled Pollen Sample 005 + 007	2007-04-12 & 13	< LOD	< LOD	< LOD
<b>Treatment (OSR grown in clothianidin treated soil)</b>				
Sampling ID	Sampling date	clothianidin	TZNG	TZMU
Pooled Nectar Sample 001 + 004	2007-04-10 & 11	< LOQ	< LOD	< LOD
Nectar Sample 006	2007-04-12	< LOQ	< LOD	< LOD
Nectar Sample 008	2007-04-13	< LOQ	< LOD	< LOD
Pooled Pollen Sample 002 + 003	2007-04-10 & 11	< LOQ	< LOD	< LOD
Pollen Sample 005	2007-04-12	1.0	< LOD	< LOD
Pollen Sample 007	2007-04-13	< LOQ	< LOD	< LOD

Limit of Quantitation (LOQ) = 1 µg/kg for all test items; Limit of Determination (LOD) = 0.3 µg/kg for all test items

Under still conservative, but more realistic use conditions (long-term background concentration applied via Clothianidin FS 250 spray application on bare soil, followed by immediate incorporation into the soil and by the additional sowing of clothianidin-dressed winter barley at a rate of 80 g clothianidin/ha before sowing of clothianidin-untreated winter-OSR), the maximum residue of clothianidin in bee relevant matrices of a flowering and bee attractive succeeding crop (nectar and pollen of winter-OSR) was found to be 1.0 µg a.s./kg; all other residue values of clothianidin in the treatment groups were either < LOQ or < LOD; residues of metabolites of clothianidin (TZMU and TZNG) in nectar & pollen in the treatment groups were always < LOD.

*Transfer of soil-borne clothianidin residues in bee relevant matrices, residue levels of clothianidin in bee relevant matrices via seed-treatment and corresponding risk evaluation for honey bees*

In a study simulating a continuous maize mono-culture scenario (Doc.-No.: M-256564-01-1 and M-256474-01-1) it has been demonstrated that an analytically verified soil concentration of 18.0 and 19.2 µg clothianidin/kg soil (day of drilling) did not result in a quantifiable up-take and translocation of clothianidin into pollen of undressed maize; clothianidin residues in pollen from clothianidin-dressed maize in clothianidin-pre-treated soil were nearly identical (difference 0.1 µg a.s./kg) compared to the residues in maize pollen from clothianidin-dressed maize in untreated soil. Nonetheless, it needs to be considered that in this study a soil background concentration has been established via freshly incorporated clothianidin, which under practical use conditions would take several years of continuous clothianidin use before this level is reached. Thus, the entire long-term soil background concentration established in this study can be expected to be (nearly) fully bio-available due to an un-realistic worst case aging period of only 55 and 42 days.

In an investigation of the adsorption behaviour of clothianidin in soil (Doc.-No.: M-032226-01-2), it was shown that  $K_{OC}$  values of clothianidin increased by a factor 2.84 (sandy loam) and 3.46 (silt loam), respectively, during ageing of residues in soil for 99 days. These data indicate that clothianidin will be considerably less bio-available in soil due to an increased sorption to the soil matrix over time. Thus, the employed aging period of freshly incorporated clothianidin soil residues of only 55 and 42 days (see above) can be considered to represent (i) an unrealistic worst case regarding normal continuous use conditions of clothianidin-seed-treated maize and to represent (ii) realistically the situation of crop failure (i.e. re-planting of clothianidin-dressed maize seeds in a soil which recently received clothianidin-dressed maize seeds that failed to emerge), as 50 g clothianidin/ha results in a  $PEC_{initial}$  of 17 µg clothianidin/kg soil, considering a soil depth of 20 cm (as the soil needs to be processed to re-prepare the seed bed for a further maize-planting).

However, it needs to be considered that generally maize follows maize after 1 year, which is even  $\approx 3$  times longer than the aging period of 99 days as investigated in aged sorption study with clothianidin (Doc.-No.: M-032226-01-2), which already led to an increase of the sorption behaviour of clothianidin to the soil matrix by a factor of  $\approx 3$ . Thus, after one year of aging in soil, undegraded clothianidin can be expected to be less bio-available and therefore less prone for plant up-take than the fresh clothianidin employed for the study.

Based on the germination requirements of maize seeds, i.e. rather high soil temperature required, spring-OSR will not be planted in case of maize crop failure, as spring-OSR needs to be sown under short-day conditions, preferably in March, in order to develop enough bastard branches to assure sufficient yield (if planted too late in the year, too little vegetative biomass is produced before entering the reproductive phase). Even winter-OSR planting in the year of maize crop-failure is unlikely, as farmers do usually not change their crop rotations due to soil nutrition and soil pest control. Thus, in case of maize crop failure, the most likely scenario is re-planting of maize, which is covered by the study simulation a continuous maize mono-culture scenario (Doc.-No.: M-256564-01-1 and M-256474-01-1). In further studies (Doc.-No.: M-291950-01-1 and M-291947-01-1) which investigated the transfer of soil-borne clothianidin residues into nectar and pollen of succeeding flowering crops under more realistic, but still conservative use conditions (i.e. application and incorporation of fresh clothianidin into the soil immediately before planting of clothianidin-dressed winter barely which was followed after cereal harvest by clothianidin-untreated winter-OSR), except of one single measurements, all residue data were below the LOQ (=1.0 µg/kg); in one single measurements, clothianidin has been determined at a level of 1.0 µg/kg.

Even after the un-realistically short aging period of freshly incorporated clothianidin soil residues of only 23 days (M-256718-01-1), which can be considered to represent an un-realistic worst case regarding normal succeeding-crop conditions for clothianidin-seed-treated maize (maize followed by OSR in the following year), the freshly incorporated clothianidin resulted on average in a residue level of 3.5 µg clothianidin/kg pollen and of 2.2 µg clothianidin/kg nectar, which is significantly below the acute or chronic threshold levels.

**Accounting for the field-relevant NOEC for honey bee colonies of 20 µg clothianidin/kg food matrix, it can be concluded that both, systemic residues of clothianidin after seed-treatment in maize as well the uptake of soil-borne clothianidin residues by succeeding crops does not pose an unacceptable risk to honey bees.**

### 3.4 SENSITIVITY OF APHIDS AGAINST CLOTHIANDIN AS COMPARED TO HONEY BEES

For imidacloprid, also a nitro-substituted neonicotinoid insecticide as clothianidin, it has been demonstrated that pest aphids are much more susceptible than honey bees (imidacloprid-LD50 for aphids: 0.54 pg/aphid; see Doc.-No.: M-110655-01-1). In the DAR of clothianidin (B.3 Data on application and further information; B.3.1.6 Effects achieved - mode of action (Annex IIA 3.4.3, 3.5.1) the following information is provided:

*“...The inhibition of [<sup>3</sup>H]imidacloprid binding to nAChR in housefly head membrane preparations by the compounds is expressed as pI<sub>50</sub>-value. pI<sub>50</sub>-values (= - log M) correspond to the concentration of cold ligand displacing 50% of bound [<sup>3</sup>H]imidacloprid from housefly head membranes:*

<b>Compound</b>	<b>(n)</b>	<b>[<sup>3</sup>H]imidacloprid pI<sub>50</sub></b>
<i>Imidacloprid</i>	4	9.1
<i>Clothianidin</i>	3	9.2
<i>Acetamiprid</i>	3	8.7
<i>Nitenpyram</i>	3	8.6
<i>Thiacloprid</i>	3	9.3

*...Mixed populations of three aphid species were treated by dipping aphid infested plant leaves into insecticide solutions of different concentrations. Furthermore one leaf-hopper species was also tested by transferring untreated insects to treated rice seedlings. Both methodologies are standardized screening procedures used for many years. The resulting dose response data were subjected to probit analysis in order to calculate LC<sub>90</sub>-values 6d after treatment...considering the dip-test results it can be concluded that the biological efficacy of clothianidin against the sucking pests tested is roughly comparable to that of imidacloprid and thiacloprid...*

<b>Compound</b>	<b><i>Myzus persicae</i></b>	<b><i>Aphis fabae</i></b>	<b><i>Aphis gossypii</i></b>	<b><i>N. cincticeps</i></b>
<i>Clothianidin</i>	1.54	8.8	2.47	0.052
<i>Imidacloprid</i>	3.18	10	2.5	0.54
<i>Thiacloprid</i>	6.95	2.1	3.1	2.8

...”

As such, when comparing the above mentioned data on clothianidin and imidacloprid, it can be concluded that clothianidin exhibits a similar intrinsic affinity to nAChR-binding sites than imidacloprid at a similar to even higher efficacy against aphids. Thus it can be concluded that the sensitivity difference between pest aphids and honey bees is even larger for clothianidin as it is for imidacloprid.

## 4. REFERENCES

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5.1.2.e	2001	Effects of TI 435 techn. residues in sunflower honey on the development of small lbee colonies and on behavior and mortality of honey bees. Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: January 18, 2001, Document No.: M-031695-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	2001	Effects of TI 435 techn. residues in pollen on the development of small bee colonies and on behavior and mortality of honey bees. Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: January 18, 2001, amended: March 20, 2001 and February 21, 2003 Document No.: M-031689-03-1 GLP, unpublished	Yes	BCS
5.1.2.e	2001a	Effects of diet (sugar solution) spiked with TI 435 techn. on behavior and mortality of honey bees ( <i>Apis mellifera</i> ) and on the weight development of bee colonies under field conditions. Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: January 18, 2001 Document No.: M-031717-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	2001	Effects of residues of imidacloprid in maize pollen from dressed seeds on honey bees ( <i>Apis mellifera</i> ) Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: April 17, 2001 Document No.: M-052637-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	2002	Evaluation of the effects of residues of imidacloprid FS 600 in maize pollen from dressed seeds on honeybees ( <i>Apis mellifera</i> ) in the semifield Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: March 07, 2002 Document No.: M-052238-01-1 GLP, unpublished	Yes	BCS

Author(s)	Year	Title, Source, Company Name, Owner, Date Document-No., GLP status (where relevant), published or not	Data protection claimed	Owner
5.1.2.e	2005	Determination of Residue Levels of Clothianidin, TZNG and TZMU in Maize Pollen in a Succeeding Crop Scenario at 5.1.2.e 5.1.2.e Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: August 26, 2005 Document No.: M-256474-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	2005	Determination of Residue Levels of Clothianidin, TZNG and TZMU in Maize Pollen in a Succeeding Crop Scenario at 5.1.2.e " 5.1.2.e Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: August 31, 2005 Document No.: M-256564-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	2005	Determination of residue levels of Clothianidin, TZMU and TZNG in bee-relevant matrices of summer rape in a succeeding crop scenario at 5.1.2.e 5.1.2.e Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: September 02, 2005 Document No.: M-256718-01-1 GLP, unpublished	Yes	BCS
Oi, M.	1999	Time-dependent sorption of imidacloprid in two different soils J. Agric. Food Chem. 1999, 47, 327-332 Document No.: M-023945-01-1 Published	Not applicable	
5.1.2.e	2007	Determination of residue levels of clothianidin, TZMU and TZNG in bee-relevant matrices of winter rape in a cereal succeeding crop scenario at 5.1.2.e 5.1.2.e Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: August 29, 2007 Document No.: M-291950-01-1 GLP, unpublished	Yes	BCS

Author(s)	Year	Title, Source, Company Name, Owner, Date Document-No., GLP status (where relevant), published or not	Data protection claimed	Owner
5.1.2.e	2007	<p>Determination of residue levels of clothianidin, TZMU and TZNG in bee-relevant matrices of winter rape in a cereal succeeding crop scenario at 5.1.2.e</p> <p>5.1.2.e</p> <p>Generated by: 5.1.2.e</p> <p>5.1.2.e</p> <p>Owner: Bayer CropScience Date: August 29, 2007 Document No.: M-291947-01-1 GLP, unpublished</p>	Yes	BCS
5.1.2.e	1999	<p>Residue levels of imidacloprid and imidacloprid metabolites in nectar, blossoms and pollen of sunflowers cultivated on soils with different imidacloprid residue levels and effects of these residues on foraging honeybees. Test location: 5.1.2.e</p> <p>1999</p> <p>Generated by: 5.1.2.e</p> <p>5.1.2.e</p> <p>Owner: Bayer CropScience Date: September 27, 1999 Document No.: M-016820-01-1 GLP, unpublished</p>	Yes	BCS
5.1.2.e	1999	<p>Residue levels of imidacloprid and imidacloprid metabolites in nectar, blossoms and pollen of sunflowers cultivated on soils with different imidacloprid residue levels and effects on these residues on foraging honeybees. Test location: 5.1.2.e</p> <p>5.1.2.e 1999</p> <p>Generated by: 5.1.2.e</p> <p>5.1.2.e</p> <p>Owner: Bayer CropScience Date: September 28, 1999 Document No.: M-016827-01-1 GLP, unpublished</p>	Yes	BCS
5.1.2.e	1999	<p>Residue levels of imidacloprid and imidacloprid metabolites in nectar, blossoms and pollen of summer rape cultivated on soils with different imidacloprid residue levels and effects of these residues on foraging honeybees. Test location: 5.1.2.e</p> <p>5.1.2.e 1999</p> <p>Generated by: 5.1.2.e</p> <p>5.1.2.e</p> <p>Owner: Bayer CropScience Date: September 28, 1999 Document No.: M-016828-01-1 GLP, unpublished</p>	Yes	BCS

Author(s)	Year	Title, Source, Company Name, Owner, Date Document-No., GLP status (where relevant), published or not	Data protection claimed	Owner
5.1.2.e	1999	Residue levels of imidacloprid and imidacloprid metabolites in pollen of maize plants cultivated on soils with different imidacloprid residue levels. Test location: 5.1.2.e 1999 Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: September 28, 1999 Document No.: M-016836-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	1999	Residue levels of imidacloprid and imidacloprid metabolites in nectar, blossoms and pollen of summer rape cultivated on soils with different imidacloprid residue levels and effects of these residues on foraging honeybees. Test location: 5.1.2.e 1999 Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: September 28, 1999 Document No.: M-016842-02-1 GLP, unpublished	Yes	BCS
5.1.2.e	1999	Residue levels of imidacloprid and imidacloprid metabolites in pollen of maize plants cultivated on soils with different imidacloprid residue levels. Test location: 5.1.2.e 1999 Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: September 28, 1999 Document No.: M-016830-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	1999	Residues of imidacloprid and imidacloprid metabolites in nectar, blossoms, pollen and honey bees sampled from a French summer rape field and effects of these residues on foraging honeybees Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: April 30, 1999 Document No.: M-006815-01-1 GLP, unpublished	Yes	BCS

Author(s)	Year	Title, Source, Company Name, Owner, Date Document-No., GLP status (where relevant), published or not	Data protection claimed	Owner
5.1.2.e	1999	Residues of imidacloprid and imidacloprid metabolites in nectar, blossoms, pollen and honey bees sampled from a summer rape field in Sweden and effects of these residues on foraging honeybees Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: May 21, 1999 Document No.: M-006811-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	1999	Residues of imidacloprid and imidacloprid metabolites in nectar, blossoms, pollen and honey bees sampled from a British summer rape field and effects of these residues on foraging honeybees Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: June 24, 1999 Document No.: M-040023-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	1999	Residues of imidacloprid and imidacloprid metabolites in sunflower blossoms sampled in Argentina Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: September 25, 1998 Document No.: M-018436-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	1999	Effects of imidacloprid residues in sunflower honey on the development of small bee colonies under field exposure conditions Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: September 21, 1999 Document No.: M-016832-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	1999	Effects of imidacloprid residues in maize pollen on the development of small bee colonies under field exposure conditions Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: September 21, 1999 Document No.: M-016845-01-1 GLP, unpublished	Yes	BCS

Author(s)	Year	Title, Source, Company Name, Owner, Date Document-No., GLP status (where relevant), published or not	Data protection claimed	Owner
5.1.2.e	2003	Residue levels of imidacloprid and imidacloprid metabolites in sunflower pollen, sunflower honey and bees from Gaucho treated sunflowers in the field Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: January 21, 2003 Document No.: M-075630-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	2001	Tunnel Test: Assessment of Side Effects of Confidor SL 200 on the Honey Bee ( <i>Apis mellifera L.</i> ) in Apple Orchard Following Application before flowering (Mouse-Ear Stage) of the Crop Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: November 09, 2001 Document No.: M-084030-01-1 GLP, unpublished	Yes	BCS
5.1.2.e	2005	Carry-over of clothianidin from spiked bee bread to honeybee royal jelly. Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: September, 1998 Document No.: M-258354-01-1 Non-GLP, unpublished	Yes	BCS
5.1.2.e	2005	Effect of clothianidin in honeybee food gland development. Microscopic analysis. Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: October, 1998 Document No.: M-259087-01-1 Non-GLP, unpublished	Yes	BCS
5.1.2.e	2001	Time-dependent sorption of TI-435 in two different soils. Generated by: 5.1.2.e 5.1.2.e Owner: Bayer CropScience Date: January 17, 2001 Document No.: M-032226-01-2 GLP, unpublished	Yes	BCS

Author(s)	Year	Title, Source, Company Name, Owner, Date Document-No., GLP status (where relevant), published or not	Data protection claimed	Owner
Vacante, V.	1997	The influence of Imidacloprid on the impollination of the tomato with <i>Bombus terrestris</i> Generated by: University of Tuscia, Viterbo, Italy Owner: Bayer CropScience Date: Year 1997 Document No.: M-304435-01-2 Non-GLP, published	Yes	BCS