Imidacloprid Bee Studies

Compilation of Study Summaries

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Bayer AG, Crop Science Division, Monheim, Germany

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Imidacloprid Bee Studies – Compilation of Study Summaries

01 - Metabolism

01.01 - Plant

Report:	01.01/01; [redacted]; 1988; M-024270-01-3
Title:	Absorption and translocation of 14C-NTN 33893 in eggplants and rice plants
Report No.:	NR1273
Document No.:	M-024270-01-3
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**no**

<<M-024270-01-3@S-602974-01-1

The absorption and translocation of NTN 33893 {imidacloprid (under application to ISO), l-(2-chloro-5-pyridinylmethyl)-2- nitroiminoimidazolidine} in young eggplants and rice plants were investigated over a period of 8 days following application of[pyridinyl-^{14}C-methyl] NTN 33893 by painting to the aerial parts and addition to the nutrient solution. The behavior of ^{14}C-NTN 33893 was similar between the two plants. Following application to the aerial parts, ^{14}C penetrated into the plants and exhibited significant acropetal translocation. The distribution of -^{14}C applied to leaf blade, petiole (eggplants) and leaf sheath (rice plants) was almost restrictive to the applied leaf, especially to its marginal area, and was small in the other parts. In the case of stem application (eggplants) in which ^{14}C penetrated via the lower part of the plants than in the cases above, ^{14}C was distributed rapidly to all the upper parts of plants. The amount of unchanged NTN 33893 in leaf wash was greater in lower surface application than that in upper surface application. On the contrary, the rates of penetration and conversion were larger in upper surface application, suggesting the great contribution of photodegradation in foliar application of NTN 33893. Further, part of photodegradation products were assumed to possess more leaf-penetrability and volatility. In nutrient solution application, ^{14}C was absorbed via roots and translocated rapidly to the aerial parts, and accumulated to the leaf margins. Although NTN 33893 was metabolized in plant tissues after uptake via roots, the parent compound was still the main component of labelled residue in plants.

>>M-024270-01-3@S-602974-01-1

Report:	01.01/02; [redacted]; 1989; M-024273-01-3
Title:	Isolation and identification of metabolites of NTN 33893 in rice by water culture
Report No.:	NR1282
Document No.:	M-024273-01-3
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-024273-01-3@S-602982-01-1

Metabolites of NTN 33893 in rice plants were investigated by applying ^{14}C and ^{13}C labeled and non-labeled chemicals in hydroponic solution (ca.50 mg/L). The 7-leaf stage rice plants were grown in the hydroponic solution for 21 days and then were used for the study. Absorption of NTN 33893 into the rice plants was estimated to be 95% of applied dose. The absorbed chemicals dominantly located in the aerial part (99% of radioactivity in the whole plants). The methanol extracts (85% of the dose) were fractionated into dichloromethane (46% of the dose) and aqueous fraction (37%). Non-labeled extracts (dose: 500 rag) were fortified with 1AC-labeled extracts for isolation of metabolites. Within ten isolated components, seven were identified by MS, NMR and co-chromatography with authentic standards. Major components were unchanged NTN 33893 (I, 34% of the dose), des-nitro derivative (= imine, NTN 38014, II, 31%). The other minor metabolites were hydroxylated one (WAK 4103, III, 4%), reduced compound (WAK 3839, IV, 3%), cyclic urea (NTN 33519, V, 1%), olefinic metabolite (NTN 35884, VI, 0.4%) and 6-chloronicotinic acid (VII, 0.3%). Identification percent was ca.74% of the dose and ca.86% in the extractable fraction. Bound fraction (9% of the dose) was exhaustively extracted and ca.88% of ^{14}C was solubilized. Metabolite [II] was detected in the soluble fraction. This suggested some part of the bound

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residue contained original skeleton of the parent compound. The intake of 1AC into the natural constituents seemed to be small. Crude extracts of rice plants which were treated with ^{13}C-labeled NTN 33893 were analyzed by ^{13}C-NMR. Metabolites [I], [II], [IV] and [V] were detected on ^{13}C-NMR spectra. Especially compound [II] was known to be a dominant metabolite at the early stage of this study and this information contributed to develop the analytical procedure.

>>M-024273-01-3@S-602982-01-1

Report:	01.01/03; [redacted]; 1991; M-024279-01-3
Title:	Metabolism of (pyridinyl-14C-methyl) NTN 33893 in rice plants (nursery box application)
Report No.:	NR1284
Document No.:	M-024279-01-3
Guideline(s):	EPA Guideline [Subdivision O, Section 171-4(a)]
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-024279-01-3@S-602991-01-1

The absorption, translocation and metabolism of [pyridinyl-^{14}C-methyl] NTN33893 in rice plants were investigated in a laboratory study. The total terminal residues in rice grain and straw were also characterized. The application rates were normal (0.32kg a.i./ha) and exaggerated four-fold (1.26kg AI/ha). The normal dose corresponds to the maximum application rate by nursery box treatment.

Approximately 4% of applied dose was translocated to immature rice shoot within 65 days posttreatment. The level of translocation did not increase appreciably afterwards and only 4.4% of applied dose was found in the aerial part harvested at 124 days posttreatment. Rice grain contained trace amounts of radioactive residues, while 98% of the radioactive residues in the aerial part remained in straw. The total terminal residues in grain were 0.014 ppm (normal dose) and 0.064 ppm (exaggerated dose) ^{14}C-NTN33893 equivalents.

In the shoot and straw, 7 compounds were identified including unchanged NTN33893. The metabolites were NTN38014, WAK3839, WAK4103, NTN35884, NTN33519 and CNA (6-chloronicotinic acid). NTN38014 was the major component in both shoot and straw, accounting for 53% and 46% of the total radioactivities, respectively, while the quantity of NTN33893 was 9%. Of the other metabolites, WAK3839, WAK4103 and NTN35884 were less than 2% respectively. NTN33519 (11 - 12%) and CNA (4 - 6%) were primarily found in the unextractable fraction by stringent extraction. NTN33519 released from the unextractable fraction was considered to be an artifact.

NTN33893 was the major component in the extractable fraction from grain, accounting for 12% of the total terminal residues. Metabolites in the extractable fraction included WAK4103 (3.5%), NTN35884 (2.0%) and trace amounts of NTN38014, CNA and WAK3839. About 70% of the radioactivity in grain was unextractable bound residues. The crude starch contained 67% (48% of total ^{14}C) of the bound residues. The glucose obtained by glycolysis of the starch was revealed to be radiolabeled with a constant specific radioactivity, suggesting that ^{14}C-carbon dioxide derived from ^{14}C-NTN33893 was incorporated into natural constituents.

The percentages of metabolites identified in shoot, straw and grain were 79.2%, 73.8% and 83.8%, respectively. The metabolic pathway of NTN33893 in rice plants was proposed on the basis of metabolites identified in this study.

>>M-024279-01-3@S-602991-01-1

Report:	01.01/04; [redacted]; 1992; M-024334-01-2
Title:	Metabolism of NTN 33893 in eggplant by planting hole application
Report No.:	NR1290
Document No.:	M-024334-01-2
Guideline(s):	EPA Guidelines Subdivision 0 Section 171-4(a)2
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-024334-01-2@S-603083-01-1

Metabolism of NTN 33893 (pyridylmethy-^{14}c-label) in eggplant was done under the GLP regulations. The objective of this study was to clear absorption, translocation and degradation of NTN 33893 in the plant after 1 % granule (0.94 % a.i.) was applied to soil at a maximum commercial rate of 2 g per a young plant (8 leaves stage) by planting hole application. The radioactivities were accounted for 2.72% of the applied radioactivity in aerial parts (stem and leaves) at 14 days after the application and an average of 1.64% in aerial parts (foliage and fruit) at 69 days. Since more than 88 % of the radioactivities were found in the leaves, the absorbed radioactivities seemed to be translocated acropetally to leaves. In the edible parts sampled at 49, 53 and 67 days after the application, 0.01 to 0.02 % of the applied radioactivity (0.032 to 0.053 mg/kg, an average of 0.043 mg/kg in NTN 33893 equivalent) were found.

Similar metabolites were found both in the leaves and edible parts. WAK 4103, NTN 35884, NTN 33519, WAK 3839, NTN 38014, RBN 1114 and CNA were found as metabolite in the foliage at 69 days after the application. Major metabolites in the foliage were NTN 38014 (an average of 24.6 % of the radioactivity found in the foliage), RBN 1114 (5.6%) and WAK 4103 (3.6 %). Unchanged parent compound and nonextracts were accounted for 10.2 % and 9.3 % of the found radioactivity, respectively.

In the edible parts, NTN33893 (an average of 18.9 % of the radioactivity found, an average of 0.0081 mg/kg), NTN 38014 (14.0 %, 0.0049 mg/kg), RBN 1114 (13.0 %, 0.0066 mg/kg), CNA (13.4 %, 0.0035 mg/kg), WAK 4103 (3.2 %, 0.0015 m
g/kg), NTN 35884(0.2 %, 0.0005 mg/kg) and WAK 3839 (0.1 %, <0.0005 mg/kg) were found. Major metabolites in the edible parts were NTN 38014, RBN 1114 and CNA. The amount of nonextracts was accounted for 6.5% (0.0028 mg/kg in NTN 33893 equivalent) and unknown metabolites accounted for greater than 10 % of the radioactivity were not present in the edible part.

The results of this study showed major metabolic pathways of NTN 33893 in eggplant are elimination of nitro moiety, hydroxylation of imidazolidine ring and cleavage of C-N bond between pyridylmethyl moiety and imidazolidine ring.

>>M-024334-01-2@S-603083-01-1

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Report:	01.01/05; [redacted]; 1991; M-026229-02-2
Title:	NTN 33893 - Metabolism in tomatoes - Addendum to NTN 33893 tomato report PF no.: 3257 (study numbers M 173 0 237-3 and M 173 0238-4) - Investigation on the metabolism of NTN 33893 after application to tomatoes
Report No.:	PF3257
Document No.:	M-026229-02-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-026229-02-2@S-602558-01-1

The metabolism of the insecticide NTN 33893 (I) was investigated after application of [pyridinyl-^{14}C-methyl] NTN 33893 to tomatoes.

14 days prior to the main harvest, an 0.2% spray solution of a 25 WP was applied to the surface of immature fruits until run-off. The fruits were harvested 4, 7, 14 and 21 days (= postharvest sample) after application.

A total residue of 0.85 mg/kg was present on day 14. 0.75 mg/kg accounted for intact parent compound (I), more than 0.59 mg/kg were located on the surface and could be washed off with methanol.

0.071 mg/kg·of the residue were shared by at least 8 metabolites which resulted from hydroxylation of the parent compound and/or hydrolysis and conjugation of hydrolysis products. Of these, the following compounds were identified by cochromatography with reference standards:

0.022 mg/kg "guanidine metabolite"	(II)
0.016 mg/kg "urea metabolite"	(III)
0.015 mg/kg "monohydroxy metabolite"	(IV, V)
0.004 mg/kg "olefin metabolite"	(VI)
0.006 mg/kg "nitrosimine metabolite"	(VIII)
< 0.001 mg/kg "chloropicolyl glucoside"	(X) and
0.007 mg/kg "chloropicolyl gentiobioside"	(XI)

Structural elucidation of NTN metabolites from tomato plants was achieved by spectroscopic methods after isolation of the compounds in a special model experiment set up for this purpose. In this case ^{13}C- and ^{14}C-labelled parent compound was used besides unlabelled NTN 33893 to facilitate the structure investigations. The application was made by stem injection. Sufficient amounts of the substances could be obtained because of the good metabolization - 24% of the 14c-radioactivity accounted for polar metabolites. The identified compounds are presented in a metabolic pathway (Fig. 40).

In an additional translocation experiment it could be shown that NTN 33893 or its metabolites do not get into the fruit via the foliage. Thus, the level of the total residue is determined by the spray deposit on the tomatoes.

These studies were conducted between December, 1987, and October, 1989 at the Institute for Metabolism Research of Bayer AG, Leverkusen, FRG.

<<M-026229-02-2@S-602558-01-1

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Report:	01.01/06; [redacted]; 1992; M-024320-01-2
Title:	Metabolism of NTN 33893 in corn after seed dressing
Report No.:	PF3673
Document No.:	M-024320-01-2
Guideline(s):	171-4 Nature of Residue (Metabolism) - Plants
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-024320-01-2@S-603082-01-1

The metabolism of the insecticide NTN 33893 (I)was investigated in corn after seed dressing with [pyridiny1-14c-methyl]NTN 33893. The active ingredient was formulated as a 70 WS and applied at a rate equivalent to 721 g a.i./100 kg of corn seed. The corn plants were grown in a greenhouse and harvested as immature corn (33 and 61 days after planting) and as mature plants (day 134). The mature plants were separated into dry grain, fodder, husks and cobs. The total residue, expressed in active ingredient equivalents, amounted to in immature corn 5.84 mg/kg (day 33) and 1.52 mg/kg (day 61), in dry grain (day 134) 0.04 mg/kg, in fodder (day 134) 3.08 mg/kg, in husks (day 134) 0.21 mg/kg and in cobs (day 134) 0.12 mg/kg.

By thin-layer chromatographic comparison (2 dimensional) with reference compounds and other methods the following compounds were identified in grain and fodder (amounts given in per cent of the radioactivity and in mg/kg active ingredient equivalents in the respective plant parts).

1. Grain

Unchanged parent compound	(I)	25.2%	0.010 mg/kg
Olefine compound	(VI)	13.1%	0.005 mg/kg
5-Hydroxy compound	(IV)	9.3%	0.004 mg/kg
Dihydroxy compound	(VII)	4.4%	0.002 mg/kg
6-Chloropicolylalcohol	(XIII)	4.4%	0.002 mg/kg
Guanidine compound	(II)	2.0%	<0.001 mg/kg
6-Chloronicotinic acid	(XII)	traces	traces
further components in lower concentrations		15.4%	ca.0.006 mg/kg

2. Fodder

Unchanged parent compound	(I)	22.2%	0.68 mg/kg
Guanidine compound	(II)	10.9%	0.34 mg/kg
5-Hydroxy compound	(IV)	5.0%	0.15 mg/kg
Olefine compound	(VI)	2.2%	0.07 mg/kg
Nitrosimine compound	(VIII)	1.8%	0.06 mg/kg
Ring opened guanidine compound	(XV)	1.6%	0.05 mg/kg
6-Chloronicotinic acid	(XII)	1.3%	0.04 mg/kg
6-Chloropicolylalcohol	(XIII)	1.1%	0.03 mg/kg
5-Hydroxy compound conjugate	(IV)	ca.1.0%	ca.0.03 mg/kg
Dihydroxy compound	(VII)	0.5%	0.02 mg/kg
Urea compound	(III)	traces	traces
further components in lower concentrations		20.3%	0.62 mg/kg

Exhaustive extraction of the solids of dry grain, after conventional extraction (26.2 %), released a further ca. 1.2% of the radioactivity as unchanged parent compound (I) or the urea compound (III) and 1.0% as the olefine compound (VI). At least 6 % of the radioactivty remaining in the solids (26.2 %) after conventional extraction was incorporated into glucose.

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Exhaustive extraction of the solids of fodder, after conventional extraction (32.1%), released unchanged parent compound I (4.4%, 0.14 mg/kg), the guanidine compound II (2.3%, 0.07 mg/kg), the olefine compound VI (0.3%, 0.009 mg/kg) and the urea compound III (7.0%, 0.22 mg/kg), probably as an artifact of the parent compound.

The identified compounds are shown in a proposed degradation pathway (Figure 27).
>>M-024320-01-2@S-603082-01-1

Report:	01.01/07; [redacted]; 1992; M-024315-01-2
Title:	Metabolism of (14C) NTN 33893 in apples
Report No.:	PF3676
Document No.:	M-024315-01-2
Guideline(s):	171-4 Nature of Residue (Metabolism), Plants
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-024315-01-2@S-603064-01-1
The metabolism of the insecticide NTN 33893 (I) was investigated in potatoes after application of [pyridinyl-^{14}C-methyl] NTN 33893. An 0.2% spray fluid of a 25 WP was applied to the foliage of potato plants 64 days before harvest. Vines and tubers were harvested 7, 28 and 64 days after application. At the time of harvest (day 64) the vines were withered and largely dry like under practical conditions; in this case the total residue amounted to 1.35 mg/kg.

0.90 mg/kg of this could be identified by chromatographic comparison with reference standards and by other physical methods:

0.51 mg/kg "NTN 33893"	(I)
0.17 mg/kg "Guanidine-metabolite"	(II)
0.095 mg/kg "Hydroxy-metabolite"	(IV)
0.034 mg/kg "Olefine-metabolite"	(VI)
0.036 mg/kg "Dihydroxy-metabolite"	(VII)
0.030 mg/kg "Nitrosimine-metabolite"	(VIII)
0.026 mg/kg "Chloropicolyl-glucoside"	(X)

The identified compounds are represented in a degradational pathway (Fig. 19).

The ^{14}C-radioactivity in the potato tuber corresponded to a total residue of 0.009 mg/kg. This is distributed among an unextractable residue (0.001 mg/kg), polar portions (0.007 mg/kg) and organosoluble ^{14}C-radioactivity (< 0.001 mg/kg) which could be assigned chromatographically to NTN 33893 (I). Approx. 0.003 mg/kg of the polar portions consisted of 6-chloronicotinic acid.

This study was conducted from July, 1987 to January, 1990 at the Institute for Metabolism Research of Bayer AG, Leverkusen, FRG.
>>M-024315-01-2@S-603064-01-1

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Report:	01.01/08; [redacted]; 1992; M-024277-02-2
Title:	Investigation of the metabolism of NTN 33893 in potatoes following granular application
Report No.:	PF3628
Document No.:	M-024277-02-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-024277-02-2@S-605095-01-1

The metabolism of the insecticide NTN 33893 was investigated in potatoes after application of [pyridinyl-^{14}C-methyl] NTN 33893. An in-furrow application of 5% granules at a rate of 0.05 g active ingredient per running meter was made at the time of planting the potatoes. The vines and tubers were harvested 129 days after application. At the time of harvest the vines were withered and mostly dry as would be under practical conditions.

The total residues, expressed in a.i. equivalents, were 5.76 mg/kg in vines and 0.091 mg/kg in tubers. Of the radioactivity applied to the soil 2.2% was taken up by the vines and 0.3% by the tubers.

By chromatographic comparison with reference compounds and other physical methods the following could be identified (amounts given in per cent radioactivity and in mg/kg a.i. equivalents in vines and tubers respectively):

1. Vines

Unchanged parent compound	(I)	26.7%	(1.53 mg/kg)
5-Hydroxy compound	(IV)	4.6%	(0.26 mg/kg)
Dihydroxy compound	(VII)	0.3%	(0.02 mg/kg)
Olefine compound	(VI)	3.3%	(0.19 mg/kg)
Nitrosimine compound	(VIII)	2.6%	(0.15 mg/kg)
Guanidine compound	(II)	8.2%	(0.48 mg/kg)
6-Chloronicotinic acid	(XII)	8.3%	(0.48 mg/kg)
Glucoside of 6-chloropicolyl alcohol	(X)	1.4%	(0.08 mg/kg)

Another 14 unknown metabolites were detected in lower concentrations which in total amounted to 16.1%, 0.93 mg/kg. The non-extractable residue corresponded to 26.4%, 1.52 mg/kg.

2. Tubers

Unchanged parent compound	(I)	48.3%	(0.044 mg/kg)
5-Hydroxy compound	(IV)	8.0%	(0.007 mg/kg)
Olefine compound	(VI)	3.1%	(0.003 mg/kg)
Guanidine compound	(II)	11.3%	(0.010 mg/kg)
6-Chloronicotinic acid	(XII)	9.4%	(0.009 mg/kg)

Another 5 unknown metabolites occurred in very low concentrations and in total amounted to 13.1%, 0.012 mg/kg. The non-extractable residue was 6.4%, 0.006 mg/kg.

The identified compounds are shown in a proposed degradation pathway (Figure 15).

>>M-024277-02-2@S-605095-01-1

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Report:	01.01/09; [redacted]; 1992; M-024289-01-2
Title:	Study on the metabolism of NTN 33893 after spray application to potatoes
Report No.:	PF3678
Document No.:	M-024289-01-2
Guideline(s):	171-4 Nature of Residue (Metabolism) - Plants
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-024289-01-2@S-603017-01-1

The metabolism of the insecticide NTN 33893 (I) was investigated in potatoes after application of [pyridinyl-^{14}C-methyl] NTN 33893. An 0.2% spray fluid of a 25 WP was applied to the foliage of potato plants 64 days before harvest. Vines and tubers were harvested 7, 28 and 64 days after application. At the time of harvest (day 64) the vines were withered and largely dry like under practical conditions; in this case the total residue amounted to 1.35 mg/kg.

0.90 mg/kg of this could be identified by chromatographic comparison with reference standards and by other physical methods:

0.51 mg/kg "NTN 33893"	(I)
0.17 mg/kg "Guanidine-metabolite"	(II)
0.095 mg/kg "Hydroxy-metabolite"	(IV)
0.034 mg/kg "Olefine-metabolite"	(VI)
0.036 mg/kg "Dihydroxy-metabolite"	(VII)
0.030 mg/kg "Nitrosimine-metabolite"	(VIII)
0.026 mg/kg "Chloropicolyl-glucoside"	(X)

The identified compounds are represented in a degradational pathway (Fig. 19).

0.19 mg/kg, corresponding to 14.1% of the total residue, accounted for unextractable ^{14}C-radioactivity. Hydrolysis experiments indicated a partial incorporation into lignin constituents of the potato vines.

The ^{14}C-radioactivity in the potato tuber corresponded to a total residue of 0.009 mg/kg. This is distributed among an unextractable residue (0.001 mg/kg), polar portions (0.007 mg/kg) and organosoluble ^{14}C-radioactivity (< 0.001 mg/kg) which could be assigned chromatographically to NTN 33893 (I). Approx. 0.003 mg/kg of the polar portions consisted of 6-chloronicotinic acid.

This study was conducted from July, 1987 to January, 1990 at the Institute for Metabolism Research of Bayer AG, Leverkusen, FRG.

>>M-024289-01-2@S-603017-01-1

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Report:	01.01/10: [redacted]; 1993; M-024294-02-2
Title:	Metabolism of NTN 33893 in cotton after seed treatment
Report No.:	PF3675
Document No.:	M-024294-02-2
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-024294-02-2@S-605105-01-1

Intrinsic Study Summary of report addendum:

The extracts of cotton seeds of the soil drench experiment from the original NTN 33893 metabolism study, report No. 3675, were further investigated.

The major metabolite (metabolite 15, 27.1 % of the radioactivity in the seeds, 2.54 mg/kg) in the methanol/6N HCl reflux extract was identified as 6-hydroxynicotinic acid methyl ester by co-chromatography with the authentic reference compound using two dimensional TLC and GC/MS after methylation. Metabolite 16 (1.6 %, 0.15 mg/kg) was identified as 6-hydroxynicotinic acid and metabolite 19.1 (0.7 %, 0.06 mg/kg) as 6-chloronicotinic acid methyl ester by co-chromatography with the corresponding authentic reference compound using two-dimensional TLC.

Furthermore, the residues in the methanol/water phase (19.9 %, 1.86 mg/kg) and in the methanol reflux extract (44.5 %, 4.16 mg/kg) were characterized as being based mostly on 6-chloronicotinic acid, 91 % and 87 % of the radioactivity, respectively.

Intrinsic Study Summary of original report:

The metabolism of the insecticide NTN 33893 was investigated in potatoes after application of [pyridinyl-^{14}C-methyl] NTN 33893. An in-furrow application of 5% granules at a rate of 0.05 g active ingredient per running meter was made at the time of planting the potatoes. The vines and tubers were harvested 129 days after application. At the time of harvest the vines were withered and mostly dry as would be under practical conditions.

The total residues, expressed in a.i. equivalents, were 5.76 mg/kg in vines and 0.091 mg/kg in tubers. Of the radioactivity applied to the soil 2.2% was taken up by the vines and 0.3% by the tubers.

By chromatographic comparison with reference compounds and other physical methods the following could be identified (amounts given in per cent radioactivity and in mg/kg a.i. equivalents in vines and tubers respectively):

1. Vines

Unchanged parent compound	(I)	26.7%	(1.53 mg/kg)
5-Hydroxy compound	(IV)	4.6%	(0.26 mg/kg)
Dihydroxy compound	(VII)	0.3%	(0.02 mg/kg)
Olefine compound	(VI)	3.3%	(0.19 mg/kg)
Nitrosimine compound	(VIII)	2.6%	(0.15 mg/kg)
Guanidine compound	(II)	8.2%	(0.48 mg/kg)
6-Chloronicotinic acid	(XII)	8.3%	(0.48 mg/kg)
Glucoside of 6-chloropicolyl alcohol	(X)	1.4%	(0.08 mg/kg)

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Another 14 unknown metabolites were detected in lower concentrations which in total amounted to 16.1%, 0.93 mg/kg. The non-extractable residue corresponded to 26.4%, 1.52 mg/kg.

2. Tubers			
Unchanged parent compound	(I)	48.3%	(0.044 mg/kg)
5-Hydroxy compound	(IV)	8.0%	(0.007 mg/kg)
Olefine compound	(VI)	3.7%	(0.003 mg/kg)
Guanidine compound	(II)	11.3%	(0.010 mg/kg)
6-Chloronicotinic acid	(XII)	9.4%	(0.009 mg/kg)

Another 5 unknown metabolites occurred in very low concentrations and in total amounted to 13.1%, 0.012 mg/kg. The non-extractable residue was 6.4%, 0.006 mg/kg.

The identified compounds are shown in a proposed degradation pathway (Figure 15).

>>M-024294-02-2@S-605105-01-1

Report:	01.01/11; [redacted]; 1994; M-024356-01-2
Title:	Admire (2.5 Granular) - Residues in field rotational crops
Report No.:	105153
Document No.:	M-024356-01-2
Guideline(s):	EPA Ref.: 165-2 Field Rotational Crops (Limited)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-024356-01-2@S-603096-01-1

Field rotational crop studies were conducted in Benoit, MS; Stanley, KS; and Fresno, CA to determine the residue levels of imidacloprid [ADMIRE, NTN33893, 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-lH-imidazol-2-amine] and metabolites in field crops at 1, 4, 8, and 11-month plant-back intervals following a single soil application of ADMIRE 2.5% Granular at the rate of 0.29 to 0.32 lb ai/acre. Representative rotational crops were planted at all three locations at the specified rotational intervals. These crop groups included (1) a cereal grain crop (wheat or sorghum), (2) a root crop (turnips), and (3) a leafy vegetable crop (spinach or mustard green). All crops were harvested at normal maturity. In addition, immature wheat or sorghum green forage was collected for analysis at 45 days post-planting in each interval.

In cereal grain crops, residues of imidacloprid were 0.12 ppm in wheat forage and 0.19 ppm in wheat straw at the 8-month plant-back interval in Fresno, CA. When extrapolated, residues of imidacloprid in wheat forage and straw were <0.05 ppm at the 11-month plant back interval in Fresno, CA. Residues of imidacloprid were also <0.05 ppm in sorghum forage and straw or wheat/sorghum forage and straw at the 11-month plant-back interval in Benoit, MS and Stanley, KS, respectively. Imidacloprid residues in cereal grain were <0.05 ppm at all plant-back intervals at all three test locations.

In root crops, residues of imidacloprid were 0.58 ppm in the turnip tops and 0.07 ppm in turnip roots at the 8-month plant-back interval in Fresno, CA. When extrapolated, residues of imidacloprid in turnip tops were <0.05 ppm at the 11-month plant-back interval in Fresno, CA. Based on having residues of 0.07 ppm in turnip roots at 8 months in Fresno, CA, the residues at an 11- month plant-back interval were anticipated to be <0.05 ppm. With the exception of 0.13 ppm residues of imidacloprid in turnip tops at the 1-month plant-back interval at the Benoit, MS location, residues of imidacloprid were <0.05 ppm in turnip tops and roots at all plant-back intervals in Benoit, MS and Stanley, KS.

In leafy vegetable crops, residues of imidacloprid were 0.32 ppm in spinach leaves at the 8-month plant-back interval in Fresno, CA. When extrapolated, residues of imidacloprid in spinach leaves were

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<0.05 ppm at the 11-month plant-back interval in Fresno, CA. Imidacloprid residues were <0.05 in mustard leaves at the 11-month plant-back interval in Benoit, MS and Stanley, KS.
>>M-024356-01-2@S-603096-01-1

Report:	01.01/12; [redacted]; 1996; M-010590-01-2
Title:	Admire 2F - Magnitude of the residue in field rotational crops
Report No.:	107133
Document No.:	M-010590-01-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-010590-01-2@S-603095-01-1
Field rotational crop trials were conducted in Benoit, MS; Stanley, KS; and Fresno, CA to determine the levels of imidacloprid [ADMIRE, NTN33893, 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine] residues in field crops at a 1-month plant-back interval following a single soil application of ADMIRE 2F at the rate of 0.3 lb ai/acre. Representative rotational crops were planted at all three locations at the 1-month plant-back interval. These crop groups included (1) cereal crop grain (sweet corn and corn grain), (2) cereal crop forage and straw (corn green forage, corn green forage with ears, and corn dry fodder), (3) legume vegetable crops (soybeans, beans, and peas), (4) foliage of legumes (soybean forage and hay), and (5) safflower seeds. All crops were harvested at normal maturity.

All residues of imidacloprid were converted to a common analyte and derivatized prior to injection on a gas chromatograph equipped with a mass selective detector (gc/msd). The limit of quantitation (LOQ) was 0.05 ppm.

The highest residue values were <0.05 ppm for cereal crop grain; 0.26 ppm for cereal crop forage and straw, 0.22 ppm for legume vegetable crop, 2.1 ppm for legume foliage, and <0.05 ppm for safflower seeds.
>>M-010590-01-2@S-603095-01-1

Report:	01.01/13; [redacted]; 1997; M-024331-01-4
Title:	Photolysis of imidacloprid (NTN 33893) on leaf surfaces of tomato plants
Report No.:	PF4277
Document No.:	M-024331-01-4
Guideline(s):	US EPA OCSPP Guideline Number: 860.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-024331-01-4@S-602559-01-1
NTN 33893 is a systemic insecticide with good activity as a contact and stomach poison. The active ingredient was assigned the proposed common name Imidacloprid.

With [methylene-^{14}C]NTN 33893 the photodegradation on tomato leaves under field conditions was investigated. The average total recovery of the individual samples ranged from 94.7 to 105.8% of the applied radioactivity in the course of the study.

The DT-50 value of NTN 33893 on leaf surfaces depended very much on the global radiation. Since there was only little degradation under dark control conditions, the global radiation of cloudy and sunny days in September and October in Monheim (51°4` latitude North, 45 m above NN) led to DT-50 values of 1.4 and 0.7 days. The radiation on leaf surfaces was measured and the mean daily values were 0.04 and 0.26 kJ/cm².

In total, up to ≥ 14metabolites were detected in the leaf extracts along with parent compound. Besides three well known plant metabolites, no typical photoproduct with ≥ 5% was formed. The imidazolidine ring was metabolised stepwise.

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The proposed degradation pathway of NTN 33893 on tomato leaf surfaces is shown in *Appendix 18*.
>>M-024331-01-4@S-602559-01-1

Report:	01.01/14; [redacted]; 1999; M-016760-02-4
Title:	Residues of 14C-NTN 33893 (imidacloprid) in blossoms of sunflower (Helianthus annuus) after seed dressing
Report No.:	MR-550/99
Document No.:	M-016760-02-4
Guideline(s):	US EPA OCSPP Guideline Number: 860.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-016760-02-4@S-602423-01-1
The occurrence of residues of the insecticide NTN 33893 (imidacloprid) and its metabolites in nectar and pollen of sunflower was investigated after seed dressing in a greenhouse experiment. [Methylene-^{14}C]imidacloprid was formulated as a WS 70 (equivalent to "Gaucho"). The application conditions projected for this experiment simulated the practice conditions of 150 g WS 70/unit sunflower seeds (1 unit = 150,000 grains), equivalent to 105 g a.i./unit. In the experiment, each sunflower seed was coated with ca. 1.0 mg of formulation, equivalent to ca. 0.7 mg a.i. A total of 22 sunflower plants (variety „Fleury") were separately grown in 34-L pots (ca. 40 cm diameter) in the greenhouse, subdivided into two rows of 11 plants each.

During flowering, nectar was collected every day with a capillary from the florets that were in the female stage. In total, ca. 1.7 g nectar/row was collected during a period of 2 weeks. Pollen was collected with the aid of plastic boxes that were installed underneath the inflorescence. The pollen freely trickled into the plastic boxes. In total, ca. 4.8 g pollen/row was collected.

The total radioactive residues (TRR) of both rows (nectar and pollen) were almost identical and averages are presented. On average, the TRR in nectar amounted to 0.0019 mg/kg and 0.0039 mg/kg in pollen. In total, 85.8 % of the TRR in the pollen was extractable with methanol/water (3:1, v/v) and methanol. Only 14.2 % of the TRR (0.0006 mg/kg) was not extractable and remained in the solids. Due to the very low radioactivity content, the solids were not further investigated.

The nectar and the pollen extracts were purified using an Oasis® resin SPE cartridge (Waters) and analyzed by 2-dimensional thin-layer chromatography as well as AMD co-chromatography. Imidacloprid was the only residue observed in the nectar and pollen extracts (0.0019 mg/kg and 0.0033 mg/kg, respectively). No metabolites of imidacloprid were observed in either nectar or pollen of sunflower.
>>M-016760-02-4@S-602423-01-1

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Report:	01.01/15; [redacted]; 2008; M-308631-01-3
Title:	Imidacloprid residue movement in plants following foliar applications and the implications for potential bee exposure
Report No.:	M-308631-01-3
Document No.:	M-308631-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 158.400(e)
Guideline deviation(s):	--
GLP/GEP:	**no**

1 BACKGROUND

On 16 September 2008, Bayer CropScience Deutschland GmbH received letters from BVL, which communicated concerns about the potential impact on bees for imidacloprid products that can be applied as a foliar spray to outdoor plants. Because imidacloprid is known to exhibit systemic behavior following seed or soil treatments, questions have been raised about the potential for imidacloprid residues resulting from foliar sprays to move throughout a plant into nectar and /or pollen for some weeks after pre-flowering applications are made. In addition, potential exposure to bees by nectar and/or pollen of plants exposed by spray drift is also questioned.

2 OBJECTIVE

The objectives of this paper are:

1) to summarize available information concerning the systemicity and translocation of imidacloprid in plants to demonstrate that imidacloprid residues in nectar or pollen will be negligible following foliar sprays made to crops or ornamental plants according to label directions and
2) to summarize key studies that can be used in risk assessment to address potential bee exposure from off-crop drift.

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01.02 - Soil and water

Report:	01.02/01: [redacted]; 1991; M-023983-01-2
Title:	Terrestrial field dissipation for NTN 33893 in California soil
Report No.:	MR101989
Document No.:	M-023983-01-2
Guideline(s):	EPA Guideline Ref. No.: 164-1 Soil Field Dissipation
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-023983-01-2@S-602920-01-1

NTN 33893 (a broad spectrum, systemic insecticide) was applied to a tomato plot near Fresno, California, on June 19, 1990, to evaluate mobility and persistence in soil. Soil at the site was characterized as a sandy loam in the 0-42-inch soil horizon. NTN 33893 240FS formulated as a 23.3% active ingredient liquid suspension was applied broadcast to tomatoes in a single application at the highest recommended rate of 0.5 pound active ingredient per acre. Soil core samples were taken to a depth of 12 inches immediately post-application (Day 0) and to a depth of 48 inches for all other sampling intervals through 18 months (546 days) post-application. A total of 15 core samples were taken per sampling interval. Each core was sectioned into 0-6-in. (A), 6-12-in. (B), 12-18-in. (C), 18-24-in. (D), 24-30-in. (E), 30-36-in. (F), and 36-48-in. (G) layers. The 15 core samples were composited by depth into three replicates prior to analyses.

The soil samples were analyzed for the parent NTN 33893 by gradient high performance liquid chromatography. The half-life ($t_{1/2}$) and first order rate constant (k) for the dissipation of NTN 33893 from Day 0 to Day 91 was 53 days (r = -0.96) and -0.013, respectively. The $t_{1/2}$ and k for the dissipation of NTN 33893 from Day 0 to Day 364 was 146 days (r = -0.52) and -0.0048, respectively. No residues were detected at or above the detection limit below the 0-6-inch depth. These data indicate that NTN 33893 does not leach.

Total accumulated rainfall for the study period through June 18, 1991 (Day 364) was 9.25 inches, which was 25 % below a National Oceanic and Atmospheric Administration (NOAA) 10-year mean for the same time period. Total irrigation for the study period was 51.43 inches for a combined total accumulated rainfall and irrigation amount of 60.68 inches. Air and soil temperatures during the study did not differ significantly from a NOAA 10-year mean.

>>M-023983-01-2@S-602920-01-1

Report:	01.02/02: [redacted]; 1991; M-024011-01-2
Title:	Terrestrial field dissipation for NTN 33893 in Georgia soil
Report No.:	MR101987
Document No.:	M-024011-01-2
Guideline(s):	EPA Guideline Ref. No.: 164-1 Soil Field Dissipation
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-024011-01-2@S-602963-01-1

NTN 33893 (a broad spectrum, systemic insecticide) was applied to a bare ground plot near Tifton, Georgia, on April 16, 1990, to evaluate mobility and persistence in soil. Soil at the site was characterized as a loamy sand in the 0-30-inch soil horizon and as a sandy loam in the 30-42-inch soil horizon. NTN 33893 240FS formulated as a 23.3% active ingredient liquid suspension was applied broadcast to bare ground in a single application at the highest recommended rate of 0.5 pound active ingredient per acre. Soil core samples were taken to a depth of 12 inches immediately post-application (Day 0) and to a depth of 48 inches for all other sampling intervals through 18 months (546 days) post-application. A total of 15 core samples were taken per sampling interval. Each core was sectioned into 0-6-in. (A), 6-12-in. (B), 12-

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18-in. (C), 18-24-in. (D), 24-30-in. (E), 30-36-in. (F), and 36-48-in. (G) layers. The 15 core samples were composited by depth into three replicates prior to analyses.

The soil samples were analyzed for the parent NTN 33893 by gradient high performance liquid chromatography. The half-life was 12 days (r= -0.95) and first-order rate constant was -0.058 for the dissipation of NTN 33893 from Day 0 through Day 14. The data from Day 28 through Day 364 were not used in the dissipation analysis due to a nonlinear decline in residues. No residues were detected at or above the detection limit below the 0-6-inch depth. These data indicate that NTN 33893 does not leach.

Total accumulated rainfall for the study period through October 14, 1991 (Day 546) was 78.58 inches, which was 12% above a National Oceanic and Atmospheric Administration (NOAA) 10-year mean for the same time period. Total irrigation for the study period was 15.83 inches for a combined total accumulated rainfall and irrigation amount of 94.41 inches. Air and soil temperatures during the study did not differ significantly from a NOAA 10-year mean.
>>M-024011-01-2@S-602963-01-1

Report:	01.02/03; [redacted]; 1991; M-023988-01-2
Title:	Terrestrial field dissipation for NTN 33893 in Minnesota soil
Report No.:	MR101988
Document No.:	M-023988-01-2
Guideline(s):	EPA Guideline Ref. No.: 164-1 Soil Field Dissipation
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-023988-01-2@S-602958-01-1

NTN 33893 (a broad spectrum, systemic insecticide) was applied in 1990 at a site near Hollandale, Minnesota to evaluate the mobility and persistence in soil. Soil at the site was characterized as a sandy loam in the 0-6 inch soil horizon. The site received one application at the highest seasonal rate of 0.5 lb a.i./A. The formulation (240 FS) was applied as a broadcast treatment to field corn. Soil core samples were taken to a depth of 6 inches immediately post-application (Day 0) and then to a depth of 48 inches for all remaining sample intervals. A total of 15 core samples was taken per sampling interval. Bach core was sectioned into 0-6-in. (A), 6-12-in. (B), 12-18-in. (C), 18-24-in. (D), 24-30-in. (E), 30-36-in. (F), and 36-48-in. (G) layers. The 15 core samples were composited to three replicates prior to analysis.

The resultant soil samples were analyzed for parent NTN 33893 by gradient high performance liquid chromatography. The half-life was 7 days (r= -0.97) and first-order rate constant was -0.096 for the dissipation of NTN 33893 from Day 0 through Day 28. The data from Day 61 through Day 365 were not used in the dissipation analysis due to an overall accumulation of residues. No residues were detected at or above the detection limit below the 0-6 in. depth. These data indicate that NTN 33893 does not leach.

Accumulated rainfall for the study period through August 1991 (Day 365) was 57.17 inches, which was 15% above a National Oceanic and Atmospheric Administration (NOAA) ten-year mean for the same time period. Total irrigation for the study period was 4.18 inches for a combined total accumulated rainfall and irrigation amount of 61.35 inches. Air temperatures during the study did not differ significantly from a NOAA ten-year mean.
>>M-023988-01-2@S-602958-01-1

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Report:	01.02/04; [redacted]; 1991; M-023514-01-2
Title:	Metabolism of (pyridinyl-14C-methylene) NTN 33893 in sandy loam under aerobic conditions
Report No.:	PF3433
Document No.:	M-023514-01-2
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-023514-01-2@S-602954-01-1

The metabolism of NTN 33893 in soil was investigated in a laboratory study according to the general protocols of the respective EPA and BBA Guidelines.

[Pyridiny1-14c-methylene]NTN 33893 was applied to a sandy loam from Kansas, USA. The samples were incubated in the dark at 20 ± 2·c and 75 % of 1/3 bar moisture level under aerobic conditions. The application rate of 0.33 mg/kg was based on the recommended maximum use rate of 200 g/ha. Sampling times were 0, 1, 3, 7, 14, 30, 59, 100, 120, 182, 274 and 366 days.

The amount of radioactivity extracted at room temperature de- creased gradually with time and represented 66.9 % of the applied radioactivity after an incubation time of 366 days. Parent compound accounted for 61.6 % of the applied radioactivity in the soil extracts 366 days posttreatment.

The amount of radioactivity bound to the soil increased gradually with time and attained a maximum of 25.6 % of the applied dose 274 days posttreatment.

A total of 7 metabolites was observed in the soil extracts along with parent compound. Six metabolites were identified by spectroscopic methods and comparison with authentic reference substances. One additional metabolite was detected by reverse isotope dilution analysis. The degradates represented a total of 3.4 % of the applied radioactivity after 366 days. No single degradate accounted for more than 1.7 % of the applied dose (0.006 ppm) at any time.

The degradation of NTN 33893 in soil proceeded via cleavage and oxidation of the dihydro-imidazole-ring and via loss of the nitro group from the intact heterocyclic ring to the key intermediate 6-chloronicotinic acid and the ultimate metabolite carbon dioxide. During the incubation time of 366 days the equivalent of 7.4 % of the applied radioactivity was transformed into carbon dioxide.

The average total recovery ranged from 94.4 to 98.9 % of the applied radioactivity in the course of the study.

The computation of the half-life was based on the initial 100 days of incubation. According to statistical interpretation of the data a first order root function proved to be the best fit. Extrapolation resulted in a half-life (DT-50) >1 year.

>>M-023514-01-2@S-602954-01-1

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Report:	01.02/05; [redacted]; 1992; M-006742-02-2
Title:	Metabolism of (pyridinyl-14C-methylene) NTN 33893 in loamy soil BBA 2.2 under aerobic conditions
Report No.:	PF3321
Document No.:	M-006742-02-2
Guideline(s):	EPA Pesticide Assessment Guidelines, Subdivision N: 6.162-1(1982) BBA Ref.: Guideline IV, 4-1 (1986)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-006742-02-2@S-605115-01-1

In a laboratory study [pyridinyl-^{14}C-methylene]NTN 33893 was applied to the loamy sand soil BBA 2.2 and maintained aerobically in the dark at 20 ± 2°C. The application rate of 0.33 mg/kg was based on the recommended maximum use rate of 200 g/ha. Sampling times were 0, 1, 3, 7, 14, 35, 62 and 100 days.

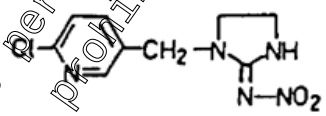
The amount of radioactivity extracted at room temperature decreased gradually with time and represented 68.6 % of the applied radioactivity after an incubation time of 100 days. Parent compound accounted for 63.2 % of the applied radioactivity in the soil extracts 100 days post treatment. The degradation kinetics could be described best by a reaction order of 2. Extrapolation resulted in a half life (DT-50) of 188 ± 25 days.

The amount of radioactivity bound to the soil increased gradually with time and reached a maximum of 21.6 % of the applied. One hundred days post treatment 7.7 % of the applied radioactivity were released from the soil by reflux extraction, 7.4 % of which were identified as parent compound.

Six metabolites were identified by spectroscopic methods and comparison with authentic reference substances. One additional metabolite was detected by reverse isotope dilution analysis. Neither of them accounted for more than 2.2 % of the applied radioactivity at any time. The degradation of NTN 33893 in soil proceeded via cleavage and oxidation of the dihydro-imidazole-ring and via loss of the nitro group from the intact heterocyclic ring to the key intermediate 6-chloronicotinic acid and the final metabolite carbon dioxide. During the incubation time of 100 days the equivalent of 10.0 % of the applied radioactivity was transformed into carbon dioxide.

The total recovery ranged from 99.4 to 103.8 % of the applied radioactivity in the course of the study.

Not a single soil borne metabolite surpassed the concentration of 0.01 ppm in the soil: only the parent compound remained at a level above 0.01 ppm.



NTN 33893

>>M-006742-02-2@S-605115-01-1

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Report:	01.02/06; [redacted]; 1992; M-006740-02-2
Title:	Degradation of [pyridinyl-14C-methylene] NTN 33893 in silt soil Hoefchen under aerobic conditions
Report No.:	PF3322
Document No.:	M-006740-02-2
Guideline(s):	Guidelines for the Official Testing of Pesticides, Part IV, 4-1 (1986)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-006740-02-2@S-605112-01-1

In a laboratory study [pyridinyl-^{14}C-methylene] NTN 33893 was applied to the silt soil Höfchen and maintained aerobically in the dark at 20 ± 2·c. The application rate of 0.36 mg/kg was based on the recommended maximum use rate of 200 g/ha. Sampling times were 0, 1, 3, 7, 14, 30, 59 and 100 days.

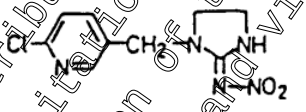
The amount of radioactivity extracted at room temperature decreased gradually with time. After an incubation time of 100 days 71.5% of the applied radioactivity were extracted. Parent compound accounted for 66.8 % of the applied radioactivity in the soil extracts 100 days post treatment.

The degradation kinetics was described best by a reaction order of 2. Extrapolation resulted in a half life (DT-50)of 248 ± 50 days.

Slowly increasing amounts of radioactivity remained bound to the soil with increasing incubation time. After 100 days the bound residues represented an average of 21.6 % of the applied radioactivity. Reflux extraction with acetonitrile released 8.5 % of the applied radioactivity 100 days post treatment, 8.1 % of which were identified as parent compound.

During the incubation time of 100 days up to 6.4 % of the applied radioactivity were transformed into the final metabolite carbon dioxide.

The total recovery ranged from 98.7 to 103.6 % of the applied radioactivity in the course of the study.



NTN 33893

>>M-006740-02-2@S-605112-01-1

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Report:	01.02/07; [redacted]; 1992; M-006728-02-2
Title:	Degradation of [pyridinyl-14C-methylene] NTN 33893 in sandy loam Monheim I under aerobic conditions
Report No.:	PF3434
Document No.:	M-006728-02-2
Guideline(s):	Guidelines for the Official Testing of Pesticides, Part IV, 4-1 (1986)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-006728-02-2@S-605110-01-1

The degradation of NTN 33893 in soil was investigated in a laboratory study according to the general protocol of the respective BBA guidelines.

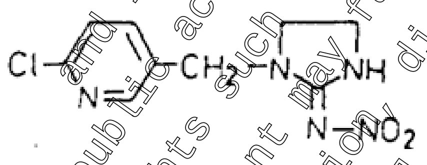
[Pyridiny1-14c-methylene]NTN 33893 was applied to the sandy loam Monheim 1. The samples were incubated in the dark at 20 ± 2°C and 40 % of the maximum water capacity of the soil under aerobic conditions. The application rate of 0.33 mg/kg was based on the recommended maximum use rate of 200 g/ha. Sampling times were 0, 1, 3, 7, 14, 35, 62, 100, 125, 183, 274 and 366 days.

The amount of radioactivity extracted at room temperature decreased gradually with time. After an incubation time of 100 days an average of 73.2 % of the applied dose was extracted. Parent compound accounted for 69.5 % of the applied radioactivity in the soil extracts 100 days posttreatment.

The statistical interpretation of the degradation behavior was based on the data obtained for the initial 100 days posttreatment. The degradation kinetics was described best by a first order root function. Extrapolation resulted in a half-life (DT-50) of 341 ± 153 days.

Slowly increasing amounts of radioactivity remained bound to the soil with increasing incubation time. After 100 days the bound residues represented an average of 25.1 % of the applied dose. During the incubation time of 100 days up to 2.7 % of the applied radioactivity were transformed into the final metabolite carbon dioxide.

The average total recovery ranged from 98.6 to 103.1 % of the applied radioactivity in the course of the study.



NTN 33893

>>M-006728-02-2@S-605110-01-1

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Report:	01.02/08; [redacted]; 1992; M-023828-01-2
Title:	Soil/sediment adsorption-desorption of 14C-imidacloprid
Report No.:	MR103816
Document No.:	M-023828-01-2
Guideline(s):	EPA Ref.: 163-1, Adsorption/Desorption
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-023828-01-2@S-602914-01-1

Aqueous solutions of ^{14}C-imidacloprid were equilibrated with four soil types and the adsorption and desorption coefficients and constants were determined. Liquid scintillation counting analysis was employed to measure the test material concentrations in the aqueous phases. Following desorption, combustion-radioanalysis was used to demonstrate ^{14}C-mass balance.

The definitive soil adsorption-desorption study was conducted at 25 ± 1 °C in the dark with ^{14}C-imidacloprid and four soils (sand #396, loamy sand #398, loam #318, silt loam #307, and silt loam #307 with sodium azide). The nominal concentrations of ^{14}C-imidacloprid for all soil types were 250, 187.5, 125, and 25 ppm. The soil-to-water ratio was 1:3.

The mean percent of compound adsorbed to the test soils during the definitive study was 11.1, 16.8, 35.7, 33.5, and 29.5% for sand #396, loamy sand #398, silt loam #307, silt loam #307 (with sodium azide), and loam #318, respectively. The mean percent of compound desorbed from the test soils during the definitive study was 83.4% for #396 sand, 44.3 % for #398 loamy sand, 41.5 % for #307 silt loam, 44. 8 % for #307 silt loam (with sodium azide), and 44.1% for #318 clay loam.

Although only 3 of the soil types tested were within the desired 20-80 % for which the Freundlich model is typically defined; the Freundlich adsorption isotherms for all of the soil types were calculated and demonstrated a high degree of linear correlation for a plot of ln (Ce) versus ln (x/m). Correlation coefficients (r) of the adsorption isotherms of sand #396, loamy sand #398, silt loam #307, silt loam #307 (with sodium azide), and loam #318 were 0.950, 0.987, 0.993, 0.988, and 0.987, respectively, implying that all of the soils adequately fit the model for this compound. Desorption isotherms were also calculated.

High-performance liquid chromatography and thin-layer chromatography were used to measure the stability of the test compound under the test conditions. Greater than 95 % of the ^{14}C activity found in the aqueous supernatants was identified as parent compound.

The mean ^{14}C-mass balance of the test compound from the sand #396, loamy sand #398, silt loam #307, silt loam #307 (with sodium azide), and loam #318 was 99.9, 93.5, 96.7, 99.1, and 100%, respectively.

The Freundlich constants and mobility class were determined as summarized below:

		Adsorption		Desorption		
Soil Identification	% Organic Carbon	K_d	K_{oc}	K_d	K_{oc}	Mobility Class
Sand #396	0.233	0.956	411	0.662	285	Medium Mobility
Loamy Sand #398	0.349	1.02	292	0.542	155	Medium Mobility
Silt Loam #307	1.51	4.18	277	4.68	310	Medium Mobility
Silt Loam #307 (with sodium azide)	1.51	4.76	315	3.38	224	Medium Mobility
Loam #318	1.16	3.45	296	4.40	378	Medium Mobility

>>M-023828-01-2@S-602914-01-1

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Report:	01.02/09; [redacted]; 1992; M-024377-01-2
Title:	Soil/sediment adsorption-desorption of 14C-NTN 33823
Report No.:	MR103817
Document No.:	M-024377-01-2
Guideline(s):	EPA Ref.: 163-1, Adsorption/Desorption
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-024377-01-2@S-603090-01-1

Aqueous solutions of ^{14}c-NTN-33823 were equilibrated with four soil types and the adsorption and desorption coefficients and constants were determined. Liquid scintillation counting analysis was employed to measure the test material concentrations in the aqueous phases. Following desorption, combustion-radioanalysis was used to demonstrate ^{14}C-mass balance.

The definitive soil adsorption-desorption study was conducted at 25 ± 1 °C in the dark with ^{14}c-NTN-33823 and four soils (sand #396, loamy sand #398, silt loam #307, and loam #318). The nominal concentrations of ^{14}C-NTN-33823 for all soil types were 250, 187.5, 125, and 25 ppm. Sand #396, loamy sand #398, and loam #318 bad a soil-to-water ratio of 1:3, and silt loam #307 had a soil-to-water ratio of 1:5.

Tue mean percent of compound adsorbed to the test soils during the definitive study was 38.5, 57.8, 73.3, and 63.8% for sand #396, loamy sand #398, silt loam #307, and loam #318, respectively. The mean percent of compound desorbed from the test soils during the definitive study was 59.1, 37.4, 20.3, and 24.6% for sand #396, loamy sand #398, silt loam #307, and loam #318, respectively. The percent adsorbed for all soil types tested was within the desired 20-80 % for which the Freundlich model is typically defined.

The Freundlich adsorption isotherms for all of the soil types were calculated and demonstrated a high degree of linear correlation for a plot of 1n (Ce) versus 1n (x/m). Correlation coefficients of the adsorption isotherms of sand #396, loamy sand #398, silt loam #307, and loam #318 were 0.9993, 0.9995, 0.9994, and 0.9998, respectively, implying that all of the soils adequately fit the model for this compound. Desorption isotherms were also calculated.

High-performance liquid chromatography and thin-layer chromatography were used to measure the stability of the test compound under the test conditions. Greater than 93 % of the ^{14}C activity found in the aqueous supernatants was identified as parent compound.

The mean ^{14}C-mass balance of the test compound from the sand #396, loamy sand #398, silt loam #307, and loam #318 was 101, 94.2, 105, and 105%, respectively.

The Freundlich constants and mobility class were determined as summarized below:

		Adsorption		Desorption		
Soil Identification	**% Organic Carbon**	**K_d**	**K_{oc}**	**K_d**	**K_{oc}**	**Mobility Class**
Sand #396	0.233	0.761	327	0.456	196	Medium Mobility
Loamy Sand #398	0.349	2.91	833	2.45	702	Low Mobility
Silt Loam #307	1.51	14.2	942	16.9	1120	Low Mobility
Loam #318	1.16	10.1	866	12.0	1034	Low Mobility

>>M-024377-01-2@S-603090-01-1

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Report:	01.02/10; [redacted]; 1998; M-023911-01-3
Title:	Long-term soil dissipation study with Zelmone 350 FS in Great Britain following seed dressing of winter barley
Report No.:	MR-196/98
Document No.:	M-023911-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 835.6100
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-023911-01-3@S-603800-01-1

Long-term field trials with repeated single annual application of Zelmone 350 FS in winter barley were carried out to investigate the behaviour of imidacloprid in soil over several years. Winter barley was chosen as test system for this study as a typical crop with potential annual use of Imidacloprid in Great Britain. Winter barley seed was dressed with Zelmone 350 FS. The application rates were 100 (=35 g.a.i.) and 200 (=70 g.a.i.) ml per 100 kg seed. Two test sites were selected. At each test site both application rates were investigated in parallel on adjacent plots. At each site one untreated plot served as control. The dressed seed was analysed for concentrations of imidacloprid. About 71 to 96 % of the theoretical amount was determined on the seeds, showing excellent performance of the dressing process.

Soil cores were collected before the first application (0-20 cm) and after harvest just before drilling for the next year (0-40 cm) (0-50 cm at the last sampling date) from the treated and untreated plots using a pushing sampling device ('Wacker Hammer'). The soil cores were segmented into 10 cm layers and carefully homogenized to ensure a representative laboratory sample.

The residues of Imidacloprid in soil were determined according to Bayer residue method no. 2 70 ([redacted], 1992). Residues were extracted with boiling methanol followed by column chromatography on silica gel. The quantitation was performed via HPLC with UV-detection. The limit of quantitation (LOQ) was 6 µg/kg, while the limit of detection was 2 µg/kg.

The analytical method was validated by running recovery experiments before and concurrently with sample analysis at different fortification levels. The recovery data obtained demonstrate the validity of the method.

Before the first application traces of imidacloprid (< 6 µg/kg) were detected in soil samples from the untreated and treated plots at Bury St. Edmunds. It is unclear whether these residues really show the presence of imidacloprid on the plots or are due to an interference from the matrix. However, at later sampling intervals and also in all control samples from Wellesbourne no residues were detected.

At the test site in Bury St. Edmunds the residues were significantly higher than at Wellesbourne. This indicates a faster dissipation of imidacloprid on the Wellesbourne plots.

In broad terms, the application rate of 200 ml per 100 kg seed gave around double the residues than the 100 ml/100 kg rate.

The maximum residues in soil were observed in the upper 20 cm layer. This was expected, since the upper soil layers were mixed by ploughing and harrowing.
In the 20-30 cm layer residues below 6 µg/kg were occasionally detected from the plots with the lower application rate, while from the plots with the higher application rate residues of ca. 6 µg/kg occurred in the 20 - 30 cm samples after the third trial year. It is possible that cultivation activity also led to some minor mixing of soil containing imidacloprid residues also into the 20-30 cm layer.

In the 30-50 cm layer residues were not detected.

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These results indicate very little, if any, movement of imidacloprid into deeper soil layers during the study.
The normalised residues in the 0-30 cm layer increased gradually during the first three years of the testing period, as would be expected from the known dissipation behaviour of imidacloprid in soil. After the 4th trial year the residues reached a plateau level and remained constant (within experimental error).

However, the overall residue levels throughout these trials were extremely low, and no harmful effects are to be expected from these residues.
>>M-023911-01-3@S-603800-01-1

Report:	01.02/11: [redacted]; 1998; M-023928-01-3
Title:	Long-term soil dissipation study with Confidor 70 WG in apple orchards in Germany following spray application
Report No.:	MR-758/98
Document No.:	M-023928-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 835.6100
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-023928-01-3@S-604672-01-1
Long-term field trials with repeated single seasonal application of Confidor 70 WG in apple orchards were carried out to investigate the behaviour of imidacloprid in soil over several years. In these trials the total amount of the product, corresponding to the annual application rate of 0.15 kg/ha, was sprayed directly to the ground in order to control exposure of the soil with imidacloprid. The applications were carried out at the end of May. The application rate and the uniformity of application was monitored using filter papers. The filter papers were analysed individually. The results showed that about 70 to 100 % of the theoretical amount was found on the filter papers with a standard deviation of about 10 %.

The trials were performed at three test sites in apple growing regions in Northern (Burscheid) and Southern (Bechtolsheim, Freinsheim) Germany in typical apple orchards. On the test plots the soil between the tree rows is covered with grass (mulch band, about 1/2 to 2/3 of total area), while under the trees there is a strip of bare soil. Soil samples were collected from the grass covered as well as from the bare soil area and combined as a common field sample. The grass was cut down as short as possible before taking soil cores. Soil cores (0-30 cm), (0-50cm since 1996) were taken at –several intervals after application using a pushing sampling device ("Wacker Hammer"). The soil cores were segmented into 10 cm layers and carefully homogenised to ensure a representative laboratory sample.

The residues of imidacloprid in soil were determined according to Bayer residue method no. 270 ([redacted], 1992). Residues are extracted with boiling methanol followed by column chromatography on silica gel. The quantitation is performed via HPLC with UV-detection. The limit of quantitation (LOQ) was 6 µg/kg while the limit of detection was 2 µg/kg.

Grass samples were analysed using Bayer residue method no. 300 ([redacted] and [redacted], 1992).

This method allows the determination of parent compound and total residues of imidacloprid from a single extract. The total residue method is a common moiety method comprising the parent compound and all its metabolites containing the 6-chloropicolyl moiety as 6-chloronicotinic acid (6-CNA).

The residues are extracted from plant material with a mixture of methanol/water. The extract is- cleaned-up by XAD 4 column chromatography.

For determination of the parent compound the eluate of the XAD 4 column is partitioned against dichloromethane and further cleaned-up by column chromatography on Florisil. The residues are quantified by HPLC with UV-detection.

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For determination of the total residues, the eluate of the XAD 4 column or an aliquot is oxidised with potassium permanganate yielding 6-CNA. After silylation the 6-CNA is determined by GC/MS. The LOQ for the parent compound was 0.01 mg/kg and for the total residue 0.05 mg/kg.

The methods were validated by running recovery experiments before and concurrently with sample analysis at different fortification levels. The recovery data obtained demonstrate the validity of the methods.

Generally, the maximum residues in soil were observed directly after an application and declined to about 50 % of the initial value within 3-4 months. The degradation was fastest in Höfchen and was slowest in Freinsheim.

In the first three years the residues remaining in the soil (0-30 cm) until the next application increased. After three years the residues reached a plateau at all three test sites. From the known dissipation behaviour of imidacloprid in soil gradually increasing residues over several years were expected before a plateau is formed.

Residues above the LOQ, i.e. > 6 µg/kg, occurred in the upper 10 cm soil layer. In the 10-20 cm layer maximum residues were about the LOQ in a very few samples, but for most of these samples the residues were either < 2µg/kg or < 6 µg/kg.

In the 20-30 cm layer no residues were detected in nearly all the samples.

These results indicate very little movement of imidacloprid into deeper soil layers during the study. Considering the 2-3 X overdose in terms of soil exposure as compared to practical application conditions, this represents an absolute worst case scenario. Under practical use conditions probably no residues would be detectable in deeper soil layers.

With respect to movement of imidacloprid into deeper soil layers preferential flow might play a role. In general, in orchard crops preferential flow conditions are more likely to occur than under arable farming conditions. Especially at the test sites Freinsheim and Bechtolsheim some of the factors promoting preferential flow, e.g. soil cracking, worm holes, root holes, etc., were observed.

In grass samples residues of about 5 to 10 mg/kg were determined at day 0 after application. The residues dissipated very rapidly and before the next application in general no residues were detected in the grass samples.

Summarising the results obtained, it must be considered that the overall residue level in soil is very low and no harmful effects are to be expected from these residues. In the six years only very little movement of imidacloprid into soil layers below 0-10 cm occurred, and this movement is considered attributable to preferential flow.

>>M-[illegible]928-01-5[illegible]S-604672-01-1

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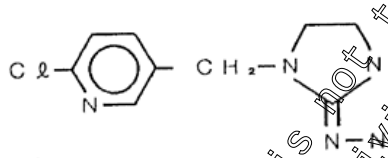
Report:	01.02/12; [redacted]; 1989; M-024064-01-2
Title:	Hydrolysis of NTN 33893
Report No.:	NR1276
Document No.:	M-024064-01-2
Guideline(s):	EPA Guideline Subdivision N Section 161-1 (1982) Hydrolysis Studies in Water
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-024064-01-2@S-602970-01-1

The hydrolysis study of NTN 33893 in the buffers adjusted to pH 5, 7, and 9 was carried out according to the EPA Guideline Subdivision N Section 161-1 (1982)1). The test solutions were prepared with radio-labeled parent compound (pyridinyl-14Cmethyl) at a concentration of 5 mg/L. The solutions were incubated for maximum of 30 days under sterile conditions in the dark at 25 °C, and the sampling intervals were 0, 5, 10, 14, 18, 22, 26 and 30 days. NTN 33893 degraded slowly in the buffer adjusted to pH 9. The experimental half-life of NTN 33893 in pH 9 was calculated to be 355 days by first order. At the end of the incubation for 30 days, NTN 33893 was accounted for 93.0% of the radioactivity found in the pH 9 solution. NTN 33893 was stable in pH 5 and 7 and no hydrolysis product was found in pH 5 and 7 at any sampling periods during the incubation for 30 days.



NTN 33893 (imidacloprid)

>>M-024064-01-2@S-602970-01-1

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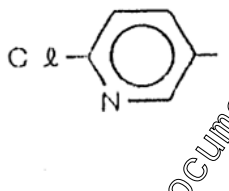
Report:	01.02/13; [redacted]; 1990; M-024040-01-3
Title:	Photodegradation of NTN 33893 on soil
Report No.:	MR100249
Document No.:	M-024040-01-3
Guideline(s):	EPA Guidelines Subdivision N Section 161-3 (1982) Photodegradation Studies on Soil
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-024040-01-3@S-604673-01-1

The photolysis study of NTN 33893 on soil was carried out under continuous irradiation for maximum of 15 days by using an artificial light source at 25 ± 2°C. The study was done according to the EPA Guidelines Subdivision N Section 161-3 (1982).[1]) The radio-labeled parent compound (pyridinyl-^{14}C-methyl) was applied onto the soil layer at a concentration of 48.5 mg/kg. The sampling intervals were 0,6 hrs, 1, 2, 3 and 5 days (TEST I) and 0,7, 12 and 15 days (TEST II). At the end of the irradiation for 15 days (TEST II), 91.6 % of the radioactivity applied was recovered and 61.5 % of the radiocarbon was found to be unchanged parent compound. The half-life of NTN 33893 was calculated by linear regression analysis to be 38.9 days (Rate constant K = 1.78 X 10^{-2} day^{-1}) under the conditions. The amount of radioactivity that could not be extracted from the soil ranged from 0.3 % to 11.0 % of the applied radioactivity. WAK 4103 was identified as the major photoproduct at the end of the irradiation period. It represented as much as 6.3 % of the applied radioactivity. None of the other extractable photoproducts was formed in amounts greater than 5 % of the applied radioactivity at any time during the irradiation.



NTN 33893 (imidacloprid)

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Report:	01.02/14; [redacted]; 1988; M-024286-01-2
Title:	Photodegradation of NTN 33893 in water
Report No.:	PF3517
Document No.:	M-024286-01-2
Guideline(s):	EPA Ref.: 161-2, Photodegradation Studies in Water
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-024286-01-2@S-603010-01-1

The photodegradation of NTN 33893 in buffer pH 7.0 was investigated with artificial sunlight under sterile conditions. The study was conducted according to the respective EPA- Guidelines in compliance with the current GLP requirements.

[Pyridinyl-14c-methyl]NTN 33893 was used. During the irradiation period of 2 hours the radioactivity balance was 100.2 ± 1.9 % of the amount at zero time.

At a concentration of 5.4 mg/l and a temperature of 23-24.5 °C NTN 33893 was degraded rapidly with a half-life of 57 min. The corresponding rate constant was 0.012 min-1. The environmental half-life was calculated to be 4.2 hours.

Under natural sunlight 60 % of the compound were degraded after 4 hours.

A large number of photoproducts of different light stability was formed. Two of the major photoproducts were identical with the reference substances NTN 33519 and NTN 33014. Both photoproducts were stable under the conditions of the experiment. After 120 min of irradiation they represented 9.8 and 17.2 % of the radioactivity according to HPLC.

>>M-024286-01-2@S-603010-01-1

Report:	01.02/15; [redacted]; 2002; M-039425-01-2
Title:	Imidacloprid - Small scale prospective ground-water monitoring study, Montcalm County, Michigan, 1996
Report No.:	110773
Document No.:	M-039425-01-2
Guideline(s):	-
Guideline deviation(s):	-
GLP/GEP:	**yes**

<<M-039425-01-2@S-603092-01-1

A small-scale prospective ground-water monitoring study was conducted approximately 3 miles north-northwest of Vestaburg, Michigan. The Test Site consisted of an approximately 3-acre Test Plot and a 0.5-acre Control Plot. Surficial soils (0-6 inches) were consistent across the study site, and consisted of loamy sand with approximately 82% sand, 12% silt, 6% clay and 1.0% organic matter, pH 5.8, cation exchange capacity of 3.5 meq/100 g, and bulk density of 1.5 g/cc. Deeper soils consisted of sand with layers of loamy sand, and a few discontinuous layers of sandy loam. Individual soils from the deeper depths contained more than 77% sand, less than 22% silt, less than 17% clay, and approximately 0.1% organic matter (0.5% at 6-12 inches). The average pH across the site increased with soil depth and ranged from 6.5 to 8.6. Depth to water was 16-18 feet below ground surface (bgs) at the time of well installation.

Imidacloprid was applied to the Test Plot as an in-furrow application of Admire 2F on May31, 1996, at a target rate of 0.34 lb imidacloprid per acre (110% of the label rate of 0.31 lb a.i. per acre for potatoes; Bayer Label 422-8686.BLD, dated 12/05/95). Application verification containers containing soil indicated the application was made at 105% of the label rate. Potassium bromide was applied on the same day as a 50% band at 50 lb/acre as a tracer of water movement.

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The Test Plot contained six instrument clusters, each containing suction lysimeters at 3.5, 6, 9, and 12 feet bgs, for monitoring soil-pore water. Each cluster also contained a shallow well screened to intercept the water table, and a deep well placed to allow sampling five feet below the shallow well, for monitoring ground water. A single instrument cluster was installed in the Control Plot. Shallow soil samples (< 24 inches bgs) were collected at 7 intervals through 31 days after treatment (DAT), and soil-pore water and ground water were collected at 39 intervals over a 4.5-year period (1647 days).

Imidacloprid residues (imidacloprid and its degradates - imidacloprid guanidine, imidacloprid guanidine olefin, and imidacloprid urea) were measured in soil by high performance liquid chromatography with a Limit of Quantitation (LOQ) of 0.01 mg/kg. For water samples, high performance liquid chromatography and electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS) was used for quantitation with an LOQ of 0.1 µg/L. The Method Detection Limits (MDLs) in soil ranged from 0.001 mg/kg to 0.002 mg/kg depending on the analyte. In water, the MDLs ranged from 0.02 µg/L to 0.04 µg/L, depending on the analyte and matrix (soil-pore water or ground water). Bromide was quantified in soil with an LOQ of 0.1 mg/kg, and in water with an LOQ of 0.2 mg/L.

Bromide moved rapidly through the soil profile and into ground water, indicating the soils were very permeable, and that sufficient water was applied to the site (209 inches of precipitation and irrigation). Imidacloprid degraded slowly during the 34 days of soil sampling with a half-life of 76 days ($r = 0.33$), and low concentrations of the degradates were observed. Imidacloprid residues were not detected below 12 inches. Imidacloprid residues (primarily imidacloprid) were detected in soil-pore water samples, with maximum concentrations of 2.1 µg/L at 3.5 feet (474 DAT), 1.3 µg/L at 6 feet (1201DAT),0.5 µg/L at 9 feet (1201 DAT), and 1.3 µg/L at 12 feet (1201 DAT). The mean soil-pore water concentrations (average of 6 lysimeters from the same depth) did not exceed 1.7 µg/L at 3.5 feet, and were less than 0.5 µg/L at the deeper depths. Imidacloprid was detected in ground water in only one of the six well clusters. Ground-water concentrations did not exceed 0.24 µg/L in the shallow well, or 0.14 µg/L in the deep well. Mean concentrations across the Test Plot did not exceed 0.04 µg/L in the shallow wells, or 0.02 µg/L in the deep wells. Imidacloprid detections in ground water continued through the end of the study, at low concentrations.

The study results indicate that imidacloprid residues have limited leaching potential under the conditions of this study. In highly vulnerable soils under conditions of very high precipitation and irrigation, imidacloprid may leach to ground water at very low concentrations.

>>M-039425-01-2@S-603092-01-1

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Report:	01.02/16; [redacted].; 2002; M-107157-01-2
Title:	Imidacloprid prospective groundwater monitoring study Monterey County, California
Report No.:	110889
Document No.:	M-107157-01-2
Guideline(s):	EPA Ref.: 166-1, Small-Scale Prospective Ground Water Monitoring
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-107157-01-2@S-602557-01-1

A small-scale prospective groundwater monitoring study was conducted approximately 11 miles southwest of Salinas in Monterey County, California. The test site consisted of a 4-acre test plot and a 1-acre control plot. The surficial soils 0 to 6 inches below ground surface (bgs) were relatively uniform across the study area and consisted of sandy loam with approximately 58% sand, 29% silt, 13% clay, and 0.9% organic matter. Deeper soils also consisted of sandy loam and sand. Discontinuous zones of silt loam were present in the upper 6 feet beneath the site. In addition, a deeper layer of sandy loam soil was found in the northern portion of the test site between 28-34 feet bgs. Depth to water was 19-24 feet bgs at the time of well installation.

Imidacloprid was applied to the test plot as an in-furrow application of Admire 2F on July 9, 1996, at a target rate of 0.45 lb imidacloprid per acre (120% of the label rate of 0.375 lb a.i. per acre for broccoli). Potassium bromide was applied on the same day in a 50% band at 50 lb/acre.

The treated plot contained six dusters of suction lysimeters for monitoring soil-pore water. The suction lysimeters were installed at a depth of 3.5, 6, 9, and 12 feet bgs. An additional slit trench lysimeter duster was installed on the treated plot with 5-foot screens which sampled at 1-4.8 ft, 4.8–8.6 ft, 8.6-12.4 ft, and 12.4-16.2 ft bgs. Twenty groundwater monitoring wells were installed on the treated plot. Eight well dusters contained a shallow well screened to intercept the water table and a medium depth well placed below the shallow well. Four well dusters contained a shallow and medium depth well plus an additional deep well in case the groundwater level dropped severely due to regional use. A single instrument duster consisting of lysimeters and wells (shallow and medium) was installed in the Control Plot (Cluster 7). Shallow soil samples (< 24 inches bgs) were collected at 7 intervals within 30 days after treatment (DAT) and again when the field phase was completed, (1520 DAT). Soil-pore water and ground water samples were collected at 57 and 56 intervals, respectively, over a 4.5-year period (1,654 days).

Soil was analyzed for imidacloprid and its degradates, imidacloprid guanidine, imidacloprid guanidine olefin, and imidacloprid urea using high performance liquid chromatography. The Limit of Quantitation (LOQ) of the method was 0.01 mg/kg. The Method Detection Limit (MDL) for imidacloprid, imidacloprid guanidine, imidacloprid guanidine olefin, and imidacloprid urea in soil was 0.005, 0.003, 0.002, and 0.001 mg/kg, respectively. The average concentration of imidacloprid in top soil (0-24 inches) decreased from 0.945 mg/kg to 0.248 mg/kg within 30 days of the application. Imidacloprid residues were not detected below 12 inches. The half-life for imidacloprid was less than 25 days.

Soil-pore water was analyzed for imidacloprid and its degradates, imidacloprid guanidine, imidacloprid guanidine olefin, and imidacloprid urea using high performance liquid chromatography and electrospray ionization tandem mass spectrometry (LC/ESVMS/MS). The LOQ for the mass spectrometry method was 0.05 µg/L. The method detection limit for imidacloprid and metabolites in soil-pore water ranged from 0.02 µg/L to 0.05 µg/L. Limited imidacloprid residues were detected in soil-pore water. A maximum concentration of 0.26 µg/L was found 666 days after treatment in a 9-foot lysimeter. Imidacloprid-guanidine, infrequently detected in soil-pore water, had a maximum concentration of 0.77 µg/L 182 days after treatment. Imidacloprid-olefin was also infrequently detected, with a maximum concentration of 0.05 µg/L found 31 and 756 days after treatment. Imidacloprid-urea detections in the lysimeters were infrequent with a maximum concentration of 0.75 µg/L found 182 days after treatment.

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Groundwater was analyzed for imidacloprid and its degradates, imidacloprid guanidine, imidacloprid guanidine olefin, and imidacloprid urea using high performance liquid chromatography and electrospray ionization tandem mass spectrometry (LC-ESVMS/MS). The LOQ for the mass spectrometry method was 0.05 µg/L. The method detection limit for imidacloprid and metabolites in ground water ranged from 0.04 µg/L to 0.06 µg/L. Imidacloprid was not found in the ground water. Imidacloprid urea was detected in two groundwater samples collected 210 days after application. The concentration of both detections in ground water was 0.05 µg/L, just above the method detection limit (MDL).

Bromide in soil was quantified using ion-selective electrode technology. The MDL was 0.4 mg/kg and the LOQ was 2.5 mg/kg. Bromide in ground water and soil-pore water was quantified by high performance liquid chromatography following chemical derivitization. The LOQ was 0.30 mg/L. The MDL was 0.02 mg/L in soil-pore water and 0.1 mg/kg in ground water. Bromide moved rapidly through the soil profile and into ground water, indicating the soils were very permeable, and that sufficient water (23.6 feet) was applied to the site to promote aquifer recharge.

A careful review of the water balance during the study shows that imidacloprid residues are not mobile. A total of 18 feet of excess water (total water applied minus crop evapotranspiration) was applied to the test plot following the chemical treatment. After the application of 18 feet of excess water imidacloprid was not found in any of the 3.5-foot lysimeters. The fact that bromide moved quickly through the soil profile while imidacloprid residues did not is a reflection of the lack of mobility of the insecticide in the sandy (vulnerable) soil profile. The study results indicate that imidacloprid residues have little or no leaching potential under the conditions of this study.

>>M-107157-01-2@S-602557-01-1

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02 - Honey bees

02.01 - Effects

02.01.01 - Lab Studies

02.01.01.01 - Active substance

Report:	02.01.01.01/01; [redacted]; 1990; M-048394-01-3
Title:	Examination of the bee toxicity for registration purposes. Laboratory testing
Report No.:	900239
Document No.:	M-048394-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-048394-01-3@S-604320-01-1

This 3 pages report does not contain a study summary.
A manually prepared study summary might be provided at a later stage.
>>M-048394-01-3@S-604320-01-1

Report:	02.01.01.01/02; [redacted]; 1990; M-048413-01-3
Title:	Examination of the bee toxicity for registration purposes - laboratory testing
Report No.:	900240
Document No.:	M-048413-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-048413-01-3@S-604322-01-1

This 3 pages report does not contain a study summary.
A manually prepared study summary might be provided at a later stage.
>>M-048413-01-3@S-604322-01-1

Report:	02.01.01.01/03; [redacted]; 1994; M-006940-02-3
Title:	The acute oral and contact toxicity to honey bees of compound NTN 33893 technical
Report No.:	BAY 158/901384
Document No.:	M-006940-02-3
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-006940-02-3@S-602931-01-1

This study was conducted on behalf of Bayer UK Ltd., to determine the acute toxicity to honey bees of NTN33893 technical by the United Kingdom Control of Pesticides Regulations 1986 protocol.

This protocol also satisfies the EPA Pesticide Assessment Guidelines for Non-target Insects, Subdivision L, Series 141-1.

Preliminary dose range finding tests indicated that NTN.33893 was highly toxic to bees with an oral LD_{50} of less than 0.1 µg/bee and a contact LD_{50} of about 0.1 µg/bee.

This was confirmed in a final test using 2 groups of 10 bees each at concentrations of 0.0015 - 0.025 µg/bee for the oral route and 0.025 - 0.40 µg/bee for the contact route.

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The 48-hour LD_{50}S with 95% confidence limits were found to be:

Oral LD_{50}	0.0037 µg/bee (limits 0.0026 - 0.0053)
Contact LD_{50}	0.081 µg/bee (limits 0.055 - 0.12)

It is concluded that NTN.33893 technical is highly toxic to bees by both oral and contact routes.
>>M-006940-02-3@S-602931-01-1

Report:	02.01.01.01/04; [redacted]; 1999; M-016792-01-4
Title:	Honeybee (Apis mellifera L.) oral toxicity study in the laboratory with imidacloprid techn.
Report No.:	AH99.4.22.4
Document No.:	M-016792-01-4
Guideline(s):	US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-016792-01-4@S-602478-01-1

The purpose of the toxicity study was to examine the effects of imidacloprid techn. on honeybees when applied in the laboratory. Per concentration 10 honeybees were fed with 100 µl sucrose solution 50% containing a range of concentrations of imidacloprid techn. By sharing the food (trophallaxis), each honeybee gets about 10 µl.

The sponsor indicated that the oral LD_{50} was between 5 and 20 ng / honeybee. That is why the concentration range of about 4 ng to 20 ng Imidacloprid techn. / honeybee was tested.

Per concentration 10 honeybees were fed with 100 µl sucrose solution 50% containing respectively: 21.22 ng imidacloprid techn., 16.78 ng imidacloprid techn, 13.18 ng imidacloprid techn, 8.63 ng imidacloprid techn. and 4.09 ng imidacloprid techn. per 10 µl.
The treatment was compared to a 50 % sucrose-solution (negative control) and a Dimethoate positive control.

The concentrations of imidacloprid techn. fed to the bees in this test, did not cause mortality of the honeybees. However effects were observed. The most significant effect was the "frozen behaviour" at which the honeybees are motionless except for a little trembling of body parts like abdomen, antennae or tarsus. Some honeybees, which had taken in about 20 ng, showed spasms and were paralysed.
As there are no data on mortality, the LD_{50} of imidacloprid techn. could not be determinated. The lethal concentration is more than 21 ng/bee
The ED_{50} of imidacloprid techn. after 24 hours, calculated with the linear regression is 34 ng / honeybee. (r2 = 0.50).
The data on effect vary a lot but the effect is clear. Amounts of 4.39 ng / honeybee or less do not result in any effect. Amounts of 6.83 ng imidacloprid techn. or higher result more or less in the described frozen behaviour.
>>M-016792-01-4@S-602478-01-1

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Report:	02.01.01.01/05; [redacted]; 1999; M-017133-01-4
Title:	Honey bee (Apis mellifera L.) contact toxicity study in the laboratory with imidacloprid technical
Report No.:	AH99.4.22.3
Document No.:	M-017133-01-4
Guideline(s):	US EPA OCSPP Guideline no. 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-017133-01-4@S-602498-01-1

The purpose of the toxicity study was to examine the effects of imidacloprid techn. on honeybees when applied in the laboratory. Individual honeybees were exposed to imidacloprid techn. by way of administration of imidacloprid techn., dissolved in acetone, on the ventral part of the thorax.

The sponsor indicated that the contact LD_{50} was between 40 and 200 ng / honeybee. That is why a concentration range of about 40 ng to 200 ng imidacloprid / honeybee was tested.

Per concentration honeybees were treated with 1 µl acetone containing respectively: 207 ng imidacloprid techn., 166 ng imidacloprid techn., 125 ng imidacloprid techn, 85 ng imidacloprid techn. and 42 ng imidacloprid techn. per 1 µl.
The treatment was compared to an acetone treatment (negative control) and a Dimethoate positive control.

The concentrations imidacloprid techn. administered to the honeybees in this test caused mortality of the honeybees. Mortality was preceded by effect. The most significant effect was the "frozen behaviour" at which the honeybees are motionless except for a little trembling of body parts like abdomen, antennae or tarsus. The first signs of effect were observed within 30 minutes after administration of imidacloprid techn. Mortality continued during the observation period.
The LD_{50} of Imidacloprid techn. based on the linear regression is:
LD_{50} (72 hours): 129 ng imidacloprid techn. ($r^2 = 0.42$)
The ED_{50}, of Imidacloprid techn. based on the linear regression is:
ED_{50} (72 hours): 131 ng imidacloprid techn. ($r^2 = 0.38$)
The effect of imidacloprid techn. administered in the concentrations from 40 to 200 ng / honeybee is clear. The typical "frozen behaviour" is observed in all concentrations tested. Mortality continued during the test period and because honeybees that are immobilised for several days eventually die, after 72 hours the LD_{50} and the ED_{50} are in the same range.

>>M-017133-01-4@S-602498-01-1

Report:	02.01.01.01/06; [redacted]; 1999; M-016942-01-4
Title:	Laboratory testing for toxicity (acute oral LD50) of NTN 33893 on honey bees (Apis mellifera L.) (Hymenoptera, Apidae)
Report No.:	6400036
Document No.:	M-016942-01-4
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-016942-01-4@S-602480-01-1

Material and methods: test substance: NTN 33893, purity: 99.4%, batch number: M00680; under laboratory conditions, starved honey bees *(Apis mellifera,* 3 groups of 10 bees per dose) received a single oral dose of either 40.9, 22.9, 12.2, 6.0, 3.1, 1.5, 0.8 or 0.1 ng per bee in ca. 20 mg sugar solution. Subsequently, honey bees were observed over a period of 96 hrs for behavioural impairments and survival rate. The test was prolonged up to 96 hours because of increasing mortality between 24 and 48 hours. The reference treatment (0.2 µg dimethoate per bee) caused a 100 % mortality (the facility-specific LD_{50} dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

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Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	NTN 33893
Test object	*Apis mellifera*
Application rates ng product/bee	40.9*, 22.9*, 12.2*, 6.0*, 3.1*, 1.5*, 0.8* and 0.1*
Exposure	oral (sugar solution)
LD_{50} ng product/bee (48 and 96h)	approximately 40.9

* values based on actual intake of the test substance

Observations: the observation period was extended for 48 hours because of delayed mortality in the higher dose groups. No treatment-related mortalities or behavioural impacts were recorded at oral doses of 1.5 ng/bee and lower. Oral doses of 3.1 ng bee and higher caused treatment-related mortalities and behavioural impacts such as apathy and exaggerated/discoordinated movements. The behavioural impacts lasted dose-related up to 48 hours; hi the control, three of 30 bees (3.3%) died whereas all bees died in the groups treated with the toxic standard.

>>M-016942-01-4@S-602480-01-1

Report: 02.01.01.01.07; [redacted]; 2000; M-068009-01-3
Title: Substance A - Acute contact toxicity to honey bees (Apis mellifera)
Report No.: HT0400a
Document No.: M-068009-01-3
Guideline(s): US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s): not specified
GLP/GEP: no

<<M-068009-01-3@S-602704-01-1

Tests were carried out to determine the acute contact toxicity of Substance A to adult honey bees (*Apis mellifera* L.). The protocol followed the EPPO guidelines (1992) and are in accordance with the draft EPA Ecological Effects Test Guidelines (OPPTS 850.3020 Honey Bee Acute Contact Toxicity) and OECD guideline 214 Honeybees, Acute Contact Toxicity (September 1998). All doses and toxicity data for the test substance refer to Substance A as the active ingredient.

Three batches of bees, in groups of 10 bees, were topically dosed on the thorax with 1 µl drops containing 140, 110, 78, 56, or 40 ng Substance A /bee in acetone. Mortality and sub-lethal effects were assessed at 4, 24 and 48 hours after dosing. Results indicated that the 24-hour contact LD_{50} of Substance A is greater than 140 ng/bee but by 48 hours and 72 hours this had decreased to 50 ng/bee and 49 ng/bee respectively. There were significant sublethal effects in all doses at 4 hrs with recovery or death by 48 hrs.

M-068009-01-3@S-602704-01-1

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Report:	02.01.01.01/08; [redacted]; 2000; M-067996-01-3
Title:	Substance A - Acute oral toxicity to honey bee Apis mellifera
Report No.:	HT0400b
Document No.:	M-067996-01-3
Guideline(s):	US EPA OCSPP Guideline no.: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-067996-01-3@S-602702-01-1

Tests were carried out to determine the acute oral toxicity of Substance A to adult honey bees (*Apis mellifera* L.). The protocol followed the EPPO guidelines (1992) and OECD guideline 213 Honeybees: Acute Oral Toxicity Test (September 1998). All doses and toxicity data for the test substance refer to Substance A as the active ingredient.

Three batches of bees, in groups of 10 bees, were offered the equivalent doses of 73.6, 24.6, 8.2, 2.8, 0.94 ng /bee Substance A in 50% w/v aqueous sucrose solution, the test substance having first been dissolved in acetone. At the highest treatment level the mean dose consumed was 45 ng /bee Substance A, 39% less than the actual dose offered. This lowered intake may be due to repellency or to the large numbers of bees observed as knocked down at 4 hrs, the bees were on their feet but immobile and therefore unable to feed.

Mortality was assessed at 4 hours after dosing. Glass test feeders were then removed and further assessments made at 24 and 48 hours after removal of the glass test feeders. Results indicated that the 24-hour and 48-hour oral LD_{50} of Substance A is greater than 45 ng /bee. Significant sub-lethal effects (50-100% knockdown) were observed at 4 hrs in the highest two doses but only 10% knockdown was observed in the highest dose at 24 hrs.

>>M-067996-01-3@S-602702-01-1

Report:	02.01.01.01/09; [redacted]; 2000; M-068023-01-3
Title:	Acute toxicity of substance A to the honeybee Apis mellifera L. under laboratory conditions
Report No.:	00 10 48 0501
Document No.:	M-068023-01-3
Guideline(s):	EPPO Standard PP 1/170(2) (1999); OECD 213 (1998), OECD 214 (1998) US EPA OCSPP Guideline no 850.SUPP
Guideline deviation(s):	not specified
GLP/GEP:	**no**

<<M-068023-01-3@S-602709-01-1

Results:
The test endpoints were mortality and behaviour of the honeybees in comparison with the control. Contact exposure to substance A caused the following mortalities:

	Mortality/Corrected mortality according to Abbott (%)			
Substance A ng/bee	24h	48	72	96
control	3.3/-	3.3/-	6.7/-	6.7/-
153.7	76.6/75.9	80.0/79.3	80.0/78.6	80.0/78.6
109.8	60.0/58.6	73.3/72.4	73.3/71.4	80.0/78.6
78.4	26.7/24.2	50.0/48.3	50.0/46.4	56.6/53.6
56.0	23.3/20.7	30.0/27.6	30.0/25.0	36.6/32.2
40.0	30.0/27.6	33.3/31.1	33.3/28.6	36.6/32.2
LD_{50}(contact) ng/bee	**97.7**	**74.9**	**78.4**	**69.0**
Confidence limits				
lower	79.08	61.77	64.70	56.06
upper	120.73	90.90	94.99	85.0
Slope b	2.56	2.63	2.75	2.6

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Therefore it is concluded that the LD_{50} for contact exposure was 74.9 ng substance A per bee in the contact toxicity test after 48 hours of exposure. The study was prolonged because mortality increased between 24 h and 48 h. The LD_{50} after 72 and 96 hours was 78.4 and 69.0 ng substance A per bee.

In all contact treatments apathy, discoordinated movements and immobility were observed after up to 48 hours after application. 72 h and 96 h after application the surviving bees had recovered and exhibited no further behavioural anomalies.

Oral uptake caused the following results:

The oral uptake of the test substance at doses of 81, 27, 9, 3 and 1 ng test substance per bee caused 46.7 %, 10 %, 20.0 %, 3.3 % and 6.7 % mortality after 48 h.

Therefore it is concluded that the LD_{50} (48 h) is 74.9 ng substance A per bee in the contact toxicity test and slightly higher as the highest provided dose of 81 (70.3 consumed) ng test substance A per bee in the oral toxicity test.

In all contact treatments and in the 81 ng a.i./bee and 27 ng a.i./bee oral treatments apathy, discoordinated movements and immobility were observed after 24 hours after application. 48 hours after application the surviving bees had recovered and exhibited no further behavioural anomalies.

The validity criterion - mortality in the control ≤ 10% - was accomplished (being 3.3 % in the contact and 3.3 % in the oral toxicity tests after 48 hours).

>>M-068023-01-3@S-602709-01-1

Report:	02.01.01.01/10; [redacted]; 2012; M-418424-02-3
Title:	Report amendment no. 1 to study S11-01962 - Imidacloprid (tech.) - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding test
Report No.:	S11-01962
Document No.:	M-418424-02-3
Guideline(s):	850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-418424-02-3@S-602251-01-1

Materials and Methods:

Test item: Name: Imidacloprid (tech.) Batch: AE F106464-01-44 Customer Order No.: TOX 09352-00
Content of a.i. analysed: 99.4 % (w/w)

The chronic effects of the test item imidacloprid (tech.) on the honey bee, *Apis mellifera* L., in a 10 days continuous feeding in the laboratory were assessed.

Over a period of 10 days, honey bees were exposed to 50 % (w/v) aqueous sucrose feeding solution, containing nominally 10, 20, 50 and 100 µg a.i./L of the test item imidacloprid (tech.) by continuous and *ad libitum* feeding. The control group was exposed for the same period of time under identical exposure conditions to untreated 50 % (w/v) aqueous sucrose feeding solution. Mortality, sublethal effects and behavioural observations were assessed every day throughout the 10 days exposure period. Furthermore, the daily food uptake was determined.
Samples and retain samples of all feeding solutions and the stock solution were taken for chemical analysis.

Dates of work: 21 June 2011 – 01 July 2011

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Findings
After 10 days of continuous exposure, mortality at all test item treatment levels was not statistically significantly increased compared to the control group.
The cumulative control mortality accounted to 2.67 %, as determined at the final assessment (day 10). At the highest test item treatment level of 100 µg a.i./L, the cumulative mortality at the final assessment (day 10) accounted to 4.00 % (corrected 1.37 %). Based on mortality, the highest test item treatment level of 100 µg a.i./L was determined to be the NOEC (No Observed Effect Concentration).

Starting on the fifth day (d5) of continuous feeding and lasting until the final assessment (d10), the bees in the highest test item treatment group of 100 µg a.i./L were observed to be very calm and inactive compared to the bees in the lower test item treatment groups and the control group, respectively.

The mean daily consumption of the sucrose feeding solution was statistically significantly reduced at the test item treatment level of 20, 50 and 100 µg a.i./L during the entire test period respectively (day-byday comparison). In the lowest test item treatment group of 10 µg a.i./L, the mean daily consumption of the sucrose feeding solution was statistically significantly reduced compared to the control group on day 2, 6, 7 and 10.

The overall mean daily consumption of the sucrose feeding solution (i.e. average value over 10 days) was 47.1, 37.7, 39.8 and 33.3 mg/bee in the test item treatment groups of 10, 20, 50 and 100 µg a.i./L, respectively. These overall mean daily consumption values were statistically significantly lower in all test item treatment groups compared to the control group (54.2 mg/bee).

After 10 days of continuous exposure, the accumulated nominal intake of the test item imidacloprid (tech.) via imidacloprid - treated sucrose solution was 0.00397, 0.00638, 0.01674 and 0.02820 µg a.i./bee at the test item treatment level of 10, 20, 50 and 100 µg a.i./L, respectively.

Table 1: Mean consumption of feeding solution, mean intake of test item accumulated over all test days and cumulative mortality at the final assessment on day 10

Treatment Level [1]	Control	Test Item			
		10	20	50	100
		[µg a.i./L]			
Overall mean daily consumption of aqueous sucrose feeding solution [mg/bee] [2]	54.2	47.1*	37.7*	39.8*	33.3*
Mean intake accumulated over test days [µg a.i./bee]	-	0.00397	0.00638	0.01674	0.02820
Cumulative mortality [%]	2.67	4.00	0.00	1.00	4.00 [3]
Corrected cumulative mortality [%]	-	1.37	-2.74	-1.72	1.37

[1] The control group was fed with untreated 50 % (w/v) aqueous sucrose feeding solution; the test item treatment group was fed with imidacloprid-treated 50 % (w/v) aqueous sucrose feeding solution
[2] The mean values per cage over the test period were used as basis for the calculation of the overall mean daily consumption of the aqueous sucrose feeding solution per treatment over the test period
[3] Determined to be the NOEC based on mortality (not significantly different compared to the control; Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, $p \leq 0.05$))
* Food consumption significantly lower compared to the control group (Bonferroni-U test; one-sided, $p \leq 0.05$)

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Conclusions

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item imidacloprid (tech.) at the treatment levels of 10, 20, 50 and 100 µg a.i./L caused no adverse effect regarding mortality. Based on mortality, the highest test item treatment level of 100 µg a.i./L was determined to be the NOEC (No Observed Effect Concentration). At the highest concentration level of 100 µg a.i./L, from d5 to d10, bees were observed to be very calm and inactive as compared to the lower test item treatment groups and the control group, respectively.
The feeding of imidacloprid (tech.) at the dose rates of 20, 50 and 100 µg a.i./L resulted in a statistically significantly reduced mean daily food uptake during the entire test period, when compared to the untreated control group (day-by-day comparison). Moreover, also the overall mean (i.e. average value over 10 days) daily food uptake was statistically significantly lower in all test item treatment groups when compared to the untreated control group.

>>M-418424-02-3@S-602251-01-1

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Report: 02.01.01.01/11;[redacted]; 2000; M-067751-01-3
Title: Substance A - Acute effects on the honeybee Apis mellifera (Hymenoptera, Apidae), non-GLP
Report No.: IBA7240N
Document No.: M-067751-01-3
Guideline(s): US EPA OCSPP Guidelineno: 850.SUPP
Guideline deviation(s): not specified
GLP/GEP: **no**

<<M-067751-01-3@S-602698-01-1

Material and methods: The test item Substance A was applied at nominal concentrations of 1, 3, 9, 27, 81 ng a.i. per bee for oral application and of 40, 56, 78, 110, 154 ng a.i. per bee (dissolved in acetone) for contact application under laboratory conditions.
Control item in oral application mode was 50 % aqueous sucrose solution with same content of acetone as test item. For the contact application acetone was used as control.

Apis mellifera (only worker bees), were kept in ventilated stainless steel cages, in 3 replicates of 10 individuals for each treatment. Mortality was assessed after a 4, 24 and 48 h exposure period and additional after 96 h in oral application mode.

Dates of work: May 05 to 08, 2000

Findings: summarized in Tables 1-2.

Table 1 **Oral Application Mode: Mortality Values of Substance A**

Concentration [ng a.i./Biene]		Mortality [%]
Nominal	Real*	48 h
Control	0.00	0
1	0.94	0
3	2.81	3
9	7.01	0
27	17.80	7
81	34.70	17
LD_{50} [ng a.i./bee] after 48 h		> 34.70

* Application rate based on actual ingestion of the test item

Table 2 **Contact Application Mode: Mortality Values of Substance A**

Concentration [ng a.i./Biene]	Mortality [%] after 48 h
Control	0
40	43
56	63
78	73
110	90
154	87
LD_{50} [ng a.i./bee]after 48 h	42.92
Confidence interval	34.64 – 53.19

>M-067751-01-3@S-602698-01-1

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Report:	02.01.01.01/12; [redacted]; 2011; M-414619-02-4
Title:	Imidacloprid tech.: Effects of exposure to spiked diet on honeybee larvae (Apis mellifera carnica) in an in vitro laboratory testing design
Report No.:	E 318 4110-8
Document No.:	M-414619-02-4
Guideline(s):	US EPA OCSPP guideline # 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-414619-02-4@S-604959-01-1

Material and methods: Test item: Imidacloprid, tech. (Development-code: NTN 33893; TOX-No.: 09352-00; Specification No.: 102000006766; Batch code: AE F106464-01-44; LIMS No.: 1109587; content of a.s. (analysed): 99.4% w/w). Principle of the testing procedure: At day +1*, first instar bee larvae (Apis mellifera carnica) were transferred from their bee hive into an artificial in vitro testing system. The bee larvae were fed with standardised amounts of untreated artificial diet at day +1 and day +3. On day +4, +5 and +6, the bee larvae in the test item treatment groups were fed with standardised amounts of test item spiked artificial exposure diet. On day +4, the bee larvae in the reference item treatment group were fed with standardised amounts of reference item spiked artificial exposure diet. Concurrently, the bee larvae in the control group (on day +4, +5 and +6) and in the reference item group (on day +5 and +6) received untreated artificial exposure diet, respectively. In the test item treatment groups, imidacloprid tech. was incorporated into the artificial exposure diet at the nominal test concentrations of 5, 10, 20 and 40 µg a.s./kg diet. The actual concentration of imidacloprid in the test item spiked exposure diet was determined according of Modification M002 to analytical method 00537 (MR-06/144, 2006-11-02, R. [redacted]) by using High Performance Liquid Chromatography, coupled with tandem mass spectrometry.

(*) Day 0 was the anticipated day of larval hatching.

During the development of the honeybee larvae, the larvae were incubated at about +35 °C. From day +1 to +8, the relative humidity inside the incubator was on average about 95 ± 5% and from day +8 to +22 the mean relative humidity was about 80 ± 5%. Mortality was determined on day +5, +6, +7, +8, +11, +13, +15 and +22. Dead test animals were discarded for sanitary reasons.

Dates of experimental work: May 04, 2011 - July 27, 2011

Results: In total, four independent test runs were conducted. In all test runs, the validity criteria as stated in the INRA - method for testing pesticide toxicity to honeybee brood in laboratory conditions (January 2008) and proposed by the recommendations of the honeybee larvae laboratory ring-test group (AUPINEL *et al.*, 2009) were met (i.e. mortality in the control group < 15% and in the reference group > 50% until day +7). In addition to the validity criteria as proposed by the ring-test group, an additional self-set validity criterion was employed (i.e. mortality in the control group < 30% until day +22). This self-set validity criterion was applied in order to exclude test runs from which it is difficult to derive biologically meaningful information due to elevated mortality levels.

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Table 1 Control performance in the individual test runs and associated validity Criteria

Validity criteria	Origin of validity criteria	Validity threshold	Obtained results			
			Test run No. 1	Test run No. 2	Test run No. 3	Test run No. 4
Mortality in the control group until day +7	INRA - method for testing pesticide toxicity to honeybee brood in laboratory conditions (January 2008)	≤ 15%	2.2%	3.1%	14.3%	2.1%
Mortality in the reference group until day +7 (Abbott)		≥ 50%	82.2%	63.8%	61.5%	68.1%
Mortality in the control group until day +22	Self-set	≤ 30%	37.0% ‡	18.8%	16.7%	14.6%

‡ Actual control performance at the end of the test has not met the self-set validity criterion

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Table 2 Control and test item performance and associated statistical evaluation

Test object	Honeybee larvae *(Apis mellifera carnica)*					
	Control (untreated exposure diet)	**Test item** (imidacloprid, spiked exposure diet)				**Reference item** (dimethoate, spiked exposure diet)
Test concentration [µg a.s./kg diet]	---	5	10	20	40	3.0 [µg a.s./larva]
Test run No. 1 [a]						
Mortality until day +22 [%]	37.0 [a]	47.8	47.8	32.6	41.3	95.7
Abbott-corrected mortality until day +22 [%]	0.0	17.2	17.2	-6.9	6.9	93.1
Test run No. 2						
Mortality until day +22 [%]	18.8	16.7	20.8	33.3	8.3	97.9
Abbott-corrected mortality until day +22 [%]	0.0	-2.6	2.6	17.9	-12.8	97.4
Test run No. 3						
Mortality until day +22 [%]	16.7	46.7	20.0	30.0	16.7	100.0
Abbott-corrected mortality until day +22 [%]	0.0	36.0	4.0	16.0	0.0	100.0
Test run No. 4						
Mortality until day +22 [%]	14.6	20.8	16.7	12.5	22.9	100.0
Abbott-corrected mortality until day +22 [%]	0.0	7.3	2.4	-2.4	9.8	100.0
Test runs No. 2, 3 and 4 combined [b]						
Mortality until day +22 [%]	16.7	25.4	19.0	24.6	16.7	99.2
Abbott-corrected mortality until day +22 [%]	0.0	10.5	2.9	9.5	0.0	99.0
Statistical comparison to the control [c]	---	n.s.	n.s.	n.s.	n.s.	---
NOEC [c]	≥ 40 µg a.s./kg diet					---
LOEC [c]	> 40 µg a.s./kg diet					---

[a] Although control performance met the validity criteria as stated in the INRA - method for testing pesticide toxicity to honeybee brood in laboratory conditions (January 2008), the self-set validity criterion for control performance at the end of the test (i.e. ≤ 30 %) was not met; no distinct differences in larval mortality can be observed at concentrations of up to and including 40 µg imidacloprid a.s./kg diet (as the self-set validity criterion was not met, no detailed statistical evaluation is presented; however, when subjecting the data to statistical analysis, there is no statistical significance up to and including 40 µg a.s./kg diet; Chi^2 Test [Bonferroni-Holms corrected, one-sided, $\alpha = 0.05$])

[b] All larvae, dead and alive, in the test item treatment groups and in the control group of the test runs No. 2, 3 and 4, respectively, were combined

[c] Chi^2 Test, (Bonferroni-Holms corrected, one-sided, $\alpha = 0.05$)
n.s.: mean value not statistically significantly different compared to the control

The analytical determination of the imidacloprid concentration in the spiked exposure diets of the test item treatment groups revealed for all four test runs [test runs No. 2, 3 and 4] the following results:

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5 µg a.s./kg diet - treatment level: nominal	On average 98 - 105% [test runs 2, 3 and 4 98-105%] of
10 µg a.s./kg diet - treatment level: nominal	On average 103 -110% [test runs 2, 3 and 4 103-110%] of
20 µg a.s./kg diet - treatment level: nominal	On average 101 -115% [test runs 2, 3 and 4 101-115%] of
40 µg a.s./kg diet - treatment level: nominal	On average 102-111% [test runs 2, 3 and 4 102-111%] of

Conclusions:

All four independent test runs, as performed during the course of this *in vitro* honeybee larvae study, comply with the validity criteria as proposed by the INRA-method (January, 2008) for testing pesticide toxicity to honeybee brood in laboratory conditions (i.e. until day +7, < 15% mortality in the control group and > 50% mortality in the reference group), three independent test runs (test runs No. 2, 3 and 4) fulfilled both, the validity criteria as proposed by the INRA-method (January 2008) and the self-set validity criterion(i.e. < 30% mortality in the control group until day +22). The analytical determination of imidacloprid in the exposure diets of the test item treatment group revealed that the actual concentrations were well in line with the nominal concentrations. The statistical processing of the combined data as obtained in the test runs No. 2, 3 and 4 revealed no statistically significant effects on mortality of exposed honeybee larvae until day +22 (end of the test, emergence) at concentrations of up to and including 40 µg imidacloprid a.s./kg diet (Chi2 Test, Bonferroni-Holms corrected, one-sided, a = 0.05). The outcome of this statistical evaluation is further supported by the findings of the test run No. 1.

Overall, it can be concluded that the No Observed Effect Concentration (NOEC) as determined in this *in vitro* honeybee larvae study is > 40µg imidacloprid a.s./kg diet.

>>M-414619-02-4@S-604959-01-1

Report: 02.01.01.01/13; [redacted]; 1998; M-110203-01-3
Title: Study of the sublethal effects of Imidacloprid and Endosulfan on olfactory learning in the honeybee Apis mellifera
Report No.: M-110203-01-3
Document No.: M-110203-01-3
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

<<M-110203-01-3@S-603263-01-1

The foraging activity of the domestic honeybee *Apis mellifera* L is based on an associative learning process [1] during the course of which the insect associates the presence of food (nectar, pollen) with the characteristics of the flower (colour, shape, volatile emissions). The fragrance-food association proves most efficient when detecting floral food sources. This individual learning process is linked to the gathering of fellow creatures at the heart of the hive, which then allows floral food sources to be exploited collectively. The main purpose of plant protection products in systems of agriculture, which is to protect crops, cannot always be reconciled with the optimum exploitation of food sources. For example, it is known that certain insecticides affect the behaviour of bees. Sublethal doses of parathion (organophosphate) affect the gathering of other honeybees by interfering with the parameters of the vibrating dance [2;3;4;5;6]. Honeybees use the vibrating dance to communicate information about the distance, direction and quality of food sources at distances of more than 100 metres from the nest [7]. To do so, honeybees are able to convert the angle between rays of the sun and the direction of the resources into an angle between the direction of the dance and gravity. The anti-cholinesterase activity of parathion [8] interferes with this ability [2;3].It also interferes with the bees' biological clock [6]. Sublethal doses of permethrin and deltamethrin (pyrethroids) disturb the honeybees' sense of direction.

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Finally, apiarists blame a new insecticide used to treat sunflower seeds (trade name Gaucho®) for causing a sensitive reduction in their harvest of honey from sunflowers. This occurred when hives emptied during the flowering period of this crop. According to bee experts, honeybees suffer behavioural aberrations with the result that they are unable to find their way back to the hive. These data suggest that the neurotoxic substances may have sublethal effects and, in particular, cause disturbances in individual learning processes in foraging activity. The aim of this work was to test this hypothesis in laboratory experiments.

It is possible to study individual foraging activity by olfactory conditioning of the reflex to extend the proboscis (bee's tongue) which was achieved in individual bees kept in an immobilised state. This experimental process was used in our study to evaluate the effects of weak doses of imidacloprid and endosulfan on the bee's olfactory learning abilities. Research has been done into the sublethal effects on behaviour in the short term and long term. The potential chronic effects of treatment on the life span of worker bees has also been studied.
Using the same biological test, it was shown that learning ability depreciates in workers bees which have been exposed to pyrethroids [11;12] and to dicofol [13], a chemical similar to DDT. Our analysis of behaviour was combined with electrophysiological analysis aimed at evaluating the concomitant modifications of peripheral olfactory sensitivity resulting from contamination. We also began recording electroantennograms (EAG), this technique having already been used in bees to study relations between behavioural reflexes and peripheral sensitivity [14;15;16;17]. Moreover, among numerous arthropods, measuring receptor potentials has allowed evaluation of the neurobiological impact of certain pyrethroids [18;19].
>>M-110203-01-3@S-603265-01-1

Report:	02.01.01.01/14; [redacted]; [redacted]; 1999; M-017079-01-4
Title:	Residue levels of imidacloprid and imidacloprid metabolites in honeybees orally dosed with imidacloprid in standardized toxicity tests (EPPO 170)
Report No.:	SXR/AM 013
Document No.:	M-017079-01-4
Guideline(s):	US EPA OPPTS: N/A (EPPO guideline 170)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-017079-01-4@S-602155-01-1

Material and methods: test substance specification: imidacloprid techn., batch no. M00680, purity 99.4%. Adult honeybees were orally dosed with either 0.0001, 0.0008, 0.0015, 0.0031, 0.006, 0.012, 0.023 or 0.041 µg honeybee imidacloprid techn.. Honeybees which died during the study were removed from the test boxes at each evaluation and stored at -20°C. At study termination, alive honeybees were killed by CO_2 asphyxiation and retained also at -20°C till residue analysis. After shipping the honeybee samples to Bayer AG, they were analysed for residues of imidacloprid and toxicologically relevant metabolites , i.e. olefin- and hydroxy-imidacloprid.

Dates of biological work: July 6-10,1999 (IBACON study 6400036).
Dates of analysis of biological samples: September 15-17, 1999.

Findings: Residues in honeybees orally dosed with imidacloprid techn.:

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Dose Applied [ng/bee]	Time to Death [h]	Sample weight [g]	Hydroxy-NTN [mg/kg]	Olefin-NTN [mg/kg]	Imidacloprid [mg/kg]
0.1	-- **	3.8	n.d.	n.d.	n.d.
0.8	-- **	4.1	n.d.	n.d.	n.d.
1.5	-- **	3.7	n.d.	n.d.	n.d.
3.1	4	0.3	n.d.	n.d.	< LOQ
	24	0.6	< LOQ	n.d.	n.d.
	-- **	2.7	< LOQ	n.d.	n.d.
6.0	24	0.7	< LOQ	n.d.	n.d.
	-- **	2.7	< LOQ	n.d.	n.d.
12.2	24	0.5	< LOQ	n.d.	n.d.
	-- **	2.8	0.006	n.d.	n.d.
22.9	24/48	0.4	0.010	< LOQ	< LOQ
	-- **	3.3	0.010	< LOQ	n.d.
40.9	1/1.15	0.2	n.d.	n.d.	< LOQ
	24	0.6	0.006	n.d.	0.017
	48/72	0.7	0.040	0.010	< LOQ
	-- **	1.8	0.006	< LOQ	n.d.

* Limit of quantitation: 0.005 mg/kg (imidacloprid & hydroxy-metabolite, 0.01 mg/kg (olefin-metabolite); n.d. = below limit of detection (0.0015 mg/kg and 0.003 mg/kg, respectively)
** Honeybees were asphyxiated by CO_2 at study termination

Observations: Oral doses of 1.5 ng/bee or less had no observable adverse effects on honeybees and no residues of imidacloprid or the olefin- and hydroxy-metabolite could be detected in those bees. All other doses caused adverse effects and residues of imidacloprid or the olefin and hydroxymetabolite could be detected in the respective honeybee samples. In most cases, the highest residue level was found for the hydroxy-metabolite which may be a suitable indicator for a significant exposure of honeybees to imidacloprid.

>>M-017079-01-4@5-502138-01-1

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Report:	02.01.01.01/15; [redacted]; 1992; M-008940-01-2
Title:	NTN 33893: Toxicity to honey bees on alfalfa treated foliage
Report No.:	103938
Document No.:	M-008940-01-2
Guideline(s):	FIFRA Guideline 141-2 Hazard Evaluation: Nontarget Insects
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-008940-01-2@S-602467-01-1

TEST SUBSTANCE: NTN 33893 240FS

WASHINGTON STATE UNIVERSITY PROJECT NO.92-004

STUDY: NTN 33893/Honey Bees Toxicity of Residues on Foliage

RESULTS:

Residue bioassay of NTN 33893 240FS (0.045 lb(AI)/acre)
Bioassay on Apis mellifera L., order Hymenoptera.
The percent mortality with 2 hour old residues was 5.6, with 8 hour old residues 7.2 and with 24 hour old residues 11.9.

Residue bioassay of NTN 33893 240FS (0.167 lb(AI)/acre)
Bioassay on Apis mellifera L., order Hymenoptera.
The percent mortality with 2 hour old residues was 11.7, with 8 hour old residues 16.1 and with 24 hour old residues 15.9.

Residue bioassay of NTN 33893 240FS (0.5 lb(AI)/acre)
Bioassay on Apis mellifera L., order Hymenoptera.
The percent mortality with 2 hour old residues was 11.8, with 8 hour old residues 23.1 and with 24 hour old residues 20.8.

CONCLUSION

NTN 33893 240FS (0.045 lb(AI)/acre) was non-hazardous to honey bees if applied in early morning or late evening when bees are not foraging.

NTN 33893 240FS (0.167 lb(AI)/acre was non-hazardous to honey bees if applied in late evening when bees are not foraging.

NTN 33893 240FS (0.5 lb(AI)/acre) was moderatly hazardous to honey bees if applied in late evening.

TEST DATES:
First Test:
Experimental Start - 9 September 1992
Experimental Termination - 11 September 1992
Second Test:
Experimental Start - 14 September 1992
Experimental Termination - 16 September 1992

STUDY COMPLETION: 16 September 1992
>>M-008940-01-2@S-602467-01-1

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Report: 02.01.01.01/16; [redacted]; 2000; M-110229-01-3
Title: Impact of imidacloprid and its main metabolites on the honeybee Apis mellifera L.: effect of chronic exposure on mortality and learning
Report No.: M-110229-01-3
Document No.: M-110229-01-3
Guideline(s): not specified
Guideline deviation(s): not specified
GLP/GEP: **no**

<<M-110229-01-3@S-604666-01-1

Crop protection treatments applied to nectar-producing plants in flower can affect the survival of behaviour of bees. In contrast to acute lethal effects, which are investigated by means of toxicology tests before products are placed on the market, there is currently no objective way of detecting the sub-lethal effects of pesticides on bee behaviour or of evaluating their chronic toxicity.

During the national study programme carried out in 1998 to evaluate the effects of Gaucho® sunflower seed dressing on bees we studied the chronic toxicity of the active ingredient in this product (imidacloprid) and its effects on the olfactory learning capacity of worker bees, which is still a matter of some dispute. Olfactory learning processes are vital in enabling bees to recognise flowers as they forage. At that time we observed significant mortality compared to the control group at concentrations of 8 and 40 ppb after 11 days ingestion of imidacloprid. Furthermore, after the 11 days administration of concentrations of 4, 8 and 40 ppb we observed a significant decline in learning performance compared to untreated individuals when we performed a Pavlovian olfactory conditioning procedure. However, we did not find any concentration-response relationship or any no-effect concentration. It should be noted that the concentrations of imidacloprid used in 1998 were not all investigated on the same day. In addition, the 1998 results were based on only two repeats. The purpose of this investigation is therefore to find out more about the sub-lethal effects of imidacloprid on bees subjected to Pavlovian conditioning. In order to do this we attempted to define concentration-response relationships and threshold concentrations by using a wide range of experimental concentrations. We also evaluated the possible effects of the two main metabolites of imidacloprid (olefin and hydroxy-imidacloprid) on learning ability. The acute concentrations tested on learning were determined on the basis of acute toxicity test results which we carried out beforehand in order to define the sensitivity of our own biological material.

>>M-110229-01-3@S-604666-01-1

Report: 02.01.01.01/17; [redacted]; 2015; M-514897-01-3
Title: [methylene-^{14}C]Imidacloprid: Storage stability in honeybees (Apis mellifera) after oral exposure
Report No.: EnSa-15-0140
Document No.: M-514897-01-3
Guideline(s): US EPA OCSPP 850.3020
Guideline deviation(s): none
GLP/GEP: **no**

<<M-514897-01-3@S-603093-01-1

The storage stability of parent [methylene-^{14}C]Imidacloprid and related residues was investigated in dead honeybees (*Apis mellifera*). The test compound was orally administered in commercially available sugar syrup (Apiinvert, 50%) at a dose of 40.0 ng a.s. per 20 mg diet, which represents the amount of sugar necessary for one honeybee per day (adjusted on the basis of OECD guideline 213 [7]). The honeybees received the sugar syrup containing the radiolabelled test compound for approx. 3.5 hours *ad libitum*, following a two hours starvation period. The concentration of Imidacloprid in the diet was empirically selected to induce test compound related mortality in at least 200 honeybees (of a total population of 800 honeybees) within a reasonably short period of time and to obtain sufficient a.s. uptake into the honeybees for subsequent analytical procedures. Throughout the experiment, the honeybees were housed in steel cages. The dead honeybees were collected immediately during the application period and stored at

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room temperature. Total radioactive residues (TRR) were determined in triplicate batches for each storage time period (day 0, day 1, day 2, day 4 and day 8 after application).

Residues were extracted from dead honeybees by mechanical homogenisation using two aliquots of acetonitrile and an acetonitrile/water mixture. The combined acetonitrile extract was subjected to a liquid/liquid distribution using n-heptane, followed by phase separation. The extraction efficiency ranged from 78.7% (0.145 mg/kg) of the TRR to 91.1% (0.169 mg/kg) of the TRR. The decline in extractable residues over time was most likely due to incorporation into natural matrices.

The total amount of radioactivity in dead honeybees was stable over all time points and ranged from 0.128 mg/kg to 0.169 mg/kg.

Parent [methylene-^{14}C]Imidacloprid was found to be the main compound throughout the entire storage period (8 days) and amounted to 0.118 mg/kg at day 0, 0.093 mg/kg at day 1, 0.099 mg/kg at day 2, 0.097 mg/kg at day 4 and 0.096 mg/kg at day 8. An overview of the stability of parent Imidacloprid and the detected metabolites is given in the following table.

Storage duration	Day 0		Day 1		Day 2		Day 4		Day 8	
Residues	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Parent Imidacloprid	63.3	0.118	59.4	0.093	56.6	0.099	51.6	0.097	51.7	0.096
Unknown	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	0.001	0.4	0.001
6-Chloronicotinic acid	1.4	0.003	2.3	0.004	3.7	0.006	4.4	0.008	5.5	0.010
4/5 Hydroxy-Imidacloprid	10.3	0.019	7.2	0.011	8.5	0.015	8.1	0.015	7.4	0.013
4,5 Dihydroxy-Imidacloprid and Imidacloprid-olefin	16.2	0.029	14.3	0.021	14.1	0.024	15.9	0.030	13.7	0.025
Total analysed	91.1	0.169	83.2	0.128	82.9	0.144	80.7	0.150	78.7	0.145

TRR: Total Radioactive Residue
n.d.: not determined

Over the entire period of investigation the maximum decline of Imidacloprid was < 20%. After 4 days of continuous storage at ambient temperatures, parent Imidacloprid did not show a further decline/dissipation in dead honeybees. When accounting for the entire storage period of 8 days at room temperature, parent Imidacloprid showed only a slight decline/dissipation.

Overall, it can be concluded that the decrease of the parent Imidacloprid in dead honeybees is limited and potential residues can be quantified even after storage at ambient temperatures, if the original exposure amounted to the limit of quantification (LOQ).

>>M-514897-01-3@S-603093-01-1

02.01.01.02 - Metabolites

Report:	02.01.01.02/01; [redacted]; 1999; M-032645-01-2
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 3772 on honey bees (Apis mellifera L.) (Hymenoptera, Apidae)
Report No.:	6330036
Document No.:	M-032645-01-2
Guideline(s):	EPPO No.170
Guideline deviation(s):	Temperatur: 29 C; relative humidity: 68-70% instead of 25°C + 2°C and relative humidity of 60-70 % as indicated in the guideline
GLP/GEP:	**yes**

<<M-032645-01-2@S-602607-01-1

Material and methods: test substance: WAK 3772, purity: 95%, batch number: MCO 136; under laboratory conditions, starved honey bees *(Apis mellifera,* 3 groups of 10 bees per dose) received a single oral dose of either 48.5, 23.9, 12.0, 6.0, 3.1, 1.5, 0.7 or 0.1 ng per bee in 20 mg sugar solution. Subsequently, honey bees were observed over a period of 48 hrs for behavioural impairments and survival rate. The reference treatment (0.2 µg dimethoate per bee) caused a 100 % mortality (the facility-specific LD5o dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	WAK 3772
Test object	*Apis mellifera*
Application rates ng product/bee	48.5*, 23.9*, 12.0*, 6.0*, 3.1*, 1.5*, 0.7* and 0.1*
Exposure	oral (sugar solution)
LD_{50} ng product/bee (24 and 48h)	> 48.5

* values based on actual intake of the test substance

Observations: One of 30 bees died after application of an oral dose with 23.9, 12.0 and 3.1 ng WAK 3772 per bee, respectively. No behavioural abnormalities were observed for the 48 hours of the experimental time.
Three of 30 bees died in the control and all bees died after treatment with Dimethoate.

>>M-032645-01-2@S-602607-01-1

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Report:	02.01.01.02/02; [redacted]; 1999; M-017095-01-3
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 4168 on honey bees (Apis mellifera L.) (Hymenoptera, Apidae) - limit test -
Report No.:	6370036
Document No.:	M-017095-01-3
Guideline(s):	GLP compliant study based on EPPO 170 (1992)
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-017095-01-3@S-602487-01-1

Material and methods: test substance: WAK 4168, purity: 99.0%, batch number: 9601k8ELB01, under laboratory conditions, starved honey bees *(Apis mellifera,* 3 groups of 10 bees per dose) received a single oral dose of either 99.5 or 1.2 µg per bee in 20 mg sugar solution. Subsequently, honey bees were observed over a period of 48 hrs for behavioural impairments and survival rate. The reference treatment (0.2 µg dimethoate per bee) caused a 100 % mortality (the facility-specific LD_{50} dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	WAK 4168
Test object	*Apis mellifera*
Application rates µg product/bee	99.5* and 1.2*
Exposure	oral (sugar solution)
LD_{50} µg product/bee (24 and 48h)	> 99.5

* values based on actual intake of the test substance

Observations: Obviously the test substance appeared to have a repellent effect in the 99.5 µg/bee dosage group indicated by the long period of uptake by the bees in this dosage group, although bees were previously starved for 60 minutes. 11 of 30 (36.7 %) bees died after application of an oral dose with 99.5 µg WAK 4168 per bee. One of the 30 (3.3 %) bees died after application of an oral dose with 1.2 µg WAK 4168 per bee. Behavioural abnormalities like apathy or discoordinated movements occurred in the 99.5 µg/bee dosage group. No behavioural abnormalities were observed in the 1.2 µg/bee dosage group for the 48 hours of the experimental time.
No bee died in both, pure syrup and water/syrup control groups. All bees died after treatment with Dimethoate.

>>M-017095-01-3@S-602487-01-1

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Report:	02.01.01.02/03; [redacted]; 1999; M-017098-01-3
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 4140 on honey bees (Apis mellifera L.) (Hymenoptera, Apidae) - Limit test
Report No.:	6360036
Document No.:	M-017098-01-3
Guideline(s):	EPPO 1992: Guideline on test methods for evaluating the side-effects of plant protection products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215 1992, No. 170
Guideline deviation(s):	Temperature: 29 °C; relative humidity: 64 - 70 % instead of 25 °C ± 2 °C and relative humidity of 60 -70 % as indicated in the guideline
GLP/GEP:	**yes**

<<M-017098-01-3@S-602491-01-1

Material and methods: test substance: WAK 4140, purity: 97.9%, batch number: 960308ELB01; under laboratory conditions, starved honey bees *(Apis mellifera,* 3 groups of 10 bees per dose) received a single oral dose of either 93.2 or 1.2 µg per bee in 20 mg sugar solution. Subsequently, honey bees were observed over a period of 48 hrs for behavioural impairments and survival rate. The reference treatment (0.2 µg dimethoate per bee) caused a 100 % mortality (the facility-specific LD_{50} dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	WAK 4140
Test object	*Apis mellifera*
Application rates µg product/bee	93.2* and 1.2*
Exposure	oral (sugar solution)
LD_{50} µg product/bee (24 and 48h)	approximately 93.2

* values based on actual intake of the test substance

Observations: Obviously the test substance appeared to have a repellent effect in the 93.2 µg/bee dosage group indicated by the long period of uptake by the bees in this dosage group, although bees were previously starved for 60 minutes. 16 of 30 (53.3 %) bees died after application of an oral dose with 93.2 µg WAK 4140 per bee. None of the 30 bees died after application of an oral dose with 1.2 µg WAK 4140 per bee. Behavioural abnormalities (discoordinated movement and apathy) of two bees during the 24 hours check occurred after ingestion of 93.2 µg/bee. No behavioural abnormalities were observed in the 1.2 µg/bee dosage group for the 48 hours of the experimental time.
No bee died in both, pure syrup and water/syrup control groups. All bees died after treatment with Dimethoate.

>>M-017098-01-3@S-602491-01-1

Report:	02.01.01.02/04; [redacted]; 1999; M-017134-01-3
Title:	Laboratory testing for toxicity (acute oral LD50) of BNF 5119B on honey bees (Apis mellifera L.) (Hymenoptera, Apidae) - Limit test
Report No.:	6380036
Document No.:	M-017134-01-3
Guideline(s):	EPPO 1992: Guideline on test methods for evaluating the side-effects of plant protection products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215 1992, No. 170
Guideline deviation(s):	Temperature: 29 °C; relative humidity: 64 - 70 % instead of 25 ° C +/- 2 ° C and relative humidity of 60 -70 % as indicated in the guideline
GLP/GEP:	**yes**

<<M-017134-01-3@S-602500-01-1

Material and methods: test substance: BNF 5119B, purity: 99.6%, batch number: 870922ELB06; under laboratory conditions, starved honey bees *(Apis mellifera)* 3 groups of 10 bees per dose) received a single oral dose of either 121.5, 11.3 or 1.2 µg per bee in 20 mg sugar solution. Subsequently, honey bees were observed over a period of 48 hrs for behavioural impairments and survival rate. The reference treatment (0.2 µg dimethoate per bee) caused a 100 % mortality (the facility-specific LD_{50} dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	BNF 5119B
Test object	*Apis mellifera*
Application rates µg product/bee	121.5*, 11.3* and 1.2*
Exposure	oral (sugar solution)
LD_{50} µg a.i./bee (24 and 48h)	> 121.5

* values based on actual intake of the test substance

Observations: One of 30 bees died after application of an oral dose with 121.5 µg BNF 5119B per bee. No bee died after application of an oral dose with 11.3 µg BNF 5119B per bee and two bees died after application of 1.2 µg BNF 5119B per bee. No behavioural abnormalities were observed for the 48 hours of the experimental time.
No bee died in neither the acetone and water, nor in the pure syrup control. All bees died after treatment with Dimethoate.

>>M-017134-01-3@S-602500-01-1

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Report:	02.01.01.02/05; [redacted]; 1999; M-018622-01-4
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 3745 on honey bees (Apis mellifera L.) (Hymenoptera, Apidae)
Report No.:	6320036
Document No.:	M-018622-01-4
Guideline(s):	US EPA OCSPP Guideline no. 850.SUPP EPPO 1992: Guideline on test methods for evaluating the side-effects of plant protection products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215,1992, No. 170
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-018622-01-4@S-602525-01-1

Material and methods: test substance: WAK 3745, purity: 98%, batch number: M90804; under laboratory conditions, starved honey bees *(Apis mellifera,* 3 groups of 10 bees per dose) received a single oral dose of either 35.7, 17.9, 10.3, 5.6, 2.4, 1.2, 0.6 or 0.1 ng per bee in ca. 20 mg sugar solution. Subsequently, honey bees were observed over a period of 96 hrs for behavioural impairments and survival rate. The test was prolonged up to 96 hours because of increasing mortality between 24 and 48 hours. The reference treatment (0.2 µg dimethoate per bee) caused a 83.3 % mortality (the facility-specific LD_{50} dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	WAK 3745
Test object	*Apis mellifera*
Application rates ng product/bee	35.7*, 17.9*, 10.3*, 5.6*, 2.4*, 1.2*, 0.6* and 0.1*
Exposure	oral (sugar solution)
LD_{50} ng product/bee (48 and 96h)	> 35.7

* values based on actual intake of the test substance

Observations: the observation period was extended for 48 hours because of delayed mortality in the highest dose groups. No mortalities or behavioural impacts were recorded at oral doses of 1.2 ng/bee and lower. Oral doses of 0.6, 2.4, and 10.3 ng/bee caused 6.7 % mortality, 23.3 % mortality was found after ingestion of 35.7 ng/bee. Since mortality pattern did not follow a dose-response relationship, the two death in the 10.3 ng/bee and lower dosing groups with WAK 3745 were considered as incidental rather than treatment-related.
Behavioural impacts such as apathy and nervousness were recorded after oral doses of 5.6 ng and higher. The behavioural impacts lasted dose-related up to 24 hours. In the control, none of the 30 bees died, whereas 25 of the 30 bees (83.3 %) died in the groups treated with the toxic standard.

>> M-018622-01-4@S-602525-01-1

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Report:	02.01.01.02/06; [redacted]; 1999; M-018647-01-4
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 4103 on honey bees (Apis mellifera L.) (Hymenoptera, Apidae)
Report No.:	6340036
Document No.:	M-018647-01-4
Guideline(s):	US EPA OCSPP Guideline no. 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-018647-01-4@S-602527-01-1

Material and methods: test substance: WAK 4103, purity: 99.4%, batch number: 930323ELB03; under laboratory conditions, starved honey bees *(Apis mellifera,* 3 groups of 10 bees per dose) received a single oral dose of either 159.2, 81.9, 39.1, 19.0, 10.4, 4.6 or 1.2 ng per bee in ca. 20 mg sugar solution. Subsequently, honey bees were observed over a period of 96 hrs for behavioural impairments and survival rate. The test was prolonged up to 96 hours because of increasing mortality between 24 and 48 hours. The reference treatment (0.2 µg dimethoate per bee) caused a 83.3 % mortality (the facility-specific LD_{50} dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	WAK 4103
Test object	*Apis mellifera*
Application rates ng product/bee	159.2*, 81.9*, 39.1*, 19.0*, 10.4*, 4.6* and 1.2*
Exposure	oral (sugar solution)
LD_{50} ng product/bee (96h)	approximately 159.2

* values based on actual intake of the test substance

Observations: the observation period was extended for 48 hours because of delayed mortality in the highest dose groups. No mortalities occurred after ingestion of 19.0 ng/bee. Oral doses of 1.2, 4.6, and 10.4 ng/bee caused 3.3% mortality. A mortality rate of 6.7, 40.0 and 53.3 % was found for oral doses of 39.1, 81.9 and 159.2 ng/bee, respectively.
Behavioural impacts such as apathy, discoordinated movements and nervousness were recorded after oral doses of 4.6 ng and higher. The behavioural impacts lasted dose-related up to 24 hours. No behavioural impacts were recorded at oral doses of 1.2 ng/bee. In the control, none of the 30 bees died, whereas 25 of the 30 bees (83.3 %) died in the groups treated with the toxic standard.

>>M-018647-01-4@S-602527-01-1

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Report:	02.01.01.02/07; [redacted]; 1999; M-018470-01-3
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 3839 on honey bees (Apis mellifera L.) (Hymenoptera, Apidae) - Limit test
Report No.:	6390036
Document No.:	M-018470-01-3
Guideline(s):	EPPO 1992: Guideline on test methods for evaluating the side-effects of plant protection products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215 1992, No. 170
Guideline deviation(s):	Temperature: 28 - 29 °C; relative humidity: 52 - 86 % instead of 25 ° C ± 2 ° C and relative humidity of 60 -70 % as indicated in the guideline
GLP/GEP:	**yes**

<<M-018470-01-3@S-602521-01-1

Material and methods: test substance: WAK 3839, purity: 99 %, batch number: 950411ELB02, (test substance was obtained in a 0.1 % ethanol solution; WAK 3839 was extracted by evaporating of the ethanol solution); under laboratory conditions, starved honey bees (*Apis mellifera*, 3 groups of 10 bees per dose) received a single oral dose of either 21.8, 0.3 or 0.08 µg per bee in 4-10 mg sugar solution. Subsequently, honey bees were observed over a period of 96 hrs for behavioural impairments and survival rate. The test was prolonged up to 96 hours because of increasing mortality between 24 and 48 hours at 0.08 µg/bee. The reference treatment (0.2 µg dimethoate per bee) caused a 100 % mortality (the facility-specific LD_{50} dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	WAK 3839
Test object	*Apis mellifera*
Application rates µg product/bee	21.8*, 0.3* and 0.08*
Exposure	oral (sugar solution)
LD_{50} µg /bee (48 and 96h)	ca. 0.08

* values based on actual intake of the test substance

Observations: the observation period was extended for 48 hours because of delayed mortality in the lowest dose groups. Although bees were previously starved for 60 minutes the bees ingested only 22, 30 and 80 % of the provided sugar solution in dosing group of 100, 1 and 0.1 µg/bee, respectively. This food rejection indicates a strong antifeedant effect of the test substance. In the highest dosage groups, immediately after uptake, the bees were strongly affected (apathy, discoordinated movements). Therefore, the bees were unable to take in the whole amount of offered contaminated food. Oral doses of 21.8 µg/bee and 0.3 led to 100 % mortality during the first 4 and 24 hours, respectively. After application of an oral dose with 0.08 µg WAK 3839 per bee, 46.7 % of the bees died during 48 hours after the application. No further mortality occurred during 72 and 96 hours. Behavioural impairments like discoordinated movement and apathy in this dose group were observed for the first 24 hours. No more behavioural abnormalities occured until the end of the experiment.
No bee died in neither the acetone, nor in the pure syrup control. All bees died after treatment with Dimethoate.

<<M-018470-01-3@S-602521-01-1

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Report:	02.01.01.02/08; [redacted]; 2000; M-019352-01-2
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 5074 on honey bees (Apis mellifera L.) (Hymenoptera, Apidae) - Limit test
Report No.:	7150036
Document No.:	M-019352-01-2
Guideline(s):	EPPO No. 170
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-019352-01-2@S-602529-01-1

Material and methods: test substance: WAK 5074, purity: 98%, batch number: DIJ11371; under laboratory conditions, starved honey bees *(Apis mellifera)* 3 groups of 10 bees per dose) received a single oral dose of either 119.8 or 1.2 µg per bee in ca. 25 mg sugar solution. Subsequently, honey bees were observed over a period of 48 hrs for behavioural impairments and survival rate. The reference treatment (0.2 µg dimethoate per bee) caused a 100 % mortality (the facility-specific LD_{50} dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	WAK 5074
Test object	*Apis mellifera*
Application rates µg product/bee	119.8* and 1.2*
Exposure	oral (sugar solution)
LD_{50} µg product/bee (24 and 48h)	> 119.8

* values based on actual intake of the test substance

Observations: None of 30 bees died after application of an oral dose with 119.8 µg or 1.2 µg WAK 5074 per bee. No behavioural abnormalities were observed for the 48 hours of the experimental time. No bee died in neither the water, nor in the pure syrup control. All bees died after treatment with Dimethoate.

>>M-019352-01-2@S-602529-01-1

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Report:	02.01.01.02/09; [redacted]; 2000; M-068030-01-3
Title:	Acute oral toxicity of substance B to the honeybee Apis mellifera L. under laboratory conditions prolonged for 10 days
Report No.:	00 10 48 0502b
Document No.:	M-068030-01-3
Guideline(s):	EPPO Standard PP 1/170(2) (1999); OECD 213 (1998); US EPA OCSPP Guideline no 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-068030-01-3@S-602713-01-1

Results:

During a 10-day test period the bees consumed sucrose solution containing 0.1, 1 and 10 ppb Substance B. The amount of consumed sucrose solution was summed up for the whole test duration. The total amount of sucrose solution containing the test substance was used to determine the total amount of test substance consumed per bee. The test endpoints were mortality and behaviour of the honeybees in comparison with the control.

House bees

No statistically significant effects on honeybee mortality were observed after oral exposure to Substance B at concentrations of 0.1, 1.0 and 10 ppb test substance per bee. The test substance at concentrations of 0.1, 1.0 and 10.0 ppb Substance B per bee caused 10.0 %, 8 % and 12 % mortality after 10 days. Therefore it is concluded that providing the test substance sucrose solution containing Substance B up to 10 ppb (equivalent to 7.266 ng Substance B consumed/bee) over the prolonged test duration of 10 days bad no impact on bee mortality. No effects on the behaviour of the bees (or other sublethal effects) were observed in comparison with the control bees.

Field bees

No statistically significant effects on honeybee mortality were observed after oral exposure to Substance B at concentrations of 0.1, 1.0 and 10.0 ppb Substance B per bee. The test substance at concentrations of 0.1, 1.0 and 10 ppb Substance B per bee caused 26 %, 36 % and 36 % mortality after l 0 days. The increasing mortality observed starting with day 7 was observed for all treatment groups including the control. The sensability of field bees (including the control treatment) compared to house bees was significantly higher. Therefore a higher overall mortality was observed in the field bee oral toxicity test.

Therefore it is concluded that providing the test substance sucrose solution containing Substance B up to 10 ppb (equivalent to 7.301 ng Substance B/bee) over the prolonged test duration of 10 days bad no significant impact on bee mortality compared to control.

No effects on the behaviour of the bees (or other sublethal effects) were observed in comparison with the control bees.

Control bees:

The mortality in the control was 4 % for the house bees and 44 % for the field bees in the oral toxicity tests after 10 days.

The increasing mortality of the field bee control was observed starting with 14 % (day 7) up to 16 % (day 8), 30 % (day 9) and 44 % (day 10).

The validity criterion - mortality in the control ≤ 10 % - was accomplished for the whole test duration of 10 days for the house bee test (4 %) and for field bees up to day 6 (8 %).

>>M-068030-01-3@S-602713-01-1

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Report:	02.01.01.02/10; [redacted]; 2000; M-068056-01-3
Title:	Substance B: feeding test on the honey bees (Apis mellifera), non-GLP
Report No.:	IBA7241N
Document No.:	M-068056-01-3
Guideline(s):	US EPA OCSPP Guideline no. 850.SUPP EPPO No. 170 according to the Guideline No. 170 of the European and Mediterranean Plant Protection Organisation (EPPO)
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-068056-01-3@S-602719-01-1

Material and methods: A feeding test was conducted over 10 days with the test item Substance B with the test concentrations of 0.1, 1.0, 10 ppb. *Apis mellifera*, foraging bees and young worker bees were kept in ventilated stainless steel cages, in 3 replicates of 10 individuals for each treatment. Control item was 50 % aqueous sucrose solution.

Mortality was assessed after 2, 4, 6, 8 and 10 days exposure period. The control and test item solutions were exchanged every 2 days and the ingested test item amount was calculated.

Dates of work: May 11 to 21, 2000

Findings: summarized in Tables 1-2

Table 1 **Mortality Values of Substance B, Worker Bees**

Test item concentration [ppb]	Ingested test item amount [ng a.i./bee]	Mortality [%] after 10 days	Corrected mortality [%] after 10 days
Control		10	
0.1	0.046	37	30
1.0	0.394	3	-7
10	3.672	63	59

Table 2 **Mortality Values of Substance B, Foraging Bees**

Test item concentration [ppb]	Ingested test item amount [ng a.i./bee]	Mortality [%] after 10 days	Corrected mortality [%] after 10 days
Control		30	
0.1	0.039	60	43
1.0	0.483	50	29
10	4.477	60	43

M-068056-01-3@S-602719-01-1

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Report:	02.01.01.02/11; [redacted]; 2000; M-068043-01-3
Title:	Substance B: feeding study with honey bees (Apis mellifera)
Report No.:	HT0400c
Document No.:	M-068043-01-3
Guideline(s):	US EPA OCSPP Guideline No.: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-068043-01-3@S-602715-01-1

Tests were carried out to determine the effect of feeding Substance B on mortality of adult honey bees (*Apis mellifera* L.) over a 10 day period. All doses and toxicity data for the test substance refer to Substance B as the active ingredient.

Five batches of bees, in groups of 10 bees, were offered 10, 1.0 and 0.1 ng/ml Substance B in 50% w/v aqueous sucrose solution.

Mortality was assessed daily after dosing. Glass test feeders were removed and weighed and replaced with fresh feed each day.

Results indicated that the Substance B had no significant effect on mortality.

>>M-068043-01-3@S-602715-01-1

Report:	02.01.01.02/12; [redacted]; 2000; M-068120-01-3
Title:	Substance B: Assessment of side effects in a ten days feeding test on the honey bee, Apis mellifera L. in the laboratory - hive bees (< 5 days)
Report No.:	20001148/01-BLEU 2.
Document No.:	M-068120-01-3
Guideline(s):	US EPA OCSPP Guideline No.: 850.SUPP
Guideline deviation(s):	not specified
GLP/GEP:	**no**

<<M-068120-01-3@S-602722-01-1

Young honey bees (1-5 days old) were fed over a ten days period with sucrose solution mixed with Substance B. The feeding test was carried out with three different concentrations of the test substance and with five replicates.

To obtain bees of approx. the same age, combs with bee brood, deriving from a healthy colony, were incubated in the laboratory for five days. The bees which hatched within five days were used for this feeding test. The young bees only fed the honey which was found in the combs, until the test started.

The mortality in the Substance B treatment groups rose up to 8 %, observed in the treatment fed with the lowest concentrated test substance solution of 0.1 µg/L which corresponded to an actual intake of 0.04458 ng/bee after ten days.

No mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance B (actual intake: 4.316 ng/bee)

No mortality was observed in the control group after the ten days exposure period.

>>M-068120-01-3@S-602722-01-1

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Report:	02.01.01.02/13; [redacted]; 2000; M-068060-01-2
Title:	Substance B: Assessment of side effects in a ten days feeding test on the honeybee, Apis mellifera L. in the laboratory - Foraging bees (= 22-32 days)
Report No.:	20001148/01-BLEU
Document No.:	M-068060-01-2
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-068060-01-2@S-602721-01-1

Worker honey bees (age: approx. 22 - 32 days) were fed over a four days period with sucrose solution mixed with Substance B. The feeding test was carried out with three different concentrations of the test substance and with five replicates. Due to a high mortality which occurred in the control group the test was terminated after four days instead of a ten days exposure period.

The mortality in the Substance B treatment group rose up to 34 %, observed in the treatment fed with the lowest concentrated test substance solution of 0.1 µg/L which corresponded to an actual intake of 0.02873 ng/bee after four days.

A 16% mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance B (actual intake: 2.881 ng/bee).

In the control group a 20 % mortality was observed after the four days exposure period.

>>M-068060-01-2@S-602721-01-1

Report:	02.01.01.02/14; [redacted]; 2000; M-068127-01-3
Title:	Acute oral toxicity of substance C to the honeybee Apis mellifera L. under laboratory conditions prolonged for 10 days
Report No.:	00 10 48 0502c
Document No.:	M-068127-01-3
Guideline(s):	EPPO Standard PP 1/170(2) (1999); OECD 213 (1998) US EPA OCSPP Guideline no 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-068127-01-3@S-602725-01-1

Results:

During a 10- day test period the bees consumed sucrose solution containing 0.1, 1 and 10 ppb Substance C. The amount of consumed sucrose solution was summed up for the whole test duration. The total amount of sucrose solution containing the test substance was used to determine the total amount of test substance consumed per bee. The test endpoints were mortality and behaviour of the honeybees in comparison with the control.

House bees:

No statistically significant effects on honeybee mortality were observed after oral exposure to Substance C at concentrations of 0.1, 1.0 and 10 ppb test substance per bee.
The test substance at concentrations of 0.1, 1.0 and 10.0 ppb Substance C per bee caused 10.0 %, 4 % and 6 % mortality after 10 days.
Therefore it is concluded that providing the test substance sucrose solution containing Substance C up to 10 ppb (equivalent to 8.056 ng test substance C consumed/bee) over the prolonged test duration of 10 days bad no impact on bee mortality.
No effects on the behaviour of the bees (or other sublethal effects) were observed in comparison with the control bees.

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Field bees

No statistically significant effects on honeybee mortality were observed after oral exposure to Substance C at concentrations of 0.1, 1.0 and 10.0 ppb Substance C per bee.
The test substance at concentrations of 0.1, 1.0 and 10 ppb Substance C per bee caused 30 %, 40 % and 32 % mortality after l 0 days. The increasing mortality observed starting with day 7 was observed for all treatment groups including the control. The sensibility of field bees (including the control treatment) compared to house bees was significantly higher. Therefore a higher overall mortality was observed in the field bee oral toxicity test.

Therefore it is concluded that providing the test substance sucrose solution containing the Substance C up to 10 ppb (equivalent to 8.056 ng Substance C/bee) over the prolonged test duration of 10 days had no significant impact on bee mortality compared to control.

No effects on the behaviour of the bees (or other sublethal effects) were observed in comparison with the control bees.

Control bees:

The mortality in the control was 4 % for the house bees and 44 % for the field bees in the oral toxicity tests after 10 days.
The increasing mortality of the field bee control was observed starting with 14 % (day 7) up to 16 % (day 8), 30 % (day 9) and 44 % (day 10).

The validity criterion - mortality in the control ≤ 10 % - was accomplished for the whole test duration of 10 days for the house bee test (4 %) and for field bees up to day 6 (8 %).

>>M-068127-01-3@S-602725-01-1

Report:	02.01.01/02/15-[redacted]; 2000; M-068131-01-3
Title:	Substance C: feeding study with honey bees (Apis mellifera)
Report No.:	HT0400d
Document No.:	M-068131-01-3
Guideline(s):	US EPA OCSPP Guideline No.: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-068131-01-3@S-602727-01-1

Tests were carried out to determine the effect of feeding Substance C on mortality of adult honey bees (*Apis mellifera* L.) over a 10 day period. All doses and toxicity data for the test substance refer to Substance C as the active ingredient.

Five batches of bees, in groups of 10 bees, were offered 10, 1.0 and 0.1 ng/ ml Substance C in 50% w/v aqueous sucrose solution.

Mortality was assessed daily after dosing. Glass test feeders were removed and weighed and replaced with fresh feed each day.

Results indicated that the Substance C had no significant effect on mortality.

>>M-068131-01-3@S-602727-01-1

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Report:	02.01.01.02/16; [redacted]; 2000; M-068147-01-3
Title:	Substance C: Assessment of side effects in a ten days feeding test on the honey bee, Apis mellifera L. in the laboratory - Hive bees (< 5 days)
Report No.:	20001149/01-BLEU
Document No.:	M-068147-01-3
Guideline(s):	US EPA OCSPP Guideline No.: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-068147-01-3@S-602783-01-1

Young honey bees (1-5 days old) were fed over a ten days period with sucrose solution mixed with Substance C. The feeding test was carried out with three different concentrations of the test substance and with five replicates.

To obtain bees of approx. the same age, combs with bee brood, deriving from a healthy colony, were incubated in the laboratory for five days. The bees which hatched within five days were used for this feeding test. The young bees only fed the honey which was found in the combs, until the test started.

In the treatments with Substance C the mortality rose up to 4 % observed at a test substance concentration of 1 µg/L (actual intake: 0.4585 ng/bee) after 10 days.

No mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance C (actual intake: 4.6769 ng/bee).

No mortality was observed in the control group after the ten days exposure period.

>>M-068147-01-3@S-602783-01-1

Report:	02.01.01.02/17; [redacted]; 2000; M-068155-01-3
Title:	Substance C: Assessment of side effects in a ten days feeding test on the honey bee, Apis mellifera L. in the laboratory - Foraging bees (= 22-32 days)
Report No.:	M-068155-01-3
Document No.:	M-068155-01-3
Guideline(s):	US EPA OCSPP Guideline No.: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-068155-01-3@S-602787-01-1

Worker honey bees (age: approx. 22 - 32 days) were fed over a four days period with sucrose solution mixed with Substance C. The feeding test was carried out with three different concentrations of the test substance and with five replicates. Due to a high mortality which occurred in the control group the test was terminated after four days instead of a ten days exposure period.

In the treatment with Substance C the mortality rose up to 10 % observed at a test substance concentration of 1 µg/L after four days.

A 6 % mortality occured in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance C (actual intake: 2.731 ng/bee).

In the control group a 20 % mortality was observed after the four days exposure period.

>>M-068155-01-3@S-602787-01-1

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Report:	02.01.01.02/18; [redacted]; 2000; M-068134-03-3
Title:	Repeat test: Substance C: feeding test on the honeybee Apis mellifera L. (Hymenoptera, Apidae), non-GLP
Report No.:	IBA7242N
Document No.:	M-068134-03-3
Guideline(s):	US EPA OCSPP Guideline no 850.SUPP according to the Guideline No. 170 of the European and Mediterranean Plant Protection Organisation (EPPO)
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-068134-03-3@S-602774-01-1

Material and methods: A feeding test was conducted over 10 days with the test item Substance C with the test concentrations of 0.1, 1.0, 10 ppb. Young worker bees (*Apis mellifera*) were kept in ventilated stainless steel cages, in 3 replicates of 10 individuals for each treatment. Control item was 50 % aqueous sucrose solution.

Mortality was assessed after 2, 4, 6, 8 and 10 days exposure period. The control and test item solutions were exchanged every 2 days and the ingested test item amount was calculated.

Dates of work: July 11 to 21, 2000

Findings: summarized in Table 1:

Table 1: **Mortality Values of Substance C, Worker Bees**

Test item concentration [ppb]	Ingested test item amount [ng a.i./bee]	Mortality [%] after 10 days	Corrected mortality [%] after 10 days
Control		7	-
0.1	0,059	10	3
1.0	0,551	7	0
10	5,801	7	0

>>M-068134-03-3@S-602774-01-1

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Report:	02.01.01.02/19; [redacted]; 2000; M-110229-01-3
Title:	Impact of imidacloprid and its main metabolites on the honeybee Apis mellifera L.: effect of chronic exposure on mortality and learning
Report No.:	M-110229-01-3
Document No.:	M-110229-01-3
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**no**

<<M-110229-01-3@S-604666-01-1

Crop protection treatments applied to nectar-producing plants in flower can affect the survival or behaviour of bees. In contrast to acute lethal effects, which are investigated by means of toxicology tests before products are placed on the market, there is currently no objective way of detecting the sub-lethal effects of pesticides on bee behaviour or of evaluating their chronic toxicity.

During the national study programme carried out in 1998 to evaluate the effects of Gaucho® sunflower seed dressing on bees we studied the chronic toxicity of the active ingredient in this product (imidacloprid) and its effects on the olfactory learning capacity of worker bees, which is still a matter of some dispute. Olfactory learning processes are vital in enabling bees to recognise flowers as they forage. At that time we observed significant mortality compared to the control group at concentrations of 8 and 40 ppb after 11 days ingestion of imidacloprid. Furthermore, after the 11 days administration of concentrations of 4, 8 and 40 ppb we observed a significant decline in learning performance compared to untreated individuals when we performed a Pavlovian olfactory conditioning procedure. However, we did not find any concentration-response relationship or any no-effect concentration. It should be noted that the concentrations of imidacloprid used in 1998 were not all investigated on the same day. In addition, the 1998 results were based on only two repeats. The purpose of this investigation is therefore to find out more about the sub-lethal effects of imidacloprid on bees subjected to Pavlovian conditioning. In order to do this we attempted to define concentration-response relationships and threshold concentrations by using a wide range of experimental concentrations. We also evaluated the possible effects of the two main metabolites of imidacloprid (olefin and hydroxy-imidacloprid) on learning ability. The acute concentrations tested on learning were determined on the basis of acute toxicity test results which we carried out beforehand in order to define the sensitivity of our own biological material.

>>M-110229-01-3@S-604666-01-1

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02.01.01.03 - Formulations

Report:	02.01.01.03/01; [redacted]; 1995; M-032525-01-2
Title:	Laboratory testing for toxicity (acute contact and oral LD50) of Confidor SC 200 to honey bees (Apis mellifera L.) (Hymenoptera, Apidae)
Report No.:	790036
Document No.:	M-032525-01-2
Guideline(s):	EPPO 170 (1992)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-032525-01-2@S-602595-01-1

The contact and oral LD_{50} (24 h and 48 h) of Confidor SC 200 to honey bees were tested according to EPPO 170 (1992) and GLP regulations. Confidor SC 200 was applied in five dosages (contact and oral toxicity test), one solvent control, one untreated negative control (contact test) and one positive control with toxic standard (Dimethoate 0.2 µg a.i./bee). The following dosages of the test substance were tested in three replicates often bees each:

Contact toxicity test			Oral toxicity test		
Test Substance Dosage (nominal) µg / bee	Mortality 24 h (%)	48 h (%)	Test Substance Dosage µg / bee	Mortality 24 h (%)	48 h (%)
5.0	20.0	86.7	0.169	40.0	80.0
1.0	60.0	86.7	0.083	20.0	23.3
0.5	50.0	66.7	0.0173	6.7	10.0
0.1	16.7	16.7	0.0083	0.0	0.0
0.05	20.0	20.0	0.0048	0.0	0.0
calculated LD_{50} µg/bee (95 % cofidence range)	0.75 (0.38 to 1.30)	0.29 (0.19 to 0.45)	calculated LD_{50} µg/bee (95 % cofidence range)	> 0.169	0.103 (0.073 to 0.144)
Controls	Mortality 24 h (%)	48 h (%)	Controls	Mortality 24 h (%)	48 h (%)
untreated Control	0.0	0.0	-	-	-
solvent control	0.0	0.0	negative control	0.0	3.3
positive control	73.3	73.3	positive control	96.7	100.0

The results of this study show toxic effects of Confidor SC 200 to honey bees in the contact and oral toxicity test. The acute contact LD_{50} (48h) was calculated to be 0.29 µg/bee and the acute oral LD_{50} (48h) was calculated to be 0.103 µg/bee.

>>M-032525-01-2@S-602595-01-1

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Report: 02.01.01.03/02; [redacted]; 1995; M-032532-01-2
Title: Laboratory testing for toxicity (acute contact and oral LD50) of Confidor WG 70 to honey bees (Apis mellifera L.) (Hymenoptera, Apidae)
Report No.: 780036
Document No.: M-032532-01-2
Guideline(s): EPPO 170 (1992)
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-032532-01-2@S-602599-01-1

The contact and oral LD_{50} (24 h and 48 h) of Confidor WG 70 to honey bees were tested according to EPPO 170 (1992) and GLP regulations. Confidor WG 70 was applied in five dosages (contact and oral toxicity test), one solvent control, one untreated negative control (contact test) and one positive control with toxic standard (Dimethoate 0.2 µg a.i./bee). The following dosages of the test substance were tested in three replicates often bees each:

Contact toxicity test			Oral toxicity test		
Test Substance Dosage (nominal) µg / bee	Mortality 24 h (%)	48 h (%)	Test Substance Dosage µg / bee	Mortality 24 h (%)	48 h (%)
1.0	30.0	76.7	0.085	36.7	76.7
0.5	13.3	60.0	0.017	53.3	56.7
0.1	20.0	23.3	0.0085	40.0	40.0
0.05	3.3	3.3	0.0017	0.0	6.7
0.01	0.0	0.0	0.0009	0.0	0.0
calculated LD_{50} µg/bee (95 % cofidence limits)	> 1	0.35 (0.25 to 0.51)	calculated LD_{50} µg/bee (95 % cofidence limits)	≥ 0.085	0.0167 (0.0105 to 0.0264)
Controls	Mortality 24 h (%)	48 h (%)	Controls	Mortality 24 h (%)	48 h (%)
untreated Control	0.0	6.7	-	-	-
solvent control	0.0	3.3	negative control	0.0	3.3
positive control	96.7	96.7	positive control	96.7	100.0

The results of this study show toxic effects of Confidor WG 70 to honey bees in the contact and oral toxicity test. The acute contact LD_{50} (48h) was calculated to be 0.35 µg/bee and the acute oral (48h) was calculated to be 0.0167 µg/bee.

>>M-032532-01-2@S-602599-01-1

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Report: 02.01.01.03/03; [redacted]; 2001; M-060864-01-2
Title: Acute effects of imidacloprid AE 0.025 to Apis mellifera (Hymenoptera, Apidae), tested as imidacloprid-AE VL 0.0625
Report No.: IBA73871
Document No.: M-060864-01-2
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

<<M-060864-01-2@S-602153-01-1

Material and methods: The insecticide Imidacloprid AE VL 0.0625 (presolution of Imidacloprid AE 0.025 (purity: 0.062 g/L, specification: article no.: 00 004434447, formulation no.: 07553/0006.00010) was applied at nominal doses of 0.00032 - 0.001 - 0.0032 - 0.01 - 0.032 - 0.1 µg a.i. per bee for oral and topical application under laboratory conditions.
As control 50 % aqueous sucrose solution in oral mode and CO_2-paralysation as well as CO_2- paralysation + acetone in contact mode was used.
ADIMETHOAT 40 EC (Dimethoate: 0.046 - 0.1 - 0.22 - 0.46 µg a.i./bee (oral and contact mode)) was used as reference treatment.
Apis mellifera (only worker bees), were kept in stainless steel cages, in 3 groups of 10 individuals for each treatment. Mortality was assessed after 4, 24, 48, 76 and 96 h exposure period. The LD_{50}-value after 24 h in the reference treatment was 0.11 µg a.i./bee (oral) and 0.14 µg a.i./bee (contact).

Dates of work: August 23 to 27, 2000 (oral) August 29 to September 02, 2000 (contact)

Findings:

Table 1: **Toxicity to Honeybees, Laboratory Tests**

Test item		Imidacloprid-AE VL 0.0625 (presolution of Imidacloprid AE 0.025, article no. 04434447)	
Test object		*Apis mellifera*	
Exposure		oral	contact
LD_{50} [µg a.i. / bee] (95 % confidence interval)	24 h	0.056* (0.041 – 0.076)	0.021 (0.018 – 0.024)
	48 h	0.041* (0.031 – 0.053)	0.010 (0.006 – 0.017)
	72 h	0.025* (0.019 – 0.034)	0.003 (0.002 – 0.005)
	96 h	0.011* (0.009 – 0.014)	0.002 (0.0016 – 0.0022)

* = Dose based on actual ingestion of the test item

Observations:
In the **oral test mode** sublethal effects were observed in the following actual consumptions:

- 0.00030 µg a.i./bee: after 4 h no sublethal effects were observed. After 24 h and 48 h 1 bee showed slow motions and had coordination problems. After 72 and 96 h no sublethal effects were observed.
- 0.0009 µg a.i./bee: after 4 h 13 bees showed slow motions and had coordination problems. After 24 h 10 bees showed slow motions and had coordination problems and 1 bee had problems in standing up. After 48 h 3 bees showed slow motions and had coordination problems. After 72 and 96 h no sublethal effects were observed.
- 0.0022 µg a.i./bee: after 4 h 27 bees, after 24h 23 bees, after 48 h 18, after 72 h 5 bees and after 96 h 14 bees showed slow motions and coordination problems.

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- 0.008 µg a.i./bee: after 4 h and 24 h 28 bees showed slow motions and coordination problems and 1 bee had problems in standing up after 24 h. After 48 h 25 bees showed slow motions and had coordination problems and 1 bee had problems in standing up. After 72 h 8 bees and after 96 h 11 bees showed slow motions and coordination problems.
- 0.018 µg a.i./bee: After 4 h 28 bees showed slow motions and had coordination problems and 1 bee had problems in standing up. After 24 h 1 bee had problems in standing up and 26 bees showed slow motions and coordination problems. After 48 h 19 bees showed slow motions and had coordination problems and 3 bees had problems in standing up. After 72 h 15 bees showed slow motions and had coordination problems and 5 bees had problems in standing up. After 96 h 8 bees showed slow motions and coordination problems.
- 0.056 µg a.i./bee: after 4 h 9 bees had problems in standing up and 19 bees showed slow motions and had coordination problems. After 24 h 11 bees had problems in standing up and 7 bees showed slow motions and had coordination problems. After 48 h 7 bees had problems in standing up and 3 bees showed slow motions and had coordination problems. After 72 h 4 showed slow motions and had coordination problems, 2 bees had problems in standing up. After 96 h 1 bee showed slow motions and coordination problems.

For details see Table 4.

In comparison to the control there were a significantly lower food uptake (t-test, $p < 0.05$) in the test item groups with the nominal doses of 0.032 and 0.1 µg a.i./bee.

Contact test mode:
In the **contact test mode** sublethal effects were observed at the test item doses ≥ 0.00032 µg a.i./bee:

- 0.00032 µg a.i./bee: no sublethal effects were observed during the test.
- 0.001 µg a.i./bee: after 4 h 8 bees showed slow motions and had coordination problems. After 24 h 3 bees, after 48 h 4 bees and after 72 h and 96 h 2 bees showed slow motions and coordination problems.
- 0.0032 µg a.i./bee: after 4 h 2 bees had problems in standing up and 28 bee showed slow motions and coordination problems. After 24 h 1 bee had problems in standing up and 19 bees showed slow motions and coordination problems. After 48 h only 8 bees showed slow motions and coordination problems, but mortality increased from 10 % (24 h) to 67 %. After 72 h 3 bees and after 96 h 1 bee showed slow motions and coordination problems.
- 0.01 µg a.i./bee: after 4 h 2 bees had problems in standing up and 28 bees showed slow motions and coordination problems. After 24 h 26 bees showed slow motions and coordination problems. After 48 h 25 bees showed slow motions and coordination problems. After 72 h only 3 bees showed slow motions and coordination problems but mortality increased from 13 % (48 h) to 67 %. After 96 h only 1 bee showed slow motions and coordination problems but 90 % of the bees were dead.
- 0.032 µg a.i./bee: after 4 h 3 bees had problems in standing up and 26 bees showed slow motions and coordinating problems. After 24 h 4 bees had problems in standing up and 4 bees showed slow motions and coordination problems. 73 % of the bees were dead. After 48 h 3 bees showed slow motions and coordination problems. After 72 h only 1 bee had problems in standing up. After 96 h all bees were dead.
- 0.1 µg a.i./bee: after 4 h 13 bees had problems in standing up and 10 bees showed slow motions and coordination problems.

For details see Table 6.

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Report:	02.01.01.03/04; [redacted]; 2001; M-060872-01-2
Title:	Imidacloprid AL 0.125 - Acute effects on the honeybee Apis mellifera (Hymenoptera, Apidae)
Report No.:	IBA73231
Document No.:	M-060872-01-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-060872-01-2@S-602155-01-1

Material and methods: The insecticide Imidacloprid AL 0.125 (purity: 0.12 g/L specification: development no.: 30-00232397, formulation no.: 06944/0032 (0071)) was applied at nominal doses of 0.001 – 0.0032 – 0.01 – 0.032 – 0.1 – 0.32 – 1 µg a.i. per bee corresponding to actual consumptions of 0.001 – 0.0031 – 0.0083 – 0.029 – 0.083 – 0.17 – 0.51 for oral (feeding) and at the test item doses of 0.001 – 0.0032 – 0.01 – 0.032 – 0.1 – 0.32 µg a.i. per bee for topical (contact) application under laboratory conditions.
As control 50 % aqueous sucrose solution (oral), CO_2-paralysation + acetone and CO_2-paralysation only (contact) was tested.
Adimethoat 40 EC (Dimethoate: 0.046 – 0.1 – 0.22 – 0.46 µg a.i./bee (oral and contact mode)) was used as reference treatment.

Apis mellifera (only worker bees), were kept in stainless steel cages, in 3 groups of 10 individuals for each treatment. Mortality was assessed after 4, 24, 48, 76 and 96 h exposure period. LD_{50} after 24 h in the reference treatment was 0.11 µg a.i./bee (oral) and 0.20 µg a.i./bee (contact).

Dates of work: August 08 to 12, 2000 (oral)
August 14 to 18, 2000 (contact)

Findings: summarized in Table 1.

Table 1: **Toxicity to Honeybees, Laboratory Tests**

Test item		Imidacloprid AL 0.125	
Test object		*Apis mellifera*	
Exposure		oral*	contact
LD_{50} [µg a.i. / bee] (95 % confidence interval)	24 h	0.291 (0.222 – 0.380)	0.029 (0.015 – 0.055)
	48 h	0.191 (0.166 – 0.221)	0.017 (0.011 – 0.026)
	72 h	0.127 (0.114 – 0.142)	0.018 (0.011 – 0.030)
	96 h	0.071 (0.050 – 0.101)	0.015 (0.008 – 0.027)

* LD_{50}-value based on actual ingestion of the test item

Observations:

In the oral mode sublethal effects were observed in the following actual consumptions:

- 0.001µg a.i./bee: after 4, 24 and 48 h no sublethal effects were observed. After 72 h 1 bee showed slow motions and had problems concerning coordination. After 96 h 3 bees showed slow motions and had problems concerning coordination.

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- 0.0031 µg a.i./bee: after 4 h 5 bees showed slow motions and had problems concerning coordination. After 24 h 23 bees showed slow motions and had problems concerning coordination. After 48 h no sublethal effects were observed. After 72 h 3 bees and after 96 h 4 bees showed slow motions and had problems concerning coordination.
- 0.0083 µg a.i./bee: after 4 h 19 bees showed slow motions and had problems concerning coordination and 1 bee had problems in standing up. After 24 h 30 bees showed slow motions and had problems concerning coordination. After 48 h 21 bees showed slow motions and had problems concerning coordination. After 72 h 9 bees and after 96 h 3 bees showed slow motions and had problems concerning coordination.
- 0.029 µg a.i./bee: after 4 h 21 bees showed slow motions and had problems concerning coordination and 1 bee had problems in standing up. After 24 h 1 bee had problems in standing up and 27 bees showed slow motions and had problems concerning coordination. After 48 h 27 bees showed slow motions and had problems concerning coordination. After 72 h 24 bees and after 96 h 21 bees showed slow motions and had problems concerning coordination.
- 0.083 µg a.i./bee: After 4 h 26 bees showed slow motions and had problems concerning coordination. After 24 h and 48 h 3 bees had problems in standing up and 26 bees showed slow motions and had problems concerning coordination. After 72 h 25 bees and after 96 h 14 bees showed slow motions and had problems concerning coordination. After 96 h 3 bees had problems in standing up.
- 0.17 µg a.i./bee: after 4 h 5 bees had problems in standing up and 23 bees showed slow motions and had problems concerning coordination. After 24 h 11 bees had problems in standing up and 12 bees showed slow motions and had problems concerning coordination. After 48 h 5 bees had problems in standing up and 10 bees showed slow motions and had problems concerning coordination. After 72 h 6 bees and after 96 h 3 bees showed slow motions and had problems concerning coordination. 4 bees had problems in standing up. After 96 h 1 bee had problems in standing up.
- 0.506 µg a.i./bee: after 4 h 17 bees had problems in standing up and 13 bees showed slow motions and had problems concerning coordination. After 24 h 5 bees had problems in standing up and 2 bees showed slow motions and had problems concerning coordination. After 48 h 1 bee had problems in standing up. After 72 h all bees were dead.

For details see Table 4.

In the **contact mode** sublethal effects were observed in the following nominal doses:

- 0.001 µg a.i./bee: after 4 h 2 bees had problems in standing up and 1 bee showed slow motions with coordination problems. After 24 h 1 bee had problems in standing up and after 48 h 8 bees showed slow motions and problems concerning coordination. After 72 h 1 bee and after 96 h 3 bees showed slow motions and problems concerning coordination.
- 0.0032 µg a.i./bee: after 4 h 3 bees had problems in standing up. After 24 h 25 bees showed slow motions and problems concerning coordination. After 48 h no sublethal effects were observed. After 72 h 5 bees and after 96 h 7 bees showed slow motions and problems concerning coordination.
- 0.01 µg a.i./bee: after 4 h 4 bees had problems in standing up and 1 bee showed slow motions with coordination problems. After 24 h 16 bees showed slow motions and problems concerning coordination. After 48 h only 2 bees showed slow motions and problems concerning coordination. After 72 h 3 bees and after 96 h 1 bee showed slow motions and problems concerning coordination.
- 0.032 µg a.i./bee: after 4 h 5 bees had problems in standing up. After 24 h 1 bee had problems in standing up and 13 bees showed slow motions and problems concerning coordination. After 48 h 4 bees showed slow motions and problems concerning coordination. After 72 h 4 bees and after 96 h 7 bees showed slow motions and problems concerning coordination and 1 bee had problems in standing up (96 h).

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- 0.1 µg a.i./bee: after 4 h 7 bees had problems in standing up and 4 bees showed slow motions and problems concerning coordination. After 24 h 10 bees showed slow motions and problems concerning coordination. 20 bees were dead. After 48 h only 1 bee showed slow motions and problems concerning coordination, 29 bees were already dead.
- 0.32 µg a.i./bee: after 4 h 5 bees had problems in standing up and 3 bees showed slow motions and problems concerning coordination. After 24 h 7 bees had problems in standing up and 7 bees showed slow motions and problems concerning coordination. After 48 h 5 bees showed slow motions and problems concerning coordination. 25 bees were already dead. After 72 h mortality was 100 %. For details see Table 6.

>>M-060872-01-2@S-602155-01-1

Report:	02.01.01.03/05; [redacted]; 2001; M-081923-01-3
Title:	Acute toxicity of imidacloprid SL 200 to the honeybee Apis mellifera L. under laboratory conditions
Report No.:	01 10 48 048
Document No.:	M-081923-01-3
Guideline(s):	OECD 213 (1998), OECD 214 (1998) US EPA OCSPP Guideline no 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-081923-01-3@S-602211-01-1

Material and methods:

Test species:	Apis mellifera carnica L.
Test system:	oral toxicity and contact toxicity test of Imidacloprid SL 200 on honeybees
Treatments:	control, test item and toxic standard (Dimethoate EC 400)
Test item treatment levels:	the test item was applied at the following doses: oral toxicity test: 0.0064, 0.0128, 0.0256, 0.0512 and 0.1025 µg a.i./bee contact toxicity test: 0.0029, 0.0057, 0.0114, 0.0229, 0.0457 and 0.0914 µg a.i./bee
Toxic standard:	Dimethoate EC 400 was applied at the following doses: oral toxicity test: 0.074, 0.089, 0.104, 0.126, 0.149 µg a.i./bee contact toxicity test: 0.012, 0.023, 0.046, 0.093, 0.186 µg a.i./bee
Dates of work:	August 28 – September 10, 2001

The insecticide Imidacloprid SL 200 (purity: 200.9 g/l; specification: Development No.: 3000249869, TOX No.: 05752-00, Formulation No.: 03833/0818 (0753) was tested under laboratory conditions on the honeybee A. mellifera after oral and contact exposure. Endpoints were mortality and behaviour of the bees compared to control up to 96 h after application. Mortality values were used to provide a regression line and calculate the median lethal dose value (LD_{50}) expressed in µg of active ingredient or product per bee.

Findings:

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Table: Oral and contact toxicity LD_{50} values of bees treated with Imidacloprid SL 200

Test item	Imidacloprid SL 200						
Test object	Honeybee *Apis mellifera L.*						
Exposure	oral / contact						
Treatment		LD_{50}					
Test item	time	oral toxicity test			contact toxicity test		
Imidacloprid SL 200		µg a.i./bee	slope b	µg product/bee	µg a.i./bee	slope b	µg product/bee
	24 h 95 %-cl lower upper	n.d.		n.d.	n.d.		n.d.
	48 h 95 %-cl lower upper	**0.066** 0.045 0.098	1.72	**0.361** 0.246 0.536	**0.056** 0.042 0.074	2.32	**0.306** 0.230 0.404
	72 h 95 %-cl lower upper	**0.056** 0.040 0.077	1.89	**0.306** 0.219 0.421	**0.048** 0.036 0.065	2.03	**0.262** 0.197 0.355
	96 h 95 %-cl lower upper	**0.053** 0.038 0.074	1.84	**0.290** 0.208 0.404	**0.045** 0.034 0.060	2.09	**0.246** 0.186 0.328

cl: confidence limits n.d.: not defined

Table (continued): Oral and contact toxicity LD_{50} values of bees treated with Imidacloprid SL 200

Treatment		LD_{50}					
Reference item	time	oral toxicity test			contact toxicity test		
Dimethoate EC 400		µg a.i./bee	slope b	µg product/bee	µg a.i./bee	slope b	µg product/bee
	24 h 95 %-cl lower upper	**0.133** 0.123 0.144	8.85	**0.358** 0.331 0.388	**0.113** 0.084 0.152	2.22	**0.304** 0.226 0.409
	48 h 95 %-cl lower upper	**0.129** 0.120 0.139	8.17	**0.347** 0.323 0.374	**0.102** 0.078 0.134	2.37	**0.275** 0.210 0.361

cl: confidence limits

No statistically significant effects on survival were observed at doses of 0.0064 and 0.0128 µg a.i. per bee in the oral toxicity test (0 and 13.3 % mortality, respectively) during 48 hours. Statistically significant effects on survival were observed at doses of 0.0256, 0.0512 and 0.1025 µg a.i. per bee in the oral toxicity test (23.3, 36.7 and 66.7 % mortality, respectively) during 48 hours. The calculated LD_{50} (48 h) is 0.066 µg a.i. per bee in the oral toxicity test (equivalent to 0.361 µg product/bee based on analysed content of a.i.).

In the contact toxicity test no statistically significant effects on survival were observed at doses of 0.0029, 0.0057, 0.0114 and 0.0229 µg a.i. per bee (0, 0, 10 and 13.3 % mortality, respectively) during 48 hours. Statistically significant effects on survival were observed only at doses of 0.0457 and 0.0914 µg a.i. per bee in the contact toxicity test (33.3 and 75.0 % mortality, respectively) during 48 hours. Therefore the calculated LD_{50} (48 h) is 0.056 µg a.i. per bee in the contact toxicity test (equivalent to 0.306 µg product/bee based on analysed content of a.i.).

Before bees died in the test item treatments apathy and immobility were observed.

The test period was prolonged up to 96 h because progressive mortality of the bees was observed at some doses between 24 and 48 hours in both the oral and contact toxicity tests. The prolongation of the study resulted in a statistically significantly increased mortality in the oral and the contact toxicity tests for the test item doses including and above 0.0256 and 0.0229 µg a.i./bee after 96 h. The calculated LD_{50} (96 h) are 0.053 and 0.045µg a.i. per bee in the oral and the contact toxicity tests (equivalent to 0.290 and 0.246 µg product/bee, respectively, based on analysed content of a.i.).

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The LD_{50} of the reference item Dimethoate was 0.133 µg a.i./bee in the oral toxicity test after 24 hours. This value was also within the preferred range of 0.10-0.35 µg a.i./bee cited in the OECD Guideline 213. The LD_{50} of the reference item was 0.113 µg a.i./bee in the contact toxicity test after 24 hours. This value corresponds to the expected range for the oral 24h - LD_{50} (0.10-0.30 µg *a.i./bee*) published in the OECD Guideline 214.

In the reference treatments apathy, discoordinated movements and immobility were observed before bees died.

The study was performed in compliance with the GLP principles.
The validity criterion - mortality in the control ≤ 10 % - was accomplished (being 0 % in the oral and in the contact toxicity tests after 48 hours).
The LD_{50} -24 h values for the toxic standard of 0.1-0.35 µg a.i./bee (oral) and 0.1-0.30 µg a.i./bee (contact) were accomplished (being 0.133 µg a.i./bee and 0.113 µg a.i./bee in the oral and the contact toxicity test, respectively).
>>M-081923-01-3@S-602211-01-1

Report:	02.01.01.0306; [redacted]; 2001; M-084112-01-2
Title:	Effects of imidacloprid SL 200 (acute contact and oral LD50) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	9981036
Document No.:	M-084112-01-2
Guideline(s):	GLP compliant study based on OECD 213 and 214 (1998) and the recent recommendations of the ICPBR group, held in Avignon, France, 1999
Guideline deviation(s):	none
GLP/GEP:	yes

<<M-084112-01-2@S-602212-01-1

Material and methods: Imidacloprid SL 200 (NTN 33893 200 SL), purity: NTN 33893: 194 g/L; (specification: Article No.: 0004958608; Batch No.: 233925888; Tox-No.: 5428-00); under laboratory conditions *Apis mellifera* (30 worker bees per treatment) were exposed for 96 hours to doses of 98.7, 38.5, 11.1, 5.6 and 1.2 ng a.i. per bee for feeding (oral, value based on the actual intake of the test item) and to doses of 800, 400, 200, 100 and 50 ng a.i. per bee for topical application (contact).
The oral and the contact test were prolonged up to 96 hours because of increasing mortality between 24 and 48 hours. The LD50 of the reference item was 0.19 µg dimethoate per bee in the oral and 0.18 µg Dimethoate per bee in the contact exposure after 24 hours.

Dates of experimental work: June 19 to August 3, 2001

Findings: Toxicity of Imidacloprid SL 200 to Honey Bees, Laboratory Tests

Table 1. Summary of mortality of the honey bees in the oral and contact toxicity test

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Test item	Imidacloprid SL 200	
Test object	*Apis mellifera*	
Application rates ng a.i./bee	98.7*, 38.5*, 11.1*, 5.6* and 1.2*	800, 400, 200, 100 and 50
Exposure	oral (50% sugar solution)	contact (solution in water + 1 % wetting agent)
LD_{50} ng a.i/bee after 48 h and 96 h (95 % Confidence Limits)	48 h: 5.6* (3.3 to 9.4) 96 h: 5.3* (3.4 to 8.4)	48 h: 42.2 (20.9 to 85.0) 96 h: statistics not applicable

* values based on actual intake of the test item

Observations:
In both, the oral and the contact test the observation period was extended for 48 hours because of delayed mortality.
Behavioural impairments (*e.g.* apathy or discoordinated movements) were observed for the first 4 hours in the 98.7, 38.5, 11.1 and 5.6 ng a.i./bee dose group. No behavioural impairments were observed in the 1.2 ng a.i. per bee dose group for the whole experimental time. After 24 hours apathy was observed in the 98.7. 38.5 and 11.1 ng a.i. dose group. No further behavioural impairments occurred in the 5.6 ng a.i./bee group. 48 hours following the application three and two bees showed apathy and moving coordination problems in the 98.7 and 38.5 ng a.i. dose group, respectively. After 72 hours two bees were apathetic or showed a discoordinated movement in the group dosed with 98.7 and 38.5 ng a.i./bee. In the 98.7 ng a.i. dose group two bees were found apathetic at the 96 hours check.
In the contact test behavioural impairments of the surviving bees like apathy, vomiting and discoordinated movements occurred in all groups dosed with Imidacloprid SL 200 during the whole experimental time of 96 hours.
3.3 % control mortality was found after 96 hours in the contact test and oral test, respectively

Conclusions:
The LD_{50} (contact) 48 hrs was determined to be 42.2 ng a.i./bee. The LD_{50} (oral) was determined to be 5.6 ng a.i./bee after 48 hrs and 5.3 ng a.i./bee after 96 hrs.
>>M-084112-01-1@S-6022-12-01-1

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Report:	02.01.01.03/07; [redacted]; 2004; M-121776-01-2
Title:	Laboratory bioassays to determine acute oral and contact toxicity of Confidor SL 200 to the honeybee, Apis mellifera
Report No.:	BAY-03-9
Document No.:	M-121776-01-2
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-121776-01-2@S-602879-01-1

Materials and methods:

Confidor 200 SL (Development No 30-00325832; Batch No 03833/0914(0912); TOX No 06313-00), nominally containing 200 g/L imidacloprid (NTN 33893), was provided both orally and topically to honeybees *(Apis mellifera* L.). Following preliminary range-finding tests, for the contact toxicity test, Confidor 200 SL was evaluated in a definitive rate-response test at six dose rates, equivalent to 1.225, 0.583, 0.278, 0.132, 0.063 and 0.030 /µg a.i.(NTN 33893)/bee (based on the measured content of a.i.); for the oral toxicity test Confidor 200 SL was evaluated in a definitive rate-response test at six dose rates, equivalent to 0.451, 0.270, 0.114, 0.101, 0.029 and 0.015µg a.i. (NTN 33893)/bee (based on the actual amount of test item consumed).

For topical dosing, the test item was dissolved in a 0.05% v/v solution of Farmon Blue, a wetting agent. For oral dosing, the test item was dispersed in a 50% w/v sucrose solution. Control treatments of deionised water and an untreated solution of Farmon Blue (0.05% v/v) (both topically applied) and untreated sucrose (administered orally), were included in the experiment. In order to establish whether the bees used for the test were of an acceptable sensitivity in accordance to testing guidelines, dose response tests (both oral and contact (topical)) were conducted using dimethoate. This was applied at nominal rates of 0.200, 0.175, 0.150, 0.125 and 0.100µg a.i./bee.

Worker bees (approximately 2 weeks old) were obtained from a hive of a commercial bee keeper. In preparation for the tests, the bees were lightly anaesthetised with humidified CO_2 gas and were then transferred in groups of ten into test cages of stainless steel netting of 2.0-2.5 mm mesh size. These cages were cylindrical, measuring 140 mm deep by 40 mm in diameter, and were closed at both ends with bungs of polyurethane foam. Feeding tubes containing 50% w/v sucrose solution were provided for the bees intended to receive the contact dose. The bees used in the oral bioassays were deprived of food prior to dosing.

For the topical (contact) application of treatments, the bees were lightly anaesthetised using humidified CO_2 gas and 1 µL of test solution was placed on the dorsal thorax of each bee using a Rainin EDP-2 motorised micropipette. For the oral application of doses, cages of bees were presented with glass feeding tubes containing 0.22 ml of a 50% w/v sucrose solution containing the appropriate treatment. The bees took the treated sugar solution from the open end of the tube. It was assumed that the bees in a cage share the test solution and so each should have received a dose of approximately 20 µL.The tubes were inspected at hourly intervals following provision of the doses. At each inspection, any apparently empty feeding tubes were removed and were replaced for the remainder of the bioassay with tubes containing untreated 50% w/v sucrose solution. For the cages in which the treated syrup had not been consumed within 6 h (this was the case for all of the cages treated with the test item solution, with the exception of replicate 2 at the dose rate of 0.132 µg a.i./20µl), the tubes were removed at this time and reweighed on a four decimal place balance, so that the precise amount of treated food consumed could be calculated for each replicate.

For the definitive test, 3 replicate cages of bees (i.e. 30 insects) were treated topically and orally with six dose rates of the test item, five dose rates of the dimethoate and with the control treatments. In both the contact and oral exposure bioassays, assessments of the condition of bees were made at approximately 2,

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4, 24, 48, 72 and 96 h after their exposure to the treatments. For the dimethoate, assessments ceased at 48 h.

For the toxic reference (dimethoate), the 24-h LD_{50} values derived for the contact and oral methods of application were 0.129 and 0.139 µg a.i./bee, respectively and these are in line with published values (Gough *et al.,* 1994). These results indicated that the test insects were of an acceptable sensitivity.

Dates of experimental work: between 28 August and 5 September 2003.

Findings:

Test item	Confidor SL 200		
Test object	*Apis mellifera*		
Exposure	Contact and Oral		
Mortality (%) at 96 h			
Contact exposure		Oral exposure	
Water control	0	Sucrose control	0
FB control	0		
0.030 µg a.i./bee	43	0.015 µg a.i./bee	10
0.063 µg a.i./bee	30	0.029 µg a.i./bee	10
0.132 µg a.i./bee	70	0.101 µg a.i./bee	80
0.278 µg a.i./bee	97	0.114 µg a.i./bee	67
0.583 µg a.i./bee	90	0.270 µg a.i./bee	100
1.225 µg a.i./bee	100	0.451 µg a.i./bee	97
LD_{50} (µg a.i./bee)	0.061	LD_{50} (µg a.i./bee)	0.060
95% confidence limits (µg a.i./bee)	0.019 – 0.114	95% confidence limits (µg a.i./bee)	0.040 – 0.086

All treatment rates of Confidor SL 200 expressed as µg a.i./bee, based on the measured content of imidacloprid

FB = 0.05% solution of Farmon Blue

>>M-121776-01-2@S-601879-01-1

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Report:	02.01.01.03/08; [redacted]; 2004; M-121772-01-2
Title:	Laboratory bioassays to determine acute oral contact toxicity of NTN 33893 200 OD to the honeybee, Apis mellifera
Report No.:	BAY-03-7
Document No.:	M-121772-01-2
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-121772-01-2@S-602856-01-1

Materials and methods:

NTN 33893 200 OD (Development No 30-00284249; Batch No 0779/0060(0036); TOX N9 06445-00), nominally containing 200 g/L imidacloprid (NTN 33893), was provided both orally and topically to honeybees (*Apis mellifera* L.). Following preliminary range-finding tests, for the contact toxicity test, NTN 33893 200 OD was evaluated in a definitive rate-response test at six dose rates, equivalent to 0.919, 0.427, 0.199, 0.092, 0.043 and 0.020 /µg a.i.(NTN 33893)/bee (based on the measured content of a.i.); for the oral toxicity test NTN 33893 200 OD was evaluated in a definitive rate-response test at six dose rates, equivalent to 0.343, 0.145, 0.113, 0.045, 0.032 and 0.015 µg a.i.(NTN 33893)/bee (based on the actual amount of test item consumed).

For topical dosing, the test item was dissolved in a 0.05% v/v solution of Farmon Blue, a wetting agent. For oral dosing, the test item was dispersed in a 50% w/v sucrose solution. Control treatments of deionised water and an untreated solution of Farmon Blue (0.05% v/v) (both topically applied) and untreated sucrose (administered orally), were included in the experiment. In order to establish whether the bees used for the test were of an acceptable sensitivity in accordance to testing guidelines, dose response tests (both oral and contact (topical)) were conducted using dimethoate. This was applied at nominal rates of 0.200, 0.175, 0.150, 0.125 and 0.100 µg a.i./bee.

Worker bees (approximately 2 weeks old) were obtained from a hive of a commercial bee keeper. In preparation for the tests, the bees were lightly anaesthetised with humidified CO_2 gas and were then transferred in groups of ten into test cages of stainless steel netting of 2.0-2.5 mm mesh size. These cages were cylindrical, measuring 140 mm deep by 40 mm in diameter, and were closed at both ends with bungs of polyurethane foam. Feeding tubes containing 50% w/v sucrose solution were provided for the bees intended to receive the contact dose. The bees used in the oral bioassays were deprived of food prior to dosing.

For the topical (contact) application of treatments, the bees were lightly anaesthetised using humidified CO_2 gas and 1 µL of test solution was placed on the dorsal thorax of each bee using a Rainin EDP-2 motorised micropipette. For the oral application of doses, cages of bees were presented with glass feeding tubes containing 0.22 ml of a 50% w/v sucrose solution containing the appropriate treatment. The bees took the treated sugar solution from the open end of the tube. It was assumed that the bees in a cage share the test solution and so each should have received a dose of approximately 20µL. The tubes were inspected at hourly intervals following provision of the doses. At each inspection, any apparently empty feeding tubes were removed and were replaced for the remainder of the bioassay with tubes containing untreated 50% w/v sucrose solution. For the cages in which the treated syrup had not been consumed within 6 h (this was the case for all of the cages treated with the test item solution, with the exception of replicates 2 and 3 at the dose of 0.015 µg a.i./20µl_ and replicate 1 at the dose of 0.032 µg a.i./20µl_), the tubes were removed at this time and reweighed on a four decimal place balance, so that the precise amount of treated food consumed could be calculated for each replicate.

For the definitive test, 3 replicate cages of bees (i.e. 30 insects) were treated topically and orally with six dose rates of the test item, five dose rates of the dimethoate and with the control treatments. In both the contact and oral exposure bioassays, assessments of the condition of bees were made at approximately 2,

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4, 24, 48, 72 and 96 h after their exposure to the treatments. For the dimethoate, assessments ceased at 48 h.

For the toxic reference (dimethoate), the 24-h LD_{50} values derived for the contact and oral methods of application were 0.119 and 0.144 µg a.i./bee, respectively and these are in line with published values (Gough et al., 1994). These results indicated that the test insects were of an acceptable sensitivity.

Dates of experimental work: between 28 August and 8 September 2003.

Findings:

Test item	NTN 33893 200 OD		
Test object	*Apis mellifera*		
Exposure	Contact and Oral		
Mortality (%) at 96 h			
Contact exposure		Oral exposure	
Water control	0	Sucrose control	3
FB control	0	-	-
Corrected mortality (%) at 96 h			
0.020 µg a.i./bee	7	0.015 µg a.i./bee	7
0.043 µg a.i./bee	40	0.032 µg a.i./bee	28
0.092 µg a.i./bee	50	0.045 µg a.i./bee	31
0.199 µg a.i./bee	93	0.113 µg a.i./bee	93
0.427 µg a.i./bee	83	0.145 µg a.i./bee	79
0.919 µg a.i./bee	97	0.343 µg a.i./bee	79
LD_{50} (µg a.i./bee)	0.078	LD_{50} (µg a.i./bee)	0.057
95% confidence limits (µg a.i./bee)	0.044 – 0.123	95% confidence limits (µg a.i./bee)	0.020 – 0.128

All doses are based on the measured amount of a.i.

FB = 0.05% solution of Farmon Blue

>>M-121[illegible]-01-2@S-602856-01-1

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Report: 02.01.01.03/09; [redacted]; 2004; M-060078-01-2
Title: Acute toxicity of NTN 33893 75 OD & AE F032640 10 to the honeybee Apis mellifera L. under laboratory conditions
Report No.: 03 10 48 067
Document No.: M-060078-01-2
Guideline(s): OECD 213 (1998), OECD 214 (1998)
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-060078-01-2@S-602151-01-1

Material and methods: Apis mellifera carnica P.

Test species: oral toxicity and contact toxicity tests with NTN 33893 75 OD & AE F032640 10 on honeybees

Treatments: control, test item and reference item Dimethoate EC 400)

Test item treatment levels: the test item in the contact and oral toxicity tests was applied at the following doses:

Contact toxicity			Oral toxicity		
µg test item/bee	µg a.i./bee		µg test item/bee	µg a.i./bee	
	NTN 33893	AE F032640		NTN 33893	AE F032640
12.5	0.938	0.129	12.5 (7.4)	0.938 (0.552)	0.129 (0.0759)
6.25	0.469	0.064	6.25 (4.06)	0.469 (0.305)	0.064 (0.0419)
3.125	0.234	0.032	3.125 (2.182)	0.234 (0.164)	0.032 (0.0225)
1.563	0.117	0.016	1.563 (1.520)	0.117 (0.114)	0.016 (0.0157)
0.781	0.059	0.008	0.781 (0.774)	0.059 (0.058)	0.008 (0.00797)
0.391	0.029	0.004	0.391 (0.391)	0.029 (0.029)	0.004 (0.00403)

Values in brackets based on the actually consumed amount of sucrose solution

Toxic standard: Dimethoate EC 400 was applied at the following doses:

Contact toxicity		Oral toxicity	
µg product/bee	µg a.i./bee	µg product/bee	µg a.i./bee
0.663	0.250	0.663	0.250
0.332	0.125	0.331	0.125
0.166	0.062	0.166	0.062
0.083	0.031	0.083	0.031

Dates of work: July 08-July 11 ,2003

The test item NTN 33893 75 OD & AE F032640 10 (content: 73.95 g/l NTN 33893 & 10.16 g/l Deltamethrin, specification: Development No.:30-00317155, Batch: 08137/0023(0019), TOX No.: 06314-00, density: 0.986 g/cm^3) was tested under laboratory conditions on the honeybee *A. mellifera* after oral and contact exposure. Endpoints were mortality and behaviour of the bees compared to control up to 48 h after application. Mortality values were used to provide a regression line and calculate the median lethal dose value (LD_{50}) expressed in µg of active ingredient or product per bee.

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Findings:

Table: Oral and contact toxicity LD_{50} values of bees treated with NTN33893 75 OD & AE F032640 10

Test item	NTN 33893 75 OD & AE F032640 10				
Test object	Honeybee *Apis mellifera L.*				
Exposure	contact / oral				
Treatment	LD_{50}				
Test item	time	contact toxicity test		oral toxicity test	
		µg test item/bee	slope b	µg test item/bee	slope b
NTN33893 75 OD & AE F032640 10	**24 h** 95 %-cl lower upper	2.554 2.111 3.091	3.326	2.504 1.991 3.151	7.105
	48 h 95 %-cl lower upper	2.218 1.795 2.741	2.798	2.401 2.068 2.788	8.666
Reference item	time	µg a.i./bee	slope b	µg a.i./bee	slope b
Dimethoate EC 400	**24 h** 95 %-cl lower upper	0.161 0.138 0.189	5.521	0.148 0.126 0.173	6.049
	48 h 95 %-cl lower upper	0.159 0.136 0.185	5.468	0.138 0.109 0.175	3.725

cl: confidence limits

No statistically significant effects of the test item NTN 33893 75 OD & AE F032640 10 on survival were observed at the doses of 0.391 and 0.781 µg test item per bee in the contact toxicity test (3.3 and 6.7 % mortality, respectively) during 48 hours. For the tested doses of 1.563, 3.125, 6.25 and 12.5 µg test item per bee statistically significant effects of the test item on survival were observed (33.3, 66.7, 90.0 and 100 % mortality, respectively) during 48 hours. The calculated LD_{50} (48 h) was 2.218 µg test item per bee in the contact toxicity test.
In the oral toxicity test no statistically significant effects on survival were observed at consumed doses of 0.391, 0.774 and 1.520 µg test item per bee (0, 0 and 3.3 % mortality, respectively) during 48 hours. For the tested oral exposure doses of 2.182, 4.063 and 7.365 µg test item per bee statistically significant effects of the test item on survival were observed (96.7, 100 and 100 % mortality, respectively) during 48 hours. Therefore, the calculated LD_{50} (48 h) was 2.401 µg test item per bee in the oral toxicity test.
Before bees died in the test item treatments, apathy and immobility were observed shortly after application until the 24 hour assessment.

The LD_{50} of the reference item Dimethoate EC 400 was 0.161 µg a.i. per bee in the contact toxicity test after 24 hours. This value was within the preferred range of 0.10- 0.30 µg a.i./bee cited in the OECD Guideline 214.
The LD_{50} of the reference item Dimethoate EC 400 was 0.148 µg a.i. per bee in the oral toxicity test after 24 hours. This value corresponded also to the expected range for the oral 24 h - LD_{50} (0.10-0.35 µg a.i./bee) published in the OECD Guideline 213.
In the reference treatments apathy, discoordinated movements and immobility were observed before bees died.

The study was performed in compliance with the GLP principles.
The validity criterion - mortality in the control ≤ 10 % - was accomplished (being 0 % in the contact and oral toxicity tests after 48 hours).
The LD_{50} - 24 h values for the toxic standard of 0.1-0.30 µg a.i./bee (contact) and 0.1-0.35 µg a.i./bee (oral) were accomplished (being 0.161 µg a.i./bee and 0.148 µg a.i./bee in the contact and the oral toxicity tests, respectively).
>>M-060078-01-2@S-602151-01-1

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Report: 02.01.01.03/10; [redacted]; 2011; M-411260-01-2
Title: Effects of imidacloprid + prothioconazole FS 200 (175+25) G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.: 63941035
Document No.: M-411260-01-2
Guideline(s): OECD 213 and 214 (1998)
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-411260-01-2@S-602249-01-1

Material and Methods:
Imidacloprid + prothioconazole FS 200 (175+25) G; imidacloprid (NTN 33893): 15.8 % w/w (176.8 g/L), prothioconazole (JAU 6476): 2.26 % w/w (25.29 g/L), (all values analytical); Batch ID.: 2011-001084, Sample Description: TOX09319-00; Material No.: 80184883; Specification No.: 102000025004-01; density: 1.119 g/mL (20°C).
Under laboratory conditions Apis mellifera 30 worker bees were exposed for 96 hours to doses of 5.0, 2.5, 1.3, 0.63, 0.31 and 0.16 µg product per bee by topical application (contact dose response test) and 30 worker bees per treatment were exposed for 72 hours to doses of 0.49, 0.34, 0.14, 0.12, 0.058, 0.027 and 0.017 µg product per bee by feeding (oral dose response test; value based on the actual intake of the test item). The contact toxicity test was prolonged for 48 hours due to increasing mortality between 24 and 72 hours, up to a maximum of 96 hours. The oral toxicity test was prolonged for 24 hours due to increasing mortality between 24 and 48 hours, up to a maximum of 72 hours.

Findings:
Table 1. Toxicity to Honey Bees; laboratory tests

Test Item	Imidacloprid + prothioconazole FS 200 (175+25) G	
Test object	*Apis mellifera*	
Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (sugar solution)
Application rate µg product/bee	5.0, 2.5, 1.3, 0.63, 0.31 and 0.16	0.49, 0.34, 0.14, 0.12, 0.058, 0.027 and 0.017
Equivalent to: Application rate µg a.i. imidacloprid/bee	0.79, 0.40, 0.21, 0.10, 0.049 and 0.025	0.077, 0.054, 0.022, 0.019, 0.009, 0.0043 and 0.0027
LD_{50} µg product/bee	24 hours: 4.5 48 hours: 3.2 72 hours: 0.31 96 hours: 0.21	24 hours: 0.42 48 hours: 0.21 72 hours: 0.19
Equivalent to: LD_{50} µg a.i. imidacloprid/bee	24 hours: 0.71 48 hours: 0.51 72 hours: 0.05 96 hours: 0.05	24 hours: 0.066 48 hours: 0.033 72 hours: 0.029
NOEC µg product/bee:	24 hours: n.d. 48 hours: n.d. 72 hours: n.d. 96 hours: n.d.	24 hours: 0.017 48 hours: 0.017 72 hours: 0.017

A LD_{10} and a LD_{20} could not be calculated.

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.24 and 0.13 µg a.i./bee, respectively.

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Observations:
Contact Test:
The contact test was prolonged for a further 48 hours up to 96 hours due to increasing mortality between 24/48 and 48/72 hours, respectively. Mortality occurred in all dose levels in a dose related manner from 90.0 % to 30.0 % at the end of the test (96 hours). 6.7 % mortality occurred in the control group (water + 0.5 % Adhäsit).
During the entire time of the experiment, behavioural abnormalities (e.g. discoordinated movements and/or apathy) were observed amongst the dose levels. There was a dose and time related pattern discernible.

Oral Test:
The oral test was also prolonged for a further 24 hours up to 72 hours due to increasing mortality between 24/48 hours. In the oral test, the maximum nominal dose level of the test item (1.0, 0.5 and 0.25 µg product/bee) could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of 6 hours. Oral doses of 0.49, 0.34, 0.14, 0.12, 0.068 and 0.027 µg product per bee resulted in mortality ranging from 80.0 % to 16.7 % at the end of the test (72 hours after application). No mortality occurred in the 0.017 µg per bee - dose group and in the control group (50% sugar solution), respectively.
Like in the contact test, behavioural abnormalities (e.g. discoordinated movement and apathy) were observed in a dose and time related manner over the time of the experiment.

Conclusion:
The toxicity of imidacloprid + prothioconazole FS 200 (175+25) G was tested in both, an acute contact toxicity test and an acute oral toxicity test on honey bees.
The LD_{50} (24, 48, 72 and 96 h) of the test item was determined to be 4.5, 3.2, 0.31 and 0.31 µg product/bee (equivalent to 0.71, 0.51, 0.05 and 0.05 µg a.i. imidacloprid/bee) in the contact toxicity test, respectively.
The LD_{50} (24, 48 + 72 h) was 0.42, 0.21 and 0.19 µg product/bee (equivalent to 0.066, 0.033 and 0.029 µg a.i. imidacloprid/bee) in the oral toxicity test, respectively.
>>M-411260-01-2@S-602249-01-2

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Report: 02.01.01.03/11; [redacted]; 2014; M-500305-01-3
Title: Effects of imidacloprid FS 350A G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.: 89281035
Document No.: M-500305-01-3
Guideline(s): OECD 213 and 214 (1998)
US EPA OCSPP Guideline No. 850.3080
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-500305-01-3@S-602282-01-1

Material and Methods:
Imidacloprid FS 350A G: imidacloprid (NTN 33893) 30.4 % w/w; 355.2 g/L analytical; Batch ID:EDFL020681; Sample Description: TOX10231-00; Workoder: 13011454; Material No.: 04817397; Specification No.: 102000007262; density: 1.169 g/mL (20 °C).

Under laboratory conditions *Apis mellifera* 30 worker bees per treatment level were exposed for 96 hours to doses of 500.0, 250.0, 125.0, 62.5, 31.3, 15.6 and 7.8 ng a.i. per bee by topical application (contact dose response test) and 30 worker bees per treatment level were exposed also for 96 hours to doses of 91.7, 72.5, 37.8, 17.7, 10.0, 7.2 and 3.5 ng a.i. per bee by feeding (oral dose response test, value based on the actual intake of the test item). Due to increasing mortality between 24/48 and 48/72 hours the contact and oral tests were prolonged for further 48 hours up to 96 hours.

Findings:
Table 1. Toxicity to Honey Bees; laboratory tests

Test Item	Imidacloprid FS 350A G	
Test Species	*Apis mellifera*	
Exposure	contact (solution in Adhäsit (0.5 % v/water)	oral (50 % w/v sucrose solution)
Application rate ng a.i./bee	500.0, 250.0, 125.0, 62.5, 31.3, 15.6 and 7.8	91.7, 72.5, 37.8, 17.7, 10.0, 7.2 and 3.5
LD_{50} ng a.i./bee	24 hours: 154.0; 48 hours: 60.0; 72 hours: 49.5; 96 hours: 47.6	24 hours: n.d.**; 48 hours: 53.7 72 hours: 29.3; 96 hours: 26.5
LD_{20} ng a.i./bee	24 hours: 39.8; 48 hours: 23.7; 72 hours: 23.9; 96 hours: 24.9	24 hours: n.d.**; 48 hours: 6.9; 72 hours: 7.6; 96 hours: 9.0
LD_{10} ng a.i./bee	24 hours: 19.6; 48 hours: 14.6; 72 hours: 16.3; 96 hours: 17.7	24 hours: n.d.**; 48 hours: 2.4; 72 hours: 3.8; 96 hours: 5.1
NOED ng a.i./bee*	24 hours: 31.0; 48 hours: 16.0; 72 hours: 16.0; 96 hours: 16.0	24 hours: < 3.5; 48 hours: 7.2; 72 hours: 7.2; 96 hours: 10.0

* The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).
** n.d.: not determined.

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The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.22 and 0.23 µg a.i./bee, respectively.

Observations:

Contact Test:
The contact toxicity test was prolonged for a further 48 hours up to 96 hours due to increasing mortality between 24/48 and 48/72 hours. Dose levels of 500.0, 250.0, 125.0, 62.5, 31.3 and 15.6 ng a.i./bee led to mortality of 100.0, 96.7, 90.0, 73.3, 16.7 and 13.3 % at test termination (96 hours). No mortality occurred in the 7.8 ng a.i./bee dose group and the control group (water + 0.5 % Adhäsit).
During the first 4 hours behavioural abnormalities (*e.g.* moribundity, movement coordination problems and/or apathy) were observed in all treatment groups. 24 hours following the application, the same symptoms were found in all dose groups except in the lowest dose group (7.8 ng a.i./bee). During the 48 hours assessment some bees in the four highest dose groups (500.0, 250.0, 125.0 and 62.5 ng a.i./bee) showed moribundity and discoordination movements. After 72 hours only one survived single bee in the 500.0 ng a.i./bee dose group showed a discoordinated movement. At the 96 hours assessment, no behavioural abnormalities were found any more. All other surviving bees appeared normal.

Oral Test:
The oral toxicity test was also prolonged for a further 48 hours up to 96 hours due to increasing mortality between 24/48 and 48/72 hours. The maximum nominal dose levels of the test item in the five highest dose groups (200.0, 100.0, 50.0, 25.0 and 12.5 ng a.i./bee) could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of six hours. Mortality occurred at all dose levels. Actual oral doses of 91.7, 72.5, 37.8, 17.7, 10.0, 7.2 and 3.5 ng a.i./bee resulted in mortality ranging from 90.0 % to 10.0 % at the end of the test (96 hours after application). There was 6.7 % mortality in the control group (sucrose 50 % w/v solution = 500 g sucrose/L tap water).
During the 4 hours assessment movement coordination problems, moribundity, cramp and/or apathy were observed in all treatment groups (91.7, 72.5, 37.8, 17.7, 10.0, 7.2 and 3.5 ng a.i./bee). After 24 hours discoordinated movements, moribundity and/or apathy were found in the 91.7, 72.5, 37.8 and 17.7 ng a.i./bee groups. 48 hours following the application, some bees in the 91.7, 72.5 and 37.8 ng a.i./bee dose groups showed a moving coordination problem and apathy. After 72 hours a few bees in the two highest dose groups (91.7 and 72.5 ng a.i./bee) and after 96 hours only one single bee in the highest (91.7 ng a.i./bee) showed moving coordination problems.

Conclusion:
The toxicity of imidacloprid FS 350A G was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} values (24 + 48 + 72 + 96 h) of imidacloprid FS 350A G were determined to be 154.0, 60.0, 49.5 and 47.6 ng a.i./bee, respectively. The oral LD_{50} values (48 + 72 + 96 h) were 53.7, 29.3 and 24.4 ng a.i./bee, respectively.
>>M-500304-01-3@S-602282-01-1

Report:	02.01.01.03/12; [redacted]; 2014; M-503109-01-3
Title:	Effects of imidacloprid + pencycuron FS 370 (120+250) G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	89661035
Document No.:	M-503109-01-3
Guideline(s):	GLP compliant study based on OECD 213 and 214 (1998) US EPA OCSPP Guideline No. 850.3020
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-503109-01-3@S-602312-01-1

Material and Methods:

Imidacloprid + pencycuron FS 370 (120+250) G: imidacloprid (NTN 33893): 10.4 % w/w (119.8 g/L), pencycuron (NTN 19701): 21.9 % w/w (252.0 g/L), (all values analytical); Batch ID: EC14101025; Sample Description: TOX09865-00; Material No.: 05866316; Specification No.: 102000008024 - 02; density: 1.151 g/mL (20°C).

Under laboratory conditions *Apis mellifera* 30 worker bees were exposed for 96 hours to doses of 4.0, 2.0, 1.0, 0.50, 0.25 and 0.13 µg product per bee by topical application (contact dose response test) and 30 worker bees per treatment were exposed for 96 hours to doses of 0.75, 0.39, 0.23, 0.14 and 0.07 µg product per bee by feeding (oral dose response test, value based on the actual intake of the test item). Both toxicity tests were prolonged for 48 hrs due to increasing mortality between 24 and 72 hours, up to a maximum of 96 hours.

Findings:

Table 1. Toxicity to Honey Bees; laboratory tests

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Test Item	Imidacloprid + pencycuron FS 370 (120+250) G	
Test Species	*Apis mellifera*	
Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (50 % w/v sucrose solution)
Application rate µg product/bee	4.0, 2.0, 1.0, 0.50, 0.25 and 0.13	0.75, 0.39, 0.25, 0.14 and 0.07
Equivalent to: Application rate µg a.i. imidacloprid/bee	0.416, 0.208, 0.104, 0.052, 0.026 and 0.014	0.0780, 0.0406, 0.0260, 0.0146 and 0.0073
LD_{50} µg product/bee	24 hours: 2.50 48 hours: 0.54 72 hours: 0.42 96 hours: 0.38	24 hours: > 0.75 48 hours: > 0.75 72 hours: 1.04 96 hours: 0.96
LD_{20} µg product/bee	24 hours: 0.33 48 hours: 0.18 72 hours: 0.22 96 hours: 0.20	24 hours: < 0.07 48 hours: 0.040 72 hours: 0.062 96 hours: 0.089
LD_{10} µg product/bee	24 hours: 0.11 48 hours: 0.11 72 hours: 0.13 96 hours: 0.14	24 hours: 0.07 48 hours: < 0.07 72 hours: < 0.07 96 hours: < 0.07
NOED µg product/bee*	24, 48, 72, 96 hours: 0.26	24, 48, 72, 96 hours: < 0.07
Equivalent to: LD_{50} µg a.i. imidacloprid/bee	24 hours: 0.260 48 hours: 0.056 72 hours: 0.044 96 hours: 0.040	24 hours: > 0.078 48 hours: > 0.078 72 hours: 0.108 96 hours: 0.100
Equivalent to: LD_{20} µg a.i. imidacloprid/bee	24 hours: 0.034 48 hours: 0.019 72 hours: 0.023 96 hours: 0.021	24 hours: < 0.0073 48 hours: 0.004 72 hours: 0.006 96 hours: 0.009
Equivalent to: LD_{10} µg a.i. imidacloprid/bee	24 hours: 0.011 48 hours: 0.011 72 hours: 0.013 96 hours: 0.015	24 hours: < 0.0073 48 hours: < 0.0073 72 hours: < 0.0073 96 hours: < 0.0073
Equivalent to: NOED µg a.i. imidacloprid/bee*	24, 48, 72, 96 hours: 0.026	24, 48, 72, 96 hours: < 0.0073

* The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, α = 0.05).

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.22 and 0.23 µg a.i./bee, respectively.

Observations:

Contact Test:
The contact test was prolonged for a further 48 hours up to 96 hours due to increasing mortality between 24 and 72 hours. Application of 4.0, 2.0, 1.0, 0.50, 0.25 and 0.13 µg/bee of imidacloprid + pencuron FS 370 (120+250) G on the honey bee thorax led to mortalities of 100.0 % to 10.0 % at the end of the test (*i.e.* after 96 hours). No mortality occurred in the control group (water + 0.5 % Adhäsit).
During the 4 and 24-hours assessments, behavioural abnormalities (*e.g.* bees were affected, moribund,

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apathetic or show cramps) were observed at the 4.0, 2.0, 1.0, 0.50 and 0.25 µg/bee dose levels. The surviving bees in the 4.0 and 2.0 µg/bee dose groups were found to be affected or moribund during the 48-hours assessment. 72 hours following treatment, one and two bees were found affected in the 4.0 and 0.50 µg/bee dose groups, respectively. At the last assessment (96 hours following application) one or two bees were still affected in the 2.0, 1.0 and 0.50 µg/bee dosing groups. No behavioural impairments occurred at the 0.13 µg/bee dose group at any time.

Oral Test:

The oral test was also prolonged for a further 48 hours up to 96 hours due to increasing mortality between 24 and 72 hours. In the oral test, the maximum nominal dose level of the test item (1.0, 0.50 and 0.25 µg product/bee) could not be achieved, because the bees did not ingest the full volume of treated 50 % w/v sucrose solution even when offered over a period of 6 hours. The resulting measured oral doses of 0.75, 0.39, 0.23, 0.14 and 0.07 µg product per bee resulted in mortality ranging from 53.3 % to 16.7 % at the end of the test (*i.e.* 96 hours after application). 6.7 % mortality occurred in the control group (50 % w/v sucrose solution = 500 g sucrose/L tap water.

Behavioural abnormalities (*e.g.* bees were affected, moribund or apathetic) were observed in all dose groups during the 4-hours assessment. 24 and 48 hours following treatment bees were affected or apathetic in the 0.75, 0.39 and 0.23 µg/bee dose levels. During the 72-hours assessment 5 bees were still affected in the 0.75 µg/bee treatment and during the 96 hours assessment one bee was found to be affected in the 0.75 and 0.23 µg/bee dose levels, respectively.

Conclusion:

The toxicity of imidacloprid + pencycuron FS 370 (120+250) G was tested in both, an acute contact toxicity test and an acute oral toxicity test on honey bees.
The LD_{50} (24, 48, 72 and 96 h) of the test item was determined to be 2.50, 0.54, 0.42 and 0.38 µg product/bee (equivalent to 0.260, 0.056, 0.044 and 0.040 µg a.i. imidacloprid/bee) in the contact toxicity test, respectively.
The LD_{50} (72 + 96 h) was 1.04 and 0.96 µg product/bee (equivalent to 0.108 and 0.10 µg a.i. imidacloprid/bee) in the oral toxicity test.

>>M-503109-01-3@S-602312-01-1

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Report:	02.01.01.03/13; [redacted]; 2014; M-501653-01-3
Title:	Effects of clothianidin + imidacloprid FS 275 (100+175) G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	89691035
Document No.:	M-501653-01-3
Guideline(s):	GLP compliant study based on OECD 213 and 214 (1998) US EPA OCSPP Guideline No. 850.3020
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-501653-01-3@S-604671-01-1

Material and Methods:

Clothianidin + imidacloprid FS 275 (100+175) G: clothianidin (TI-435): 8.95 % w/w (100.3 g/L), imidacloprid (NTN 33893): 15.8 % w/w (176.7 g/L) (all analytical values); Batch-ID: 2013-001347, Material No.: 80529651; Sample Description: TOX10068-00; Specification No.: 102000025006 - 01; density: 1.121 g/mL (20 °C).

Under laboratory conditions *Apis mellifera* 30 worker bees per treatment level were exposed for 48 hours to doses of 1.0, 0.50, 0.25, 0.13, 0.063 and 0.031 µg product per bee by topical application (contact dose response test) and 30 worker bees per treatment level were exposed for 48 hours to doses of 0.17, 0.11, 0.053, 0.027 and 0.013 µg product per bee by feeding (oral dose response test; value based on the actual intake of the test item).

Findings:

Table 1. Toxicity to Honey Bees; laboratory tests

Test Item	Clothianidin + imidacloprid FS 275 (100+175) G	
Test species	*Apis mellifera*	
Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (sugar solution)
Application rate µg product/bee	1.0, 0.50, 0.25, 0.13, 0.063 and 0.031	0.17, 0.11, 0.053, 0.027 and 0.013
LD_{50} µg product/bee	24 hours: 0.39 48 hours: 0.29	24 hours: 0.062 48 hours: 0.058
LD_{20} µg product/bee	24 hours: 0.101 48 hours: 0.093	24 hours: 0.034 48 hours: 0.030
LD_{10} µg product/bee	24 hours: 0.050 48 hours: 0.051	24 hours: 0.025 48 hours: 0.021
NOED µg product/bee*	24 hours: 0.063 48 hours: 0.063	24 hours: 0.027 48 hours: 0.027

* The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

The contact and oral LD50 (24 h) values of the reference item (dimethoate) were calculated to be 0.28 and 0.14 µg a.i./bee, respectively.

Observations:

Contact Test:

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Test item dose levels of 1.0, 0.50, 0.25, 0.13, 0.063 and 0.031 µg product/bee led to dose dependent mortality, ranging from 73.3 % to 3.3 % at test end (48 hrs following treatment). No mortality occurred in the control group (water + 0.5 % Adhäsit).
Behavioural abnormalities (*e.g.* moribund or affected bees, cramps) were observed in all dose level groups during the 4-hours assessment. Behavioural abnormalities were also observed during the 24- hours assessment in the 1.0, 0.5, 0.25 and 0.13 µg product/bee treatment groups. 48 hours following the application, five bees were found to be affected in the 1.0 µg product/bee dosing group. No further behavioural abnormalities were found in the other dosing groups. All other surviving bees appeared normal.
Oral Test:
Mortality occurred in all test item treated dose levels. Actual oral doses of 0.17, 0.11, 0.053, 0.027 and 0.013 µg product/bee resulted in mortality ranging from 96.7 % to 6.7 % at the end of the test (48 hours after application). No mortality occurred in the control group (sucrose 50 % w/v solution = 500 g sucrose/L tap water).
Behavioural abnormalities (*e.g.* moribund bees or affected bees) were found during the 4-hours assessment in the 0.17, 0.11, 0.053 and 0.027 µg product/bee treatment groups. A few bees were behaving abnormal 24 hours following treatment in the 0.17, 0.11 and 0.053 µg/bee dose levels and one and 6 bees were found to be affected during the 48-hours assessment in the 0.17 and 0.11 µg/bee treatment group, respectively. No behavioural abnormalities were found in the 0.013 µg product/bee dosing group during the test.

Conclusion:

The toxicity of clothianidin + imidacloprid FS 275 (100+175) G was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD50 values (24 and 48 h) of clothianidin + imidacloprid FS 275 (100+175) G were determined to be 0.39 and 0.29 µg product/bee, respectively. The oral LD50 value (24 h – 48 h) was 0.062 and 0.058 µg product/bee, respectively.
>>M-501653-01-3@S-604671-01-1

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02.01.02 - Semi-field

Report:	02.01.02/01; [redacted]; 1988; M-038201-01-4
Title:	Tolerability of seed treatments to bees (bee tunnel I)
Report No.:	VAZ 4/88
Document No.:	M-038201-01-4
Guideline(s):	US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-038201-01-4@S-604650-01-1

One possible indication for the root systemic active ingredients **NTN 33893** and JKU 0337 is the seed treatment of rape. The first of these two active ingredients was tested last year. Ten weeks after the summer rape was sown, there was an unmistakable reduction in flower visits and an increased number of deaths (see report VAZ 13/87). We have now repeated this trial with winter rape. There were 230 days (33 weeks) between sowing (= seed treatment) and flowering.

No danger to bees was associated with this more realistic trial design. Winter rape can be seed treated with oftanol, NTN 33 893 and JKU 0337 without any risk to bees.

>>M-038201-01-4@S-604650-01-1

Report:	02.01.02/02; [redacted]; 1997; M-005376-01-3
Title:	Bees I: systemic nature of insecticidal seed treatment
Report No.:	DVG 10/97
Document No.:	M-005376-01-3
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-005376-01-3@S-602104-01-2

There is the theoretical possibility that systemic active ingredients may appear in nectar. The active ingredient imidacloprid, which is dangerous to bees, is used as a seed treatment for winter rape. The question to be investigated was whether there is a risk to bees at the time of flowering, i.e. 7 to 8 months after sowing. In this trial, the seed-treated winter rape was sown on 10.9.96 and started to flower on 5.5.97, i.e. 237 days after sowing. The bee colonies (three-comb nucleus colonies) were placed in the gauze tunnels covering 50 m^2 of ground on 6.5.97. The bee colonies were removed from the tunnels after 24 days, on 30.5.97, when flowering ended. In addition to an untreated control, the following seed treatments were used:

No. 2) Beta cyfluthrin & imidacloprid 080 & 420 FS 2500 g/dt = 200 & 1050 g a.i./dt
No. 3) Beta cyfluthrin & imidacloprid 080 & 420 FS 5000 g/dt = 400 & 2100 g a.i./dt
No. 4) AKD 1022 70 WS 1500 g/dt = 1050 g a.i./dt
No. 5) TI 435 70 WS 1500 g/dt = 1050 g a.i./dt

>>M-005376-01-3@S-602104-01-2

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Report:	02.01.02/03; [redacted]; 1999; M-086651-01-4
Title:	Observations in a tunnel trial with bees following seed treatment of summer rape
Report No.:	DVG 7/98
Document No.:	M-086651-01-4
Guideline(s):	US EPA OCSPP Guideline no. 850.SUPP
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-086651-01-4@S-604659-01-1

The effect of seed treatments on bees at the time of flowering of summer rape was investigated in a tunnel trial with two series separated in time (staggered sowing of seed). Poncho 2.5 and 5.0 (single and double quantities), TI 435 and fipronil had no effect on mortality, flower visits, hive weight, colony strength, food supply and brood. They can therefore be assumed to be not dangerous to bees.

In the case of KKO 3334, the suspicion arose that there was a reaction by the bees as the hive weight and colony strength decreased, the nectar supply could not be maintained and egg laying was reduced, resulting in a reduction in the size of the brood nest and a striking increase in the proportion of empty cells. Although these effects were minor, they occurred in both series.

>>M-086651-01-4@S-604659-01-1

Report:	02.01.02/04; [redacted]; 1999; M-075504-01-3
Title:	1999 Evaluation of: Gaucho seed dressing applied to canola on the honey bee,(Apis mellifera Linnaeus) at Indian head, Saskatchewan (Indian head research station site)
Report No.:	M-075504-01-3
Document No.:	M-075504-01-3
Guideline(s):	US EPA OCSPP Guideline 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-075504-01-3@S-604324-01-1

Conclusions:
Gaucho treated canola did not show any obvious or measured adverse affects on colony development. Brood rearing was with limits considered to be normal, worker bee survival was within expected limits, worker bee populations increased while the colonies were confined to the pollination cages, dead bees were not observed in front of the colonies, and foraging activity was similar in both colonies.

>>M-075504-01-3@S-604324-01-1

Report:	02.01.02/05; [redacted]; 2001; M-084030-01-2
Title:	Tunnel test: Assessment of side effects of Confidor SL 200 on the honey bee (Apis mellifera L.) in apple orchard following application before flowering (mouse-ear stage) of the crop
Report No.:	20011099/01-BZE01
Document No.:	M-084030-01-2
Guideline(s):	Based on EPPO Guideline No. 170
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-084030-01-2@S-602823-01-1

Materials and Methods

Test substance:
Name: Confidor SL 200;
purity: 194 g/L (nominal: 200 g/L)

The following study was designed to determine the effects of Confidor SL 200 on the honey bee (*Apis mellifera* L.) under semi-field conditions in an apple orchard. The study was carried out in Germany near

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Karlsruhe at the test location Augustenberg. The test substance Confidor SL 200 was tested at an application rate of 0.105 kg a.s./ha in 500 L water/ha (amount of water was adapted to the tree height). The application was performed at the mouse-ear stage of the apple trees (BBCH-code 10, 30MAR2001). Untreated orchard plots with apple trees served as control.

This GLP compliant study was conducted based on the guideline of the European and Mediterranean Plant Protection Organisation No. 170 (EPPO, 1992).

After the application of the test substance before the start of flowering (04APR2001) 3 tunnel tents for the test substance treatment were build up over the treated plots of apple trees. In the control 3 tunnels were set-up over untreated plots of apple trees from the same variety. At the start of full flowering (23APR2001) one small bee colony was placed in each tunnel of the test substance treated apple plots and the untreated apple plots for the control.

Mortality, foraging activity, behaviour, and condition of the colonies and the development of the bee brood were assessed over a period of 7 days.

The influence of the test substance Confidor SL 200 was evaluated by comparing the bees in the pesticide-treated tunnels to those in the control tunnels regarding the following observations:

- Mortality at the edge of the treated area and in the bee traps.
- Flight intensity in the crop (number of flying bees/tree/minute).
- Flight intensity in front of the hives (number of bees leaving/entering the hive/minute).
- Behaviour of the bees on the crop and around the hive.
- Development of the bee brood.

Dates of work: 30MAR2001 - 30APR2001

Findings

Effects on honey bee mortality:

No increased number of dead bees in the dead bee traps and on the linen at the edge of the treated area could be noticed in the test substance treatment in comparison to the control. The daily average of dead bees in the dead bee trap was 4.8 dead bees/tent in the test substance treatment and 7.3 dead bees/tent in the control. During evaluation day 1 - 7 the daily average of dead bees recorded on the linen was 7.9 dead bees/tent in the test substance treatment compared to 12.4 dead bees/tent in the control. The total daily average of dead bees per tent was 12.7 dead bees/tent in the test substance treatment and 19.7 dead bees/tent in the control.

Effects on honey bee flight intensity:

During the 7 days of assessments the daily average flight intensity in the crop ranged from 0.04 - 20.89 forager bees/tree/minute/tent in the test substance treatment and from 0.00 - 20.22 forager bees/tree/minute/tent in the control. The overall daily average of flight intensity on the apple trees during the period of assessments was similar in both treatments with 10.05 forager bees/tree/minute/tent in the test substance treatment compared to 9.24 forager bees/tree/minute/tent in the control.

The daily average number of forager bees leaving/entering the hive per minute was 10.31 bees/tent in the test substance treatment and 10.47 bees/tent in the control during the period of assessments.

Conditions of the colonies and effects on honey bee brood development:

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The conditions of the colonies and the bee brood development showed no abnormal difference which could be attributed to the influence of the test substance.

Effects on behaviour of the bees:

No abnormal difference in behaviour of the bees was observed between the test substance treatments and the control treatments at any time during the period of assessment.

Conclusion:

The treatment of apple trees at the mouse-ear stage with Confidor SL 200 at an test rate of 0.105 kg a.s./ha in 500 L water/ha did not cause adverse effects to honey bee mortality, flight intensity in the crop or the brood development of the colonies in this semi-field study.

>>M-084030-01-2@S-602823-01-1

Report:	02.01.02/06; [redacted] 2001; M-089338-01-3
Title:	Confidor SL 200: a multiple rate cage study to determine effects on honeybees, Apis mellifera L, when applied to flowering Phacelia tanacetifolia
Report No.:	B074AMS
Document No.:	M-089338-01-3
Guideline(s):	US EPA OCSPP Guideline No. 850.SLPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-089338-01-3@S-603080-01-1

A multiple rate cage study with the insecticide Confidor SL 200 was performed in a fully replicated semi-field cage test design for honeybees, *Apis mellifera* L. Honeybees were exposed to flowering *Phacelia tanacetifolia* (fiddleneck) treated at several rates of the test product. The following nominal test application rates were used: 14 g a.i./ha, 9 g a.i./ha, 4 g a.i./ha, 2 g a.i./ha, 1.2 g a.i./ha and 0.6 g a.i./ha. The overall test design was in agreement with OEPP/EPPO-guidelines (EPPO, 1992) for cage studies with honeybees.

Small, standardised honeybee colonies were placed in meshed cages of 4 x 5 meter and 2 meter high. Each cage contained approximately 108 untreated flowering *Phacelia*-plants. Honeybees gained foraging experience for four days before exposure. During this period mortality was assessed after every period of honeybee flight. During the final two days before exposure, foraging activity was monitored on six moments during the day.

After this initial 4-day period, the exposure phase started by applying the test product to the *Phacelia* present inside the tents in the morning after the onset of the honeybee flight. All treatment groups were tested simultaneously and compared to a water treated control and a reference item (PennCap M, a 240 g/l CS formulation of methylparathion, at 1000 g a.i./ha). For each treatment there were four replicates. Foraging activity and mortality of the honeybees were assessed during 4 days after initiation of exposure.

Treatment effects were evaluated both by within-colony comparison of foraging activity and mortality before and after exposure (pre-post design) and by among colony comparison of different treatment groups to the water treatment.

>>M-089338-01-3@S-603080-01-1

Please click on the hyperlink to order a Study Report.

Report:	02.01.02/07; [redacted]; 2003; M-090327-01-3
Title:	A multiple-rate cage test to study effects of Confidor SL 200 on honeybee (Apis mellifera L.) when applied to flowering Phacelia tanacetifolia 24, 48 and 96 hours before bee exposure
Report No.:	B075AMS
Document No.:	M-090327-01-3
Guideline(s):	OEPP/EPPO 1992: Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honeybees. Bulletin OEPP-EPPO Bulletin 22, 203-215 US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-090327-01-3@S-604661-01-1

Materials and methods:

The insecticide Confidor SL 200 (active ingredient NTN 33893, content: 196 g/l, TOX no.: 6037-00, Art. no: 0004958808, Batch no.: 233026473) was applied to flowering *Phacelia tanacetifolia* plants (Fiddleneck), approximately 24, 48 and 96 hours before bee exposure at two nominal rates, 21 and 35 g a.i./ha at an application volume equivalent to 200 l/ha. The control was treated with deionised water. PennCap M at a rate of 5 g product per liter (i.e. 1000 g product/ha) was used as toxic reference. For each treatment there were four replicate groups. Five days before exposure in the evening, small, standardised honeybee colonies were placed in meshed cages of 4 x 5 meter and 2 meter high, each containing 36 pots with untreated flowering *Phacelia* plants. During the next four days mortality was assessed after every period of honeybee flight. During the last two days before exposure, foraging activity was recorded for all cages at six moments during the day. After this initial 4-day period, exposure was initiated by replacing the plants inside the cages with a second series of treated plants in all tents. Before treatment, these plants had been growing under identical conditions. The timing of treatments was such that at the start of exposure, i.e. the beginning of bee flight following plant exchange, groups of plants had been treated 24, 48 or 96 hours before. Foraging activity and mortality of the honeybees were assessed during 4 days after initiation of exposure. The number of flowers was counted at the first day and the fourth day of the exposure period.

Effects on foraging activity were analysed using repeated measures ANCOVA, with the number of flowers as a covariate. Treatments were compared to the deionised water control using linear contrasts.

Effects on mortality were analysed, using a covariance alternated to repeated measured ANOVA. The cumulative number of bees that died in the last 2 days before exposure was used as a covariate. Treatments were compared to the deionised water control using linear contrasts.

Dates of work (biological part): 27 July 2002 - 5 August 2002.

Findings:

Foraging activity and low mortality in the deionised water control indicated that the trial was valid for the purposes to which it was designed. High mortality in the toxic reference treatment (about 10 times higher than the in deionised water control) showed that the test set-up was sufficiently sensitive and that potential adverse effects of exposure to test item residues could be detected. Due to sub-optimal weather conditions, overall foraging activity one day after initiation of exposure was low. Therefore findings concerning foraging behaviour pertaining to this day are not considered for the evaluation.
On the day of exposure and two days later, foraging activity in the cages with plants treated one day earlier with Confidor SL 200 at a rate of 35 g a.i./ha was reduced and significantly different from foraging in the water control. Foraging was also reduced and significantly different from foraging in the water control when plants were treated 4 days before exposure with Confidor SL 200 at a rate of 21 g a.i./ha.

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Reductions in foraging activity were not observed in any of the other groups treated with Confidor SL 200.

Overall mortality in the treatments where Confidor SL 200 was applied at 21 g a.i./ha two and four days before exposure and the treatment where Confidor was applied at 35 g a.i./ha four days before exposure, was about one fifth of the toxic reference treatment and two times higher than in the deionised water control. These differences were statistically significant. In the other Confidor SL 200 treatments mortality was roughly equal to or 1.5 times higher than in the deionised water control and no significant effect on mortality was observed.

A summary of findings is given in Table 1.1 (foraging data) and Table 1.2 (mortality data).

Table 1.1 Summary of findings foraging data

Treatment	Pre-exposure average day -2-1	Exposure day 1	day 2 (see note)	day 3	day 4
Deionised water	79.4 ± 16.8	67.5 ± 8.5	5.3 ± 1.8	69.5 ± 12.7	33.3 ± 5.3
Confidor SL 200 (hours before application)					
21 g a.i./ha (24)	88.1 ± 8.5	58.8 ± 14.5	5.0 ± 2.2	59.5 ± 6.9	28.5 ± 1.7
21 g a.i./ha (48)	97.9 ± 10.2	54.0 ± 6.3	2.5 ± 0.6 *	53.0 ± 1.1	30.0 ± 4.3
21 g a.i./ha (96)	81.1 ± 8.1	104.2 ± 17.6 *	3.3 ± 1.7	77.5 ± 18.7	32.3 ± 2.1
35 g a.i./ha (24)	82.5 ± 18.5	38.3 ± 15.3 *	1.0 ± 0.7 **	32.0 ± 7.9	25.0 ± 2.4
35 g a.i./ha (48)	64.5 ± 4.3	52.8 ± 16.2	1.0 ± 0.4 *	46.3 ± 7.4	28.3 ± 2.8
35 g a.i./ha (96)	90.5 ± 6.6	85.0 ± 12.0	0.8 ± 0.5 *	50.8 ± 5.4	22.3 ± 4.3
PennCap M	60.9 ± 7.1	34.8 ± 8.3	0.3 ± 0.3 **	33.5 ± 4.6 **	25.5 ± 3.0

*= P<0.05; ** = P<0.01 (Difference with water control; ANCOVA followed by linear contrasts)

note: due to sub-optimal weather conditions foraging activity on this day was low and and observed effects are suspected and should not be taken as biologically meaningful

Table 1.2 Summary of findings mortality data

Treatment	Pre-exposure average day -2-1	Exposure cumulative
Deionised water	3.6 ± 0.5	18.5 ± 3.0
Confidor SL 200 (hours before application)		
21 g a.i./ha (24)	4.0 ± 0.8	21.8 ± 2.1
21 g a.i./ha (48)	2.8 ± 0.4	35.0 ± 3.5 *
21 g a.i./ha (96)	5.3 ± 1.1	36.8 ± 4.0 *
35 g a.i./ha (24)	4.5 ± 1.2	28.3 ± 5.5
35 g a.i./ha (48)	3.5 ± 1.3	27.5 ± 5.0
35 g a.i./ha (96)	5.5 ± 0.9	40.0 ± 8.8 *
PennCap M	3.9 ± 1.2	216.3 ± 34.8 ***

*= P<0.05; *** = P<0.001 (Difference with water control; ANCOVA followed by linear contrasts)

>>M-0903[illegible]1-3@S-604661-01-1

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Report:	02.01.02/08; [redacted]; 2003; M-116136-01-3
Title:	Evaluation of the effects of a soil treatment of ornamental plants with Imidacloprid WG 5 on nectar and pollen sampling honeybees (Apis mellifera) in the semifield (test plants: Erica and Lobelia)
Report No.:	M-116136-01-3
Document No.:	M-116136-01-3
Guideline(s):	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

<<M-116136-01-3@S-602223-01-1

Material and methods: In a tunnel test ornamental plants, *Lobelia erinus* and *Erica gracilis,* received soil treatment at a rate of 0.015 g a.i./l soil substrate at full blossom with Imidacloprid WG 5 (NTN 33893: Article-No.: 0005439280, Batch-No.: PFOOOOOOREC, content of a.i.: 5.5%, TOX No. 6135-00). Control plants received no treatment.
5 treatments with two replicates for each treatment were defined by different proportions of treated and untreated plants with a proportion of 50% of the ground covered with untreated and 50% covered with treated plants for the treatments A and B and a proportion of 10% of the ground covered with treated and 90% covered with untreated plants for the treatments C and D. The numbers of plants for the treatments and the control were as follows:

K: control: no treatment, 300 untreated *Lobelia erinus* (equivalent to 50%) and 300 untreated *Erica gracilis* (equivalent to 50%) in the tunnel

A: 15 mg a.i./l soil substrate, 300 treated *Lobelia erinus* (equivalent to 50%) and 300 untreated *Erica gracilis* (equivalent to 50%) in the tunnel

B: 15 mg a.i./l soil substrate, 300 treated *Erica gracilis* (equivalent to 50%) and 300 untreated *Lobelia erinus* (equivalent to 50%) in the tunnel

C: 15 mg a.i./l soil substrate, 65 treated *Lobelia erinus* (equivalent to 10%)* and 535 untreated *Erica gracilis* (equivalent to 90%) in the tunnel

D: 15 mg a.i./l soil substrate, 65 treated *Erica gracilis* (equivalent to 10%)* and 535 untreated *Lobelia erinus* (equivalent to 90%) in a tunnel

* for an easier arrangement of the treated plants between the untreated plants, the number of treated plants was increased to 65 instead of 60 plants

The plants were placed inside tunnels (floor space 10 m x 5 m) on the experimental farmland "Höfchen". In each tunnel one honeybee (*Apis mellifera*) colony (containing approx. 3000 honeybees) was allocated. The honeybees were once daily observed for the parameters mortality and foraging and flight activity during a period of 17 days.

Dates of biological work: 2002-09-02 to 2002-09-19
Findings:
Findings for the treatments are presented in table 1.

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Table 1: Summary

Treatment	K	A	B	C	D
Average daily mortality per treatment between the potted plants and in front of the bee hive [n]	11.2	31.2	29.0	23.2	27.8
Average number of honeybees found at the ceiling per treatment and assessment [n]	32.3	36.7	43.8	55.0	41.5
Average number of honeybees found foraging per treatment and assessment [n]	221.9	99.2	90.6	167.4	130.9
Average number of foraging honeybees per treatment and assessment on untreated plants [n]	-	89.9	68.3	*	*
Average number of foraging honeybees per treatment and assessment on treated plants [n]	-	9.3	22.3	*	*

* no quantitative data were recorded in the treatments C and D since the treated plants were set up in between the untreated plants

Conclusion:
Mortality was slightly higher in each of the treatments than in the control; however, absolute mortality was not high, neither in control nor in treatment. There were no differences recognisable in mortality, irrespective which proportion and which of the plant species was treated or untreated, respectively.

Flight activity was at a comparable level in all treatment groups.

Overall foraging activity was distinctly higher in the control than in each of the treatment groups, whereas in the 90:10 (untreated : treated) group the foraging activity was slightly higher than in the 50:50 (untreated : treated) group.

In the replicates with treated plants, the foraging honeybees clearly preferred the untreated plants and obviously avoided visiting the treated ornamentals.

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Report:	02.01.02/09; [redacted]; 2008; M-308625-01-2
Title:	On the relevant endpoint of the study of Bakker (2001) Confidor SL 200, a multiple rate cage study to determine effects on honeybees, Apis mellifera L., when applied to flowering Phacelia tanacetifolia
Report No.:	M-308625-01-2
Document No.:	M-308625-01-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

1. Introduction

For the honey bee off-crop risk assessment on spray applications of products containing the active ingredient Imidacloprid, the study of BAKKER (2001) is of relevance, since in this study offcrop drift rates of Imidacloprid SL 200 were tested on honey bees under semi-field condition. In the following, the results of the study are discussed with respect to the endpoint relevant for the ecotoxicological risk assessment which can be derived from the study.

2 Summary of the study results

In order to determine the effects of an imidacloprid spray application on honeybees, a semi-field study was conducted in which small bee colonies were exposed to a spray treatment of imidacloprid SL 200 onto a bee attractive crop, *Phacelia*, under caged conditions (Bakker 2001), *Phacelia* being chosen because it ensures a high foraging activity of honeybees. Six application rates were tested, 0.6, 1.2, 2.0, 4.0, 9.0, and 14.0 g a.s./ha, with each treatment group replicated four times. A toxic reference standard, PennCap M, was also included in the study at a rate of 5 mg a.s./ha as well as a water control. Critical endpoints monitored in the study were mortality and foraging activity, starting 2 days before application through 4 days after mortality was assessed on a daily basis and foraging activity several times per day. Hive weight and brood development of the colonies were also assessed.

No increased mortality was observed in any imidacloprid treatment group relative to control during the post-application period whereas the toxic standard did show a significantly increased mortality rate. Treatment with imidacloprid at rates of 0.6 and 1.2 g a.s./ha showed no effect on foraging whereas at rates of 2.0, 4.0 and 9.0 g a.s./ha slight reductions in foraging activity on the day of application only were observed. Reductions were not particularly high in comparison to the pre-application figures, but were statistically significant. This reduction of foraging activity lasted through the second day after application in the 14.0 g a.s./ha group. However, even here the reduction was not numerically large standard (see table below).

Average number ± SE (n=4) of foraging bees per day

Treatment	Pre-treatment		Post-treatment			
g a.s./ha	11-jun-01	12-jun-01	13-jun-01	14-jun-01	15-jun-01	16-jun-01
De-ionized water	59.0 ± 6.7	74.3 ± 11.1	96.0 ± 19.3	87.3 ± 21.2	87.8 ± 22.0	74.0 ± 17.8
Imidacloprid 0.6	47.3 ± 3.6	47.0 ± 16.4	69.3 ± 12.2	77.5 ± 8.6	76.5 ± 16.1	61.8 ± 12.4
Imidacloprid 1.2	38.7 ± 16.8	54.0 ± 22.5	75.8 ± 10.0	85.0 ± 16.1	76.0 ± 11.4	67.8 ± 13.5
Imidacloprid 2.0	56.5 ± 8.5	64.3 ± 12.2	57.5 ± 3.0*[a]	89.5 ± 12.6	74.0 ± 7.7	74.0 ± 16.7
Imidacloprid 4.0	50.0 ± 9.2	65.5 ± 12.5	50.5 ± 9.4*	59.0 ± 6.7	64.0 ± 16.8	48.5 ± 16.6
Imidacloprid 9.0	46.0 ± 6.6	72.0 ± 9.4	43.8 ± 6.0*	58.5 ± 6.8	64.0 ± 5.5	51.3 ± 8.9
Imidacloprid 14.0	52.3 ± 6.3	61.0 ± 10.4	40.8 ± 3.0*	48.0 ± 12.8*	56.5 ± 12.5*	49.5 ± 12.4
PennCap 5.0	65.3 ± 17.4	84.3 ± 16.6	31.0 ± 3.1*	2.5 ± 1.0*[b]	10.8 ± 4.2*	11.0 ± 5.2*

* Statistically significantly different from control (P<0.05 ANCOVA followed by Fisher's LSD test)
[a] Exclusion of colonies with reduced foraging activity in the pre-exposure period, identified as outliers in the startistical analysis, led to statistically significant conclusion
[b] The very high mortality observed from day of treatment onward is considered to contribute to conspicuous reduction in foraging days 2 to 4 post-application

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Report: 02.01.02/10;[redacted]; 2001; M-052637-01-3
Title: Effects of residues of imidacloprid in maize pollen from dressed seeds on honey bees (Apis mellifera)
Report No.: M-052637-01-3
Document No.: M-052637-01-3
Guideline(s): US EPA OPPTS 850.3040
Guideline deviation(s): not specified
GLP/GEP: **yes**

<<M-052637-01-3@S-602655-01-1

Material and methods: test substance: Gaucho WS 70, residues in maize pollen from dressed seeds, dressing rate: 49 g/unit a.i Residues of imidacloprid in the pollen were found to be below limit of quantitation (LOQ = 0.005 mg/kg). No olefine and hydroxy metabolites could be detected (limit of detection: 0.003 mg/kg and 0.0015 mg/kg, respectively).

Small bee colonies (appr. 700 honeybees) were confined in tent cages (ca. 20 m2) on short grass meadows and exclusively fed with maize pollen which was harvested from plants, the seeds of which were dressed with Gaucho WS 70 or which were untreated (control). Sunflower honey was provided as carbohydrate source. The small bee colonies were examined for treatment-related impacts over a period of 38 days. In particular, the following endpoints were evaluated: mortality, comb cell production, food consumption, storage behavior, hive weight increase, egg laying activity, breeding success, colony strength, foraging intensity and behavioral anomalies.

Dates of biological work: 2000-08-21 to 2000-09-28.

Findings: Effects of Gaucho WS 70 residues in maize pollen on small honeybee colonies

Testing Endpoint	Control A	Control B	Treatment A	Treatment B
Mortality (no. of dead bees in front of bee hives)	32	27	20	30
Mortality (no of dead bees at the tent edges)	146	141	139	151
Foraging intensity (no. of bees at the pollen feeder)	1	15	29	2
Foraging intensity (no. of bees at the honey feeder)	267	253	274	255
Bee activity (no. of bees at the tent roof)	180	203	196	185
Pollen collected [g]	16	58	43	26
Honey collected [g]	736	853	819	877
Comb cell production [cm²]	606	618	660	664
Honey storage area at study termination [cm²]	434	254	417	399
Hive weight increase [% of the initial weight]	9.8	6.6	12.4	16.6
Egg laying activity [cm² comb area containing eggs at study termination)	19	63	15	18
Colony strength [cm² comb area covered with bees at study termination)	279	249	253	263

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Observations: There were no treatment-related effects in the testing endpoints foraging activity, orientation, honey and pollen consumption, comb cell, production, honey storage, hive weight increase, population development, mortality, breeding activity, and breeding success. There are no hints that imidacloprid residues in pollen from maize seeds treated with Gaucho at the rate recommended might have any adverse effects to honey bee colonies.
>>M-052637-01-3@S-602655-01-1

Report:	02.01.02/11; [redacted]; 2002; M-052238-01-3
Title:	Evaluation of the effects of residues of imidacloprid FS 600 in maize pollen from dressed seeds on honeybees (Apis mellifera) in the semifield
Report No.:	M-052238-01-3
Document No.:	M-052238-01-3
Guideline(s):	not applicable
Guideline deviation(s):	The following procedures were not carried out under GLP: seed dressing, sowing of the seeds, analysis of soil contents of the field where seeds were sown, harvesting of the maize panicles, sieving and drying of the pollen.
GLP/GEP:	**yes**

<<M-052238-01-3@S-602148-01-1
Material and methods: Test substance: maize pollen with grown residues of Imidacloprid FS 600 (seeds dressed with commercially available product at a rate of 4 g a.i. / 1000 seeds). Small honeybee colonies (approx. 500 honeybees) were confined on oat plots (50 m^2, drilled on 2001-05-03) in tunnels and fed with maize pollen containing grown residues of Imidacloprid or untreated control pollen. For treatment and control, three replicates were set up each. Sunflower honey was provided as carbohydrate source. The small bee colonies were examined for treatment-related effects over a period of 52 days. In particular, the endpoints mortality and foraging intensity were evaluated. Likewise the endpoints comb cell production, food consumption, pollen and honey stores, egg laying activity, breeding success, colony strength and hive weight development were assessed and statistically analysed using a t-Test.
Behavioural anomalies were also assessed.

Dates of biological work: 2001-06-21 to 2001-08-12.
Dates of analytical work: 2001-03-14 to 2001-06-05

Findings: Effects of residues of Imidacloprid FS 600 in pollen on small honeybee colonies

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Table 1: Summary

Testing Endpoint	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
Mortality (Total No. of dead bees in front of the bee hives) [n]	1	1	0	5	1	3
Mortality (Total No. of dead bees at the tunnel edge) [n]	28	31	25	50	34	50
Cumulative comb cell production at study termination [cm²]	768	708	675	692	651	706
Cumulative honey collected [g]	702	694	677	621	661	668
Cumulative pollen collected [g]	12.2	8.9	9.6	21.0	39.8	27.1
Honey storage area at study termination [cm²]	194	234	116	136	172	226
Pollen storage area at study termination [cm²]	41	13	18	17	25	27
Egg laying activity [cm² comb area containing cells with eggs at study termination]	177	98	130	182	125	161
Larval abundance [cm² comb area containing cells with larvae at study termination]	92	79	79	89	67	120
Pupal abundance [cm² comb area containing cells with pupae at study termination]	113	92	149	86	110	125
Colony strength [cm² comb area covered with bees at study termination]	266	183	183	269	222	260
Hive weight increase [%]	25.5	27.6	27.1	17.3	23.6	21.6
Foraging activity [Average No. of bees at the pollen feeder / assessment]	0.7	0.7	0.7	0.8	1.1	0.6
Foraging activity [Average No. of bees at the honey feeder / assessment]	7.3	8.1	7.7	7.3	8.2	7.9

Observations: There were no significant differences between control and treatment in comb cell production (t=-0.478, p=0.641), honey consumption (t=2.530, p=0.065), hive weight increase (t=1.720, p=0.161), pollen stores (t=-0.360, p=0.725) and honey stores (t=0.086, p=0.933), egg deposition (t=-0.176, p=0.863), larval abundance (t=-0.328, p=0.749), pupal abundance (t=-0.288, p=0.778) and abundance of adult bees (t=-0.068, p=0.947). The pollen consumption was significantly higher in the treatment.

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02.01.03 - Field and Monitoring

Report:	02.01.03/01; [redacted]; 1991; M-048426-01-2
Title:	Integrated pest and pollinators investigations 1991 (including hony bee toxicity of NTN 33893)
Report No.:	103815
Document No.:	M-048426-01-2
Guideline(s):	Ecological Effects Requirements: Subdivision E 40 CFR 158.145 Supplemental to Guidelines 141-1 and 141-2
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-048426-01-2@S-604653-01-1

Pollinating bees were exposed to NTN treated alfalfa foliage to evaluate poisoning risk. NTN 33893 240 FS was sprayed onto second growth alfalfa at 0.025, 0.05, and 0.1 lb. AI/A. Foliage samples were placed in petri dishes, part with 2 hour old residue and part with 8 hour old residue. Pollinating bees were placed in the petri dishes and % mortality recorded after 24 hours. Four replications each of three different bees were made. The pollinators included honey bee workers (APISME), alfalfa leafcutting bees (MEGCRO), and alkali bees (Nomia melanderi). Bee repellency was not evaluated in this study.

RESLUTS: There appeared to be no separation of % mortality by rate. The alkali bee was the only one of the three to show increased mortality from the 2 hr old residue vs the 8 hr old residue. Mortality, after 24 hr exposure ranged as follows: alkali bee 2 - 28%, leafcutting bee 9 - 18%, honey bee 12 - 20%, and UTC 0 - 4%.

WSU guidelines suggest when 8 hour residues cause less than 25% mortality the compound is probably safe to use around bees if applied in late evening after bees have quit foraging for the day. NTN as tested would qualify as non-hazardous to these three pollinating bees if applied in late evening.

NTN 33893 240 FS Batch No. = 4033004

>>M-048426-01-2@S-604653-01-1

Report:	02.01.03/02; [redacted]; 1995; M-008517-01-3
Title:	Bee VII: Bud burst spray treatment, upper Italy
Report No.:	VAZ 35/95
Document No.:	M-008517-01-3
Guideline(s):	--
Guideline deviation(s):	
GLP/GEP:	**no**

<<M-008517-01-3@S-604653-01-1

At Upper Italy four trials were conducted in apple with bud burst spray treatments at stage 54 (mouse-ear) with Confidor 0.01 % + Oliocin 2.4 %. At blossom bee colonies were placed in the middle of the plots.

A mortality referred to the insecticide could not be detected (summarizing table). Foraging was not impaired. Reduced foraging was, however, observed at spray treatment of Confidor 8 days prior to blossom at red bud stage. At this plot the behaviour of the bees was also slightly irritated. The activity of the bee colonies was, however, equally high at all sites. Also at the "red bud" plot the bees evaded to untreated areas where they collected pollen and nectar. The efficiency of the bees during foraging can be taken from the colour of the stamen. The treatment with Confidor at bud burst did not disturb this efficiency. At "red bud" it was still stated to be within the tolerance. The weight of bee hives increased at one location and remained unchanged at two others. The bad weather did not allow a differentiation. The bud burst spray treatment with Confidor did result in a good fruit set of 30 %, which could be compared to the one of the control of 27 %. The fruit set at "red bud" was slightly lower.

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There are no objections towards application of Confidor at mouse-ear stage. The safety period of more than 10 days prior to blossom should, however, be considered. The trial showed that effects on foraging cannot be excluded at Confidor treatment 8 days prior to blossom.
>>M-008517-01-3@S-604649-01-1

Report:	02.01.03/03; [redacted]; 1998; M-006826-01-4
Title:	The impact of Gaucho 70 WS seed treated sunflower seeds on honey bees
Report No.:	BF 1/98
Document No.:	M-006826-01-4
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-006826-01-4@S-602891-01-1
Sunflower seeds were dressed with Gaucho 70 WS (0.7 mg a.i. per seed) and sown on 8.5.98. Four bee colonies were introduced to the 1.25 ha trial field 75 days later when the plants were in flower. The same process was carried out using undressed seed on a control field of the same size 4 kilometres away, where the same parameters were measured.

The use of Gaucho seed dressing did not lead to increased bee mortality.

Treatment with Gaucho did not reduce foraging visits to sunflowers.

Bees collected large amounts of pollen from both sunflower fields.

Colony weights remained almost unchanged at both sites. This is not unusual as weight depends on the site, variety and weather conditions.

A bee counter allowed us to accurately determine the number of bees returning to the hive from the treated field. No evidence of bee disorientation was found.

No residues of imidacloprid or its main metabolites were found in the honey bladders after preparation or in the remaining bees.

Our final conclusion is that at the time when sunflowers are in flower no relevant residues of the treatment product remain in the nectar that could affect bees.
>>M-006826-01-3@S-602891-01-1

Report:	02.01.03/04; [redacted]; 2009; M-031852-02-3
Title:	The effects of sublethal doses of imidacloprid, dihydroxy-imidacloprid and olefine-imidacloprid on the behaviour of honeybees
Report No.:	110634
Document No.:	M-031852-02-3
Guideline(s):	US EPA OPPTS: N/A
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-031852-02-3@S-604935-01-1
Sublethal effects of imidacloprid and two of its metabolites, olefine-imidacloprid and dihydroxy-imidacloprid, on the behaviour of honeybees were studied in laboratory as well as field experiments. In the field, sucrose solutions containing olefine-imidacloprid were fed to honeybee foragers and possible effects on foraging activity and communication behaviour analyzed. The behavioural effects of olefine-imidacloprid are found to be similar to those of imidacloprid itself. However, the effects are much less pronounced. The only effect, which was significant in the range of concentrations tested, was an increase in the frequency of tremble dances. No significant disorientation could be found in the dances of olefine

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imidacloprid treated bees and no significant effect was found on the foraging activity up to 100 ppb. The effects of imidacloprid, olefine-imidacloprid and dihydroxy-imidacloprid 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-imidacloprid and dihydroxy-imidacloprid, did not significantly affect the learning performance at 100ppb. However, with olefine-imidacloprid effects were found at 500ppb, with dihydroxy-imidacloprid at 2 ppm. In addition, long term effects of feeding sucrose solutions containing 10 ppb imidacloprid to young bees kept in an incubator ad.lib. for 10-12 days were investigated. No effect on the learning performance in the proboscis extension reflex paradigm was found. Imidacloprid is a chloronicotinyl insecticide developed by Bayer. It acts on nicotinic acetylcholine receptors. Previous studies indicated that imidacloprid has sublethal effects on learning and memory as well as orientation and communication behaviour of honeybees. The aims of the present study were to extend a previous investigation of effects on orientation and communication behaviour of imidacloprid to two of its metabolites in treated plants, dihydroxy-imidacloprid and olefine-imidacloprid, and to investigate effects of imidacloprid and its metabolites on the learning performance of honeybees.

>>M-031852-02-3@S-604935-01-1

Report:	02.01.03/05; [redacted]; 1999; M-032341-01-3
Title:	Field test of Gaucho 350 FS seeddressed sunflowers on honeybee colonies
Report No.:	3103/99
Document No.:	M-032341-01-3
Guideline(s):	US EPA OCSPP Guideline no. 850.3040
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-032341-01-3@S-602554-01-1

Test item. Gaucho 350 FS
Test crop: sunflower (Helianthus annuus)
Seedressing dose. 6.3 l/150,000 seeds
Test species: Honey bee (Apis mellifera carnica)
Colony number: 30, 15 on the treated field and 15 on the control field
Placing: The colonies were allocated in multiple-store hives of 4 suppers and were placed at the edge of the fields.
Test fields: The treated field of 45 hectares and the control field of 35 hectares belonged to "Gold ear" Agricultural Producers' Co-operative. On both field variety Alexandra sunflower seeds were sown in clay-loamy soil. The sowing conditions, the plant growth and the pesticide treatments were the same in both fields.
Objective of the study: The field test should prove, that the active ingredient of Gaucho 350 FS does harm/influence or not the foraging bees during the flowering period.

Test dates:
Issue of the study plan: 31 March 1999

Experimental phase of the study: 05 July - 20 July 1999

Issue of the final report: 16 December 1999

Results:

Foraging activity and behavior of the bees
Except the last two days the foraging activity was intensive during the whole period of the experiment. In the experimental period in average 76 foragers were counted on 400 sunflowerheads on the treated field and 43 on the control field.

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On the treated field the average bee ingress was 23.9 bees per minutes, and 26.6 bees per minutes in the control field. On days 1 and 2 and on the last four days of the experiment just few bees were observed returning to the hives with pollenloads. The pollen gathering was characteristic between 09 - 15 July.

On both fields in average 1.9 bees per minutes were observed entering the hives with orangered pollenloads.

No abnormal behavior of the bees was observed during foraging and around the hives.

Weight gain of the hives
The weight gain of the hives on the treated field was 12.5 % and 23.1 % on the control fields.

Strength of the colonies
Initially, the strength of the colonies has been slightly higher in the control. Number of the inhabited combs increased by 2.8 % on the treated field and by 6.5 % on the control field.

Brood status and behavior of the queens
The number of the combs with brood and the total area of all brood stages increased at the bee colonies placed on the treated field. In contrast the brood of the bee colonies placed on the control field decreased. The number of the combs with brood in the control bee colonies also decreased.
Some empty cells were found at the end of the experiment in case of the 100 marked brood cells/colony which was designated for observation at the starting of the experiment. This was attributed to the marking frame which disturbed the bees. The unharmed brood development was normal.
In the experimental period 2 natural requeening was observed in case of the colonies on the treated field and 1 in case of the colonies on control field.
The behavior and eggs laying of the queens were normal in case of the other bee colonies.

Mortality
Except 2 cases in the control bee colonies the bee mortality did not exceed the accepted natural mortality level which is less than 100 dead bees/colony/day. The mortality of the drones was not significant during the whole period of the experiment.
In front of the hives on the treated field 44-365 dead bees per day and 15-972 dead bees per day on the control field were found. The mortality of the drones was not significant during the whole period of the experiment.

Weather conditions and soil moisture
During the experiment sunny days and no winds characterised the weather conditions. Most of the rainfalls were registered during the night, which was 90 mm on the treated and 109 mm on the control field.
The soil moisture of the treated field was 17.12 - 32.53 % and 16.26 - 34.08 % on the control field.

Evaluation made by the beekeeper
At the initiation of the experiment lot of rainfalls and high relative humidity were registered. This why the sunflower produced thin nectar. That was one of the reasons for slight weight gain of the hives. As the weather changed for the better, greater nectar input was registered at the second part of the experiment.
In case of the bee colonies with no requeening, activity of the queens was totally normal. The eggs laying dynamism of the queens was according to the season on both fields.

Conclusion
The honey production of the bees was generally poor in this year in Hungary. Under the conditions of the experiment the weight gain of bee hives on the field sown with Gaucho 350 FS treated seeds at a dose of 0.3 l/150,000 seeds was less than the weight gain of bee hives on the control field. This could be corroborated by a higher energy demand of the the bee colonies placed on the treated field which

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produced substantially more brood. The seeddressing product had no adverse effect on the forager bees, the queens and the brood.
>>M-032341-01-3@S-602554-01-1

Report:	02.01.03/06; [redacted]; 1998; M-038723-01-4
Title:	Flower visits to sunflowers seed-treated with Gaucho
Report No.:	Bees 1/98
Document No.:	M-038723-01-4
Guideline(s):	US EPA OCSPP Guideline no. 850.3040
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-038723-01-4@S-604651-01-1
Sunflowers were seed-treated with Gaucho 70 WS 0.7 and 1.4 mg a.i./grain. The four colonies of bees were installed on the 1 ha fields at the time of flowering. Flower visits on the treated sunflowers were even slightly better and more intensive than on the untreated ones. Throughout the flowering period, which lasted for 11 to 13 days, it did not subside so that it can be concluded that nectar harvesting was consistent throughout. However, the foraging activity of the bees was not reflected in a weight gain by the colonies. The weight of the colonies decreased slightly at all three sites, most of all at the untreated site, so that any connection with the Gaucho seed treatment can be ruled out. The return of the bees to the hive was observed on the landing board. This did not decrease during the period of the trial. Weakening of the colony strength as a result of disorientation cannot be inferred. Without exception, the pollen-carrying bees came from the sunflower field. Any other source of nectar flow could be ruled out because of the orangey-red colour of the pollen.
>>M-038723-01-4@S-604651-01-1

Report:	02.01.03/07; [redacted]; 2015; M-038733-01-4
Title:	Bees VI: Flower visits after seed treatment
Report No.:	VAZ 34a/95
Document No.:	M-038733-01-4
Guideline(s):	US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-038733-01-4@S-604651-01-1
Two fields in the Sologne area of France, each of 1.5 ha, were sown with sunflowers on 22 May 1995, the seeds of one field having been treated with Gaucho 0.7 g a.i./grain. A nearby field, which had also been treated with Gaucho 49 g a.i./ha, was also included in the trial. At the start of flowering on 22 July 1995 (= 61 days after sowing), 6 bee colonies were installed in the middle of each field (only 4 on the regular field).

The sunflowers on all the fields were visited by the bees, with little difference between visits to the untreated and seed-treated sunflowers. There did not appear to be any Gaucho seed treatment-induced inhibition.

The activity of the bees counted on the landing board was even higher on the Gaucho field than on the control field.

The progress of flowering was better on the Gaucho field, i.e. quicker than on the control field, which is only possible following intensive, undisturbed flower visits.

The colonies on the Gaucho field increased their weight in 12 days to 119.4% of their initial weight, i.e. 9 kg increase per colony. In contrast, the weight of the colonies on the control field remained constant (-1%). The Gaucho seed treatment had no inhibitory effect on honey production in this trial.

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A mini-sample of nectar was obtained 64 days after sowing and tested with aphids in the laboratory. It contained no detectable levels of imidacloprid, as the aphids survived.

Analysis of the pollen which was collected provided no constructive results as no pollen could be obtained in the control. The bees in the Gaucho field obtained their pollen mainly from mustard and maize, but collected nectar from sunflowers.

The yield from the sunflower harvest was one and a half times as high in the Gaucho field as in the control field, which is only possible following perfect pollination by bees, together with good fertilization and watering of the crop.

Following this extensive field trial, it is inconceivable that treatment of the sunflower seeds with Gaucho can affect the bees and their honey production.

>>M-038733-01-4@S-604940-01-1

Report:	02.01.03/08; [redacted]; 1998; M-064758-01-3
Title:	Side effects of Confidor SL 200 on bees following one application to apple trees at the mouse-ear stage
Report No.:	ITA-98-901
Document No.:	M-064758-01-3
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-064758-01-3@S-602156-01-1

In 1998, a field trial on apple trees was performed to assess the risks to honey bees associated with use of Confidor 200 SL. Apple-growing represents a realistic worst-case scenario for such a risk assessment for the following reasons:

1. Apple trees blossom shortly after the point at which it is recommended that treatment takes place, i.e. the mouse-ear stage.
2. Apples are a crop that is usually pollinated by bees.
3. Bee colonies are in the early stages of development and are therefore more susceptible to interfering factors.
4. At the envisaged time of use, few other treatments are applied and so the risk of interference from other products is minimised.

>>M-064758-01-3@S-602156-01-1

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Report: 02.01.03/09; [redacted]; 2002; M-066846-01-3
Title: Field test: Side effects of oil-seed rape grown from seeds dressed with imidacloprid and beta-cyfluthrin FS 500 on the honey bee (Apis mellifera L.)
Report No.: 99398/01-BFEU
Document No.: M-066846-01-3
Guideline(s): US EPA OCSPP Guideline Number: 850.304
Guideline deviation(s): not specified
GLP/GEP: **yes**

<<M-066846-01-3@S-602206-01-1

Procedures
Materials and methods:
Fields with oil-seed rape *(Brassica napus,* variety Cirajet) dressed with 1051.17 g a.i. & 187.31 g a.i./100 kg seeds Imidacloprid & Beta- Cyfluthrin FS 500 (dressed seeds: article number 02 0094819 A, product used for dressing: development number 0195939, formulation number 0055, tox number 4867-00) and the fungicide Thiram were used as test substance treatment group. Plots with oil-seed rape dressed only with Thiram served as control.
The effect of the test substance was examined on bee colonies placed next to the fields at the begin of the full flowering stage of *Brassica napus L.* The study was carried out with one replicate (one field) per treatment group. Two groups of three hives were placed next to each field. One group served as test colonies, the other for the collection of nectar, pollen and honey. The bees were exposed to the flowering oilseed rape from the 27/04/2000 until the 12/05/2000 (BBCH 61-62, start of blooming until BBCH 69, end of flowering).
From the 28/04/2000 until the 11/05/2000 mortality and foraging activity of the bees were assessed once a day. The strength of the colonies and the development of the bee brood were assessed 4 times during the study. Additionally the weight from the bee hives of the first group was recorded continuously.
Samples of pollen, nectar and honey were collected during the study, for analysis of residues of the test substance and metabolites of the test substance.
The influence of the test substance Imidacloprid & Beta-Cyfluthrin FS 500 was evaluated by comparing the bees of the test field to the bees of the control field.

Dates of work: 23/08/1999 - 13/06/2000
Biological Findings:

Test substance	Imidacloprid & Beta-Cyfluthrin FS 500			
Test organism	*Apis mellifera*			
Exposure	Oil-seed rape			
Endpoints	Control field		Test substance field	
Dead bees in the bee traps and in front of bee hives	504		350	
Dead bees in the field	2		11	
Mean flight activity	2.3 bees/m²/min		3.3 bees/m²/min	
Colony strength described by the weight of the test colonies	hive 76	+ 31.7 kg (54.7 %)	hive 35	+ 24.3 kg (44.6 %)
	hive 90	+ 26.3 kg (49.0 %)	hive 124	+ 27.7 kg (52.5 %)
	hive 15	+ 24.8 kg (44.5 %)	hive 19	+ 24.4 kg (47.6 %)

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Analytical Findings:

Test substance	Imidacloprid & Beta-Cyfluthrin FS 500					
Test organism	*Apis mellifera*					
Exposure	Oil-seed rape					
Sample material	Control field			Test substance field		
Analysed for [mg/kg]	Hydroxy-Imida-cloprid	Olefin-Imida-cloprid	Imida-cloprid	Hydroxy-Imida-cloprid	Olefin-Imida-cloprid	Imida-cloprid
Nectar from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d. to < LOQ
Pollen from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Honey from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nectar from the blossoms	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for Imidacloprid and Hydroxy-Metabolite, 0.01 mg/kg for the Olefin-Metabolite
< 0.005 and < 0.010 = Residues below the limit of quantitation (< LOQ)
Limit of detection: 0.0015 mg/kg for Imidacloprid and Hydroxy-Metabolite, 0.003 mg/kg for the Olefin-Metabolite
n.d.: Residues below the limit of detection

Observations:

There were no adverse affects of the treatment on foraging activities of the bees, colony weight and development or mortality. No behavioural impacts (e.g. apathy, exaggerated motility, discoordinated movements) were observed on the honey bees collecting rape, nectar and pollen on the test substance field compared to the control. The development of bee brood was not affected by the test substance and was nearly similar in the hives exposed to the test substance field or to the control field. Likewise, in the analytical part of this study no residues of metabolites of the test substance were found in pollen, nectar or honey. In the nectar collected out of the combs residues of the test substance below the limit of quantitation (< 0.005 mg/kg) were found. In the other samples (pollen and honey from the combs and nectar from the blossoms) no residues of the test substance were found.

>>M-066846-01-3

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Report:	02.01.03/10; [redacted]; 2009; M-074400-01-4
Title:	The effects of sublethal doses of imidacloprid on the foraging behaviour and orientation ability of honeybees
Report No.:	M-074400-01-4
Document No.:	M-074400-01-4
Guideline(s):	US EPA OPPTS: N/A
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-074400-01-4@S-602208-01-1

This paper examines the possible effects of sublethal doses of the insecticide imidacloprid on the behaviour and orientation performance of foraging honeybees. Sucrose solutions containing imidacloprid was fed to bees, and changes in behaviour were found for imidacloprid concentrations of 20 ppb to 100 ppb after comparison with the control groups. No effect was observed at 10 ppb. In the sublethal concentration range indicated above, imidacloprid causes a reduction in the foraging activity of the treated bees and induces trembling dances by which the foraging bees discourage other worker bees from foraging, which in turn reduces the foraging activity of the bees in the nest. In addition, the effectiveness of the waggling dances used to attract bees to such food sources is reduced as the direction and the distance information as communicated by the waggling dance is less precise. Although these effects on the behaviour of the bees were observed to start at imidacloprid concentrations of 20 ppb, no damage to the test populations was observed for the range of concentrations tested up to 100 ppb.
Although this experiment did not examine whether the observed effects will affect the population development, such effects appear not very likely unless bee hives without any food stores are exposed to such food sources at concentrations where the foraging activity decreases. Should concentrations above 20 ppb occur in nectar, it has to be verified whether or not a decrease in honey yield is observed under practical conditions.

Imidacloprid is a chloronicotinyl insecticide which was developed by Bayer. Imidacloprid acts on various types of nicotinic acetylcholine receptors. It is used amongst others as a seed dressing agent to control pest species. Following reports from French beekeepers of 'disoriented' honey bees that had been foraging in treated sunflower fields, and preliminary trials carried out by Bayer which showed possible effects on foraging behaviour of bees fed with an 100 ppb imidacloprid sucrose solution, further specifically designed experiments were performed in summer 1998 to find out whether feeding of imidacloprid in the sublethal concentration range from 10 ppb to 100 ppb could affect the foraging behaviour and orientation ability of honey bees.

Comprehensive research has been undertaken on various aspects of the foraging behaviour of honeybees. In contrast to many other insect species that feed on flowering plants, foraging behaviour of honey bees is to a large extent regulated by social interactions with the dance communication system as the main element in regulating the collection of nectar and pollen (surveys in von Frisch 1965, Seeley 1995, Kirchner 1997). This means that potential effects observed on the foraging intensity for nectar or pollen observed at the population level may not solely be based on direct effects on the foraging behaviour of individual bees but may also be triggered by the social communication system. In other words, if a reduction in foraged food is recorded, this may possibly be due to the fact that the frequency and/or duration of round and waggling dances used to attract conspecifics in the hive to food sources are reduced.

Indications of such complex effect arised from observations in preliminary trials carried out by Bayer that trembling dances appeared to be more frequent at high concentrations of imidacloprid in sugar solution. The honey bee trembling dance, whose function was not understood for a long time (von Frisch 1965), regulates the balance between the amount of nectar brought in by foraging bees and the amount accepted and processed by worker bees inside the hive (Seeley 1992, Nieh 1993, Kirchner 1993, Kirchner and Lindauer 1994, Seeley et al. 1996). If so much nectar is brought in that the foraging bees have to wait for a long time in the hive before they can deliver the food, some of these foraging bees start to perform

trembling dances. These dances reduce the number of recruited foraging bees (foraging activity) due to a decreased frequency of waggling dances and increase the recruitment of hive bees which take the nectar from the foraging bees. Kirchner and Lindauer (1994) found that even when there was not an oversupply of food, trembling dances could be triggered in experiments by a wide range of conditions which caused longer waiting times for nectar delivery. Reports from Schneider (1949) and Schick (1953) state that feeding various toxic substances also triggered trembling dances. It therefore seemed that a detailed investigation of the effects of imidacloprid on dance behaviour and the frequency of trembling dances would be a sensible approach to explaining the fall in foraging activity observed at the population level if high concentrations of the compound are fed to the bees.

At the same time, a detailed investigation of bee dance behaviour will also allow us to characterise any impairment of orientation more accurately. When honeybees find a good source of food they learn its smell, colour and visual appearance, and also its position relative to the hive (von Frisch 1965, Seeley 1995). They do not only return on a direct route from the food source to the hive and find the food source directly when they next leave the hive, but they also communicate the direction and distance between the hive and the food source to their conspecifics in the hive via dancing. Any impairment of solar compass orientation, estimation of distance and route integration can therefore be quantified by assessing the direction and distance information coded in the bee dance (Kirchner and Braun 1994).

The purpose of our study was therefore to quantify the possible effects of imidacloprid on the behaviour and orientation ability of individual bees, and in particular the behaviour of individually marked bees returning to the hive from a food source. The concentrations of the active ingredient examined were limited to a range from 10 ppb to 100 ppb.

Materials and methods

The experiments were performed on two honeybee populations of the strain *Apis mellifera carnica.* Each population contained about 5,000 bees. The test hives were placed in twin-comb observation hives as described by von Frisch (1965). One of the populations had access to a flight room at the beginning of the experiment. Later on in the experiments both colonies were given the opportunity to forage out-doors. All bees returning to the hive were directed to one side of the comb so that all individually marked foraging bees could be observed.

The tests in the flight room were performed between April and June and the out-door tests from June to the end of August. In the flight room, groups of individually marked foraging bees were fed one metre from the hive with a solution that contained either 2M sucrose solution or 2 M sucrose solution mixed with 100 ppb (w/v) of imidacloprid (calculated on the basis of the 70% concentration of Gaucho WS 70 used in this series of tests) or with 0.5 M table salt (as an additional control). Records were made of the frequency of trembling dances according to the method described in Kirchner and Lindauer 1994, the search time until a foraging bee met a hive bee which accepted the harvested food, and the number of trophallactic contacts.

These experiments were continued out-door with the same population and a food source 10 metres from the hive. In this set of experiments imidacloprid was used at concentrations ranging from 10 ppb to 100 ppb derived from Confidor (containing 98.3% imidacloprid). The observations also covered the frequency of waggling dances (for the traditional distinction between round dances and waggling dances at close distances see Kirchner et al. 1988).

The second colony was used to investigate the precision in the communication of direction and distance as given in the waggling dances. The food source was located 500 metres away from the hive. The tests were performed using imidacloprid concentrations ranging from 10 ppb to 100 ppb derived from imidacloprid (98.3% a.i. content). The dances of the returning foraging bees were recorded in the dark (room lit only with a red darkroom light that is invisible to bees) on an infrared-sensitive video camera. Subsequent evaluation of the dances allowed us to determine the direction information communicated

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with each waggling dance to the nearest 1°, and the speed of waggling movements (which codes the distance of the food source) to the nearest 20ms. A calibration method derived from previous tests was used to calculate the distance indicated by the speed of the waggling movements.

For detecting any persistent effects, control runs were conducted before and after each test ran and temporal trends were analyzed. However, the relative low longevity of fouragier bees restrict the possibility to monitor chronic effects. In the field, the average longevity of fouragier bees is about 8 to 10 days. Fouragier bees which were marked on the food source will, therefore, live on average only further 4 - 5 days. In the experiments examining the frequency of trembling dances individual bees were tested for up to 10 days. In the experiments examining the precision of communication, the imidacloprid-containing sucrose solutions were fed typically over three subsequent days. The days before and after the feeding period were used to perform the control runs.

The preparation of the test solution was done according to the following procedure: 100 mg a.i. (i.e. either 142.8 mg Gaucho (WS 70 uncoloured, NTN 33893 70 WS) or 101.7 mg imidacloprid tech. (98.3%) was pre-solved in 1 L A. dest. and stirred for 4 hrs (results in 100 ppm). 10 ml of this solution was then diluted with 490 ml (2 ppm). A lot of either 2.5 ml, 5 ml, 12.5 ml or 25 ml of this dilution was then filled up to 500 ml into a 2 molar sucrose solution (resulting in 10, 20, 50 and 100 ppb (w/v) imidacloprid in 2 M sucrose solution). The ready-to-use 2 M sucrose solutions were stored in a cooler at 4°C and used for a maximum of 1 week.

Circular statistical methods were used in the statistical evaluation of direction informations coded in the bee dances (Batschelet 1981).
>>M-074400-01-4@S-602208-01-1

Report:	02.01.03/11; [redacted]; 2001; M-084721-01-3
Title:	The impact of Gaucho and TI-435 seed-treated Canola on honey bees, Apis mellifera L
Report No.:	110403
Document No.:	M-084721-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-084721-01-3@S-602840-01-1
GAUCHO® (Bayer Corp.) is a seed treatment containing the 'chloronicotinyl insecticide known as imidacloprid. Imidacloprid is first compound in the chloronicotinyl family to act on an insect's nicotinic acetylcholine receptors (Leicht, 1993). Since its initial registration in France 1991, imidacloprid has become widely used receiving acknowledgment for its biological activity on a broad range of homopteran insect pest including aphids, leafhoppers, planthoppers, thrips and whiteflies (Elbert et al,, 1991; [redacted], 1999). In addition, this compound has been found to be active against some species in the orders Coleoptera, Diptera and Lepidoptera (Elbert et al, 1991). Today, imidacloprid is registered for use in many countries, having considerable agricultural importance as a broad spectrum multi formulation insecticide that can be used on a wide variety of crops.

lmidacloprid is highly water-soluble with considerable molecule mobility in the xylem of treated plants (Elbert et al, 1998). These systemic characteristics make imidacloprid particularly suited for seed treatment and soil application. Imidacloprid´s systemicity is enhanced by its residual activity, which in seed treatments, has been established.at up to 60days after planting of the seed (Tröltzsch, 1995; [redacted], 1999).Therefor, imidacloprid as a seed treatment can be used with confidence on crops, such as canola, that bloom >60days after planting and are pollinated by insects such as the honey bee (*Apis mellifera* L) Further more, the systemic nature and residual activity of imidacloprid make it a valuable tool in integrated pest management programs for many agricultural insect pests.

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Despite worldwide recognition of imidacloprid as an efficacious agricultural chemical, claims were made by French beekeepers 1997 that GAUCHO® treated sunflowers were responsible for the decline and subsequent failure of French honey bee colonies. French beekeepers maintain that honey bees from hives placed in GAUCHO® treated sunflower fields display high rates of mortality, disorientation and low honey production all leading to a severe decrease in colony strength and in some instances colony death. Recent studies European studies examined the effects of GAUCHO® seed treated sunflowers and contaminated sugar syrup on honey bees, and found no evidence to support the claims made by French beekeepers (Schmidt and [redacted], 2000)

With the registration of GAUCHO® in Canada 1998 and the U.S. in 1997 it was important to determine whether honey bee colonies used to pollinate the massive expanses of canola grown in these two countries would be negatively impacted by this new seed treatment. The objectives of this study were to: 1) determine whether GAUCHO® and TI 435, a new unregistered second generation chloronicotinyl, seed Treated canola grown in Ontario, Canada and Minnesota, USA had any effect on the honey producing ability, and foraging and hive behavior of honey bees; and 2) determine whether pollen and nectar collected by honey bees from seed treated canola blossoms contained residues of imidicloprid plus two metabolites, olefin-imidicloprid and hydroxy-imidicloprid, or TI 435 above the "no observable adverse effect concentration" (NOAEC) of 20ppb (0.02ppm) (Schmidt and [redacted], 2000).

>>M-084721-01-3@S-602840-01-1

Report:	02.01.03/12; [redacted]; 2001; M-084752-01-3
Title:	Evaluation of effects on the foraging activity of bee population in the sunflower field of Western France - Is Gaucho seed dressing (active ingredient: imidacloprid) responsible for the effects?
Report No.:	110635
Document No.:	M-084752-01-3
Guideline(s):	US EPA OCSPP Guideline no. 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-084752-01-3@S-604658-01-1

Since 1996, beekeepers in the West of France have been observing massive depopulation of their apiaries during the sunflower honey harvest, accompanied by characteristic symptoms. The beekeepers are accusing a crop protection product of causing these problems: Gaucho, an insecticide used to treat sunflower seed and marketed by Bayer.

However, while none of the results of field studies provide any evidence that Gaucho affects bees, there are other plausible explanations for these symptoms, particularly diseases such as viral diseases promoted by infestations with Varroa, a parasite which is spreading following the development of resistance to varroacides (acaricides) in these regions since 1996, or infections with spiroplasms, which caused such problems in the South-West of France fifteen years ago.

These other possibilities were ignored in 1998, when a vast research programme was put in place to examine the relationship between Gaucho and these phenomena.

The results of the 1998 trials, evaluated in accordance with the methods accepted and practiced in bee ecotoxicology, do not indicate that Gaucho, used on sunflower seeds, presents a risk to bees.

>>M-084752-01-1@S-604658-01-1

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Report:	02.01.03/13; [redacted]; 2001; M-088167-01-2
Title:	Assessment of side effectsc of Confidor SL 200 on the honey bee (Apis mellifera L.) in apple orchard following application before flowering (mouse-ear stage) of the crop
Report No.:	20011099/01-BFEU
Document No.:	M-088167-01-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-088167-01-2@S-602848-01-1

Materials and Methods

Test substance: Name: Confidor SL 200;
purity: 194 g/L (nominal: 200 g/L)

The following study was designed to determine the effects of Confidor SL 200 on the honey bee (Apis mellifera L.) under field conditions in an apple orchard. The study was carried out in Germany near Karlsruhe at the test location Augustenberg. The test substance Confidor SL 200 was tested at an application rate of 0.105 kg a.s./ha in 500 L water/ha (the amount of water was adapted to the height and the growth stage of the trees according to Good Agricultural Practice). The application was performed at the mouse-ear stage of the apple trees (BBCH-code 10) on 30MAR2001. An untreated orchard of apple trees from the same variety served as control. At the start of full flowering (29APR2001) four colonies were placed between the rows of each test field.

Mortality, foraging activity, behaviour, and condition of the colonies, the development of the bee brood and the weight changes of the colonies were assessed over a period of 7 days.

The influence of the test substance Confidor SL 200 was evaluated by comparing the bees in the pesticide-treated field to those in the control field regarding the following observations:

- Mortality in the bee traps
- Flight intensity in the crop (number of flying bees/tree/minute)
- Flight intensity in front of the hives (number of bees leaving/ entering the hive/minute)
- Behaviour of the bees on the crop and around the hive
- Development of the bee brood

Weight changes of the colonies

Dates of work: 30MAR2001 - 12JUN2001

Findings

Effect on honey bee mortality:

In the Confidor SL 200 treated group as well as in the control group the mean mortality increased from ED 2 until the end of the observation period (ED 7). The mean mortality rose up to a mean maximum of 25.3 dead bees per colony/day in the Confidor SL 200 treated group compared to a mean maximum of 43.5 dead bees/colony/day in the control group, both observed on ED 7. On every assessment day the mean values of mortality observed in the test substance treatment group were lower than in the control group.

Effects on honey bee flight intensity:

During the entire exposure period the mean flight intensity in the test substance treated group was similar or on a higher level compared to the control. By comparing the overall mean of flight intensity a value of 5.0 bees/tree/minute was found in the test substance treated group compared to 3.6 bees visiting the flowers in the control group.

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The mean flight intensity observed in front of the hives increased during the first three assessment days (ED 1 to 3) in both treatment groups and remained on a high level from ED 3 to 5. On day 6 and 7 after start of exposure the mean flight intensity observed at the front of the hives was on a lower level compared to the previous days. Only a slight difference between the test substance treated group and the control group occurred concerning the mean flight intensity in front of the hives over the entire test period (33.62 bees leaving/entering the hive per minute in the treated group and 37.92 bees leaving/entering the hive per minute in the control group).

Effects on honey bee brood development:

In the bee brood development no abnormal difference which could be attributed to the influence of the test substance were observed between the test substance and control treatment.

Behaviour of the Bees:

No abnormal difference in behaviour of the bees was observed between the test substance treatments and the control treatments at any time during the period of assessment.

Weight of the colonies

No remarkable observations were made regarding the weight changes of the Confidor SL 200 treated hives compared to the control hives.

Conclusion:

The treatment of apple trees at the mouse-ear stage with Confidor SL 200 at the test rate of 0.105 kg a.s./ha in 500 L water/ha did not cause adverse effects to honey bee mortality, flight intensity in the crop or the brood development of the colonies in this field study.

>>M-088167-01-2@S-602848-01-1

Report:	02.01/03/14; [redacted]; 1990; M-090324-01-4
Title:	Bee IV: Soil application of NTN 33893 at sowing time
Report No.:	VAZ 10/90
Document No.:	M-090324-01-4
Guideline(s):	US EPA OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-090324-01-4@S-604660-01-1

In the case of such a perfect and long-acting soil systemic aphicide as NTN 33893, the question arises as to whether the active ingredient appears in the flowers and whether it affects bees. The question is especially controversial as according to BBA [Federal Biological Institute] test guidelines, even a double dose must be tolerated by bees.

Three dicotyledonous crops were sown in spring either as treated seed or as granules. When they started to flower, a tunnel was erected over them and small colonies of bees installed.

Flowering and start of the trial in days after sowing:

Field beans	83 = 12 weeks
Summer rape	99 = 14 weeks
Sunflowers	80 = 11.5 weeks

>>M-090324-01-4@S-604660-01-1

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Report:	02.01.03/15; [redacted]; 2000; M-090720-01-2
Title:	Field evaluation in Argentina of possible risk for honey bees from the product Gaucho on sunflowes
Report No.:	LPE-41/00
Document No.:	M-090720-01-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-090720-01-2@S-602221-01-1

The possible risk that the product Gaucho may present to honey bees is assessed in this paper through a field test. The evolution of hives that were exposed to flowering sunflower from seeds treated with Gaucho was qualitatively and quantitatively evaluated. Variables sensitive to factors that have an impact on bees such as: weight of hive, honey yield, nectar, pollen and brood were recorded, as well as field activity, incoming pollen in hives and mortality.

In order to validate this paper and to extrapolate it to other tests that have been done in various European countries, LPE, MACN, and CONICET drafted a test protocol based on the guidelines of BBA (1980) and OEPP/EPPO (1992), that was approved by the *"Working group for the reevaluation of Imidacloprid for possible negative effects on bees"* (SENASA) at the 01/10/2000 meeting. As required by the *Good Laboratory Practices* (GLP), *Standardized Operation Procedures* (SOP) were added for each of the actions related to the test; as well as the *Amendments*, aimed at including the necessary corrections in order to obtain, at the end of the testing, a validated protocol; and the *Deviations,* which permitted to overcome specific features related to the imponderables of this particular test.

Since this study is multidisciplinary, LPE - MACN - CONICET, as scientific coordinator of the study, invited several members of university academic sector in Argentina as well as institutes and researchers of the Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), to proceed to analytical chemical, statistical, palinologic, and other tests, whose reports support the conclusions in this paper. SENASA -directly or through the appointment of an auditor (1NTA)-, BAYER S.A. -manufacturer of the product Gaucho, and LPE - MACN - CONICET, in charge of the scientific coordination, have all been involved in the field work and samplings, from sowing of sites to the last evaluation of the beehives.

The test formally started with the treatment of sunflower seeds with the product Gaucho, according to label recommendation, and with the installation of 32 beehives, 16 of which were randomly selected for the test. From that moment on, a permanent follow up of the sunflower crop and the plant health treatments was made, as well as the follow up of wild flora in adjoining sites. Sunflower test sites were culturally managed based on good agricultural practices in Argentina. Special attention was paid to the assessment of the phonological condition of sunflower in order to adapt the hive exposure to the terms of the usual pollination practices. Basic meteorological data were recorded while hives remained on the sunflower sites. According to apicultural recommendations a program of hive sanitarian treatments was developed with different products to prevent varroa and nosema diseases.

The samples during the test were taken in triplicate and immediately distributed to SENASA, BAYER S.A. and LPE - MACN - CONICET, the latter being used specifically for the test and the rest being kept as counter-samples. Samples were taken of seeds, soil, sunflower inflorescences, wax, honey and pollen to determine Imidacloprid residues; on the other hand, samples of honey and pollen were taken for palinologic tests.

The complete study includes: original protocol, amendments, deviations, study protocol, scope, materials and methods, results and discussion, conclusions and annexes. In order to obtain a picture of the time structure of the study, activities developed at each of the evaluation times are summarized in Table 9 (p. 17). For the same reason, the results of the analytical tests were summarized in Table 11 (p. 48), and those concerning population in Table 10 (p. 47), where differences between hives on the treated site and those on the control site are highlighted for each of the variables tested.

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From the results of this study, it may be concluded that:

Concerning plant density and the phenologic condition of sunflower in test sites, at transfer of hives to sunflower sites (at time T2) plant density of the site treated with Gaucho was higher than that of the control site, a fact that probably has to do with seed treatment with the tested product. The number of flowering plants was similar in both sites. However, towards the middle of flowering and throughout the flowering period a higher proportion of plants without pollen was observed in the control site as compared with the treated site.

As for bees activity and mortality in test sites between dates T2 and T 3, field activity was significantly higher in the treated site as compared with the control. No significant differences were observed on bees with pollen entering hives from both sites. Mortality measured in front of hives of both test sites was not statistically different.

In pollen counts made on honey samples taken in T3 a high percentage of sunflower pollen (> 20%) is observed as it can be expected for a test under field conditions and in conformity with literature information *(Maurizio & Louveaux, 1963; Ricciardelli d'Albore, 1997)*. Furthermore, honeys were identified *in situ* according to their origin as "sunflower honey" in accordance to the organoleptic properties of the samples obtained.

On the other hand, when exposure to sunflower (date T2) began, composition and structure of the population in the hives were uniform, weights and frame area percentages filled with honey, nectar, pollen and brood, did not show significant differences. At the end of the exposure period of hives to sunflower (date T3), increases in hives of the control site and hives of the treated site were observed for the following parameters: average weight of hives, amount of honey and nectar in top supers and amount of pollen and brood in bottom supers. However, increases of these parameters were significantly higher for hives in the treated site.

At date T4, 24 days after removing the hives from sunflower, the amount of pollen, nectar and honey stocks in hives that were exposed to the treated site was significantly higher as compared with those of the control site.

In samples of sunflower seeds treated with Gaucho that were obtained before sowing, an average content of 0.2458 mg Imidacloprid/seed was determined. That is in agreement with the treatment of seed that was applied. As for the Imidacloprid residue tests, no quantifiable Imidacloprid residues were found in samples of soil and sunflower heads at date T2. No quantifiable Imidacloprid residues were found in samples of either pollen, honey or wax at dates T3 and T4.

It can be considered that, during the stay of hives in sunflower sites, hives of the site treated with Gaucho developed more rapidly than those in the control site. However, 24 days after their removal from sunflower, both hive groups (control and treated), reached a similar level of population development, even if honey and pollen production was higher for the hives that were in the treated site. Differences in hive development of both sites may be related to differences observed in field activity and with the different proportion of plants with available pollen that were present in both sunflower sites during flowering.

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Report:	02.01.03/16; [redacted]; 1998; M-105190-01-4
Title:	Feeding test with bees in field conditions
Report No.:	MO-03-010457
Document No.:	M-105190-01-4
Guideline(s):	US EPA OCSPP Guideline # 850.SUPP
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-105190-01-4@S-604662-01-1

FEEDING TEST WITH BEES IN FIELD CONDITIONS

This test consists in comparing the behaviour of two beehives, one was fed with sugar syrup, the other with the same syrup containing 20 ppb of imidacloprid. The trial was conducted from June 23rd, to July 8th, 1998. Two hives were placed in two sites, about 5 km apart. The bees were used to feeding in a feeder containing sugared water and at a 150 m distance of the hive. After contamination of the sugar syrup in one of the two sites, the feeder attendance was noted, as well as the quantity of consumed syrup and the return activity to the beehive.

>>M-105190-01-4@S-604662-01-1

Report:	02.01.03/17; [redacted]; 1999; M-110240-02-3
Title:	Effects of crop protection products on bees, effects of Gaucho seed dressing on losses of foraging bees with comments on the summary report from Gaelle Curé and Bernard Amboiet, 16.11.1998
Report No.:	M-110240-02-3
Document No.:	M-110240-02-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-110240-02-3@S-604948-01-1

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

>>M-110240-02-3@S-604948-01-1

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Report:	02.01.03/18; [redacted]; 2003; M-116169-01-2
Title:	Assessment of side effects of imidacloprid & deltamethrin OD 85 on the honey bee (Apis mellifera L.) in the field following application after bee-flight
Report No.:	20031216/01-BFEU
Document No.:	M-116169-01-2
Guideline(s):	OEPP/EPPO Guideline No. 170 (3) and BBA Guideline VI, 23-1
Guideline deviation(s):	Yes, but acceptable
GLP/GEP:	**yes**

<<M-116169-01-2@S-602224-01-1

Material and methods

Test substance: Name: Imidacloprid & Deltamethrin OD 85; Development No.: 30-00317155; Batch: 08137/0023(0019); Tox-No.: TOX06314-00; purity: NTN 33893 (imidacloprid): 73.95 g/L (75 g/L nominal), AE F032640 (deltamethrin): 10.16 g/L (10 g/L nominal).

The effects of Imidacloprid & Deltamethrin OD 85 were tested on the honey bee (*Apis mellifera* L.) under field conditions following the guideline of the European and Mediterranean Plant Protection Organisation No. 170(3) (OEPP/EPPO, 2001) and partly based on the guideline for the testing of plant protection products for registration of the Federal Biological Research Centre for Agriculture and Forestry, Federal Republic of Germany (BBA), part VI, 23-1 (STUTE *etal.* 1991).

The study comprised one trial which was carried out in Germany, near Hechingen. The test substance Imidacloprid & Deltamethrin OD 85 was tested at an application rate of 1 L product/ha in 400 L water/ha. The application was carried out in the evening after daily flight activity of the bees and before full flowering (before BBCH stage 65) of the oil-seed spring rape (*Brassica napus*) field. A field of untreated oilseed spring rape was used as control treatment. According to the OEPP/EPPO guideline No. 170 (3) the use of a toxic standard for field studies is optional as long as the exposition of the bees is proved by monitoring foraging activity. In this study no toxic standard was used and the option of documentation of the exposition of the bees by proving the foraging activity was chosen. Four Commercial bee colonies were placed near each test field 2 days before the application. To insure that the bees are exposed to the test field detailed assessments of foraging activity were done before as well as after the application. Mortality and foraging activity of the bees was checked prior to (1 day) and after application (10 days). The conditions of the colonies and the bee brood were assessed 2 days before and 10 days (control) respective 11 days (test substance) and 4 weeks after the application.

The influence of the test substance on the honey bees was evaluated by comparing the results of the test substance treatment to those of the control treatment. The following points were assessed:

- Mortality in front of the hives and in the crop
- Foraging activity (number of foraging bees/m² flowering oil-seed spring rape crop)
- Condition of the colonies and development of the bee brood
- Behaviour of the bees in front of the hives and in the crop

Dates of work: 13JUN2003 to 10JUL2003

Findings: Toxicity to Honey Bees, field test

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Test substance		Imidacloprid & Deltamethrin OD 85	
Test object		*Apis mellifera*	
Exposure		Spray treatment in the evening after foraging activity of the bees in flowering oil-seed spring rape	
Treatment group		Test substance (Imidacloprid & Deltamethrin OD 85)	Control (untreated)
Application rate [in 400 L water/ha]		1 L (0.986 kg product)	-
Average Mortality rate [dead bees/ hive/day]	pre:	10.2	9.3
	post [1]:	43.8	5.0
	post [1-10]:	22.5	6.7
	$Q_{M(average)}$:	2.2	0.7
Average Flight intensity [foraging bees/ m²/day]	pre:	8.6	10.5
	post [1]:	1.1	8.2
	post [1-10]:	1.9	4.7

pre = average values for day T_1 and T_{2a}
post [1] = day after application (T_{1a});
post [1-10] = average value for day T_1 – T_{10} after application;
$Q_{M(average)}$ = Average mortality per day before application divided by average mortality per day after application.

Observations

Effect on honey bee mortality:

The application of the test substance in the evening after daily bee flight activity of the bees caused a significant increase in the average honey bee mortality on the first two assessment days after application (T_1 43. 8 dead bees/hive, T_2: 100.5 dead bees/hive). In the control treatment the average mortality was on a low level during the entire post-application period. The average daily post-application mortality was 22.5 dead bees/hive in the test substance treatment compared to 6.7 dead bees/hive in the control treatment.

Effects on honey bee flight intensity:

After the application the flight intensity in the test substance treatment was distinctly decreased from T_1 to T_4 compared to the values recorded in the control treatment (test substance: 0.7 - 5.4 bees/m²/day, control: 8.2 - 11.0 bees/m²/day). From T_5 to T_{10} the number of forager bees was on a low level in both treatments due to a decreased attractiveness of the rape fields after full flowering up to end of flowering of the crop.

The average daily post-application level of flight intensity was 1.9 bees/m² in the test substance treatment and 4.7 bees/m² in the control treatment.

Effects on honey bee brood development:

Regarding the colony strength and the bee brood development no differences attributable to the influence of the test substance were observed between the test substance colonies and the control colonies.

Conclusions

It was concluded that the application of Imidacloprid & Deltamethrin OD 85 on a bee-attractive flowering crop such as oil-seed spring rape resulted in a significant increase of honey bee mortality which was noticed over two days after application. Additionally the application of Imidacloprid & Deltamethrin OD 85 resulted in a distinctly reduced flight intensity which was observed during the first four days after application. No impact of the application of Imidacloprid & Deltamethrin OD 85 was noticed regarding the condition of the honey bee colonies and the brood development during the observation period.

>>M-116169-01-2@S-602224-01-1

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Report:	02.01.03/19; [redacted]; 2005; M-428629-01-3
Title:	Monitoring of depopulation and mortality events of bees in beehives with different agricultural destinatuions in the region Emilia Romagna - Final report 2005
Report No.:	M-428629-01-3
Document No.:	M-428629-01-3
Guideline(s):	not specified
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-428629-01-3@S-605921-01-1

Taking into account the bees mortality observed in the last years in several EU Countries including Italy, the reason of which should hypothetically be the use of different agricultural practices, it has been considered suitable to check in open field the mechanism of this phenomenon and particularly its possible causes. The attention has been focused on different possible factors related both to agricultural and environmental practices, weather conditions, bee-practices etc. It has been considered as an important factor the synergy coming from different combinations. According to the indications of bee-losses from the bee-keepers, it has been supposed a possible relationship between bee-hives depopulation and corn sowing. Therefore a field protocol has been processed to be used in a corn-area where corn was considered the most important crop; as control another area without corn fields has been selected. Furthermore, in this study a third area has been included, with mixed crops and without a preponderance of maize.

>>M-428629-01-3@S-605921-01-1

Report:	02.01.03/20; [redacted]; 2005; M-428632-01-3
Title:	Monitoring about possible events of decline of bee populations and mortality in different-cultivated areas in the Region Veneto - Report 2005
Report No.:	M-428632-01-3
Document No.:	M-428632-01-3
Guideline(s):	not specified
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-428632-01-3@S-605933-01-1

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In particular according to the indications of bee-losses from the bee-keepers, it has been supposed a possible relationship between bee-hives depopulation and corn sowing, taking into account that these two events occur at the same time. Therefore a field protocol has been processed to be used in a corn-area where corn was considered the most important crop; as control an area without corn has been selected. Furthermore, in this study a third area has been included, with mixed crops and without a preponderance of maize.

>>M-428632-01-3@S-605933-01-1

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Report:	02.01.03/21; [redacted]; 2006; M-428630-01-3
Title:	Monitoring of depopulation and mortality events of bees in beehives with different agricultural destinations in the region Emilia Romagna - Final report 2006
Report No.:	M-428630-01-3
Document No.:	M-428630-01-3
Guideline(s):	not specified
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-428630-01-3@S-605927-01-1

Bees and plant protection products

The insecticide applications, particularly frequent in the last decades, can provoke out-and-out hecatomb of bees and wild pollinators. The use of poorly selective active ingredients together with a long lasting toxic activity and the lack of expertise showed by farmers in different occasions, are some of the causes of the bee-intoxication that every year occurs in our cultivated fields. There are numerous experimental trials carried out in laboratory and field, to investigate the activity of PPPs towards bees, but the market introduction of new modern molecules requires continuously checking activities about their possible side effects.

Chloro-derivates, phosphorganic, carbammates and pyrethroids
Generally phosphorganic and carbammate products show a strong knock-down activity while chlor-derivates have a more diluted activity towards insects. Many PPPs, in addition to the fact of killing foraging bees, show also a very negative effect towards bee-broods and "home bees" (i.e. those that remain in the beehive before becoming foraging bees themselves) that normally are contaminated by residues of products introduced in the beehive.

The effects of some PPPs can be different and may depend, for example, on the age of bees. Young bees are more sensitive towards carbaryl, while adult bees are more sensitive towards malathion and methyl-parathion (Johansen, 1979). Following the exposure to phosphorganics and pyretroids the bees seem to be more aggressive and regurgitate the content of honey bag, while coming into contact with carbaryl causes a slow luck of mobility and a numb behaviour, but they can die also after 3 days (Johansen, 1984). Parathion in sub-lethal doses could instead negatively influence the communication through the bee dance. In particular foraging bees communicate a different angle from that indicated by non contaminated bees even if kept under the same conditions (Schricker et Stephan, 1970; Schricker 1974).

Systemic products
It has been demonstrated that many systemic products (dimethoate, acephate, methomyl, methamidophos, monocrotophos, etc.) applied in pre-flowering can later contaminate the nectar provoking, depending on the imported quantity, serious damages to beehive and particularly to the brood, and, in some cases, even the death of the whole family (Fiedler, 1987).

Microincapsulated
Microincapsulated methylparathion is one of the product provoking devastating effects on bees because the microcapsule containing the product has similar dimension (from 30 to 50 µm) as pollen collected by foraging bees and then transported into beehive (Selkirk, 1976; Burgett e Fisher, 1977; Atkins e Kellum, 1984). This product constituted by microcapsules with a dimension of some tens of µm, has been studied to slowly release the inside active ingredient in the environment - when the water film wrapping the capsules dries itself, in order to reduce the number of PPPs applications; but even if the idea is good, the field application is not good in the same extent. In fact the microcapsules brought inside the beehive prolong the toxicity and therefore the brood and home bees mortality for a very long time: till to 19 months (Barker *el al.* 1979). In last years many other products realized with a new generation of microcapsules (reduced size and manufactured by using new materials) appeared on the market. The trials on effects on bees of different microincapsulated formulations give contradictory results. Some trials

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indicate that there are no differences between the different microincapsulated products and between these products and the traditional formulations, while other reports show the contrary. Anyway it has been demonstrated that the capsule dimension, the microincapsulation and the used material play a fundamental role for the safeguard of our precious pollinator. In any case considering also the recent serious bee deaths, in which microincapsulated products were concerned, it has to be underlined the necessity to apply products far from flowering and to cut the spontaneous flowering weeds eventually present.

Growth regulators
Fenoxycarb, a growth regulator which became famous some years ago due to very bad and insidious effects on useful entomofauna (particularly on silkworm), has been recognised as dangerous for bees too. This product provokes alterations to the metamorphosis processes in the young stages of bee and malformations on adult working bees (de Ruijter e van der Steen, 1987). The observed anomalies are different: eyes without pigments or with a typical half lunar streak, short and small thorax more or less pigmented, wings wrapped up in the pupal exuvia, deformed and not suitable to fly, teguments with uncompleted skeleton and abdomen differently pigmented (Gerig, 1991; Marletto *et al*., 1992; Nitsch, 1992). Colonies treated with fenoxycarb (Insegar®) showed a rapid decline during the season and a reduction of surviving of queens in next spring, confirming therefore that the molecule has an activity on colonies both on short and long terms. On the contrary, Diflubenzuron (Dimilin®) demonstrated a negative effect on the strength of colony (number of adults and larvae) in the short time, but a minor impact on long terms and no effect on survivals of queens (Thompson e Wilkins, 2002).

Neonicotinoids (imidacloprid)
Among the active ingredients recently introduced in the market, imidacloprid (Gaucho®, Confidor®, etc.), a systemic insecticide used for seed dressing of different crops and to control sucking pests, has provoked stark stress between beekeepers and the the producer company. Imidacloprid is a molecule with a very high toxicity against bees, the residues of which can not be easily detected in dead bees. Some researches carried out in laboratory and field have pointed out that in case bees come into contact with this molecule at sublethal rates, they can be disoriented and have difficulties in coming back to the beehive.

Bees which received the molecule showed a clear reduced activity with regard to mobility in comparison to the untreated ones. The negative effect was noticed only for some time after the treatment (30-60 minutes) and disappeared after some hours. Imidacloprid therefore acts as an inhibitor on insects even if only for a limited time. The time during which the insects behaviour is alterated could be fatal to foraging bees (Medrzycki *et al*., 2003; Bortolotti *et al*., 2003). Similar active ingredients which are nowadays on the market and for which similar effects are expected, are fipronil, thiamethoxan, clothianidin.

Synergic effects
Another shifty bee intoxication mechanism is the synergic effect of two or more active ingredients which, if used separately, are not lethal or in any case less toxic. This is for example the case of deltamethrin, a pyrethroid insecticide, and the nitrogen-organic fungicide prochloraz, which show a higher toxicity if used in mixture than if used in sequence or straight (Belzunces *et al*., 1993). This phenomenon seems to be related to the inhibition of microsomial monoossigenase activity, and particularly to citocrome *P*-450III, that enters in the metabolism of the pyretroid detoxification (Pilling, 1993); but this theory has been put under discussion in the last years through precise trials carried out by using models which simulate the deltamethrin's pharmacokinetic in presence on not of prochloraz (Chalvet-Monfray *et al*., 1996).
Treatments against varroa (*Varroa destructor* Anderson e Trueman) can make bees more sensible to some pesticides with more evident effects in comparison to untreated beehives. This is what has been pointed out by Dustmann and Lienau (1993) with a preliminary study, checking the synergic activity of coumaphos towards some phosphorganics such as dimethoate and phosalone. The cause should be the inhibition of some enzymes.

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Influence of the Environment
Also temperature has a high influence on the toxicity and danger to bees of an active ingredient. Treatments carried out during the hottest hours are generally more dangerous than those after sunset or during the night. Nevertheless mevinphos increases its toxicity at low nightly temperatures, so that it is recommended to apply it in summer and not in spring (Benedek, 1975); it is the same for fluvalinate that is 4 times more toxic at 20°C in comparison to 32°C (Niijima *et al*., 1985). On the contrary Malathion is often dangerous for bees in the hot climatic conditions of California, but not in the fresh climatic conditions of Washington State (Johansen, 1979). Treatments should not be done if a sensible decrease of temperature is expected because, in addition to a slower product degradation, the following dew building makes the active ingredient sprayed the day before available for a larger number of bees (Johansen, 1979).
>>M-428630-01-3@S-605927-01-1

Report:	02.01.03/22; [redacted]; 2006; M-428631-01-3
Title:	Monitoring of depopulation and mortality events of bees in beehives with different agricultural destinations in the region Veneto - Report 2006
Report No.:	M-428631-01-3
Document No.:	M-428631-01-3
Guideline(s):	not specified
Guideline deviation(s):	--
GLP/GEP:	**no**

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Microincapsulated

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>>M-428631-01-3@S-605930-01-1

Report:	02.01.03/23; [redacted]; 2012; M-429243-01-3
Title:	Summary of key findings and conclusions of investigations to evaluate bee exposure levels at Southern California citrus groves previously treated with imidacloprid
Report No.:	EBNTL056-7
Document No.:	M-429243-01-3
Guideline(s):	US OCSPP Guideline 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-429243-01-3@S-605233-01-1
A series of field investigations were conducted in 2010 and 2011 to determine exposure levels of honey bees foraging on spring flowers of citrus trees previously treated with imidacloprid. Annual reports previously finalized that contain the detailed findings of each year's investigations are attached (Appendices A and B). The purpose of this document is to provide an overall summary of the key findings and conclusions.
>>M-429243-01-3@S-605233-01-1

Report:	02.01.03/24; [redacted]; 2013; M-463556-01-2
Title:	Bee monitoring task force: Survey study on pollination practices and their impact on bee health in the Flemish region - Study 2012 -2013
Report No.:	M-463556-01-2
Document No.:	M-463556-01-2
Guideline(s):	US EPA OCSPP 870.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-463556-01-2@S-603071-01-1
The purpose of this study was to evaluate if crop protection agents (neonicotinoids) used for insect control in fruit growing do have an impact on the colony development/health of honeybees that are used to pollinate fruit crops. Therefore we examined if there is a difference in honeybee decline/winter mortality between bees that are used for pollination or come into contact with commercial fruit plantations on the one hand, and bees that never forage on commercial fruit plantations at the other hand, by conducting a large-scale survey amongst Flemish beekeepers.
>>M-463556-01-2@S-603071-01-1

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Report:	02.01.03/25; [redacted]; 2012; M-442872-01-2
Title:	Assessment of exposure of honey bees (Apis mellifera) to imidacloprid in controlled feeding study, interim report
Report No.:	M-442872-01-2
Document No.:	M-442872-01-2
Guideline(s):	US EPA OCSPP Guideline Number: 850.3030 (Ecological Effects
Guideline deviation(s):	none
GLP/GEP:	no

This interim report is superseeded by the final report (M-442868-02-2) below.

Report:	02.01.03/26; [redacted]; 2013; M-442868-02-2
Title:	Pilot study: Honey bee brood and colony level effects following imidacloprid intake via treated artificial diet in a field study in North Carolina
Report No.:	S12-01341
Document No.:	M-442868-02-2
Guideline(s):	US EPA Ref.: OPPTS 850.3040 (Ecological Effects)
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-442868-02-2@S-605065-01-1

A full-field trial was conducted to determine feasibility of evaluating the potential for colony- level effects on honey bees (*Apis mellifera* L.) during and after forced dietary consumption of Imidacloprid. Thirty hives were chosen for the study based on Colony Condition Assessments (CCA's) prior to study initiation. Parameters for choosing a hive included having all stages of brood, a laying queen, 7 frames of "colony components" consisting of drawn comb with food and brood, and 3 frames of empty or drawn frames for expansion. Hives were randomly assigned to a treatment group. The hives were placed in a mostly non-agricultural county in central North Carolina, in an area with only scattered patches of cropland, none of which were bee-attractive. Samples of trapped pollen collected at four different time intervals over the course of the study and analyzed by the USDA Analytical Laboratory (Gastonia, NC) for presence of >200 agrochemicals and metabolites confirmed lack of exposure of these hives to any agrochemicals.

The exposure part of the study was conducted by exposing honey bee colonies under field conditions to Imidacloprid-fortified, artificial nectar (50% sugar solution) or pollen diets. Pollen patties were prepared from Mega Bee powder by combining 500 mL water, 500 mg of sugar and 700 mg of Mega Bee powder. Honey bees were exposed to the treated diets at target rates and timings as shown below.

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Target Application Summary

Treatment Group	Code	Application Timing	Amount a.i.	Application Volume
Treatment 1 : UTC Sugar syrup	T1	Twice a week (12 total)	0 ppb (control)	1000 mL
Treatment 2 : UTC Pollen patty	T2	Twice a week(12 total)	0 ppb (control)	300 g
Treatment 3 : Low rate Sugar syrup	T3	Twice a week(12 total)	50 ppb	1000 mL
Treatment 4 : Low rate Pollen patty	T4	Twice a week(12 total)	50 ppb	300 g
Treatment 5: High rate Sugar syrup	T5	Twice a week(12 total)	200 ppb	1000 mL
Treatment 6: High Rate Pollen patty	T6	Twice a week(12 total)	200 ppb	300 g

Treatment groups 1, 3 and 5 were fed artificial nectar and allowed to forage freely for pollen. Treatment groups 2, 4 and 6 were fed artificial pollen patties, were prevented from bringing significant amounts of natural pollen into the hive by placement of pollen traps on the hive entrance, and were allowed to forage freely for nectar. Individual colony consumption rates for artificial nectar ranged from 8,660mL to 12,000mL for the 6-week period. Treatment 1 and Treatment 3 consumed the entire amount of artificial nectar provided for the duration of the exposure. Treatment 5 consumption of artificial nectar ranged from 8,660mL to 11,750mL. Pollen-fed colonies were provided a total of 3,600 grams of artificial pollen over the course of the study. Individual colony consumption rates for the pollen patties ranged from 621.5g to 1716.1g for the 6-week period. Treatment 2 (control) consumed an average of 1,370.5g. Treatment 4 (50 ppb) consumed 1,301.1g and Treatment 6 (200 ppb) consumed 821.4g.

Colony strength and general health were monitored via standardized colony condition assessments (CCAs) that were made every two weeks beginning the week prior to initiation of the 6-week dietary exposure and continuing until two weeks after this exposure ended. Counting the first week of dietary exposure as week 1, CCA's were conducted during weeks -1, 2, 4, 6, 7 and 8. An assessment on week 7 was conducted in addition to the regular schedule of CCA's made every other week in order to obtain colony measurements immediately after the end of the exposure period. Additional CCAs were made in mid- September and mid-October. During CCAs, colony strength was assessed as the total area (cm^2) occupied by adult bees, open brood (eggs and larvae), capped brood, stored pollen, and stored honey and the status of the queen was determined. Intrahive mortality was assessed one day each week of the exposure period using dead bee traps. Varroa mite and Nosema infestation levels were sampled at the start and end of the exposure period. No treatments for these bee health factors were applied prior to or during the exposure phase of the study. By September, Varroa populations were high in some of the colonies and treatment with Apiguard® was administered.

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The study methodology was sensitive in demonstrating colony-level differences between the treatment groups fed the artificial nectar diets, but not the groups fed the artificial pollen diets. The nectar fed groups exhibited a dose-dependent reduction in stored pollen and brood area during the exposure period, and adult bee population after the exposure period. The effect was obvious in the high exposure (200 ppb) group. All of the colonies fed the artificial pollen diets, even the controls, appeared stressed by the conditions of the test which (a) did not include supplemental feeding of syrup during a dearth period for natural nectar, and (b) used pollen traps to prevent forager bees from bringing in significant amounts of natural pollen. A clear effect of the test compound was not evident in these groups. On the basis of this study, a definitive test in which replicate test colonies are fed artificial nectar diets spiked with the test substance appears to be a feasible and sensitive method for determining colony-level responses of honey bees to dietary exposures.

>>M-442868-02-2@S-605065-01-1

Report:	02.01.03/27; [redacted] 2014; M-478404-02-2
Title:	Honey bee colony feeding study, evaluating the effects of Imidacloprid-fortified artificial nectar diet on long term colony health in a field study in North Carolina: Colony condition assessment data & statistics - Interim report
Report No.:	M-478404-02-2
Document No.:	M-478404-02-2
Guideline(s):	US EPA OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

This interim report is superseeded by the final report M-501299-01-2 below

<<M-478404-02-2@S-602258-01-1

A colony-feeding study was conducted with honey bee colonies (*Apis mellifera* L.) in a field setting with free-foraging colonies, exposes through sucrose solution dosed with different concentrations of imidacloprid. The purpose of this study was to evaluate the potential of imidacloprid exposure to result in adverse effects on the long-term health of honey bee colonies. At treatment levels of 50 ppb and above, numerous endpoints were repeatedly affected, with pollen stores and capped brood initially being affected. The no observable adverse effect level (NOAEL) for this study is 25 ppb.

>>M-478404-02-2@S-602258-01-1

Report:	02.01.03/28; [redacted] 2014; M-501299-01-2
Title:	Honey bee brood and colony level effects following Imidacloprid intake via treated artificial diet in a field study in North Carolina
Report No.:	SI3-03176
Document No.:	M-501299-01-2
Guideline(s):	US EPA OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-501299-01-2@S-602258-01-1

A colony-feeding study was conducted with honey bees (*Apis mellifera* L.) in a field setting with free-foraging colonies, exposed through sucrose solution dosed with imidacloprid at nominal rates of 12.5 ppb, 25 ppb, 50 ppb, 100 ppb or 200 ppb. The purpose of this study was to evaluate the potential of imidacloprid exposure to result in adverse effects on the long-term survival and condition of honey bee colonies. Treatment solutions were placed inside hives and renewed twice weekly over a six week exposure period. Assessments were made to evaluate the overall colony performance at several time points during and after the exposure period, as well as in the fall and following spring.

Analyses of the colony condition assessment (CCA) data indicate apparent effects on colony endpoints at the 50 ppb, and more pronounced at the 100 and 200 ppb treatment level, although colony survival was

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only affected at the 100 and 200 ppb levels. These effects were observed consistently at multiple time points and exhibit dose response relationships. The largest effect observed was a reduction in pollen stores. Not only did this endpoint have the largest reductions with almost no pollen stores present at the 200 ppb level, but these effects occurred at the earliest time point mid-way through the exposure period.

At the 100 and 200 ppb treatment level, consistent effects were also observed on endpoints related to brood. These effects followed a similar trend to the reduction in pollen stores. As brood development is dependent on pollen, reductions in brood cells could be a downstream effect from the reduced pollen stores. Egg cell counts were generally more variable and not as sensitive.

Effects on adult bees and nectar stores were also observed at the 50 ppb, and more pronounced at the 100 and 200 ppb treatment level, however these effects occurred after effects on other parameters were observed. This suggests that these effects are a downstream of effects on brood and/or pollen stores, and represent an overall reduction in colony performance. Hive weights, which incorporate to varying degrees all of these factors, was as sensitive as any other endpoint. At all assessments after the exposure was initiated, a significant reduction was observed at the 50 ppb, and more pronounced at the 100 and 200 ppb treatment level.

Increased overwintering losses (i.e. colony deaths) were only observed at the 100 and 200 ppb treatment level. While the same percentage of colonies survived overwintering in the 50 ppb treatment as in the controls, the 50 ppb treatment colonies were significantly weaker with respect to most of the endpoints evaluated.

In conclusion, at treatment levels of 50 ppb and above, numerous endpoints were repeatedly affected. The lowest observable adverse effect level (LOAEL) for this study is 50 ppb. The no observable adverse effect level (NOAEL) for this study is 25 ppb. With respect to colony survival, the LOAEL for this study was 100 ppb and the NOAEL was 50 ppb. There are no indications that exposure to imidacloprid resulted in a higher susceptibility of colonies to Varroa and Nosema infestation.

>>M-501299-01-2@S-602885-01-1

Report: 02.01-03/29; [redacted]; 2016; M-553526-02-3
Title: Report amendment 01 - Bayer CropScience sentinel hive study-Eastern Canada - Final report
Report No.: CETIN005
Document No.: M-553526-02-3
Guideline(s): US EPA OCSPP Guideline Number 850.SUPP
Guideline deviation(s): none
GLP/GEP: **no**

<<M-553526-02-3@S-605078-01-1

A monitoring study was set up starting in the spring of 2013 to track a set of 12 honey bee colonies in corn and soybean growing area for changes in health. Initially, these colonies were in 4 apiaries operated by 4 beekeepers in southern Ontario. An additional beekeeper in Quebec with three more hives was added in the fall of 2013 and the study was continued until the spring of 2015. All colonies were in rural agricultural areas where the corn-soybean-wheat crop rotation is common, and all were close to corn and soybean fields. Mapping of the land use in the area around the apiaries showed mainly corn-soybean wheat agricultural rotation but a great diversity of other vegetation was also present. There was no statistically significant correlation between honey yield and % of the area within 2 km that was planted with corn (Pearson's R = 0.02, p=0.9195, n=28). The actual food sources used by the bees were determined at a series of times by collecting pollen for palynology assessment. The results showed little use of corn, dandelion or soybean pollen and a preference for between 1 and 3 dominant food sources at a time during the growing season.

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The weather, and the temperature and relative humidity in the brood area of the hive were recorded. The sensor was placed in the upper box when 2 brood boxes were present. At five times during the season: pre-plant, at plant, post plant, mid-summer and early fall, the hives were inspected and the brood boxes were assessed, frame by frame (colony condition assessment). During these inspections, samples of live adult bees were taken for diagnostic analysis using molecular methods to determine the presence of common bee viruses, diseases and parasites. Samples of live and dead adult bees, honey, nectar, pollen and wax were collected for analysis to determine the presence and concentration of neonicotinoids acetamiprid (ACM), clothianidin (CLT), thiamethoxam (TMX) and metabolite TZNG. Imidacloprid (IMI) was added in 2014 and 2015. For the 2014 and 2015 samples, it became possible to simultaneously analyse virus diseases and neonicotinoids in small samples. Impaired bees and black bees, which had symptoms attributed to pesticide poisoning, were added to the sample types. Larvae were also added at this time.

In most cases, the colonies were considered to be healthy, but 6 colonies were lost during the study. The main causes were swarming, extreme winter weather and late season queen loss. In the latter case, aggressive robbing and wasp attacks were seen, which may have led to the queen loss.

The in-hive temperature results showed that the bees maintained the temperature in the brood area of the hive at close to 35 °C when brood was present, even when the outside temperature changed rapidly. They maintained some control over the relative humidity as well. The diagnostic results showed the presence of *Varroa* mites, occasionally at levels above the action threshold. European and American foul brood were insignificant. Viruses were the most prominent health issue. In one case, intervention was required to save the colony. 100% of the samples collected in 2013 contained at least one virus, detected using the qualitative Agriculture Canada National Bee Diagnostic Centre method. Over 50% had more than 3 viruses, with the most common virus being sacbrood. Impaired bees collected in front of the hives showed symptoms of viruses and an infection was confirmed by diagnosis. Qualitative virology showed sacbrood and black queen cell virus were most frequently detected. Quantigene® virology used for the 2014 and 2015 samples showed that deformed wing virus was dominant, often without morphological symptoms. The frequency of detection of at least one virus was 85.4, 85.7 and 32.1% in, brood area bees, foragers and larvae, respectively, using the Quantigene method. The pattern of occurrence of individual viruses was quite different from the qualitative results obtained in 2013, which may reflect differences in sensitivity of the method.

The neonicotinoid analytical results were assessed for both the individual compounds and using the aggregate exposure for all compounds for each type of bee (brood area bees, foragers larvae). Neither assessment showed any risk of bee loss. The aggregate assessment was done by assuming additive toxicity and calculating the sum of toxic units (TU). The TU for each compound was the total exposure relative to the NOEL for acute lethality, which was the main concern when the study was initiated. A value of 1.0 for the aggregate TU corresponds to the NOEL for acute lethality. The aggregate TU can be considered equivalent to a risk quotient.

The results showed that a significant amount of exposure occurred at the pre-plant time, indicating that planting must have already started in the area around the apiaries at the time the samples were taken. The maximum aggregate TU values were 0.755, 0.701 and 0.362 for brood area bees, forager bees, and larvae respectively. The corresponding 95th percentiles of the aggregate TU data for these bees were 0.149, 0.214 and 0.081. These results show that there was no likelihood of acute lethal toxicity to adult bees or larvae. The correlation between the aggregate TU values for brood area bees in 2013 and 2014 and honey yield was not statistically significant (Pearson's R 0.1556, p=0.4383, n=27). The correlation between the aggregate TU and overwintering survival was also not significant (Z score 0.6405, p=0.5222, n=129).

Bees with the symptoms listed for pesticide toxicity by the Government of Ontario through the Ontario Beekeepers found to be suffering from virus diseases. The black bees had no detectable residues. The impaired bees had several detections, but mostly at trace levels although one sample contained 4.28 µg/kg (0.428 ng/bee).

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Beewatch® hive scale data made it possible to determine the weight gain from each hive from the start of weight gain in the early spring until the end of the spring build-up. This spring hive weight gain is a colony level parameter that is sensitive to many factors, both lethal and sublethal, including queen fecundity, forager success and survival, brood development, growth and survival, and immune capacity. Included in the forager success and survival factor, is the potential loss of foragers that die without returning to the hive. While bee colonies are quite resilient to the loss of foragers, if the loss is significant, hive weight gain will be reduced. The spring weight gain in 2014, when IMI was included and weight gain was available through the spring build-up was compared to the average of pre-plant, at plant and post plant aggregate TU values for brood area bees and foragers, no statistically significant correlation was found (brood area bees: Pearson's R 0.4959, p=0.0713, n=14; foragers: Pearson's R 0.4919, p=0.074, n=14). Therefore the effect of loss of foragers in the field on the colonies was not significant and there were no detectable colony level effects during the spring build-up for the bee colonies.

Since the residues were highest in pollen the bees collected from willows and fruit trees in spring, these residues are likely the result of abraded seed dust generated during planting, and drifting into trees which are in bloom. Nectar samples at this time do not contain significant concentrations of neonicotinoids. Mitigation of this route of exposure can be achieved through use of improvements in dust control from planting treated seeds. An improved fluency agent has been developed and modifications to the air exhaust system for air seeders have been developed to accomplish this.

>>M-553526-02-3@S-605078-01-1

Report:	02.01.03/30; [redacted], 2016; M-555888-01-2
Title:	The importance of the green industry in reliably and sustainably protecting the natural beauty of our landscapes against destructive pests
Report No.:	USO564
Document No.:	M-555888-01-2
Guideline(s):	US EPA OCSPP Guideline no 850.SUPP
Guideline deviation(s):	--
GLP/GEP:	**no**

Under real-world environmental conditions, not every plant in a landscape is treated with imidacloprid and of those treated many are not attractive to honey bees. Additionally, honey bee colonies living in urban and suburban landscapes are not exposed to levels of imidacloprid that adversely affect their populations. New data from the real-world environment demonstrates a low likelihood and magnitude of exposure to individual honey bees and the overall colony. Over the years, the Green Industry has been committed to numerous stewardship actions and label refinements for imidacloprid. When the risks of non-agricultural uses for imidacloprid are reviewed by the U.S. Environmental Protection Agency, its benefits must also be taken into consideration.

Report:	02.01.03/31; [redacted], 2017; M-581863-01-2
Title:	Pollinator field study evaluating chronic effects of seed, in-furrow at planting and a pre-foliar application of imidacloprid to cotton, Gossypium barbadense L. - Final report
Report No.:	M-581863-01-2
Document No.:	M-581863-01-2
Guideline(s):	US EPA OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-581863-01-2@S-602903-01-0

A field study was conducted to evaluate the potential long-term effects of imidacloprid exposure to honey bee hives, which were placed within or at the edge of treated and untreated commercial cotton fields in the California Central Valley during the summer of 2015. The honey bee hives were established from 3-lb packages in new hive equipment, with sister queens, in the vicinity of Orland, CA mid-April 2015. After two screening steps, study hives were selected and randomly assigned in a stratified manner to either

imidacloprid-treated cotton field sites (n=4) or reference cotton field sites (n=4). The initial study plan included 5 reference and 5 imidacloprid-treated cotton fields; however, 2 fields were treated by the same grower with imidacloprid outside of the protocol scope and were therefore excluded from the study, thus resulting in a final replication of 4 reference and 4 imidacloprid-treated cotton fields. Eight study hives and one monitoring hive were assigned to each cotton field. Imidacloprid-treated cotton fields were planted with imidacloprid-treated seed, and received an in-furrow application at planting and one or two foliar applications prior to the exposure period. The study hives were placed in their assigned fields at the beginning of the cotton blooming period and remained at the cotton fields for 6 weeks. Thereafter, hives were relocated to a post-exposure apiary near Lost Hills, CA. Two collections of non-Apis bees were conducted during the mid-exposure and late-exposure periods, using bee bowl traps containing soapy water. Colony condition assessments (CCA) were conducted with digital photography at critical time periods including pre-exposure, exposure and post-exposure periods. The overwintering survival was evaluated in early March 2016, and the field phase of the study completed in late March 2016 when the last of two post-winter planned colony assessment was concluded. Samples of soil, crop matrices, in-hive matrices, monitoring hives, and bees were collected at critical time periods to characterize exposure to imidacloprid and other pesticides, floral resources and overall health status of the hives throughout the study. This final report includes results for the following honey bee hive parameters: adult bee counts, capped brood cell counts, bee bread cell counts, hive weights, and overwinter survival. In addition, the report also includes results from the non-Apis bee surveys, residues of imidacloprid and other pesticides, and identification of floral resources. The results indicate that there were no significant differences in capped brood, pollen counts, and overwinter survival between the hives that were placed at untreated and imidacloprid-treated cotton fields. The adult bee counts differed between imidacloprid-treated and reference plots at two CCAs: at CCA4 hives at imidacloprid-treated sites had higher adult bee counts, while at CCA6 hives from reference-treated sites had higher adult bee counts. However, at the end of the study there were no significant differences between treatment groups for this parameter. There were also no differences in non-Apis abundance between imidacloprid-treated and untreated cotton fields. The overall conclusion from this study is that honey bee colonies placed at the edge of blooming cotton treated with imidacloprid developed and survived as well as colonies placed at the edge of untreated reference blooming cotton during the same period.

>>M-581863-01-2@S-602903-01-1

Report: 02.01.03/32; [redacted] x 2011; M-408424-01-3
Title: Determination of exposure levels of honey bees foraging on flowers of citrus trees previously treated with imidacloprid
Report No.: EBNTL056-7
Document No.: M-408424-01-3
Guideline(s): US EPA OCSPP 850.SUPP
Guideline deviation(s): --
GLP/GEP: **no**

<<M-408424-01-3@S-602921-01-1

A series of field investigations were undertaken to determine to what extent honey bees foraging on citrus blossoms may be exposed to imidacloprid when citrus trees are treated with systemic applications (soil treatments) of this insecticide.

Tunnel Cage Study (Section 2)

- The objective of this component of the study was to examine citrus groves that were treated with a soil application of imidacloprid systemic insecticide, to understand the levels of imidacloprid that occurred in (a) nectar extracted by hand from citrus flowers, (b) nectar collected by forager honey bees and transported back to the hive, and (c) nectar or “uncapped honey” deposited by bees in cells of the brood comb
- Concentrations of imidacloprid, 5-hydroxy imidacloprid and imidacloprid olefin in nectar collected by hand from citrus flowers were similar to those in stomachs of bees foraging on the same trees confined within tunnels.

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- The highest residue levels from the 3 nectar sources were measured in the nectar deposited within the new comb (stored nectar). Compared to the concentrations in the honey bee stomach extracts, the levels of imidacloprid and 5-hydroxy imidacloprid in the stored nectar extracts were about 3-fold higher while the levels of imidacloprid olefin were 5-fold higher. The higher measurements in the stored nectar may be because comb nectar has lower water content and higher sugar content compared with unprocessed nectar, although our results are not conclusive based on refractometry measurements.

Open Field Study (Section 3)

- The objective of this component of the study was to examine citrus groves that were treated with a soil application of imidacloprid systemic insecticide, to understand the levels of imidacloprid that occurred in (a) nectar extracted by hand from citrus flowers, (b) nectar collected by forager honey bees and transported back to the hive, (c) nectar or "uncapped honey" deposited by bees in cells of the brood comb, and (d) pollen retrieved from pollen traps in the same hives used for the nectar studies.
- Concentrations in nectar extracted from the stomachs of free-ranging bees were somewhat lower than for samples collected directly from flowers of nearby trees. This may reflect a "dilution effect" from bees foraging on other (untreated) flower types. Mean imidacloprid residues in nectar sampled from the trees were less than 7 ppb.
- Residue concentrations in stored nectar samples were somewhat greater than in flower nectar. This may be because comb nectar has lower water content and higher sugar content compared with unprocessed nectar, although our results are not conclusive based on refractometry measurements.
- The imidacloprid concentrations measured in the limited pollen available for analysis were equal to those in the stored nectar samples collected from the same hives.

Citrus Nectar Collections from Field Sites Treated In One Year with 1X and 2X Label Rates of Imidacloprid (Section 4)

- The objective of this component of the study was to determine to what extent increasing the imidacloprid application rate would impact residues in the nectar
- Concentrations in flower nectar samples appear to be linearly related to application rate, based on *ca.* 2-fold increases in residue levels with a doubling of application rate.

Citrus Nectar Collections from Field Sites Treated in Successive Years with Imidacloprid (Section 5)

- The objective of this component of the study was to determine to what extent imidacloprid residues might persist and/or accumulate in citrus trees from year-to- year following multi-year applications.
- Based on experiments at the Hemet site, imidacloprid residues in spring flower nectar appear to be a function of the rate applied at the most recent application only, and appear to be independent of applications made in prior years. This conclusion is based on a period of 1 year between applications, which would be the normal use under the current label recommendation for citrus in California. There was a suggestion of some carryover between years with the 2X label rate treatments, although the result was not statistically significant.
- Nectar samples were obtained from 11 sites (citrus blocks in the Temecula region and at Lindcove Research and Extension Center) where the 1X soil application rate of imidacloprid had been made in two successive years (2008, 2009) prior to sampling in April 2010. Residue levels at these 11 sites averaged 8 ppb and ranged from 1 to 18 ppb.

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- The application timing (fall vs. spring) appears to be an important factor in determining residue levels in flower nectar the following year. Fall (Sept) applications resulted in about 2-fold higher residue concentrations compared with Spring (April-June) applications.
- Our conclusion is that at current label rates the residues of imidacloprid detected in the nectar during Spring bloom reflect the imidacloprid rate used during the most recent application, with limited impact from imidacloprid treatments conducted in prior seasons.

>>M-408424-01-3@S-605221-01-1

Report:	02.01.03/33;[redacted]; 2015; M-534355-01-2
Title:	Weight of evidence assessment of higher tier studies on the toxicity and risks of imidacloprid in honeybees
Report No.:	M-534355-01-2
Document No.:	M-534355-01-2
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-534355-01-2@S-602403-01-1

A weight of evidence methodology was used to assess a number of higher-tier studies on the effects of imidacloprid (IMD) on honeybees. The methodology was used to characterize the strength and quality of the available studies and to assess their relevance to potential or measured adverse effects. The higher tier studies focussed on exposures of honeybees via several matrices to IMD as measured in the field as well as effects in experimentally-controlled field studies and some ecoepidemiology studies.

Assessment endpoints were population size and stability of commercially managed bees; and, for the latter, quantity and quality of hive products. The field exposures were compared to the results of a higher-tier field toxicology study that used a number of hive-relevant responses of honeybees. This study reported a NOAEC of 25 µg IMD/L equivalent to an oral NOAED (7.3 ng/bee/d) for all responses measured. The LOAEC was 50 ng IMD/L, equivalent to an oral LOAED of 14.6 ng/bee/d. These toxicity values were expressed in doses per bee to allow normalization from different sources of exposure.

Reports provided by Bayer CropScience and papers from the open literature were assessed in detail, using pre-defined criteria for quality and relevance to develop scores (on a relative scale of 0-4) to separate the higher quality from the lower quality studies and the relevant from the non-relevant results. These WoE analyses are presented in the detailed supplemental information (SI). These scores were summarized graphically to illustrate the strength of the studies and their relevance.

Potential risks from exposures of honeybees to IMD via various matrices sampled in the field were characterized. Some studies were stronger than others and the overall mean for strength of methods (SoM) was 2.82 ± SE of 0.10. The mean and SE for relevance was 0.17 ± 0.12 suggesting consistent lack of relevant effects in studies that were generally strong. The overall weight of evidence suggests that there is little or no risk to bees from exposure to IMD from its use as a seed treatment. For exposures via treatments other than seeds (soil drench and foliar applications), some studies were stronger than others and the overall mean for SoM was 2.74 ± SE of 0.16. The mean and SE for relevance was 1.17 ± 0.29, suggesting greater variance in relevant effects in studies that were generally strong. These data suggest that some soil and foliar treatments might result in concentrations above the hazard values for IMD in nectar and pollen; however, the exact conditions resulting in this could not be identified. However, these conclusions are inherently conservative as it is assumed that bees are only exposed to nectar and pollen from treated crops. These results need to be considered in the light of the lack of effects observed in field studies where bees were exposed under actual conditions of use in the field. Exposures via dusts from currently-used seed coatings present a *de minimis* risk to honeybees via uptake in plants that are a source of pollen or nectar for honeybees.

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The WoE analysis of field studies of the effects on seed treatments with IMD on honeybees showed no relevant effects in a range of studies of variable strength. The WoE clearly demonstrates that environmentally realistic concentrations of IMD result in no adverse effects to honey bees at the colony level of biological organization when used appropriately. Four studies were available to assess the relationship between strengths and relevance of potential effects of IMD to honeybees exposed via artificial diets formulated with IMD-amended syrup or pollen patties. Overall, there was little relevance associated with the responses and the WoE supports that negative impacts to honey bee populations occur when exposure concentrations are environmentally unrealistic (e.g., 20 µg/L or kg). For other forms of application, the studies available did not show adverse effects on honeybees. Two studies examined the potential impacts or effects to honey bees exposed via drift of dust with residues during and immediately following sowing of dressed seed. The impact of dust residues on bees foraging and flying honey bees in adjacent fields with flowering plants indicated that there was minimal impact to honey bees. The SoM was 1.48 ± SE of 0.10 which was similar to that of the seed-dressing application. Low relevance was associated with the responses associated with the other types of applications.

With respect to honeybees, there were fewer higher-tier observational (ecoepidemiological) studies conducted with IMD. As for other responses, some studies were stronger than others; the overall mean for SoM was 1.88 ± SE of 0.19. The mean and SE for relevance to adverse effects was 0.11 ± 0.11. In general, weaknesses were related to lack of full consideration of potential confounders, either in terms of exposures to other pesticides, weather, and diseases. Overall, the weight of evidence does not support a causal relationship between exposure to IMD and adverse effects in bees. All the stronger studies did not identify adverse effects and the results of all but one of the weaker studies were consistent. The lack of effects in these studies is likely due to a combination of low exposures and label directions to minimize exposures to bees.

Considering all the lines of evidence, strength of the studies included in this analysis was variable but the results of the studies were consistent and point to the same conclusion. The overall weight of evidence based on a large number of studies thus does not falsify the main null hypothesis being tested, i.e., that IMD has no negative impacts on colony viability and no adverse effects on survival of the hive. Thus, the overall conclusion is that imidacloprid, as currently used in good agricultural practices, does not present a significant risk to honeybees at the level of the hive.

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Report:	02.01.03/34; [redacted]; 1999; M-006815-01-3
Title:	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
Report No.:	SXR/AM 001
Document No.:	M-006815-01-3
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-006815-01-3@S-602053-01-1

Material and methods: Poncho FS 500, a.i. content: 78.3 g/L Beta-cyfluthrin & 428.2 g/l Imidacloprid; specification (formulation No.: 030 based on 062000029, developmental No.: 00195939); under field conditions small beehives (appr. 5,000 honeybees) were caged on flowering summer rape plots (drilling rate: 5 kg/ha) as a sampling device for rape nectar and rape pollen. Nectar was also directly sampled from flowers via micropipettes. In addition, flowers were sampled by hand. The honeybees used as sample collectors were observed for signs of behavioral impacts. All samples including the honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.
Dates of biological work: June 15 - 18, 1998.
Dates of analytical work: June 30 - July 22, 1998.

Findings: Residues in rape plant matrices and in the foraging honeybees

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	--	--	--
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees	--	--	--
Treatment Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees	< 0.01	< 0.01	< 0.01

* Limit of quantitation: 0.01 mg/kg

Observations: No behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees used for collecting rape nectar and rape pollen. At the time of sampling, aphids were observed on the rape plants.

<<M-006815-01-3@S-602053-01-1

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Report:	02.01.03/35; [redacted]; 1999; M-006811-01-3
Title:	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
Report No.:	SXR/AM 002
Document No.:	M-006811-01-3
Guideline(s):	Internal Testing Method
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

<<M-006811-01-3@S-601942-01-1

Material and methods: Poncho FS 500, a.i. content: 78.3 g/L Beta-Cyfluthrin & 428.2 g/l Imidacloprid; specification (formulation No.: 030 based on 06200/0029, developmental No.: 00195939); under field conditions small beehives (appr. 5,000 honeybees) were caged on flowering summer rape plots (drilling rate: 5 kg/ha) as a sampling device for rape nectar and rape pollen. Nectar was also directly sampled from flowers via micropipettes. In addition, flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples including the honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 2 - 6, 1998.
Dates of analytical work: July 9 - 29, 1998

Findings: Residues in rape plant matrices and in the foraging honeybees

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Honeybees after exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees **	--	--	--
Treatment Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Honeybees after exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees **	--	--	--

* Limit of quantitation: 0.01 mg/kg. ** Amount insufficient for residue analysis

Observations: No behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees used for collecting rape nectar and rape pollen. At the time of sampling, aphids were observed on the rape plants.

M-006811-01-3@S-601942-01-1

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Report: 02.01.03/36; [redacted]; 1999; M-016820-01-3
Title: 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. 'Hoefchen' 1999
Report No.: SXR/AM 006
Document No.: M-016820-01-3
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

<<M-016820-01-3@S-602058-01-1

Material and methods: sunflower seed (variety "Fleury") either dressed with 150 g/U Gaucho WS 70 (a.i. content: 72.5% imidacloprid; batch no. 233 614 749, developmental no. 04 175 778) or imidacloprid-free were drilled on 10 May 1999 in soils with different imidacloprid residue levels. Soil samples for an analytical determination of the imidacloprid residue level were taken immediately before drilling. Drilling rate was 0.5 U/ha. During peak flowering of the sunflowers (end of July) small bee colonies (2,000 to 3,000 honeybees) were caged on these plots (appr. 50 m²) as a sampling device for sunflower nectar and pollen. In addition, some pollen and flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples and a small sample of honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 25 – August 3, 1999.
Dates of soil analysis: August 8 – 13, 1999.
Dates of analysis of biological samples: September 25 – 29, 1999.

Findings: Residues in soil and in sunflower plant matrices planted as succeeding crop (detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (south of field number 502) – imidacloprid-free seed in imidacloprid-free soil			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid. n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

[i] 1 U (Unit) = 150,000 seed

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Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1997“ (field number 502) – imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	0.018	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1998“ (field number 507) – imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	< LOQ	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1999“ (south of field number 502) – Gaucho-dressed seed in imidacloprid-free soil			
Soil sample (0-20 cm)	n.d.	--	--
Leaves (produced latest)	0.007	n.d.	< LOQ
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid, n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid, n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

Observations: No behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees used for collecting sunflower nectar and pollen.

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Report:	02.01.03/37; [redacted]; 1999; M-016827-01-3
Title:	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. 'Laacher Hof' 1999
Report No.:	SXR/AM 007
Document No.:	M-016827-01-3
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-016827-01-3@S-602071-01-1

Material and methods: sunflower seed (variety "Fleury") either dressed (= variant "1999) with 150 g/U Gaucho WS 70 (a.i. content: 72.5% imidacloprid; batch no. 233 614 749; developmental no. 04 175 778) or imidacloprid-free (control, variants "1997, 1998 and 1998 (2x)" were drilled on 12 May 99 in soils with different imidacloprid residue levels and treatment history. Soil samples for an analytical determination of the imidacloprid residue level were taken immediately before drilling. Drilling rate was 0.58 U/ha. During peak flowering of the sunflowers (21 and 26 July) small bee colonies (2,000 to 3,000 honeybees) were caged on these plots (appr. 50 m2) as a sampling device for sunflower nectar and pollen. In addition, some pollen and flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples and a small sample of honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 23 - August 3, 1999.
Dates of soil analysis: August 9-11, 1999.
Dates of analysis of biological samples: August 25 - September 21, 1999.

Findings: Residues in soil, in sunflower plant matrices planted as succeeding crop and in honeybees used as sampling device, (detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (field number 7/11) – imidacloprid-free seed in imidacloprid-free soil			
Soil sample (0–30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.

*Limit of quantitation (LOQ) for soil samples: 0.006 mg/kg (imidacloprid); n.d.=below limit of detection (0.002 mg/kg)
LOQ for biological samples: 0.005 mg/kg (imidacloprid & hydroxy-metabolite), 0.01 mg/kg (olefin-metabolite);
n.d. = below limit of detection (0.0015 mg/kg and 0.003 mg/kg, respectively)

[i] 1 U (Unit) = 150,000 seed

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Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1997" (field number 710) – imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	0.016	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1998" (field number 702) - imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	0.016	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1998 (2x)" (field number A XII) - imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	0.016	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1999" (field number 711) – Gaucho-dressed seed in imidacloprid-free soil			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	0.006	n.d.	< LOQ
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.

*limit of quantitation (LOQ) for soil samples: 0.006 mg/kg (imidacloprid); n.d.=below limit of detection (0.002 mg/kg)
LOQ for biological samples: 0.005 mg/kg (imidacloprid & hydroxy-metabolite), 0.01 mg/kg (olefin-metabolite);
n.d. = below limit of detection (0.0015 mg/kg and 0.003 mg/kg, respectively)

Observations: No treatment-related behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybee colonies used for collecting sunflower nectar and pollen. A colony check on day 8 after the first exposure (for test variant „1998 (2x)" on day 13) did also reveal no abnormalities in either colony strength or brood status.

>>M-016827-01-3@S-602071-01-1

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Report:	02.01.03/38; [redacted]; 1999; M-016832-01-5
Title:	Effects of imidacloprid residues in sunflower honey on the development of small bee colonies under field exposure conditions
Report No.:	SXR/Am 004
Document No.:	M-016832-01-5
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-016832-01-5@S-602121-01-1

Material and methods: *test substance:* imidacloprid techn., *purity:* 98.6%, *identity:* article no. 04145852, formulation/batch no. 230 824 088, no. of certificate TOX-No. 4941-00. Under field exposure conditions small bee colonies (appr. 500 honeybees) were confined on oat plots (50 m², drilled on 1 April 1999) and exclusively fed with sunflower honey which was fortified with either 0, 2, 5, 10 or 20 µg/kg imidacloprid. One colony received comb cells produced by honeybees during a previous feeding experiment with a 10 µg/kg sucrose solution. Pollen of the Mediterranean bush was provided as a protein source. The small bee colonies were examined for treatment related impacts over a period of 39 days. In particular, the following endpoints were evaluated: mortality, comb cell production, food consumption, storage behavior, hive weight increase egg laying activity, breeding success, colony strength, foraging intensity and behavioral anomalies.

Dates of biological work: May 28 - July 7, 1998.
Findings: Effects of imidacloprid residues in sunflower honey on small honeybee colonies

Testing Endpoint	Control	2 µg/kg	5 µg/kg	10 µg/kg	10 µg/kg*	20 µg/kg
Mortality (no. of dead bees in front of bee hives)	14	8	5	8	7	5
Mortality (no of dead bees at the tent margin)	24	20	21	18	18	26
Foraging intensity (no. of bees at the Honey feeder)	117	113	114	135	143	121
Foraging intensity (no. of bees at the pollen feeders)	26	26	22	24	31	36
Honey consumption [g]	546	546	581	566	616	546
Pollen consumption [g]	73	76	80	53	63	65
Comb cell production at study termination [cm²]	559	568	603	610	583	576
Honey storage area at study termination [cm²]	199	109	252	201	313	165
Hive weight increase at study termination	240	200	205	235	270	220
Egg laying activity[cm² comb area containing eggs] at study termination	120	115	143	208	60	148
Colony strength [cm² comb area covered with bees] at study termination	177	252	231	213	210	351

* Fed with comb cells from a previous feeding experiment.

Observations: There were no differences between the control and the treatment groups in any of the evaluated test parameters. In addition, no behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees of the treatment groups.
>>M-016832-01-5@S-602121-01-1

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Report: 02.01.03/39; [redacted]; 1999; M-016845-01-4
Title: Effects of imidacloprid residues in maize pollen on the development of small bee colonies under field exposure conditions
Report No.: SXR/AM 005
Document No.: M-016845-01-4
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

<<M-016845-01-4@S-602132-01-1

Material and methods: *test substance:* imidacloprid techn., *purity:* 98.6%, *identity:* article no. 04145852, formulation/batch no. 230 824 088, no. of certificate TOX-No. 4941-00. Under field exposure conditions small bee colonies (appr. 500 honeybees) were confined on oat plots (50 m 2, drilled on 1 April 1999) and exclusively fed with maize pollen which was fortified with either 0.2, 5, 10 or 20 µg/kg imidacloprid. Sunflower honey was provided as carbohydrate source. The small bee colonies were examined for treatment-related impacts over a period of 39 days. In particular, the following endpoints were evaluated: mortality, comb cell production, food consumption, storage behavior, hive weight increase egg laying activity, breeding success, colony strength, foraging intensity and behavioral anomalies.

Dates of biological work: May 28 - July 6, 1998.

Findings: Effects of imidacloprid residues in maize pollen on small honeybee colonies

Testing Endpoint	Control	2 µg/kg	5 µg/kg	10 µg/kg	20 µg/kg
Mortality (no. of dead bees in front of bee hives)	10	5	6	8	7
Mortality (no of dead bees at the tent margin)	22	21	22	21	30
Foraging intensity (no. of bees at the pollen feeder)	22	19	23	37	24
Foraging intensity (no. of bees at the honey feeder)	104	124	123	130	128
Pollen consumption [g]	35	29	32	39	34
Honey consumption [g]	491	541	521	500	543
Comb cell production [cm²]	528	551	579	584	563
Honey storage area at study termination [cm²]	177	201	186	147	174
Hive weight increase	180	230	215	200	200
Egg laying activity [cm² comb area containing eggs at study termination)	144	153	181	205	153
Colony strength [cm² comb area covered with bees at study termination)	217	258	305	314	221

Observations: There were no differences between the control and the treatment groups nor a concentration-related trend among the treatment groups for any of the evaluated test parameters. In addition, no behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees of the treatment groups.

>>M-016845-01-4@S-602132-01-1

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Report:	02.01.03/40; [redacted]; 1999; M-040023-01-3
Title:	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
Report No.:	SXR/AM 003
Document No.:	M-040023-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-040023-01-3@S-602143-01-1

Material and methods: Poncho FS 500, a.i. content: 78.3 g/L Beta-Cyfluthrin & 428.2 g/l Imidacloprid; specification (formulation No.: 030 based on 06200/0029, developmental No.: 00195939); test product: rape seed dressed with 2.5 l/dt Poncho FS 500; drilling rate: 5 kg/ha. Under field conditions small beehives (appr. 5,000 honeybees) were caged on flowering summer rape plots (60 m^2, drilled on 20 March 98) as a sampling device for rape nectar and rape pollen. Nectar was also directly sampled from flowers via micropipettes. In addition, flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples including the honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: June 22 - 24, 1998 (soil analysis: September 25 - 29, 1998).
Dates of analytical work: June 30 - July 28, 1998.

Findings: Residues in rape plant matrices and in the foraging honeybees

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Honeybees after exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees	< 0.01	< 0.01	< 0.01
Treatment Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Honeybees after exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees	< 0.01	< 0.01	< 0.01

* Limit of quantitation: 0.01 mg/kg.

Observations: No behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees used for collecting rape nectar and rape pollen.

>>M-040023-01-3@S-602143-01-1

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Report:	02.01.03/41; [redacted]; 2006; M-451677-01-3
Title:	Assessment of effects of imidacloprid WG 70 on foraging activity and mortality of honey bees and bumblebees after drenching application under field conditions on shrubs of the species Rhododendron catawbiense grandiflorum surrounded by other ornamental plant species
Report No.:	M-451677-01-3
Document No.:	M-451677-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-451677-01-3@S-604680-01-1

Material and methods:

Shrubs of the species Rhododendron catawbiense grandiflorum located at the experimental farmland "Laacher Hof" near Monheim (40789 Monheim, Nordrhein Westfalen, Germany) received soil treatment with Imidacloprid WG 70 dissolved in water at an application volume of 1 L per shrub in winter of 2005 (2005-01-13) at the application rates given in Table 1. Control shrubs (treatment group 1) received no treatment. Each treatment group consisted of 3 parallel rows of 10 Rhododendron plants.

Table 1: Summary: Treatment Groups and Rates

Treatment group	1	2	3
Application rates	control	4.3 g a.s./m plant size* = 2.58 g a.s./shrub = 3.68 g product/shrub	2.15 g a.s./m plant size* = 1.29 g a.s./ shrub = 1.84 product/shrub
Water volume rate per plant	1 L tap water		

* plants were 0.6 m high/wide

Between the rows of Rhododendron catawbiense grandiflorum, a mixture of bee attractive. potted ornamentals in watering trays was set upon the linen sheets between the Rhododendron rows an 2005-05-19. The species composition of the ornamentals was as follows: Fuchsia sp.: variety "Beacon", strawberry plant: variety "Fragoo", Alyssum sp., Lantana camara and Lobelia sp. In the near surroundings of the study site no other flowering crops were located.

One hive colony of honey bees Apis mellifera and 3 colonies of bumblebees Bombus terrestris were placed next to the Rhododendron catawbiense grandiflorum shrubs on 2005-05-20 {honey bees) and 2005-05-21 (bumblebees). Assessments on foraging activity of the honey bees and bumblebees were conducted on 10 days during flowering of the Rhododendron catawbiense grandiflorum shrubs from 2005-05-21 to -25 (5 consecutive days), an 2005-05-27 (1 day) and from 2005-05-30 to -06-02 (5 consecutive days) once in the morning and once in the afternoon separately on the Rhododendron plants and the surrounding ornamentals. The mortality of honey bees and bumblebees was assessed in front of the hives/colonies and on linen sheets laid out between the Rhododendron rows.

Blossom samples were collected from 15 Rhododendron plants per treatment group on 2005-05-19 (126 days after the application) and stored at -18°C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99).

Dates of biological work: 2005-01-13 to 2005-06-02
Dates of analytical work: 2005-06-21 to 2005-07-13

Findings:

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In Table 2 the results of the residue analyses of blossom samples are summarised.

Table 2: Summary: Results of Residue Analysis

Treatment Group	Sampling Date	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
1 (untreated control)	2005-05-19	126	< LOQ**	< LOQ**	< LOQ**
2 (4.3 g a.s./m plant size= 2.58 g a.s./shrub)	2005-05-19	126	0.488 – 1.996	0.073 – 0.215	< LOQ – 0.027
3 (2.15 g a.s./m plant size= 1.29 g a.s./shrub)	2005-05-19	126	0.092 – 0.812	0.014 – 0.060	< LOQ – 0.014

* DAT: days after treatment
** In 1 of 15 control samples residues were detected. No identification of the origin of this contamination was found.
Imidacloprid and Hydroxy-Metabolite: LOQ = 0.005 mg/kg LOD = 0.0015 mg/kg
Olefin-Metabolite: LOQ = 0.010 mg/kg LOD = 0.003 mg/kg

In Table 3 the results of the foraging activity assessments are summarized:

Table 3: Summary: Foraging Activity of Bumblebees (BB) and Honey Bees (B)

Treatment group	Rhododendron am		Ornamentals am		Rhododendron pm		Ornamentals pm	
	BB	B	BB	B	BB	B	BB	B
Control	120	0	3 (Fuchsia)	1 (strawberry)	126	1	2 (Fuchsia)	2 (strawberry, *Lobelia* sp.)
2.15 g a.s./m plant size	72	0	1 (Fuchsia)	0	59	0	2 (Fuchsia)	1 (strawberry)
4.3 g a.s./m plant size	70	0	0	0	63	0	0	1 (*Lobelia* sp.)

The foraging activity of bumblebees on the Rhododendron plants was comparable between the morning and the afternoon assessments. The highest numbers of foraging bumblebees were found in the control. The foraging activity of bumblebees was lower in the treatment groups 2 and 3 but with comparable numbers in both treatment groups. The ornamental plants were only scarcely visited by the bumblebees in the morning and in the afternoon.

Throughout the study only one honey bee was observed foraging on a Rhododendron plant (control). In none of the other treatment groups visits on this plant species occurred. Also the ornamental plants were only scarcely visited by the honey bees. Honey bees were observed to forage on strawberry and Lobelia sp. The beekeeper noticed that bees returning to the hive carried yellow pollen, which probably originated from plants other than the ornamentals set up in this study. However, in the near surrounding of the study site no other flowering crops were located.

No dead honey bees worker bees or bumblebees were found throughout the study on the individually labelled linen sheets laid out between the Rhododendron catawbiense grandiflorum rows and the rows of the surrounding potted ornamental plants and the linen sheets placed in front of the bee hive and the bumblebee colonies.

Conclusion:
In this field study no effects on mortality were observed on bumblebees and honey bees foraging on Rhododendron catawbiense grandiflorum plants surrounded by a species composition of ornamental

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plants (Fuchsia sp., strawberry plant, Alyssum sp., Lantana camara and Lobelia sp. The Rhododendron catawbiense grandiflorum plants had received a soil drench treatment 126 days before the start of the study with lmidacloprid WG 70 at either 4.3 g a.s./m plant size (2.58 g a.s./shrub = 3.68 g product/shrub) resulting in residues in blossoms up to 1.996 mg imidacloprid/kg or at 2.15 g a.s./m plant size (1.29 g a.s./ shrub = 1.84 product/shrub) resulting in residues in blossoms up to 0.812 mg imidacloprid/kg.

Untreated Rhododendron catawbiense grandiflorum plants were visited more frequently by the bumblebees than the treated ones, but frequency of visits was within a comparable order of magnitude between the sets of Rhododendron treated at different rates. Alternative ornamental plants were visited only very scarcely.

No behavioural anomalies were observed.

>>M-451677-01-3@S-604680-01-1

Report:	02.01.03/42; [redacted], 2007; M-451681-01-3
Title:	Assessment of effects of a drench application of imidacloprid WG 70 to shrubs of Rhododendron sp and to Hibiscus syriacus on foraging activity and mortality of honey bees and bumblebees under field conditions
Report No.:	M-451681-01-3
Document No.:	M-451681-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

>>M-451681-01-3@S-604681-01-1

Material and methods:

The study was carried out in 2 parts: the first part was conducted in spring 2006, during flowering of Rhododendron, and the second part in summer 2006, during flowering of Hibiscus.

Shrubs of the two species Rhododendron sp. and Hibiscus syriacus located at the area of Bayer CropScience AG (40789 Monheim, Nordrhein-Westfalen, Germany) received soil treatment with Imidacloprid WG 70 dissolved in water at an application volume of 2 L per shrub on 2006-04-12 at the application rates given In Table 1 (treatment groups 2 and 4). Control shrubs (treatment groups 1 and 3), located in a distance of 200 m received no treatment.

Table 1: Summary: Treatment Groups and Rates

Treatment group	1	2	3	4
Treatment name	Rhododendron, untreated	Rhododendron, treated	Hibiscus, untreated	Hibiscus, treated
Application rates	-	4.3 g a.s./m average plant width*	-	4.3 g a.s./m average plant height*
	-	5.2 g a.s./shrub = 7.37 g product/shrub	-	4.3 g a.s./shrub = 6.14 g product/shrub

* To describe the size of the Rhododendron shrubs the parameter shrub width was used for fixing the application rate. For Hibiscus the parameter shrub height was used for fixing the application rate.

Each treatment group consisted of 3 parallel rows of 6 shrubs each, Rhododendron and Hibiscus respectively. At the exterior sides of the 2 outer rows with Rhododendron sp. and Hibiscus syriacus a mixture of bee-attractive potted ornamentals was planted or sown in flower beds. The composition of ornamental plants intends to reflect typical conditions as to be expected in North American home gardens. Between the shrub rows further ornamental plants [Pelargonium sp. and Surfinia sp.) were set up in flower boxes on the linen sheets with which the ground around the rows was covered. Ornamental species composition for the Rhododendron part Fragaria sp.f Pulmonaria officinalis, Fuchsia sp. hybrids,

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Centaurea montana, Lobelia erinus and Lupinus sp. During the Rhododendron study period no other flowering bee-attractive plants were located in the near surroundings of both study plots. Ornamental species composition for the Hibiscus part: Lavendula augustifolia, Calluna vulgaris, Centaurea montana, Phacelia tanacetifolia, Lobelia erinus, Helianthus sp. And Fragaria sp. Near the control plot (treatment group 3) Mayweed was growing on a field and next to the treatment plot (treatment group 4) flowering Gladiolus (not attractive for honeybees), Snapdragons and Larkspur (approx. 20% open blossoms, minimally bee attractive) were present during the study period.

In approx. 20-25 m distance to each plot 1 beehive (consisting of 11 combs at the start of the study and containing approx. 10,000 honeybees and a queen) was located. Two colonies of bumblebees (Bombus terrestris) per study part were placed next to each plot at the beginning of shrub flowering. Honeybees and bumblebees were observed for foraging activity and mortality for 10 days (39 days after the application in Rhododendron and 103 days after the application in Hibiscus). Assessments on foraging activity of the honeybees and bumblebees were conducted once in the morning and once in the afternoon on 10 days during flowering of the Rhododendron shrubs, each time on the Rhododendron shrubs and the surrounding ornamentals separately from 2006-05-21 to 2006-05-24 (4 consecutive days) and from 2006-05-28 to 2006-06-01 (5 consecutive days). Due to the weather conditions on 2006-05-26 only one assessment in the morning was conducted; on 2006-06-02 the last afternoon assessment was made. Foraging assessments on the Hibiscus syriacus shrubs and the surrounding ornamentals were separately conducted once in the morning and once in the afternoon from 2006-07-25 to 2006-07-27 (3 consecutive days), from 2006-07-31 to 2006-08-04 (5 consecutive days) and from 2006-08-07 to 2006-08-09 (2 consecutive days). The mortality of honeybees and bumblebees was assessed in front of the hives/colonies and on linen sheets laid out between the shrub rows. Blossom samples were collected from 18 treated and 9 untreated plants during flowering of the respective shrub species. For Rhododendron this was conducted 35 days after the application and for Hibiscus 106-117 days after the application. Samples were stored at -18°C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS were performed according to method 01010 (MR-06/127). Dead honeybees and bumblebees found on the linen sheets between the plants and in front of the bee hives and bumblebee colonies were also subjected to residue analysis for residues of Imidacloprid and its Olefin- and Hydroxy-Metabolites. Extraction and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS was performed according to method 00537/M002 (MR-6/144).

Findings:
In the Tables 2 and 3 the results of the foraging activity assessments in Rhododendron and Hibiscus are summarised.

Table 2: Summary: Foraging Activity of Honeybees and Bumblebees on Rhododendron

	Total number per species observed per plot [n]			
	Honeybees		Bumblebees	
Treatment group	Rhododendron	Ornamentals	Rhododendron	Ornamentals
1: Control	23	64	608	238
2: Treatment	10	104	107	87

Only few honeybees were observed foraging on Rhododendron shrubs on the control and treatment plot respectively, but more on the control than on the treatment plots.

Foraging activity of honeybees on the surrounding ornamentals was higher than on the Rhododendron plants, but higher on the treated than on the control plot. The foraging activity of bumblebees on the

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Rhododendron plants was significantly higher on the untreated compared to the treated plants. The ornamental plants on the treated plot were likewise significantly less visited than those on the control plot.

Table 3: Summary: Foraging Activity of Honeybees and Bumblebees on Hibiscus

	Total number per species observed per plot [n]			
	Honeybees		Bumblebees	
Treatment group	Hibiscus	Ornamentals	Hibiscus	Ornamentals
3: Control	10	192	233	937
4: Treatment	5	108	9	623

Again only few honeybees were observed foraging on Hibiscus shrubs on the control and on the treatment plot respectively. Foraging activity of honeybees on the surrounding ornamentals was lower on the treated plot compared with the control. The foraging activity of bumblebees on the Hibiscus plants was distinctly higher on the control plot compared with the treated plot. The number of foraging bumblebees on the surrounding ornamentals was slightly higher on the control than on the treated plot. Mortality observed is depicted in Tables 4 and 5. In the Rhododendron part of the study, in total 27 dead honeybees were found in the treatment group, while in the control group 2 dead honeybees were found. In the Hibiscus part, no dead honeybees were found at all. Dead bumblebees were not found in the control replicates, neither in the Rhododendron nor in the Hibiscus part. In the treatment replicates, in total 2 dead bumblebees were found in the Rhododendron part, and 14 dead bumblebees in the Hibiscus part.

Table 4: Summary: Mortality of Honeybees

	Rhododendron		Hibiscus	
	Total number [n]			
Treatment group	on the plot	in front of hive	on the plot	in front of hive
Control	0	2	0	0
Treatment	2	25	0	0

Table 5: Summary: Mortality of Bumblebees

	Rhododendron		Hibiscus	
	Total number [n]			
Treatment group	on the plot	in front of hive	on the plot	in front of hive
Control	0	0	0	0
Treatment	1	1	12	2

Colony health and condition of the honeybee colonies was not different before and after the study, neither in the control nor in the treatment. Colony health and condition of the bumblebee colonies after the Hibiscus part of the study were not different between treatment and control.[1]

In Table 6 the results of the residue analysis of the Rhododendron and Hibiscus blossom samples and the residues in honeybees and bumblebees are summarised.

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Table 6: Summary: Results of Residue Analysis

Treatment Group	Sample description	Study part	Sampling Date	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
1: Control	blossoms	Rh	2006-05-17	35	< LOQ	< LOQ	< LOQ
2: Treatment	blossoms	Rh	2006-05-17	35	0.09 – 0.79	0.01 – 0.04	< LOQ – 0.01
3: Control	blossoms	H	2006-07-27	106	< LOQ	< LOQ	< LOQ
4: Treatment	blossoms	H	2006-07-27 to 2006-08-07	106 - 117	0.76 – 5.01	< LOQ – 0.45	< LOQ – 0.33
1: Control	2 honey-bees (colony)	Rh	2006-05-29	47	0.005 – 0.022	< LOQ – 0.008	0.001 – 0.019
2: Treatment	25 honey-bees (colony)	Rh	2006-05-21 to 2006-05-31	39 - 49	< LOQ – 0.016	< LOQ – 0.001	< LOQ – 0.001
	2 honey-bees (plot)	Rh	2006-05-21 to 2006-05-31	39 - 49	0.002 – 0.091	< LOQ – 0.018	< LOQ – 0.001
	1 bumble-bee (colony)	Rh	2006-05-29	47	0.001	0.001	0.005
	1 bumble-bee (plot)	Rh	2006-05-31	49	0.005	0.003	0.003
4: Treatment	2 bumble-bees (colony)	H	2006-07-26	105	0.003 – 0.004	0.001 – 0.003	0.004 – 0.009
	12 bumble-bees (plot)	H	2006-07-25 to 2006-08-08	104 - 118	0.077- 1.663	0.019 - 0.196	0.031 – 0.405

* DAT: days after treatment Rh Rhododendron H Hibiscus
Blossom samples: Imidacloprid, Hydroxy-Metabolite, Olefin-Metabolite: LOQ = 0.010 mg/kg
Insect samples: Imidacloprid, Hydroxy-Metabolite, Olefin-Metabolite: LOQ = 0.001 mg/kg

Residue levels in the Rhododendron blossoms were 0.09 - 0.79 mg imidacloprid/kg and in Hibiscus blossoms 0.76 - 5.01 mg imidacloprid/kg in the treated replicates. In the blossoms of the untreated plants, residue levels were < LOQ. Residue levels found in dead honeybees in the control groups were 0.005 - 0.022 mg imidacloprid/kg. In dead honeybees in the treatment groups, residue levels were between < LOQ and 0.091 mg imidacloprid/kg. Residue levels found in dead bumblebees in the treatment groups were 0.001 -1.663 mg imidacloprid/kg.

[1] Inspection of the bumblebee colonies exposed in the Rhododendron part of the study after end of exposure could not be conducted. Inspection of bumblebee colonies before start of exposure is generally not feasible technically.

>>M-451688-01-3@S-604681-01-1

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Report: 02.01.03/43; [redacted]; [redacted]; [redacted]; 2007; M-016828-02-3
Title: 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. Laacher Hof 1999
Report No.: SXR/AM 008
Document No.: M-016828-02-3
Guideline(s): 850.3040
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-016828-02-3@S-602076-01-1

Material and methods: summer rape seed (variety "Lisonne") either dressed with 25 ml/kg Poncho FS 500 (a.i. content: 79.7 g/L beta-Cyfluthrin and 427.4 g/L imidacloprid; batch no. 6200/0055 A according to formulation no. 6200/0059, developmental no. 00195939) or imidacloprid-free were drilled on 12 May 99 in soils with different imidacloprid residue levels. Soil residue levels were analytically determined immediately before drilling. Drilling rate was 3.25 kg/ha. During peak flowering of the summer rape (mid of July) small bee colonies (2,000 to 3,000 honeybees) were caged on these plots (appr. 50 m ²) as a sampling device for summer rape nectar and pollen. In addition, some nectar and flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples and a small sample of honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 12 – 19, 1999.
Dates of soil analysis: August 8 – 13, 1999.
Dates of analysis of biological samples: September 25 – 29, 1999.

Findings: Residues in soil, in summer rape plant matrices planted as succeeding crop and in honeybees used as sampling device. (detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (field number 711)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid. n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

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Therefore

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1997“ (field number 710)			
Soil sample (0-30 cm)	0.016	--	--
Leaves (produced latest)	< LOQ	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1998“ (field number 702)			
Soil sample (0-30 cm)	0.015	--	--
Leaves (produced latest)	< LOQ	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	< LOQ	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1998 (2x)“ (field number A XII)			
Soil sample (0-30 cm)	0.014	--	--
Leaves (produced latest)	< LOQ	n.d.	< LOQ
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	< LOQ	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1999“ (field number 711)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	< LOQ	n.d.	< LOQ
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	< LOQ	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid; n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

Observations: No treatment-related behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybee colonies used for collecting summer rape nectar and pollen. The small colonies were remained till 3 September. The final check on this day did also reveal no abnormalities in either colony strength or brood status.

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Report:	02.01.03/44; [redacted]; [redacted]; [redacted]; 2007; M-016842-02-3
Title:	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 residue on foraging honeybees. 'Hoefchen' 1999
Report No.:	SXR/AM 010
Document No.:	M-016842-02-3
Guideline(s):	US EPA OCSPP Giuideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-016842-02-3@S-604919-01-1

Material and methods: summer rape seed (variety "Lisonne") either dressed with 25 ml/kg Poncho FS 500 (a.i. content: 79.7 g/L beta-Cyfluthrin and 427.4 g/L imidacloprid; batch no. 6200/0055* A according to formulation no. 6200/0059, developmental no. 00195939) or imidacloprid-free were drilled on 11 May 99 in soils with different imidacloprid residue levels. Soil samples for an analytical determination of the imidacloprid residue level were taken immediately before drilling. Drilling rate was 7 kg/ha. During peak flowering of the summer rape (mid of July) small bee colonies (2,000 to 3,000 honeybees) were caged on these plots (appr. 50 m^2) as a sampling device for summer rape nectar and pollen. In addition, some nectar and flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples and a small sample of honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 12-19, 1999
Dates of soil analysis: August 9-11, 1999
Dates of analysis of biological samples: August 27 - September 21, 1999

Findings: Residues in soil, in summer rape plant matrices planted as succeeding crop and in honeybees used as sampling device. (detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (south of field number 502)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid. n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

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Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1997“ (field number 502)			
Soil sample (0-30 cm)	0.018	--	--
Leaves (produced latest)	< LOQ	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1998“ (field number 507)			
Soil sample (0-30 cm)	< LOQ	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1999“ (south of field number 502)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	< LOQ	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	< LOQ	n.d.	n.d.
Pollen sampled from hives and bees	< LOQ	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid. n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

Observations: No treatment-related behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or increased mortality was observed on the honeybee colonies used for collecting summer rape nectar and pollen. The final check at study termination did also not reveal any abnormality in either colony strength or brood status.

>>M-016842-03-3@S-6049/9-01-1

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Report:	02.01.03/45; [redacted]; 2017; M-542796-03-2
Title:	Pollinator full field study evaluating chronic effects of a post seeding application of imidacloprid in pumpkins (Curcubita pepo pepo) - Final report
Report No.:	13798.4145
Document No.:	M-542796-03-2
Guideline(s):	US EPA OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-542796-03-2@S-605072-01-1

A field study was conducted to evaluate the potential long-term effects of imidacloprid exposure to honey bee and bumble bee colonies, which were placed in imidacloprid-treated and reference pumpkin fields in central South Dakota during the summer of 2015. Pumpkins were direct seeded into large fields (40 acres) and imidacloprid was applied a sub-surface side dress at 0.38 lb/acre once pumpkins had attained the six true leaf stage (BBCH16). Fields were located in areas for which grassland pasture and wheat fields were the predominant land use.

The honey bee hives were established from 4-lb packages in new hive equipment, with sister queens, in North Carolina on 11 Apr 2015 and transported to South Dakota on 25 Jul 2015. Study hives were selected and randomly assigned in a stratified manner to either imidacloprid-treated (n=5) or untreated reference (n=5) pumpkin fields. Nine study honey bee hives and one monitoring hive were assigned to each pumpkin field. Nine bumble bee nests and two monitoring bumble bee nests were randomly assigned to each pumpkin field. Honey bee hives and bumble bee nests were moved into the fields once sufficient blooming of the pumpkins had occurred. The hives remained in the pumpkin fields for 6 weeks. Thereafter, hives were relocated to a post- exposure apiary near Durand, WI.

Samples for residue analysis were collected from field soils pre-treatment and indicated very low, background levels (0-19 ppb) of imidacloprid, clothianidin, and thiamethoxam. Nectar and pollen samples were collected from pumpkin blossoms and analyzed for clothianidin and two metabolites as well as clothianidin and thiamethoxam. In nectar samples, only imidacloprid in treated fields were detected; however, levels were very low (0.8, 2.1, and 1.2 ppb median residues for the three time points). In pollen samples, there was one sample with detectable level of clothianidin, but no thiamethoxam detected in any sample. There were some, very low detections of imidacloprid in reference pollen samples. In treated fields, however, imidacloprid was consistently detected, although at low levels (3.4, 7.0, and 4.7 ppb median residues for the three time points.

Hive matrices (capped honey and bee bread) were collected from hives before being moved into pumpkin fields with a few hives having detections for imidacloprid. During the pumpkin field phase of the study, uncapped nectar and bee bread were sampled from study hives. Most uncapped nectar samples did not have any detectable imidacloprid residues in either the reference or treated fields. Imidacloprid residues, however, were more consistently detected in bee bread samples in the treated fields and demonstrate the largest difference in residues between reference and treated fields. After overwintering, no imidacloprid residues were detected in capped honey samples collected from either reference or treated fields.

Colony condition assessments showed no statistical differences between reference and treated fields for numbers of adult bees, capped brood cells, or bee bread cells for any assessment. Overall colony survival, including overwintering was 60% for reference fields and 56% for treated fields. There were no significant differences in *Nosema* or *Varroa* infection detected except for *Varroa* counts after overwintering. However, this difference was not considered treatment-related based on previous studies and the very low levels of *Varroa* detected across all hives.

Three surveys of non-*Apis* bees were conducted during the pumpkin bloom period using bee bowl traps containing soapy water. Large numbers of bees were collected across both reference and imidacloprid-treatment sites and no significant differences were observed amongst well-represented species and

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diversity indices. Bumble bee colonies performed very poorly in both reference and imidacloprid-treated sites likely due to the late time of the year or being outside of their normal range. Performance of the bumble bee colonies was not sufficient to compare between reference and treated fields.

Overall, no adverse effects were observed in honey bee colonies and non-Apis bee surveys between reference and imidacloprid treated fields. There were no statistical differences in numbers of adult bees, capped brood cells, nor bee bread cells which previously were observed to be sensitive endpoints for chronic imidacloprid exposure.

>>M-542796-03-2@S-605072-01-1

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02.02 – Exposure

02.02.01 - Nectar and Pollen

Report:	02.02.01/01; [redacted]; 2012; M-018436-01-4
Title:	Residues of imidacloprid and imidacloprid metabolites in sunflower blossoms sampled in Argentinia
Report No.:	SXR/AM 002a
Document No.:	M-018436-01-4
Guideline(s):	US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-018436-01-4@S-602140-01-1

Material and methods: Gaucho WS 70 (700 g/L imidacloprid) was dressed on sunflower seeds of different varieties (i.e. Rigasol, Albena, Tournesol cv Rigasol, Jaguar) at a rate of 0.7 mg a.i./seed. The target rate was verified by an analytical check of treated seed samples. The actual seed dressing rates ranged between 79 and 119% of the nominal value. The treated sunflower seeds were drilled in 56 m^2 plots established within a conventionally managed sunflower field. The sunflower field was located in the vicinity of San Gregorio, Argentinia. When the sunflowers were in full blossom flowers were harvested from different zones of the sunflower heads (i.e. "early" and "late" flowers). These flower samples were frozen on dry ice and stored at -20°C prior to analysis. The samples were then analytically examined for the presence of the parent compound and the Olefin- and Hydroxy-Metabolite. The limit of quantification (LOQ) was at 0.01 mg/kg.

Dates of work: February 6 to 17, 1998

Findings: *Residues of Imidacloprid and Imidacloprid Metabolites in Sunflower Blossoms*

Position of the collected flowers	Residue level [mg/kg]		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Inner zone (= „early" flowers)	< 0.01	< 0.01	< 0.01
Central zone	< 0.01	< 0.01	< 0.01
Outer zone („late" flowers)	< 0.01	< 0.01	< 0.01

Observations: At the quantification limit of 0.01 mg/kg, no residues of either the parent compound or of the Olefin- and Hydroxy-Metabolite could be detected in blossoms of Gaucho treated sunflowers.

>>M-018436-01-4@S-602140-01-1

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Report: 02.02.01/02; [redacted]; 1999; M-006815-01-3
Title: 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
Report No.: SXR/AM 001
Document No.: M-006815-01-3
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

<<M-006815-01-3@S-602053-01-1

Material and methods: Poncho FS 500, a.i. content: 78.3 g/L Beta-Cyfluthrin & 428.2 g/l Imidacloprid, specification (formulation No.: 030 based on 062000029, developmental No.: 00195939); under field conditions small beehives (appr. 5,000 honeybees) were caged on flowering summer rape plots (drilling rate: 5 kg/ha) as a sampling device for rape nectar and rape pollen. Nectar was also directly sampled from flowers via micropipettes. In addition, flowers were sampled by hand. The honeybees used as sample collectors were observed for signs of behavioral impacts. All samples including the honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.
Dates of biological work: June 15 - 18, 1998.
Dates of analytical work: June 30 - July 22, 1998.

Findings: Residues in rape plant matrices and in the foraging honeybees

Type of Sample	Residue Level [mg/kg]*		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	--	--	--
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees		--	--
Treatment Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees	< 0.01	< 0.01	< 0.01

* Limit of quantitation: 0.01 mg/kg

Observations: No behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees used for collecting rape nectar and rape pollen. At the time of sampling, aphids were observed on the rape plants.

<<M-006815-01-3@S-602053-01-1

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Report:	02.02.01/03; [redacted]; 1999; M-006811-01-3
Title:	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
Report No.:	SXR/AM 002
Document No.:	M-006811-01-3
Guideline(s):	Internal Testing Method
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

<<M-006811-01-3@S-601942-01-1

Material and methods: Poncho FS 500, a.i. content: 78.3 g/L Beta-Cyfluthrin & 428.2 g/l Imidacloprid; specification (formulation No.: 030 based on 06200/0029, developmental No.: 00195939); under field conditions small beehives (appr. 5,000 honeybees) were caged on flowering summer rape plots (drilling rate: 5 kg/ha) as a sampling device for rape nectar and rape pollen. Nectar was also directly sampled from flowers via micropipettes. In addition, flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples including the honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 2 - 6, 1998.
Dates of analytical work: July 9 - 29, 1998

Findings: Residues in rape plant matrices and in the foraging honeybees

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Honeybees after exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees **	--	--	--
Treatment Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Honeybees after exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees **	--	--	--

* Limit of quantitation: 0.01 mg/kg. ** Amount insufficient for residue analysis

Observations: No behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees used for collecting rape nectar and rape pollen. At the time of sampling, aphids were observed on the rape plants.

M-006811-01-3@S-601942-01-1

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Report: 02.02.01/04; [redacted]; 1999; M-016820-01-3
Title: 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. 'Hoefchen' 1999
Report No.: SXR/AM 006
Document No.: M-016820-01-3
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

<<M-016820-01-3@S-602058-01-1

Material and methods: sunflower seed (variety "Fleury") either dressed with 150 g/U[i] Gaucho WS 70 (a.i. content: 72.5% imidacloprid; batch no. 233 614 749, developmental no. 04 175 778) or imidacloprid-free were drilled on 10 May 1999 in soils with different imidacloprid residue levels. Soil samples for an analytical determination of the imidacloprid residue level were taken immediately before drilling. Drilling rate was 0.5 U/ha. During peak flowering of the sunflowers (end of July) small bee colonies (2,000 to 3,000 honeybees) were caged on these plots (appr. 50 m²) as a sampling device for sunflower nectar and pollen. In addition, some pollen and flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples and a small sample of honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 25 – August 3, 1999.
Dates of soil analysis: August 8 – 13, 1999.
Dates of analysis of biological samples: September 25 – 29, 1999.

Findings: Residues in soil and in sunflower plant matrices planted as succeeding crop (detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (south of field number 502) – imidacloprid-free seed in imidacloprid-free soil			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid. n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

[i] 1 U (Unit) = 150,000 seed

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Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1997“ (field number 502) – imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	0.018	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1998“ (field number 507) – imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	< LOQ	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1999“ (south of field number 502) – Gaucho-dressed seed in imidacloprid-free soil			
Soil sample (0-20 cm)	n.d.	--	--
Leaves (produced latest)	0.007	n.d.	< LOQ
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid, n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid, n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

Observations: No behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees used for collecting sunflower nectar and pollen.

>>M-008820-01-3(2)S-602058-01-1

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Report:	02.02.01/05; [redacted]; 1999; M-016827-01-3
Title:	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. 'Laacher Hof' 1999
Report No.:	SXR/AM 007
Document No.:	M-016827-01-3
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-016827-01-3@S-602071-01-1

Material and methods: sunflower seed (variety "Fleury") either dressed (= variant "1999") with 150 g/U Gaucho WS 70 (a.i. content: 72.5% imidacloprid; batch no. 233 614 749; developmental no. 04 175 778) or imidacloprid-free (control, variants "1997, 1998 and 1998 (2x)" were drilled on 12 May 99 in soils with different imidacloprid residue levels and treatment history. Soil samples for an analytical determination of the imidacloprid residue level were taken immediately before drilling. Drilling rate was 0.58 U/ha. During peak flowering of the sunflowers (21 and 26 July) small bee colonies (2,000 to 3,000 honeybees) were caged on these plots (appr. 50 m2) as a sampling device for sunflower nectar and pollen. In addition, some pollen and flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples and a small sample of honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 23 - August 3, 1999.
Dates of soil analysis: August 9-11, 1999.
Dates of analysis of biological samples: August 25 - September 21, 1999.

Findings: Residues in soil, in sunflower plant matrices planted as succeeding crop and in honeybees used as sampling device
(detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (field number 711) – imidacloprid-free seed in imidacloprid-free soil			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.

*Limit of quantitation (LOQ) for soil samples: 0.006 mg/kg (imidacloprid); n.d.=below limit of detection (0.002 mg/kg)
LOQ for biological samples: 0.005 mg/kg (imidacloprid & hydroxy-metabolite), 0.01 mg/kg (olefin-metabolite);
n.d. = below limit of detection (0.0015 mg/kg and 0.003 mg/kg, respectively)

i 1 U (Unit) = 150,000 seed

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Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1997" (field number 710) – imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	0.016	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1998" (field number 702) - imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	0.016	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1998 (2x)" (field number A XII) - imidacloprid-free seed in imidacloprid-contaminated soil			
Soil sample (0-30 cm)	0.016	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.
Variant „1999" (field number 711) – Gaucho-dressed seed in imidacloprid-free soil			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	0.006	n.d.	< LOQ
Flowers (male / female flowers)	n.d.	n.d.	n.d.
Nectar sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the hive combs	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Honeybees exposed to the sunflowers	n.d.	n.d.	n.d.

*limit of quantitation (LOQ) for soil samples: 0.006 mg/kg (imidacloprid); n.d.=below limit of detection (0.002 mg/kg)
LOQ for biological samples: 0.005 mg/kg (imidacloprid & hydroxy-metabolite), 0.01 mg/kg (olefin-metabolite);
n.d. = below limit of detection (0.0015 mg/kg and 0.003 mg/kg, respectively)

Observations: No treatment-related behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybee colonies used for collecting sunflower nectar and pollen. A colony check on day 8 after the first exposure (for test variant „1998 (2x)" on day 13) did also reveal no abnormalities in either colony strength or brood status.

>>M-016827-01-3@S-602071-01-1

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Report:	02.02.01/06; [redacted]; 1999; M-016830-01-3
Title:	Residue levels of imidacloprid and imidacloprid metabolites in pollen of maize plants cultivated on soils with different imidacloprid residue levels Test location: farmland 'Hoefchen' - 1999
Report No.:	SXR/AM 011
Document No.:	M-016830-01-3
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-016830-01-3@S-602112-01-1

Material and methods: maize seed (variety "Ilias") either dressed with 70 g/U1 Gaucho WS 70 (a.i. content: 72.5% imidacloprid; batch no. 233 614 749, developmental no. 04 175 778) or imidacloprid-free were drilled on 10 May 99 in soils with different imidacloprid residue levels. Soil samples for an analytical determination of the imidacloprid residue level were taken immediately before drilling. Drilling rate was 2 U/ha. During peak flowering of the maize plants (end of July) pollen was harvested from the male flowers. These pollen samples were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 22 - 29, 1999
Dates of soil analysis: August 9-11, 1999
Dates of analysis of biological samples: August 31 - September 22, 1999

Findings: Residues in soil, and in pollen of maize planted as succeeding crop, (detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (south of field field number 502)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Variant „1997“ (field number 502)			
Soil sample (0-30 cm)	0.018	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.003 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid. n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

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Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1998“ (field number 507)			
Soil sample (0-30 cm)	< LOQ	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Variant „1999“ (south of field number 502)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	0.011	n.d.	< LOQ
Pollen sampled from the plants	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid. n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

Observations: No residue levels at or above the limit of detection could be detected in pollen of maize planted as succeeding crop in soil previously cropped with Gaucho-dressed plants. Even in pollen of seed-dreesed maize plants, no residues of imidacloprid above the limit of detection were found. In the latest leaf stages, a residue level of 11 µg/kg imidacloprid and traces of the hydroxy-metabolite (< LOQ) were detected.

>>M-016830-01-3@S-602112-01-1

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Report: 02.02.01/07; [redacted]; 1999; M-016832-01-5
Title: Effects of imidacloprid residues in sunflower honey on the development of small bee colonies under field exposure conditions
Report No.: SXR/Am 004
Document No.: M-016832-01-5
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

<<M-016832-01-5@S-602121-01-1

Material and methods: *test substance:* imidacloprid techn., *purity:* 98.6%, *identity:* article no. 04145852, formulation/batch no. 230 824 088, no. of certificate TOX-No. 4941-00. Under field exposure conditions small bee colonies (appr. 500 honeybees) were confined on oat plots (50 m², drilled on 1 April 1999) and exclusively fed with sunflower honey which was fortified with either 0, 2.5, 10 or 20 µg/kg imidacloprid. One colony received comb cells produced by honeybees during a previous feeding experiment with a 10 µg/kg sucrose solution. Pollen of the Mediterranean bush was provided as a protein source. The small bee colonies were examined for treatment related impacts over a period of 39 days. In particular, the following endpoints were evaluated: mortality, comb cell production, food consumption, storage behavior, hive weight increase egg laying activity, breeding success, colony strength, foraging intensity and behavioral anomalies.

Dates of biological work: May 28 - July 7, 1998.
Findings: Effects of imidacloprid residues in sunflower honey on small honeybee colonies

Testing Endpoint	Control	2 µg/kg	5 µg/kg	10 µg/kg	10 µg/kg*	20 µg/kg
Mortality (no. of dead bees in front of bee hives)	14	8	5	8	7	5
Mortality (no of dead bees at the tent margin)	24	20	21	18	18	26
Foraging intensity (no. of bees at the Honey feeder)	117	113	134	135	143	121
Foraging intensity (no. of bees at the pollen feeders)	26	26	22	24	31	36
Honey consumption [g]	546	546	581	566	616	546
Pollen consumption [g]	73	76	80	53	63	65
Comb cell production at study termination [cm²]	559	568	603	610	583	576
Honey storage area at study termination [cm²]	199	109	252	201	313	165
Hive weight increase at study termination	240	200	205	235	270	220
Egg laying activity [cm² comb area containing eggs] at study termination	120	115	143	208	60	148
Colony strength [cm² comb area covered with bees] at study termination	177	252	231	213	210	351

* Fed with comb cells from a previous feeding experiment.

Observations: There were no differences between the control and the treatment groups in any of the evaluated test parameters. In addition, no behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees of the treatment groups.

>>M-016832-01-5@S-602121-01-1

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Report: 02.02.01/08; [redacted]; 1999; M-016836-01-3
Title: Residue levels of imidacloprid and imidacloprid metabolites in pollen of maize plants cultivated on soils with different imidacloprid residue levels. Test location: farmland 'Laacher Hof' - 1999
Report No.: SXR/AM 009
Document No.: M-016836-01-3
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

<<M-016836-01-3@S-602125-01-1

Material and methods: maize seed (variety "Ilias") either dressed with 70 g/U[i] Gaucho WS 70 (a.i. content: 72.5% imidacloprid; batch no. 233 614 749, developmental no. 04 175 778) or imidacloprid-free were drilled on 12 May 99 in soils with different imidacloprid residue levels. Soil samples for an analytical determination of the imidacloprid residue level were taken immediately before drilling. Drilling rate was 2 U/ha. During peak flowering of the maize plants (end of July) pollen was harvested from the male flowers. These pollen samples were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 22-29, 1999.
Dates of soil analysis: August 9-11, 1999.
Dates of analysis of biological samples: August 31 - September 22, 1999.

Findings: Residues in soil, and in pollen of maize planted as succeeding crop, (detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (field number 711)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Variant „1997“ (field number 710)			
Soil sample (0-30 cm)	0.016	--	--
Leaves (produced latest)	< LOQ	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.002 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid, n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

[i] 1 U (Unit) = 50,000 seed

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Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1998“ (field number 702)			
Soil sample (0-30 cm)	0.013	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Variant „1998 (2x)“ (field number A XII)			
Soil sample (0-30 cm)	0.014	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Pollen sampled from the plants	n.d.	n.d.	n.d.
Variant „1999“ (field number 711)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	0.010	n.d.	< LOQ
Pollen sampled from the plants	< LOQ	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid, n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

Observations: No residue levels at or above the limit of detection could be detected in pollen of maize planted as succeeding crop in soil previously cropped with Gaucho-dressed plants. In pollen of seed-dressed maize plants, some residues of imidacloprid were found. The residue level, however, was below the limit of quantitation, i.e. less than 5 µg/kg. In the latest leaf stages, a residue level of 10 µg/kg imidacloprid and traces of the hydroxy-metabolite (< LOQ) were detected.

>>M-016836-01-3@S-602125-01-1

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Report: 02.02.01/09; [redacted]; 1999; M-016845-01-4
Title: Effects of imidacloprid residues in maize pollen on the development of small bee colonies under field exposure conditions
Report No.: SXR/AM 005
Document No.: M-016845-01-4
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

<<M-016845-01-4@S-602132-01-1

Material and methods: *test substance:* imidacloprid techn., *purity:* 98.6%, *identity:* article no. 04145852, formulation/batch no. 230 824 088, no. of certificate TOX-No. 4941-00. Under field exposure conditions small bee colonies (appr. 500 honeybees) were confined on oat plots (50 m 2, drilled on 1 April 1999) and exclusively fed with maize pollen which was fortified with either 0.2, 5, 10 or 20 µg/kg imidacloprid. Sunflower honey was provided as carbohydrate source. The small bee colonies were examined for treatment-related impacts over a period of 39 days. In particular, the following endpoints were evaluated: mortality, comb cell production, food consumption, storage behavior, hive weight increase egg laying activity, breeding success, colony strength, foraging intensity and behavioral anomalies.

Dates of biological work: May 28 - July 6, 1998.

Findings: Effects of imidacloprid residues in maize pollen on small honeybee colonies

Testing Endpoint	Control	2 µg/kg	5 µg/kg	10 µg/kg	20 µg/kg
Mortality (no. of dead bees in front of bee hives)	10	5	6	8	7
Mortality (no of dead bees at the tent margin)	22	21	22	21	30
Foraging intensity (no. of bees at the pollen feeder)	22	19	23	37	24
Foraging intensity (no. of bees at the honey feeder)	104	124	123	130	128
Pollen consumption [g]	35	29	32	39	34
Honey consumption [g]	491	541	521	500	543
Comb cell production [cm²]	528	551	579	584	563
Honey storage area at study termination [cm²]	177	201	186	147	174
Hive weight increase	180	230	215	200	200
Egg laying activity[cm² comb area containing eggs at study termination)	144	153	181	205	153
Colony strength [cm² comb area covered with bees at study termination)	217	258	305	314	221

Observations: There were no differences between the control and the treatment groups nor a concentration-related trend among the treatment groups for any of the evaluated test parameters. In addition, no behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees of the treatment groups.

>>M-016845-01-4@S-602132-01-1

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Report:	02.02.01/10; [redacted]; 1999; M-040023-01-3
Title:	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
Report No.:	SXR/AM 003
Document No.:	M-040023-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-040023-01-3@S-602143-01-1

Material and methods: Poncho FS 500, a.i. content: 78.3 g/L Beta-Cyfluthrin & 428.2 g/l Imidacloprid; specification (formulation No.: 030 based on 06200/0029, developmental No.: 00195939); test product: rape seed dressed with 2.5 l/dt Poncho FS 500; drilling rate: 5 kg/ha. Under field conditions small beehives (appr. 5,000 honeybees) were caged on flowering summer rape plots (60 m^2, drilled on 20 March 98) as a sampling device for rape nectar and rape pollen. Nectar was also directly sampled from flowers via micropipettes. In addition, flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples including the honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: June 22 - 24, 1998 (soil analysis: September 25 - 29, 1998).
Dates of analytical work: June 30 - July 28, 1998.

Findings: Residues in rape plant matrices and in the foraging honeybees

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Honeybees after exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees	< 0.01	< 0.01	< 0.01
Treatment Samples			
Honeybees before exposure	< 0.01	< 0.01	< 0.01
Honeybees after exposure	< 0.01	< 0.01	< 0.01
Rape nectar sampled by bees	< 0.01	< 0.01	< 0.01
Rape nectar sampled with micro-capillaries from the flowers	< 0.01	< 0.01	< 0.01
Rape blossoms	< 0.01	< 0.01	< 0.01
Rape pollen sampled by bees	< 0.01	< 0.01	< 0.01

* Limit of quantitation: 0.01 mg/kg.

Observations: No behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybees used for collecting rape nectar and rape pollen.

>>M-040023-01-3@S-602143-01-1

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Report:	02.02.01/11; [redacted]; 2001; M-052637-01-3
Title:	Effects of residues of imidacloprid in maize pollen from dressed seeds on honey bees (Apis mellifera)
Report No.:	M-052637-01-3
Document No.:	M-052637-01-3
Guideline(s):	US EPA OPPTS 850.3040
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-052637-01-3@S-602655-01-1

Material and methods: test substance: Gaucho WS 70, residues in maize pollen from dressed seeds, dressing rate: 49 g/unit a.i Residues of imidacloprid in the pollen were found to be below limit of quantitation (LOQ = 0.005 mg/kg). No olefine and hydroxy metabolites could be detected (limit of detection: 0.003 mg/kg and 0.0015 mg/kg, respectively).

Small bee colonies (appr. 700 honeybees) were confined in tent cages (ca. 20 m2) on short grass meadows and exclusively fed with maize pollen which was harvested from plants, the seeds of which were dressed with Gaucho WS 70 or which were untreated (control). Sunflower honey was provided as carbohydrate source. The small bee colonies were examined for treatment-related impacts over a period of 38 days. In particular, the following endpoints were evaluated: mortality, comb cell production, food consumption, storage behavior, hive weight increase, egg laying activity, breeding success, colony strength, foraging intensity and behavioral anomalies.

Dates of biological work: 2000-08-21 to 2000-09-28.

Findings: Effects of Gaucho WS 70 residues in maize pollen on small honeybee colonies

Testing Endpoint	Control A	Control B	Treatment A	Treatment B
Mortality (no. of dead bees in front of bee hives)	32	22	20	30
Mortality (no of dead bees at the tent edges)	146	141	139	151
Foraging intensity (no. of bees at the pollen feeder)	1	15	29	2
Foraging intensity (no. of bees at the honey feeder)	267	253	274	255
Bee activity (no. of bees at the tent roof)	180	203	196	185
Pollen collected [g]	16	58	43	26
Honey collected [g]	736	853	819	877
Comb cell production [cm²]	696	618	660	664
Honey storage area at study termination [cm²]	314	254	417	399
Hive weight increase [% of the initial weight]	9.8	6.6	12.4	16.6
Egg laying activity [cm² comb area containing eggs at study termination]	19	63	15	18
Colony strength [cm² comb area covered with bees at study termination]	279	249	253	263

Observations: There were no treatment-related effects in the testing endpoints foraging activity, orientation, honey and pollen consumption, comb cell, production, honey storage, hive weight increase, population development, mortality, breeding activity, and breeding success. There are no hints that imidacloprid residues in pollen from maize seeds treated with Gaucho at the rate recommended might have any adverse effects to honey bee colonies.

>>M-052637-01-3@S-602655-01-1

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Report:	02.02.01/12; [redacted]; 2002; M-052238-01-3
Title:	Evaluation of the effects of residues of imidacloprid FS 600 in maize pollen from dressed seeds on honeybees (Apis mellifera) in the semifield
Report No.:	M-052238-01-3
Document No.:	M-052238-01-3
Guideline(s):	not applicable
Guideline deviation(s):	The following procedures were not carried out under GLP: seed dressing, sowing of the seeds, analysis of soil contents of the field where seeds were sown, harvesting of the maize panicles, sieving and drying of the pollen.
GLP/GEP:	**yes**

<<M-052238-01-3@S-602148-01-1

Material and methods: Test substance: maize pollen with grown residues of Imidacloprid FS 600 (seeds dressed with commercially available product at a rate of 1 g a.i. /1000 seeds). Small honeybee colonies (approx. 500 honeybees) were confined on oat plots (50 m^2, drilled on 2001-05-03) in tunnels and fed with maize pollen containing grown residues of Imidacloprid or untreated control pollen. For treatment and control, three replicates were set up each. Sunflower honey was provided as carbohydrate source. The small bee colonies were examined for treatment-related effects over a period of 52 days. In particular, the endpoints mortality and foraging intensity were evaluated. Likewise the endpoints comb cell production, food consumption, pollen and honey stores, egg laying activity, breeding success, colony strength and hive weight development were assessed and statistically analysed using a t-Test.
Behavioural anomalies were also assessed.

Dates of biological work: 2001-06-21 to 2001-08-12.
Dates of analytical work: 2001-03-14 to 2001-06-05.

Findings: Effects of residues of Imidacloprid FS 600 in pollen on small honeybee colonies

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Table 1: Summary

Testing Endpoint	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
Mortality (Total No. of dead bees in front of the bee hives) [n]	1	1	0	5	1	3
Mortality (Total No. of dead bees at the tunnel edge) [n]	28	31	25	50	34	50
Cumulative comb cell production at study termination [cm²]	768	708	675	692	651	706
Cumulative honey collected [g]	702	694	677	621	661	668
Cumulative pollen collected [g]	12.2	8.9	9.6	21.0	39.8	27.1
Honey storage area at study termination [cm²]	194	234	116	136	172	226
Pollen storage area at study termination [cm²]	41	13	18	17	25	27
Egg laying activity [cm² comb area containing cells with eggs at study termination]	177	98	130	182	125	161
Larval abundance [cm² comb area containing cells with larvae at study termination]	92	79	79	89	67	120
Pupal abundance [cm² comb area containing cells with pupae at study termination]	113	92	149	86	110	125
Colony strength [cm² comb area covered with bees at study termination]	266	183	183	269	222	260
Hive weight increase [%]	25.5	27.6	23.1	17.3	23.6	21.6
Foraging activity [Average No. of bees at the pollen feeder / assessment]	0.7	0.7	0.7	0.8	1.1	0.6
Foraging activity [Average No. of bees at the honey feeder / assessment]	7.3	8.1	7.7	7.3	8.2	7.9

Observations: There were no significant differences between control and treatment in comb cell production (t=-0.478, p=0.641), honey consumption (t=2.530, p=0.065), hive weight increase (t=1.720, p=0.161), pollen stores (t=-0.360, p=0.725) and honey stores (t=0.086, p=0.933), egg deposition (t=-0.176, p=0.863), larval abundance (t=-0.328, p=0.749), pupal abundance (t=-0.288, p=0.778) and abundance of adult bees (t=-0.068, p=0.947). The pollen consumption was significantly higher in the treatment

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Report:	02.02.01/13; [redacted]; 2001; M-052524-02-3
Title:	Determination of residues of imidacloprid and relevant metabolites in nectar, pollen and honey of winter rape
Report No.:	MR-147/01
Document No.:	M-052524-02-3
Guideline(s):	Equivalent to US EPA OPPTS 850.3040
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-052524-02-3@S-604946-01-1

Rape flowers, pollen, nectar and honey samples obtained from a German trial station were analysed for residues of Imidacloprid and its olefin- and Hydroxy metabolites. The results are summarized in the table below. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-metabolite by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99). The limit of quantitation was 0.005 mg/kg for Imidacloprid and the Hydroxy-metabolite and 0.01 mg/kg for the Olefin-metabolite. The limit of detection was 0.0015 mg/kg for Imidacloprid and the Hydroxy-metabolite and 0.003 mg/kg for the Olefin-metabolite.

>>M-052524-02-3@S-604946-01-1

Report:	02.02.01/14; [redacted]; 2003; M-075630-01-3
Title:	Residue levels of imidacloprid and imidacloprid metabolites in sunflower pollen, sunflower honey and bees from Gaucho treated sunflowers in the field
Report No.:	MR-710/99
Document No.:	M-075630-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-075630-01-3@S-602816-01-1

Sunflower honey, pollen and bee samples obtained from the German trial station "Ahrweiler/Mayen" were analysed for residues of imidacloprid and its olefin- and hydroxy metabolites. The results are summarized in the table below. Extraction, sample clean up and determination of imidacloprid, hydroxy- and olefin-metabolite by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99). The limit of quantitation was 0.005 mg/kg for imidacloprid and the hydroxy-metabolite and 0.01 mg/kg for the olefin-metabolite. The limit of detection was 0.0015 mg/kg for imidacloprid and the hydroxy-metabolite and 0.003 mg/kg for the olefin-metabolite.

>>M-075630-01-3@S-602816-01-1

Report:	02.02.01/15; [redacted]; 2004; M-451697-01-3
Title:	Residues of imidacloprid WG 5 in blossom samples of Rhododendron sp. (variety Nova Zembla) after soil treatment in the field - 2003,
Report No.:	M-451697-01-3
Document No.:	M-451697-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-451697-01-3@S-604694-01-1

Material and methods: Eight year old *Rhododendron* plants (variety "Nova Zembla") growing at the experimental farmland "Höfchen" near Burscheid, Germany received pre and post-flowering soil treatment with Imidacloprid WG 5 in two replicates (A and B: 8 plants each) per treatment group. Soil application with Imidacloprid WG 5 (article No.: 0005439280, Batch No.: PF00000REC, TOX No. 6135-001 purity: 5.5%) dissolved in water at an application volume of 2 l per plant was carried out on 2003-05-09 (pre-flowering treatment) and 2003- 06-05 (post-flowering treatment) at the application rates shown below. Control plants (treatment 1) received no treatment.

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Treatment-No.	Application rate / 50 cm plant height	Sampled material
1	Control	**Blossoms**: 2003-05-20 and 26 (2nd time only replicate A) Leaves: 2003-05-20 and 26, 2003-07-22, 2003-09-02 *
2	2500 mg a.i. pre-flowering	**Blossoms**: 2003-05-20 and 26 Leaves: 2003-05-20 and 26, 2003-07-22, 2003-09-02 *
3	2500 mg a.i. post-flowering	Leaves: 2003-05-20 and 26, 2003-07-22, 2003-09-02 *
4	1250 mg a.i. pre-flowering	**Blossoms**: 2003-05-20 and 26 Leaves: 2003-05-20 and 26, 2003-07-22, 2003-09-02 *
5	1250 mg a.i. post-flowering	Leaves: 2003-05-20 and 26, 2003-07-22, 2003-09-02 *
6	100 pre- plus 200 mg a.i. post-flowering	**Blossoms**: 2003-05-20 Leaves: 2003-05-20, 2003-07-22, 2003-09-02 *

*** sampled *Rhododendron* leaves were not analysed**

Rhododendron blossoms were collected from all pre-flowering treatment groups 11 and 17 days after application (except for treatment group 6) and stored at -20° C for approximately four months prior to analysis. The blossoms were analysed for residues of Imidacloprid and its Olefin- and Hydroxy-Metabolites. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99) by R. [redacted].

Dates of biological work: 2003-05-09 to 2003-09-02
Dates of analytical work: 2003-09-05 to 2003-09-22

Findings: In the following table the results of the residue analyses of blossom samples from the pre-flowering treatment are summarised.

Pre-flowering treatment	DAT	Imidacloprid in µg/kg	Hydroxy-Imidacloprid in µg/kg	Olefin-Imidacloprid in µg/kg
Control	11	<LOD – 12.5	<LOD - <LOQ	<LOD
	17*	<LOD – 12.7	<LOD - <LOQ	<LOD - <LOQ
Treatment 2 (2500 mg a.i./50 cm plant height)	11	<LOD – 20.0	< LOD – 6.3	< LOD
	17	<LOQ – 23.2	< LOQ – 8.7	< LOD
Treatment 4 (1250 mg a.i./50 cm plant height)	11	<LOD – 11.4	<LOD - <LOQ	< LOD
	17	<LOD – 13.6	<LOD - <LOQ	< LOD
Treatment 6 (100 mg a.i./50 cm plant height)	17	<LOD – 16.8	<LOD - <LOQ	< LOD

Imidacloprid and Hydroxy-Metabolite: LOQ = 5 µg/kg LOD = 1.5 µg/kg
Olefin-Metabolite: LOQ = 10 µg/kg LOD = 3 µg/kg
* only replicate A analysed

Conclusion:
Imidacloprid and its Hydroxy and Olefin metabolites were detected in both treated and control blossom samples. The residues found in the control samples are considered to originate from efficacy trials carried out with these plants between 1997-2000 which included drenching treatment before planting out in the field. The possibility of contamination occurring during sampling, storage or analytical work, has been investigated and could be ruled out. Since the residue levels lie within the same range in control and all treatment groups, the treatments carried out in 2003 do obviously not significantly contribute to the residue levels detected.

>>M-451697-01-3@S-604694-01-1

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Report: 02.02.01/16; [redacted]; 2004; M-451701-01-3
Title: Determination of the residue levels of imidacloprid and its relevant metabolites in nectar, pollen and other plant material of chestnut trees (Aesculus hippocastanum) after soil treatment application and sampling 2001
Report No.: AM021
Document No.: M-451701-01-3
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **no**

<<M-451701-01-3@S-604670-01-1

Material and methods: Four Horse Chestnut trees (Aesculus hippocastanum) (T1 - T4) received soil treatment with Imidacloprid WG 70 (commercially available product: Article No. 00044211898, Batch No. 233914158*0, 1, No. of sample: FAR00802-00) on 2001-03-13 at an application rate of 0.28 g a.i./cm stem diameter (=0.4 g product/cm stem diameter at a height of 1.3 m) at a water application rate of 2L/tree. The 4 control trees (C1 - C4) received no treatment.

During flowering of the trees, blossoms were collected. Nectar was sampled from the control group. Leaf samples w re taken five times throughout the vegetation period and fruits were sampled once at the end of the vegetation period. All samples were subjected to a residue analysis for Imidacloprid and its relevant metabolites.

Residue analysis was carried out on leaves and blossoms using the analytical method RA 00537 (1999, R. [redacted]). Fruits were analysed using the method RA 00200 (PF-Nachrichten 1993/2, [redacted], E. [redacted]).

Dates of biological work: 2001-03-13 to 2001-09-30
Dates of analytical work: until 2002-11-09

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Findings: Summary of residues in leaves, blossom and nectar samples of 4 treatment and 4 control trees:

Treatment	Sample material	DAT	Imidacloprid in µg/kg	Hydroxy-Imidacloprid in µg/kg	Olefin-Imidacloprid in µg/kg
Control (C1-C4)	leaves	58 (2001-05-10)	n.d. - <LOQ	n.d.	n.d.
		90 (2001-06-11)	n.d. - <LOQ	n.d.	n.d.
		118 (2001-07-09)	n.d. - <LOQ	n.d.	n.d.
		153 (2001-08-13)	n.d.	n.d.	n.d.
		181 (2001-09-10)	n.d. - <LOQ	n.d.	n.d.
Treatment (T1-T4)	leaves	58 (2001-05-10)	n.d.	n.d.	n.d.
		90 (2001-06-11)	33 - 121	16 - 53	n.d. - 15
		118 (2001-07-09)	73 - 339	29 - 165	5 – 12
		153 (2001-08-13)	126 - 282	57 - 98	14 – 17
		181 (2001-09-10)	59 - 250	40-116	<LOQ - 16
Control (C1-C4)	blossoms	58 (2001-05-10)	n.d. - <LOQ	n.d.	n.d.
		64 (2001-05-16)	n.d. - 8	n.d.	n.d.
Treatment (T1-T4)	blossoms	58 (2001-05-10)	n.d. - <LOQ	n.d.	n.d.
Control (C2)*	nectar	62 (2001-05-14)	n.d.	n.d.	n.d.

Imidacloprid and Hydroxy-Metabolite: LOQ = 5 µg/kg LOD = 1.5 µg/kg
Olefin-Metabolite: LOQ = 10 µg/kg LOD = 3 µg/kg
* It was technically not feasible to sample the necessary amount of nectar for a residue analysis from all trees.

Residues in fruit samples:

Treatment	Sample material	DAT	Total residue of Imidacloprid in µg/kg
Control (C1-C4)	fruits	181 (2001-09-10)	n.d.
Treatment (T1-T4)	fruits	181 (2001-09-10)	n.d. - <LOQ

Total residue: LOQ = 50 µg/kg LOD = 15 µg/kg

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Report:	02.02.01/17; [redacted]; 2004; M-451703-01-3
Title:	Determination of the residue levels of imidacloprid and its relevant metabolites in nectar, pollen and other plant material of horse chestnut trees (Aesculus hippocastanum) after trunk injection application and sampling 2001
Report No.:	AM023
Document No.:	M-451703-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-451703-01-3@S-604699-01-1

Material and methods: Four Horse Chestnut trees *(Aesculus hippocastanum)* (T1 - T4) were treated by trunk injection with Imidacloprid SL 200 (commercially available product:: Article No. 000495860, Batch No. 0594*0.25, No. of sample: FAR00801-00) on 2001-05-09 at an application rate of 0.06 g a.i./cm stem diameter (=0.3 mL product/cm stem diameter in 42.6 mL water/cm stem diameter at a height of 1.3 m). The 4 control trees (C1 - C4) received no treatment.

During flowering of the trees, blossoms were collected from all treatment groups and nectar was sampled from the control group. Leaf samples were taken six times throughout the vegetation period and fruits were sampled once at the end of the vegetation period. All samples were subjected to a residue analysis for Imidacloprid and its relevant metabolites.

Residue analysis was carried out on leaves and blossoms using the analytical method RA 00537 (1999, R. [redacted]). Fruits were analysed using the method RA 00300 (PF-Nachrichten 1993/2, [redacted], E. [redacted]).

Dates of biological work: 2001-05-09 to 2001-09-30

Dates of analytical work: until 2003-11-09

Findings: Summary of residues in leaves, blossom and nectar samples of 4 treatment and 4 control trees:

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Treatment	Sample material	DAT	Imidacloprid in µg/kg	Hydroxy-Imidacloprid in µg/kg	Olefin-Imidacloprid in µg/kg
Control (C1-C4)	leaves	1 (2001-05-10)	n.d. - <LOQ	n.d.	n.d.
		33 (2001-06-11)	n.d. - <LOQ	n.d.	n.d.
		61 (2001-07-09)	n.d. - <LOQ	n.d.	n.d.
		96 (2001-08-13)	n.d. - <LOQ	n.d.	n.d.
		124 (2001-09-10)	n.d. - <LOQ	n.d.	n.d.
Treatment (T1-T4)	leaves	1 (2001-05-10)	n.d. - <LOQ	n.d.	n.d.
		2 (2001-05-11)	14-1300	n.d.-48	n.d. - <LOQ
		33 (2001-06-11)	1205 – 1996	302-1106	54-143
		61 (2001-07-09)	155 – 2200	118 - 2313	74 – 259
		96 (2001-08-13)	17 – 1571	19 - 791	<LOQ - 139
		124 (2001-09-10)	173 - 419	164 - 611	17 - 56
Control (C1-C4)	blossoms	1 (2001-05-10)	n.d. - <LOQ	n.d.	n.d.
		7 (2001-05-16)	n.d. – 8	n.d.	n.d.
Treatment (T1-T4)	blossoms	2 (2001-05-11)	n.d. - 7	n.d.	n.d.
		7 (2001-05-16)	5 - 283	n.d. 7	n.d. - <LOQ
Control (C2)*	nectar	5 (2001-05-14)	n.d.	n.d.	n.d.

Imidacloprid and Hydroxy-Metabolite: LOQ = 5 µg/kg LOD = 1.5 µg/kg
Olefin-Metabolite: LOQ = 10 µg/kg LOD = 3 µg/kg
* It was technically not feasible to sample the necessary amount of nectar for a residue analysis from all trees.

Residues in fruit samples:

Treatment	Sample material	DAT	Total residue of Imidacloprid in µg/kg
Control (C1-C4)	Fruits	124 (2001-09-10)	n.d.
Treatment (T1-T4)	fruits	124 (2001-09-10)	n.d. - <LOQ
Total residue:		LOQ = 50 µg/kg	LOD = 15 µg/kg

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Report: 02.02.01/18; [redacted]; 2004; M-451700-01-2
Title: Determination of the residue levels of imidacloprid and its metabolites hydroxy-imidacloprid and olefin-imidacloprid in leaves and blossoms of horse chestnut trees (Aesculus hippocastanum) after soil treatment - Application 2001 and sampling 2002
Report No.: M-451700-01-2
Document No.: M-451700-01-2
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **no**

<<M-451700-01-2@S-603148-01-1

Material and methods: Four Horse Chestnut Trees *(Aesculus hippocastanum)* (T1 - T4) received soil treatment with Imidacloprid WG 70 (Article No. 000211898, Batch No. 233914158*0,1, No. of sample: FAR00802-00) on 2001-03-13 at an application rate of 0.28 g a.s./cm stem diameter (=0.4 g product/cm stem diameter at a height of 1.3 m) at a water application rate of 2 L/tree. The 4 control trees (C1 - C4) received no treatment.
Sampling was carried out in 2002, one year after the application had been carried out. During flowering of the trees, blossoms and leaves were collected. Additional leaf samples were taken four times throughout the vegetation period. All samples were subjected to a residue analysis for Imidacloprid and its metabolites Hydroxylmidacloprid and Olefin-Imidacloprid. Residue analysis was carried out on leaves and blossoms using the analytical method RA 00537 (1999, R. [redacted]).

Dates of biological work: 2001 -03-13 to 2002-09-30

Dates of analytical work: 2002-03-25 to 2003-03-10

Findings: Summary of residues in leaves, blossom and nectar samples of 4 treatment and 4 control trees:

Treatment	Sample material	DAT	Imidacloprid [µg/kg]	Hydroxy-Imidacloprid [µg/kg]	Olefin-Imidacloprid [µg/kg]
Control (C1 - C4)	leaves	407 - 412 (2002-04-24 - 29)	n.d.	n.d.	n.d.
		441 (2002-05-28)	n.d. - <LOQ	n.d.	n.d.
		476 (2002-07-02)	n.d.	n.d.	n.d.
		504 (2002-07-30)	n.d.	n.d.	n.d.
		539 (2002-09-03)	n.d. - <LOQ	n.d.	n.d.
Treatment (T1 - T4)	leaves	408 - 412 (2002-04-25 - 29)	26 - 40	13 - 30	<LOQ
		441 (2002-05-28)	115 - 308	n.d. - 115	29 – 101
		476 (2002-07-02)	176 - 492	n.d. -161	119 – 114
		504 (2002-07-30)	161 - 532	n.d. - 177	34 – 229
		539 (2002-09-03)	80 - 185	63 - 107	19 - 52
Control (C1 – C4)	blossoms	407 - 412 (2002-04-24 - 29)	n.d.	n.d.	n.d.
Treatment (T1 - T4)	blossoms	408 - 412 (2002-04-25 - 29)	n.d. - <LOQ	n.d.	n.d.

Imidacloprid and Hydroxy-Metabolite: LOQ = 5 µg/kg LOD = 1.5 µg/kg
Olefin-Metabolite: LOQ = 10 µg/kg LOD = 3 µg/kg
n.d. = not detected

>>M-451700-01-2@S-603148-01-1

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Report: 02.02.01/19; [redacted]; 2004; M-451696-01-2
Title: Determination of the residue levels of imidacloprid and its metabolites hydroxy-imidacloprid and olefin-imidacloprid in leaves and blossoms of horse chestnut trees (Aesculus hippocastanum) after trunk injection - Application 2001 and sampling 2002
Report No.: M-451696-01-2
Document No.: M-451696-01-2
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **no**

<<M-451696-01-2@S-603097-01-1

Material and methods:
Four Horse Chestnut Trees *(Aesculus hippocastanum)* (T1 - T4) were treated by trunk injection with Imidacloprid SL 200 (Article No. 0004958608, Batch No. 0594*0.25, No. of sample: FAR00801-00) on 2001-05-09 at an application rate of 0.06 g a.s./cm stem diameter (=0.3 ml product/cm stem diameter in 42.6 mL water/cm stem diameter at a height of 1.3 m). The 4 control trees (C1 - C4) received no treatment. Sampling was carried out in 2002, one year after the application had been carried out. During flowering of the trees, blossoms and leaves were collected. Additional leaf samples were taken four times throughout the vegetation period. All samples were subjected to a residue analysis for Imidacloprid and its metabolites Hydroxy- Imidacloprid and Olefin-Imidacloprid.

Residue analysis was carried out on leaves and blossoms using the analytical method RA 00537 (1999, R. [redacted]).

Dates of biological work: 2001-05-09 to 2002-09-30

Dates of analytical work: 2002-03-25 to 2003-03-10

Findings: Summary of residues in leaves, blossom and nectar samples of 4 treatment and 4 control trees:

Treatment	Sample material	DAT	Imidacloprid [µg/kg]	Hydroxy-Imidacloprid [µg/kg]	Olefin-Imidacloprid [µg/kg]
Control (C1 - C4)	leaves	350 – 355 (2002-04-24 - 29)	n.d.	n.d.	n.d.
		384 (2002-05-28)	n.d. - <LOQ	n.d.	n.d.
		419 (2002-07-02)	n.d.	n.d.	n.d.
		447 (2003-07-30)	n.d.	n.d.	n.d.
		482 (2002-09-03)	n.d. - <LOQ	n.d.	n.d.
Treatment (T1 - T4)	leaves	350 – 355 (2002-04-24 - 29)	<LOQ - 29	n.d. – 14	n.d. - <LOQ
		384 (2002-05-28)	15 - 190	<LOQ - 58	n.d. – 11
		419 (2002-07-02)	7 - 92	<LOQ - 47	n.d. - <LOQ
		447 (2003-07-30)	16 - 53	7 - 49	n.d. – 10
		482 (2002-09-03)	12 - 20	<LOQ - 19	n.d. – 10
Control (C1 – C4)	blossoms	350 – 355 (2002-04-24 - 29)	n.d.	n.d.	n.d.
Treatment (T1 - T4)	blossoms	350 – 355 (2002-04-24 - 29)	n.d. - <LOQ	n.d.	n.d.

Imidacloprid and Hydroxy-Metabolite: LOQ = 5 µg/kg LOD = 1.5 µg/kg
Olefin-Metabolite: LOQ = 10 µg/kg LOD = 3 µg/kg
n.d. = not detected

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>>M-451696-01-2@S-603097-01-1

Report:	02.02.01/20; [redacted]; 2004; M-451667-01-3
Title:	Residues of imidacloprid WG 5 in blossom samples of Rhododendron sp. (variety Nova Zembla) after soil treatment in the field - Application: Spring 2003, sampling: 2003 and 2004
Report No.:	M-451667-01-3
Document No.:	M-451667-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-451667-01-3@S-604678-01-1

Material and methods: Eight year old *Rhododendron* plants (variety "Nova Zembla") growing at the experimental farmland "Höfchen" near Burscheid (Nordrhein-Westfalen, Germany) received pre- and post-flowering soil treatment with Imidacloprid WG 5 in two replicates (A and B; 8 plants each) per treatment group.

Soil application with Imidacloprid WG 5 (article No.: 0005439280, Batch No.: PF00000REC, TOX No. 6135-00, purity: 5,5%) dissolved in water at an application volume of 2 L per plant was carried out on 2003-05-09 (pre-flowering treatment) and 2003-06-05 (post-flowering treatment) at the application rates shown below. Control plants (treatment group 1) received no treatment.

Treatment Group	Application Rate per 50 cm Plant Height	Application Date	Sampling Date of Blossoms
1	Control		2003-05-20 and 26 (2nd time only replicate A), 2004-05-26 (only replicate A)
2	2500 mg a.s. pre-flowering	2003-05-09	2003-05-20 and 26
3	2500 mg a.s. post-flowering	2003-06-05	2004-05-26
4	1250 mg a.s. pre-flowering	2003-05-09	2003-05-20 and 26
5	1250 mg a.s. post-flowering	2003-06-05	2004-05-26
6	100 pre- plus 200 mg a.s. post-flowering	2003-05-09 and 2003-05-09	2003-05-20, 2004-05-26

*** sampled *Rhododendron* leaves were not analysed**

Samples of blossoms and leaves of *Rhododendron* sp. were collected from the control and the pre-flowering treatment groups 2, 4 and 6, eleven and 17 days after application (except for treatment group 6, with only 1 sampling date) and stored at -18° C until residue analysis.

After sample preparation, the blossoms were analysed for residues of Imidacloprid and its Olefin- and Hydroxy-Metabolites. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99) by R. [redacted].

In the year 2004 samples of blossoms and leaves of *Rhododendron* sp. were collected from the control and the post-flowering treatment groups 3, 5 and 6, 356 days after application. Samples were stored as in 2003 and blossoms analysed using the same method.

Dates of biological work:	2003-05-09 to 2004-05-26
Dates of analytical work:	2003-09-05 to 2003-09-22, 2004-07-21 to 2004-08-06

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Findings: In the following table the results of the residue analyses of **blossom** samples summarised.

Treatment group	DAT	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
1: Control	11	<LOD – 0.0125	<LOD - <LOQ	<LOD
	17*	<LOD – 0.0127	<LOD - <LOQ	<LOD - <LOQ
	356*	<LOD – 0.0188	<LOD - <LOQ	<LOD
2: (2500 mg a.s. per 50 cm plant height, pre-flowering)	11	<LOD – 0.0200	<LOD – 0.0063	< LOD
	17	<LOQ – 0.0232	<LOQ – 0.0087	< LOD
3: (2500 mg a.s. per 50 cm plant height, post-flowering)	356	0.1101 – 1.4396	0.0234 – 0.1575	<LOQ – 0.0298
4: (1250 mg a.s. per 50 cm plant height, pre-flowering)	11	<LOD – 0.0114	<LOD - <LOQ	< LOD
	17	<LOD – 0.0136	<LOD - <LOQ	< LOD
5: (1250 mg a.s. per 50 cm plant height, post-flowering)	356	0.0164 – 0.5430	0.0070 – 0.0682	<LOD – 0,0122
6: (100 mg a.s. per 50 cm pre-flowering, 200 mg a.s. per 50 cm plant height, post-flowering)	11	<LOD – 0.0168	<LOD - <LOQ	<LOD
	356	0.0518 – 0.1804	0.0082 – 0.0291	<LOD - <LOQ

DAT: day after application
Imidacloprid and Hydroxy-Metabolite: LOQ = 0.005 mg/kg LOD = 0.0015 mg/kg
Olefin-Metabolite: LOQ = 0.010 mg/kg LOD = 0.003 mg/kg
* only replicate A analysed as only this replicate was sampled

Conclusion:
Imidacloprid and its Hydroxy and Olefin metabolites were detected in both treated and control blossom samples in 2003. The residues found in the control samples are considered to originate from efficacy trials carried out with these plants between1997- 2000 which included drenching treatment before planting out in the field. The possibility of contamination occurring during sampling, storage or analytical work, has been investigated and can be ruled out. Since the residue levels lie within the same range in control and all pre-treatment groups, the treatments carried out in 2003 did obviously not significantly contribute to the residue levels detected.

In the high dose pre-flowering treatment group (application rate: 2500 mg a.s./50 cm plant height) residues up to 0.0232 mg lmidacloprid/kg, 0.0087 mg Hydroxy-Imidacloprid/ kg and 0.003 mg Olefin-lmidacloprid/kg were found 17 days after treatment.

For flowers from the low dose pre-flowering treatment group (application rate: 1250 mg a.s./50 cm plant height) residues up to 0.0136 mg lmidacloprid/kg, 0.0015 mg Hydroxy Imidacloprid/ kg and 0.003 mg Olefin-lmidacloprid/kg were found 17 days after treatment.

In the high dose post-flowering treatment group (application rate: 2500 mg a.s./50 cm plant height) residues up to 1.440 mg lmidacloprid/kg, 0.158 mg Hydroxy-Imidacloprid/ kg and 0.03 mg Olefin-lmidacloprid/kg were found 356 days after treatment.

For flowers from the low dose post-flowering treatment group (application rate: 1250 mg a.s./50 cm plant height) residues up to 0.543 mg lmidacloprid/kg, 0.068 mg Hydroxy-lmidacloprid/kg and 0.012 mg Olefin-lmidacloprid/kg were found 356 days after treatment.

In the group that had received pre-flowering treatment at 100 mg a.s. plus postflowering treatment 200 mg a.s./50 cm plant height, residues up to
0.180 mg lmidacloprid/kg, 0.029 mg Hydroxy-lmidacloprid/kg and below
0.010 mg Olefin-lmidacloprid/kg were found 356 days after treatment.

Residue levels in the control were at a comparable level as in the previous year.
>>M-451667-01-3@S-604678-01-1

Report:	02.02.01/21; [redacted]; 2004; M-451691-01-3
Title:	Residues of imidacloprid WG 5 in blossom samples of lime trees (Tilia europaea) after soil treatment in the field - Application: 2003, sampling: 2004
Report No.:	P672034513
Document No.:	M-451691-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-451691-01-3@S-604682-01-1

Dates of biological work: 2006-05-21 to 2006-08-08
Dates of analytical work: 2006-07-05 to 2006-10-19

Material and methods:
The study was carried out in 2 parts: the first part was conducted in spring 2006, during flowering of Rhododendron, and the second part in summer 2006, during flowering of Hibiscus.
Shrubs of the two species Rhododendron sp. and Hibiscus syriacus located at the area of Bayer CropScience AG (40789 Monheim, Nordrhein-Westfalen, Germany) received soil treatment with Imidacloprid WG 70 dissolved in water at an application volume of 2L per shrub on 2006-04-12 at the application rates given In Table 1 (treatment groups 2 and 4). Control shrubs (treatment groups 1 and 3), located in a distance of 200 m received no treatment.

Table 1: Summary: Treatment Groups and Rates

Treatment group	1	2	3	4
Treatment name	Rhododendron, untreated	Rhododendron, treated	Hibiscus, untreated	Hibiscus, treated
Application rates	-	4.3 g a.s./m average plant width*	-	4.3 g a.s./m average plant height*
		5.2 g a.s./shrub = 7.37 g product/shrub		4.3 g a.s./shrub = 6.14 g product/shrub

* To describe the size of the Rhododendron shrubs the parameter shrub width was used for fixing the application rate. For Hibiscus the parameter shrub height was used for fixing the application rate.

Each treatment group consisted of 3 parallel rows of 6 shrubs each, Rhododendron and Hibiscus respectively. At the exterior sides of the 2 outer rows with Rhododendron sp. and Hibiscus syriacus a mixture of bee-attractive potted ornamentals was planted or sown in flower beds. The composition of ornamental plants intends to reflect typical conditions as to be expected in North American home gardens. Between the shrub rows further ornamental plants (Pelargonium sp. and Surfinia sp.) were set up in flower boxes on the linen sheets with which the ground around the rows was covered.
Ornamental species composition for the Rhododendron part Fragaria sp.f Pulmonaria officinalis, Fuchsia sp. hybrids, Centaurea montana, Lobelia erinus and Lupinus sp. During the Rhododendron study period no other flowering bee-attractive plants were located in the near surroundings of both study plots.
Ornamental species composition for the Hibiscus part: Lavendula augustifolia, Calluna vulgaris, Centaurea montana, Phacelia tanacetifolia, Lobelia erinus, Helianthus sp. And Fragaria sp. Near the control plot (treatment group 3) Mayweed was growing on a field and next to the treatment plot (treatment group 4) flowering Gladiolus (not attractive for honeybees), Snapdragons and Larkspur (approx. 20% open blossoms, minimally bee attractive) were present during the study period.
In approx. 20-25 m distance to each plot 1 beehive (consisting of 11 combs at the start of the study and containing approx. 10,000 honeybees and a queen) was located. Two colonies of bumblebees (Bombus terrestris) per study part were placed next to each plot at the beginning of shrub flowering.

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Honeybees and bumblebees were observed for foraging activity and mortality for 10 days (39 days after the application in Rhododendron and 103 days after the application in Hibiscus)
Assessments on foraging activity of the honeybees and bumblebees were conducted once in the morning and once in the afternoon on 10 days during flowering of the Rhododendron shrubs, each time on the Rhododendron shrubs and the surrounding ornamentals separately from 2006-05-21 to 2006-05-24 (4 consecutive days) and from 2006-05-28 to 2006-06-01 (5 consecutive days). Due to the weather conditions on 2006-05-26 only one assessment in the morning was conducted; on 2006-06-02 the last afternoon assessment was made. Foraging assessments on the Hibiscus syriacus shrubs and the surrounding ornamentals were separately conducted once in the morning and once in the afternoon from 2006-07-25 to 2006-07-27 (3 consecutive days), from 2006-07-31 to 2006-08-04 (5 consecutive days) and from 2006-08-07 to 2006-08-09 (2 consecutive days). The mortality of honeybees and bumblebees was assessed in front of the hives/colonies and on linen sheets laid out between the shrub rows. Blossom samples were collected from 18 treated and 9 untreated plants during flowering of the respective shrub species. For Rhododendron this was conducted 35 days after the application and for Hibiscus 106-117 days after the application. Samples were stored at -18° C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy and Olefin-Metabolites by HPLC-MS/MS were performed according to method 01010 (MR-06/127).
Dead honeybees and bumblebees found on the linen sheets between the plants and in front of the bee hives and bumblebee colonies were also subjected to residue analysis for residues of Imidacloprid and its Olefin- and Hydroxy-Metabolites. Extraction and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS was performed according to method 00537/M002 (MR-6/144).

Findings:
In the *Tables* 2 and 3 the results of the foraging activity assessments in Rhododendron and Hibiscus are summarised.

Table 2: Summary: Foraging Activity of Honeybees and Bumblebees on Rhododendron

	Total number per species observed per plot [n]			
	Honeybees		Bumblebees	
Treatment group	Rhododendron	Ornamentals	Rhododendron	Ornamentals
1: Control	23	64	608	238
2: Treatment	10	104	107	87

Only few honeybees were observed foraging on *Rhododendron* shrubs on the control and treatment plot respectively, but more on the control than on the treatment plots.

Foraging activity of honeybees on the surrounding ornamentals was higher than on the Rhododendron plants, but higher on the treated than on the control plot.
The foraging activity of bumblebees on the *Rhododendron* plants was significantly higher on the untreated compared to the treated plants. The ornamental plants on the treated plot were likewise significantly less visited than those on the control plot.

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Table 3: Summary: Foraging Activity of Honeybees and Bumblebees on Hibiscus

	Total number per species observed per plot [n]			
	Honeybees		Bumblebees	
Treatment group	Hibiscus	Ornamentals	Hibiscus	Ornamentals
3: Control	10	192	233	837
4: Treatment	5	108	9	623

Again only few honeybees were observed foraging on *Hibiscus* shrubs on the control and on the treatment plot respectively. Foraging activity of honeybees on the surrounding ornamentals was lower on the treated plot compared with the control.
The foraging activity of bumblebees on the *Hibiscus* plants was distinctly higher on the control plot compared with the treated plot. The number of foraging bumblebees on the surrounding ornamentals was slightly higher on the control than on the treated plot.
Mortality observed is depicted in *Tables 4* and *5*. In the Rhododendron part of the study, in total 27 dead honeybees were found in the treatment group, while in the control group 2 dead honeybees were found. In the Hibiscus part, no dead honeybees were found at all. Dead bumblebees were not found in the control replicates, neither in the Rhododendron nor in the Hibiscus part. In the treatment replicates, in total 2 dead bumblebees were found in the Rhododendron part, and 14 dead bumblebees in the Hibiscus part.

Table 4: Summary: Mortality of Honeybees

	Rhododendron		Hibiscus	
	Total number [n]			
Treatment group	on the plot	in front of hive	on the plot	in front of hive
Control	0	2	0	0
Treatment	2	25	0	0

Table 5: Summary: Mortality of Bumblebees

	Rhododendron		Hibiscus	
	Total number [n]			
Treatment group	on the plot	in front of hive	on the plot	in front of hive
Control	0	0	0	0
Treatment	1	1	12	2

Colony health and condition of the honeybee colonies was not different before and after the study, neither in the control nor in the treatment. Colony health and condition of the bumblebee colonies after the Hibiscus part of the study were not different between treatment and control.[1]

In Table 6 the results of the residue analysis of the Rhododendron and Hibiscus blossom samples and the residues in honeybees and bumblebees are summarised.

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Table 6: Summary: Results of Residue Analysis

Treatment Group	Sample description	Study part	Sampling Date	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
1: Control	blossoms	Rh	2006-05-17	35	< LOQ	< LOQ	< LOQ
2: Treatment	blossoms	Rh	2006-05-17	35	0.09 – 0.79	0.01 – 0.04	< LOQ – 0.01
3: Control	blossoms	H	2006-07-27	106	< LOQ	< LOQ	< LOQ
4: Treatment	blossoms	H	2006-07-27 to 2006-08-07	106 - 117	0.76 – 5.01	< LOQ – 0.45	< LOQ – 0.33
1: Control	2 honey-bees (colony)	Rh	2006-05-29	47	0.005 – 0.022	< LOQ – 0.008	0.001 – 0.019
2: Treatment	25 honey-bees (colony)	Rh	2006-05-21 to 2006-05-31	39 - 49	< LOQ – 0.016	< LOQ – 0.001	< LOQ – 0.001
	2 honey-bees (plot)	Rh	2006-05-21 to 2006-05-31	39 - 49	0.002 – 0.091	< LOQ – 0.018	< LOQ – 0.001
	1 bumble-bee (colony)	Rh	2006-05-29	47	0.001	0.001	0.005
	1 bumble-bee (plot)	Rh	2006-05-31	49	0.006	0.003	0.003
4: Treatment	2 bumble-bees (colony)	H	2006-07-26	105	0.003 – 0.004	0.001 – 0.003	0.004 – 0.009
	12 bumble-bees (plot)	H	2006-07-25 to 2006-08-08	104 - 118	0.077 - 1.663	0.019 - 0.196	0.031 – 0.405

* DAT: days after treatment; Rh = Rhododendron; H = Hibiscus
Blossom samples: Imidacloprid, Hydroxy-Metabolite, Olefin-Metabolite; LOQ = 0.010 mg/kg
Insect samples: Imidacloprid, Hydroxy-Metabolite, Olefin-Metabolite; LOQ = 0.001 mg/kg

Residue levels in the Rhododendron blossoms were 0.09 - 0.79 mg imidacloprid/kg and in Hibiscus blossoms 0.76 - 5.01 mg Imidacloprid/kg in the treated replicates. In the blossoms of the untreated plants, residue levels were < LOQ. Residue levels found in dead honeybees in the control groups were 0.005 - 0.022 mg imidacloprid/kg. In dead honeybees in the treatment groups, residue levels were between < LOQ and 0.091 mg imidacloprid/kg. Residue levels found in dead bumblebees in the treatment groups were 0.001 - 1.663 mg imidacloprid/kg.

[1] Inspection of the bumblebee colonies exposed in the Rhododendron part of the study after end of exposure could not be conducted. Inspection of bumblebee colonies before start of exposure is generally not feasible technically.

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Report:	02.02.01/22; [redacted]; 2004; M-451699-01-3
Title:	Residues of imidacloprid WG 5 in blossom and leaf samples of apple trees after soil treatment in the field - Application: 2003, Sampling: 2004
Report No.:	P672034511
Document No.:	M-451699-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-451699-01-3@S-604696-01-1

Material and methods: Apple trees (variety James Grieve) growing at the Bayer CropScience AG experimental farmland "Höfchen", in the vicinity of Burscheid (Germany, Nordrhein-Westfalen), received soil treatment in autumn 2003 with Imidacloprid WG 5 in two replicates (A and B; 8 trees each) per treatment group.

Soil application with Imidacloprid WG 5 (active substance: NTN 33893; purity: 5.3%; material No.: 02295087 batch No.: PF000006PD) dissolved in water at an application volume of 2 L per tree was carried out on 2003-10-30 at the application rates given in the table below. Control trees received no treatment. In treatment group 2, 0.28 g a.s./cm stem diameter were applied and in treatment group 3, 0.14 g a.s./cm stem diameter.

The average stem diameter at a tree height of 1.3 m was 11 cm.

Treatment Group	Application Rate per Tree
1	untreated control
2	3.08 g a.s./tree = 58.08 g product/tree
3	1.54 g a.s./tree = 29.04 g product/tree

Blossom and leaf samples were collected once in spring 2004:
•blossoms at flowering on 2004-04-28 (181 days after application)
•leaves after flowering on 2004-05-14 (197 days after application)

The samples of blossoms and leaves were stored at -18° C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99) by R. [redacted].

Dates of biological work: 2003-10-30 to 2004-05-14
Dates of analytical work: 2004-05-04 to 2004-08-15

Findings: In the following table the results of the residue analyses of blossom and leave samples are summarised.

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Sampled Material	Treatment Group	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
Blossoms	1 (untreated control)	181	<LOD – <LOQ**	<LOD	<LOD
	2 (3.08 g a.s./tree = 58.08 g product/tree)	181	<LOD	<LOD	<LOD
	3 (1.54 g a.s./tree = 29.04 g product/tree)	181	<LOD	<LOD	<LOD
Leaves	1 (untreated control)	197	<LOQ – <LOD	<LOD	<LOD
	2 (3.08 g a.s./tree = 58.08 g product/tree)	197	<LOD – 0.012	<LOD – 0.013	<LOQ – <LOD

Imidacloprid and Hydroxy-Metabolite: LOQ = 0.005 mg/kg LOD = 0.0015 mg/kg
Olefin-Metabolite: LOQ = 0.010 mg/kg LOD = 0.003 mg/kg

* DAT: days after treatment

** In 1 of 16 control samples residues <LOD were detected. No identification of the origin of this contamination was found.

>>M-451699-01-3@S-604696-01-1

Report: 02.02.01/23; [redacted]; 2004; M-451694-01-3
Title: Residues of imidacloprid WG 5 in blossom samples of Rhododendron sp. after soil treatment in the field - Application: Autumn 2003, sampling: 2004
Report No.: P67203451
Document No.: M-451694-01-3
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **no**

<<M-451694-01-3@S-604692-01-1

Material and methods:
Shrubs of the species *Rhododendron* sp. planted in 2003 at the experimental farmland "Höfchen" near Burscheid (Nordrhein-Westfalen, Germany) received soil treatment in autumn 2003 with Imidacloprid WG 5 in two replicates (A and B; 8 shrubs each) per treatment group.
Soil application with Imidacloprid WG 5 (active substance: NTN 33893, purity: 5.3%, material No.: 02295087, batch No.: PF000006P01) dissolved in water at an application volume of 1.5 L per shrub was carried out on 2003-11-26 at the application rates given in the table below. Control shrubs received no treatment. In treatment group 2 4.3 g a.s./m shrub height were applied and in treatment group 3, 2.15 g a.s./m shrub height.

The average shrub height was 0.4 m.

No. of Treatment Group	Application Rate per Shrub
1	untreated control
2	1.72 g a.s./shrub = 32.5 g product/shrub
3	0.86 g a.s./shrub = 16.2 g product/shrub

Blossom and leaf samples were collected once at flowering on 2004-05-19 (175 days after application). The sampled Rhododendron leaves were not analysed.

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The samples of blossoms and leaves were stored at -18°C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99) by R. [redacted].

Dates of biological work: 2003-11-26 to 2004-05-19
Dates of analytical work: 2004-06-03 to 2004-07-06

Findings: In the following table the results of the residue analyses of blossom samples are summarised.

Sampled Material	Treatment Group	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
Blossoms	1: untreated control	175	< LOD	< LOD	< LOD
	2: 1.72 g a.s./shrub = 32.5 g product/shrub	175	0.027 – 0.85	<LOQ – 0.064	< LOD – 0.0082
	3: 0.86 g a.s./shrub = 16.2 g product/shrub	175	0.012 – 0.37	< LOQ – 0.043	< LOD - <LOQ

* DAT: days after treatment
Imidacloprid and Hydroxy-Metabolite: LOQ = 0.005 mg/kg LOD = 0.0015 mg/kg
Olefin-Metabolite: LOQ = 0.010 mg/kg LOD = 0.003 mg/kg

>>M-451694-01-3@S-604692-01-1

Report: 02.02.01/24; [redacted]; 2005; M-451662-01-3
Title: Residues of Imidacloprid WG 5 in blossom samples of Cornus mas after soil treatment in the field - Application: 2003, sampling: 2005
Report No.: M-451662-01-3
Document No.: M-451662-01-3
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **no**

<<M-451662-01-3@S-604693-01-1

Material and methods: Shrubs of the species Cornus mas growing at the market garden "Selders" (Elberfelderstr. 217, 42781 Haan) in Germany (Nordrhein-Westfalen), received soil treatment in autumn with Imidacloprid WG 5 in two replicates (A and B: 8 shrubs each) per treatment group.

Soil application with Imidacloprid WG 5 (active substance: NTN 33893, purity: 5.3%, material No.: 02295087, batch No.: PF00006PD) dissolved in water at an application volume of 1.5 L per shrub was carried out on 2003-10-31 at the application rates given in the table below. Control shrubs (treatment group 1) received no treatment. In treatment group 2,4.3 g a.s./m shrub height were applied and in treatment group 3, 2.15 g a.s./m shrub height. The average shrub height was 1.2 m.

No. of Treatment Group	Application Rate per Shrub
1	untreated control
2	5.16 g a.s./shrub = 97.4 g product/shrub
3	2.58 g a.s./shrub = 48.7 g product/shrub

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Blossom and leaf samples were collected once in spring 2005:

- blossoms at flowering on 2005-03-17 (505 days after application)
- leaves after flowering on 2005-04-21 (540 days after application)

The samples of blossoms and leaves were stored at -18° C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites on the blossoms were carried out. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites were performed by HPLC-MS/MS.

Dates of biological work: **2005-03-17 to 2005-04-21**

Dates of analytical work: **2005-06-23 to 2005-06-30**

Findings: In the following table the results of the residue analyses of samples of blossoms are summarised.

Sampled Material	Treatment Group	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
Blossoms	1: untreated control**	505	< LOQ	< LOQ	< LOQ
	2: 5.16 g a.s./shrub = 97.4 g product/shrub***	505	1.038 – 2.816	0.029 – 0.088	0.009 – 0.030
	3: 2.58 g a.s./shrub = 48.7 g product/shrub****	505	1.537 – 3.374	0.087 – 0.155	0.014 – 0.063

* DAT: days after treatment
** number of samples in total: 3
*** number of samples in total: 3
**** number of samples in total: 6
Imidacloprid and Hydroxy-Metabolite: LOQ = 0.005 mg/kg LOD = 0.0015 mg/kg
Olefin-Metabolite: LOQ = 0.010 mg/kg LOD = 0.003 mg/kg

The sampled leaves were not analysed.

>>M-451662-01-1 >>S-604677-01-1

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Report:	02.02.01/25; [redacted]; 2005; M-451656-01-3
Title:	Residues of imidacloprid WG 5 in blossom and leaf samples of Amelanchier sp. after soil treatment in the field - Application: 2003, sampling: 2004 and 2005
Report No.:	M-451656-01-3
Document No.:	M-451656-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-451656-01-3@S-604676-01-1

Material and methods: Shrubs of the species Amelanchier sp. growing at the market garden "Selders" (Elberfelderstr. 217, 42781 Haan) in Germany (Nordrhein-Westfalen), received soil treatment in autumn with Imidacloprid WG 5 in two replicates (A and B: 8 shrubs each) per treatment group.

Soil application with Imidacloprid WG 5 (active substance: NTN 33893, purity: 5.3%, material No.: 02295087, batch No.: PF000006PD) dissolved in water at an application volume of 1.5 L per shrub was carried out on 2003-10-31 at the application rates given in the table below. Control shrubs (treatment group 1) received no treatment. In treatment group 2, 4.3 g a.s./m shrub height were applied and in treatment group 3, 2.15 g a.s./m shrub height. The average shrub height was 1.6 m.

No. of Treatment Group	Application Rate per Shrub
1	untreated control
2	6.88 g a.s./shrub = 129.8 g product/shrub
3	3.44 g a.s./shrub = 64.9 g product/shrub

Blossom and leaf samples were collected once in spring 2004:

- blossoms at flowering on 2004-04-14 (166 days after application)
- leaves after flowering on 2004-05-04 (186 days after application)

The samples of blossoms and leaves were stored at -18° C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites were performed by HPLC-MS/MS.

Blossom and leaf samples were collected once in spring 2005:

- blossoms at flowering on 2005-04-21 (540 days after application)
- leaves after flowering on 2005-06-14 (594 days after application)

The samples of blossoms and leaves were stored at -18°C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. The sampled leaves from 2005 were not analysed. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin Metabolites were performed by HPLC-MS/MS. The leaf samples were not analysed.

Dates of biological work: 2003-10-31 to 2004-05-04
and 2005-04-20 to 2005-06-14

Dates of analytical work: 2004-04-21 to 2004-05-29

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and 2005-06-27 to 2005-07-12

Findings: In the following table the result of the residue of the analyses of samples of blossoms and leaves are summarized.

Sampled Material	Treatment Group	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
Blossoms	1: untreated control	166	< LOD	< LOD	< LOD
	2: 6.88 g a.s./shrub = 129.8 g product/shrub	166	< LOD	< LOD	< LOD
	3: 3.44 g a.s./shrub = 64.9 g product/shrub	166	< LOD	< LOD	< LOD
Leaves	1: untreated control	186	< LOD	< LOD	< LOD
	2: 6.88 g a.s./shrub = 129.8 g product/shrub	186	0.56 – 3.2	0.086 – 0.47	0.018 – 0.15
	3: 3.44 g a.s./shrub = 64.9 g product/shrub	186	0.79 – 2.8	0.11 – 0.45	0.014 – 0.16
Blossoms	1: untreated control	540	< LOQ	< LOQ	< LOQ
	2: 6.88 g a.s./shrub = 129.8 g product/shrub	540	0.70 – 4.56	0.22 – 1.31	0.12 – 0.79
	3: 3.44 g a.s./shrub = 64.9 g product/shrub	540	0.66 – 2.84	0.19 – 0.60	0.15 – 0.53

* DAT: days after treatment
Imidacloprid and Hydroxy-Metabolite: LOQ = 0.005 mg/kg LOD = 0.0015 mg/kg
Olefin-Metabolite: LOQ = 0.010 mg/kg LOD = 0.003 mg/kg

The sampled leaves from 2005 were not analysed.

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Report: 02.02.01/26; [redacted]; 2005; M-451673-01-3
Title: Residues of imidacloprid WG 5 in blossom samples of shrubs of different sizes of the species Rhododendron sp after drenching application in he field - Application 2004, Sampling: 2005
Report No.: M-451673-01-3
Document No.: M-451673-01-3
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **no**

<<M-451673-01-3@S-604679-01-1

Material and methods:
Shrubs of the species *Rhododendron* sp. growing on the area of a market garden in Rastede near Bad Zwischenahn (Niedersachsen, Germany) received soil treatment in autumn 2004 with Imidacloprid WG 5.in 3.treatment groups defined by plant size. Each, treatment group consisted of 4 sub-treatment groups defined by application rate in two replicates per sub-treatment group. Nine shrubs were used per replicate.

Soil application with Imidacloprid WG 5 (active substance: NTN 33893, purity: 5%, material Nos. 0249273, batch No.: PF000006PD) dissolved in water at an application volume of 2 L per shrub was carried out on 2004-10-28 at the application rates given in the table below. Control shrubs received no treatment. In sub-treatment group 2, 4.3 g a.s./m shrub size were applied, in sub-treatment group 3 2.15 g a.s./m shrub size and in sub-treatment 4:1.075 g a.s./m shrub size. The shrub size was defined by the shrub width. The shape of the plants was approx. spherical.

Treatment Group/ Plant Size	Sub-Treatment	Application Rate per Shrub
1 / 0.5 m	1	untreated control
	2	2.15 g a.s./shrub = 43 g product/shrub
	3	1.075 g a.s./shrub = 21.5 g product/shrub
	4	0.538 g a.s./shrub = 10.75 g product/shrub
2 / 1 m	1	untreated control
	2	4.3 g a.s./shrub = 86 g product/shrub
	3	2.15 g a.s./shrub = 43 g product/shrub
	4	1.075 g a.s./shrub = 21.5 g product/shrub
3 / 1.5 m	1	untreated control
	2	6.45 g a.s./shrub = 129 g product/shrub
	3	3.225 g a.s./shrub = 64.5 g product/shrub
	4	1.611 g a.s./shrub = 32.25 g product/shrub

Blossom samples were collected from all plants of the treatment group 2 on 2005-06-01 (216 days after the application) and only from those plants which were flowering in treatment group 1 (in total 19 plants). Blossoms of plant in treatment group 2 were already at a final flowering stage. From ail other plants of treatment group 1 and from all plants of treatment group 3 no sampling of blossoms was possible as due to the cold winter all flower buds were frozen to death.
Samples of leaves were collected once for all plants on 2005-05-31 (215 days after the application) for treatment group 3 and on 2005-06-01 (216 days after the application) for the treatment groups 1 and 2.

Young shoots were collected after flowering on 2005-06-20 (235 days after application) for treatment group 3 and on 2005-06-21 (236 days after application) for treatment groups 1 and 2.

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The sample boxes containing the blossoms, leaves and young shoots were stored at approx. -80°C on dry ice after sampling. After arrival in Monheim the samples were transferred into a freezer and stored at -18° C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99).

The sampled *Rhododendron* leaves and the young shoots were not analysed.

Dates of biological work: 2004-10-28 to 2005-06-21
Dates of analytical work: 2005-06-22 to 2005-07-13

Findings: In the following table the results of the residue analyses of blossom samples and samples of young shoots are summarised. Sampled leaves were not analysed.

Treatment Group/ Plant Size	Sub-Treatment	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
1 / 0.5 m, blossoms	2 (4.3 g a.s./m shrub size)**	216	0.108 – 0.512	0.011 – 0.056	< LOQ
	3 (2.15 g a.s./m shrub size)***	216	0.060 – 0.274	0.007 – 0.032	< LOQ
	4 (1.075 g a.s./m shrub size)****	216	0.017 – 0.234	< LOQ – 0.018	< LOQ
2 / 1 m, blossoms	1 (Control)	216	< LOQ	< LOQ	< LOQ
	2 (4.3 g a.s./m shrub size)	216	0.017 – 0.121	< LOQ – 0.014	< LOQ
	3 (2.15 g a.s./m shrub size)	216	0.031 – 0.205	< LOQ – 0.022	< LOQ
	4 (1.075 g a.s./m shrub size)	216	0.013 – 0.105	< LOQ – 0.013	< LOQ

* DAT: days after treatment
** total number of samples: 8
*** total number of samples: 8
**** total number of samples: 8
Imidacloprid and Hydroxy-Metabolite: LOQ = 0.005 mg/kg LOD = 0.0015 mg/kg
Olefin-Metabolite: LOQ = 0.010 mg/kg LOD = 0.003 mg/kg

>>M-451673-01-3@S-604679-01-1

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Report:	02.02.01/27; [redacted]; 2006; M-451677-01-3
Title:	Assessment of effects of imidacloprid WG 70 on foraging activity and mortality of honey bees and bumblebees after drenching application under field condition on shrubs of the species Rhododendron catawbiense grandiflorum surrounded by other ornamental plant species
Report No.:	M-451677-01-3
Document No.:	M-451677-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-451677-01-3@S-604680-01-1

Material and methods:

Shrubs of the species Rhododendron catawbiense grandiflorum located at the experimental farmland "Laacher Hof" near Monheim (40789 Monheim, Nordrhein-Westfalen, Germany) received soil treatment with Imidacloprid WG 70 dissolved in water at an application volume of 1 L per shrub in winter of 2005 (2005-01-13) at the application rates given in Table 1. Control shrubs (treatment group 1) received no treatment. Each treatment group consisted of 3 parallel rows of 10 Rhododendron plants.

Table 1: Summary: Treatment Groups and Rates

Treatment group	1	2	3
Application rates	-	4.5 g a.s./m plant size*	2.15 g a.s./m plant size*
	control	2.58 g a.s./shrub = 3.68 g product/shrub	1.29 g a.s./ shrub = 1.84 product/shrub
Water volume rate per plant	1 L tap water		

* plants were 0.6 m high/wide

Between the rows of Rhododendron catawbiense grandiflorum, a mixture of bee attractive. potted ornamentals in watering trays was set up on the linen sheets between the Rhododendron rows an 2005-05-19. The species composition of the ornamentals was as follows: Fuchsia sp.: variety "Beacon", strawberry plant: variety "Fragaria", Alyssum sp., Lantana camara and Lobelia sp. In the near surroundings of the study site no other flowering crops were located.

One hive colony of honey bees Apis mellifera and 3 colonies of bumblebees Bombus terrestris were placed next to the Rhododendron catawbiense grandiflorum shrubs on 2005-05-20 {honey bees) and 2005-05-21 (bumblebees). Assessments on foraging activity of the honey bees and bumblebees were conducted on 10 days during flowering of the Rhododendron catawbiense grandiflorum shrubs from 2005-05-21 to -25 (5 consecutive days), an 2005-05-27 (1 day) and from 2005-05-30 to -06-02 (5 consecutive days) once in the morning and once in the afternoon separately on the Rhododendron plants and the surrounding ornamentals. The mortality of honey bees and bumblebees was assessed in front of the hives/colonies and on linen sheets laid out between the Rhododendron rows.

Blossom samples were collected from 15 Rhododendron plants per treatment group on 2005-05-19 (126 days after the application) and stored at -18°C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99).

Dates of biological work: 2005-01-13 to 2005-06-02
Dates of analytical work: 2005-06-21 to 2005-07-13

Findings:

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In Table 2 the results of the residue analyses of blossom samples are summarised.

Table 2: Summary: Results of Residue Analysis

Treatment Group	Sampling Date	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
1 (untreated control)	2005-05-19	126	< LOQ**	< LOQ**	< LOQ**
2 (4.3 g a.s./m plant size= 2.58 g a.s./shrub)	2005-05-19	126	0.488 – 1.996	0.073 – 0.215	< LOQ – 0.027
3 (2.15 g a.s./m plant size= 1.29 g a.s./shrub)	2005-05-19	126	0.092 – 0.812	0.014 – 0.060	< LOQ – 0.014

* DAT: days after treatment
** In 1 of 15 control samples residues were detected. No identification of the origin of this contamination was found.
Imidacloprid and Hydroxy-Metabolite: LOQ = 0.005 mg/kg LOD = 0.0015 mg/kg
Olefin-Metabolite: LOQ = 0.010 mg/kg LOD = 0.003 mg/kg

In Table 3 the results of the foraging activity assessments are summarized:

Table 3: Summary: Foraging Activity of Bumblebees (BB) and Honey Bees (B)

Treatment group	Rhododendron am		Ornamentals am		Rhododendron pm		Ornamentals pm	
	BB	B	BB	B	BB	B	BB	B
Control	120	0	2 (Fuchsia)	1 (strawberry)	126	1	2 (Fuchsia)	2 (strawberry, *Lobelia* sp.)
2.15 g a.s./m plant size	72	0	1 (Fuchsia)	0	59	0	2 (Fuchsia)	1 (strawberry)
4.3 g a.s./m plant size	70	0	0	0	63	0	0	1 (*Lobelia* sp.)

The foraging activity of bumblebees on the Rhododendron plants was comparable between the morning and the afternoon assessments. The highest numbers of foraging bumblebees were found in the control. The foraging activity of bumblebees was lower in the treatment groups 2 and 3 but with comparable numbers in both treatment groups. The ornamental plants were only scarcely visited by the bumblebees in the morning and in the afternoon.

Throughout the study only one honey bee was observed foraging on a Rhododendron plant (control). In none of the other treatment groups visits on this plant species occurred. Also the ornamental plants were only scarcely visited by the honey bees. Honey bees were observed to forage on strawberry and Lobelia sp. The beekeeper noticed that bees returning to the hive carried yellow pollen, which probably originated from plants other than the ornamentals set up in this study. However, in the near surrounding of the study site no other flowering crops were located.

No dead honey bees worker bees or bumblebees were found throughout the study on the individually labelled linen sheets laid out between the Rhododendron catawbiense grandiflorum rows and the rows of the surrounding potted ornamental plants and the linen sheets placed in front of the bee hive and the bumblebee colonies.

Conclusion:

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In this field study no effects on mortality were observed on bumblebees and honey bees foraging on Rhododendron catawbiense grandiflorum plants surrounded by a species composition of ornamental plants (Fuchsia sp., strawberry plant, Alyssum sp., Lantana camara and Lobelia sp. The Rhododendron catawbiense grandiflorum plants had received a soil drench treatment 126 days before the start of the study with lmidacloprid WG 70 at either 4.3 g a.s./m plant size (2.58 g a.s./shrub = 3.68 g product/shrub) resulting in residues in blossoms up to 1.996 mg imidacloprid/kg or at 2.15 g a.s./m plant size (1.29 g a.s./ shrub = 1.84 product/shrub) resulting in residues in blossoms up to 0.812 mg imidacloprid/kg.

Untreated Rhododendron catawbiense grandiflorum plants were visited more frequently by the bumblebees than the treated ones, but frequency of visits was within a comparable order of magnitude between the sets of Rhododendron treated at different rates. Alternative ornamental plants were visited only very scarcely.

No behavioural anomalies were observed.

>>M-451677-01-3@S-604680-01-1

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Report:	02.02.01/28; [redacted]; 2007; M-451681-01-3
Title:	Assessment of effects of a drench application of imidacloprid WG 70 to shrubs of Rhododendron sp and to Hibiscus syriacus on foraging activity and mortality of honey bees and bumblebees under field conditions
Report No.:	M-451681-01-3
Document No.:	M-451681-01-3
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-451681-01-3@S-604681-01-1

Material and methods:
The study was carried out in 2 parts: the first part was conducted in spring 2006, during flowering of Rhododendron, and the second part in summer 2006, during flowering of Hibiscus.
Shrubs of the two species Rhododendron sp. and Hibiscus syriacus located at the area of Bayer CropScience AG (40789 Monheim, Nordrhein-Westfalen, Germany) received soil treatment with Imidacloprid WG 70 dissolved in water at an application volume of 2 L per shrub on 2006-04-12 at the application rates given In Table 1 (treatment groups 2 and 4). Control shrubs (treatment groups 1 and 3), located in a distance of 200 m received no treatment.

Table 1: Summary: Treatment Groups and Rates

Treatment group	1	2	3	4
Treatment name	Rhododendron, untreated	Rhododendron, treated	Hibiscus, untreated	Hibiscus, treated
Application rates	-	4.3 g a.s./m average plant width*	-	4.3 g a.s./m average plant height*
	-	5.2 g a.s./shrub = 7.37 g product/shrub	-	4.3 g a.s./shrub = 6.14 g product/shrub

* To describe the size of the Rhododendron shrubs the parameter shrub width was used for fixing the application rate. For Hibiscus the parameter shrub height was used for fixing the application rate.

Each treatment group consisted of 3 parallel rows of 6 shrubs each, Rhododendron and Hibiscus respectively. At the exterior sides of the 2 outer rows with Rhododendron sp. and Hibiscus syriacus a mixture of bee-attractive potted ornamentals was planted or sown in flower beds. The composition of ornamental plants intends to reflect typical conditions as to be expected in North American home gardens. Between the shrub rows further ornamental plants (Pelargonium sp. and Surfinia sp.) were set up in flower boxes on the linen sheets with which the ground around the rows was covered. Ornamental species composition for the Rhododendron part Fragaria sp., Pulmonaria officinalis, Fuchsia sp. hybrids, Centaurea montana, Lobelia erinus and Lupinus sp. During the Rhododendron study period no other flowering bee-attractive plants were located in the near surroundings of both study plots.
Ornamental species composition for the Hibiscus part: Lavendula augustifolia, Calluna vulgaris, Centaurea montana, Phacelia tanacetifolia, Lobelia erinus, Helianthus sp. And Fragaria sp. Near the control plot (treatment group 3) Mayweed was growing on a field and next to the treatment plot (treatment group 4) flowering Gladiolus (not attractive for honeybees), Snapdragons and Larkspur (approx. 20% open blossoms, minimally bee attractive) were present during the study period.

In approx. 20-25 m distance to each plot 1 beehive (consisting of 11 combs at the start of the study and containing approx. 10,000 honeybees and a queen) was located. Two colonies of bumblebees (Bombus terrestris) per study part were placed next to each plot at the beginning of shrub flowering. Honeybees and bumblebees were observed for foraging activity and mortality for 10 days (39 days after the application in Rhododendron and 103 days after the application in Hibiscus) Assessments on foraging activity of the honeybees and bumblebees were conducted once in the morning and once in the afternoon

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on 10 days during flowering of the Rhododendron shrubs, each time on the Rhododendron shrubs and the surrounding ornamentals separately from 2006-05-21 to 2006-05-24 (4 consecutive days) and from 2006-05-28 to 2006-06-01 (5 consecutive days). Due to the weather conditions on 2006-05-26 only one assessment in the morning was conducted; on 2006-06-02 the last afternoon assessment was made. Foraging assessments on the Hibiscus syriacus shrubs and the surrounding ornamentals were separately conducted once in the morning and once in the afternoon from 2006-07-25 to 2006-07-27 (3 consecutive days), from 2006-07-31 to 2006-08-04 (5 consecutive days) and from 2006-08-07 to 2006-08-09 (2 consecutive days). The mortality of honeybees and bumblebees was assessed in front of the hives/colonies and on linen sheets laid out between the shrub rows. Blossom samples were collected from 18 treated and 9 untreated plants during flowering of the respective shrub species. For Rhododendron this was conducted 35 days after the application and for Hibiscus 106-117 days after the application. Samples were stored at -18°C until the sample preparation and eventually residue analysis for Imidacloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy-and Olefin-Metabolites by HPLC-MS/MS were performed according to method 01010 (MR-06/127). Dead honeybees and bumblebees found on the linen sheets between the plants and in front of the bee hives and bumblebee colonies were also subjected to residue analysis for residues of Imidacloprid and its Olefin- and Hydroxy-Metabolites. Extraction and determination of Imidacloprid, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS was performed according to method 00537/M002 (MR-6/144).

Findings:
In the Tables 2 and 3 the results of the foraging activity assessments in Rhododendron and Hibiscus are summarised.

Table 2: Summary: Foraging Activity of Honeybees and Bumblebees on Rhododendron

	Total number per species observed per plot [n]			
	Honeybees		Bumblebees	
Treatment group	Rhododendron	Ornamentals	Rhododendron	Ornamentals
1: Control	23	64	608	238
2: Treatment	10	104	107	87

Only few honeybees were observed foraging on Rhododendron shrubs on the control and treatment plot respectively, but more on the control than on the treatment plots.

Foraging activity of honeybees on the surrounding ornamentals was higher than on the Rhododendron plants, but higher on the treated than on the control plot. The foraging activity of bumblebees on the Rhododendron plants was significantly higher on the untreated compared to the treated plants. The ornamental plants on the treated plot were likewise significantly less visited than those on the control plot.

Table 3: Summary: Foraging Activity of Honeybees and Bumblebees on Hibiscus

	Total number per species observed per plot [n]			
	Honeybees		Bumblebees	
Treatment group	Hibiscus	Ornamentals	Hibiscus	Ornamentals
3: Control	10	192	233	837
4: Treatment	5	108	9	623

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Again only few honeybees were observed foraging on Hibiscus shrubs on the control and on the treatment plot respectively. Foraging activity of honeybees on the surrounding ornamentals was lower on the treated plot compared with the control. The foraging activity of bumblebees on the Hibiscus plants was distinctly higher on the control plot compared with the treated plot. The number of foraging bumblebees on the surrounding ornamentals was slightly higher on the control than on the treated plot. Mortality observed is depicted in Tables 4 and 5. In the Rhododendron part of the study, in total 27 dead honeybees were found in the treatment group, while in the control group 2 dead honeybees were found. In the Hibiscus part, no dead honeybees were found at all. Dead bumblebees were not found in the control replicates, neither in the Rhododendron nor in the Hibiscus part. In the treatment replicates, in total 2 dead bumblebees were found in the Rhododendron part, and 14 dead bumblebees in the Hibiscus part.

Table 4: Summary: Mortality of Honeybees

	Rhododendron		Hibiscus	
	Total number [n]			
Treatment group	on the plot	in front of hive	on the plot	in front of hive
Control	0	2	0	0
Treatment	2	25	0	0

Table 5: Summary: Mortality of Bumblebees

	Rhododendron		Hibiscus	
	Total number [n]			
Treatment group	on the plot	in front of hive	on the plot	in front of hive
Control	0	0	0	0
Treatment	1	1	12	2

Colony health and condition of the honeybee colonies was not different before and after the study, neither in the control nor in the treatment. Colony health and condition of the bumblebee colonies after the Hibiscus part of the study were not different between treatment and control.[1]

In Table 6 the results of the residue analysis of the Rhododendron and Hibiscus blossom samples and the residues in honeybees and bumblebees are summarised.

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Table 6: Summary: Results of Residue Analysis

Treatment Group	Sample description	Study part	Sampling Date	DAT*	Imidacloprid [mg/kg]	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]
1: Control	blossoms	Rh	2006-05-17	35	< LOQ	< LOQ	< LOQ
2: Treatment	blossoms	Rh	2006-05-17	35	0.09 – 0.79	0.01 – 0.04	< LOQ – 0.01
3: Control	blossoms	H	2006-07-27	106	< LOQ	< LOQ	< LOQ
4: Treatment	blossoms	H	2006-07-27 to 2006-08-07	106 - 117	0.76 – 5.01	< LOQ – 0.45	< LOQ – 0.33
1: Control	2 honey-bees (colony)	Rh	2006-05-29	47	0.005 – 0.022	< LOQ – 0.008	0.001 – 0.019
2: Treatment	25 honey-bees (colony)	Rh	2006-05-21 to 2006-05-31	39 - 49	< LOQ – 0.016	< LOQ – 0.001	< LOQ – 0.001
	2 honey-bees (plot)	Rh	2006-05-21 to 2006-05-31	39 - 49	0.002 – 0.091	< LOQ – 0.018	< LOQ – 0.001
	1 bumble-bee (colony)	Rh	2006-05-29	47	0.001	0.001	0.005
	1 bumble-bee (plot)	Rh	2006-05-31	49	0.005	0.003	0.003
4: Treatment	2 bumble-bees (colony)	H	2006-07-26	105	0.003 – 0.004	0.001 – 0.003	0.004 – 0.009
	12 bumble-bees (plot)	H	2006-07-25 to 2006-08-08	104 - 118	0.077- 1.663	0.019 - 0.196	0.031 – 0.405

* DAT: days after treatment Rh Rhododendron H Hibiscus
Blossom samples: Imidacloprid, Hydroxy-Metabolite, Olefin-Metabolite: LOQ = 0.010 mg/kg
Insect samples: Imidacloprid, Hydroxy-Metabolite, Olefin-Metabolite: LOQ = 0.001 mg/kg

Residue levels in the Rhododendron blossoms were 0.09 - 0.79 mg imidacloprid/kg and in Hibiscus blossoms 0.76 - 5.01 mg Imidacloprid/kg in the treated replicates. In the blossoms of the untreated plants, residue levels were < LOQ. Residue levels found in dead honeybees in the control groups were 0.005 - 0.022 mg imidacloprid/kg. In dead honeybees in the treatment groups, residue levels were between < LOQ and 0.091 mg imidacloprid/kg. Residue levels found in dead bumblebees in the treatment groups were 0.001 -1.663 mg imidacloprid/kg.

[1] Inspection of the bumblebee colonies exposed in the Rhododendron part of the study after end of exposure could not be conducted. Inspection of bumblebee colonies before start of exposure is generally not feasible technically.

>>M-451688-01-3@S-604681-01-1

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Report:	02.02.01/29;[redacted];[redacted];[redacted]; 2007; M-016828-02-3
Title:	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. Laacher Hof 1999
Report No.:	SXR/AM 008
Document No.:	M-016828-02-3
Guideline(s):	850.3040
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-016828-02-3@S-602076-01-1

Material and methods: summer rape seed (variety "Lisonne") either dressed with 25 ml/kg Poncho FS 500 (a.i. content: 79.7 g/L beta-Cyfluthrin and 427.4 g/L imidacloprid; batch no. 6200/0055 A according to formulation no. 6200/0059, developmental no. 00195939) or imidacloprid-free were drilled on 12 May 99 in soils with different imidacloprid residue levels. Soil residue levels were analytically determined immediately before drilling. Drilling rate was 3.25 kg/ha. During peak flowering of the summer rape (mid of July) small bee colonies (2,000 to 3,000 honeybees) were caged on these plots (appr. 50 m^2) as a sampling device for summer rape nectar and pollen. In addition, some nectar and flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples and a small sample of honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 12 – 19, 1999.
Dates of soil analysis: August 8 – 13, 1999.
Dates of analysis of biological samples: September 25 – 29, 1999.

Findings: Residues in soil, in summer rape plant matrices planted as succeeding crop and in honeybees used as sampling device. (detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (field number 711)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid. n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

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Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1997“ (field number 710)			
Soil sample (0-30 cm)	0.016	--	--
Leaves (produced latest)	< LOQ	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1998“ (field number 702)			
Soil sample (0-30 cm)	0.015	--	--
Leaves (produced latest)	< LOQ	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	< LOQ	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1998 (2x)“ (field number A XII)			
Soil sample (0-30 cm)	0.014	--	--
Leaves (produced latest)	< LOQ	n.d.	< LOQ
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	< LOQ	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1999“ (field number 711)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	< LOQ	n.d.	< LOQ
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	< LOQ	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid; n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

Observations: No treatment-related behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortality was observed on the honeybee colonies used for collecting summer rape nectar and pollen. The small colonies were remained till 3 September. The final check on this day did also reveal no abnormalities in either colony strength or brood status.

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Report:	02.02.01/30; [redacted]; [redacted]; [redacted]; 2007; M-016842-02-3
Title:	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 residue on foraging honeybees. 'Hoefchen' 1999
Report No.:	SXR/AM 010
Document No.:	M-016842-02-3
Guideline(s):	US EPA OCSPP Giuideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-016842-02-3@S-604919-01-1

Material and methods: summer rape seed (variety "Lisonne") either dressed with 25 ml/kg Poncho FS 500 (a.i. content: 79.7 g/L beta-Cyfluthrin and 427.4 g/L imidacloprid; batch no. 6200/0055 A according to formulation no. 6200/0059, developmental no. 00195939) or imidacloprid-free were drilled on 11 May 99 in soils with different imidacloprid residue levels. Soil samples for an analytical determination of the imidacloprid residue level were taken immediately before drilling. Drilling rate was 7 kg/ha. During peak flowering of the summer rape (mid of July) small bee colonies (2,000 to 3,000 honeybees) were caged on these plots (appr. 50 m ^{2}) as a sampling device for summer rape nectar and pollen. In addition, some nectar and flowers were sampled by hand. The honeybees used as samplers were observed for signs of behavioral impacts. All samples and a small sample of honeybees were subjected to a residue analysis for imidacloprid and its relevant metabolites.

Dates of biological work: July 12-19, 1999
Dates of soil analysis: August 9-11, 1999
Dates of analysis of biological samples: August 27 - September 21, 1999

Findings: Residues in soil, in summer rape plant matrices planted as succeeding crop and in honeybees used as sampling device. (detects above the LOQ are highlighted):

Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (south of field number 502)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid, n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

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Type of Sample	Residue Level [mg/kg] *		
	Imidacloprid	Olefin-NTN	Hydroxy-NTN
Variant „1997“ (field number 502)			
Soil sample (0-30 cm)	0.018	--	--
Leaves (produced latest)	< LOQ	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1998“ (field number 507)			
Soil sample (0-30 cm)	< LOQ	--	--
Leaves (produced latest)	n.d.	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	n.d.	n.d.	n.d.
Pollen sampled from hives and bees	n.d.	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.
Variant „1999“ (south of field number 502)			
Soil sample (0-30 cm)	n.d.	--	--
Leaves (produced latest)	< LOQ	n.d.	n.d.
Flowers	n.d.	n.d.	n.d.
Nectar sampled from the flowers	< LOQ	n.d.	n.d.
Pollen sampled from hives and bees	< LOQ	n.d.	n.d.
Honeybees exposed to the summer rape	n.d.	n.d.	n.d.

* Limit of quantitation for soil samples: 0.006 mg/kg for imidacloprid; n.d. = below limit of detection (0.002 mg/kg)
Limit of quantitation for biological samples: 0.005 mg/kg for imidacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-imidacloprid. n.d. = below limit of detection (0.0015 and 0.003 mg/kg).

Observations: No treatment-related behavioral impacts (e.g. apathy, exaggerated motility, discoordinated movements) or increased mortality was observed on the honeybee colonies used for collecting summer rape nectar and pollen. The final check at study termination did also not reveal any abnormality in either colony strength or brood status.

>>M-016842-03-3@S-6049/9-01-1

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Report:	02.02.01/31; [redacted]; 2011; M-401652-01-2
Title:	Determination of residues of imidacloprid OD 200 and its metabolites applied via drip irrigation in watermelon in the semi-field in Spain in 2009
Report No.:	S09-00075
Document No.:	M-401652-01-2
Guideline(s):	IVA (1992), EU (1999)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-401652-01-2@S-602564-01-1

Material and methods

Test item:	Name: Imidacloprid OD 200 A G Analyzed content of active ingredient:19.6 % w/w Active ingredients: Imidacloprid (NTN 33893) Batch: 2008-009969

The following study was designed to determine the residues of Imidacloprid OD 200 in bee-relevant matrices of watermelon following an application by drip irrigation in the semi-field in Spain.
This GLP compliant study encompassed the objectives of Commission Directive 96/68/EC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market, Oct.21, 1996 and "Commission Working Document 1607/VI/97 Rev. 2; General Recommendations for the Design, Preparation and Realisation of Residue Trials, July 22, 1999" and the "IVA-Leitlinie – Rückstandsversuche, Prüfungen an Pflanzen, Teil 1A und 1B", Industrieverband Agrar e. V., Frankfurt/Main, 1992.
Particular attention was directed to the residues in young plants, flowers, pollen and nectar.
The study comprised one trial which was carried out in watermelon in Spain. Commercially grown young plants were purchased and then transplanted into the field, as typical for commercial agricultural practice. There was one test item (Imidacloprid OD 200) treatment group and one control group. Application of the test item was performed once via drip irrigation at the growth stage of BBCH 15 (after transplanting), corresponding to 200 g imidacloprid a.s./ha. The control group remained imidacloprid-untreated.

Before the watermelons approached their flowering period, gauzecovered tunnels of approx. 5 m x 50 m surface area were set-up in the respective watermelon fields. The test item treatment group comprised 3 replicates (tunnels), the control group comprised 1 replicate (tunnel).
Thereafter, small honey bee colonies were placed in the tunnels as soon as enough flowers were present to allow foraging of the bees. The honey bees were used as a sampling device for nectar and pollen.
Samples of young watermelon plants were taken at the time of transplanting from the greenhouse to the field. Additionally, flowers from the plants as well as freshly collected nectar/honey and freshly collected pollen from combs was sampled at several dates, starting at beginning of flowering and continuing during flowering period of the crop. The collected samples were immediately stored in dry-ice in the field and kept deep-frozen thereafter, to be analysed for potential residues of the test item.

Dates of work: 30 April 2009 to 16 December 2009

Findings (Residue Analysis):

Residues of imidacloprid (NTN 33893) and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin were analysed by High Performance Liquid Chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). The Limit of Quantification (LOQ) for imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefin, defined as the lowest validated fortification level, was 0.001 mg/kg and the Limit of Detection (LOD) was 0.0003 mg/kg, respectively.
No quantifiable residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefin were found in any of the young watermelon plant, pollen and honey/nectar samples (i.e. residues were always below the LOQ).

Residues of imidacloprid in flowers ranged between 0.0017 mg/kg to 0.0460 mg/kg. Residues of imidacloprid-olefin in flowers ranged between <LOQ to 0.0041 mg/kg and residues of imidacloprid-5-hydroxy ranged between <LOQ and 0.0108 mg/kg.
Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefin in control samples were always below the LOQ.

Conclusions
No quantifiable residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefin have been revealed in young watermelon plants immediately before transplanting to the designated treatment (i.e. before test item application) and control field plots.
Residues of imidacloprid in flowers ranged between 0.0017 mg/kg to 0.0460 mg/kg. Residues of imidacloprid-olefin in flowers ranged between <LOQ to 0.0041 mg/kg and residues of imidacloprid-5-hydroxy ranged between <LOQ and 0.0108 mg/kg.
The study revealed no quantifiable residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefin in pollen and honey/ nectar, collected from honey bee colonies, exposed under confined conditions to flowering watermelon plants, which have been treated with Imidacloprid OD 200 via drip irrigation at BBCH 15, at a rate corresponding to 1 x 200 g imidacloprid a.s./ha.
>>M-401652-01-2@S-602564-01-1

Report:	02.02.01/32; [redacted]; 2011; M-404577-01-3
Title:	Determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from cotton, grown at locations treated with imidacloprid at least once per year during two successive years
Report No.:	EBNT1056-01
Document No.:	M-404577-01-3
Guideline(s):	US EPA Regl.: OPPTS 850.SUPP (Ecological Effects)
Guideline deviation(s):	The field and sampling phase of this study were not conducted to meet the requirements of EPA Good Laboratory Practice Standards (40 SFR part 160; FR, August 17, 1989). The anlytical phase of this study was conducted to meet GLP standards. The preparation of the field fortification samples was not conducted under GLP but their analyses met GLP standards.
GLP/GEP:	**yes**

<<M-404577-01-3@S-602564-01-1
Five trials were conducted in clay soils classified as 'heavy' to determine the residues of imidacloprid and its metabolites (5-hydroxy imidacloprid and imidacloprid olefin) in nectar and leaves collected from cotton plants grown at locations treated with imidacloprid at least once per year for two years. All soils had received previous application(s) of Admire Pro by chemigation at rates ranging from 0.18 to 0.38 lb ai/A in the prior year(s) and received one aerial foliar spray application of imidacloprid (Provado 1.6 F, 17.4% imidacloprid by weight) in 2010 during flowering (BBCH61, beginning of flowering to BBCH67, flowering finished, majority of flowers faded).

Composite samples of cotton nectar and cotton leaves were collected seven to two days prior to the 2010 imidacloprid application (pre-application) and six days following the 2010 application (post-application). Nectar and leaves were collected from the same cotton plants.

The residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

The limits of quantitation (LOQs) are shown below.

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Matrix	Analyte	LOQ (ppm)
Cotton Nectar	Imidacloprid	0.001
	5-hydroxy imidacloprid	0.001
	Imidacloprid olefin	0.001
	Total Imidacloprid	0.001
Cotton Leaves	Imidacloprid	0.002
	5-hydroxy imidacloprid	0.002
	Imidacloprid olefin	0.002
	Total Imidacloprid	0.002

Transit stability samples (control nectar and leaves fortified with imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) monitored the stability of the analytes during sampling, transit, and storage. The average recovery of all analytes in these samples ranged from 72% to 95%, demonstrating that residues were stable under the practices used in this study. The maximum storage period of frozen samples in this study was 122 days for cotton nectar and 149 days for cotton leaves.

A summary of the residues is shown in the table below.
Summary of Residue Data for Total Imidacloprid in Nectar and Leaves from Cotton Grown in Heavy Soils.

Commodity	Plot Type [a]	Application Rate lb ai/A (oz FP/A) [b]	Days After Treatment (DAT) [c]	Total Imidacloprid Residue Levels (ppm) [d]						
				n	Min	Max	Highest Average Site Residue	Median	Mean	Standard Deviation
Cotton Nectar	Heavy Soil	NA [e]	Pre-App (-7 to -2 DAT)	10	0.0010	0.0043	0.0042	0.0027	0.0028	0.0010
Cotton Nectar	Heavy Soil	0.063 (5.0)	Post-App (6 DAT)	10	0.013	0.066	0.056	0.021	0.029	0.018
Cotton Leaves	Heavy Soil	NA [e]	Pre-App (-7 to -2 DAT)	10	0.0031	0.029	0.023	0.014	0.014	0.0078
Cotton Leaves	Heavy Soil	0.063 (5.0)	Post-App (6 DAT)	10	0.15	1.9	1.7	0.36	0.77	0.67

a Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service. "Heavy" class represents soil with slow drainage capacity.
b Although all plots had received applications of imidicloprid in previous year(s) at rates ranging from 0.18 to 0.38 lb ai/A, the application rate cited refers to application of Provado 1.6F in 2010 only.
c Duplicate samples of nectar and leaves from the five trials were analyzed at pre- and post-application sample intervals.
d Abbreviations used are as follows: Min is the lowest treated residue value; Max is the highest treated residue value; Median is the geometric median of the treated residue values; Mean is the mathematical average of the treated residue values; Standard Deviation is the standard deviation for a small population of n samples.
e NA= Not Applicable.

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Report:	02.02.01/33; [redacted]; 2012; M-424399-01-3
Title:	Imidacloprid - Determination of residues of imidacloprid in pollen, extrafloral nectar fluids and nectar of cotton plants grown from imidacloprid-treated seeds in two cotton growing areas in Greece 2011
Report No.:	S11-02885
Document No.:	M-424399-01-3
Guideline(s):	EU 1999: 1607/VI/97 SANCO/3029/99 rev. 4 Directive 2010/21/EU US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-424399-01-3@S-602309-01-1

Material and methods

Test item: Pella site Name: Cotton plants grown from seeds treated with Imidacloprid FS 350 Active ingredient: Imidacloprid Analyzed content of active ingredient: 484.67 g/100 kg seeds Batch: EA 385 11 10 23

Larissa site Name: Cotton plants grown from seeds treated with Imidacloprid FS 350 Active ingredient: Imidacloprid Analyzed content of active ingredient: 555.07 g/100 kg Batch: EA 385 11 10 27

The field study was conducted in Greece, one trial in Giannitsa in the vicinity of Pella (trial S11-02885-01) and a second trial in Glafki in the vicinity of Larissa (trial S11-02885-02).

The purpose of the study was the determination of residues of imidacloprid in pollen, nectar and extra floral nectar in cotton plants grown from imidacloprid-treated seeds. Cotton seeds (variety Flora and Carmen) were pre-treated with Imidacloprid FS 350. The sowing had taken place on 12MAY2011 (Pella site) and on 13MAY2011 (Larissa site). The sizes of the plots on which the cotton was grown were 10416m² (Pella site) and 10032m² (Larissa site).
After emergence of the crop, samples were taken for pollen, nectar and extrafloral nectar.
Sampling of pollen, nectar and extrafloral nectar was carried out consecutively three times, at different BBCH stages (Meier 2001), on both study locations, respectively.

Dates of work: 01AUG2011 (Pella site) and 04AUG2011 (Larissa site) (start of field work) to 21OCT2011 (end of residue analysis).

Findings (Residue Analysis)
Residues of imidacloprid were detected in four of the six cotton pollen samples. In two samples the residues were <LOQ (i.e. <1 µg/kg). The mean residue level of the six pollen samples was 2µg/kg (range: <1 - 5 µg/kg).
Residues of imidacloprid were detected in five of the six cotton nectar samples, except of the last sample (BBCH 65-69) of the Larissa site, where the residue level of imidacloprid was <LOQ (i.e. <1 µg/kg). The mean residue level of all nectar samples was 2µg/kg (range: <1 – 4µg/kg).
In extrafloral nectar samples, residues of imidacloprid were found in five of the six samples. In one sample (BBCH 61-64), low residues were detected (<LOQ, i.e. <1 µg/kg). The mean residue level of all extrafloral nectar samples was 3µg/kg (range: <1 – 5µg/kg).

Results of Analysis of Pollen Samples

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Sample	Growth Stage	Imidacloprid Residues [µg/kg]
L11-02885-01-001A	BBCH 61-64	1
L11-02885-01-004A	BBCH 63-66	2
L11-02885-01-007A	BBCH 65-69	<1
L11-02885-02-001A	BBCH 61-64	2
L11-02885-02-004A	BBCH 63-66	<1
L11-02885-02-007A	BBCH 65-69	5
Mean		**2**

LOQ Imidacloprid: 1 µg/kg
For calculation of the arithmetic mean, values below LOQ were set to the LOQ (LOQ = 1 µg/kg)

Results of Analysis of Nectar Samples

Sample	Growth Stage	Imidacloprid Residues [µg/kg]
L11-02885-01-003A	BBCH 61-64	3
L11-02885-01-006A	BBCH 63-66	3
L11-02885-01-009A	BBCH 65-69	1
L11-02885-02-003A	BBCH 61-64	2
L11-02885-02-006A	BBCH 63-66	4
L11-0288502-009A	BBCH 65-69	<1
Mean		**2**

LOQ Imidacloprid: 1 µg/kg
For calculation of the arithmetic mean, values below LOQ were set to the LOQ (LOQ = 1 µg/kg)

Results of Analysis of Extra Floral Nectar Samples

Sample	Growth Stage	Imidacloprid Residues [µg/kg]
L11-02885-01-002A	BBCH 61-64	<1
L11-02885-01-005A	BBCH 63-66	4
L11-02885-01-008A	BBCH 65-69	1
L11-02885-02-002A	BBCH 61-64	5
L11-02885-02-005A	BBCH 63-66	3
L11-02885-02-008A	BBCH 65-69	3
Mean		**3**

LOQ Imidacloprid: 1 µg/kg
For calculation of the arithmetic mean, values below LOQ were set to the LOQ (LOQ = 1 µg/kg)

Imidacloprid residue levels in plant matrices grown from imidaclopridtreated cotton seeds ranged from <1 to 4 µg/kg in nectar, from <1 to 5 µg/kg in pollen and from <1 to 5 µg/kg in extrafloral nectar.

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Report:	02.02.01/34; [redacted]; 2012; M-428259-01-2
Title:	Imidacloprid OD 200: A semi-field study in Spain 2011 to determine residues in tomato pollen collected by forager bumble bees following drip application
Report No.:	S10-03119
Document No.:	M-428259-01-2
Guideline(s):	IVA (1992), 7029/VI/95 (EU, 1997), 1607/VI/97 (EU, 1999), 1107/2009 (EU, 2009), 544/2011 (EU, 2011a) and 545/2011 (EU, 2011b)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-428259-01-2@S-603000-01-1

Material and methods

Test item: Name: Imidacloprid OD 200 Analysed content of active ingredient: 203.1 g/L Active ingredients: Imidacloprid Batch: ECE5101280

The purpose of the study was to determine the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in tomato (*Lycopersicon esculentum*) pollen, collected by bumble bees, *Bombus terrestris* L., under confined semi-field (tunnel tents) conditions, following single or repeated drip applications of Imidacloprid OD 200.

Before start of the test, commercially grown, young tomato plants were transplanted from pots to open, natural soil as usual for commercial open field tomato cultivation. The planting density was 25,000 tomato plants per ha. Shortly before onset of flowering, gauze tunnels were set up.

The study comprised four treatment groups. There were three test item groups (T1, T2, T3) and one untreated control group (C). In T1, one application corresponding to 150 g a.s./ha was carried out at BBCH 13- 14, i.e. just after transplanting. In T2, there was one application corresponding to 200 g a.s./ha at BBCH 13-14. In T3, there were two sequential applications: a first application corresponding to 200 g a.s./ha at BBCH 13- 14, followed by a second application, also corresponding to 200 g a.s./ha, 14 days after the first application, at BBCH 21-22.

The applications in T1 – T3 were performed by using a water rate of 1.0 L/m2 via drip irrigation, respectively. The control group remained untreated. Set-up of the bumble bee hives was at start of flowering at BBCH 62- 63, just when enough flowers were present to allow foraging of the bumble bees. Forager bumblebee pollen samples for residue analysis were taken 10 times after start of flowering at BBCH 62-63.

Dates of work: 2nd run: 25 May 2011 (start of field work) to 13 Dec 2011 (end of residue analysis)

Findings:
Residue Analysis:

At the first sampling (DAE 0, i.e. the first day of bumble bee pollen collection inside the tunnels (exposure), just when the first flowers opened at BBCH 62 - 63), only one single sample from one of the three replicate tunnels (T1) barely met the required minimum amount of 0.1 g pollen for residue analysis, all other samples comprised only tiny amounts.

The overall maximum amount of total imidacloprid residues was determined on DAE 4/5 (i.e. 4/5 days after start of bumble bee pollen collection) in one replicate of treatment group T3. Total imidacloprid residues in tomato pollen declined over time in all treatment groups under investigation.

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A summary of the results is displayed below. A detailed analytical phase report is presented in the Appendix A4.

Imidacloprid OD 200 Final Report S10-03112

Sample type	Sampling				Residue [µg/kg]		
	Sampling date	DAE	DA(f)A	Treatment group	Imidacloprid	Imidacloprid-5-hydroxy	Imidacloprid-olefin
Tomato Pollen	07 Jul 2011	0	42	C	-	-	-
				T1	49	2.7	2.6
				T2	-	-	-
				T3	-	-	-
	11/12 Jul 2011	4/5	46/47	C	< LOD	< LOD	< LOD
				T1	27 - 44	1.5 - 3.1	0.9 - 1.5
				T2	38 - 62	2.6 - 3.6	1.4 - 1.6
				T3	106 - 139	6.5 - 8.6	3.5 - 3.6
	15 Jul 2011	8	50	C	< LOD	< LOD	< LOD - < LOQ
				T1	29 - 30	1.6 - 2.1	1.5 - 2.2
				T2	35 - 54	2.2 - 3.2	2.0 - 5.5
				T3	53 - 90	3.6 - 6	1.1 - 1.6
	19/20 Jul 2011	12/13	54/55	C	< LOD	< LOD	< LOD - < LOQ
				T1	13 - 20	< LOQ - 1.1	< LOD
				T2	15 - 24	< LOQ - 1.9	< LOQ
				T3	25 - 34	1.7 - 2.3	< LOQ
	22 Jul 2011	15	57	C	< LOD - < LOQ	< LOD	< LOD - < LOQ
				T1	16 - 21	< LOQ - 1.3	< LOD - 1
				T2	20 - 32	1.1 - 2.4	< LOD - < LOQ
				T3	41 - 66	2.9 - 4.6	< LOQ - 1.5
	27/28 Jul 2011	20/21	62/63	C	< LOD	< LOD	< LOD
				T1	14 - 16	< LOQ - 1.3	< LOD - < LOQ
				T2	18 - 23	< LOQ - 1.6	< LOQ
				T3	31 - 62	2 - 4.1	< LOQ - 1.8
	02/03 Aug 2011	26/27	68/69	C	< LOD	< LOD	< LOD
				T1	8.4 - 12	< LOD - < LOQ	< LOD - < LOQ
				T2	8.9 - 14	< LOD - LOQ	< LOD - < LOQ
				T3	18 - 47	1.3	< LOD - < LOQ
	09/10 Aug 2011	33/34	75/76	C	< LOD	< LOD	< LOD
				T1	6.1 - 10	< LOD - < LOQ	< LOD
				T2	9.8 - 21	< LOD	< LOD - 1.3
				T3	23 - 31	< LOD - 2.2	< LOD - < LOQ
	17/18 Aug 2011	41/42	83/84	C	< LOD	< LOD	< LOD
				T1	11 - 13	< LOD	< LOD
				T2	8.4 - 14	< LOD - < LOQ	< LOD
				T3	23 - 36	< LOD – 1.7	< LOD
	31 Aug 2011	55	97	C	-	-	-
				T1	5.6	< LOD	< LOD
				T2	-	-	-
				T3	-	-	-

LOQ = Limit of Quantitation = 1 µg/kg
LOD = Limit of Detection = 0.5 µg/kg
C: Control (untreated)
T1: Treatment 1 (test item applied at a rate of 1 x 150 g a.s./ha at BBCH 13-14)
T2: Treatment 2 (test item applied at a rate of 1 x 200 g a.s./ha at BBCH 13-14)
T3: Treatment 3 (test item applied at a rate of 2 x 200 g a.s./ha, 14 days interval, 1st application at BBCH 13-14)
" – " : not analysed due to a weight below 0.1 g
DAE = Days after start of bumble bee pollen collection inside the respective tunnels
DA(f)A = Days after (first) application

Conclusions

The study revealed that drip application(s) of Imidacloprid OD 200 to tomato plants at a growth stage typical for early transplanting (BBCH 13-14) resulted in total imidacloprid residues (i.e. imidacloprid + imidacloprid-5-hydroxy + imidacloprid-olefin) in tomato pollen which followed a dose dependant pattern. The highest imidacloprid residues were found during the first two weeks after flower emergence and the residues declined thereafter.

>>M-428259-01-2@S-603000-01-1

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Report:	02.02.01/35; [redacted]; 2012; M-429087-01-2
Title:	Determination of exposure levels of honey bees foraging on flowers of citrus trees previously treated with imidacloprid
Report No.:	EBNTL056-7a
Document No.:	M-429087-01-2
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

<<M-429087-01-2@S-605225-01-1

The objective of this study was to determine if residues of imidacloprid and its important metabolites could persist and/or accumulate in nectar from year-to-year in situations where the insecticide was used on the same trees in successive years. Also, because imidacloprid uptake into trees can be affected by soil type, sites were chosen to reflect the variety of soil types where citrus is grown in California. In our report submitted to the CaDPR in April 2011, we provided residue data for several sites where citrus was grown in soils that ranged from sandy loam to loam. The CaDPR requested additional data for citrus growing in heavier clay soils. In response to this request, and also to supplement the residue data from citrus grown in lighter soils, we conducted the following work in Spring 2011. At all sites, nectar was extracted from flowers by hand during bloom and imidacloprid, imidacloprid olefin and 5-OH imidacloprid were quantified by LC/MS/MS. The full methodology for the collection and analyses are described in the April 2011 report.

- We collected nectar from six citrus groves in Tulare County where the soil was classified as Porterville clay (clay content of 40%). At 5 of these locations, the citrus had been treated with the full label rate of imidacloprid for at least the past 3 years, and at the sixth site for the past 2 years. Two composite samples were collected from each grove.
- We collected nectar from 6 groves in the Temecula Valley (Riverside County) where the trees had been treated for 3 successive years with the full label rate of imidacloprid. In 2010, we had collected from these sites after 2 years of imidacloprid applications, and these data were presented in the April 2011 report. The soil type at these sites was sandy loam. Two composite samples were collected from each grove.
- We collected nectar from 5 citrus blocks at the Lindcove Research and Extension Center (LREC). The trees had been treated in September 2008, 2009 and 2010 with the full label rate of imidacloprid. In 2010, we had collected nectar from these same blocks to determine imidacloprid levels after 2 successive years of applications, and these data were presented in the April 2011 report. The soil type throughout LREC is classified as a loam (20% clay). Two composite samples were collected from each block.
- We collected nectar from a lemon grove in Ventura County where the trees had been treated with the full label rate of imidacloprid at different timings during the season. The treatment timings were in May, July and September 2010. These trees had not been treated in 2009. The soil type was determined by the UC Davis Analytical Laboratory to be 23% clay/35% sand. Two composite nectar samples were collected from trees treated at Timings 1 and 3, and one composite sample was collected from trees treated at Timing 2.
- We collected nectar from a citrus block on the farm at UCR (Agricultural Operations) where the trees had been treated with the full label rate of imidacloprid in October 2010. The soil type was classified as a loam. Sixteen composite samples were collected from the trees at this site.

>>M-429087-01-2@S-605225-01-1

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Report:	02.02.01/36; [redacted]; 2012; M-445207-01-3
Title:	Determination of the residue of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from strawberries, grown at locations treated with imidacloprid at least once per year during two successive years
Report No.:	EBNTL056-04
Document No.:	M-445207-01-3
Guideline(s):	US EPA Ref.: OPPTS 850.SUPP (Ecological Effects)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-445207-01-3@S-604668-01-1

Blossom and leaf samples were collected from seven treated field sites in either a sand soil ("light"; 3 sites) or a loam soil ("medium"; 4 sites) to determine the residues of imidacloprid and its metabolites (5-hydroxy imidacloprid and imidacloprid olefin) in blossoms, anthers, pollen and leaves collected from strawberry plants grown at locations treated with imidacloprid at least once per year for two years. All soils had received previous application(s) of either Alias 4F or Admire Pro at a rate of 0.5 lb ai/A in the prior year as well as an application of imidacloprid in 2010.

Duplicate composite samples of strawberry blossoms for direct analysis, strawberry blossoms for anther samples, strawberry blossoms for pollen samples and strawberry leaves were collected at a BBCH ranging from 61 to 69 (flowering) at each field site.

The residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

The limits of quantitation (LOQs) are shown below.

Matrix	Analyte	LOQ (ppm)
Strawberry Blossom	Imidacloprid	0.005
	5-hydroxy imidacloprid	0.005
	Imidacloprid olefin	0.005
	Total Imidacloprid	0.005
Strawberry Anthers	Imidacloprid	0.005
	5-hydroxy imidacloprid	0.005
	Imidacloprid olefin	0.005
	Total Imidacloprid	0.005
Strawberry Pollen	Imidacloprid	0.010
	5-hydroxy imidacloprid	0.010
	Imidacloprid olefin	0.010
	Total Imidacloprid	0.010
Strawberry Leaves	Imidacloprid	0.010
	5-hydroxy imidacloprid	0.010
	Imidacloprid olefin	0.010
	Total Imidacloprid	0.010

Transit stability samples (control pollen samples fortified with imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) monitored the stability of the analytes during sampling, transit, and storage. The average recovery of all analytes in these samples ranged from 93% to 104%, demonstrating that residues were stable under the practices used in this study. The maximum storage period of frozen samples in this study was 445 days.

A summary of the residues is shown in the table below.

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Summary of Residue Data for Total Imidacloprid in Strawberry Blossoms, Strawberry Anthers, Strawberry Pollen and Strawberry Leaves.

Commodity	Soil Type	Total Imidacloprid Residue Levels (ppm) [d]						
		n	Min	Max	Highest Average Site Residue	Median	Mean	Standard Deviation
Strawberry Blossoms	Light	6	0.21	0.54	0.50	0.38	0.36	0.13
Strawberry Anthers	Light	6	0.081	0.30	0.25	0.20	0.18	0.082
Strawberry Pollen	Light	6	0.078	0.32	0.28	0.21	0.19	0.095
Strawberry Leaves	Light	6	1.7	2.8	2.4	2.1	2.2	0.41
Strawberry Blossoms	Medium	8	<0.0050	0.031	0.028	0.0064	0.0094	0.0091
Strawberry Anthers	Medium	8	0.011	0.033	0.023	0.013	0.018	0.0079
Strawberry Pollen	Medium	8	<0.010	0.010	<0.010	0.010	<0.010	<0.010
Strawberry Leaves	Medium	8	<0.010	0.018	0.017	0.011	0.011	<0.010

[a] Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service.

[b] Abbreviations used are as follows: Min is the lowest treated residue value; Max is the highest treated residue value; Median is the geometric median of the treated residue values; Mean is the mathematical average of the treated residue values; Standard Deviation is the standard deviation for a small population of n samples.

[c] NA= Not Applicable.

>>M-445207-01-3@S-604668-01-1

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Report:	02.02.01/37; [redacted]; 2013; M-404588-02-2
Title:	Determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from tomato, a fruiting vegetable, grown at locations treated with imidacloprid at least once per year during two successive years (amended)
Report No.:	EBNTL056-05-1
Document No.:	M-404588-02-2
Guideline(s):	US EPA Ref.: OPPTS 850.SUPP (Ecological Effects)
Guideline deviation(s):	The field and sampling phase of this study were not conducted to meet the requirements of EPA Good Laboratory Practice Standards (40 CFR part 160; FR, August 17, 1989). The anlytical phase of this study was conducted to meet GLP standards. The preparation of the field fortification samples was not conducted under GLP but their analyses met GLP standards.
GLP/GEP:	**yes**

<<M-404588-02-2@S-604953-01-1

Nine trials were conducted in clay or loam soils classified as “heavy” or “medium” to determine the residues of imidacloprid and its metabolites (5-hydroxy imidacloprid and imidacloprid olefin) in anthers (pollen) and leaves collected from tomato plants grown at locations treated with imidacloprid at least once per year for two years. All soils had received previous chemigation application(s) of Admire Pro at total rates ranging from 0.18 to 0.25 lb ai/A (5.0 to 7.0 fl oz formulated product/A) in 2009.

Each trial received application(s) of Admire Pro in 2010 at the same rates as in 2009. The six sites located in Kings County received two applications of Admire Pro at 3.5 fl oz FP/A/application (0.13 lb imidacloprid/A/application) for a total seasonal rate of 7.0 fl oz FP/A (0.25 lb ai/A). The first applications were made at or closely following transplanting with the second applications 52 to 57 days following the first applications. The three sites located in Kern County received a single application of Admire Pro at 0.18 lb imidacloprid/A (5.0 fl oz FP/A) 2 to 25 days following transplanting.

The growth stages of the plants at the times of applications were not documented but likely occurred at growth stages of BBCH 21 to 51 (first primary shoot to first inflorescence visible) for the first applications and BBCH61 to BBCH69 (flowering but prior to fruiting) for the second applications. All applications were made through drip chemigation (buried lines).

Composite samples of tomato anthers (pollen) and tomato leaves were collected from tomato plants 72 to 102 days following the last treatment (DALT) at indeterminate flowering and fruiting growth stages (BBCH6X to BBCH7X) and analyzed for residues of imidacloprid.

The residue(s) of imidacloprid, 5-hydroxy imidacloprid and imidacloprid olefin were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

The limits of quantitation (LOQs) are shown below.

Matrix	Analyte	LOQ (ppm)
Tomato Anthers (pollen)	Imidacloprid	0.002
	5-hydroxy imidacloprid	0.002
	Imidacloprid olefin	0.002
	Total Imidacloprid	0.002
Tomato Leaves	Imidacloprid	0.002
	5-hydroxy imidacloprid	0.002
	Imidacloprid olefin	0.002
	Total Imidacloprid	0.002

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Transit stability samples (control anthers and leaves fortified with imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) monitored the stability of the analytes during sampling, transit, and storage. The average recovery of all analytes in these samples ranged from 79% to 99%, demonstrating that residues were stable under the practices used in this study. The maximum storage period of frozen samples in this study was 158 days for tomato anthers and 164 days for tomato leaves.

A summary of the residues is shown in the table below.

Summary of Residue Data for Total Imidacloprid in Anthers and Leaves from Tomatoes Grown in Heavy and Medium Soils.

Commodity	Plot Type [a]	Application Rate lb ai/A (oz FP/A) [b]	Days After Last Treatment (DALT) [c]	Total Imidacloprid Residue Levels (ppm) [d]						
				n	Min	Max	Highest Average Site Residue	Median	Mean	Standard Deviation
Tomato Anthers	Heavy Soil	0.25 (7.0)	72 - 79	8	0.014	0.030	0.027	0.022	0.021	0.005
Tomato Anthers	Medium Soil	0.18 – 0.25 (5.0 – 7.0)	79 - 102	10	0.016	0.064	0.046	0.036	0.034	0.012
Tomato Leaves	Heavy Soil	0.25 (7.0)	72 - 79	8	0.057	0.14	0.12	0.089	0.093	0.026
Tomato Leaves	Medium Soil	0.18 – 0.25 (5.0 – 7.0)	79 - 102	10	0.038	0.23	0.20	0.10	0.11	0.061

[a] A total of nine tomato trials were conducted, four in "heavy" soils and five in "medium" soils. Ten trials were scheduled; however, one commercial processor harvested the plot before trial samples could be harvested. Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service. "Heavy" class represents soil with slow drainage capacity and "medium" class represents soil with moderate draining capacity.

[b] Although all plots had received applications of imidicloprid in the previous year (2009) at rates ranging from 0.18 to 0.38 lb ai/A, the application rate cited refers to applications in 2010 only.

[c] All trials received one or two applications of Admire Pro. DALT are the days following the last application when two applications were made.

[d] Abbreviations used are as follows: Min is the lowest treated residue value; Max is the highest treated residue value; Median is the geometric median of the treated residue values; Mean is the mathematical average of the treated residue values; Standard Deviation is the standard deviation for a small population of n samples.

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Report: 02.02.01/38; [redacted]; 2013; M-444526-02-2
Title: Determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from melons grown at locations treated with imidacloprid at least once per year during two successive years
Report No.: EBNTL056-02-1
Document No.: M-444526-02-2
Guideline(s): US EPA Ref.: OPPTS 850.SUPP (Ecological Effects)
Guideline deviation(s): yes, see report
GLP/GEP: **yes**

<<M-444526-02-2@S-605035-01-1

Ten trials were conducted in California in soils classified as either "heavy (fine-textured)", or "medium (medium-textured)" to determine the residues of imidacloprid and its metabolites (5-hydroxy imidacloprid and imidacloprid olefin) in bee collected nectar (hive deposited), bee collected pollen (pollen traps), and leaves (hand collected) from melon plants (cucurbits) grown at locations treated previously with imidacloprid.

Imidacloprid application rates and application methods for the trial locations during the years of 2008 through 2011 were collected from grower communications (see below). Individual application rates ranged from 0.23 to 0.38 lb imidacloprid A/application (0.26 to 0.43 kg imidacloprid/ha/application). Applications in 2011 were made at or near transplant of the melons.

Application History[a]

Field Number	Field Identification	Location (City, State, NAFTA Region)	Soil Type[b]	Application Method	Year	Date	Rate (lb a.i./A)[c]
1	NT209	Imperial County, CA, NAFTA Region 10	Holtville Silty Clay (Heavy)	Injected	2011	Jan 10	0.36
				None	2010	NA [d]	NA
				Injected	2009	Oct. 30	0.36
				Injected	2008	Oct 5	0.31
2	NT210	Imperial County, CA, NAFTA Region 10	Holtville Silty Clay (Heavy)	Injected	2011	Jan 10	0.36
				None	2010	NA	NA
				Injected	2009	Oct. 30	0.36
				Injected	2008	Oct 5	0.31
3	NT201	Imperial County, CA, NAFTA Region 10	Holtville Silty Clay (Heavy)	Injected	2011	Jan 7	0.29
				Injected	2010	Jan 25	0.29
				None	2009	NA	NA
4	NT202	Imperial County, CA, NAFTA Region 10	Meloland Very Fine Sandy Loam (Medium)	Injected	2011	Jan 10	0.29
				None	2010	NA	NA
				Injected	2009	Jan 24	0.29
5	NT203	Imperial County, CA, NAFTA Region 10	Holtville Silty Clay (Heavy)[e]	Injected	2011	Jan 10	0.29
				None	2010	NA	NA
				Injected	2009	Jan 24	0.29
6	NT208	Imperial County, CA, NAFTA Region 10	Imperial-Glenbar Silty Clay (Medium)	Seed Line	2011	Jan 3	0.36
				None	2010	NA	NA
				Seed Line	2009	Oct 4	0.30

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Field Number	Field Identification	Location (City, State, NAFTA Region)	Soil Type[b]	Application Method[b]	Year	Date	Rate (lb a.i./A)[c]
7	NT206	Imperial County, CA, NAFTA Region 10	Imperial-Glenbar Silty Clay (Medium)	None	2011	NA	NA
				Seed Line	2010	Oct 21	0.28
				Seed Line	2009	Nov 21	0.23
				Seed Line	2009	March 2	0.28
8	NT205	Imperial County, CA, NAFTA Region 10	Meloland Very Fine Sandy Loam (Medium)	Seed Line	2011	Jan 9	0.38
				None	2010	NA	NA
				None	2009	NA	NA
9	NT204	Imperial County, CA, NAFTA Region 10	Holtville Silty Clay (Heavy)	Seed Line	2011	Jan 3	0.36
				None	2010	NA	NA
				None	2009	NA	NA
10	NT207	Imperial County, CA, NAFTA Region 10	Meloland Very Fine Sandy Loam (Medium)	Injected	2011	Jan 20	0.36
				None	2010	NA	NA
				Injected	2009	Jan 20	0.36

[a] All rates, methods and dates were collected from verbal communications with the growers and could not be confirmed.
[b] Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service. "Heavy" class represents soil with slow drainage capacity.
[c] Applications were made either by injection to a drip irrigation system or with a drench application over the seed line.
[d] NA – Per grower information an imidacloprid application was not made during the year.
[e] One tent was in Holtville Silty Clay. The other tent in this site was divided between Holtville Silty Clay and Imperial-Glenbar Silty Clay Loam. The trial was designated 'heavy' since the majority of the sampling was from Holtville Silty Clay.

Within each field site, two plots were established, each with a bee-tight, ventilated mesh tent (24 ft x 100 ft x 10 ft tall) for sample collection. One normally developed, apparently healthy and queen-right honey bee colony was placed in each tented area shortly after the beginning of flowering when enough blossoms were open to allow orientation and foraging of the bees.

Bee collected nectar (hive deposited) and pollen (pollen traps), as well as, leaves were collected from each plot. Nectar samples were composite samples collected by syringe at several sampling intervals over several days. Sampling was from several cells of a brood frame, as no nectar was stored in an empty frame placed next to the brood frame. Composite pollen samples were collected from each hive in hive-entrance mounted, plastic pollen traps over a period of two to three weeks. Composite leaf samples were collected from each plot near the middle of the nectar and pollen sampling period.

The residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue. The limits of quantitation (LOQs) are shown below:

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Summary of LOQs

Matrix	Analyte	LOQ (ppm, parent equivalents)
Melon nectar	Imidacloprid	0.001
	5-hydroxy imidacloprid	0.001
	Imidacloprid olefin	0.001
	Total imidacloprid	0.001
Melon pollen and leaves	Imidacloprid	0.010
	5-hydroxy imidacloprid	0.010
	Imidacloprid olefin	0.010
	Total imidacloprid	0.010

Transit stability samples (control nectar and pollen fortified with imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) monitored the stability of the analytes during sampling, transit, and storage. The average recovery of both analytes in these samples ranged from 86% to 95%, demonstrating that residues were stable under the sample storage/transport practices used in this study. The maximum storage period of frozen samples in this study was 598 days (20 months).

A summary of the residues is shown in the table below.

Summary of Residue Data for Total Imidacloprid

Sample Name	Plot Type [a]	Total Imidacloprid Residue Levels (ppm) [b,c]						
		n	Min	Max	Highest Average Site Residue	Median	Mean	Standard Deviation
Bee collected (hive deposited) melon nectar	Heavy Soil	10	0.0012	0.0053	0.0039	0.0024	0.0030	0.0015
Bee collected (hive deposited) melon nectar	Medium Soil	10	0.0016	0.0080	0.0049	0.0030	0.0039	0.0025
Bee collected (pollen traps) melon pollen	Heavy Soil	10	<0.010	0.012	0.011	<0.010	<0.010	0.0028
Bee collected (pollen traps) melon pollen	Medium Soil	10	<0.010	0.032	0.019	<0.010	0.013	0.0086
Melon leaves	Heavy Soil	10	<0.010	0.028	0.027	0.013	0.016	0.0067
Melon leaves	Medium Soil	10	<0.010	0.071	0.055	0.010	0.027	0.025

[a] Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service. "Heavy" class represents soil with slow drainage capacity.

[b] Total imidacloprid is the sum of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in parent equivalents.

[c] Abbreviations used are as follows: Min is the lowest residue value; Max is the highest residue value; 'Highest average site residue' is the highest average of the two replicates from each site; Median is the geometric median of the residue values; Mean is the mathematical average of the residue values; Standard Deviation is the standard deviation for a small population of n samples.

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Report:	02.02.01/39; [redacted]; 2013; M-260729-01-3
Title:	Determination of the residues of imidacloprid, NTN33893-5-hydroxy and NTN33893-olefin metabolites in field sample of rape (blossom, nectar, daily honey, bee bread, pollen, and soil)
Report No.:	MR-128/05
Document No.:	M-260729-01-3
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, point 6 and Annex III, part A, point 8 Residues in or on Treated Products, Food and Feed US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-260729-01-3@S-604674-01-1

Field samples of rape blossoms, honey (nectar), bee bread and pollen of seed-treated winter rape were collected from different locations in a “Chemical Monitoring Program” requested by the German Regulatory Authority. The purpose of the study is to determine the residues of imidacloprid and its metabolites NTN33893-5-hydroxy and NTN33893-olefin in rape (blossom, nectar, daily honey, bee bread, pollen, and soil) after seed treatment with imidacloprid.

Residues of imidacloprid, NTN33893-5-hydroxy and NTN33893-olefin in bee relevant matrices were determined according to method 00537/M001.

The individual recovery values for imidacloprid with method 00537/M001 ranged from 90 to 106% with overall recoveries between 97 and 99% and with relative standard deviations (RSDs) between 2.7 and 8.8% (n = 4 to 8). For NTN33893-5-hydroxy recoveries ranged from 85 to 113% with overall recoveries between 99 and 100% and with RSDs between 6.4 and 11.1% (n = 4 to 8). Recoveries for NTN33893-olefin were between 84 and 106%, overall recoveries between 88 and 104% with RSDs between 1.8 and 5.1% (n = 4 to 8). All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

The limit of quantitation (LOQ) was 0.001 mg/kg for imidacloprid, NTN33893-5-hydroxy and NTN33893-olefin for all bee relevant matrices.

Residues of imidacloprid in soil were determined according to method 00790/M001.

The individual recovery values for imidacloprid with method 00790/M001 ranged from 88 to 110% with an overall recovery of 101% and with a relative standard deviation (RSDs) of 10.6% (n = 4).

All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully. The limit of quantitation (LOQ) of the method was 0.005 mg/kg for imidacloprid. The limit of detection (LOD) of the method was 0.002 mg/kg.

Residue values of imidacloprid were all below the LOQ (0.001 mg/kg) in all bee relevant sample materials with expect two of 17 bee bread samples from Celle where the residues were 0.001 mg/kg for imidacloprid. No residues of NTN33893-5-hydroxy and NTN33893-olefin above the LOQ were found in any sample.

Imidacloprid residues in soil samples from Kirchhain and Münster had positive detects (≤ 0.005 and 0.007 mg/kg), confirming a seed treatment of sampled winter rape plants with Imidacloprid

M-260729-01-3@S-604674-01-1

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Report:	02.02.01/40; [redacted]; 2014; M-475297-02-2
Title:	Amended report - Interim progress report for imidacloprid residue studies in cotton and tomato. Preliminary residue results in bee relevant matrices collected from 6 of 9 trials in year-1 of the 2-year cotton study
Report No.:	US0401-1
Document No.:	M-475297-02-2
Guideline(s):	OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-475297-02-2@S-605040-01-1

Bayer CropScience (BCS) is conducting residue studies to measure potential residues in pollen and nectar to support a pollinator risk assessment for imidacloprid. These required studies were designed to measure the magnitude of residues of imidacloprid and its metabolites 5-Hydroxy imidacloprid and imidacloprid Olefin. The study sites include fields at multiple locations with varying soil types in California. Crops include cotton, tomato, pome fruit and stone fruit. Matrices include pollen, nectar and leaves of cotton, stone fruit and pome fruit, and pollen and leaves of tomato. All target crops were treated at maximum seasonal use rates (0.5 lb ai/acre) and most use patterns include both soil and foliar applications to reach this worst-case rate. These are 2-year studies with imidacloprid applications over two consecutive seasons. For cotton and tomato, year-1 of both test material application and sample collection occurred in 2013, and year-2 will occur in 2014. For stone fruit and pome fruit, test material applications were made post-bloom in 2013 (as per the label) and the first opportunity for pollen, nectar and leaf sample collection will occur during bloom in 2014. The pome and stone fruit studies will continue with additional applications in 2014 and sampling in 2015.

As per a letter from the California Department of Pesticide Regulation (CDPR) (ID# 254696) dated September 20, 2012, interim reports are to be submitted for the first year sampling on cotton and tomato on or before January 31, 2014. This interim report provides an update on the status of the tomato study, and a summary of the preliminary residues measured in floral and extrafloral nectar from 6 of the 9 cotton sites (3 sites have not yet been analyzed). Please note that these are preliminary values and should not be used until definitive data are available from the 2-year cotton study and a final report is complete.

>>M-475297-02-2@S-605040-01-1

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Report: 02.02.01/41; [redacted]; 2014; M-500863-01-2
Title: Determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from seed treated field corn during two successive years and in white clover planted after seed treated field corn
Report No.: EBNTY009
Document No.: M-500863-01-2
Guideline(s): US EPA OPPTS/OCSPP 850.SUPP, Ecological Effects
Guideline deviation(s): not specified
GLP/GEP: **yes**

<<M-500863-01-2@S-602294-01-1

Executive Summary, Part A

A total of three field trials were conducted each year for two successive years to measure the magnitude of imidacloprid residues in bee-relevant corn pollen samples and in/on leaves, tassels, and soil from corn plants grown from seed treated with Gaucho 600 Flowable for two years consecutively, and to measure the magnitude of the same residues in/on bee-relevant white clover pollen and nectar samples and in flowers, leaves, and soil from white clover plants grown at locations where corn was grown from Gaucho 600 Flowable treated seed the previous year. Gaucho 600 Flowable is a flowable concentrate seed treatment formulation containing 600 g/L imidacloprid. Gaucho 600 Flowable was applied to field corn seeds at target rates as shown below.

Target Application Summary

			Target Rate/Application						Soil Loading Rate	
			Formulated Product (fp)		Active Ingredient (ai)			Seeds		
Plot ID[a]	Year[b]	Test Subs.	fl oz fp/seed	ml/fp/seed	Name of ai	mg ai/seed	lb ai/seed	seeds/A	lb ai/A	kg ai/ha
UTCA	1, 2	NA[c]	NA	NA	NA	NA	NA	40,250	NA	NA
UTCB	1, 2	NA	NA	NA	NA	NA	NA	40,250	NA	NA
TRTSTA	1, 2	Treated seeds	7.55E-05	0.0022	Imidacloprid	1.34	2.95E-6	40,250	0.119	0.133
TRTSTB	1	Treated seeds	7.55E-05	0.0022	Imidacloprid	1.34	2.95E-6	40,250	0.119	0.133
TRTSTB	2	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a] Plot ID: UTCA = Untreated control plot receiving untreated field corn seed in years 1 and 2.
UTCB = Untreated control plot receiving untreated field corn seed in year 1 and untreated forage crop (white clover) in year 2.
TRTSTA = Treated plot receiving field corn seed treated with Gaucho 600 Flowable in years 1 and 2.
TRTSTB = Treated plot receiving field corn seed treated with Gaucho 600 Flowable in year 1 and untreated forage crop (white clover) in year 2.
[b] A fresh batch of treated seed was used for the second year's planting of plot TRTSTA.
[c] NA = Not applicable.

Plot TRTSTA received field corn seed treated with Gaucho 600 Flowable in years 1 and 2 of the study (2012 and 2013, respectively). Plot TRTSTB received field corn seed treated with Gaucho 600 Flowable in year 1 and untreated forage crop (white clover) seed in year 2. All plots were tilled or disked at least once prior to the year 2 planting of corn or clover. For plot TRTSTA, the seed planting rate ranged from 36,440 to 41,480 seeds/A across both years. Soil application rates due to seed treatment for TRTSTA ranged from 0.115 to 0.122 lb imidacloprid/A (0.129 to 0.137 kg imidacloprid/ha) in year 1 and from 0.108 to 0.121 lb imidacloprid/A (0.120 to 0.135 kg imidacloprid/ha) in year 2. For plot TRTSTB, the treated seed planting rate ranged from 38,820 to 41,330 seeds/A in year 1. Soil application rates due to seed treatment for TRTSTB ranged from 0.115 to 0.122 lb imidacloprid/A (0.129 to 0.137 kg imidacloprid/ha) in year 1.

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In all trials, the following sample collections were targeted. Five composite treated samples (separate runs through the plot) of field corn leaves, tassels, and pollen were collected by hand per sample period from treated plot TRTSTA in years 1 and 2 and from treated plot TRTSTB in year 1. Five composite samples (separate runs through the plot) of white clover flowers and leaves were collected by hand and five composite samples of nectar and pollen were collected from the hives of tented bees in treated plot TRTSTB in year 2. Samples of field corn tassels, field corn pollen, and white clover flowers were collected at four sampling periods per year of collection, when the corn plants were at growth stages of BBCH 63, 65, 67, and 69 (corn BBCH 63 male: beginning of pollen shedding; corn BBCH 63 female: tips of stigmata visible; corn BBCH 69: end of flowering, stigmata completely dry); (clover BBCH 63: about 30% of flowers open; clover BBCH 69: end of flowering). Samples of field corn and white clover leaves were collected at six sampling periods per year, when the plants were at growth stages of BBCH 59, 63, 65, 67, 69, and 71 (corn BBCH 59: end of tassel emergence, tassels fully emerged and separated; corn BBCH 71: beginning of grain development, kernels at blister stage, about 16% dry matter); (clover BBCH 59: first petals visible, flowers/buds still closed; clover BBCH 71: 10% of pods have reached typical length). Nine soil samples were collected using a soil sampling device prior to seed planting and at the end of the growing season per year from treated plots TRTSTA and TRTSTB.

Two composite samples of all field corn and clover matrices and nine composite samples of soil were collected from the control plots UTCA and UTCB, at the same sampling periods as used for the treated samples of that sample type.

In some trials, not enough matrix material was present to allow for the full number of target samples to be collected (see Appendix 1).

The residues of Gaucho 600 Flowable (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The limit of detection (LOD) for the total residue is the highest LOD value for an individual analyte in a particular matrix. The limit of quantification (LOQ) for the total residue is the highest LOQ value for an individual analyte in a particular matrix.
The LOQs and LODs are shown below.

Summary of LODs and LOQs

Matrix	Analyte	LOQ[a] (ppm)	LOD[a] (ppm)
Field corn tassels/anthers	Imidacloprid	0.010	0.0009
	5-hydroxy Imidacloprid	0.010	0.0015
	Imidacloprid olefin	0.010	0.0019
	Total Residue	**0.010**	**0.0019**

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Matrix	Analyte	LOQ[a] (ppm)	LOD[a] (ppm)
Field corn leaves	Imidacloprid	0.010	0.0016
	5-hydroxy Imidacloprid	0.010	0.0017
	Imidacloprid olefin	0.010	0.0022
	Total Residue	**0.010**	**0.0022**
Field corn pollen (hand-collected)	Imidacloprid	0.001	0.0004
	5-hydroxy Imidacloprid	0.001	0.0005
	Imidacloprid olefin	0.001	0.0003
	Total Residue	**0.001**	**0.0005**
Clover flowers	Imidacloprid	0.010	0.0030
	5-hydroxy Imidacloprid	0.010	0.0024
	Imidacloprid olefin	0.010	0.0016
	Total Residue	**0.010**	**0.0030**
Clover leaves	Imidacloprid	0.010	0.0017
	5-hydroxy Imidacloprid	0.010	0.0027
	Imidacloprid olefin	0.010	0.0020
	Total Residue	**0.010**	**0.0027**
Clover pollen (hive-collected)	Imidacloprid	0.001	0.0004
	5-hydroxy Imidacloprid	0.001	0.0005
	Imidacloprid olefin	0.001	0.0003
	Total Residue	**0.001**	**0.0005**
Clover nectar (hive-collected)	Imidacloprid	0.001	0.0003
	5-hydroxy Imidacloprid	0.001	0.0007
	Imidacloprid olefin	0.001	0.0006
	Total Residue	**0.001**	**0.0007**
Soil	Imidacloprid	0.005	0.0012
	5-hydroxy Imidacloprid	0.005	0.0015
	Imidacloprid olefin	0.005	0.0012

[a] Soil LODs and LOQs are reported in individual analyte equivalents, and no total imidacloprid residue is calculated. All other matrix analyte LODs and LOQs are reported in parent equivalents.

Storage stability studies and transit spikes indicate that the imidacloprid residues would have been stable during frozen storage for at least 741 days (24 months) in field corn and clover matrices and for at least 793 days (26 months, imidacloprid) or 1281 days (42 months, imidacloprid olefin and 5-hydroxy imidacloprid) in soil matrices prior to analysis (Section 5.0). The maximum storage period of frozen samples in this study for imidacloprid was 214 days for clover leaves, 210 days for clover nectar (hive-collected), 156 days for clover pollen (hive-collected), 226 days for clover flowers, 559 days for corn leaves, 499 days for corn tassels, 734 days for corn pollen (hand-collected), and 728 days for soil prior to extraction.

The imidacloprid residues in corn leaves, tassels, and pollen; clover leaves, flowers, pollen, and nectar; and soil are given in Table 8 (SP C.3.). An analysis of the total imidacloprid residues in the bee-relevant matrices of pollen and nectar is described in Section 3.6.

An analysis of the total imidacloprid residues in soil is given in Section 3.7. The imidacloprid residues in soil were variable, but showed higher concentrations in the second year of the study for the corn/corn plot (TRTSTA), and low concentrations in the corn/clover plot (TRTSTB), indicating residues were available for potential uptake by the clover.

Executive Summary, Part B

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For corn and for clover, there was no consistent trend in the magnitude of the pollen or nectar residues and the growth stage. There was no significant difference between early, mid, and late pollen shed or the bloom phase in white clover. There was no increase in pollen residues from year 1 to year 2 after re-planting seed treated corn. In fact, corn pollen residues were significantly higher in the first year of the trial than in the second year.

There were significant differences observed in corn pollen residues between years and trials. Therefore each trial year was considered separately with respect to summary statistics. The highest median and 90th percentile values for total residues in corn pollen were 7 ppb and 17 ppb, respectively.

For clover pollen and nectar residues, there were very few residues detected. There were no differences among the trials in 2013, and the results were combined for summary statistics. The median and 90th percentile values for clover pollen were less than the LOQ (<LOQ) and 3 ppb, respectively. The median and 90th percentile values for clover nectar were less than the LOD (<LOD) and <LOQ, respectively.

For the statistical analysis summarized in the table below, the LOD/LOQ for the total imidacloprid residue (sum of imidacloprid, imidacloprid olefin, and 5-hydroxy imidacloprid) is taken to be the sum of the individual analyte LODs/LOQs.

Total Imidacloprid Residues in Corn Pollen, Clover Pollen, and Clover Nectar

Matrix	Selected Summary Statistic (Source):[a]	Imidacloprid Olefin (ppb)	5-Hydroxy Imidacloprid (ppb)	Imidacloprid (ppb)	Total Residue (ppb)[b]
Corn Pollen	Median	<0.3[c]	<0.5[c]	6.5[e]	6.5 – 7.3[e]
	90th Percentile	<0.3[c]	<0.5[c]	16[e]	16 – 17[e]
Clover Pollen	Median	<0.3[c]	<0.5[c]	<1.0[d]	<1.8[d]
	90th Percentile	<0.3[c]	<0.5[c]	2.1	2.1 – 2.9[e]
Clover Nectar	Median	<0.6[c]	<0.7[c]	<0.3[b]	<1.6[b]
	90th Percentile	<0.6[c]	<0.7[c]	<1.0[d]	<2.3[d]

[a] Corn pollen statistical values are the highest values from any trial. Clover pollen and nectar statistical values are the values calculated across all trials.

[b] Median and 90th percentile summary statistics for each analyte were summed to estimate the value (or possible range of values) for that summary statistic for total residue (imidacloprid plus metabolites). These total residue median and 90th percentile values were considered <LOD if the corresponding summary statistic was <LOD for all three analytes, <LOQ if that summary statistic was<LOQ for all three analytes but ≥LOD for at least one analyte, and quantifiable if that summary statistic was ≥LOQ for at least one analyte. In the total residue ranges of values, the lower value is the sum of all quantifiable residues; the upper value is the sum of all residues, with non-quantifiable residues summed in at the shown LOD or LOQ value.

[c] <LOD

[d] <LOQ

[e] ≥LOQ

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Report: 02.02.01/42; [redacted]; 2014; M-501306-01-2
Title: Determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from treated cotton during two successive years and in white clover planted after treated cotton
Report No.: EBNTY010
Document No.: M-501306-01-2
Guideline(s): US EPA OPPTS/OCSPP 850.SUPP, Ecological Effects
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-501306-01-2@S-602302-01-1

Executive Summary, Part A

A total of three field trials were conducted each year for two successive years to measure the magnitude of imidacloprid residues in bee-relevant cotton pollen and nectar samples and in/on leaves, blossoms, and soil from cotton plants grown from seed treated with Gaucho 600 Flowable and sprayed with Admire Pro Systemic Protectant for two years consecutively, and to measure the magnitude of the same residues in/on bee-relevant white clover pollen and nectar samples and in blossoms, leaves, and soil from white clover plants grown at locations where cotton plants were grown from Gaucho 600 Flowable treated seed and sprayed with Admire Pro the previous year. Gaucho 600 Flowable is a flowable concentrate seed treatment formulation containing 600 g/L imidacloprid. Admire Pro Systemic Protectant is a suspension concentrate formulation for foliar spray use containing 550 g/L imidacloprid. Admire Pro Systemic Protectant and Gaucho 600 Flowable were applied to cotton seeds at target rates as shown below.

Target Application Summary

Plot ID[a]	Year[b]	Test Subs.	No. of Apps	Target Rate/Application					Target App. Interval (days)	Target PBI[c] (days)
				Formulated Product (fp)		Active Ingredient (ai)				
						Name of ai	lb ai/A	kg ai/ha		
UTCA	1, 2	NA[d]	NA	NA	NA	NA	NA	NA	NA	NA
UTCB	1, 2	NA	NA	NA	NA	NA	NA	NA	NA	NA
TRTDA	1, 2	Treated seeds (Gaucho)	1	2.1E-5 fl oz fp/seed	6E-4 ml fp/seed	Imidacloprid	0.048 (1.3E-5 oz ai/seed, 58,000 seeds/A)	0.054 (0.375 mg ai/seed, 58,000 seeds/A)	NA	NA
		Admire Pro	5	1.7 fl oz fp/A	124 ml fp/ha	Imidacloprid	0.061	0.068	5–8	14
TRTDB	1	Treated seeds (Gaucho)	1	2.1E-5 fl oz fp/seed	6E-4 ml fp/seed	Imidacloprid	0.048 (1.3E-5 oz ai/seed, 58,000 seeds/A)	0.054 (0.375 mg ai/seed, 58,000 seeds/A)	NA	NA
		Admire Pro	5	1.7 fl oz fp/A	124 ml fp/ha	Imidacloprid	0.061	0.068	5–8	14
TRTDB	2	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a] Plot ID: UTCA = Untreated control plot receiving untreated cotton seed in years 1 and 2.
UTCB = Untreated control plot receiving untreated cotton seed in year 1 and untreated forage crop (white clover) in year 2.
TRTDA = Treated plot receiving cotton seed treated with Gaucho 600 Flowable and 5 foliar applications of Admire Pro Systemic Protectant in years 1 and 2.
TRTDB = Treated plot receiving cotton seed treated with Gaucho 600 Flowable and 5 foliar applications of Admire Pro Systemic Protectant in year 1 and untreated forage crop (white clover) in year 2.

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[b] A fresh batch of treated seed was used for the second year's planting of plot TRTDA. Study year 1 was 2012, and study year 2 was 2013.
[c] PBI = Pre-bloom interval, the interval between the last application and the beginning of blooming.
[d] NA = Not applicable.

The actual application rates and spray volumes for imidacloprid are summarized in the table below. All spray applications were made using ground-based equipment with adjuvant (Dyne-Amic 0.25% v/v). Plot seeding rates ranged from 57,088 to 60,002 seeds/A. All plots were tilled prior to year 2 seed planting.

Actual Application Summary for Imidacloprid

Plot ID	Year	Application Type	App. No.	BBCH Growth Stage[a]	Individual Spray Volumes		Individual Rates per Application		Total Rates		App. Interval (days)[b]
					GPA	LPH	lb ai/A	kg ai/ha	lb ai/A	kg ai/ha	
UTCA	1, 2	NA[c]	NA	NA	NA	NA	NA	NA	NA	NA	NA
UTCB	1, 2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TRTDA	1	Treated seeds	1	00	NA	NA	0.047–0.048	0.053	0.35	0.39	NA
		Foliar Spray	1–5	19–60	9.9–10.1	93–94	0.060–0.061	0.067–0.069			5–8
TRTDA	2	Treated seeds	1	00	NA	NA	0.048–0.050	0.054–0.056	0.35	0.39–0.40	NA
		Foliar Spray	1–5	24–59	9.8–10.2	92–95	0.060–0.062	0.067–0.069			5–6
TRTDB	1	Treated seeds	1	00	NA	NA	0.047–0.048	0.053	0.35	0.39	NA
		Foliar Spray	1–5	19–60	9.9–10.1	93–94	0.060–0.061	0.067–0.069			5–8
TRTDB	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a] BBCH 19: nine or more leaves unfolded; BBCH 24: four side shoots detectable; BBCH 59: first petals visible, many individual flower buds still closed; BBCH 60: first flowers open.
[b] First foliar spray applications were made 38–61 days after planting.
[c] NA = Not applicable.

Composite samples (separate runs through the plot) of cotton leaves, blossoms for direct analysis, and blossoms to be processed for nectar and pollen were collected by hand per sample period from plots UTCA and TRTDA in years 1 and 2 and from plots UTCB and TRTDB in year 1. Cotton pollen, floral nectar, and extrafloral nectar samples were extracted by hand from the processed blossoms at the field trial site. In year 2, clover seeds were planted on plots UTCB and TRTDB (previously treated in year 1); however, no clover grew in trial NT013-12ZA, so no clover samples could be collected and no bees were used. In trials NT014-12HA and NT015-12ZA, clover samples were collected from within erected bee tents; one tent was placed per plot, and inside each tent one honey bee (Apis mellifera) hive was placed several days prior to clover sampling. Composite samples of white clover leaves and blossoms for direct analysis were collected by hand, and composite samples of nectar and pollen were collected from the bee hives.

Sample collection began 13 to 15 days after the last application (DAA). Samples of cotton leaves, blossoms for direct analysis, and blossoms to be processed for nectar and pollen were collected at five sampling periods per year of collection, targeted for when the cotton plants were at growth stages of BBCH 60, 61, 65, 67, and 69 (cotton BBCH 60: first flowers opened, sporadically within the population; BBCH 69: end of flowering). Samples of white clover leaves, blossoms, nectar (hive-collected), and pollen (hive-collected) were collected at four sampling periods per year, targeted when the clover plants were at growth stages of BBCH 61, 63, 65, and 67 (clover BBCH 61: flowers open on first raceme; BBCH 67: flowering declining). Nine soil samples were collected from all plots using a soil sampling device before cotton planting in year 1, before cotton planting in year 2, and after all sampling was complete in year 2.

For all plant-based (non-soil) matrices, five samples were targeted for collection from treated plots and two samples from untreated control plots at each sampling interval. In some trials, not enough matrix

material was present to allow for the full number of target samples to be collected (see Appendix 1). Additionally, because cotton and clover are continuously blooming plants, the sampling target BBCH growth stages were not always met; in such cases, samples were taken at approximate 1-week intervals.

The residues of imidacloprid, 5 hydroxy imidacloprid, and imidacloprid olefin were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The limit of detection (LOD) for the total residue is the highest LOD value for an individual analyte in a particular matrix. The limit of quantification (LOQ) for the total residue is the highest LOQ value for an individual analyte in a particular matrix.

The LOQs and LODs are shown below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ[a] (ppm)	LOD[a] (ppm)
Cotton blossoms for direct analysis	Imidacloprid	0.0100	0.0017
	5-hydroxy Imidacloprid	0.0100	0.0019
	Imidacloprid olefin	0.0100	0.0020
	Total Residue	**0.0100**	**0.0020**
Cotton leaves	Imidacloprid	0.0100	0.0049
	5-hydroxy Imidacloprid	0.0100	0.0029
	Imidacloprid olefin	0.0100	0.0017
	Total Residue	**0.0100**	**0.0049**
Pollen (cotton and clover)	Imidacloprid	0.0010	0.0004
	5-hydroxy Imidacloprid	0.0010	0.0005
	Imidacloprid olefin	0.0010	0.0003
	Total Residue	**0.0010**	**0.0005**
Nectar (cotton and clover)	Imidacloprid	0.0010	0.0003
	5-hydroxy Imidacloprid	0.0010	0.0007
	Imidacloprid olefin	0.0010	0.0006
	Total Residue	**0.0010**	**0.0007**
Clover blossoms	Imidacloprid	0.0100	0.0005
	5-hydroxy Imidacloprid	0.0100	0.0015
	Imidacloprid olefin	0.0100	0.0027
	Total Residue	**0.0100**	**0.0027**
Clover leaves	Imidacloprid	0.0100	0.0015
	5-hydroxy Imidacloprid	0.0100	0.0025
	Imidacloprid olefin	0.0100	0.0025
	Total Residue	**0.0100**	**0.0025**
Soil	Imidacloprid	0.0050	0.0006
	5-hydroxy Imidacloprid	0.0050	0.0026
	Imidacloprid olefin	0.0050	0.0016

[a] Soil LODs and LOQs are reported in individual analyte equivalents, and no total imidacloprid residue is calculated. All other matrix analyte LODs and LOQs are reported in parent equivalents.

Storage stability studies and transit spikes indicate that the imidacloprid residues would have been stable during frozen storage for at least 741 days (24 months) in cotton and clover matrices and for at least 793 days (26 months, imidacloprid) or 1281 days (42 months, imidacloprid olefin and 5-hydroxy imidacloprid) in soil matrices prior to analysis (Section 5.0). The maximum storage period of frozen samples in this study for imidacloprid was 621 days for cotton blossoms, 595 days for cotton leaves, 413 days for cotton extrafloral nectar, 409 days for cotton floral nectar, 544 days for cotton pollen, 218 days

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for clover blossoms, 223 days for clover leaves, 245 days for clover nectar, 250 days for clover pollen, and 734 days for soil prior to extraction.

The imidacloprid residues in cotton leaves, blossoms, pollen, floral nectar, and extrafloral nectar; clover leaves, blossoms, pollen, and nectar; and soil are given in Table 8 (SP C.3.). A statistical evaluation of the total imidacloprid residues in blossoms, leaves, and the bee-relevant matrices of pollen and nectar is described in Section 3.6.

A discussion of the total imidacloprid residues in soil is presented in Section 3.7. The imidacloprid residues in soil showed higher concentrations in the second year of the study for the cotton/cotton plot (TRTDA) and lower residues in the second year of the study for the cotton/clover plot (TRTDB, which received no year 2 application), indicating residues were available for potential uptake by the clover.

Executive Summary, Part B

The total imidacloprid residues in/on cotton extrafloral nectar, floral nectar, and pollen declined with time after the last foliar application of Admire Pro to the cotton plants in both years of the study. These results indicate imidacloprid residues are likely to be highest shortly after a foliar spray treatment, and residues will decrease rapidly with time.

The total imidacloprid residues in/on clover nectar and clover pollen were very low, near the LOD of the analytical methods. No pattern of decline or increase could be identified. Similar residues were seen in the control samples from the trial sites.

The median and 90th percentile values (summary statistics) for treated cotton pollen, floral nectar, and extrafloral nectar and for rotational crop clover pollen and nectar were compiled by trial or by trial and year. The highest summary statistics for each matrix are given in the table below. These values are recommended for exposure risk assessment.
For the statistical analysis summarized in the table below, the LOD/LOQ for the total imidacloprid residue (sum of imidacloprid, imidacloprid olefin, and 5-hydroxy imidacloprid) is taken to be the sum of the individual analyte LODs/LOQs.

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Overall Results of Summary Statistics

Matrix	Selected Summary Statistic (Source)	Imidacloprid Olefin (ppb)	5-Hydroxy Imidacloprid (ppb)	Imidacloprid (ppb)	Total Residue (ppb)[a]
Cotton Extrafloral Nectar	Median (NT013-12ZA, year 2)	<0.64 [b]	<0.65 [b]	10 [d]	10 - 11 [d]
	90th Percentile (NT013-12ZA, year 2)	<0.64 [b]	1.7 [d]	18 [d]	20 - 21 [d]
Cotton Nectar	Median (NT015-12ZA, year 2)	<0.64 [b]	<0.65 [b]	11 [d]	11 - 12 [d]
	90th Percentile (NT015-12ZA, year 2)	1.3 [d]	1.3 [d]	28 [d]	30 [d]
Cotton Pollen	Median (NT013-12ZA, year 2)	<0.33 [b]	<0.48 [b]	2.4 [d]	2.4 - 3.2 [d]
	90th Percentile (NT014-12HA, year 2)	<0.33 [b]	<0.48 [b]	19 [d]	19 - 20 [d]
Clover Nectar	Median (NT015-12ZA)	<0.64 [b]	<0.65 [b]	<0.53 [b]	<1.6 [b]
	90th Percentile (NT015-12ZA)	<0.64 [b]	<0.65 [b]	1.0 [d]	1.0 - 2.3 [d]
Clover Pollen	Median (NT015-12ZA)	<0.33 [b]	<0.48 [b]	<1.0 [c]	<1.8 [c]
	90th Percentile (NT015-12ZA)	1.0 [c]	<0.48 [b]	2.2 [d]	2.2 - 3.7 [d]

[a] Median and 90th percentile summary statistics for each analyte were summed to estimate the value (or possible range of values) for that summary statistic for total residue (imidacloprid plus metabolites). These total residue median and 90th percentile values were considered <LOD if the corresponding summary statistic was <LOD for all three analytes, <LOQ if that summary statistic was<LOQ for all three analytes but ≥LOD for at least one analyte, and quantifiable if that summary statistic was ≥LOQ for at least one analyte. In the total residue ranges of values, the lower value is the sum of all quantifiable residues; the upper value is the sum of all residues, with non-quantifiable residues summed in at the shown LOD or LOQ value.

[b] <LOD

[c] <LOQ

[d] ≥LOQ

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Report: 02.02.01/43; [redacted]; 2014; M-503101-01-2
Title: Admire Pro - Magnitude of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from citrus trees following foliar applications of imidacloprid over two successive years
Report No.: EBNTY007
Document No.: M-503101-01-2
Guideline(s): US EPA OPPTS/OCSPP 850.SUPP, Ecological Effects
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-503101-01-2@S-602311-01-1

Executive Summary, Part A

A total of three field trials were conducted each year for two successive years to measure the magnitude of imidacloprid residues in bee-relevant pollen and nectar samples and in/on flowers, leaves, and soil from citrus trees following two foliar applications per year of ADMIRE PRO Systemic Protectant. ADMIRE PRO is a suspension concentration containing 550 g/L imidacloprid. ADMIRE PRO was applied to citrus trees at target rates and timings as shown below.

Target Application Summary

Plot ID[a]	Year	Test Subs.	No. of Apps.	Target Rate/Application						Target App. Interval (days)	Target PBI (days)[b]	Spray Volume	
				Formulated Product (fp)			Active Ingredient (ai)					GPA min.	LPHA min.
				fl oz fp/A	ml fp/ha	Name of ai	lb ai/A	g ai/ha					
UTC	1, 2	NA[c]	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA
TRTD	1, 2	Admire Pro	2	7.0	512	imidacloprid	0.25	280.5		8-10	10	50	468

[a] Plot ID: UTC = untreated control plot.
TRTD = Treated plot with two pre-bloom foliar spray applications of ADMRIE PRO with an appropriate additive.
[b] PBI = Pre-bloom interval. If necessary, samples may be collected after the second application when the blooms start to open.
[c] NA = Not applicable.

Plot TRTD received two foliar applications of ADMIRE PRO® Systemic Protectant in years 1 and 2 of the study (2012 and 2013, respectively). Individual application rates ranged from 0.2818 to 0.2913 kg imidacloprid/ha/application (0.2514 to 0.2599 lb imidacloprid/A/application) in year 1 and from 0.2841 to 0.2908 kg imidacloprid/ha/application (0.2535 to 0.2594 lb imidacloprid/A/application) in year 2. Total seasonal application rates ranged from 0.565 to 0.577 kg imidacloprid/ha (0.504 to 0.515 lb imidacloprid/A) in year 1 and from 0.569 to 0.580 kg imidacloprid/ha (0.507 to 0.517 lb imidacloprid/A) in year 2. All applications were made between BBCH growth stages 51 and 59 (BBCH 51: inflorescence buds swelling: buds closed, light green scales visible; BBCH 59: most flowers with petals forming a hollow ball) in year 1 and between BBCH growth stages 31 and 61 (BBCH 31: beginning of shoot growth, axes of developing shoots visible; BBCH 61: beginning of flowering, about 10% of flowers open) in year 2. In both years, the intervals between applications were 8 to 10 days, and the spray volumes for plot TRTD ranged from 60 to 70 gal/A. All applications were made with adjuvant (Dyne-Amic, 0.25% to 0.50%) using ground-based equipment.

Due to principal field investigator (PFI) oversight, which was not known at study initiation, the plots in trial NT004-12ZA were sprayed with Provado, an insecticide containing imidacloprid, in both 2010 and 2011. Additionally, Prev, another insecticide containing imidacloprid, was used as a maintenance pesticide on both the UTC and TRTD plots in September of 2012 and 2013. Because of the additional imidacloprid added to the plots prior to the study, the residue values are notably higher in this trial.

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In all trials, five composite (separate runs through the plot) samples of citrus flowers for direct analysis, flowers to be processed for nectar and pollen, and leaves were collected by hand at each sampling period from plot TRTD in years 1 and 2. Citrus pollen and nectar samples were collected by hand at the field trial site from the flowers collected for processing.

Citrus flowers were collected at four sampling periods, when the citrus trees were at growth stages of BBCH 61, 64, 65, and 67 (BBCH 61: beginning of flowering, about 10% of flowers open; BBCH 67: flower fading, majority of petals fallen), corresponding to 4 to 38 days after the last application (DAA). Exceptions are trial NT006-12ZA year 2, when only BBCH 64 and 65 flowers were collected, and trial NT004-12ZA year 2, when samples were collected at BBCH 60 and not at BBCH 64. Citrus leaves were collected at six sampling periods, when the citrus trees were at growth stages of BBCH 59, 61, 64, 65, 67, and 69 (BBCH 59: most flowers with petals forming a hollow ball, BBCH 69: end of flowering, all petals fallen), corresponding to 3 to 49 DAA. The exceptions are trial NT004-12ZA, when leaves were collected at BBCHs of 55, 60, 61, 65, 67, and 83 (BBCH 55: flowers visible, still closed (green bud), borne on single or multiflowered leafy or leafless inflorescences; BBCH 83: fruit ripe for picking; fruit has not yet developed variety-specific color), and trial NT006-12ZA, when no BBCH 61 samples were collected in year 2. Nine soil samples were collected prior to treatment and at the end of the growing season per plot per year, except in trial NT006-12ZA, when only seven samples were collected prior to the year 2 applications.

Two composite samples of all citrus matrices and nine composite samples of soil were collected from the control plot of each trial at the same sampling periods as for the treated samples of their sample type.

The residues of ADMIRE PRO (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The limit of detection (LOD) for the total residue is the highest LOD value for an individual analyte in a particular matrix. The limit of quantification (LOQ) for the total residue is the highest LOQ value for an individual analyte in a particular matrix.

The limits of detection (LODs) are shown below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ[a] (ppm)	LOD[a] (ppm)
Citrus flowers	Imidacloprid	0.010	0.0009
	5-Hydroxy imidacloprid	0.010	0.0011
	Imidacloprid olefin	0.010	0.0033
	Total Imidacloprid	**0.010**	**0.0033**

Matrix	Analyte	LOQ[a] (ppm)	LOD[a] (ppm)
Citrus leaves	Imidacloprid	0.010	0.0027
	5-Hydroxy imidacloprid	0.010	0.0030
	Imidacloprid olefin	0.010	0.0030
	Total Imidacloprid	**0.010**	**0.0030**
Nectar	Imidacloprid	0.001	0.0003
	5-Hydroxy imidacloprid	0.001	0.0007
	Imidacloprid olefin	0.001	0.0006
	Total Imidacloprid	**0.001**	**0.0007**
Pollen	Imidacloprid	0.001	0.0004
	5-Hydroxy imidacloprid	0.001	0.0005
	Imidacloprid olefin	0.001	0.0003
	Total Imidacloprid	**0.001**	**0.0005**
Soil	Imidacloprid	0.005	0.0012
	5-Hydroxy imidacloprid	0.005	0.0015
	Imidacloprid olefin	0.005	0.0024

[a] Soil LODs and LOQs are reported in individual analyte equivalents, and no total imidacloprid residue is calculated. All other matrix analyte LODs and LOQs are reported in parent equivalents.

Storage stability studies and transit spikes indicate that the imidacloprid residues would have been stable during frozen storage for at least 1080 days (36 months) in citrus matrices and for at least 1281 days (42

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months) in soil matrices prior to analysis (Section 5.0). The maximum storage period of frozen samples in this study for imidacloprid was 790 days for citrus flowers, 787 days for citrus leaves, 532 days for citrus nectar, 770 days for citrus pollen, and 910 days for soil prior to extraction (Appendix 1).

The imidacloprid residues in citrus leaves, flowers, pollen, nectar, and soil are given in Table 8 (SP C.3.). A statistical evaluation of the total imidacloprid residues in the bee-relevant matrices of pollen and nectar is described in Section 3.7.

The total imidacloprid residues in/on citrus blossoms and citrus leaves typically declined with time after the last foliar application of Admire Pro to the citrus trees in both years of the study. A discussion fo the imdacloprid residues in leaves and blossoms is given in Section 3.6.

A discussion of the imidacloprid residues in soil is given in Section 3.8. Imidacloprid residues in the surface soil were variable with respect to time, so no clear trends could be seen, other than a lack of higher residues in the second year of the study, indicating dissipation of imidacloprid in the surface soil (movement of residues below six inches, and/or degradation).

Executive Summary, Part B
In this citrus study, three field trials were conducted for two consecutive years (2012 and 2013). In each trial, citrus trees received two foliar pre-bloom applications per year (2 x 0.25 lb ai/ac). The first foliar spray was approximately 18 days pre-bloom and the second foliar spray was approximately 10 days pre-bloom. First samples were collected at early bloom, and there were 3 more intervals of sample collection prior to petal fall. Due to differences in trials and weather over the 2 years, the first samples were collected as early as 4 days after the final application (DAA) and as late as 30 DAA.

- Nectar residues declined over the bloom interval. Of the six trial-years, five had data sets appropriate to analyze a decline. One of the five exhibited no decline in nectar residues; the remaining four exhibited a significant decline, with half-lives ranging from 4 to 7 days. Therefore, nectar acute and chronic exposure values were calculated for each trial-year based on the earliest sampled residues as a conservative estimate of potential exposure following pre-bloom foliar applications. The highest resulting exposure estimates from all of the trial-years are presented in the summary table below.
- Pollen residues among the six trial-years were more consistent during citrus bloom. In two of the six trials-years, there was a decline in pollen residue. In those cases where pollen residues did decline, the half-life was similar to nectar at approximately 4 to 7 days. Therefore, pollen acute and chronic exposure values were calculated based on the earliest sampled residues for the trial-years that exhibited significant decline and based on all sampled residues for those trial-years that did not exhibit a significant decline. The highest resulting exposure estimates from all of the trial-years are presented in the summary table below.
- Residues in both pollen and nectar were lower overall in trial-years with a longer interval between the last foliar application and first bloom. This suggests that a longer pre-bloom interval for foliar applications may result in reduced overall total imidacloprid residues in pollinator food items.

Calculated Acute and Chronic Exposure Values

Citrus (2 x 0.25 lb ai/ac)	Maximum Total Residue[a]	Trial
Pollen	4100 ppb	NT006-12ZA
Nectar	430 ppb	NT006-12ZA
	Median Total Residue[a]	
Pollen	2900 ppb	NT005-12ZA
Nectar	290 ppb	NT006-12ZA

[a] Maximum total residue represents an estimate of acute exposure to pollinators and median represents chronic. Values from trial NT004-12ZA are excluded because plots in that trial received additional imidacloprid treatments that were not part of the intended study design.

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Report: 02.02.01/44; [redacted]; 2014; M-505618-01-2
Title: Determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from cherry trees following foliar application of imidacloprid over two successive years
Report No.: EBNTY008
Document No.: M-505618-01-2
Guideline(s): US EPA OPPTS/OCSPP 850.SUPP, Ecological Effects
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-505618-01-2@S-602383-01-1

Executive Summary, Part A

A total of four field trials were conducted each year for two successive years to measure the magnitude of imidacloprid residues in bee-relevant pollen and nectar samples and in/on flowers, leaves, and soil from cherry trees following five foliar applications per year of ADMIRE PRO Systemic Protectant. ADMIRE PRO is a suspension concentration containing 550 g/L imidacloprid. ADMIRE PRO was applied to cherry trees at target rates and timings as shown below.

Target Application Summary

Plot ID[a]	Year	Test Subs.	No. of Apps.	Target Rate/Application					Target App. Interval (days)	Target DAA (days)[b]	Spray Volume	
				Formulated Product (fp)		Active Ingredient (ai)						
				fl oz fp/A	ml fp/ha	Name of ai	lb ai/A	g ai/ha			GPA min.	LPHA min.
UTC	1, 2	NA[c]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TRTD	1, 2	Admire Pro	5	2.8	205	Imidacloprid	0.1	112	8-10	5-7	50	468

[a] Plot ID: UTC12/UTC13 = Untreated control plot (UTC) initiated in 2012/2013.
TRTD12/TRTD13 = Treated plot (TRTD) with five post-bloom foliar spray applications of ADMIRE PRO with an appropriate additive made in 2012/2013.
[b] DAA = Days after application; the last application was targeted to occur 5 to 7 days prior to harvest.
[c] NA = Not applicable.

Plot TRTD received five foliar applications of ADMIRE PRO® Systemic Protectant in each of years 2012 and 2013. All applications were made with adjuvant (Dyne-Amic 0.3%) using ground-based equipment.

In 2012, individual application rates ranged from 0.1097 to 0.1149 kg imidacloprid/ha per application (0.0978 to 0.1025 lb imidacloprid/A per application). Total seasonal application rates ranged from 0.560 to 0.569 kg imidacloprid/ha (0.500 to 0.507 lb imidacloprid/A). The first applications were made after cherry harvest, at BBCH growth stage 91 (BBCH 91: shoot growth completed; foliage still fully green), and the interval between applications was 8 to 10 days. The spray volumes for plot TRTD ranged from 50 to 107 gal/A.

In 2013, individual application rates ranged from 0.1115 to 0.1145 kg imidacloprid/ha per application (0.0994 to 0.1022 lb imidacloprid/A per application). Total seasonal application rates ranged from 0.560 to 0.564 kg imidacloprid/ha (0.499 to 0.503 lb imidacloprid/A). The first applications were made prior to cherry harvest, between BBCH growth stages 73 and 75 (BBCH 73: second fruit fall; BBCH 75: fruit about half final size), and the interval between applications was 8 to 11 days. The spray volumes for plot TRTD ranged from 50 to 99 gal/A.

Samples that were treated in 2012 were harvested in 2013 (this period inclusively considered year 1 of the study), and samples treated in 2013 were harvested in 2014 (year 2 of the study). Each treated plot TRTD was divided into 5 subplots. In trials NT007-12ZA, NT008-12ZA, and NT016-12ZA, one composite (separate runs through each subplot) sample of cherry flowers for direct analysis, flowers to be processed

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for nectar and pollen, and leaves was collected by hand at each sampling period from each subplot (for 5 total samples) in both study years. In trial NT017-12ZA, no samples after the fall 2013 applications were collected because the trees were removed from the orchard by the grower. Samples were taken from the same trees at each sampling interval and in successive years. Cherry pollen and nectar samples were harvested by hand at the field trial site from the flowers collected for processing. Two composite samples of all cherry matrices were collected from the control plot of each trial at the same sampling periods as the treated samples of that sample type.

Cherry flowers were collected when the trees were at growth stages of BBCH 61 and 65 (BBCH 61: beginning of flowering, about 10% of flowers open; BBCH 65: full flowering, at least 50% of flowers open and first petals falling), corresponding to 205 to 218 days after the last application (DAA) in year 1 and 274 to 303 DAA in year 2. The exception is trial NT007-12ZA, which could not harvest BBCH 61 flowers in year 2 (2014) due to weather. Cherry leaves were collected when the trees were at growth stages of BBCH 65 and 69 (BBCH 69: end of flowering, all petals fallen), corresponding to 209 to 232 DAA in year 1 and 279 to 312 DAA in year 2.

Nine soil samples were collected twice during the first year of the study, prior to the first a.i. application in 2012 and after the last sampling per plot in 2013, with the exception of trials NT007-12ZA and NT008-12ZA, in which the last leaf collection took place after the year 1 2013 soil collection. Soil samples were also collected twice during the second year of the study, after the last application in 2013 and after the last sampling in 2014, with the exception of trial NT017-12ZA, in which the 2014 samples were not collected due to the tree removal.

The residues of ADMIRE PRO (imidacloprid, 5-hydroxy imidacloprid and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The limit of detection (LOD) for the total residue is the highest LOD value for an individual analyte in a particular matrix. The limit of quantification (LOQ) for the total residue is the highest LOQ value for an individual analyte in a particular matrix.

The LOQs and LODs are summarized in the table below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ[a] (ppm)	LOD[a] (ppm)
Cherry flowers	Imidacloprid	0.0100	0.0010
	5-Hydroxy imidacloprid	0.0100	0.0016
	Imidacloprid olefin	0.0100	0.0013
	Total Imidacloprid	**0.0100**	**0.0016**

Matrix	Analyte	LOQ[a] (ppm)	LOD[a] (ppm)
Cherry leaves	Imidacloprid	0.0100	0.0020
	5-Hydroxy imidacloprid	0.0100	0.0031
	Imidacloprid olefin	0.0100	0.0020
	Total Imidacloprid	**0.0100**	**0.0031**
Cherry nectar	Imidacloprid	0.0010	0.0003
	5-Hydroxy imidacloprid	0.0010	0.0007
	Imidacloprid olefin	0.0010	0.0006
	Total Imidacloprid	**0.0010**	**0.0007**
Cherry pollen	Imidacloprid	0.0010	0.0004
	5-Hydroxy imidacloprid	0.0010	0.0005
	Imidacloprid olefin	0.0010	0.0003
	Total Imidacloprid	**0.0010**	**0.0005**
Soil	Imidacloprid	0.005	0.0013
	5-Hydroxy imidacloprid	0.005	0.0015
	Imidacloprid olefin	0.005	0.0018

[a] Soil LODs and LOQs are reported in individual analyte equivalents, and no total imidacloprid residue is calculated. All other matrix analyte LODs and LOQs are reported in parent equivalents.

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Storage stability studies and transit spikes indicate that the imidacloprid residues would have been stable during frozen storage for at least 1080 days (36 months) in cherry flower and leaf matrices and for at least 1281 days (42 months) in soil matrices prior to analysis (Section 5.0). The maximum storage period of frozen samples in this study for imidacloprid was 452 days for cherry flowers, 462 days for cherry leaves, 129 days for cherry nectar, 500 days for cherry pollen, and 668 days for soil prior to extraction.

The imidacloprid residues in soil and cherry leaves, flowers, pollen, and nectar are given in Table 8 (SP C.3.). A statistical evaluation of the total imidacloprid residues in flowers, leaves, and the bee-relevant matrices of pollen and nectar is described in Section 3.6.

A discussion of the imidacloprid residues in soil is given in Section 3.7. Residues of imidacloprid generally increased following the year 1 and 2 applications, but declined over the last 300 days of the study, indicating imidacloprid was dissipating (movement below 6 inches, or degradation) in the soil.

Executive Summary, Part B

In this cherry study, four field trials were conducted for two consecutive years. In each trial, cherry trees received five foliar applications of ADMIRE PRO® Systemic Protectant in both 2012 and 2013 (target was 5 x 0.1 lb imidacloprid/A with 8 to 11 days between applications). All applications were made with adjuvant (Dyne-Amic 0.3%) using ground-based equipment. In 2012, applications were made after cherry harvest at BBCH growth stage 91 (BBCH 91: shoot growth completed; foliage still fully green). In 2013, applications were made prior to cherry harvest, between BBCH growth stages 73 and 75 (BBCH 73: second fruit fall; BBCH 75: fruit about half final size). Samples treated in 2012 were harvested in 2013 (this period inclusively is considered year 1 of the study), and samples treated in 2013 were harvested in 2014 (year 2 of the study).

In both years, nectar and pollen samples were collected at growth stages of BBCH 61 and 65 (BBCH 61: beginning of flowering, about 10% of flowers open; BBCH 65: full flowering, at least 50% of flowers open and first petals falling). For the year 1 samples, these growth stages occurred between 205 and 218 days after the last application (DAA). For the year 2 samples, these growth stages occurred between 274 and 303 DAA. The longer period between application and sampling in year 2 than in year 1 was largely due to the difference in application timing between the two years (i.e., post-harvest application in year 1 vs. pre-harvest application in year 2).

Within each trial and year, total imidacloprid residues in nectar and pollen were generally similar between BBCH 61 and 65. Additionally, for both nectar and pollen, total imidacloprid residues were lower and less variable in year 2 than in year 1. This difference was most likely related to the longer pre-bloom application interval in year 2.

The most conservative median and 90th percentile summary statistics from each trial and year, for each of pollen and nectar, are provided in the table below. These values are recommended as conservative dietary exposure estimates for use in pollinator risk assessments.

Calculated Acute and Chronic Exposure Values

Cherry (5 x 0.1 lb ai/ac)	90th Percentile Total Residue (ppb)[a]	Trial, Year
Pollen	660	NT008-12ZA, Year 1
Nectar	7.7	NT007-12ZA, Year 1
	Median Total Residue (ppb)[a]	
Pollen	400	NT008-12ZA, Year 1
Nectar	4.6	NT007-12ZA, Year 1

[a] 90th percentile total residue represents an estimate of acute exposure to pollinators and median represents chronic.

>>M-505618-01-2@S-602383-01-1

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Report: 02.02.01/45; [redacted]; 2014; M-506016-01-2
Title: Determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from blueberries following soil application of imidacloprid over two successive years - Admire pro systemic protectant (550 g/L) (imidacloprid SC 550 G)
Report No.: EBNTY006
Document No.: M-506016-01-2
Guideline(s): US EPA OPPTS/OCSPP 850.SUPP, Ecological Effects
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-506016-01-2@S-602385-01-1

Executive Summary, Part A

A total of three field trials were conducted each year for two successive years to measure the magnitude of imidacloprid residues in bee-relevant pollen and nectar samples and in/on flowers, leaves, and soil from blueberry plants following one post-harvest banded soil application per year of ADMIRE PRO Systemic Protectant. ADMIRE PRO is a suspension concentration containing 550 g/L imidacloprid. ADMIRE PRO was applied to blueberry plants at target rates and timings as shown below.

Target Application Summary

Plot ID[a]	Year	Test Subs.	No. of Apps.	Target Rate/Application					Target App. Interval	Target PHI[b] (days)	Spray Volume	
				Formulated Product (fp)		Active Ingredient (ai)						
				fl oz fp/A	ml fp/ha	Name of ai	lb ai/A	g ai/ha			GPA	LPHA
UTC	1, 2	NA[c]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TRTD	1, 2	Admire Pro	1	14	1023	Imidacloprid	0.50	561	NA	-3	15-50	140- 470

[a] Plot ID: UTC = untreated control plot, size sufficient to hold two 150-270 ft x 15-20 ft bee tents.
TRTD = Treated plot with one post-harvest soil application of ADMIRE PRO, size sufficient to hold five 150-270 ft x 15-20 ft bee tents.
[b] PHI = Pre-harvest interval. Single applications to occur 3 days post-harvest on target date of Oct. 1 each year.
[c] NA = Not applicable.

Plot TRTD received one post-harvest soil application of ADMIRE PRO® Systemic Protectant sprayed as an 18-inch band on each side of the row in years 1 and 2 of the study. Individual application rates ranged from 0.558 to 0.565 kg imidacloprid/ha/application (0.498 to 0.504 lb imidacloprid/A/application) in year 1 and from 0.559 to 0.564 kg imidacloprid/ha/application (0.499 to 0.503 lb imidacloprid/A/application) in year 2. The total seasonal application rates are equivalent to the individual rates. The applications were made between September 26 and October 4 of each year, when the plants were between BBCH growth stages 92 and 97 (BBCH 92: leaves begin to change color or fall; BBCH 97: plant resting or dormant). The spray volumes for plot TRTD ranged from 19.2 to 20.0 gal/A (180 to 187 L/ha) in year 1 and from 16.8 to 20.0 gal/A (158 to 187 L/ha) in year 2. All applications were made using ground-based equipment.

In all trials, five bee tunnels were erected on treated plot TRTD and two bee tunnels were erected on untreated plot UTC in years 1 and 2 of the study. In year 1 of the study, one honey bee (Apis mellifera) hive was placed in each tunnel for the collection of pollen and nectar. The honey bees could not collect sufficient blueberry pollen in year 1, so bumble bee (Bombus impatiens) colonies (1 to 4 per tunnel) were also placed in the tents in year 2 to provide additional pollen collection.

Composite samples (separate runs through the subplot) of blueberry leaves, flowers, nectar, and pollen were collected from plots UTC and TRTD in years 1 and 2 of the study, except in trial NT002-12ZA, in which no year 2 UTC samples could be collected because the UTC blueberry plants died the previous

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winter. Leaves and flowers were collected by hand. Nectar and pollen were collected using honey bees and/or bumble bees. Blueberry flowers, nectar, and pollen were to be collected at four target sampling periods between BBCH growth stages 60 and 67 (BBCH 60: first flowers open; BBCH 67: flower fading, majority of petals fallen) in both years. Actual collection took place 228 to 257 days after the last application (DAA) at BBCH 60 to 69 (BBCH 69: end of flowering, fruit set visible). Blueberry leaves were to be collected at six target sampling periods, when the blueberry plants were between BBCH growth stages 59 and 69 (BBCH 59: first flower petals visible in petalled forms) in both years. Actual collection took place 228 to 264 DAA at BBCH 59 to 74 (see Appendix 1).

All samples were collected from within the erected bee tunnels on both plots except in trial NT002-12ZA when leaf samples taken prior to and after pollen and nectar sampling were collected from the bushes on which the tunnels would be/were erected because the tunnels were not yet present. For all plant-based (non-soil) matrices, five samples were targeted for collection from treated plot TRTD and two samples from untreated control plot UTC at each sampling period, corresponding to one sample per erected bee tunnel (also referred to as a subplot). In all trials, not enough plant matrix material was present at every sampling interval to allow for the full number of target samples or sampling intervals to be collected (see Appendix 1).

Nine soil samples were collected prior to treatment and after sampling per plot per year. Exceptions were trial NT001-12ZA year 2, when the soil samples were collected before the spray application and just before sampling, and trial NT003-12ZA, when the only soil samples collected were after sampling in year 2 (no year 1 soil samples).

The residues of imidacloprid, 5-hydroxy imidacloprid and imidacloprid olefin were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The limit of detection (LOD) for the total residue is the highest LOD value for an individual analyte in a particular matrix. The limit of quantification (LOQ) for the total residue is the highest LOQ value for an individual analyte in a particular matrix.

The LOQs and LODs are shown below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ[a] (ppm)	LOD[a] (ppm)
Blueberry flowers	Imidacloprid	0.0100	0.0036
	5-Hydroxy imidacloprid	0.0100	0.0035
	Imidacloprid olefin	0.0100	0.0036
	Total Imidacloprid	**0.0100**	**0.0036**

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Matrix	Analyte	LOQ[a] (ppm)	LOD[a] (ppm)
Blueberry leaves	Imidacloprid	0.0100	0.0055
	5-Hydroxy imidacloprid	0.0100	0.0016
	Imidacloprid olefin	0.0100	0.0023
	Total Imidacloprid	**0.0100**	**0.0055**
Nectar	Imidacloprid	0.0010	0.0003
	5-Hydroxy imidacloprid	0.0010	0.0007
	Imidacloprid olefin	0.0010	0.0006
	Total Imidacloprid	**0.0010**	**0.0007**
Pollen	Imidacloprid	0.0010	0.0004
	5-Hydroxy imidacloprid	0.0010	0.0005
	Imidacloprid olefin	0.0010	0.0003
	Total Imidacloprid	**0.0010**	**0.0005**
Soil	Imidacloprid	0.0050	0.0005
	5-Hydroxy imidacloprid	0.0050	0.0013
	Imidacloprid olefin	0.0050	0.0012

[a] Soil LODs and LOQs are reported in individual analyte equivalents, and no total imidacloprid residue is calculated. All other matrix analyte LODs and LOQs are reported in parent equivalents.

Storage stability studies and transit spikes indicate that the imidacloprid residues would have been stable during frozen storage for at least 1080 days (36 months) in blueberry matrices and for at least 1281 days (42 months) in soil matrices prior to analysis (Section 5.0). The maximum storage period of frozen samples in this study for imidacloprid was 273 days for blueberry flowers, 489 days for blueberry leaves, 443 days for blueberry nectar, 429 days for blueberry pollen, and 679 days for soil prior to extraction (Appendix 1).

The imidacloprid residues in blueberry leaves, flowers, pollen and nectar are given in Table 8 (SP C.3.). A statistical evaluation of the total imidacloprid residues in the bee-relevant matrices of pollen and nectar is described in Section 3.6.

A discussion of the imidacloprid residues is presented in Section 3.7. In soil, the imidacloprid residues were higher in the second year compared to the first year, but the higher residues did not result in increased imidacloprid concentrations in nectar or pollen in the second year.

Executive Summary, Part B

For blueberry nectar, total imidacloprid residues were consistently low. Nectar residues did not show any clear trends between sampling intervals within a year, or year on year. For blueberry pollen, residues from the same trial and year were generally similar regardless of sampling interval or year.

The highest median and 90th percentile summary statistics from each trial and year for pollen and nectar are provided in the table below. These values are recommended as conservative dietary exposure estimates for use in pollinator risk assessments.

Blueberry Study Summary, Pollen and Nectar (1 x 0.5 lb ai/ac)

Matrix	Exposure Estimate Type	Selected Summary Statistic (Source)	Total Residue (ppb)
Blueberry Pollen	Chronic	Median (Trial NT003-12ZA, Year 1)	15
	Acute	90th Percentile (Trial NT003-12ZA, Year 2)	23
Blueberry Nectar	Chronic	Median (Trial NT003-12ZA, Year 1)	7
	Acute	90th Percentile (Trial NT003-12ZA, Year 1)	12

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Report: 02.02.01/46; [redacted]; 2016; M-544990-01-2
Title: Determination of the residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in bee relevant matrices collected from stone fruit trees following application of imidacloprid over two successive years
Report No.: EBNTN013
Document No.: M-544990-01-2
Guideline(s): US EPA OPPTS/OCSPP 850.SUPP (Ecological Effects)
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-544990-01-2@S-602997-01-1

A total of nine field trials were conducted to measure the magnitude of imidacloprid residues in/on cherry, plum, apricot and peach (stone fruit) nectar and pollen and in/on stone fruit leaves following one soil and two foliar applications of Admire Pro® Systemic Protectant in each of two successive years. Admire Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L imidacloprid. Admire Pro® Systemic Protectant was applied to stone fruit trees at target rates and timings as shown below.

Target Application Summary

Plot ID[a]	Test Substance	Type/ Number of App.	Rate/Application (±5%)					Target App. Interval (Days)	Target PHI[b] (Days)	Spray Volume	
			Formulated Product (fp)		Active Ingredient (a.i.)						
			fl oz fp/A	ml fp/A	Name of a.i.	lb a.i./A	g a.i./ha			GPA	LPHA
UTC	NA[c]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TRTD	Admire Pro Systemic protectant	Soil / 1	10.5	768	Imidacloprid	0.38	426	NA	21	13,500-28,000	126,358-262,076
		Foliar / 2	1.7	124	Imidacloprid	0.06	67	8-10	7	50-100	468-936

[a] UTC = Untreated control plot.
TRTD = Treated plot receiving one soil and two foliar applications (first foliar 3-5 days after soil application, second foliar 13-15 days after soil application).
[b] PHI = Pre-harvest interval. Days listed apply to 2014 normal commercial fruit harvest; in 2013, all applications were made after normal commercial fruit harvest.
[c] NA = Not applicable.

Applications were made in 2013 and 2014, post-bloom. Across both years, individual soil application rates were 0.38 lb imidacloprid/A (0.42 to 0.43 kg/ha). The interval between soil and first foliar applications was 3 to 7 days. For all foliar applications, individual rates ranged from 0.058 to 0.064 lb imidacloprid/A (0.065 to 0.071 kg/ha). The interval between first and second foliar applications was 7 to 11 days. Application volumes ranged from 13,000 to 16,600 gal/A (GPA) for the soil applications and from 53 to 90 GPA for the foliar applications. Total seasonal application rates ranged from 0.50 to 0.51 lb imidacloprid/A (0.56 to 0.57 kg/ha). In 2013, all applications were made after stone fruit harvest; at BBCH growth stages 91 to 99 (BBCH 91: shoot growth completed, foliage still fully green; BBCH 99: harvested product). In 2014, soil applications were targeted for 21 days prior to stone fruit harvest and made at BBCH growth stages 77 to 81 (BBCH 77: fruit about 70% of final size; BBCH 81: beginning of fruit coloring); the two foliar applications were targeted such that the last would occur 7 days prior to fruit harvest, with sprays made at BBCH growth stages 76 to 89 (BBCH 76: fruit about 70% of final size; BBCH 89: fruit ripe for consumption, fruit have typical taste and firmness).

All applications were made using ground-based equipment. The adjuvant Dyne-Amic was used in all foliar applications at a rate of 0.25% v/v, except in trial NT027-13ZA, when a rate of 0.025% v/v was used.

Stone fruit flower (also called blossom) and leaf samples were collected once in the spring of 2014, following the post-harvest fall 2013 applications, and once in the spring of 2015, following the pre-

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harvest fall 2014 applications. Each TRTD plot was divided into two subplots. At each sampling interval, two composite samples (one from each subplot) of cherry, plum, apricot, or peach flowers (to be hand-processed to obtain nectar and pollen) were collected by hand when the stone fruit trees were at bloom, BBCH 65 (BBCH 65: full flowering, at least 50% of flowers open, first petals falling). Two composite samples (one from each subplot) of cherry, plum, apricot, or peach leaves were collected after bloom, once the leaves had expanded, at BBCH 69 to 75 (BBCH 69: end of flowering, all petals fallen; BBCH 75: fruit about half final size) or at BBCH 19 (first leaves fully expanded). In 2014, flower samples were collected at 133 to 160 days after the last application (DAA), and leaf samples were collected at 155 to 188 DAA. In 2015, flower samples were collected at 211 to 309 DAA, and leaf samples were collected at 230 to 323 DAA.

Single composite samples of cherry, plum, apricot, or peach flowers and leaves were collected from the control plot of each trial on the same days that samples were collected from the treated plot.

After collection, stone fruit flowers were hand-processed at a facility near the field site to obtain the bee-relevant matrices nectar and pollen. The processed flowers were discarded.

The residues of Admire Pro Systemic Protectant (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

The limits of quantitation (LOQs) and limits of detection (LODs) are shown below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppm)	LOD (ppm)
Cherry, plum, apricot, and peach leaves	Imidacloprid	0.005	0.0005
	5-Hydroxy imidacloprid	0.005	0.0004
	Imidacloprid olefin	0.005	0.0016
	Total Imidacloprid	**0.005**	**0.0016**
Cherry, plum, apricot, and peach nectar	Imidacloprid	0.001	0.0003
	5-Hydroxy imidacloprid	0.001	0.0007
	Imidacloprid olefin	0.001	0.0006
	Total Imidacloprid	**0.001**	**0.0007**
Cherry, plum, apricot, and peach pollen	Imidacloprid	0.001	0.0004
	5-Hydroxy imidacloprid	0.001	0.0005
	Imidacloprid olefin	0.001	0.0003
	Total Imidacloprid	**0.001**	**0.0005**

Storage stability studies indicate that the imidacloprid residues would have been stable during frozen storage for at least 1080 days (36 months) in stone fruit leaves prior to analysis. Transit spikes showed that imidacloprid residues were stable in pollen and nectar for the duration of the study. The maximum storage period of frozen samples in this study for imidacloprid was 420 days for stone fruit leaves and 222 days for nectar and pollen.

A summary of the total imidacloprid residues grouped by year and for the overall study is shown in the table below.

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Summary of Residue Data for Imidacloprid in/on Stone Fruit, All Trials

Commodity	Plot Name	Sampling Year	DAA (days)[a]	Seasonal Application Rates (lb a.i./A)	Total Imidacloprid Residue Levels (ppm)[b]						
					n[c]	Min	Max	90th percentile	Median	Mean	Standard Deviation
Nectar	TRTD	2014	133–160	0.50–0.51	18	<LOD	0.034	0.016	0.003	0.007	0.010
		2015	211–291	0.50–0.51	16	<LOD	0.011	0.006	0.001	0.002	0.003
		2014, 2015	133-291	0.50–0.51	34	<LOD	0.034	0.009	0.002	0.005	0.008
Pollen	TRTD	2014	133–160	0.50–0.51	14	0.013	0.34	0.13	0.034	0.069	0.086
		2015	211–309	0.50–0.51	16	0.002	0.19	0.087	0.009	0.033	0.054
		2014, 2015	133-309	0.50–0.51	30	0.002	0.34	0.13	0.027	0.050	0.072
Leaves	TRTD	2014	155–188	0.50–0.51	18	0.002	0.28	0.21	0.046	0.060	0.085
		2015	230–323	0.50–0.51	18	0.006	0.20	0.13	0.021	0.058	0.073
		2014, 2015	155-323	0.50–0.51	36	0.002	0.28	0.19	0.023	0.059	0.078

[a] DAA = Days after the last application.
[b] For the purpose of calculating the total imidacloprid residues, any individual analyte value reported as <LOD was summed into the total at a default value equal to ½ the LOD.
[c] n = Number of individual treated samples analyzed. The leaf n values represent the target number of samples to be collected; where the n value is lower, samples were composited or were of insufficient size to analyze.

Differences in pollen and nectar residue levels do not appear to be related to the differences in soil types. The use pattern included both a soil drench and post-bloom foliar sprays. The magnitude of residues measured in the subsequent spring increased as the interval between the final foliar spray and bloom decreased. This interval is referred to as days after last application (DAA) in subsequent sections of this report.

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Report: 02.02.01/47; [redacted]; 2016; M-544778-01-2
Title: Determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from apple trees following soil and foliar applications of imidacloprid over two successive years
Report No.: EBNTN014
Document No.: M-544778-01-2
Guideline(s): US EPA OPPTS/OCSPP 850.SUPP, Ecological Effects
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-544778-01-2@S-602888-01-1

A total of nine field trials were conducted to measure the magnitude of imidacloprid residues in apple nectar and pollen and in/on apple leaves following one soil and two foliar applications of Admire Pro® Systemic Protectant in each of two successive years.
Admire Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L imidacloprid. Admire Pro® Systemic Protectant was applied to apple trees at target rates and timings as shown below.

Target Application Summary

Plot ID[a]	Test Substance	Type/ Number of App.	Rate/Application (±5%)					Target App. Interval (Days)	Target PHI[b] (Days)	Spray Volume	
			Formulated Product (fp)		Active Ingredient (a.i.)						
			fl oz fp/A	ml fp/A	Name of a.i.	lb a.i./A	g a.i./ha			GPA	LPHA
UTC	NA[c]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TRTD	Admire Pro Systemic protectant	Soil / 1	10.5	768	Imidacloprid	0.38	426	NA	21	13,000-27,000	121,678-252,716
		Foliar / 2	1.7	124	Imidacloprid	0.06	67	10	7	50-100	468-936

[a] Plot ID: UTC = Untreated control plot
TRTD = Treated plot
[b] PHI = Pre-harvest interval, the period between application and commercial apple harvest. For applications in 2013 only, if applications could not be made prior to commercial apple harvest, it was acceptable to apply after apple harvest using the same application intervals.
[c] NA = Not applicable

Applications were made in the fall of 2013 and 2014, post-bloom. The second year of trial NT035-13ZA could not be completed because the apple trees were removed from the plot field, so only first year data are reported from this trial.

Across both years, individual soil application rates ranged from 0.38 to 0.39 lb imidacloprid/A (0.43 to 0.44 kg/ha). The interval between soil and first foliar applications was 3 to 5 days. For all foliar applications, individual rates ranged from 0.059 to 0.064 lb imidacloprid/A (0.066 to 0.071 kg/ha). The interval between first and second foliar applications was 8 to 10 days. Application volumes ranged from 13,000 to 15,200 gal/A (GPA) for the soil applications and from 55 to 75 GPA for the foliar applications. Total seasonal application rates ranged from 0.50 to 0.52 lb imidacloprid/A (0.56 to 0.58 kg/ha). In 2013, trials NT031-13ZA and NT036-13ZA made applications prior to apple harvest, while the other trials made all applications post-harvest. Soil applications were made at BBCH growth stages 79 to 99 (BBCH 79: fruit about 90% final size; BBCH 99: harvested product), and the two foliar applications were made at BBCH growth stages 81 to 99 (BBCH 81: beginning of ripening, first appearance of cultivar-specific color) and 85 to 99 (BBCH 85: advanced ripening, increase in intensity of cultivar-specific color), respectively. In 2014, all applications were made prior to apple harvest. Soil applications were targeted for 21 days prior to apple harvest and made at BBCH growth stages 75 to 89 (BBCH 75: fruit about half final size; BBCH 89: fruit ripe for consumption, fruit have typical taste and firmness); the two foliar applications were targeted such that the last would occur 7 days prior to harvest, with sprays made at BBCH growth stages 65 to 85 (BBCH 65: full flowering, at least 50% of flowers open, first petals falling) and 67 to 89 (BBCH 67: flowers fading, majority of petals fallen), respectively.

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All applications were made using ground-based equipment. The adjuvant Dyne-Amic (0.25 % v/v) was used in all foliar applications.

Apple flower (also called blossom) and leaf samples were collected once in the spring of 2014, following the fall 2013 applications, and once in the spring of 2015, following the fall 2014 applications. The exception is trial NT035-13ZA, in which the year 2 (2015) sample collection was cancelled because the apple trees were removed from the trial field. Each TRTD plot was divided into two subplots. At each sampling interval, two composite samples (one from each subplot) of apple flowers (to be hand-processed to obtain apple nectar and pollen) and apple leaves were collected by hand when the apple trees were at bloom, BBCH 65 to 69 (BBCH 69: end of flowering, all petals fallen). Exceptions are the leaf samples collected in 2014 from trials NT034-13ZA and NT035-13ZA and in 2015 from trial NT036-13ZA, which were collected at BBCH 71 (BBCH 71: fruit size up to 10 mm, fruit fall after flowering). In 2014, apple flower samples were collected at 138 to 193 days after the last application (DAA), and apple leaf samples were collected at 151 to 214 DAA. In 2015, apple flower samples were collected at 131 to 287 DAA, and apple leaf samples were collected at 147 to 293 DAA.

Single composite samples of apple flowers and leaves were collected from the control plot of each trial on the same days that samples were collected from the treated plots.

After their collection, apple flowers were hand-processed at the field site to obtain the bee- relevant matrices of apple nectar and pollen. The processed flowers were discarded.

The residues of Admire Pro Systemic Protectant (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

The limits of quantitation (LOQs) and limits of detection (LODs) are shown below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppm)	LOD (ppm)
Apple nectar	Imidacloprid	0.001	0.0003
	5-Hydroxy imidacloprid	0.001	0.0007
	Imidacloprid olefin	0.001	0.0006
	Total Imidacloprid	**0.001**	**0.0007**
Apple pollen	Imidacloprid	0.001	0.0004
	5-Hydroxy imidacloprid	0.001	0.0005
	Imidacloprid olefin	0.001	0.0003
	Total Imidacloprid	**0.001**	**0.0005**
Apple leaves	Imidacloprid	0.005	0.0009
	5-Hydroxy imidacloprid	0.005	0.0005
	Imidacloprid olefin	0.005	0.0008
	Total Imidacloprid	**0.005**	**0.0009**

Storage stability studies indicate that the imidacloprid residues would have been stable during frozen storage for at least 1080 days (36 months) in apple leaves prior to analysis. Transit spikes showed that imidacloprid residues were stable in pollen and nectar for the duration of the study. The maximum storage period of frozen samples in this study for Admire Pro Systemic Protectant was 413 days for apple leaves and 203 days for apple nectar and pollen.

A summary of the residues grouped by year and for overall study is shown in the table below.

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Summary of Residue Data for Imidacloprid in/on Apple, All Trials

Commodity	Plot Name	Sampling Years	DAA (days)[a]	Seasonal Application Rates (lb a.i./A)	Total Imidacloprid Residue Levels (ppm)[b]					
					n[c]	Min	Max	90th percentile	Median	Mean	Standard Deviation
Nectar	TRTD	2014	138-193	0.50-0.52	17	0.001	0.036	0.003	0.001	0.004	0.008
		2015	131-287	0.50-0.52	16	<LOD	0.004	0.003	0.001	0.002	0.001
		2014, 2015	131-287	0.50-0.52	34	<LOD	0.036	0.003	0.001	0.003	0.006
Pollen	TRTD	2014	138-193	0.50-0.52	18	0.001	0.047	0.030	0.015	0.016	0.012
		2015	131-287	0.50-0.52	16	0.002	0.10	0.089	0.015	0.033	0.035
		2014, 2015	131-287	0.50-0.52	34	0.001	0.10	0.057	0.015	0.024	0.027
Leaves	TRTD	2014	151-214	0.50-0.52	18	0.001	0.060	0.031	0.009	0.014	0.017
		2015	147-293	0.50-0.52	16	0.002	3.6[d]	1.7	0.015	0.45	1.1
		2014, 2015	147-293	0.50-0.52	34	0.001	3.6	0.14	0.012	0.22	0.81

[a] DAA = Days after the last application.
[b] For the purpose of calculating the total imidacloprid residues, any individual analyte value reported as <LOD was summed into the total at a default value equal to ½ the LOD.
[c] n = Number of individual treated samples analyzed.
[d] The next highest residue from 2015 leaf samples in trial NT029-13ZA was 0.004 ppm.

Differences in pollen and nectar residue levels do not appear to be related to the differences in soil types. The use pattern included both a soil drench and post-bloom foliar sprays. The magnitude of residues measured in the subsequent spring increased as the interval between the final foliar spray and bloom decreased. This interval is referred to as days after last application in subsequent sections of this report (DAA).

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Report:	02.02.01/48; [redacted]; 2016; M-525733-02-2
Title:	Determination of the residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in bee relevant matrices collected from cotton during two successive years - Admire Pro Systemic Protectant (550 g/L) (imidacloprid SC 550 G)
Report No.:	EBNTN011-01
Document No.:	M-525733-02-2
Guideline(s):	US EPA OPPTS/OCSPP 850.SUPP (Ecological Effects)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-525733-02-2@S-602387-01-1

A total of nine field trials were conducted to measure the magnitude of imidacloprid residues in bee-relevant cotton pollen and nectar samples and in/on cotton leaves following four applications of Admire Pro Systemic Protectant in each of two successive years. Admire Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L imidacloprid. Admire Pro Systemic Protectant was applied to cotton at target rates and timings as shown below.

Target Application Summary

Plot ID[a]	Test Substance	Type/ Number of Apps.	Rate/Application (±5%)					Target App. Interval (Days)	Target DAA[b] (Days)	Spray	
			fl oz fp/A	ml fp/ha	Name of active ingredient (a.i.)	lb a.i./A	g a.i./ ha			GPA	LPHA
UTC	NA[c]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TRTD	Admire Pro Systemic Protectant	Soil / 1	9.20	673	Imidacloprid	0.329	370	NA	NA	10 - 20	94 - 188
	Admire Pro Systemic Protectant	Foliar / 3	1.59	116	Imidacloprid	0.057	64	7	14	10 - 20	94 - 188

[a] Plot ID: UTC = Untreated control plot
TRTD = Treated plot receiving an in-furrow spray application at planting followed by three foliar spray applications with an application interval of 6 to 7 days (target 7 days). The in-furrow spray was directed on or below the seed. The first foliar application occurred at a minimum of 60 days after the in-furrow application. The foliar applications were broadcast or directed sprays including the adjuvant Dyne-Amic. The same application pattern was repeated over two consecutive growing seasons.

[b] DAA = Days after application, the number of days between the most recent application and sample collection.

[c] NA = Not applicable.

Only the first year of trial NT002-13ZB could be completed and reported because the plot location was no longer available.

Plot TRTD received one soil (in-furrow) spray application of Admire Pro at planting (BBCH 00: dry seed) followed by 3 equivalent Admire Pro foliar spray applications per planting season. Individual soil application rates ranged from 0.35 to 0.38 kg imidacloprid/ha per application (0.32 to 0.34 lb/A), and spray volumes were 13 to 15 gal/A. The interval between the soil and first foliar application was 75 to 99 days. Individual foliar application rates ranged from 0.063 to 0.067 kg imidacloprid/ha/application (0.056 to 0.060 lb/A). All foliar applications were made between BBCH growth stages 61 and 72 (BBCH 61: beginning of flowering; BBCH 72: about 20% of bolls have attained their final size). The interval between foliar applications was 6 to 8 days. The foliar spray volumes ranged from 14 to 20 gal/A. Total seasonal application rates ranged from 0.55 to 0.57 kg imidacloprid/ha (0.49 to 0.51 lb/A). All applications were made using ground-based equipment. The adjuvant Dyne-Amic (0.25% v/v) was used in all foliar applications.

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Cotton leaf and flower samples were collected at three sampling intervals: 4 to 5 days prior to the first foliar application (70 to 95 days after the soil application), 4 to 5 days after the last foliar application, and 10 to 14 days after the last foliar application. At each sampling interval, duplicate composite samples (two separate runs through the plot) of cotton flowers (to be hand-processed to obtain cotton pollen, floral nectar, and extrafloral nectar) and cotton leaves were collected from the treated plots when the plants were at bloom, BBCH 61 (begin flowering, early bloom) to BBCH 73 (about 30% of bolls have attained their final size). Single composite samples of cotton leaves and flowers were collected from the control plot of each trial on the same days that samples were collected from the treated plots.

After their collection, cotton flowers were hand-processed at the field site to obtain the bee-relevant samples of cotton pollen, floral nectar, and extrafloral nectar. The processed flowers were discarded.

The residues of Admire Pro Systemic Protectant (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

The limits of quantitation (LOQs) and limits of detection (LODs) are shown below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppm)	LOD (ppm)
Cotton leaves	Imidacloprid	0.005	0.0012
	5-Hydroxy imidacloprid	0.005	0.0007
	Imidacloprid olefin	0.005	0.0008
	Total Imidacloprid	**0.005**	**0.0012**
Cotton extrafloral nectar	Imidacloprid	0.001	0.0003
	5-Hydroxy imidacloprid	0.001	0.0007
	Imidacloprid olefin	0.001	0.0006
	Total Imidacloprid	**0.001**	**0.0007**
Cotton floral nectar	Imidacloprid	0.001	0.0003
	5-Hydroxy imidacloprid	0.001	0.0007
	Imidacloprid olefin	0.001	0.0006
	Total Imidacloprid	**0.001**	**0.0007**
Cotton pollen	Imidacloprid	0.001	0.0004
	5-Hydroxy imidacloprid	0.001	0.0005
	Imidacloprid olefin	0.001	0.0003
	Total Imidacloprid	**0.001**	**0.0005**

Storage stability studies indicate that the imidacloprid residues would have been stable during frozen storage for at least 1080 days (36 months) in cotton leaves prior to analysis. Transit spikes showed that imidacloprid residues were stable in pollen and nectar for the duration of the study. The maximum storage period of frozen samples in this study for Admire Pro Systemic Protectant was 569 days for cotton leaves, 226 days for cotton pollen, and 211 days for cotton floral and extrafloral nectar.

A summary of the residues is shown in the table below.

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Summary of Residue Data for Imidacloprid in/on Cotton

Commodity	Plot Name	Days after the Application[a]	Application Rates (lb a.i./A)	Imidacloprid Residue Levels (ppm)[b]						
				n[c]	Min	Max	90th percentile	Median	Mean	Standard Deviation
Floral Nectar	TRTD	70 to 95 DASA (-4 to -5 DA1FA)	In-furrow application: 0.32 to 0.34	32	0.001	0.13	0.050	0.007	0.020	0.027
		4 – 5 DA3FA	Total seasonal rate: 0.49 to 0.51	36	0.012	0.13	0.13	0.070	0.075	0.043
		10 – 14 DA3FA		33	0.010	0.12	0.069	0.035	0.040	0.027
Extrafloral Nectar	TRTD	70 to 95 DASA (-4 to -5 DA1FA)	In-furrow application: 0.32 to 0.34	28	0.001	0.038	0.017	0.003	0.007	0.008
		4 – 5 DA3FA	Total seasonal rate: 0.49 to 0.51	33	0.047	2.8	1.5	0.28	0.56	0.62
		10 – 14 DA3FA		33	0.008	0.14	0.090	0.027	0.040	0.033
Pollen	TRTD	70 to 95 DASA (-4 to -5 DA1FA)	In-furrow application: 0.32 to 0.34	34	0.001	0.043	0.014	0.001	0.006	0.010
		4 – 5 DA3FA	Total seasonal rate: 0.49 to 0.51	34	0.004	2.9	0.38	0.046	0.26	0.58
		10 – 14 DA3FA		34	0.003	0.18	0.19	0.015	0.050	0.058
Leaves	TRTD	70 to 95 DASA (-4 to -5 DA1FA)	In-furrow application: 0.32 to 0.34	34	0.001	0.36	0.11	0.024	0.045	0.068
		4 – 5 DA3FA	Total seasonal rate: 0.49 to 0.51	34	0.48	3.2	2.7	1.5	1.6	0.68
		10 – 14 DA3FA		34	0.057	0.72	0.64	0.32	0.36	0.18

[a] DASA = Days after the soil (in-furrow) application; DA1FA = days after the first foliar application; DA3FA = days after the third foliar (and last) application. A negative number designates days prior to the indicated application.

[b] For the purpose of calculating the total imidacloprid residues, any individual analyte value reported as <LOD was summed into the total at a default value equal to ½ the LOD.

[c] n = Number of individual treated samples analyzed. Two samples were to be collected per sampling interval from each trial each year (trial NT002-13ZB has 1 year of data and all other trials have 2 years of data) for a total of 34 samples; in cases where n<34, samples were composited or were too small to analyze.

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Report: 02.02.01/49; [redacted]; 2016; M-525735-02-2
Title: Amended report 1 to EBNTN012 - Determination of the residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in bee relevant matrices collected from tomatoes following application of imidacloprid over two successive years - Admire Pro Systemic Protectant (550 g/L) (imidacloprid SC 550 G)
Report No.: EBNTN012
Document No.: M-525735-02-2
Guideline(s): US EPA OPPTS/OCSPP 850.SUPP (Ecological Effects)
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-525735-02-2@S-602394-01-1

A total of nine field trials were conducted to measure the magnitude of imidacloprid residues in transplanted tomato pollen and in/on transplanted tomato leaves following three applications of Admire Pro Systemic Protectant in each of two successive years. Admire Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L imidacloprid. Admire Pro Systemic Protectant was applied to tomato at target rates and timings as shown below.

Target Application Summary

Plot ID[a]	Test Substance	Type/ Number of Apps.	Rate/Application (±5%)					Target App. Interval (Days)	Target DAA[b] (Days)	Spray Volume	
			Formulated Product (fp)		Active Ingredient (a.i.)						
			fl oz/A	ml/ha	Name of a.i.	lb/A	g/ha			GPA min.	LPHA min.
UTC	NA[c]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TRTD	Admire Pro Systemic Protectant	Soil / 1	10.5	768	Imidacloprid	0.38	422	NA	See below[d]	NA	NA
	Admire Pro Systemic Protectant	Foliar / 2	1.7	124	Imidacloprid	0.06	68	5	See below[d]	50	468

[a] Plot ID: UTC = Untreated control plot.
TRTD = Treated plot receiving one soil application 5-7 days after transplant and two foliar applications, made 4-5 days apart, between the second and third sampling events.
[b] DAA = Days after application, the number of days between the application and sample collection.
[c] NA = Not applicable.
[d] The PFI permanently marked the plots before planting. The soil application occurred 5-7 days after the tomato seedlings were transplanted (June/July). The first foliar application was made at target 1 day after the second sampling of pollen and leaves (approx. 40-60 days after soil application). The second foliar application was made 4-5 days after the first (approx. 45-65 days after soil application).

Across all trials and years, plot TRTD received one soil (in-furrow) drip/drench application of Admire Pro 5 to 7 days after tomato transplantation followed by 2 equivalent Admire Pro foliar spray applications per planting season. Individual soil application rates ranged from 0.37 to 0.38 lb imidacloprid/A per application (0.42 to 0.43 kg/ha). The interval between the soil and first foliar applications was 48 to 78 days. Individual foliar application rates ranged from 0.058 to 0.062 lb imidacloprid/A/application (0.065 to 0.070 kg/ha). All foliar applications were made to flowering tomato plants, after the first two sampling events were complete. The interval between foliar applications was 4 to 5 days. The foliar spray volumes ranged from 50 to 101 gal/A, with the exception of the second foliar spray in 2013 to trial NT018-13ZA (48 gal/A). Total seasonal application rates were 0.49 to 0.50 lb imidacloprid/A (0.55 to 0.56 kg/ha).

All applications were made using ground-based equipment. The adjuvant Dyne-Amic (0.25 or 0.5 % v/v) was used in all foliar applications, with the exception of the first 2014 foliar application in trial NT017-13ZB and both 2014 foliar applications in trial NT039-13ZA.

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Each trial year, one bee tunnel was erected on untreated plot UTC, and 2 bee tunnels were erected on treated plot TRTD, except in trials NT013-13ZA, NT040-13ZA, and NT041-13ZA, when only one TRTD tunnel was erected. Bumble bee (Bombus impatiens) colonies (1 to 3 per tunnel) were placed in each tunnel for the collection of pollen. One sample was collected per bee tunnel, yielding two TRTD samples and one UTC sample at each sampling interval, except in trials NT013-13ZA, NT040-13ZA, and NT041-13ZA, when two replicate samples were collected from the single erected TRTD tunnel. Additionally, in trial NT042-13ZA, the first pollen sampling of 2015 was made by hand-collecting pollen directly from the flowers in the field due to a bee shortage.

Tomato leaf and pollen samples were collected at four sampling intervals each year: two samples were collected after the soil application, approximately 14 days apart (31 to 68 and 45 to 77 days after the soil application, respectively), and two samples were collected after the last foliar application, approximately 14 days apart (2 to 8 and 14 to 22 days after the last foliar application, respectively). At each interval, fresh bumble bee colonies were placed in each bee tunnel, and the bumble bees were allowed to forage from the tomato flowers for several days. Then, bumble bees carrying pollen were collected from the tunnels, and the pollen was removed from the bees. To ensure a large enough pollen sample for analysis, some trials collected bees over multiple days (up to 7) per sampling event. Multi-day pollen samples from the same sampling interval and bee tent were composited together into one sample vial.

During the described sampling intervals, composite samples of tomato leaves were collected from within the tunnels of the treated plots.

Composite samples of tomato pollen and leaves were collected from the control plot tunnel of each trial during the same sampling intervals and using the same methods as samples collected from the treated plots.

The residues of Admire Pro Systemic Protectant (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

The limits of quantitation (LOQs) and limits of detection (LODs) are shown in the following table.

Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppm)	LOD (ppm)
Tomato leaves	Imidacloprid	0.005	0.0022
	5-Hydroxy imidacloprid	0.005	0.0007
	Imidacloprid olefin	0.005	0.0010
	Total Imidacloprid	**0.005**	**0.0022**
Pollen	Imidacloprid	0.001	0.0004
	5-Hydroxy imidacloprid	0.001	0.0005
	Imidacloprid olefin	0.001	0.0003
	Total Imidacloprid	**0.001**	**0.0005**

Storage stability studies indicate that the imidacloprid residues would have been stable during frozen storage for at least for at least 1080 days (36 months) in tomato leaves prior to analysis. Transit stability samples showed that imidacloprid residues were stable in pollen for the duration of the study. The

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maximum storage period of frozen samples in this study for Admire Pro Systemic Protectant was 561 days for tomato leaves and 560 days for tomato pollen.

A summary of the residues is shown in the table below.

Summary of Residue Data for Imidacloprid in/on Tomato

Matrix	Plot Name	Sampling Interval , Days after the Application[a]	Application Rates (lb a.i./A)	Imidacloprid Residue Levels (ppm)[b]						
				n[c]	Min	Max	90th percentile	Median	Mean	Standard Deviation
Pollen (bee-collected)	TRTD	Interval 1: 31 to 68 DASA (-20 to -10 DA1FA)	In-furrow application: 0.37 to 0.38	30	0.007	0.29	0.18	0.041	0.074	0.077
		Interval 2: 45 to 77 DASA (-3 to -1 DA1FA)	In-furrow application: 0.37 to 0.38	27	0.002	0.14	0.096	0.030	0.040	0.035
		Interval 3: 2 to 8 DA2FA	Total seasonal application: 0.49 to 0.50	22	0.25	1.8	1.0	0.44	0.59	0.40
		Interval 4: 14 to 22 DA2FA	Total seasonal application: 0.49 to 0.50	32	0.017	0.35	0.15	0.066	0.079	0.064
Pollen[d] (hand-collected)	TRTD	Interval 1: 58 DASA (-7 DA1FA)	NT042-13ZA In-furrow 2015 application: 0.38	2	0.53	0.68	NA[e]	NA	NA	NA
Leaves	TRTD	Interval 1: 31 to 63 DASA (-20 to -7 DA1FA)	In-furrow application: 0.37 to 0.38	36	0.002	0.65	0.44	0.13	0.18	0.18
		Interval 2: 45 to 77 DASA (-3 to -1 DA1FA)	In-furrow application: 0.37 to 0.38	36	0.005	0.50	0.35	0.10	0.15	0.14
		Interval 3: 4 to 8 DA2FA	Total seasonal application: 0.49 to 0.50	36	0.045	6.2	3.3	0.73	1.2	1.3
		Interval 4: 17 to 21 DA2FA	Total seasonal application: 0.49 to 0.50	36	0.015	0.43	0.33	0.096	0.15	0.12

[a] Samples were collected in four intervals. DASA = Days after the soil (in-furrow) application; DA1FA = days after the first foliar application; DA2FA = days after the second foliar (and last) application. A negative number designates days prior to the indicated application.

[b] For the purpose of calculating the total imidacloprid residues, any individual analyte value reported as <LOD was summed into the total at a default value equal to ½ the LOD.

[c] n = Number of individual treated samples analyzed. Two samples were to be collected per sampling interval from each trial each year, for 36 target samples per interval; in cases where n<36, samples could not be collected or were too small for analysis.

[d] The first sampling interval of pollen in 2015 from trial NT042-13ZA was collected by hand due to a bee supply shortage.

[e] NA = Not applicable.

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Report:	02.02.01/50; [redacted]; 2016; M-559999-01-2
Title:	Amended final report - Field collection study to evaluate total imidacloprid residue levels (imidacloprid parent, olefin and 5-hydroxy) in pollen, nectar, and leaves of blooming bedding plants from retail garden centers
Report No.:	US0592
Document No.:	M-559999-01-2
Guideline(s):	Based on EPA et al. Guidance for Assessing Risk to Bees 2014 OCSPP 850.SUPP (Ecological Effects)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-559999-01-2@S-602419-01-1

The purpose of this study was to determine the amount, if any, of imidacloprid in the pollen, nectar, and leaves of annual or perennial flowering bedding plants in the live goods retail section of The Home Depot (Atlanta GA, 30339 USA) stores. There was no active treatment of the bedding plants with imidacloprid products included in this study; the purpose of the study was to impartially select blooming plants in the retail garden centers and analyze for potential residues. The imidacloprid total residue method includes the parent imidacloprid and the bee-relevant analytes imidacloprid olefin and 5-hydroxy imidacloprid.

The plants for sampling in this study were collected in two States: Florida and California. Five stores were visited for each trial with at least 4 different plant species purchased per store.

A summary of the residues are shown in the table below. The majority of the blooming plants collected in this study had no quantifiable residues of imidacloprid. The results for each matrix (nectar, pollen or leaf), for all trials and sites, were grouped into a single distribution to estimate potential exposure in a retail garden store during February or March. Total imidacloprid results of <LOQ were given a value of ½ the LOQ for the purposes of determining these summary statistics.

Matrix	N	Results <LOQ	Min (ppb)	Max (ppb)	90th percentile	Median	Mean	Standard deviation
Nectar	23	16	<LOQ	226	127	<LOQ	21.8	60.1
Pollen	22	12	<LOQ	229	16.4	<LOQ	14.5	48.1
Leaf	45	35	<LOQ	2947	176	<LOQ	153	561

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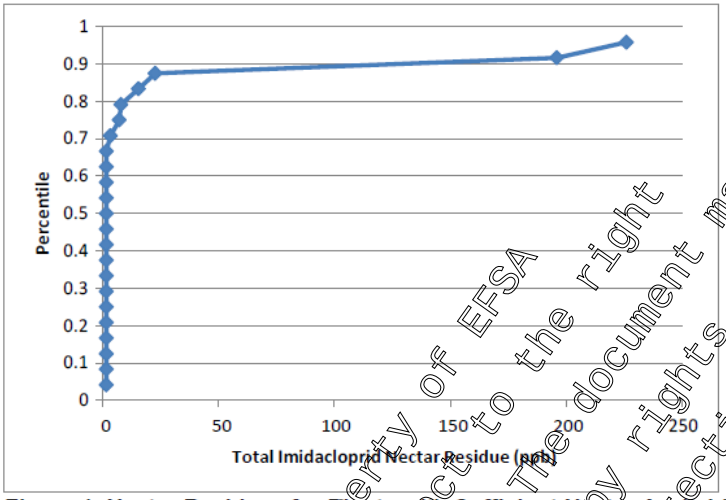

Figure 1. Nectar Residues for Plants with Sufficient Nectar Available in the Blossoms

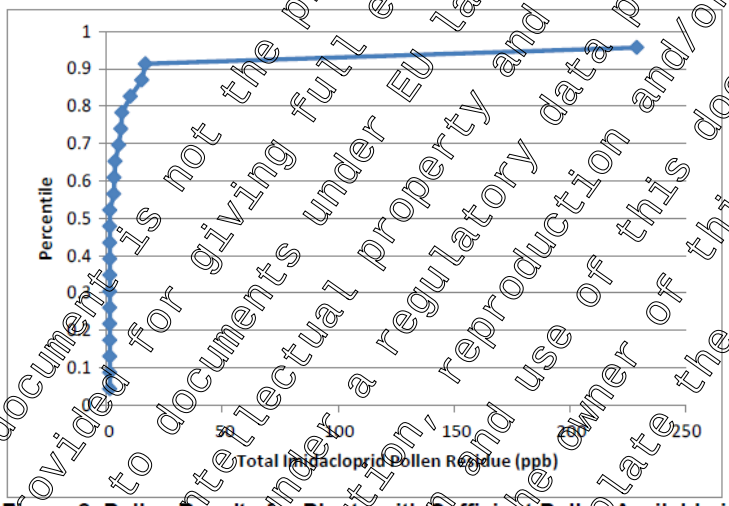

Figure 2. Pollen Results for Plants with Sufficient Pollen Available in the Blossoms

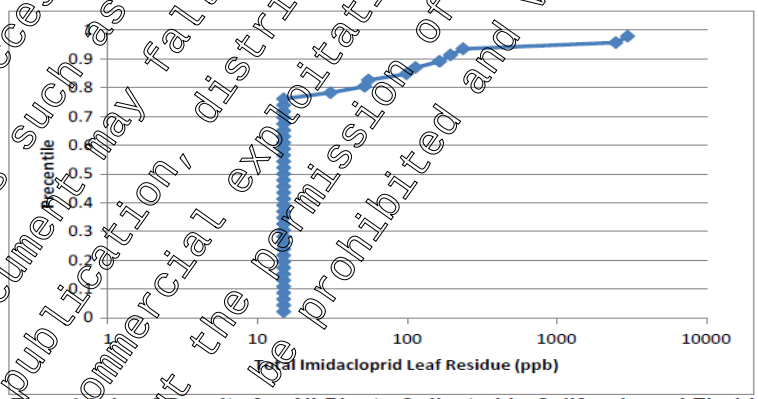

Figure 3. Leaf Results for All Plants Collected in California and Florida 2015

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Report:	02.02.01/51; [redacted]; 2016; M-559994-01-2
Title:	Amended final report - Field collection study to evaluate total imidacloprid residue levels (imidacloprid parent, olefin and 5-hydroxy) in pollen, nectar, and leaves of blooming bedding plants from retail garden centers and in field planted blooming bedding plants
Report No.:	US0593
Document No.:	M-559994-01-2
Guideline(s):	Based on EPA et al. Guidance for Assessing Risk to Bees 2014 OCSPP 850.SUPP (Ecological Effects)
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-559994-01-2@S-602409-01-1

The purpose of this study was to determine the amount, if any, of imidacloprid in the pollen, nectar, and leaves of annual or perennial flowering bedding plants in the live goods retail section of The Home Depot stores, and in those same plants after being planted and allowed to re-bloom under field conditions. There was no active treatment of the bedding plants with imidacloprid products included in this study; the purpose of the study was to impartially select blooming plants in the retail garden centers and analyze for potential residues. The imidacloprid total residue method includes the parent imidacloprid and the bee-relevant analytes imidacloprid olefin and 5-hydroxy imidacloprid.

The plants for use in this study were collected in two regions: greater Raleigh area in North Carolina and greater Kansas City area in both Kansas and Missouri. Five Home Depot stores were visited for each trial with at least 4 different plant species purchased per store during April and May 2015.

Composite samples of pollen and or nectar and leaf matrix were collected from at least 20 individual plants per species. Leaves and blossoms were collected from the canopy of the plants. Pollen was collected from the blossoms using a vacuum pump and a filter pipette tip. Nectar was collected from blossoms using a 10 or 20 µL capillary tube. The quantity of plants used to collect samples for each species was dependent on the size of the blossoms and leaves produced, and the yield of pollen and nectar from those blossoms. The plants were then transplanted and allowed to re-bloom under field conditions. The North Carolina plants were transplanted to garden plots in Clayton, NC at a Bayer site; and the Kansas and Missouri plants were transplanted to garden plots in Stilwell, KS at the SynTech site. Sampling of pollen, nectar, and leaves was then repeated approximately 4 weeks later.

Results from the study show total leaf imidacloprid residues in North Carolina retail garden centers ranging from <LOQ – 3812.3 ppb for pre-planted flowers and <LOQ – 85.7 ppb after flowers were transplanted. Nectar residues for pre-planted flowers ranged from <LOQ – 353.3 and all total imidacloprid residues for nectar in post-planted flowers were <LOQ. Pollen residues ranged from <LOQ – 35.3 ppb in pre-planted flowers and <LOQ – 6.3 ppb after flowers were planted.

Results for retail garden centers in the greater Kansas City area (Kansas and Missouri) indicated that the total imidacloprid leaf and nectar residues were <LOQ for all pre- and post-planted samples. Pollen residues ranged from <LOQ – 44.9 ppb in pre-planted flowers and <LOQ – 42.2 ppb in post-planted flowers.

A summary of the residues are shown in the table below. The pre-planting results for each matrix (nectar, pollen or leaf), for all trials and sites, were grouped into a single distribution to estimate potential exposure in a retail garden store during April and May. Total imidacloprid results of <LOQ were given a value of ½ the LOQ for the purposes of determining these summary statistics. The majority of the blooming plants collected in this study had no quantifiable residues of imidacloprid. The mean results for the bee-relevant dietary matrices (pollen and nectar) in the pre-planting samples are 19.2 ppb and 9.5 ppb, respectively. The post-planting results were evaluated in the same way to estimate potential exposure

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when the plants are transplanted by the consumer. The nectar and pollen taken from the plants that re bloomed in the garden plots indicated a decrease in residues and the mean results were <LOQ and 0.7 ppb, respectively. For those few plants that initially contained imidacloprid residues, the potential dietary exposure was even lower 4 weeks after purchase.

Matrix	N	# samples <LOQ	Min (ppb)	Max (ppb)	90th percentile (ppb)	Median (ppb)	Mean (ppb)	Standard deviation
Residues in plants purchased from retail garden centers in April or May 2015								
Nectar	20	18	<LOQ	353	3	<LOQ	19.2	78.6
Pollen	20	11	<LOQ	44.9	35	<LOQ	9.5	13.1
Leaf	40	37	<LOQ	3812	<LOQ	<LOQ	112	600
Residues in the same plants that were transplanted into garden plots and allowed to re-bloom prior to second sampling (approx. 4 weeks after purchase).								
Nectar	18	18	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0
Pollen	20	13	<LOQ	42.2	35.9	<LOQ	6.7	11.8
Leaf	40	39	<LOQ	85.7	<LOQ	<LOQ	<LOQ	11

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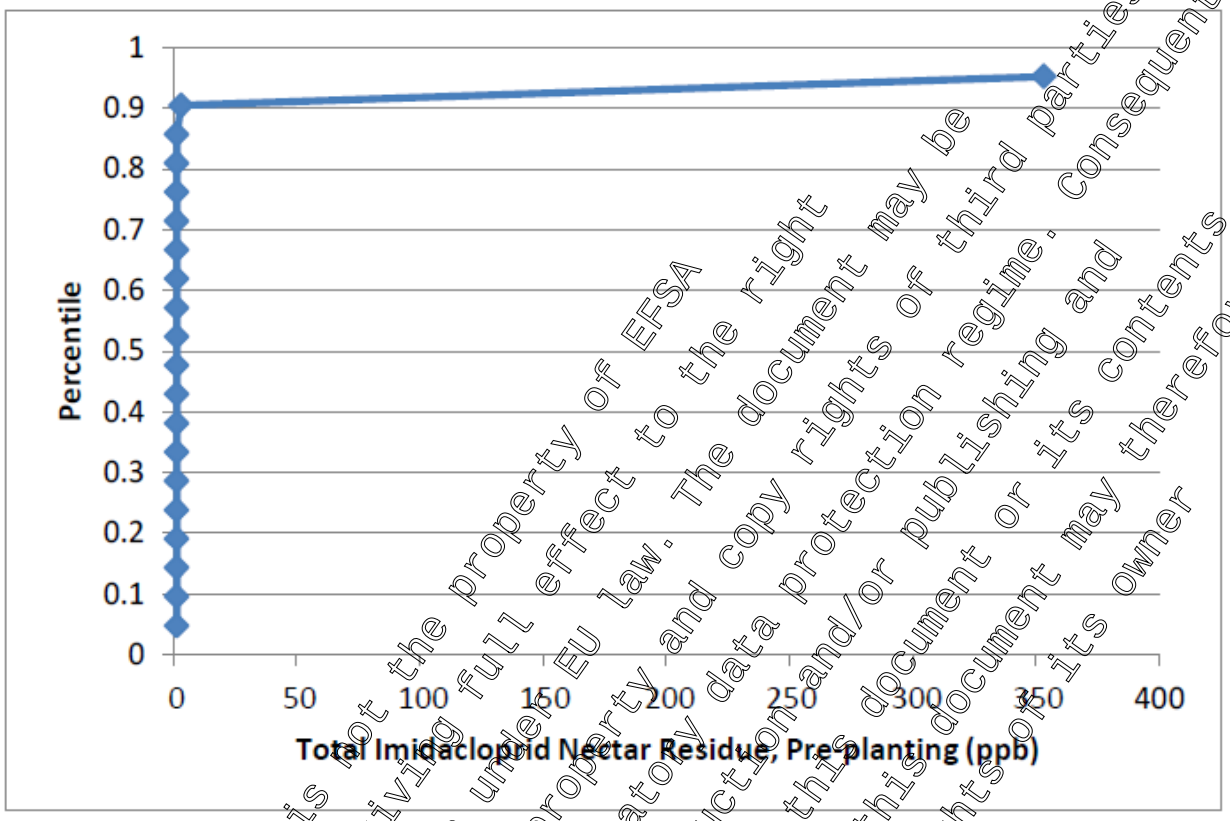

Figure 1. Nectar Residue Results, Pre-Planting

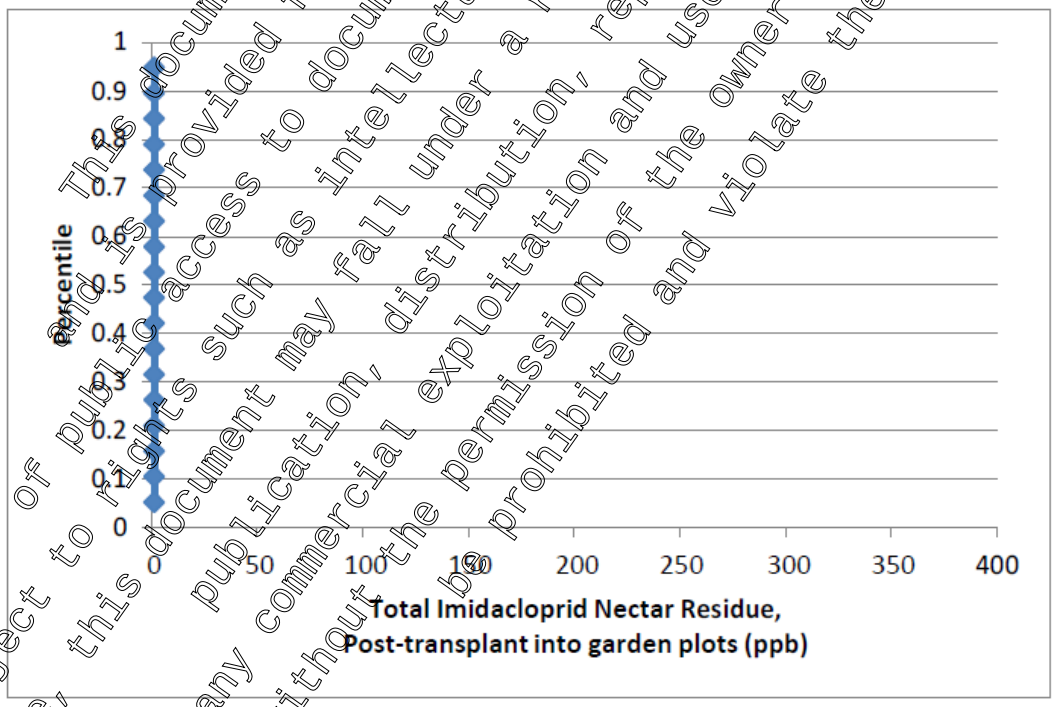

Figure 2. Nectar Residue Results after 4 weeks of growing in the garden plot

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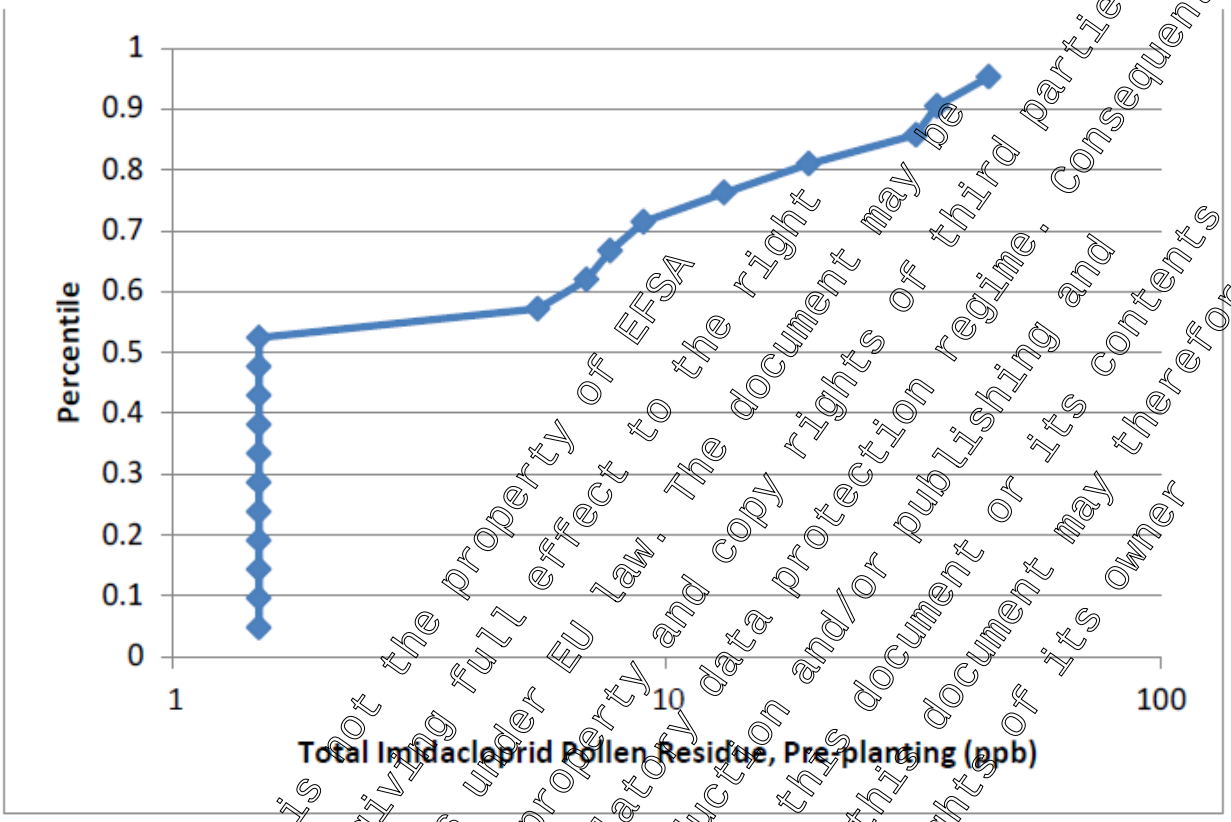

Figure 3. Pollen Residue Results, Pre-planting

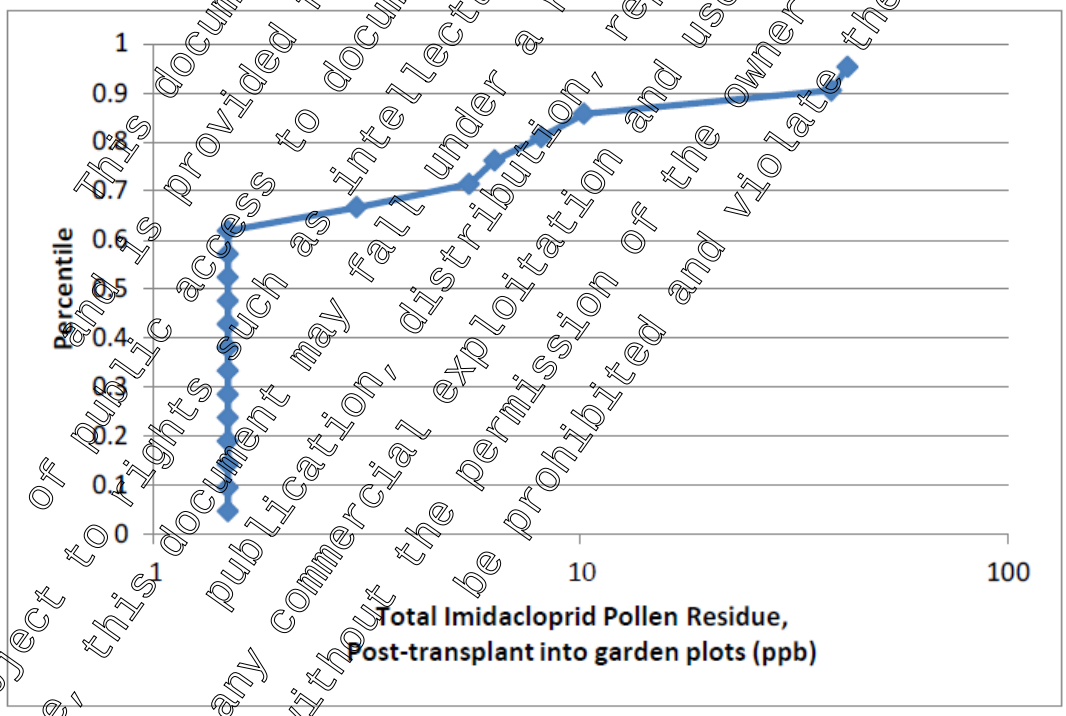

Figure 4. Pollen Residue Results after 4 weeks of growing in the garden plot

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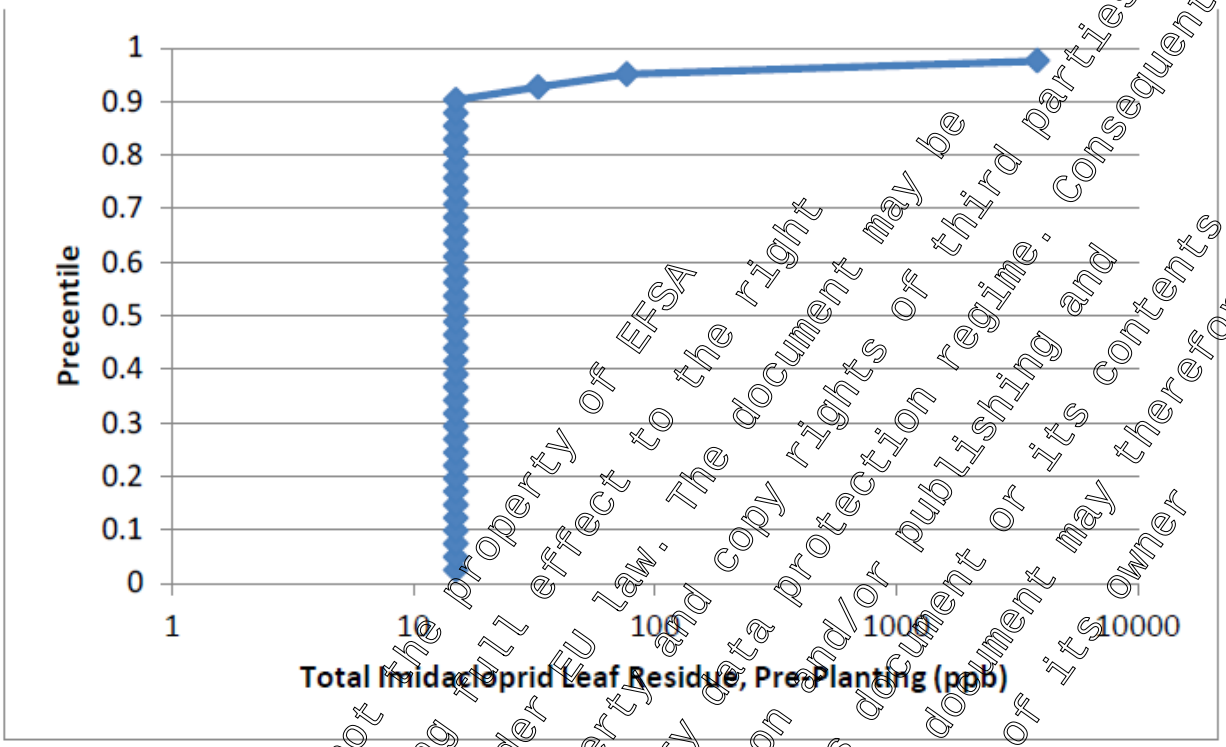

Figure 5. Leaf Residue results, Pre-planting

Figure 6. Leaf Residue results after 4 weeks of growing in the garden plot

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Report: 02.02.01/52; [redacted]; 2017; M-581858-01-2
Title: Final report: Survey of imidacloprid residues in nectar and pollen collected by honey bees in urban and suburban environments across different regions of the United States
Report No.: ESNTN015
Document No.: M-581858-01-2
Guideline(s): OCSPP 850.SUPP (Special Design)
Guideline deviation(s): none
GLP/GEP: **no**

<<M-581858-01-2@S-602901-01-1

A two-year, multi-state study was initiated in July 2014 with the goal of assessing exposure to pesticide residues in pollen and nectar collected by honey bees in urban and suburban environments. The project collaborators included Dr. Jamie Ellis (University of Florida, project coordinator), Dr. Zachary Huang (Michigan State University), Dr. Juliana Rangel and Mr. Pierre Lau (Texas A&M University), and Dr. Joseph Sullivan (Ardea Consulting). Pollen and nectar samples were collected monthly from 15 hives each in Florida (FL), Michigan (MI), California (CA) and Texas (TX). The percentage of developed area in the primary foraging area (1-mile radius) of the study hives was 56.6 - 99.6 % in FL, 58.5 - 100 % in MI, 51.6 - 99.1 % in CA and 13.1 - 99.2% in TX. The nectar and pollen samples were submitted periodically to the USDA-NSL for multi-residue analysis, which included imidacloprid parent, imidacloprid olefin and imidacloprid 5-hydroxy at levels of detection of 1, 10 and 25 ppb, respectively. A portion of the pollen samples collected between July 2014 and June 2015 served for identification of floral resources in these environments through palynological analysis. Exceptions to the sampling schedule included winter months for some regions and periods of pollen and/or nectar dearth. A total of 1,628 samples were analyzed, of those 767 were nectar and 861 were pollen samples. Imidacloprid olefin was detected at trace level in one pollen sample collected from CA, with no other detections of imidacloprid metabolites. Therefore, the results presented in this report correspond to the imidacloprid parent molecule. The percentage of nectar samples with detectable imidacloprid residues in FL, MI, CA and TX was 0.8% (n=263), 1.5% (n=194), 11.2% (n=223), and 0% (n=87), respectively. Likewise, the percentage of pollen samples with detectable imidacloprid residues in FL, MI, CA and TX was 5.5% (n=272), 4.7% (n=190), 20.7% (n=275), and 0.8% (n=124), respectively. No imidacloprid residue level exceeded the North American regulatory agencies' (EPA, PMRA, and CDPR) levels of concerns for nectar (25ppb) or pollen (100 ppb) for honey bees at the colony level. Overall, the results of this survey show that the risk to honey bee colonies in these environments during the study was minimal. In addition, trees were identified as important pollen sources in urban and suburban areas. The results from this study will be published in one or more peer-reviewed journal articles and have been presented at national and regional professional meetings, and at beekeepers associations meetings.

<<M-581858-01-2@S-602901-01-1

Report: 02.02.01/53; [redacted]; 2017; M-542796-03-2
Title: Pollinator full field study evaluating chronic effects of a post seeding application of imidacloprid in pumpkins (Curcubita pepo pepo) - Final report
Report No.: 43798.4145
Document No.: M-542796-03-2
Guideline(s): US EPA OCSPP 850.SUPP
Guideline deviation(s): none
GLP/GEP: **no**

<<M-542796-03-2@S-605072-01-1

A field study was conducted to evaluate the potential long-term effects of imidacloprid exposure to honey bee and bumble bee colonies, which were placed in imidacloprid-treated and reference pumpkin fields in central South Dakota during the summer of 2015. Pumpkins were direct seeded into large fields (40 acres) and imidacloprid was applied a sub-surface side dress at 0.38 lb/acre once pumpkins had attained the six true leaf stage (BBCH16). Fields were located in areas for which grassland/pasture and wheat fields were the predominant land use.

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The honey bee hives were established from 4-lb packages in new hive equipment, with sister queens, in North Carolina on 11 Apr 2015 and transported to South Dakota on 25 Jul 2015. Study hives were selected and randomly assigned in a stratified manner to either imidacloprid-treated (n=5) or untreated reference (n=5) pumpkin fields. Nine study honey bee hives and one monitoring hive were assigned to each pumpkin field. Nine bumble bee nests and two monitoring bumble bee nests were randomly assigned to each pumpkin field. Honey bee hives and bumble bee nests were moved into the fields once sufficient blooming of the pumpkins had occurred. The hives remained in the pumpkin fields for 6 weeks. Thereafter, hives were relocated to a post- exposure apiary near Durand, WI.

Samples for residue analysis were collected from field soils pre-treatment and indicated very low background levels (0-19 ppb) of imidacloprid, clothianidin, and thiamethoxam. Nectar and pollen samples were collected from pumpkin blossoms and analyzed for clothianidin and two metabolites as well as clothianidin and thiamethoxam. In nectar samples, only imidacloprid in treated fields were detected; however, levels were very low (0.8, 2.1, and 1.2 ppb median residues for the three time points). In pollen samples, there was one sample with detectable level of clothianidin, but no thiamethoxam detected in any sample. There were some, very low detections of imidacloprid in reference pollen samples. In treated fields, however, imidacloprid was consistently detected, although at low levels (3.4, 7.0, and 4.7 ppb median residues for the three time points.

Hive matrices (capped honey and bee bread) were collected from hives before being moved into pumpkin fields with a few hives having detections for imidacloprid. During the pumpkin field phase of the study, uncapped nectar and bee bread were sampled from study hives. Most uncapped nectar samples did not have any detectable imidacloprid residues in either the reference or treated fields. Imidacloprid residues, however, were more consistently detected in bee bread samples in the treated fields and demonstrate the largest difference in residues between reference and treated fields. After overwintering, no imidacloprid residues were detected in capped honey samples collected from either reference or treated fields.

Colony condition assessments showed no statistical differences between reference and treated fields for numbers of adult bees, capped brood cells, or bee bread cells for any assessment. Overall colony survival, including overwintering was 60% for reference fields and 56% for treated fields. There were no significant differences in *Nosema* or *Varroa* infection detected except for *Varroa* counts after overwintering. However, this difference was not considered treatment-related based on previous studies and the very low levels of *Varroa* detected across all hives.

Three surveys of non-*Apis* bees were conducted during the pumpkin bloom period using bee bowl traps containing soapy water. Large numbers of bees were collected across both reference and imidacloprid-treatment sites and no significant differences were observed amongst well-represented species and diversity indices. Bumble bee colonies performed very poorly in both reference and imidacloprid-treated sites likely due to the late time of the year or being outside of their normal range. Performance of the bumble bee colonies was not sufficient to compare between reference and treated fields.

Overall, no adverse effect were observed in honey bee colonies and non-Apis bee surveys between reference and imidacloprid treated fields. There were no statistical differences in numbers of adult bees, capped brood cells, nor bee bread cells which previously were observed to be sensitive endpoints for chronic imidacloprid exposure.

>>M-552796-03-2<< 505072-01-1

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Report:	02.02.01/54; [redacted]; 2011; M-408424-01-3
Title:	Determination of exposure levels of honey bees foraging on flowers of citrus trees previously treated with imidacloprid
Report No.:	EBNTL056-7
Document No.:	M-408424-01-3
Guideline(s):	US EPA OCSPP 850.SUPP
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-408424-01-3@S-605221-01-1

A series of field investigations were undertaken to determine to what extent honey bees foraging on citrus blossoms may be exposed to imidacloprid when citrus trees are treated with systemic applications (soil treatments) of this insecticide.

Tunnel Cage Study (Section 2)

- The objective of this component of the study was to examine citrus groves that were treated with a soil application of imidacloprid systemic insecticide, to understand the levels of imidacloprid that occurred in (a) nectar extracted by hand from citrus flowers, (b) nectar collected by forager honey bees and transported back to the hive, and (c) nectar or "uncapped honey" deposited by bees in cells of the brood comb
- Concentrations of imidacloprid, 5-hydroxy imidacloprid and imidacloprid olefin in nectar collected by hand from citrus flowers were similar to those in stomachs of bees foraging on the same trees confined within tunnels.
- The highest residue levels from the 3 nectar sources were measured in the nectar deposited within the new comb (stored nectar). Compared to the concentrations in the honey bee stomach extracts, the levels of imidacloprid and 5-hydroxy imidacloprid in the stored nectar extracts were about 3-fold higher while the levels of imidacloprid olefin were 5-fold higher. The higher measurements in the stored nectar may be because comb nectar has lower water content and higher sugar content compared with unprocessed nectar, although our results are not conclusive based on refractometry measurements.

Open Field Study (Section 3)

- The objective of this component of the study was to examine citrus groves that were treated with a soil application of imidacloprid systemic insecticide, to understand the levels of imidacloprid that occurred in (a) nectar extracted by hand from citrus flowers, (b) nectar collected by forager honey bees and transported back to the hive, (c) nectar or "uncapped honey" deposited by bees in cells of the brood comb, and (d) pollen retrieved from pollen traps in the same hives used for the nectar studies.
- Concentrations in nectar extracted from the stomachs of free-ranging bees were somewhat lower than for samples collected directly from flowers of nearby trees. This may reflect a "dilution effect" from bees foraging on other (untreated) flower types. Mean imidacloprid residues in nectar sampled from the trees were less than 7 ppb.
- Residue concentrations in stored nectar samples were somewhat greater than in flower nectar. This may be because comb nectar has lower water content and higher sugar content compared with unprocessed nectar, although our results are not conclusive based on refractometry measurements.
- The imidacloprid concentrations measured in the limited pollen available for analysis were equal to those in the stored nectar samples collected from the same hives.

Citrus Nectar Collections from Field Sites Treated In One Year with 1X and 2X Label Rates of Imidacloprid (Section 4)

- The objective of this component of the study was to determine to what extent increasing the imidacloprid application rate would impact residues in the nectar

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- Concentrations in flower nectar samples appear to be linearly related to application rate, based on *ca.* 2-fold increases in residue levels with a doubling of application rate.

Citrus Nectar Collections from Field Sites Treated in Successive Years with Imidacloprid (Section 5)

- The objective of this component of the study was to determine to what extent imidacloprid residues might persist and/or accumulate in citrus trees from year-to-year following multi-year applications.
- Based on experiments at the Hemet site, imidacloprid residues in spring flower nectar appear to be a function of the rate applied at the most recent application only, and appear to be independent of applications made in prior years. This conclusion is based on a period of 1 year between applications, which would be the normal use under the current label recommendation for citrus in California. There was a suggestion of some carryover between years with the 2X label rate treatments, although the result was not statistically significant.
- Nectar samples were obtained from 11 sites (citrus blocks in the Temecula region and at Lindcove Research and Extension Center) where the 1X soil application rate of imidacloprid had been made in two successive years (2008, 2009) prior to sampling in April 2010. Residue levels at these 11 sites averaged 8 ppb and ranged from 1 to 18 ppb.
- The application timing (fall vs. spring) appears to be an important factor in determining residue levels in flower nectar the following year. Fall (Sept) applications resulted in about 2-fold higher residue concentrations compared with Spring (April-June) applications.
- Our conclusion is that at current label rates the residues of imidacloprid detected in the nectar during Spring bloom reflect the imidacloprid rate used during the most recent application, with limited impact from imidacloprid treatments conducted in prior seasons.

>>M-408424-01-3@S-605221-01-1

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02.02.02 - Succeeding crops

Report:	02.02.02/01; [redacted]; 2002; M-061850-01-3
Title:	Imidacloprid (Admire) residue levels following in-furrow application in potato fields in Prince Edward Island and New Brunswick
Report No.:	M-061850-01-3
Document No.:	M-061850-01-3
Guideline(s):	US EPA OPPTS 850.3040
Guideline deviation(s):	not specified
GLP/GEP:	**no**

<<M-061850-01-3@S-602677-01-1

Imidacloprid (ADMIRE® 240F), is a synthetic systemic chloronicotinyl insecticide, produced for the control of Colorado potato beetles, aphids, flea beetles, and leafhoppers on potato crops (Elbert et al,, 1991; [redacted], 1999). Imidacloprid is an agonist at nicotinic acetylcholine receptors that demonstrates selective toxicity for insects over vertebrates, and has the fastest growing sales of any insecticide worldwide. Since its initial registration in France (1991), in January 1995, the Pesticide Management Regulatory Agency (PMRA) received applications requesting the registration of imidacloprid, and in April 1995, the PMRA granted temporary registration under section 17 of the Pest Control Products Regulations of Admire 240 F for the control of Colorado potato beetles in potatoes in Eastern Canada. In April 1999, imidacloprid was approved for use in potatoes across Canada and as a broad spectrum pesticide, it is presently registered in 100 countries for use on over 65 crops.

The high molecular mobility of Admire in the xylem of treated plants is due to its high water solubility (510 mg/L) (Elbert et al. 1998; Elbert et al, 1991). The molecular ability of imidacloprid makes it an ideal candidate for the use on potatoes and numerous other crops (apples, lettuce, tomatoes, mustard, canola, cucumber, com, etc.). Due to its long term action, this chloronicotinoid is highly effective and has been used extensively as an in-furrow treatment for Colorado potato beetle. In potato fields the recommended in-furrow rate of application is 850 mL to 1.3 L / ha. Due to its residual activity, imidacloprid has become the most popular control agent for Colorado potato beetle.

Despite worldwide recognition, the use of Admire® has been in question following reports by French bee keepers of "disoriented" honey bees that had been foraging in imidacloprid (Gaucho®) treated sunflower fields. The bee keepers in France also reported that the honey bees had high rates of mortality, and low honey production due to a decrease in colony strength. In Canada, the PMRA' s initial review of imidacloprid concluded that although pollinators (honey bees) could be at risk due to its high toxicity, the risk could be mitigated by a label statement contraindicating application of the product to blooming crops when bees are visiting the treatment area. Since that time, the question of whether systemic residues of imidacloprid may occur in nectar and pollen of flowering crops at concentrations harmful to honey bees has been the focus of an extensive research program. A study conducted by Schmidt and [redacted] (2000) examined the effects of imidacloprid (Gaucho®) seed treated sunflowers on honey bees and found no evidence to support the claims made by French beekeepers. In an investigation on the foraging behavior and orientation ability of honey bees by Kirchner changes in behavior were found for imidacloprid concentrations of 20 ppb (parts per billion) to 100 ppb, although no effect was observed at 10 ppb. Although the effects on the behavior of bees were observed to start at imidacloprid concentrations of 20 ppb, no damage to the test populations was observed for the range of concentrations tested up to 100 ppb.

With the release of the information from France, some bee keepers in Prince Edward Island and New Brunswick, complained of similar problems following placement of colonies near clover fields that bad been previously treated with ADMIRE®, and requested a moratorium on the use of Admire® on Prince Edward Island. With this concern expressed, it was important to determine whether imidacloprid residue levels following use in potato fields was negatively affecting honey bee health on Prince Edward Island.

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The objectives of this study were to determine if residue levels (ppb) of imidacloprid applied in furrow, plus two metabolites, (hydroxy-imidacloprid and olefin-imidacloprid), were present one and two years following application of Admire in:

1. soil, clover leaves, and clover flowers, and wild flowers
2. pollen, and nectar collected from honey bees foraging in previously treated clover fields
3. uncapped honey collected from the hives placed in previously treated clover fields

The following report is a review of the protocol and results of the project.
>>M-061850-01-3@S-602677-01-1

Report:	02.02.02/02; [redacted]; 2011; M-406075-01-3
Title:	Determination of residue levels of imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine in bee relevant matrices of winter rape in a cereal succeeding crop scenario at Bayer CropScience AG experimental farm Germany
Report No.:	E 319 3388-5
Document No.:	M-406075-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-406075-01-3@S-602303-01-1

Experimental starting/completion date: October 17, 2007 – August 25, 2009

Material and methods:
The imidacloprid containing test item (mixture of imidacloprid and fungicides), used for the purpose of this study, was fuberidazol + imazalil + imidacloprid + triadimenol FS 145.2 (7.2+8+70+60) G, TOX-No. of test item: 08068-00; analysed content of imidacloprid: 72.3 g a.s./L; density: 1.081 g/mL). In addition: imidacloprid-treated winter wheat seeds of the variety "Dekan", dressed with the above mentioned test item (TOX-No. of treated seeds: 08079-00; analysed content of imidacloprid: 70.75 g a.s. /100 kg seeds; imidacloprid-free dressed winter wheat seeds of the variety "Dekan" as well as imidacloprid-free dressed winter oil-seed rape (OSR) seeds of the variety "Adriana".

In autumn 2007 (18 October 2007), the imidacloprid-containing test item was applied and incorporated down to 20 cm soil depth on a fallow test plot (=treatment test plot) at a rate corresponding to nominally 126 g a.s. imidacloprid/ha to conservatively establish a long-term soil plateau concentration of imidacloprid, simulating the consecutive use of imidacloprid on the same field plot over several years. Incorporation was achieved by means of a power-harrow. On the same day, immediately after the establishment of the long-term soil plateau concentration of imidacloprid, imidacloprid-treated winter wheat seeds, dressed with test item (=treatment winter wheat seeds), were sown on the treatment test plot at a nominal sowing rate of 180 kg seeds/ha corresponding to nominally 126 g a.s. imidacloprid/ha. On an equivalent control test plot, imidacloprid-free dressed wheat seeds (=control winter wheat seeds) of the same variety as the treatment seeds were sown at the same day (18 October 2007). These imidacloprid-free control winter wheat seeds received the same nominal loading of active fungicidal substances as the treatment seeds. The control seeds were sown on the control test plot also at a nominal sowing rate of 180 kg seeds/ha. On the control test plot, no plateau concentration has been established, and as such, no spray application was performed.

In late summer 2008 (19 August 2008), after harvesting of the winter wheat at 30 July 2008, winter OSR seeds with an imidacloprid-free seed coating (insecticidal seed coating: Elado® (= 400 g clothianidin a.s. /L + 80 g beta-cyfluthrin a.s./L) + fungicidal seed coating "Thiram" (= 700 g thiram a.s./L)) were sown on the treatment test plot and the control test plot, respectively. No further crop was sown during the intervening period after harvesting of winter wheat and sowing of winter OSR seeds, as typical for commercial agricultural practice.

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Seven days before foraging honeybees were exposed to the flowering winter OSR crop under confined conditions, one gauze tunnel (approximately 50 m² surface areas) was set up on the treatment test plot and the control test plot, respectively (16 April 2009). Thereafter, one honeybee colony with about 3000 bees (Apis mellifera carnica) was installed inside the tunnel on the treatment test plot and the control test plot, respectively (23 April 2009). During the flowering period of winter OSR, nectar- and pollen foraging honeybees were manually collected inside the tunnels and stored deep frozen. Afterwards, the frozen honeybees were worked up by separating pollen loads from the legs of the pollen forager bees and by extracting bee-collected nectar by puncturing the honey bulbs of the nectar forager bees with an ultra-fine syringe. Thereafter, the extracted pollen and nectar was analysed to determine residue levels of imidacloprid and its metabolites imidacloprid-monohydroxy and imidacloprid-olefine.

Findings:
Imidacloprid residues in soil:
Directly after the application and incorporation of the test item into the upper 20 cm of the soil of the treatment test plot, the mean analysed imidacloprid concentration was 45.7 µg a.s./kg dry soil.

After a period of nearly 10 months, directly before sowing winter OSR seeds with an imidacloprid-free seed coating, the mean imidacloprid concentration on the treatment test plot decreased to 18.8 µg a.s./kg dry soil. The corresponding parallel soil residue analysis on the control test plot showed no residues of imidacloprid.

Analytical results for imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine in bee relevant matrices of winter OSR:

Sample Number	Sample Name	Sample Material	Treatment / Control Test Plot [T/C]	Residue [mg/kg]		
				Imidacloprid	Imidacloprid-monohydroxy	Imidacloprid-olefine
001	Pollen C1	Pollen	C	< LOD	< LOD	< LOD
003	Pollen C3		C	< LOD	< LOD	< LOD
005	Pollen C5		C	< LOD	< LOD	< LOD
002	Pollen T2		T	0.002	< LOD	< LOD
004	Pollen T4		T	0.002	< LOD	< LOD
006	Pollen T6		T	0.002	< LOD	< LOD
001	Nectar C1	Nectar	C	< LOD	< LOD	< LOD
003	Nectar C3		C	< LOD	< LOD	< LOD
005	Nectar C5		C	< LOD	< LOD	< LOD
002	Nectar T2		T	< LOD	< LOD	< LOD
004	Nectar T4		T	< LOD	< LOD	< LOD
006	Nectar T6		T	< LOD	< LOD	< LOD

Limit of quantitation (LOQ) for imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine = 0.001 mg/kg,
Limit of detection (LOD) for imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine = 0.0003 mg/kg

Conclusion:
Under still unrealistic worst case conditions (long-term imidacloprid plateau concentration conservatively simulated by fresh application and incorporation of imidacloprid into the soil at the day of sowing imidacloprid-dressed winter wheat, followed by winter OSR as a succeeding crop), residues of imidacloprid in OSR-nectar collected on the imidacloprid treatment test plot were always below the limit of detection (LOD). The imidacloprid concentration in the three pollen samples from the imidacloprid treatment test plot was determined to be 0.002 mg a.s./kg, respectively.
The imidacloprid-monohydroxy and imidacloprid-olefine concentration of all pollen and nectar samples from the treatment test plot was always below the limit of detection (LOD).

>>M-406075-01-3@S-602303-01-1

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Report:	02.02.02/03; [redacted]; 2013; M-406083-01-3
Title:	Determination of residue levels of imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine in bee relevant matrices of winter rape in a cereal succeeding crop scenario at Bayer CropScience AG experimental farm Hoefchen, Germany
Report No.:	E 319 3387-4
Document No.:	M-406083-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-406083-01-3@S-602306-01-1

Experimental starting/completion date: October 17, 2007 - August 24, 2009

Material and methods:
The imidacloprid containing test item (mixture of imidacloprid and fungicides), used for the purpose of this study, was fuberidazol + imazalil + imidacloprid + triadimenol FS 145.2 (7.2+8+70+60) G, TOX-No. of test item: 08068-00; analysed content of imidacloprid: 72.3 g a.s./L, density: 1.081 g/mL). In addition: imidacloprid-treated winter wheat seeds of the variety "Dekan", dressed with the above mentioned test item (TOX-No. of treated seeds: 08079-00; analysed content of imidacloprid: 70.75 g a.s. /100 kg seeds; imidacloprid-free dressed winter wheat seeds of the variety "Dekan" as well as imidacloprid-free dressed winter oil-seed rape (OSR) seeds of the variety "Adriana".

In autumn 2007 (19 October 2007), the imidacloprid-containing test item was applied and incorporated down to 20 cm soil depth on a fallow test plot (=treatment test plot) at a rate corresponding to nominally 126 g a.s. imidacloprid/ha to conservatively establish a long-term soil plateau concentration of imidacloprid, simulating the consecutive use of imidacloprid on the same field plot over several years. Incorporation was achieved by means of a power-harrow. On the same day, immediately after the establishment of the long-term soil plateau concentration of imidacloprid, imidacloprid-treated winter wheat seeds, dressed with test item (=treatment winter wheat seeds), were sown on the treatment test plot at a nominal sowing rate of 180 kg seeds/ha, corresponding to nominally 126 g a.s. imidacloprid/ha. On an equivalent control test plot, imidacloprid-free dressed wheat seeds (=control winter wheat seeds) of the same variety as the treatment seeds were sown at the same day (19 October 2007). These imidacloprid-free control winter wheat seeds received the same nominal loading of active fungicidal substances as the treatment seeds. The control seeds were sown on the control test plot also at a nominal sowing rate of 180 kg seeds/ha. On the control test plot, no plateau concentration has been established, and as such, no spray application was performed.

In late summer 2008 (21 August 2008), after harvesting of the winter wheat at 01 August 2008, winter OSR seeds with an imidacloprid-free seed coating (insecticidal seed coating: Elado® (= 400 g clothianidin a.s. /L + 80 g beta-cyfluthrin a.s./L) + fungicidal seed coating "Thiram" (= 700 g thiram a.s./L)) were sown on the treatment test plot and the control test plot, respectively. No further crop was sown during the intervening period after harvesting of winter wheat and sowing of winter OSR seeds, as typical for commercial agricultural practice.

Seven days before foraging honeybees were exposed to the flowering winter OSR crop under confined conditions, one gauze tunnel (approximately 50 m² surface areas) was set up on the treatment test plot and the control test plot, respectively (13 April 2009). Thereafter, one honeybee colony with about 3000 bees (*Apis mellifera carnica*) was installed inside the tunnel on the treatment test plot and the control test plot, respectively (20 April 2009). During the flowering period of winter OSR, nectar- and pollen foraging honeybees were manually collected inside the tunnels and stored deep frozen. Afterwards, the frozen honeybees were worked up by separating pollen loads from the legs of the pollen forager bees and by extracting bee-collected nectar by puncturing the honey bulbs of the nectar forager bees with an ultra-fine syringe. Thereafter, the extracted pollen and nectar was analysed to determine residue levels of imidacloprid and its metabolites imidacloprid-monohydroxy and imidacloprid-olefine.

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Findings:

Imidacloprid residues in soil

Directly after the application and incorporation of the test item into the upper 20 cm of the soil of the treatment test plot, the mean analysed imidacloprid concentration was 34.0 µg a.s./kg dry soil.
After a period of nearly 10 months, directly before sowing winter OSR seeds with an imidacloprid-free seed coating, the mean imidacloprid concentration on the treatment test plot decreased to 15.2 µg a.s./kg dry soil. The corresponding parallel soil residue analysis on the control test plot showed no residues of imidacloprid.

Analytical results for imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine in bee relevant matrices of winter OSR:

Sample Number	Sample Name	Sample Material	Treatment / Control Test Plot [T/C]	Residue [mg/kg]		
				imidacloprid	imidacloprid-monohydroxy	imidacloprid-olefine
002	Pollen C2	Pollen	C	< LOD	0.004	< LOD
004	Pollen C4		C	< LOD	< LOD	< LOD
006	Pollen C6		C	< LOD	< LOD	< LOD
008	Pollen C8		C	< LOD	< LOD	< LOD
001	Pollen T1		T	0.0003	< LOD	< LOD
003	Pollen T3		T	< LOD	< LOD	< LOD
005	Pollen T5		T	0.0003	< LOD	< LOD
007	Pollen T7		T	< LOD	< LOD	< LOD
002	Nectar C2	Nectar	C	< LOD	< LOD	< LOD
004	Nectar C4		C	< LOD	< LOD	< LOD
006	Nectar C6		C	< LOD	< LOD	< LOD
008	Nectar C8		C	< LOD	< LOD	< LOD
001	Nectar T1		T	< LOD	< LOD	< LOD
003	Nectar T3		T	< LOD	< LOD	< LOD
005	Nectar T5		T	< LOD	< LOD	< LOD
007	Nectar T7		T	< LOD	< LOD	< LOD

Limit of quantification (LOQ) for imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine = 0.001 mg/kg,
Limit of detection (LOD) for imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine = 0.0003 mg/kg

Conclusion:

Under still unrealistic worst case conditions (long-term imidacloprid plateau concentration conservatively simulated by fresh application and incorporation of imidacloprid into the soil at the day of sowing imidacloprid-dressed winter wheat, followed by winter OSR as a succeeding crop), residues of imidacloprid in OSR-pollen and OSR-nectar collected on the imidacloprid treatment test plot were always below the limit of quantitation (LOQ).
The imidacloprid concentration in two pollen samples from the treatment test plot matched the limit of detection (LOD) of 0.0003 mg a.s./kg, respectively, and in two pollen samples from the treatment test plot the imidacloprid concentration was < LOD, respectively. The imidacloprid-monohydroxy and imidacloprid-olefine concentration of all pollen samples from the treatment test plot was < LOD. The imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine concentration of all nectar samples from the treatment test plot was < LOD.

The residue finding of imidacloprid-monohydroxy in one of the pollen samples collected on the control test plot (“Pollen C2”) is suspected to result from a contamination in the analytical laboratory, as neither parent imidacloprid nor imidacloprid-olefine was detected in this particular sample.

>>M-406083-01-3@S-602306-01-1

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Report: 02.02.02/04; [redacted]; 2014; M-504801-01-3
Title: Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with Phacelia and maize in northern France
Report No.: 7SRFR13C1
Document No.: M-504801-01-3
Guideline(s): US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-504801-01-3@S-602361-01-1

Study phases

Phase	Start date	End date
Field phase	05/05/2014	03/08/2014
Maize Guttation	02/06/2014	10/07/2014
Phacelia flowering	05/05/2014	26/05/2014
Maize flowering	29/07/2014	03/08/2014

Sampling periods

Sample type	Sub plot	Sample date(s)
Soil for characterisation	Maize 1 - 3	19/07/2014
	Maize 1 - 3	19/07/2014
Soil for characterisation	Phacelia	06/05/2014
Soil for residue analysis	Phacelia	06/05/2014
Guttation	Maize 1 - 3	02/06/2014 - 10/07/2014
Maize pollen	Maize 1 - 3	29/07/2014
		02/08/2014
		03/08/2014
Phacelia pollen	Tunnels 1 -3	06/05/2014
		15/05/2014
		26/05/2014
Phacelia nectar	Tunnels 1 -3	06/05/2014
		15/05/2014
		26/05/2014

Executive summary:

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Objective:
The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen, nectar and guttation fluid) collected from untreated flowering rotational crops cultivated as succeeding crops grown in France on fields with a history of Imidacloprid use and as such with natural aged soil-residues of this active ingredient.

Study Site:
The study was conducted on a field site near Meung-sur-Loire (F-45130, France) with a known history of Imidacloprid use and such with a likelihood of natural aged soil residues of this active substance. An approximately one hectare plot located within the field was marked out, and divided into two evenly sized sub-plots. One sub-plot was sown with maize (Zea mays) the other sub-plot was sown with Phacelia (Phacelia tanacetifolia).

Material and Methods:

Crops were sown according to Good Agricultural Practice (GAP).
Maize and Phacelia without neonicotinoid seed treatment were sown in 2014, using calibrated equipment (tractor and seed drill). The target sowing rates were 10 kg seeds/ ha for Phacelia and 100,000 kernel/ ha for maize.
The plot sown with maize was later divided into three smaller sub plots, each similar in size that were large enough to have a sufficient numbers of plants available for both, sampling of guttation fluid and for maize pollen sampling.
Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the phacelia plot after successful germination. A single honeybee colony was placed into each tunnel at the start of Phacelia flowering to collect nectar and pollen.

Soil sampling:
From each of the maize sub plots and from the phacelia sowing area, two different types of soil sample were collected. These samples were used for:
1. Soil characterisation of the upper 10 cm soil layer.
2. Determination of the residues of parent imidacloprid and its metabolites in the upper 15 cm soil layer.

Soil cores used for characterisation and residue analysis were collected from each of the three maize sub plots during the guttation sampling phase of the trial and from inside of the Phacelia sowing area prior to placement of the honeybee colonies into the tunnels.

Sampling of Nectar and Pollen from Phacelia Crops:
Nectar and pollen sampling was conducted at three different time points during bloom of the Phacelia crop. Once the Phacelia started to bloom, Honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering Phacelia under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid

Sampling of Guttation fluid and Pollen from Maize:
Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand.
Sampling of guttation fluid was carried out on a regular basis over a 46-day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 11-12) until flowering (BBCH scale 65).

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Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period at each time point was approximately 30 minutes to ensure an equivalent time chronology every day
Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3.
When guttation was present it was collected from >10 plants throughout each of the sub plots. The target volume for each sample was 1 ml of guttation fluid.
Pollen sampling at three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67).
At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature overnight.
Next day, the pollen was shaken out and cleaned with two analytical sieves (mesh size 2 mm and 1 mm), to ensure a pure pollen sample. Maize pollen in the base pan was cleaned from plant or insect debris remaining in the pollen sample by hand using forceps or a fine paint brush.

Residue analysis:
All samples (soil samples, pollen, nectar and guttation fluid) were analysed for their content of imidacloprid and and its metabolites 5-hydroxy and olefin by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.
Analysis of the soil samples followed the provisions of method 00790/M001. Analysis of nectar and pollen followed the provisions of method 01433 and for the analysis of guttation liquid the analytical method 00537/M002 was used, which is based on the method 01433.
The Limit of Quantitation (LOQ) of imidacloprid, defined as the lowest validated fortification level, was 5.0 µg/kg for soil. The corresponding Limit of Detection (LOD) was 2 µg/kg.
The LOQ levels for imidacloprid were 0.6 µg/kg for pollen, 0.3 µg/kg for nectar and 1.0 µg/L for guttation liquid while the LOQ level of the metabolites was constant 1.0 µg/kg for all sample materials.
The corresponding Limit of Detections (LOD) were 0.2 µg/kg for pollen, 0.1 µg/kg for nectar and 0.3 µg/L (0.0003 mg/L) for guttation liquid, respectively for imidacloprid and 0.3 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine for all sample materials.
All results of the method validations were in accordance with the general requirements for residue analytical methods; therefore, the employed methods were validated successfully. The average recoveries were within the acceptable range of 60 - 120%. RSD values are below 20%. A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar samples are provided in the following tables:

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Residues of imidacloprid in soil

Sample material	Crop	Residue imidacloprid * [µg/kg dry soil]
Soil	Maize	43-50
Soil	Phacelia	39

LOQ = Limit of Quantitation = 5 µg/kg in/on soil samples (all analytes)
LOD = Limit of Detection = 2 µg/kg in/on soil samples (all analytes)

* Unrounded Residue Imidacloprid [µg/kg] /(1-(Moisture/100)

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in Maize guttation liquid samples

Sample material	Residue of imidacloprid [µg/L]	Residue of imidacloprid-5-hydroxy [µg/L]	Residue of imidacloprid-olefine [µg/L]
Guttation liquid (Maize)	< LOD – 5.7	< LOD – < LOQ	< LOD

LOQ = Limit of Quantitation = 1 µg/L in guttation liquid samples (all analytes)
LOD = Limit of Detection = 0.3 µg/L in guttation liquid samples (all analytes)

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in pollen from *Phacelia* and Maize and nectar from *Phacelia*

Sample material	Residue of imidacloprid [µg/kg]	Residue of imidacloprid-5-hydroxy [µg/kg]	Residue of imidacloprid-olefine [µg/kg]
Pollen (Phacelia)	< LOQ	< LOD – < LOQ	< LOD
Pollen (Maize)	< LOD – < LOQ	< LOD	< LOD
Nectar (Phacelia)*	< LOQ – 3.5	< LOD	< LOD

*: 8 out of 9 samples ≤ 0.5 µg/kg
LOQ = Limit of Quantitation = 0.3 µg/kg in nectar and 0.6 µg/kg in pollen samples for imidacloprid and 1 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine in all sample materials.
LOD = Limit of Detection = 0.1 µg/kg in nectar and 0.2 µg/kg in pollen samples for imidacloprid and 0.3 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine in all sample materials.

Conclusion:
The study was conducted on a field site near Auxy, (Meung-sur Loire, F-45130, France) with a known history of crops and imidacloprid uses as such with natural aged soil-residues of this active substance. Therefore, this study provides realistic field data on residue levels of imidacloprid within bee relevant matrices, collected from non-imidacloprid treated flowering Phacelia and maize plants cultivated as succeeding crops from a field with natural aged soil-residues of imidacloprid.

Maize:
One set of soil samples were taken from the maize sub plots during the trial. The residue levels of imidacloprid in soils ranged from 43 µg a.s./kg to 50 µg a.s./kg dry soil during guttation.
Residues analysis of guttation fluid, collected directly after emergence until early bloom of the maize plants, revealed generally low residue levels.
The residue levels of imidacloprid in guttation fluid ranged from below the LOD (<0.3 µg a.s.) to 5.7

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µg a.s./L and are thus several orders of magnitude below neonicotinoid values measured in droplets from seed treated maize plants.
The residue levels of imidacloprid in pollen, as sampled at three time points during bloom of the maize plants ranged from below the LOD (<0.2 µg a.s./kg) to below the LOQ (<0.6 µg a.s.).

Phacelia:

Soil cores used for residue analysis were taken from the entire field prior to placement of the honeybee colonies into the tunnels. The residue level of imidacloprid in the phacelia plot was 39 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of Phacelia, revealed generally low residue levels.
The residue levels of imidacloprid in pollen was always below the LOQ (<0.6 µg a.s./kg).
The residue levels of imidacloprid in nectar ranged from below the LOQ (<0.3 µg a.s./kg) to 3.5 a.s./kg. 8 out of 9 samples contained residues < 0.5 µg a.s./kg.

>>M-504801-01-3@S-602361-01-1

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Report: 02.02.02/05; [redacted]; 2014; M-504806-01-3
Title: Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with winter oil seed rape, Phacelia and maize in northern France
Report No.: 7SRFR13C2A
Document No.: M-504806-01-3
Guideline(s): US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-504806-01-3@S-602365-01-1

Study phases

Phase	Start date	End date
Field phase	27/03/2014	27/07/2014
Maize Guttation	26/05/2014	04/07/2014
Phacelia flowering	08/07/2014	22/07/2014
Maize flowering	21/07/2014	22/07/2014
Winter oil seed rape flowering	27/03/2014	14/04/2014

Sampling periods

Sample type	Sub plot	Sample date(s)
Soil for characterisation	Maize 1 - 3	17/07/2014
Soil for residue analysis	Maize 1 - 3	17/07/2014
Soil for characterisation	Phacelia	09/07/2014
Soil for residue analysis	Phacelia	09/07/2014
Soil for characterisation	W-OSR	28/03/2014
Soil for residue analysis	W-OSR	28/03/2014
Guttation	Maize 1 - 3	26/05/2014 - 04/07/2014
Maize pollen	Maize 1 - 3	21/07/2014
		25/07/2014
		27/07/2014
Phacelia pollen	Tunnels 1 -3	09/07/2014
		16/07/2014
		22/07/2014

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Phacelia nectar	Tunnels 1 -3	09/07/2014 16/07/2014 22/07/2014
W-OSR pollen	Tunnels 1 -3	28/03/2014 09/04/2014 14/04/2014
W-OSR nectar	Tunnels 1 -3	28/03/2014 09/04/2014 14/04/2014

Executive summary:

Objective:
The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen, nectar and guttation fluid) collected from flowering rotational crops cultivated as succeeding crops grown in France on fields with a history of Imidacloprid use and as such with natural aged soil-residues of this active ingredient.

Study Site:
The study was conducted on a field site near Giroux (F-36150, France) known history of Imidacloprid use and such with a likelihood of natural aged soil residues of this active substance. On this land, non imidacloprid treated Winter oil seed (Brassica napus) has been cultivated in 2013. During bloom in 2014, in total, three tunnels were setup for Winter oil seed with one bee hive per tunnel. Samples of pollen loads (collected with pollen traps) and forager honey bees (for subsequent extraction of nectar from honey stomach) were taken. The samples were analysed for residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin during the Analytical Phase.
After sample collection and prior to sowing of non-imidacloprid treated phacelia (Phacelia tanacetifolia) and maize (Zea mays) the previous crop was removed from the land.

Material and Methods:

All Crops were sown according to Good Agricultural Practice (GAP).
The maize and phacelia plots were sown using calibrated equipment (tractor and seed drill). The target sowing rates were 10 kg seeds/ ha for Phacelia and 100,000 kernel/ ha for maize.
The sub plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient numbers of plants available for both guttation fluid and for maize pollen sampling.
Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the phacelia plot after successful germination. A single honeybee colony was placed into each tunnel at the start of Phacelia flowering.

Soil sampling:
From each of the maize sub plots and from respectively the phacelia and winter oil seed rape sowing area, two different types of soil sample were collected. These samples were used for:
1. Soil characterisation of the upper 10 cm soil layer.
2. Determination of the residues of parent imidacloprid and its metabolites in the upper 15 cm soil layer.

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Soil cores used for characterisation and residue analysis were collected for winter oil seed rape shortly before start of the sampling. In addition to this, soil cores used for characterisation and residue analysis for the other crops were collected from each of the three segregated maize sub plots, during the guttation sampling phase of the trial and from inside of the Phacelia sowing area prior to placement of the honeybee colonies into the tunnels.

Sampling of Nectar and Pollen from Winter Oilseed Rape:
Nectar and pollen sampling was conducted at three different time points during bloom of the oilseed crop. Once the winter oilseed started to bloom, Honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering winter oilseed under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid

Sampling of Nectar and Pollen from Phacelia:
Nectar and pollen sampling was conducted at three different time points during bloom of the Phacelia crop. Once the Phacelia started to bloom, Honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering Phacelia under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid

Sampling of Guttation fluid and Pollen from Maize:
Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand.
Sampling of guttation fluid was carried out on a regular basis over a 40-day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 10-12) until flowering (BBCH scale 65). Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period at each time point was approximately 30 minutes to ensure an equivalent time chronology every day.
Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3.
When guttation was present it was collected from > 10 plants throughout each of the sub plots. The target volume for each sample was 1 ml of guttation fluid.
Pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67).
At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature overnight.
Next day, the pollen was shaken out and cleaned with two analytical sieves (mesh size 2 mm and 1 mm), to ensure a pure pollen sample. Maize pollen in the base pan was cleaned from plant or insect debris remaining in the pollen sample by hand using forceps or a fine paint brush.
Pollen samples during bloom as well as collected guttation fluid were analysed for residues of imidacloprid.

Residue analysis:
All samples (soil samples, pollen, nectar and guttation fluid) were analysed for their content of imidacloprid and and its metabolites 5-hydroxy and olefin by using High Performance Liquid

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Residue analysis of imidacloprid in soil samples and samples of guttation liquid, nectar and pollen was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection. Analysis of the soil samples followed the provisions of method 00790/M001. Analysis of guttation liquid, nectar and pollen followed the provisions of method 01433.

The Limit of Quantification (LOQ) of imidacloprid, defined as the lowest validated fortification level, was 5.0 µg/kg for soil. The corresponding Limit of Detection (LOD) was 2 µg/.

The LOQ levels for imidacloprid was 0.6 µg/kg for pollen, 0.3 µg/kg for nectar and 1.0 µg/L for guttation liquid while the LOQ level of the metabolites were constant 1.0 µg/kg for all sample materials. The corresponding Limit of Detections (LOD) were 0.2 µg/kg, 0.1 µg/kg and 0.3 µg/kg respectively for imidacloprid and 0.3 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine for all sample materials.

All results of the method validations were in accordance with the general requirements for residue analytical methods; therefore, the employed methods were validated successfully. The average recoveries were within the acceptable range of 60 – 120%. RSD values are below 20%. A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar samples are provided in the following tables:

Residues of imidacloprid in soil

Sample material	Crop	Residue imidacloprid * [µg/kg dry soil]
Soil	Maize	35-48
Soil	Phacelia	46
Soil	OSR	43

LOQ = Limit of Quantitation = 5 µg/kg on/from soil samples (all analytes)
LOD = Limit of Detection = 2 µg/kg on/from soil samples (all analytes)
* = [rounded Residue Imidacloprid [µg/kg] / (1-%Moisture/100)

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in Maize guttation liquid samples

Sample material	Residue of imidacloprid [µg/L]	Residue of imidacloprid-5-hydroxy [µg/L]	Residue of imidacloprid-olefine [µg/L]
Guttation liquid (Maize)	< LOD – 1.3	< LOD – < LOQ	< LOD – < LOQ

LOQ = Limit of Quantitation = 1 µg/L in guttation liquid samples (all analytes)
LOD = Limit of Detection = 0.3 µg/L in guttation liquid samples (all analytes)

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in pollen from Winter oil seed rape (OSR), Phacelia and Maize and in nectar from Winter oil seed rape (OSR) and Phacelia

Sample material	Residue of imidacloprid [µg/kg]	Residue of imidacloprid-5-hydroxy [µg/kg]	Residue of imidacloprid-olefine [µg/kg]
Pollen (OSR)	< LOQ	< LOD	< LOD
Pollen (Phacelia)*	< LOQ – 1.5	< LOD	< LOD
Pollen (Maize)*	< LOD – 2.5	< LOD	< LOD
Nectar (OSR)	< LOQ – 0.3	< LOD	< LOD
Nectar (Phacelia)	< LOD – 0.4	< LOD	< LOD

*: 8 out of 9 samples < LOQ

LOQ = Limit of Quantitation = 0.6 µg/L in pollen samples for imidacloprid, 1 µg/L for imidaclprid-5-hydroxy and imidacloprid-olefin

LOD = Limit of Detection = 0.2 µg/L in pollen samples for imidacloprid, 0.3 µg/L for imidaclprid-5-hydroxy and imidacloprid-olefin

LOQ = Limit of Quantitation = 0.3 µg/L in nectar samples for imidacloprid, 1 µg/L for imidaclprid-5-hydroxy and imidacloprid-olefin

LOD = Limit of Detection = 0.1 µg/L in nectar samples for imidacloprid, 0.3 µg/L for imidaclprid-5-hydroxy and imidacloprid-olefin

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Conclusion:

The study was conducted on a field site near Auxy, (Meung-sur Loire, F-45130, France) with a known history of crops and imidacloprid uses as such with natural aged soil-residues of this active substance. Therefore, this study provides realistic field data on residue levels of imidacloprid within bee relevant matrices, collected from non-imidacloprid treated flowering winter oilseed rape, Phacelia and maize plants cultivated as succeeding crops from a field with natural aged soil-residues of imidacloprid.

Winter Oilseed Rape:
Soil cores used for residue analysis were taken from the entire field prior to placement of the honeybee colonies into the tunnels. The residue level of imidacloprid in the field was 43 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of winter oilseed rape, revealed generally low residue levels.
The residue levels of imidacloprid in pollen was always below the LOQ (<0.6 µg a.s./kg).
The residue levels of imidacloprid in nectar ranged from below the LOQ (<0.3 µg a.s./kg) to the LOQ (<0.3 µg a.s./kg).

Maize:
One set of soil samples were taken from the maize sub plots during the trial. The residue levels of imidacloprid in soils ranged from 35 µg a.s./kg to 48 µg a.s./kg dry soil during guttation.
Residues analysis of guttation fluid, collected directly after emergence until early bloom of the maize plants, revealed generally low residue levels.
The residue levels of imidacloprid in guttation fluid ranged from below the LOD (<0.3 µg a.s.) to 1.3 µg a.s./L and are thus several orders of magnitude below values measured in droplets from seed treated maize plants.
The residue levels of imidacloprid in pollen, as sampled at three time points during bloom of the maize plants ranged from below the LOD (<0.2 µg a.s./kg) to 2.5 µg a.s./kg). 8 of 9 samples contained residues < LOQ.

Phacelia:
Soil cores used for residue analysis were taken from the entire field prior to placement of the honeybee colonies into the tunnels. The residue level of imidacloprid in the phacelia plot was 46 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of Phacelia, revealed generally low residue levels.
The residue levels of imidacloprid in pollen ranged from below the LOQ (<0.6 µg a.s./kg) to 1.5 a.s./kg. 8 of 9 samples contained residues < LOQ.
The residue levels of imidacloprid in nectar ranged from below the LOD (<0.1 µg a.s./kg) to 0.4 a.s./kg

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Report: 02.02.02/06; [redacted]; 2014; M-504836-01-3
Title: Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with Phacelia and maize in northern France
Report No.: 7SRFR13C2B
Document No.: M-504836-01-3
Guideline(s): US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-504836-01-3@S-602373-01-1

Study phases

Phase	Start date	End date
Field phase	06/05/2014	20/07/2014
Maize Guttation	06/05/2014	16/06/2014
Phacelia flowering	30/06/2014	17/07/2014
Maize flowering	18/07/2014	20/07/2014

Sampling periods

Sample type	Sub plot	Sample date(s)
Soil for characterisation	Maize 1 - 3	07/07/2014
Soil for residue analysis	Maize 1 - 3	07/07/2014
Soil for characterisation	Phacelia	01/07/2014
Soil for residue analysis	Phacelia	01/07/2014
Guttation	Maize 1 - 3	06/05/2014 - 15/06/2014
Maize pollen	Maize 1 - 3	18/07/2014 19/07/2014 20/07/2014
Phacelia pollen	Tunnels 1 -3	01/07/2014 08/07/2014 17/07/2014
Phacelia nectar	Tunnels 1 -3	01/07/2014 08/07/2014 17/07/2014

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Executive summary:

Objective:
The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen, nectar and guttation fluid) collected from flowering rotational crops cultivated as succeeding crops grown in France on fields with a history of imidacloprid use and as such with natural aged soil-residues of this active ingredient.

Study Site:
The study was conducted on a field site near Auxy (F-45340, France) with a known history of Imidacloprid use and such with a likelihood of natural aged soil residues of this active substance. An approximately one hectare plot located within the dimension of the agricultural land was marked out, and divided into two evenly sized sub-plots. One sub-plot was sown with maize (Zea mays) the other sub-plot was sown with Phacelia (Phacelia tanacetifolia).

Material and Methods:

Crops were sown according to Good Agricultural Practice (GAP).
The maize and phacelia plots were sown using calibrated equipment (tractor and seed drill). The target sowing rates were 10 kg seeds/ ha for Phacelia and 100,000 kernel/ ha for maize.
The sub plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient numbers of plants available for both guttation fluid and for maize pollen sampling.
Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the phacelia plot after successful germination. A single honeybee colony was placed into each tunnel at the start of Phacelia flowering

Soil sampling:
From each of the maize sub plots and from the phacelia sowing area, two different types of soil sample were collected. These samples were used for;
1. Soil characterisation of the upper 10 cm soil layer.
2. Determination of the residues of parent imidacloprid and its metabolites in the upper 15 cm soil layer.

Soil cores used for characterisation and residue analysis were collected from each of the three segregated maize sub plots, during the guttation sampling phase of the trial and from inside of the Phacelia sowing area prior to placement of the honeybee colonies into the tunnels.

Sampling of Nectar and Pollen from Phacelia Crops:
Nectar and pollen sampling was conducted at three different time points during bloom of the Phacelia crop. Once the Phacelia started to bloom, Honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering Phacelia under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid

Sampling of Guttation fluid and Pollen from Maize:
Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand.
Sampling of guttation fluid was carried out on a regular basis over a 42-day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 11-12) until flowering (BBCH scale 65).

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Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise.
The sampling period at each time point was approximately 30 minutes to ensure an equivalent time chronology every day.
Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3.
When guttation was present it was collected from >10 plants throughout each of the sub plots. The target volume for each sample was 1 ml of guttation fluid.
Pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67).
At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature overnight.
Next day, the pollen was shaken out and cleaned with two analytical sieves (mesh size 2 mm and 1 mm), to ensure a pure pollen sample. Maize pollen in the base pan was cleaned from plant or insect debris remaining in the pollen sample by hand using forceps or a fine paint brush.
Pollen samples during bloom as well as collected guttation fluid were analysed for residues of imidacloprid.

Residue analysis:
All samples (soil samples, pollen, nectar and guttation fluid) were analysed for their content of imidacloprid and and its metabolites 5-hydroxy and olefin by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.
Analysis of the soil samples followed the provisions of method 00790/M001. Analysis of nectar and pollen followed the provisions of method 01433 and for the analysis of guttation liquid the analytical method 00537/M002 was used, which is based on the method 01433.
The Limit of Quantitation (LOQ) of imidacloprid, defined as the lowest validated fortification level, was 5.0 µg/kg for soil. The corresponding Limit of Detection (LOD) was 2 µg/kg.
The LOQ levels for imidacloprid were 0.6 µg/kg (0.0006 mg/kg) for pollen, 0.3 µg/kg for nectar and 1.0 µg/kg for guttation liquid while the LOQ level of the metabolites were constant 1.0 µg/kg for all sample materials. The corresponding Limit of Detections (LOD) were 0.2 µg/kg for pollen, 0.1 µg/kg for nectar and 0.3 µg/kg for guttation liquid, respectively for imidacloprid and 0.3 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine for all sample materials.
All results of the method validations were in accordance with the general requirements for residue analytical methods; therefore, the employed method was validated successfully. The average recoveries were within the acceptable range of 60 – 120%. RSD values are below 20%. A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar samples are provided in the following tables:

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Residues of imidacloprid in soil

Sample material	Crop	Residue imidacloprid * [µg/kg dry soil]
Soil	Maize	41 - 59
Soil	Phacelia	52

LOQ = Limit of Quantitation = 5 µg/kg on/from soil samples (all analytes)
LOD = Limit of Detection = 2 µg/kg on/from soil samples (all analytes)

* Unrounded Residue Imidacloprid [µg/kg] /(1-(Moisture/100)

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in Maize guttation liquid samples

Sample material	Residue of imidacloprid [µg/L]	Residue of imidacloprid-5-hydroxy [µg/L]	Residue of imidacloprid-olefine [µg/L]
Guttation liquid (Maize)	< LOD – 4.1	< LOD – < LOQ	< LOQ

LOQ = Limit of Quantitation = 1 µg/L in guttation liquid samples (all analytes)
LOD = Limit of Detection = 0.3 µg/L in guttation liquid samples (all analytes)

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in pollen from *Phacelia* and Maize and nectar from *Phacelia*

Sample material	Residue of imidacloprid [µg/kg]	Residue of imidacloprid-5-hydroxy [µg/kg]	Residue of imidacloprid-olefine [µg/kg]
Pollen (Phacelia)*	< LOQ – 1.2	< LOD	< LOD
Pollen (Maize)	0.94 – 0.91	< LOD	< LOD
Nectar (Phacelia)	< LOQ – 0.4	< LOD	< LOD

*: 8 out 9 samples <LOQ

LOQ = Limit of Quantitation = 0.3 µg/kg in nectar and 0.6 µg/kg in pollen samples for imidacloprid and 1 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine in all sample materials.

LOD = Limit of Detection = 0.1 µg/kg in nectar and 0.2 µg/kg in pollen samples for imidacloprid and 0.3 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine in all sample materials.

Conclusion:

The study was conducted on a field site near Auxy, (F-45340, France) with a known history of crops and imidacloprid uses as such with natural aged soil-residues of this active substance. Therefore, this study provides realistic field data on residue levels of imidacloprid within bee relevant matrices, collected from non-imidacloprid treated flowering Phacelia and maize plants cultivated as succeeding crops from a field with natural aged soil-residues of imidacloprid.

Maize:

One set of soil samples were taken from the maize sub plots during the trial. The residue levels of imidacloprid in soils ranged from 41 µg a.s./kg to 59 µg a.s./kg dry soil during guttation.

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Residues analysis of guttation fluid, collected directly after emergence until early bloom of the maize plants, revealed generally low residue levels.
The residue levels of imidacloprid in guttation fluid ranged from below the LOD (<0.3 µg a.s.) to 4.1 µg a.s./L and are thus several orders of magnitude below values measured in droplets from seed treated maize plants.
The residue levels of imidacloprid in pollen, as sampled at three time points during bloom of the maize plants ranged from 0.64 µg a.s./kg to 0.91 µg a.s./kg.

Phacelia:
Soil cores used for residue analysis were taken from the entire field prior to placement of the honeybee colonies into the tunnels. The residue level of imidacloprid in the phacelia plot was 52 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of Phacelia, revealed generally low residue levels.
The residue levels of imidacloprid in pollen ranged from below the LOQ (<0.6 µg a.s./kg) to 1.2 µg a.s./kg. Residues in 8 out of 9 samples were <LOQ.
The residue levels of imidacloprid in nectar ranged from below the LOQ (<0.3 µg a.s./kg) to 0.4 a.s./kg.
>>M-504836-01-3@S-602373-01-1

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Report: 02.02.02/07; [redacted]; 2014; M-504810-01-3
Title: Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with winter oil seed rape in northern France
Report No.: 7SRFR13C2C
Document No.: M-504810-01-3
Guideline(s): US EPA OCSPP Guideline Number 850.SUPP
Guideline deviation(s): none
GLP/GEP: **yes**

<<M-504810-01-3@S-602369-01-1

Study phases

Phase	Start date	End date
Field phase	09/04/2014	18/04/2014
Winter oil seed rape flowering	09/04/2014	18/04/2014

Sampling periods

Sample type	Sub plot	Sample date(s)
Soil for characterisation	W-OSR	10/04/2014
Soil for residue analysis	W-OSR	10/04/2014
W-OSR pollen	Tunnels 1 -3	10/04/2014 15/04/2014 18/07/2014
W-OSR nectar	Tunnels 1 -3	10/04/2014 15/04/2014 18/07/2014

Executive summary:

Objective:
The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen and nectar) collected from flowering rotational crops cultivated as succeeding crops

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grown in France on fields with a history of imidacloprid use and as such with natural aged soil residues of this active ingredient.

Study Site:
The study was conducted on a field site near Ribeaucourt (F-55290, France) with a known history of Imidacloprid use and such with a likelihood of natural aged soil residues of this active substance. On this land, non imidacloprid treated Winter oil seed (Brassica napus) has been cultivated in 2013. During bloom on 2014, in total, three tunnels were setup for Winter oil seed with one bee hive per tunnel. Samples of pollen loads (collected with pollen traps) and forager honey bees (for subsequent extraction of nectar from honey stomach) were taken.

Material and Method:

Winter oil seed rape was sown according to Good Agricultural Practice (GAP).
Winter oil seed rape has been sown by the cooperating farmer. Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the winter oil seed rape plot prior to bloom. A single honeybee colony was placed into each tunnel at the start of winter oilseed rape flowering

Soil sampling:
From the winter oil seed rape, two different types of soil sample were collected. These samples were used for:
1. Soil characterisation of the upper 10 cm soil layer.
2. Determination of the residues of parent imidacloprid and its metabolites in the upper 15 cm soil layer.

Soil cores used for characterisation and residue analysis were collected from inside of the winter oil seed sowing area prior to placement of the honeybee colonies into the tunnels.

Sampling of Nectar and Pollen from Winter Oilseed Rape:
Nectar and pollen sampling was conducted at three different time points during bloom of the oilseed crop. Once the winter oilseed started to bloom, Honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering winter oilseed under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid

Residue analysis:
All samples (soil samples, pollen and nectar) were analysed for their content of imidacloprid and and its metabolites 5-hydroxy and olefin by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection. Analysis of the soil samples followed the provisions of method 00790/M001. Analysis of nectar and pollen followed the provisions of method 01433.
The Limit of Quantification (LOQ) of imidacloprid, defined as the lowest validated fortification level, was 5.0 µg/kg for soil. The corresponding Limit of Detection (LOD) was 2 µg/kg. The LOQ levels for imidacloprid were 0.6 µg/kg for pollen and 0.3 µg/kg for nectar. The LOQ levels of the metabolites were constant 1.0 µg/kg for nectar and pollen. The corresponding Limits of Detections (LOD) were 0.2 µg/kg for pollen and 0.1 µg/kg for nectar, respectively for imidacloprid and 0.3 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid olefine for both sample materials.
All results of the method validations were in accordance with the general requirements for residue analytical methods; therefore, the employed method was validated successfully. The average recoveries were within the acceptable range of 60 – 120%. RSD values are below 20%. A summary of the analytical results as obtained by analyzing samples of soil, pollen and nectar is provided in the following tables:

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Residues of imidacloprid in soil samples

Sample material	Crop	Residue Imidacloprid* [µg/kg dry soil]**
Soil	Winter oil seed rape	45

LOQ = Limit of Quantitation = 5 µg/kg for imidacloprid in/on soil samples
LOD = Limit of Detection = 2 µg/kg for imidacloprid in/on soil samples

* Residue imidacloprid [µg/kg] /(1-(Moisture/100); For the calculation of imidacloprid residues related to dry soil, unrounded values were used. Therefore minor deviations may occur when rounded values shown within this table are used.
** The given residue values and corresponding residue values related to dry soil are mean values of two individually extracted samples to assure maximal homogeneity.

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid olefine in oil seed rape nectar and pollen samples

Sample Material	Residue Imidacloprid [µg/kg]	Residue Imidacloprid-5-hydroxy [µg/kg]	Residue Imidacloprid olefine [µg/kg]
Nectar (oil seed rape)	< LOD – 0.4	<LOD	<LOD
Pollen (oil seed rape)	LOQ – 1.3	<LOD	<LOD

LOQ = Limit of Quantitation = 0.3 µg/kg imidacloprid in nectar samples and 0.6 µg/kg in pollen samples, 1 µg/kg for imidaclprid-5-hydroxy and imidacloprid olefine in nectar samples
LOD = Limit of Detection = 0.1 µg/kg for imidacloprid in nectar samples and 0.2 µg/kg in/on pollen samples, 0.3 µg/kg for imidaclprid-5-hydroxy and imidacloprid olefine in nectar samples.

Conclusion:

The study was conducted on a field site near Ribeaucourt (F-59290, France) with a known history of crops and imidacloprid uses as such with natural aged soil-residues of this active substance. Therefore, this study provides realistic field data on residue levels of imidacloprid within bee relevant matrices, collected from non-imidacloprid treated flowering winter oilseed rape cultivated as succeeding crops from a field with natural aged soil-residues of imidacloprid.

Winter Oilseed Rape:
Soil cores used for residue analysis were taken from the entire field prior to placement of the honeybee colonies into the tunnels. The residue level of imidacloprid in the Winter Oilseed Rape plot was 45 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of winter oilseed rape, revealed generally low residue levels.
The residue levels of imidacloprid in pollen ranged from below the LOQ (<0.6 µg a.s./kg) to 1.3 µg a.s./kg.
The residue levels of imidacloprid in nectar ranged from below the LOQ (<0.3 µg a.s./kg) to 0.7 µg a.s./kg.

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Report:	02.02.02/08; [redacted]; 2014; M-504842-01-3
Title:	Determination of the residues of imidacloprid in bee relevant matrices collected from succeeding crops following application of imidacloprid FS 600E G via soil incorporation to plateau concentration and sowing of imidacloprid-treated winter barley seeds. Field phase conducted in southern France
Report No.:	7SRFR13C3
Document No.:	M-504842-01-3
Guideline(s):	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-504842-01-3@S-602377-01-1

Aim: determination of the amount of residues which may be taken up and translocated into bee-relevant matrices (nectar, pollen) and to guttation fluid of succeeding crops after several years of use resembling a worst case scenario under agronomical practices.

Objective:
The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen, nectar and guttation fluid) collected from succeeding crops following application of IMIDACLOPRID FS 600E G via soil incorporation to plateau concentration and sowing of imidacloprid-treated winter barley seeds.

Study Site:
The study was conducted on a field site near Nîmes (F-30000, France). An approximately two hectare field located on the field site was marked out, and divided into two evenly sized plots. Three crops were cultivated on both plots of the Study Field: phacelia (*Phacelia tanacetifolia*), mustard (*Sinapis arvensis*) and maize (*Zea mays*) (each in an area of approx. 0.2 ha).

Material and Methods:

Test item and application:
The test item **imidacloprid** was applied in autumn 2013 with two different calculated plateau concentrations directly to bare soil. After incorporation of the calculated plateau concentrations, dressed winter barley seeds (again with two different seed dressing rates) were sown (see overview below):

	Application of the plateau concentration * (25.09.2013)	Sowing of treated winter barley seeds * (10.10.2013)
Low plateau concentration + low seed dressing rate (variant blue)	97.3 g a.s./ha 0.144 L product/ha	85.8 g a.s./ha 184.5 kg seeds/ha
High plateau concentration + high seed dressing rate (variant green)	154.0 g a.s./ha 0.254 L product/ha	118.5 g a.s./ha 189.5 kg seeds/ha

*Actual concentrations

In 2014, Winter barley crops were removed and untreated succeeding crops (Mustard, Phacelia and Maize) were sown on the areas withprevious imidacloprid applications.
Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the phacelia and the mustard plot after successful germination. A single honeybee colony was placed into each tunnel at the start of Phacelia, respectively mustard flowering
The sub plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient numbers of plants available for both guttation fluid and for maize pollen sampling

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Soil sampling:
From each of the maize sub plots and from the phacelia and mustard sowing areas, two different types of soil sample were collected. These samples were used for;
1. Soil characterisation of the upper 10 cm soil layer.
2. Determination of the residues of parent imidacloprid and its metabolites in the upper 15 cm soil layer.
Soil cores used for characterisation and residue analysis were collected from each of the three segregated maize sub plots, during the guttation sampling phase of the trial and from inside of the Phacelia or mustard sowing area prior to placement of the honeybee colonies into the tunnels.

Sampling of Nectar and Pollen from Phacelia or Mustard Crops:
Nectar and pollen sampling was conducted at three different time points during bloom of the corresponding crop. Once the crop started to bloom, Honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering Phacelia or Mustard under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid

Sampling of Guttation fluid and Pollen from Maize:
Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand.
Sampling of guttation fluid was carried out on a regular basis over a XYZ day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 11-12) until flowering (BBCH scale 65). Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period at each time point was approximately 30 minutes to ensure an equivalent time chronology every day
Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3.
When guttation was present it was collected from >10 plants throughout each of the sub plots. The target volume for each sample was 1 ml of guttation fluid.
Pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67).
At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature overnight.
Next day, the pollen was shaken out and cleaned with two analytical sieves (mesh size 2 mm and 1 mm), to ensure a pure pollen sample. Maize pollen in the base pan was cleaned from plant or insect debris remaining in the pollen sample by hand using forceps or a fine paint brush.
Pollen samples during bloom as well as collected guttation fluid were analysed for residues of imidacloprid.

Residue analysis:
Residue analysis of imidacloprid in soil samples and samples of guttation liquid, nectar and pollen was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection. Analysis of the soil samples followed the provisions of method 00790/M001. Analysis of guttation liquid mainly followed the provisions of method 00587/M002, except for the fact that an extraction was not necessary while guttation liquids are mainly consisting of water. Guttation liquid samples were only diluted prior to analysis. Analysis of nectar and pollen followed the provisions of method 01433.
The Limit of Quantification (LOQ) of imidacloprid, defined as the lowest validated fortification level, was 5.0 µg/kg for soil. The corresponding Limit of Detection (LOD) was 2 µg/kg. The LOQ levels for imidacloprid was 0.6 µg/kg for pollen, 0.3 µg/kg for nectar and 1.0 µg/L for guttation liquid while the

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LOQ level of the metabolites were constant 1.0 µg/kg for all sample materials. The corresponding Limit of Detections (LOD) were 0.2 µg/kg for pollen, 0.1 µg/kg for nectar and 0.3 µg/kg for guttation liquid, respectively for imidacloprid and 0.3 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine for all sample materials.
All results of the method validations were in accordance with the general requirements for residue analytical methods; therefore, the employed method was validated successfully. The average recoveries were within the acceptable range of 60 – 120%. RSD values are below 20%. A summary of the analytical results as obtained by analyzing samples of soil, guttation liquid, pollen and nectar is provided in the following tables:

Residues of imidacloprid in soil (green and blue plots)

Sample material	Variant	Residue Imidacloprid [µg/kg]** during bloom	Moisture [%]	Residue Imidacloprid * [µg/kg dry soil]* * during bloom
Soil	green plot ("high")	28 - 72	10.60 - 17.33	35 - 93
Soil	blue plot ("low")	12 - 62	10.53 - 17.55	34 - 82

LOQ = Limit of Quantitation = 5 µg/kg for imidacloprid in/on soil samples
LOD = Limit of Detection = 2 µg/kg for imidacloprid in/on soil samples

* Residue imidacloprid [µg/kg] / (1-(Moisture/100)). For the calculation of imidacloprid residues related to dry soil, unrounded values were used. Therefore minor deviations may occur when rounded values shown within this table are used.
** The given residue values and corresponding residue values related to dry soil are mean values of two individually extracted samples to assure maximal homogeneity.

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in guttation liquid samples (green and blue plots)

Sample Material	Variant	Residue Imidacloprid [µg/kg]	Residue Imidacloprid-5-hydroxy [µg/kg]	Residue Imidacloprid-olefine [µg/kg]
Guttation liquid (Maize)	green plot ("high")	<LOQ - 44	<LOD - 12	<LOQ - 2
Guttation liquid (Maize)	blue plot ("low")	<LOQ - 88	<LOD - 9	<LOQ - 2

LOQ = Limit of Quantitation = 1 µg/L for guttation liquid samples (all analytes)
LOD = Limit of Detection = 0.3 µg/L for guttation liquid samples (all analytes)

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Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in Mustard nectar samples (green and blue plots)

Sample material	Variant	Residue Imidacloprid [µg/kg]	Residue Imidacloprid-5-hydroxy [µg/kg]	Residue Imidacloprid-olefine [µg/kg]
Nectar (Mustard)	green plot (“high”)	<LOQ - 0.5	<LOD	<LOD
Nectar (Phacelia)		0.8 – 1.0	<LOD	<LOD
Nectar (Mustard)	blue plot (“low”)	0.7 - 3.9	<LOD - <LOQ	<LOD - <LOQ
Nectar (Phacelia)		<LOD – <LOQ	<LOD	<LOD

LOQ = Limit of Quantitation = 0.3 µg/kg imidacloprid in nectar samples, 1 µg/kg for imidaclprid-5-hydroxy and imidacloprid-olefine in nectar samples

LOD = Limit of Detection = 0.1 µg/kg for imidacloprid in nectar samples, 0.3 µg/kg for imidaclprid-5-hydroxy and imidacloprid-olefine in nectar samples

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in pollen samples (green and blue plots)

Sample material	Variant	Residue Imidacloprid [µg/kg]	Residue Imidacloprid-5-hydroxy [µg/kg]	Residue Imidacloprid-olefine [µg/kg]
Pollen (Mustard)	green plot (“high”)	1.6 - 4.7	<LOD - <LOQ	<LOQ - 1.2
Pollen (Maize)		<LOQ - 0.93	<LOD	<LOD - <LOQ
Pollen (Phacelia)		1.9 – 2.0	<LOD	<LOD
Pollen (Mustard)	blue plot (“low”)	1.8 - 5.1	<LOD - <LOQ	<LOQ - 1.2
Pollen (Maize)		<LOQ - 1.2	<LOD - <LOQ	<LOD
Pollen (Phacelia)		<LOQ - 0.6	<LOD	<LOD

LOQ = Limit of Quantitation = 0.6 µg/kg imidacloprid in/on pollen samples, 1 µg/kg for imidaclprid-5-hydroxy and imidacloprid-olefine in/on pollen samples

LOD = Limit of Detection = 0.2 µg/kg for imidacloprid in/on pollen samples, 0.3 µg/kg for imidaclprid-5-hydroxy and imidacloprid-olefine in/on pollen samples

Conclusion:

The study has been performed to cover various scenarios (crop rotations) of a consecutive use of Imidacloprid and to determine the potential residue level of Imidacloprid and its metabolites -5-hydroxy and –olefine in bee-relevant matrices (nectar and pollen) and guttation droplets of succeeding crops. In a model approach, two levels of Imidacloprid plateau concentrations were established (information about the rates to be applied were provided by the sponsor) on an agricultural site near Nîmes (F-30000, France). After incorporation of the calculated plateau concentrations in September 2013, dressed winter barley seeds (again with two different seed dressing rates) were sown.

Phacelia:
Residues analysis of pollen and nectar, as collected at one time during blooming of Phacelia, in three tunnels per test rate revealed in low residue levels. The residue levels of imidacloprid in nectar ranged from below the LOQ (< 0.3 µg a.s./kg) to 1.0 µg a.s./kg. Residue levels of imidacloprid in pollen ranged from below the LOQ (< 0.6 µg a.s./kg) to 2.0 µg a.s./kg.

Mustard:
Residues analysis of pollen and nectar, as collected at three time points during blooming of mustard in three tunnels per test rate revealed in low residue levels. The residue levels of imidacloprid in nectar

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ranged from below the LOQ (< 0.3 µg a.s./kg) to 3.9 µg a.s./kg. Residue levels of imidacloprid in pollen ranged from 1.6 µg a.s./kg to 5.1 µg a.s./kg.

Maize:
Residues analysis of guttation fluid, as collected from directly after emergence until early bloom of the Maize plants, revealed in generally low residues. The residue levels of imidacloprid in guttation fluid ranged from below the LOQ (< 1 µg a.s./L) to 88 µg a.s./L and are thus several orders of magnitude below values measured in droplets from neonicotinoid seed treated maize plants. The maximum residue level of imidacloprid in pollen, as sampled at three time points during bloom on three subplots ranged from below the LOQ (< 0.6 µg a.s./kg) to 1.2 µg a.s./kg.

Overall, transfer of Imidacloprid soil residues into bee-relevant matrices and guttation droplets of succeeding crops takes place on very low levels even if calculated long-term plateau concentrations are established without ageing of residues over years. Traces of Imidacloprid metabolites were only measured in single guttation or pollen samples
>>M-504842-01-3@S-602377-01-1

Report:	02.02.02/09; [redacted]; 2014; M-504854-01-3
Title:	Residues of imidacloprid in nectar and pollen of flowering rotational crops in Western Germany
Report No.:	P13068-2
Document No.:	M-504854-01-3
Guideline(s):	Regulation (EC) No 1107/2009
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-504854-01-3@S-602379-01-1

Aim
Determination of the amount of residues which may be taken up and translocated into bee-relevant matrices (nectar, pollen) and to guttation fluid of succeeding crops after several years of use resembling a worst case scenario under agronomical practices.
Objectives

- to determine residues of imidacloprid and its metabolites 5-hydroxy and olefine in nectar and pollen of flowering rotational crops (phacelia and mustard) after incorporation of imidacloprid long-term plateau soil concentrations and growing of imidacloprid seed-dressed winter barley
- to determine residues of imidacloprid and its metabolites 5-hydroxy and olefine in guttation fluid and pollen of maize plants after incorporation of imidacloprid long-term plateau soil concentrations and growing of imidacloprid seed-dressed winter barley

Study Site
The study was conducted in the vicinity of Zuelpich, North Rhine-Westphalia in Germany. Two areas of approximately 1 ha each, were established on the Study Field.
Three crops were cultivated on both variants of the Study Field: phacelia (Phacelia tanacetifolia), mustard (Sinapis arvensis) (each in an area of approx. 0.2 ha) and maize (Zea mays) (each in an area of approx. 0.1 ha).

Material and Methods
Test item and application: The test item imidacloprid was applied in two applications in autumn 2013:

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	Imidacloprid Application of the Plateau Concentration* 26.09.2013	Imidacloprid Sowing of treated winter barley seeds* 09.10.2013
Low plateau concentration + low seed dressing rate (Variant blue)	95.4 g a.s./ha 0.157 L product/ha	63.2 g a.s./ha 136 kg seeds/ha (with 46.5 g a.s./dt)
High plateau concentration + high seed dressing rate (Variant green)	173.4 g a.s./ha 0.286 L product/ha	126.3 g a.s./ha 202 kg seeds/ha (with 62.5 g a.s./dt)

*Actual concentrations

In spring 2014, untreated phacelia, mustard and maize were sown on the study plots which contained soil residues from the previous Imidacloprid applications. During flowering, nectar and pollen of phacelia and mustard were sampled by honeybees in tunnels. Maize pollen was sampled manually; the same applies to guttation droplets between maize emergence and flowering. The following ranges of Imidacloprid residues were determined:

Nectar & Pollen sampling: Honeybee colonies were placed into mesh covered tunnels erected over phacelia and mustard crops a few days prior expected bloom. Honeybees were exposed to the flowering phacelia and mustard under confined conditions and were exclusively used as a sampling device for both nectar and pollen at three times (in a period of approx. 10 days) during flowering of the respective crop.

Nectar was collected by honey bulb extraction from forager bees in mustard and phacelia crop. For each nectar sample about 800-1000 returning forager bees were collected with a modified vacuum sampler, deep-frozen and transported to the laboratory for nectar extraction. Targeted nectar amount per sample was ≥ 500 mg.

Pollen of phacelia and mustard was collected from forager bees via pollen traps attached to the bee hive entrance. The collected pollen was stored deep-frozen until residue analysis. The target sample size per tunnel and per sampling date was approximately 1.5 g pollen with a minimum requirement of approximately 750 mg.

Maize pollen was collected three times during flowering of maize plants (BBCH 63-65). The pollen, targeted were 1.5 g per sample, collected from at least 30 plants, was shaken out of the flowers into paper bags and cleaned by sieving (mesh size 2 mm and 1 mm).
Maize guttation fluid, target 1 ml per sample, was collected daily starting at emergence of the seedlings (BBCH 11) until early flowering (BBCH 55). The samplings started at sunrise (± 15 min) lasted for a maximum of 30 min.

Residue analysis:
All samples (pollen, nectar and guttation fluid) were analysed for their content of imidacloprid and its metabolites 5-hydroxy and olefine via HPLC-MS/MS. Residues are reported in terms of µg active substance/kg for pollen, nectar and soil respectively µg/L for guttation fluid.

Results and Discussion

Imidacloprid residues – Measured range for soil and bee relevant components

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Matrix	Crop	Imidacloprid	
		Variant blue (after application 95.4 g a.s./ha plateau + 63.2 g a.s./ha treated seeds)	Variant green (after application 273.4 g a.s./ha plateau + 126.3 g a.s./ha treated seeds)
Soil* [µg/kg]	2013 PEC plateau	71	140
	2014 Phacelia	9-13	16-29
	2014 Mustard	12-18	14-19
	2014 Maize	9-13	16-22
Pollen [µg/kg]	Phacelia	< LOD - < LOQ	< LOD – 0.62
	Mustard	< LOQ – 2.0	< LOQ
	Maize	< LOD	< LOD
Nectar [µg/kg]	Phacelia	< LOQ – 0.43	< LOD – 0.49
	Mustard	< LOD – 0.57	< LOD – 0.63
Guttation [µg/L]	Maize	< LOD – 13	< LOD – 26

*** calculated to dry soil**

LOQ = Limit of Quantification = 5 µg a.s./kg for soil, 0.6 µg a.s./kg for pollen, 0.3 µg a.s./kg for nectar and 1 µg a.s./L for guttation liquid samples for imidacloprid.

LOD = Limit of Detection = 2 µg a.s./kg for soil, 0.2 µg a.s./kg for pollen, 0.1 µg a.s./kg for nectar and 0.3 µg a.s./L for guttation liquid samples for imidacloprid.

Imidacloprid metabolites residues - Measured range for bee relevant components

Matrix	Crop	Imidacloprid-5-Hydroxy		Imidacloprid-olefine	
		Variant blue	Variant green	Variant blue	Variant green
Pollen [µg/kg]	Phacelia	< LOD - < LOQ	< LOD - < LOQ	< LOD	< LOD
	Mustard	< LOD	< LOD	< LOD - < LOQ	< LOD
	Maize	< LOD	< LOD	< LOD	< LOD
Nectar [µg/kg]	Phacelia	< LOD	< LOD	< LOD	< LOD
	Mustard	< LOD	< LOD	< LOD	< LOD
Guttation [µg/L]	Maize	< LOD – 2	< LOD – 11	< LOD - < LOQ	< LOD – 2

LOQ = Limit of Quantification = For the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine 1 µg a.s./kg for all matrices.

LOD = Limit of Detection = For the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine 0.3 µg a.s./kg for all matrices.

Conclusion

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The study has been performed to cover various scenarios (crop rotations) of a consecutive use of Imidaloprid and to determine the potential residue level of Imidacloprid and its metabolites -5-hydroxy and –olefine in bee-relevant matrices (nectar and pollen) and guttation droplets of succeeding crops. In a model approach, two levels of Imidacloprid plateau concentrations were established (information about the rates to be applied were provided by the sponsor) on an agricultural site near Zuelpich, Germany. After incorporation of the calculated plateau concentrations in September 2013, dressed winter barley seeds (again with two different seed dressing rates) were sown (see overview below)

Phacelia:
Residues analysis of pollen and nectar, as collected at three time points during blooming of phacelia, in three tunnels per test rate revealed in low residue levels. The residue levels of imidacloprid in nectar ranged from below the LOD (< 0.1 µg a.s./kg) to 0.49 µg a.s./kg. Residue levels of imidacloprid in pollen ranged between from below LOD (< 0.2 µg a.s./kg) to 0.62 µg a.s./kg.

Mustard:
Residues analysis of pollen and nectar, as collected at three time points during blooming of mustard in three tunnels per test rate revealed in low residue levels. The residue levels of imidacloprid in nectar ranged from below LOD (< 0.1 µg a.s./L) to 0.63 µg a.s./L. Residue levels of imidacloprid in pollen ranged between from below LOQ of <0.6 µg a.s./kg to 1 µg a.s./kg.

Maize:
Residues analysis of guttation fluid, as collected from directly after emergence until early bloom of the maize plants, revealed in generally low residues. The residue levels of imidacloprid in guttation fluid ranged from below the LOD (< 1 µg a.s./L) to 26 µg a.s./L and are thus several orders of magnitude below values measured in droplets from seed treated maize plants. Residues were primarily detected at the earliest samplings after emergence and declined over time to < LOD.
The maximum residue level of imidacloprid in pollen, as sampled at three time points during bloom on three subplots was always below the LOD (< 0.2 µg a.s./kg).
Overall, transfer of Imidacloprid soil residues into bee-relevant matrices and guttation droplets of succeeding crops takes place on very low levels even if calculated long-term plateau concentrations are established without ageing of residues over years. Traces of Imidacloprid metabolites were only measured in single guttation samples.
>>M-504854-01-1 / S-602378-01-1

Report: 02.02.02/10; [redacted] 2014; M-503458-01-3
Title: Calculation of plateau concentrations in soil for imidacloprid and clothianidin
Report No.: EnSa-14-1318
Document No.: M-503458-00-3
Guideline(s): US EPA OCSPP Guideline Number 850.SUPP
Guideline deviation(s):
GLP/GEP: **no**

Plateau concentrations in soil were calculated for the actives imidacloprid and clothianidin to assess the contribution of preceding applications of these actives to the exposure in soil. For this purpose a conservative assessment scheme was used which was recently presented by EFSA in EFSA (2010) and EFSA (2012) – in the following abbreviated as “EFSA approach”. The plateau concentrations were used to determine the application rates of the two actives which are necessary to establish these plateau concentrations at the test sites Zülpich and Nimes.

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02.02.03 - Weeds

Report:	02.02.03/01; [redacted]; 2014; M-505126-01-3
Title:	Statement - Evaluation of the occurrence of flowering weeds in agricultural crops: Cereals, sugar beet and potatoes
Report No.:	M-505126-01-3
Document No.:	M-505126-01-3
Guideline(s):	US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s):	--
GLP/GEP:	**no**

The potential uptake of neonicotinoids pesticides into flowering weeds, as route of exposure of bees, has been identified as a data gap. Due to the variation in weed species (growth, habit, flowering period), the small amounts of pollen and nectar produced by many weedy species and different growing conditions and crops, this would be very difficult to measure experimentally as no standardized methods are available.

The European Food Safety Authority (EFSA) bee risk assessment scheme requires a first tier assessment through various exposure scenarios (EFSA, 2013[1]). To date this document has not been adopted as the official European guidance and remains the guidance of EFSA. One exposure route cited in the document is through foraging on attractive weeds within the treated field. This scenario matches the stated data gap. The guidance goes on to say that if <10% of the area of use is covered in attractive weeds then the exposure route is not relevant in the 90th %-ile case. Consequently, if the situation is that <10% of the area of use is covered in attractive weeds then this data gap will have been fulfilled as no risk assessment would be required. In this statement the occurrence of flowering weeds has been investigated and therefore the potential relevance for honeybees can be assessed based on the above criteria.

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[1] European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295

02.02.04 - Honey dew

Report:	02.02.04/01; [redacted]; 2013; M-453965-01-3
Title:	Statement - Information on the occurrence or possible occurrence of the development of resistance of the plant protection product Janus Forte (for submission in Europe)
Report No.:	M-453965-01-3
Document No.:	M-453965-01-3
Guideline(s):	US EPA OCSPP Guideline Number: 850.SUPP PP1/213(2) EU Directive 91/414 EEC According to OECD format guidance for industry data submissions on plant protection products and their active substances
Guideline deviation(s):	--
GLP/GEP:	**no**

Resistance in arthropod pest species comprises a change in the genetic composition of a population in response to selection by pesticides such that control in the field may be impaired repeatedly at recommended application rates. The report includes resistance management information regarding key invertebrate pests targeted in sugar beet in countries such as Belgium, Czech Republic, France, Germany, Poland, Romania, Slovakia and Serbia by seed treatments with Janus Forte® (FS 280) containing the insecticidal ingredients clothianidin, imidacloprid and beta-cyfluthrin.

Janus Forte® is a mixture of three chemically different insecticides complementing each other in numerous properties and belonging to two distinct mode of action classes, i.e. acting on different molecular target-sites not yet shown to be involved in any cross-resistance issues globally.

Beta-cyfluthrin belongs to the chemical class of synthetic pyrethroids and is a well known contact insecticide particularly for the control of coleopteran pests, e.g. Agriotes ssp.other elaterid soil pests. Pyrethroid insecticides such as beta-cyfluthrin are classified by IRAC (Insecticide Resistance Action Committee) in mode of action class 3A, sodium channel modulators.

Resistance to pyrethroid insecticides has been described for different crop pests and the major mechanisms of resistance were identified as either metabolic (esterases and monooxygenases) or knock-down-resistance (kdr) due to a mutation in the IIS6 domain of the voltage-gated sodium channel. All of the pest insects intended to be targeted by Beta-cyfluthrin in Janus Forte® as a seed treatment are not listed as high risk pests within EPPO´s Std. PP1/213 on resistance risk analysis and haven´t been included for a detailed survey, primarily due to a lack of any resistance issues in the past.

Clothianidin and Imidacloprid are members of the neonicotinoid class of insecticides and well established tools for the control of sucking, chewing and soil pests in seed treatment applications due to their systemic properties. They specifically control a number of coleopteran pests in sugar beet such as elaterid larvae (Agriotes ssp., wireworms), weevils (Bothynoderes), flea beetles (Chaetocnema ssp.) and Atomaria linearis. Other important pests targeted in sugar beet include aphid pests such as Aphis fabae and Myzus persicae, thrips (Thrips tabaci), dipterans (Pegomyia), millipedes (e.g. Blaniulus guttulatus) and myriapodes (e.g. Scutigerella immaculata). Neonicotinoid insecticides such as clothianidin and imidacloprid are classified by IRAC in mode of action class 4A, nicotinic acetylcholine recpetor (nAChR) agonists.

However, very recently M. persicae was shown to have locally developed resistance to neonicotinoid insecticide sprays in peaches in southern France, northern Spain and northern Italy, based on a target-site mutation in the nicotinic acetylcholine receptor ß-subunit. No reports are known from any secondary host species yet, including sugar beet and vegetables.

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In sugar beet no resistance to clothianidin, imidacloprid and beta-cyfluthrin seed treatments is yet described for any of the pests or pest groups mentioned above, including aphid species such as Aphis fabae and Myzus persicae (particularly targeted by systemically acting clothianidin and imidacloprid).

General resistance management guidelines for neonicotinoid and pyrethroid insecticides as published by IRAC are usually followed with products such as Janus Forte® and regionally adapted as necessary.

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02.02.05 - Guttation

Report:	02.02.05/01; [redacted]; 2014; M-498939-01-3
Title:	Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter wheat (W-WHT), seed-treated either with an imidacloprid or a clothianidin combi-product
Report No.:	R09247-4
Document No.:	M-498939-01-3
Guideline(s):	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

<<M-498939-01-3@S-602266-01-1

Aim of the Study

The study was conducted on two separated test locations (study sites) from the beginning of October 2009 until the end of April 2010. One test location was situated in Northern Germany, the other test location was situated in Southern Germany. Honey bee colonies were set up directly adjacent to fields sown with winter wheat (W-WHT) seeds, in order to investigate the potential effects from exposure to guttating W-WHT, starting from seedling emergence in autumn 2009 until beginning of winter oil-seed flowering in the respective region in spring 2010. The study has been performed in cooperation with the State Institutes of Apiculture in Hohenheim (Dr. Rosenkranz, Baden-Württemberg) for Southern Germany and the Institute of Apiculture in Celle (Dr. von der Ohe, Lower Saxony) for Northern Germany, respectively. All bee assessments have been conducted by the cooperation partner of the corresponding region. The study comprised two treatment groups and one control group per test location. One of the two treatment groups per test location was imidacloprid-treated (=imidacloprid-treatment group) and comprised one individual study field, on which imidacloprid-treated W-WHT seeds were sown; the other treatment group per test location was clothianidin-treated (=clothianidin-treatment group) and comprised also one individual study field on which clothianidin-treated W-WHT seeds were sown. Per test location there was in addition one control group, comprising one individual study field on which non-insecticide treated (=control) W-WHT seeds were sown (seed-treated with a routine fungicide (EfA®)).

Moreover, all seeds were additionally seed-treated with commercial PENTECO®, in order to minimize dust abrasion.

As such, treatment is defined by the presence and the potential exposure of honey bees to either the systemic neonicotinoid insecticide imidacloprid or to the systemic neonicotinoid insecticide clothianidin.

All W-WHT seeds were seed-treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim, Germany. In total, two different W-WHT varieties were employed for the purpose of the study: the W-WHT variety "Manager" was used at the test location in Northern Germany and the variety "Herrmann" was used at the test location in Southern Germany. The control W-WHT seeds were of the same variety as the treatment seeds at the respective test location.

All seeds were sown by following typical commercial use conditions.

Key study objectives were to evaluate and to compare the colony development and the overwintering performance of exposed honey bee colonies in two study groups (i.e. two different treatment groups and one control group, respectively). Furthermore, the guttation behaviour of W-WHT was surveyed and it was examined whether exudation of guttation fluid of W-WHT and flight activity of honey bees occurred simultaneously.
In case flight activity and guttation coincided, the bee activity in the respective study field was surveyed. For this purpose, a specified area (= assessment area) next to the honey bee colonies was intensively monitored for bee visits. Regarding this activity, one "monitoring" is defined by an approximately thirty-five minute continuous observation of the assessment area. In addition, guttation fluid of W-WHT in the

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two treatment groups was collected in the field and analysed for residues of imidacloprid or clothianidin, respectively. Moreover, dead bee traps were installed at the entrance of the bee hives to record the number of dead bees.

Material and Methods

Test item
W-WHT seeds, either imidacloprid-treated (Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 60 + 70 + 7.2 + 8) or clothianidin-treated (Clothianidin & Beta-Cyfluthrin FS 375 + 80), respectively.

Study sites and sowing
The study was conducted in a) Northern and b) Southern Germany, at six commercially managed agricultural fields (study fields), respectively: a) test location northeast of Celle in the federal state of Lower Saxony (in the following called Celle), where the study fields were owned by two different commercial farmers and b) test location southwest of Stuttgart, near Renningen, in the federal state of Baden-Württemberg, where the study fields were located at the Ihinger Hof experimental field station for plant cultivation and protection of the University Hohenheim (in the following called Ihinger Hof).

On each of the two test locations one study field was assigned as imidacloprid-treated field (on which imidacloprid-treated W-WHT seeds were sown), one study field as clothianidin-treated field (on which clothianidin-treated W-WHT seeds were sown) and one study field was assigned as control field (on which non-insecticide treated (=control) W-WHT seeds were sown), respectively. As there were in total two test locations, the study comprised in total two imidacloprid-treated fields, two clothianidin-treated fields and two control fields, giving overall six study fields under investigation.

Set-up of honey bee hives
At each of the six study fields under investigation, five honey bee colonies were placed along a line one to eight days before sowing, either directly adjacent or within a maximum distance of 0.5 m to the W-WHT crop, depending on the actual local field situation.

Assessment area
A specified area (assessment area) in front of the honey bee colonies was intensively monitored. The whole assessment area was divided into two In-Crop Zones (Zone 0 and Zone 1) and an Off-Crop Zone. Zone 0 (width: 5 m to each side of the hives, 2 m depth into the in-crop) covered the immediate area in front of the bee hives and Zone 1 (a 2 m broad band, shaped like an inverted ‘U’, with a vertical distance of the band to the field margin of 7 m inside the crop). The bee hives were placed into the Off-Crop Zone, directly adjacent to the W-WHT crop (width: 10 m length along the field margin, 1 m depth into the off-crop). In addition, two 1 m² assessment plots were established to record the proportion of W-WHT displaying guttation and/or dew.

Honey bee mortality
Each hive was equipped with a dead bee trap. The traps were emptied daily during the monitoring period to record the number of dead honey bees. After 09 October 2010, also dead bees found on the soil surface in front of each colony, respectively, were recorded.

Guttation fluid sampling
In case guttation was observed in the morning at a respective field, up to three samples of guttation fluid, each with a volume of approximately 1 mL were collected from various plants of W-WHT. The samples were thereafter deep frozen (-20°C) for later analysis.

Monitoring
The monitoring activities started as soon as the W-WHT plants had emerged on the fields under investigation and lasted for a maximum period of four consecutive weeks until end of October 2009. The monitoring activities in the field re-started in spring 2010 with the beginning of the inflorescence of the

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Goat Willow *(Salix caprea)* and lasted for a period of four consecutive weeks until beginning of the flowering of winter oil-seed in the respective region.

During the morning, the respective assessment area on the study fields was systematically checked for occurrence of guttation fluid and/or dew. If guttation was still present at the start of honey bee activity, the numbers of honey bees resting or walking on the ground or on the W-WHT crop were counted and any potential uptake of guttation fluid or dew by the bees or any conspicuous bee behaviour was recorded. Field assessments were stopped after no more guttation fluid was present or after a maximum of four subsequent monitorings, whatever occurred earlier. During each of the seasonal monitoring sessions (autumn and spring), one observer was continuously responsible for two study plots. At the study site Ihinger Hof, the observer alternated between two study plots within one day. However, at the other study site at Celle, the observer alternated between two study plots within two days. Beyond field assessments in the morning, the study field which was monitored in the morning was also visited in the evening (at Ihinger Hof the study field which was monitored first was also monitored in the evening). During these evening assessments, the onset of guttation and the end of bee activity was recorded.
One "monitoring session" lasted for approximately 35 minutes and was defined as one complete observation cycle of the assessment area and its associated two segregated plots of 1 m², at which guttation- and honey bee assessments were conducted during the presence of guttation fluid on the W-WHT crop.

Honey bee colony strength and health assessment
At both test locations (i.e. Ihinger Hof and Celle), the colony strength and the colony development were estimated according to the Liebefeld method (Imdorf et al. 1987). The first assessment was performed shortly before (Celle) or after (Ihinger Hof) colony set-up; further assessments were performed every 21 days until end of October 2009. In spring 2010, colony development was assessed in the same manner from the beginning of inflorescence of the Goat Willow (Salix caprea) until beginning of winter oil-seed flowering in the respective region. Maintaining of the bee hives as well as all honey bee assessments have been performed by the Institute of Apiculture in Celle (Dr. von der Ohe, Lower Saxony) in Northern Germany and the State Institute of Apiculture in Hohenheim (Dr. Rosenkranz, Baden- Württemberg) in Southern Germany, respectively.

Residue analysis
Imidacloprid and clothianidin residues in the various samples were analysed by an analytical laboratory of Bayer CropScience AG.

Results
Frequency of guttation
During the assessments in the morning, guttation fluid was observed on W-WHT at 86.4 % of all observation days in autumn 2009 and at 87.9 % of the observation days in spring 2010. No remarkable coincidence of guttation of W-WHT and bee activity in the evening in autumn 2009 and spring 2010 was observed.

Duration of guttation
Whenever guttation was observed on a respective day, it was already present in the early morning. Depending on the actual weather conditions, the time when guttation ended was variable. Under foggy or misty conditions, drizzle or slight rain, guttation lasted over longer periods as compared to dry conditions. On most observations days, guttation lasted for several hours.

Honey bee activity in the assessment area
During the entire field monitoring periods in autumn 2009 and spring 2010 (comprising a total of 222 individual monitoring sessions, giving approximately 129 hours of total observation time), a total of 3,276 honey bees was observed within the assessment areas: 1,459 honey bees were resting on the soil surface, with 848 in the In-Crop Zones and 611 in the Off-Crop Zone; 1,817 honey bees were resting on plants, with 1,199 in the In-Crop Zones and 618 in the Off-Crop Zone.

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Most of the direct honey bee observations within the assessment area were made in the In-Crop Zone 0, i.e. directly in front of the hives, followed by the Off-Crop Zone and the In-Crop Zone 1.

Honey bees were observed visiting the study plots frequently. The relative proportion of honey bees observed per monitoring on plants in the respective assessment areas in both, treatments and control, was mostly higher in spring 2010 than in autumn 2009. With the exception of honey bees on soil surface: in autumn 2009 the observed relative proportion was three to four times higher in Zone 0 than in spring in the respective zone, which can obviously be explained by the cold weather. The observed relative proportion of honey bees per monitoring taking up guttation fluid and dew in both, treatment and control, was unequivocally higher in all assessment zones in spring 2010 as compared to autumn 2009. Throughout the entire field observation period in autumn 2009 and spring 2010, a total of 68 honey bees were observed taking up dew and a total of 343 honey bees were recorded taking up guttation fluid within the assessment zones (which includes the Off-Crop Zone). Most of the bees taking up dew or guttation fluid were observed in Zone 0, i.e. directly in front of/adjacent to the hives. Accounting for all honey bees directly observed during the individual monitoring sessions within the assessment area in both treatments and control, a moderate proportion of bees was observed taking up guttation fluid, i.e. 343 bees / 3,276 bees = 10.5 %. Most of the honey bees which took up guttation fluid were observed during springtime (341 of 343 bees), which gives a relative proportion of bees taking up guttation fluid in autumn of 2 bees / 404 bees = 0.5 % and of 341 bees / 2,872 bees = 11.9 % during springtime.

Residue analysis of guttation fluid

All samples of guttation fluid collected from the treatment fields were analysed either for residues of imidacloprid or clothianidin, respectively. Selected samples of guttation fluid collected from the treatment fields were additionally analysed for their content of the clothianidin metabolites TZNG and TZMU (clothianidin treatment group) or their content of the imidacloprid metabolites imidacloprid-5-hydroxy and imidacloprid-olefin (imidacloprid treatment group). Chromatography and detection by MS/MS was performed according to method 00554/M001 (clothianidin, TZNG and TZMU) or method 00537/M002 (imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin).

The Limit of Quantitation (LOQ) of each analyte in guttation fluid was 0.01 mg/L and the Limit of Detection (LOD) of each analyte was 0.001mg/L, respectively.

The residue levels of clothianidin in guttation water were within the range of < LOQ to 13.0mg/L. The residue levels of TZNG in guttation water were within the range of <LOQ to 0.49mg/L. The residue levels of TZMU in guttation water were within the range of <LOD to 0.32mg/L. The residue levels of imidacloprid in guttation water were within the range of <LOD to 6.9mg/L. The residue levels of imidacloprid-5-hydroxy in guttation water were within the range of <LOD to 0.61mg/L. The residue levels of imidacloprid-olefin in guttation water were within the range of <LOD to 0.12 mg/L.

Honey bee mortality

At both study sites, honey bee mortality in autumn was mostly low until a period of cold weather in October in all experimental groups. The increased mortality during this period was clearly correlated with the weather conditions and was not influenced by the experimental setup.
During springtime, the mortality found in the traps was generally low, but still variable from colony to colony and with higher mortality at Ihinger Hof than at Celle.

Colony development

During the autumn 2009 observation period, most colonies developed normally. Three colonies had to be removed after the last assessment before overwintering, as they had less than 5,000 bees and were therefore not considered capable for overwintering.

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During wintertime, four colonies died. During the spring 2010 observation period, the colony development in both, treatment and control, was considered to be within the normal range in most of the exposed colonies. Two colonies had to be removed during spring, one did not recover from bad overwintering and one lost its queen.

Conclusions

Guttation of W-WHT plants was a regular occurring phenomenon during the autumn and spring growth period of the crop and there is usually a time overlap between presence of guttation fluid and bee flight activity during morning hours.

Honey bees were observed visiting the study plots frequently. Most of the direct honey bee observations within the assessment areas were made directly in front of the hives. The relative proportion of honey bees observed per monitoring on plants in the respective assessment areas in both, treatments and control, was mostly higher in spring 2010 than in autumn 2009. Moreover, also the observed relative proportion of honey bees per monitoring taking up guttation fluid and dew in both, treatment and control, was mostly higher in all assessment zones in spring 2010 as compared to autumn 2009.

Accounting for all honey bees directly observed during the individual monitorings within the assessment area in both, treatments and control, respectively (i.e. 3,276 bees in total; 404 bees during autumn and 2,872 bees during springtime) overall a moderate proportion of bees was observed taking up guttation fluid, i.e. 343 bees / 3,276 bees = 10.5 %. Most of the honey bees which took up guttation fluid were observed during springtime (341 of 343 bees), which gives a relative proportion of bees taking up guttation fluid in autumn of 2 bees / 404 bees = 0.5 % and of 341 bees / 2,872 bees = 11.9 % during springtime.

The overall maximum measured concentration of clothianidin within guttation fluid, collected from the clothianidin-treated fields, was determined during the autumn growth period of the W-WHT crop and accounted for 13.0mg a.s./L. Residues of clothianidin in guttation fluid were generally higher during the autumn growth period as compared to the spring growth period. During the spring growth period, the maximum measured concentration of clothianidin within guttation fluid was 0.39 mg a.s./L. The residue levels of the clothianidin metabolites TZNG and TZMU in guttation water ranged between <LOQ to 0.49mg/L and between <LOD to 0.32mg/L, respectively.

Also for imidacloprid the overall maximum measured concentration in guttation fluid, collected from the imidacloprid-treated fields was determined during the autumn growth period of the W-WHT crop and accounted for 6.9mg a.s./L. As for clothianidin, the residues of imidacloprid in guttation fluid were also generally higher during the autumn growth period as compared to the spring growth period. During the spring growth period, the maximum measured concentration of imidacloprid within guttation fluid was 0.19 mg a.s./L. The residue levels of its metabolites imidacloprid-5-hydroxy or imidacloprid-olefin in guttation water ranged between <LOD to 0.61mg/L or between <LOD to 0.12 mg/L, respectively.

No treatment related differences in honey bee mortality, colony development in autumn and spring as well as in the overwintering performance were observed between the control and the treatment groups (imidacloprid and clothianidin treatment group respectively). Weak development in autumn, leading to discarding the colonies or winter losses can easily be explained by *varroa* loads and other diseases found in the colonies, together with the very long and cold winter 2009/10.

Overall, it is concluded that guttation fluid, exudated by winter wheat seedlings, seed-treated with nitro-substituted neonicotinoids, does not have unacceptable effects on honey bee colonies under typical commercial use conditions.

>>M-XXXX39-01-1 - S_502266-01-1

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Report:	02.02.05/02; [redacted]; 2012; M-498922-01-3
Title:	Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter barley (W-BAR), seed-treated either with an imidacloprid or a clothianidin combi-product
Report No.:	R09247-3
Document No.:	M-498922-01-3
Guideline(s):	U.S. EPA OCSPP 850.3040
Guideline deviation(s):	not specified
GLP/GEP:	**no**

<<M-498922-01-3@S-602264-01-1

Aim of the Study

The study was conducted on two separated test locations (study sites) from the end of September 2009 until the end of April 2010. One test location was situated in Northern Germany, the other test location was situated in Southern Germany. Honey bee colonies were set up directly adjacent to fields sown with winter barley (W-BAR) seeds, in order to investigate the potential effects from exposure to guttating W-BAR, starting from seedling emergence in autumn 2009 until beginning of winter oil-seed flowering in the respective region in spring 2010. The study has been performed in cooperation with the State Institutes of Apiculture in Hohenheim (Dr. Rosenkranz, Baden- Württemberg) for Southern Germany and the Institute of Apiculture in Celle (Dr. von der Ohe, Lower Saxony) for Northern Germany, respectively. All bee assessments have been conducted by the cooperation partner of the corresponding region. The study comprised two treatment groups and one control group per test location: One of the two treatment groups per test location was imidacloprid-treated (=imidacloprid-treatment group) and comprised one individual study field, on which imidacloprid-treated W-BAR seeds were sown; the other treatment group per test location was clothianidin-treaded (=clothianidin-treatment group) and comprised also one individual study field on which clothianidin-treated W-BAR seeds were sown. Per test location there was in addition one control group, comprising one individual study field on which non-insecticide treated (=control) W-BAR seeds were sown (seed-treated with a routine fungicide (EFA®)).

Moreover, all seeds were additionally seed-treated with commercial INTECO®, in order to minimize dust abrasion.

As such, treatment is defined by the presence and the potential exposure of honey bees to either the systemic neonicotinoid insecticide imidacloprid or to the systemic neonicotinoid insecticide clothianidin.

All W-BAR seeds were seed-treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim, Germany. In total, two different W-BAR varieties were employed for the purpose of the study: the W-BAR variety "Lomerit" was used at the test location in Northern Germany and the variety "Highlight" was used at the test location in Southern Germany. The control W-BAR seeds were of the same variety as the treatment seeds at the respective test location.
All seeds were sown by following typical commercial use conditions.
Key study objectives were to evaluate and to compare the colony development and the hibernation performance of exposed honey bee colonies in two study groups (i.e. two different treatment groups and one control group, respectively). Furthermore, the guttation behaviour of W-BAR was surveyed and it was examined whether exudation of guttation fluid of W-BAR and flight activity of honey bees occurred simultaneously. In case flight activity and guttation coincided, the bee activity in the respective study field was surveyed. For this purpose, a specified area (= assessment area) next to the honey bee colonies was intensively monitored for bee visits. Regarding this activity, one "monitoring" is defined by an approximately thirty-five-minute continuous observation of the assessment area. In addition, guttation fluid of W-BAR in the two treatment groups was collected in the field and analysed for residues of imidacloprid or clothianidin, respectively. Moreover, dead bee traps were installed at the entrance of the bee hives to record the number of dead bees.

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Material and Methods
Test item
W-BAR seeds, either imidacloprid-treated (Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 60 + 70 + 7.2 + 8) or clothianidin-treated (Clothianidin & Beta-Cyfluthrin FS 375 + 80), respectively.

Study sites and sowing
The study was conducted in a) Northern and b) Southern Germany, at six commercially managed agricultural fields (study fields), respectively: a) test location northeast of Celle in the federal state of Lower Saxony (in the following called Celle), where the study fields were owned by two different commercial farmers and b) test location southwest of Stuttgart, near Renningen, in the federal state of Baden-Württemberg, where the study fields were located at the Ihinger Hof experimental field station for plant cultivation and protection of the University Hohenheim (in the following called Ihinger Hof).

On each of the two test locations one study field was assigned as imidacloprid-treated field (on which imidacloprid-treated W-BAR seeds were sown), one study field as clothianidintreated field (on which clothianidin-treated W-BAR seeds were sown) and one study field was assigned as control field (on which non-insecticide treated (=control) W-BAR seeds were sown), respectively. As there were in total two test locations, the study comprised in total two imidacloprid-treated fields, two clothianidin-treated fields and two control fields, giving overall six study fields under investigation.

Set-up of honey bee hives
At each of the six study fields under investigation, five honey bee colonies were placed along a line one to eight days before sowing, either directly adjacent or within a maximum distance of 0.5 m to the W-BAR crop, depending on the actual local field situation.

Assessment area
A specified area (assessment area) in front of the honey bee colonies was intensively monitored. The whole assessment area was divided into two In-Crop Zones (Zone 0 and Zone 1) and an Off-Crop Zone. Zone 0 (width: 5 m to each side of the hives, 2 m depth into the in-crop) covered the immediate area in front of the bee hives and Zone 1 (a 2 m broad band, shaped like an inverted ‘U’, with a vertical distance of the band to the field margin of 7 m inside the crop). The bee hives were placed into the Off-Crop Zone, directly adjacent to the W-BAR crop (width: 10 m length along the field margin, 1 m depth into the off-crop). In addition, two 1 m² assessment plots were established to record the proportion of W-BAR displaying guttation and/or dew.

Honey bee mortality
Each hive was equipped with a dead bee trap. The traps were emptied daily during the monitoring period to record the number of dead honey bees. After 09 October 2010, also dead bees found on the soil surface in front of each colony, respectively, were recorded.

Guttation fluid sampling
In case guttation was observed in the morning at a respective field, up to three samples of guttation fluid, each with a volume of approximately 1 mL were collected from various plants of W-BAR. The samples were thereafter deep frozen (-20°C) for later analysis.

Monitoring
The monitoring activities started as soon as the W-BAR plants had emerged on the fields under investigation and lasted for a maximum period of four consecutive weeks until end of October 2009. The monitoring activities in the field re-started in spring 2010 with the beginning of the inflorescence of the Goat Willow *(Salix caprea)* and lasted for a period of four consecutive weeks until beginning of the flowering of winter oil-seed in the respective region.

During the morning, the respective assessment area on the study fields was systematically checked for occurrence of guttation fluid and/or dew. If guttation was still present at the start of honey bee activity,

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the numbers of honey bees resting or walking on the ground or on the W-BAR crop were counted and any potential uptake of guttation fluid or dew by the bees or any conspicuous bee behaviour was recorded. Field assessments were stopped after no more guttation fluid was present or after a maximum of four subsequent monitorings, whatever occurred earlier. During each of the seasonal monitoring sessions (autumn and spring), one observer was continuously responsible for two study plots. At the study site Ihinger Hof, the observer alternated between two study plots within one day. However, at the other study site at Celle, the observer alternated between two study plots within two days. Beyond field assessments in the morning, the study field which was monitored in the morning was also visited in the evening (at Ihinger Hof the study field which was monitored first was also monitored in the evening). During these evening assessments, the onset of guttation and the end of bee activity was recorded.
One "monitoring session" lasted for approximately 35 minutes and was defined as one complete observation cycle of the assessment area and its associated two segregated plots of 1 m², at which guttation- and honey bee assessments were conducted during the presence of guttation fluid on the W-BAR crop.

Honey bee colony strength and health assessment At both test locations (i.e. Ihinger Hof and Celle) the colony strength and the colony development were estimated according to the Liebefeld method (Imdorf et al. 1987). The first assessment was performed immediately after colony set-up; further assessments were performed every 21 days until end of October 2009. In spring 2010, colony development was assessed in the same manner from the beginning of inflorescence of the Goat Willow *(Salix caprea)* until beginning of winter oil-seed flowering in the respective region. Maintaining of the bee hives as well as all honey bee assessments have been performed by the Institute of Apiculture in Celle (Dr. von der Ohe, Lower Saxony) in Northern Germany and the State Institute of Apiculture in Hohenheim (Dr. Rosenkranz, Baden- Württemberg) in Southern Germany, respectively.

Residue analysis
Imidacloprid and clothianidin residues in the various samples were analysed by an analytical laboratory of Bayer CropScience AG.

Results
Frequency of guttation
During the assessments in the morning, guttation fluid was observed on W-BAR at 84.2 % of all observation days in autumn 2009 and at 80.7 % of the observation days in spring 2010. No remarkable coincidence of guttation of W-BAR and bee activity in the evening in autumn 2009 was observed. A coincidence during this period of time occurred, with a few exceptions only, just on those days where guttation anyhow prevailed for the whole day due to damp or rainy weather. In spring 2010, no coincidence between presence of guttation in the evening and bee activity was observed at all.

Duration of guttation
Whenever guttation was observed on a respective day, it was already present in the early morning. On dry, windy days, guttation stopped shortly after sunrise, whereas on cold, damp days with drizzle, it occasionally lasted until afternoon and on some occasions even until evening. On most observations days, guttation lasted for several hours.

Honey bee activity in the assessment area
During the entire field monitoring periods in autumn 2009 and spring 2010 (comprising a total of 264 individual monitoring sessions, giving approximately 144 hours of total observation time), a total of 3,148 honey bees was observed within the assessment areas: 1,230 honey bees were resting on the soil surface, with 911 in the In-Crop Zones and 319 in the Off-Crop Zone; 1,918 honey bees were resting on plants, with 1,386 in the In-Crop Zones and 532 in the Off-Crop Zone.
Most of the direct honey bee observations within the assessment area were made in the In-Crop Zone 0, i.e. directly in front of the hives, followed by the Off-Crop Zone and the In-Crop Zone 1.

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Honey bees were observed visiting the study plots frequently. The relative proportion of honey bees observed per monitoring on plants in the respective assessment areas in both, treatments and control, was mostly higher in spring 2010 than in autumn 2009. Moreover, also the observed relative proportion of honey bees per monitoring taking up guttation fluid and dew in both, treatment and control, was mostly higher in all assessment zones in spring 2010 as compared to autumn 2009. Throughout the entire field observation period in autumn 2009 and spring 2010, a total of 72 honey bees were observed taking up dew and a total of 334 honey bees were recorded taking up guttation fluid within the assessment areas (which includes the Off-Crop Zone). Most of the bees taking up dew or guttation fluid were observed in Zone 0 and in the Off-Crop Zone, i.e. directly in front of / adjacent to the hives. Accounting for all honey bees directly observed during the individual monitoring sessions within the assessment area in both, treatments and control, a moderate proportion of bees was observed taking up guttation fluid, i.e. 334 bees / 3,148 bees = 10.6 %. Most of the honey bees which took up guttation fluid were observed during springtime (301 of 334 bees), which gives a relative proportion of bees taking up guttation fluid in autumn of 33 bees / 1,267 bees = 2.6 % and of 301 bees / 1,881 bees = 16 % during springtime.

Residue analysis of guttation fluid

All samples of guttation fluid collected from the treatment fields were analysed either for residues of imidacloprid or clothianidin, respectively. Selected samples of guttation fluid collected from the treatment fields were additionally analysed for their content of the clothianidin metabolites TZNG and TZMU (clothianidin treatment group) or their content of the imidacloprid metabolites imidacloprid-5-hydroxy and imidacloprid-olefin. Chromatography and detection by MS/MS was performed according to method 00554/M001 (clothianidin, TZNG and TZMU) or method 00537/M002 (imidacloprid and its metabolites imidacloprid-5- hydroxy and imidacloprid-olefin).

The Limit of Quantitation (LOQ) of each analyte in guttation fluid was 0.01 mg/L and the Limit of Detection (LOD) of each analyte was 0.001 mg/L, respectively.

The residue levels of clothianidin in guttation water were within the range of < LOD to 2.3 mg/L. The residue levels of TZNG in guttation water were within the range of < LOD to 0.05 mg/L. The residue levels of TZMU in guttation water were within the range of < LOD to 0.02 mg/L. The residue levels of imidacloprid in guttation water were within the range of < LOQ to 15 mg/L. The residue levels of imidacloprid-5-hydroxy in guttation water were within the range of < LOD to 0.64 mg/L. The residue levels of imidacloprid-olefin in guttation water were within the range of < LOD to 0.05 mg/L.

Synoptic assessment of honey bee mortality and colony performance

Effects during the autumn exposure period

During the approximately 5 week's continuous autumn exposure period, none of the treatment colonies revealed adverse effects in terms of mortality rates and/or suspicious behavioural impairments, although honey bees were frequently recorded to forage within the neonicotinoid-treated barley fields. The number of honey bees exhibiting behavioural impairments, however, did not differ between treatment groups with 30, 48 and 13 impaired honey bees for the control, the imidacloprid and the clothianidin treatment, respectively. In all treatment groups, honey bee mortality in autumn was mostly low until a period of cold weather in October. The increased mortality in all experimental groups (treatments and control) during this period was clearly correlated with the weather conditions and was not influenced by the experimental setup. During springtime, the mortality found in the traps was generally low, but still variable from colony to colony.

Based on these observations, it can be concluded that guttation fluid of neonicotinoid-treated barley seedlings, although carrying an intrinsically high hazard potential, does not impair honey bee colonies - which were exposed at the field margin in direct vicinity to those fields - in a unacceptable manner.

Observations at the end of the autumn exposure period and after overwintering

The final evaluation of all experimental data revealed that the standard procedure of stochastically assigning honey bee colonies to different treatment groups caused a bias in terms of initial colony vitality in disfavour of the clothianidin treatment group. The "lessons learned" from this unfortunate experience is that the assignment of honey bee colonies in long-term trials have to be altered in such a way that all

colonies have first to be ranked alongside their vitality, i.e. colony strength, brood mass, *Varroa* infestation level. Thereafter, the random assignment to the future treatment groups must start by assigning top colonies first, followed by second quality colonies, and so on. Due to this and further experimental bias in the clothianidin treatment group (see below), no reliable conclusions can be drawn for this group concerning overwintering performance. The initial colony vitality between the control and the imidacloprid treatment groups was fairly comparable, which in turn allows a scientifically meaningful analysis of the observations during the overwintering period. Two and one colonies failed to successfully overwinter in the control and the imidacloprid treatments groups, respectively. This translates into an overwintering success (total success) rate of 80 (80)% in the control group and 89 (80)% in the imidacloprid treatment group, indicating that guttating W-BAR seedlings, carrying high levels of intrinsically bee-toxic neonicotinoid residues, have no impact on the rate of successful overwintering of adjacently located and exposed honey bee colonies. Regarding those colonies which were discontinued due to a too low colony strength after the autumn exposure period (0 colonies in control, 1 in the imidacloprid treatment group and 2 in the clothianidin treatment group), a clear correlation can be seen between colony strength in combination with available brood mass: the weaker both figures, the less the probability to reach the minimum colony strength to overwinter and/or to survive overwintering (see below).

Methodological deficiencies resulting in experimental biases, particularly for the clothianidin treatment group
The autumn- and overwintering conditions for the clothianidin treatment group were substantially less favourable as compared to the control and/or to the imidacloprid treatment group due to three key factors:

- Higher number of weak colonies at study initiation

Colonies which have a below average colony strength in autumn will have an overall lower survival rate over winter time than stronger colonies. Considering the initial pre-exposure colony vitality of all colonies across the three treatment groups, it turned out that there was an assignment bias in the number of the weakest colonies, i.e. colonies with ≤ 8,000 bees, with 2, 2 and 3 of such colonies being assigned to the control (colonies 7/2 and 7/4), the imidacloprid-treatment group (colonies 8/1 and 14/4) and the clothianidin-treatment group (colonies 9/1, 9/4 and 15/1), respectively. In the control group, one of the two weak colonies (7/4) developed badly during the course of the study and did finally not survive the winter. The second weak colony (7/2) could restore colony strength during autumn from better bee brood stores and subsequently hibernate successfully.

In the imidacloprid-treatment group one of the two weak colonies (8/1) was removed before over-wintering as from empirical experience the number of bees was evidently too low for successful overwintering. This colony could not restore colony strength due to low bee brood stores at the time of test initiation. The second weak colony (14/4) showed a weak colony strength during autumn and overwintered badly. Although it finally overwintered successfully, the restoring of this colony during springtime would have required favourable circumstances.

In the clothianidin-treatment group, two of the three colonies with insufficient brood for restoring colony strength (9/4 and 15/1) had to be removed before overwintering as from empirical experience the number of bees was too low for successful overwintering. The third of these colonies (9/1) developed slightly during autumn but remained too weak to finally survive the winter.
When comparing the colony performance of all initially weak colonies, they all showed a similar pattern across experimental groups, i.e. no restore of colony strength except forv control colony (7/2) due to better brood mass. Those colonies which could not restore colony strength from available brood stores experienced either early termination (at the end of the autumn exposure period) or failure during overwintering. The abandonment/loss of three colonies in the clothianidin-treatment group (i.e. colonies 9/1, 9/4 and 15/1) can be attributed to their rather low number of adult bees at the time of colony set-up in combination with below average brood stores.

- Higher *Varroa* infestation level

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Colonies which are infested by *Varroa* mites are heavily stressed, first, by the parasitic activity of the mites and second by the diseases vectored by the mites. It is well known that a high *Varroa* infestation rate during the autumn period significantly increases the likelihood of overwintering failure of a colony. Nonetheless, based on genetic adaptation, some colonies apparently tolerate a higher *Varroa* pressure than other colonies. Although all colonies which were employed for this study received the same anti-*Varroa* treatments (Bayvarol® before study initiation, oxalic acid (and Perizin®, additionally used in Celle) during the study), it is a matter of fact well known in apiculture that the anti-*Varroa* treatment success per individual colony is quite variable. When scrutinizing the clothianidin-treatment group with regard to *Varroa* infestation, there was one colony (9/2; study site Ihinger Hof) which showed during the pre-oxalic acid anti-*Varroa* treatment period in autumn the overall highest natural mite drop (□: 343 mites) and the overall highest mite drop after oxalic acid treatment (1,220 mites), which shows that this colony was heavily infested by *Varroa* between study initiation and overwintering. Also the colonies 15/3 and 15/5 (both: study site Celle) in the clothianidin-treatment group suffered from a high *Varroa* pressure, which became apparent during the pre-anti-Varroa treatment period in autumn.

In the control group, only one colony (13/1; study site Celle) exhibited during both, the preoxalic acid (and Perizin®) anti-*Varroa* treatment period and the time immediately after the treatment period a mite number which was higher as compared to the colonies 15/3 and 15/5. However, the mite drop in the colony 13/1 decreased more significantly after treatment as compared to the colonies 15/3 and 15/5 (period 05 – 11 NOV versus period 29 OCT – 05 NOV) which indicated a more effective *Varroa* control as compared to the colonies 15/3 and 15/5. The poor overwintering performance of the colonies 9/2, 15/3 and 15/5 in the clothianidin treatment group, which finally resulted in winter loss, could, therefore, be attributed to the high *Varroa* infestation level of these colonies rather than an effect of an exposure to potentially acute toxic guttation fluid which, however, is not stored and should therefore, not exhibit any delayed toxicity effects.

- Less favourable ambient conditions during hibernation

On top of the negatively biased colony vitality of the clothianidin treatment groups, these colonies also suffered from more unfavourable ambient conditions prevailing at the assigned study plots in comparison to the control- and the imidacloprid study sites.

At the Ihinger Hof study site, the honey bee colonies at the clothianidin study plot were significantly more exposed to the wind due to the absence of any shelter. Moreover, the hive entrances of the colonies in the clothianidin group were directed to the North (i.e. no sun), whereas the hive entrances of the colonies set-up in the two other groups were directed to the South and East. In addition, the clothianidin study plot suffered from a significantly higher soil dampness, which further contributed to an increased cold and damp microclimate.

Also on the study location Celle, environmental factors differed on the individual study locations. Particularly the clothianidin study plot was affected, as the honey bee colonies were placed in a slight landscape depression. The soil around the bee colonies was compacted, rendering the place to be damp, which became most apparent during springtime 2010, where the area was swamped and the hives had to be placed on elevated ground in order to prevent the colonies from flooding. During wintertime, also cold air could be expected to have accumulated in this landscape depression, framed by the edges of a forest. When correcting the clothianidin treatment group performance for colonies with evidently lower colony vitality at study initiation due to low colony strength, low brood stores and high *Varroa* infestation levels, the observed total performance, including overwintering performance, is not indicative for an unacceptable effect of an autumn exposure of honey bee colonies to guttating W-BAR seedlings, seed-treated with clothianidin.

The assumption of a treatment-related effect as the reason for the lower overall performance and the lower overwintering success of the clothianidin treatment group is further not supported from the following considerations:

- Intrinsic bee toxicity and exposure levels were not different between imidacloprid and clothianidin colonies

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The analysis of the residue situation of both neonicotinoid compounds, clothianidin and imidacloprid, in guttation fluid on both study locations did not reveal distinct differences, neither in the absolute maximum residue levels (imidacloprid: 15 mg a.s./L, clothianidin:2.3 mg a.s/L) nor in the residue kinetics, which gives no indication that the colonies in the two nitro-substituted neonicotinoid treatment groups were exposed differently over time. Both nitro-substituted neonicotinoid compounds share an identical intrinsic honey bee toxicity (imidacloprid – lowest LD_{50} value: 3.7 ng/bee; clothianidin – lowest LD_{50} value: 2.5 ng/bee; source: Bayer CropScience).

- Recorded symptoms during exposure to guttation exudates were comparable between imidacloprid and clothianidin colonies
The number of bees with behavioural abnormalities did not differ between the clothianidin (13 bees) and the imidacloprid treatment group (48 bees). There were also no distinct differences in the number of honey bees directly observed in the individual assessment areas taking up guttation fluid from seed-treated W-BAR plants, neither during the autumn period nor during springtime (control group – autumn/spring/total: 7/53/60 bees; imidacloprid treatment group – autumn/spring/total: 12/111/123 bees; clothianidin treatment group – autumn/spring/total: 5/58/63 bees).

Thus, when accounting for all of the above-mentioned facts, it can be concluded that the lower performance of the clothianidin treatment group as compared to the imidacloprid treatment and control group, is in fact not treatment related, but can be attributed to a combination of adverse external factors, which affected the clothianidin group, like the allocation of a higher number of weaker colonies (colony strength and brood), higher initial *Varroa* infestation levels as well as a lower suitability of the study sites.

Conclusions
Guttation of W-BAR plants was a regular occurring phenomenon during the autumn and spring growth period of the crop and there is usually a time overlap between presence of guttation fluid and bee flight activity during morning hours.
Honey bees were observed visiting the study fields frequently. Most of the direct honey bee observations within the assessment areas were made directly in front of the hives. The relative proportion of honey bees observed per monitoring on plants in the respective assessment areas in both, treatments and control, was mostly higher in spring 2010 than in autumn 2009. Moreover, also the observed relative proportion of honey bees per monitoring taking up guttation fluid and dew in both, treatment and control, was mostly higher in all assessment zones in spring 2010 as compared to autumn 2009.
Accounting for all honey bees directly observed during the individual monitorings within the assessment area in both, treatments and control, respectively (i.e. 3,148 bees in total; 1,267 bees during autumn and 1,881 bees during springtime), overall a moderate proportion of bees was observed taking up guttation fluid, i.e. 334 bees / 3,148 bees = 10.6 %. Most of the honey bees which took up guttation fluid were observed during springtime (301 of 334 bees) which gives a relative proportion of bees taking up guttation fluid in autumn of 33 bees / 1,267 bees = 2.6 % and of 301 bees / 1,881 bees = 16 % during springtime.

For imidacloprid, the overall maximum measured concentration in guttation fluid, collected from the imidacloprid-treated fields was determined during the autumn growth period of the W-BAR crop and accounted for 15 mg a.s./L. Residues of imidacloprid in guttation fluid were generally higher during the autumn growth period as compared to the spring growth period. During the spring growth period, the maximum measured concentration of imidacloprid within guttation fluid was 0.10 mg a.s./L. The residue levels of its metabolites imidacloprid-5- hydroxy or imidacloprid-olefin in guttation water ranged between < LOD to 0.64 mg/L or between < LOD to 0.05 mg/L, respectively.
The overall maximum measured concentration of clothianidin within guttation fluid, collected from the clothianidin-treated fields, was determined during the autumn growth period of the W-BAR crop and accounted for 2.3 mg a.s./L. As for imidacloprid, the residue levels of clothianidin in guttation fluid were also generally higher during the autumn growth period as compared to the spring growth period. During the spring growth period, the maximum measured concentration of clothianidin within guttation fluid was

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0.18 mg a.s./L. The residue levels of the clothianidin metabolites TZNG and TZMU in guttation water ranged between < LOD to 0.05 mg/L and between < LOD to 0.02 mg/L, respectively.
No treatment related differences in honey bee mortality, colony development in autumn and spring as well as in the overwintering performance were observed between the control and the imidacloprid treatment group. The same conclusion could be drawn for the clothianidin treatment group if appropriate corrections are made for experimental biases concerning colony vitality at study initiation.

Overall, it is concluded that guttation fluid, exudated by winter barley seedlings, seedtreated with nitro-substituted neonicotinoids, does not have unacceptable effects on honey bee colonies under typical commercial use conditions.
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Report:	02.02.05/03; [redacted]; 2014; M-501261-01-4
Title:	Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter barley (W-BAR), seed-treated with the insecticidal seed-treatment product clothianidin + imidacloprid FS 100 + 175 G in Germany in 2011/2012
Report No.:	R11130
Document No.:	M-501261-01-4
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Guideline deviation(s):	not specified
GLP/GEP:	**yes**

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Aim

The field study was conducted in winter barley (W-BAR), grown from seeds treated with the cereals seed-treatment product Clothianidin + Imidacloprid FS 100 + 175 G, in order to investigate the potential effects from exposure to guttating W-BAR, starting from seedling emergence in autumn 2011 until beginning of winter oil-seed rape (W-OSR) flowering in spring 2012. The Assessment Phase and Bee Health Phase lasted from middle of September 2011 until beginning of April 2012. The study fields were located in Hesse, Germany.

Honey bee colonies were set up at the study fields either directly adjacent to the crop or in a distance of approximately 4.5 m to the crop margin. The study comprised one treatment group and one control group: The treatment group, comprising four study fields with altogether five study plots on which W-BAR seeds, seed-treated with Clothianidin + Imidacloprid FS 100 + 175 G and a fungicide (Baytan®) were grown, and a control group, comprising also four study fields with altogether five study plots on which W-BAR seeds, seed-treated with a fungicide (Baytan®, defined as control) were grown. Moreover, all seeds (control + treatment) were additionally seed-treated with commercial INTECO® in order to reduce dust abrasion.

Treatment is defined by the presence and the potential exposure of honey bees to the systemic neonicotinoid insecticides clothianidin and imidacloprid.

All treatment seeds were seed-treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim, Germany. The seed variety was “Campanile”.

All seeds were sown by typical pneumatic cereal sowing machines under typical commercial use conditions.

Key study objectives were to assess acute honey bee mortality and to evaluate and to compare the long-term colony development along with the overwintering performance of exposed honey bee colonies in the two study groups (i.e. treatment and control). Furthermore, the guttation behaviour of W-BAR was surveyed and it was examined whether exudation of guttation fluid of W-BAR and flight activity of honey bees occurred simultaneously.

In case bee flight activity and guttation coincided, the bee activity in the respective study field was surveyed. For this purpose, a specified area (= assessment area) next to the honey bee colonies was intensively monitored for bee visits. Regarding this activity, one “monitoring” is defined by an approximately thirty-five minute continuous observation of the assessment area. In addition, guttation fluid of W-BAR in the treatment group was collected and analysed for residues of clothianidin and imidacloprid. Moreover, dead bee traps were installed at the entrance of the bee hives to record the number of dead bees.

Material and Methods

Test item

W-BAR seeds seed-treated with Clothianidin + Imidacloprid FS 100 + 175 G.

Study sites and sowing

The study was conducted in the vicinity of Gießen in Hesse, Germany, at eight commercially managed agricultural fields (study fields). On four study fields five study plots were established which were assigned as Clothianidin + Imidacloprid FS 100 + 175 G treated plots (defined as study plots sown with W-BAR seeds, seed-treated with Clothianidin + Imidacloprid FS 100 + 175 G plus a routine fungicide

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(Baytan®)) and on four study fields five study plots were established and assigned as control plots (defined as study plots sown with W-BAR seeds, seed-treated only with a routine fungicide (Baytan®)).

Set-up of honey bee hives
At each of the ten study plots (i.e. five treatment and five control plots, respectively), five honey bee colonies were placed along a line shortly before sowing (6 to 13 days), either directly adjacent or within a distance of approximately 4.5 m to the W-BAR crop, depending on the actual local field situation. In total, the treatment and the control group comprised each 25 honey bee colonies.
Assessment area
A specified area (assessment area) in front of the honey bee colonies was intensively monitored. The whole assessment area was divided into two In-Crop Zones (Zone 0 and Zone 1) and an Off-Crop Zone. Zone 0 (width: 5 m to each side of the hives, 2 m depth into the crop) covered the immediate area in front of the bee hives and Zone 1 (a 2 m broad band, shaped like an inverted 'U', with a vertical distance of the band to the field margin of 7 m inside the crop). The bee hives were placed into the Off-Crop Zone, either directly adjacent to the W-BAR crop (Off-Crop Zone: width: 10 m length along the field margin, 1 m depth into the off-crop) or in a distance of approximately 4.5 m to the W-BAR crop (Off-Crop Zone: width: 10 m length along the field margin, 5 m depth into the off-crop). Each assessemtn area had additionally four segregated areas with each 50 W-BAR plants inside in autumn 2011 respectively of one square meter in spring 2012 to record the proportion of W-BAR displaying guttation and/or dew.

Honey bee mortality
Each hive was equipped with a dead bee trap. The traps were emptied daily to record the number of dead honey bees. Additionally, also the number of dead bees from dead bee traps located on a small plot of 0.5 x 0.5 m² in front of each dead trap were recorded.

Guttation fluid sampling
In case guttation was observed in the morning at a respective treatment plot, up to three samples of guttation fluid, each with a volume of approximately 1 mL were collected from various plants of W-BAR. The samples were thereafter stored deep frozen (≤ -18 °C) for later residue analysis.

Monitoring
The monitoring activities on the respective study plots started as soon as the W-BAR plants had emerged on the study fields and the autumn exposure period lasted up to a period of four and a half consecutive weeks until end of October 2011. The monitoring activities re-started in spring 2012 with the beginning of the flowering of the goat willow (Salix caprea) at the vicinity of the exposure plots and lasted for a period of five consecutive weeks until beginning of the flowering of winter oil-seed rape (W-OSR) in the region where the study fields were located.
During morning hours, the respective assessment area on the study plots under investigation was systematically checked for the occurrence of guttation fluid and/or dew. If guttation was still present at the start of honey bee flight activity, the numbers of honey bees resting or walking on the ground or on the W-BAR crop were counted and any potential uptake of guttation fluid or dew by the bees as well as any conspicuous bee behaviour was recorded. The monitoring sessions were stopped if no more guttation fluid was present. During each of the seasonal monitoring sessions (autumn and spring), one observer was continuously responsible for two study plots. The observer alternated between two study plots within two days. The study plot which was monitored in the morning was also re-visited in the evening. During these evening assessments, the onset of guttation and the end of bee flight activity was recorded.
One "monitoring session" lasted approximately 35 minutes and was defined as one complete observation cycle of the assessment area and its associated four segregated areas, at which guttation- and honey bee assessments were conducted during the precense of guttation fluid on the W-BAR crop.

Honey bee colony strength and health assessment
The colony strength and the colony development were assessed according to the Liebefeld method (Imdorf et al. 1987). The first assessment on the study plots was performed two to three days after colony set-up; further assessments were performed every three weeks until end of October 2011. In spring 2012,

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colony development was assessed in the same manner from the beginning of flowering of the goat willow (Salix caprea) until beginning of the flowering of the winter oil-seed rape (W-OSR) in the region. From beginning of November 2011 until the start of goat willow (Salix caprea) flowering, all colonies from treatment and control plots were overwintered on a shared overwintering location. After the last assessment on the respective study plots in spring 2012, all honey bee colonies were transferred to a monitoring site with low exposure to any pesticides and were assessed three weeks later for a final time.

Residue analysis
Guttation fluid as collected throughout the Assessment Phase on the treatment plots was analysed for residues of clothianidin and imidiacloprid by using High Performance Liquid Chromatography (HPLC) chromatographied under isocratic reversed phase conditions and coupled with electrospray and tandem mass spectrometry (MS/MS) detection.
Results
Frequency of guttation
Guttation was a frequent phenomenon during the Assessment Phase. During the assessments in the morning, guttation fluid was observed on W-BAR at 100% of all observation days in autumn 2011 and at 87.6% of the observation days in spring 2012. Guttation in the herbaceous off-crop area was observed at 66.2% in autumn 2011 and at 87.0% in spring 2012. During the course of the observation days, the presence of guttation declined until it ceased, on average at about 12 p.m., both in autumn 2011 and spring 2012.

No remarkable coincidence of W-BAR guttation and bee flight activity was observed in the evening. In most cases with evening guttation in autumn 2011, the guttation lasted for the whole day, due to rainy or damp weather (24.1% on W-BAR and 9.7% in off-crop Zone). In spring 2012, there was only little guttation in the evening at all (4.7% on W-BAR and 4.1% in off-crop Zone).
Honey bee observations
Altogether 734 monitoring sessions (355 in autumn 2011 / 379 in spring 2012) were carried out, which lasted 388 hours (197 h / 191 h). In the morning, bee flight activity and guttation coincided on approximately 70% of all observation days (73.1% / 69.7%), in the evening only on 11.0% of all observation days in autumn 2011. In spring 2012, the whole observed overlap of guttation and bee flight activity lasted only 10 minutes (0.6%).
If there was an overlap between the presence of guttation and bee flight activity during morning hours, the mean overlap time in autumn 2011 was 2 h 35 min and 2 h in spring 2012. In the evening, the mean temporal overlap during autumn 2011 lasted 34 minutes. On average, honey bee flight activity started at 10:13 a.m. and at 18:22 p.m. in autumn 2011, and at 09:51 a.m. and 18:26 p.m. respectively in spring 2012.
In total, 6,973 honey bees were observed within the assessment areas. Most of the observations were made in Zone 0, i.e. directly in front of the hives, followed by the Off-Crop Zone and Zone 1. In spring 2012 most bones bee observations were made in the Off-Crop Zone followed by Zone 0 and Zone 1. In autumn 2011, honey bees taking up guttation fluid (3 times observed) or dew (nine times observed) was a rare phenomenon, while it was more common in spring 2012, were 502 honey bees were observed taking up guttation fluid, respectively 1000 taking up dew. Thus, only a small proportion of honey bees was directly observed taking up guttation fluid.

Honey bee mortality
In autumn 2011, both in control and treatment group, honey bee mortality was on the same, generally low level. With beginning of October 2011, there was a slight increase in both treatment and control group, according to increasing precipitation and decreasing temperatures. There was quite some variability in mortality, even amongst colonies at the same study plot, indicating that there are other factors than weather, location and treatment, which may influence honey bee colonies. There were no distinct, biologically relevant differences between treatment and control (irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop). This conclusion was supported by statistical analysis.

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Colony development
In autumn 2011, the control and the treatment group developed in a normal and similar way, no distinct, biologically relevant differences could be detected in both, the number of adult bees and brood cells. There were no distinct, biologically relevant differences between treatment and control (irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop). This conclusion is supported by statistical analysis. In spring 2012, at the final colony assessment, there were also no distinct, biologically relevant differences in the number of adult bees and brood cells between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop, although the average number of worker bees in the treatment colonies statistically significantly exceeded the corresponding number of the control colonies.
A detailed analysis of the control group revealed an intra-group variability in two out of the five control plots, which ranked behind the other control colonies concerning number of adult bees at the 3rd and 4th colony assessment. Statistical analysis of colony strength withouth considering the data of these 10 colonies (C1-1 to C1-5 and C5-1 to C5-5), eliminated every difference between control and treatment groups.
Development of brood cells was on an empirically normal level and comparable between control and treatment colonies in autumn 2011 and at the 1st colony assessment in March 2012. There were no distinct, biologically relevant differences between treatment and control (irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop). This conclusion is supported by statistical analysis.
From the 5th colony assessment (04 April 2012) onwards, until the end of the Bee Health Phase, treatment colonies displayed a better brood development than control colonies. This might be the result of the unsignificant, but somewhat weaker overwintering performance of some control colonies.

Overwintering performance
After overwintering, colony strength had decreased in both groups when compared to the before-winter-evaluation, which is a typical apidological phenomenon. That equates to an average overwintering index of 57.8 ± 21.1% in control colonies and to an average overwintering index of 67.0 ± 14.1% in treatment colonies. There were no distinct, biologically relevant differences between treatment and control (irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop). This conclusion is supported by statistical analysis. Only one colony (C1-4) had to be removed from the study, as on 14 March 2012 as it was detected to be queenless and was therefore deprieved in bees after overwintering (1625 bees). As a sign of good beekeeping practice, employed throughout the Assessment Phase and Bee Health Phase, no colony was lost during winter time due to scarce food supply, inefficient anti-Varroa treatment or other factors capable of being influenced by the beekeeper.
All colonies preserved colony vitality which would enable a successful further development of the colonies during the upcoming season. However, with respect to an adequate strength for prospective spring honey yield, six control colonies and one treatment colony were not too promising (one control and one treatment colony when excluding the C1 (C1-1 to C1-5) and the C5 (C5-1 to C5-5) group).

Varroa destructor
In autumn 2011, the mean daily Varroa mite fall was on a moderate level. The maximum mean was detected on the last assessment at the end of October 2011 with 16.6 ± 28.2 mites per day in the control group, and 5.1 ± 5.2 mites per day in the treatment group. There were no distinct, biologically relevant differences between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop.
The success of the oxalic acid treatment was shown at the first colony assessment in spring 2012, when no living mites were found in all colonies. At the following three assessments in spring 2012, the mite fall was on a low and comparable level for the control and the treatment group colonies, with a maximum of 0.4 ± 0.4 mites per day in the control group and 1.7 ± 3.1 mites per day in the treatment group end of April 2012 and with 0.2 ± 0.4 mites per day in the control group and 0.9 ± 2.5 mites per day in the treatment group beginning of May 2012. Again, there were no distinct, biologically relevant differences

between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop. Overall, the Varroa infestation was on a generally low level, which did not affect the colonies during this study.

Residue analysis
Residue analysis of guttation fluid, as collected throughout the duration of the Assessment Phase on the treatment plots, revealed that clothianidin and imidacloprid-residues generally peaked shortly after emergence of the dressed W-BAR crop. Residues of clothianidin and imidacloprid declined throughout the autumn observation period until end of October and were generally distinctly lower throughout the spring observation period. The maximum residue level of imidacloprid was 6.65 mg/L (01 October 2011). The maximum residue level of clothianidin was 8.51 mg/L (11 October 2011). The overall maximum observed combined residue level of imidacloprid and clothianidin was 11.78 mg/L (11 October 2011).

Conclusions
Guttation of W-BAR plants was a regular occurring phenomenon during the autumn and spring growth period of the investigated W-BAR crop. Time overlap between presence of guttation fluid and bee flight activity was a common phenomenmon during morning hours, but rarely observed in the evening (if at all, only on a few days in autumn).
Honey bees were observed visiting the study plots frequently in spring, but rarely in autumn. The relative proportion of honey bees observed per monitoring on plants in the respective assessment areas in both, treatment and control, was higher in spring 2012 than in autumn 2011. Moreover, also the observed relative proportion of honey bees per monitoring taking up guttation fluid and dew in both, treatment and control, was higher in all assessment Zones in spring 2012 as compared to autumn 2011, were it was a rare phenomenon. Most of the direct honey bee observations within the assessment areas were made directly in front of the hives.
Accounting for all honey bees, observed during the individual assessments on the study plots throughout the entire field observation period in both, treatment and control, respectively, only a small proportion of bees was directly observed taking up guttation fluid.
Residue analysis of guttation fluid, as collected throughout the duration of the study on the treatment plots, revealed that clothianidin and imidacloprid-residues generally peaked shortly after emergence of the dressed W-BAR crop. Residues of clothianidin and imidacloprid declined throughout the autumn observation period until end of October and were generally distinctly lower throughout the spring observation period. The maximum residue level of imidacloprid was about 6.65 mg a.s./L, the maximum residue level of clothianidin was 8.51 mg a.s./L; the overall maximum observed combined residue level of imidacloprid and clothianidin was 11.78 mg total a.s./L (all maximum values first half of October).
Regarding honey bee mortality, brood, and colony development, colony strength and varroa infestation levels during autumn and spring, there were no distinct, biologically relevant differences between treatment and control (irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop). This conclusion is supported by statistical analysis. There were also no distinct, biologically relevant (nor statistically significant) differences between treatment and control regarding overwintering performance. No treatment related adverse effects were observed during the entire course of the study.

Overall, it can be concluded that guttation fluid, excreted by winter barley, seed-treated with Clothianidin + Imidacloprid FS 100 + 175 G, does not have unacceptable effects on honey bee colonies under typical commercial use conditions, as there were no adverse acute, short-term or long-term effects on colony strength and -development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality, colony health, or on overwintering performance.

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Report:	02.02.05/04; [redacted]; 2014; M-500724-01-3
Title:	A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014
Report No.:	S13-00171
Document No.:	M-500724-01-3
Guideline(s):	OEPP/EPPO Guideline No. 170(4) (2010); SANCO/3029/99 rev. 4 U.S. EPA OCSPP 850.3040
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-500724-01-3@S-602286-01-1

Material and methods:

Test item:

Name: Sugar Beet Pills, prepared with clothianidin, imidacloprid and beta-cyfluthrin; TOX number: TOX10065-00; Batch: ZR02931; content of a.i. (nominal): 0.6 mg/pill clothianidin + 0.3 mg/pill imidacloprid + 0.08 mg/pill beta-cyfluthrin

The potential effects of exposure of honeybees (*Apis mellifera* L.) to guttation liquid from sugar beet plants, grown from sugar beet pills, commercially prepared with the insecticides clothianidin, imidacloprid and beta-cyfluthrin at a treatment rate corresponding to nominally 0.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrin/pill, during the first 6 weeks after emergence, were investigated under field conditions in Germany by following the OEPP/EPPO Guideline No. 170(4), 2010.
The field study consisted of two treatment groups: The test item treatment group T (sugar beet pills, prepared with clothianidin + imidacloprid + beta-cyfluthrin) and the control group C (non-insecticide-treated sugar beet pills). Commercial bee colonies were placed at the field sites shortly after emergence of the plants (T: BBCH 12, C: BBCH 12). The exposure phase started on 0DAE. The mortality of the honeybees was assessed over a period of 5 days shortly before start of exposure and daily after set-up of the colonies at the field sites from

1DAE to 42DAE. Flight intensity and behaviour as well as the number of honeybees visiting sugar beet plants and the occurrence and proportion of guttation on sugar beet plants was assessed daily after set-up of the bee colonies at the field sites from 1DAE to 42DAE. The condition of the colonies was assessed once before set-up of the colonies at the field sites and regularly thereafter after until end of overwintering. The Varroa infestation level was evaluated and samples of honeybees for bee disease and bee virus analysis as well as nectar for AFB analysis was collected to monitor colony health. Samples of guttation liquid from sugar beet plants (test item treatment group T only) were collected for residue analysis.
The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control under consideration of the results of:

- Mean number of dead bees on the linen sheets and in the dead bee traps;
- Flight intensity in the field (mean number of forager bees / 5 x 2 m² / min);
- Observation of honeybees visiting sugar beet plants displaying guttation;
- Occurrence and proportion of guttation;
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date);
- Bee health (bee disease and bee virus analysis);
- Overwintering performance

Dates of work: 15 May 2013 to 26 May 2014

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Findings

	Treatment group	Control (C)	Test Item (T)
Daily mean mortality (dead bees/colony) ± STD	5DBE to 1DBE (Pre-exposure)	21.5 ± 26.2	14.8 ± 9.8
	1DAE to 42DAE (Exposure)	12.9 ± 4.7	16.6 ± 5.4

DAE: days after start of exposure; DBE: days before start of exposure; STD: standard deviation

Mortality
During the pre-exposure period at the monitoring site (5DBE to 1DBE), the mean daily mortality, assessed by using dead bee traps, was approximately on the same level in the control group and in the test item treatment group (21.5 and 14.8 dead bees/colony/day for the control group C and treatment group T, respectively).
Throughout the entire field exposure period of the colonies, no conspicuous differences regarding the mortality levels were observed on a daily basis between the test item treatment group and the control group. During the entire exposure period at the field sites (assessed from 1DAE to 42DAE), the mean daily mortality, assessed by dead bee traps, was 12.9 and 16.6 dead bees/colony/day for the control group C and treatment group T, respectively.

On the linen sheets, spread out in the test fields (mortality within the crop area), throughout the entire exposure period, a mean of 0.3 and 0.2 dead bees/day was found in C and T, respectively.
Thus, no notable difference in mortality was observed between the control group and the test item treatment group during the entire exposure period.

Flight Intensity in the Field and Observation of Honeybees Visiting Sugar Beet Plants
The assessments of flight intensity in the field and the observation of honeybees visiting sugar beet plants were conducted in the morning after flight activity at the hive entrances had started. During the entire assessment period from 1DAE to 42DAE, a total of 5 honeybees was observed in the observation areas in the control group, whereas a total of 4 honeybees was observed in the test item treatment group. In the control group, 4 honeybees were flying over the crop and 1 honeybee was located on sugar beet plants. In the test item treatment group, 3 honeybees were flying over the crop and 1 honeybee was located on sugar beet plants. No honeybees taking up guttation liquid were observed in both, the control and the test item treatment group during the entire observation period.
Overall, the number of honeybees observed in the five in-crop assessment areas was on the same low level, in both, the control and the test item treatment group. There were no notable differences between the test item treatment group and the control group.

Behaviour of the Bees
During the assessment period from 1DAE to 42DAE, small numbers of honeybees exhibiting abnormal behaviour were observed on 5 out of 42 days in the test item treatment group and on 4 out of 42 days in the control group. On the remaining days, only normal behaviour was recorded in both treatment groups.
Clustering of large numbers of honeybees at the hive entrance was observed in single colonies on 2 out of 42 days in the test item treatment group (1 colony/assessment date), and also on 2 out of 42 days in the control (1 colony/assessment date).

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One cramping honeybee was observed on 1 out of 42 days in the test item treatment group (1 bee/8 colonies and assessment date), and also on 1 out of 42 days in the control (1 bee/8 colonies and assessment date).
On 1 out of 42 days, one honeybee in the control group showed locomotion problems. In the test item treatment group, this behaviour was not observed during the entire assessment period.

One trembling honeybee was observed on 1 out of 42 days in the test item treatment group (1 bee/8 colonies and assessment date), and also on 1 out of 42 days in the control (1 bee/8 colonies and assessment date).
Small numbers of inactive honeybees were observed on 2 out of 42 days in the test item treatment group (range: 24–31 bees/8 colonies and assessment date). No inactive honeybees were recorded in the control group.

Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behaviour was observed between the test item treatment group and the control. If abnormal behaviour was observed, it was only observed in a small number of honeybees on all assessment dates in both, in the test item treatment group and in the control group.
Thus, no test-item related adverse effects on honeybee behaviour were observed.

Occurrence of Guttation and Percentage of Plants Displaying Guttation
In the control group, guttation of sugar beet plants in the assessment areas was observed on 1 out of 42 assessment days. In the concurrently assessed off-crop area, guttation occurred on 22 out of 42 assessment days. In the test item treatment group, guttation of sugar beet plants in the assessment areas was observed on 11 out of 42 assessment days. In the concurrently assessed off-crop area, guttation occurred on 26 out of 42 assessment days.
When guttation occurred in the in-crop assessment areas in the control group, the percentage of plants exhibiting guttation per assessment area varied from 2.7 % to 5.3 %. In the test item treatment group, the percentage of plants exhibiting guttation per assessment area varied from 2.4 % to 30.0 %, when guttation was detected.
Overall, guttation occurred only infrequently in sugar beets, and if, the overall abundance of guttation droplets was rather low, particularly when compared to adjacent off-crop areas.

Condition of the Colonies
Strength of the Colonies
The mean number of bees per colony assessed during the first colony assessment on 11 Jun 2013 (2DBE) shortly before start of exposure revealed a mean colony strength of 16981 bees/colony in the control C (range: 11115 to 20605) and 17152 bees/colony in the test item treatment group T (range: 17355 to 20800).
At the second colony assessment on 03 Jul 2013 (20DAE), during exposure, the mean colony strength had increased in C (21060 bees/colony; range: 16315 to 31525) as well as in T (20914 bees/colony, range: 13910 to 31785). The increase of colony strength was approx. equal in the control and in the test item treated group.

At the third colony assessment on 25 Jul 2013 (42DAE), at the end of the exposure period, the mean colony strength had slightly decreased in C (16835 bees/colony; range: 12220 to 22165) as well as in T (18257 bees/colony; range: 13910 to 24115). The extent of decrease of colony strength was similar in the control and in the test item treated group.
At the fourth colony assessment on 20 Aug 2013 (68DAE), three to four weeks after the end of the exposure period and relocation of the colonies to the monitoring site, the mean colony strength had again slightly increased in C (19013 bees/colony; range: 11505 to 25090) as well as in T (21296 bees/colony, range: 9815 to 28600), virtually back to the level of the second colony assessment.
During the subsequent colony assessments on 17 Sep 2013 (96DAE), 15 Oct 2013 (124DAE, start of overwintering) and 13 Mar 2014 (273DAE, end of overwintering), the mean colony strength in the control and in the test item treated group followed the natural course of colony strength development,

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with a decreasing tendency from late summer to autumn and spring of the following year. At the start of overwintering in autumn 2013, the mean colony strength was 12537 bees/colony in C (range: 9425 to 16965) and 15096 bees/colony in T (range: 12285 to 19760). At the end of overwintering in early spring 2014, the mean colony strength was 8491 bees/colony in C (range: 6695 to 10205) and 8296 bees/colony in T (range: 5070 to 12025).

Throughout the entire observation period, the mean colony strength in the test item treatment group T was on the same level as or slightly higher than in the control group C.
Thus, no test-item related adverse effects on colony strength were observed during the entire course of the study.

Brood Stages and Overwintering Performance
In the colonies of the control group C and the test item treatment group T, the natural and typical changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred during the observation period. From 11 Jun 2013 (2DBE) up to and including 20 Aug 2013 (68DAE), all colonies in the control (except colony Ca on 25 Jul 2013 (42DAE); see below) and in the test item treatment group (except Th on 03 Jul 2013 (20DAE) and 25 Jul 2013 (42DAE) and Td and Tf on 20 Aug 2013 (68DAE); see below) contained all brood stages during the brood assessments.

In colony Ca, no larvae were present on 25 Jul 2013. This was most probably due to a loss of the queen during or shortly after the previous colony assessment. The absence of the queen in Ca was first noticed during a beekeeper check on 17 Jul 2013 as well as a hatched queen cell. Since Ca contained cells with eggs on 25 Jul 2013, a new queen had been raised by the colony.
In colony Th, no eggs were present on 03 Jul 2013 and no brood cells at all on 25 Jul 2013. A new queen was added to this colony on 25 Jul 2013.
In the colonies Td and Tf, no eggs and larvae were present on 20 Aug 2013. This was probably due to the loss of the respective queens either during the colony assessment and samplings for bee disease and bee virus analysis on 25 Jul 2013 or during transport to the monitoring site on 26 Jul 2013. In Td, a new queen was added on 03 Sep 2013. In Tf, a new queen had been raised by the colony. During the colony assessment on 17 Sep 2013, all brood stages were present again in the colonies Td and Tf.

In late summer and early autumn, when the natural period of breeding activity of the colonies came to an end, the number of cells with brood had notably declined in both, the control and the test item treatment group up to the colony assessment on 17 Sep 2013 (96DAE). On the last colony assessment before start of overwintering on 15 Oct 2013 (124DAE), no (Ca, Cc-Cg, Tc, Te, Tg) or only a relatively small number of cells with brood (Cb, Ch, Ta, Tb, Td, Tf, Th) were observed in C and T.
The overwintering period lasted from 15 October 2013 until 13 Mar 2014. After overwintering, all colonies of the test item treatment group and the control were viable and all were found to have resumed breeding activity.
Thus, no test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance.

Food Storage
In the colonies of the control group C and the test item treatment group T, respectively, the natural and typical changes and fluctuations in the relative amount of nectar and pollen storage cells occurred during the observation period. The control group C and the test item treatment group T showed approximately equal mean numbers of pollen and nectar storage cells throughout the entire observation period.
Thus, no test item-related adverse effects on the food storage of the exposed colonies were observed.

Colony Health
Evaluation of Varroa Infestation in the Colonies
Varroa mite occurrence in the colonies was assessed via a ‘Varroa board’ beneath the hives. The infestation level of a colony was monitored by counting dead mites on the board.

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From the first assessment on 20 Aug 2013 (Varroa board was inserted on 01 Aug 2013) to 15 Oct 2013, small or medium mean numbers of mites were detected. The mean Varroa infestation levels in the test item treatment colonies were moderately higher than in the control colonies during all assessments. However, the detailed bee disease analysis (see chapter 1.2.5.4.2) revealed that already the initial Varroa infestation level in the (future) test item treatment group (on 11 Jun 2013) was slightly to moderately higher as compared to the (future) control group before the actual set-up of the colonies on their respective exposure fields.

Bee Diseases
Samples from three sampling dates in 2013 and one sampling date in 2014 were analysed for the pathogens Nosema sp., Malpighamoeba mellificae, Varroa destructor and Paenibacillus larvae.
In the bee samples taken from the control colonies before start of exposure, Nosema sp. spores were found in colonies Cb, Cc and Ch (medium infestation level). Control colonies Ca, Cd, Ce and Cg were free of analysable spores. No bee sample was available from control colony Cf.
In the bee samples taken from control colonies at end of exposure the control colony Ca had a low infestation level and the control colony Cg had a medium infestation level with Nosema sp. spores. The control colonies Cb, Cc, Cd, Ce, Cf and Ch were free of analysable spores.
In the bee samples taken at start of overwintering no Nosema sp. spores were found in any sample taken from control colonies.

In the control bee samples taken at end of overwintering Nosema sp. spores were analysed only in control colony Ce (low infestation level). All other control colonies were free of analysable spores.
The highest infestation rate with Varroa mites was 2.5 % in the bee sample taken from the control colony Ca at end of exposure. In all other bee samples examined the Varroa infestation rate was between 0.0 % and 2.1 %.
In the bee samples taken from the test item treatment colonies before start of exposure, Nosema sp. spores were on a medium level in test item treatment colonies Ta, Tc and Tf. Test item treatment colonies Tb, Td, Te, Tg and Th were free of analysable spores.
In the bee samples taken at end of exposure, two test item treatment colonies had a low infestation level (Tf and Th), six test item treatment colonies were free of analysable spores (Ta, Tb, Tc, Td, Te and Tg).
In the samples taken at start of overwintering, test item treatment colonies Ta and Th had a medium infestation level. No Nosema sp. spores were found in any of the other test item treatment colonies (Tb, Tc, Td, Te, Tf and Tg).
In the samples taken at end of overwintering, test item treatment colonies Ta and Tg had a low infestation level and test item treatment colony Th had a medium infestation level. In all other colonies no infestation with Nosema sp. spores was analysed.

The highest infestation rate with Varroa mites in samples taken from the test item treatment colonies was found in colony Te with 10.8 %, followed by colony Tf with 7.5 % at the start of overwintering. The test item treatment colonies Te and Tf showed however normal Varroa infestation rates after overwintering. The infestation rate of all other test item treatment colonies varied between 0.0 % and 5.2 %.
No Malpighamoeba mellificae and no spores of Paenibacillus larvae were found in any of the samples taken in 2013 and 2014 neither in the control nor in the test item treatment colonies.
Overall, no distinct differences in the bee health status between the colonies of the control group and the test item treatment group could be observed.

Bee Viruses
The objective of the bee virus analysis was to determine the following bee viruses in bee samples collected at different time points of the year: DWV (deformed wing virus), SBV (sacbrood virus), ABPV (acute bee paralysis virus), CBPV (chronic bee paralysis virus), KBV (Kashmir bee virus), IAPV (Israeli acute paralysis virus), BQCV (black queen cell virus).
The bee viruses CBPV, KBV and IAPV were not detected in any of the samples taken at any time point.
BQCV was detected in five out of eight colonies of the test item treatment group, but not in the control group at the time point ‘before start of exposure’ on 11 Jun 2013. The pre-exposure BQCV infestation

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level was therefore slightly higher in the test item treatment group. At the time point 'end of exposure' on 25 Jul 2013, BQCV was detected in all eight colonies of the test item treatment group, but also in seven out of eight colonies of the control group. At the start and at the end of overwintering, the colonies of both treatment groups were free of BQCV. The BQCV infestation level in the test item treatment group after the start of exposure to the test item showed therefore no differences to the control group.

DWV was detected in two samples taken from colonies of the test item treatment group (Ta, Tg), but not in the control group at the time point 'start of overwintering' on 15 Oct 2013. At the time point 'after overwintering' in spring 2014, all colonies of the control group and the test item treatment group were free of DWV.
SBV was detected in one colony of the test item treatment group (Td) and in six colonies of the control group at the time point 'end of exposure' on 25 Jul 2013. At the start and at the end of overwintering, the colonies of the control and of the test item treated groups were free of SBV.
ABPV was detected at the time point 'end of exposure' on 25 Jul 2013 in three out of eight colonies of the test item treatment group, but not in the control. At the start and at the end of overwintering, the colonies of both treatment groups were free of ABPV.
The fact that increased infestation levels of DWV, SBV and ABPV in a small fraction of the test item treatment colonies were only observed once during the observation period and since each virus was not detectable anymore in samples from the following time point, suggests that the increased infestation levels were only a temporary phenomenon and of no notable consequences for the affected colonies.
Overall, no distinct differences in the bee health status in terms of virus infestation between the colonies of the control group and the test item treatment group could be observed.

Residue Analysis
The determined clothianidin residues in guttation liquid, as analysed in the samples collected on each day where guttation droplets were actually present on the sugar beet plants in the test item treatment group T, were within the range of 153-327, 35-57 and 36-53 µg/kg for parent clothianidin and its metabolites TZNG and TZMU, respectively. The corresponding imidacloprid residues were within the range of 18-61, 6.9-16 and 1.9-4.0 µg/kg for parent imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, respectively. Residues of beta-cyfluthrin in all guttation liquid samples were virtually inexistent.

Conclusion
The objective of this study was to determine the potential effects of exposure of honeybees (Apis mellifera L.) to guttation liquid from sugar beet plants grown from pills, commercially prepared with the insecticides clothianidin, imidacloprid and beta-cyfluthrin at a rate corresponding to nominally 0.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrin/pill during the first 6 weeks after emergence under field conditions.
Guttation in the test fields was observed on 1 out of 42 days in the test item treatment group and on 11 out of 42 days in the control. During the entire assessment period at the exposure sites, a total of 5 honeybees was observed in the assessment areas in the control group, whereas a total of 4 honeybees was observed in the test item treatment group. The number of honeybees observed in the crop was therefore on the same level in both the control and the test item treatment group. Overall, guttation occurred only infrequently in sugar beets, and if, the overall abundance of guttation droplets is rather low, particularly when compared to adjacent off-crop areas.
No test item-related adverse effects were observed on mortality and behaviour of the honeybees.

No test item-related adverse effects were observed on colony development (including colony strength, brood development and food storage of the colonies) as well as on overall colony vitality throughout the entire field exposure period and throughout the entire monitoring period until the end of overwintering in spring 2014.
No test item-related adverse effects were observed on colony health with respect to the pathogens Nosema sp., Malpighamoeba mellificae, Varroa destructor and Paenibacillus larvae as well as to all bee viruses analysed in the course of this study.

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The assessment of the Varroa infestation via Varroa boards beneath the hives indicated moderately higher Varroa infestation levels in the test item treatment group when compared to the control colonies during all assessments. A closer examination during bee disease analysis by the use of the anatomic test for the infestation of dead bees with Varroa mites revealed that two out of eight colonies of the test item group (Te, Tf) exhibited Varroa infestation levels above 7 % at the start of overwintering in late autumn, whereas all colonies of the control group showed normal infestation levels.

Varroa infestation levels above 7°% in late autumn may be regarded as critical for the overwintering performance. However, the overwintering performance of the colonies of the test item treatment group (including the colonies Te and Tf) was not adversely affected when compared to the performance of the control group. Moreover, the test item treatment colonies Te and Tf showed normal Varroa infestation rates at the end the overwintering period in early spring of the following year.
Overall, it can be concluded that the exposure of honeybee colonies to guttation liquid from sugar beet plants, grown from pills, commercially prepared with the insecticides clothianidin, imidacloprid and beta-cyfluthrin at a rate corresponding to nominally 0.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrin/pill during the first 6 weeks after emergence, did neither cause acute, short-term nor long-term adverse effects on mortality, honeybee behaviour, colony strength, colony health and vitality, brood- and food development and overwintering performance in the exposed colonies.

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Report:	02.02.05/05; [redacted]; 2014; M-500734-01-3
Title:	A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014
Report No.:	S13-00170
Document No.:	M-500734-01-3
Guideline(s):	OEPP/EPPO Guideline No. 170(4) (2010); SANCO/3029/99 rev. 4 U.S. EPA OCSPP 850.3040
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-500734-01-3@S-602289-01-1

1.1 Material and methods:

Test item:

Sugar Beet Pills, prepared with clothianidin, imidacloprid and beta-cyfluthrin; TOX number: TOX10065-00; Batch: ZR02931; content of a.i. (nominal): 0.6 mg/pill clothianidin + 0.3 mg/pill imidacloprid + 0.08 mg/pill beta-cyfluthrin

The potential effects of exposure of honeybees (Apis mellifera L.) to guttation liquid from sugar beet plants, grown from sugar beet pills, commercially prepared with the insecticides clothianidin, imidacloprid and beta-cyfluthrin at a treatment rate corresponding to nominally 0.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrin/pill, during the first approximately 6 weeks after emergence, were investigated under field conditions in Germany by following the OEPP/EPPO Guideline No. 170(4), 2010.

The field study consisted of two treatment groups: The test item treatment group T (sugar beet pills, prepared with clothianidin + imidacloprid + beta-cyfluthrin) and the control group C (non-insecticide-treated sugar beet pills). Commercial bee colonies were placed at the field sites shortly after emergence of the plants (T: BBCH 12, C: BBCH 12-14). The exposure phase started on 0DAE. The mortality of the honeybees was assessed over a period of 5 days shortly before start of exposure and daily after set-up of the colonies at the field sites from 1DAE to 40DAE. Flight intensity and behaviour as well as the number of honeybees visiting sugar beet plants and the occurrence and proportion of guttation on sugar beet plants was assessed daily after set-up of the bee colonies at the field sites from 1DAE to 40DAE. The condition of the colonies was assessed once before set-up of the colonies at the field sites and regularly thereafter until end of overwintering. The Varroa infestation level was evaluated and samples of honeybees for bee disease and bee virus analysis as well as nectar for AFB analysis was collected to monitor colony health. Samples of guttation liquid from sugar beet plants (test item treatment group T only) were collected for residue analysis.

The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control under consideration of the results of:

- Mean number of dead bees on the linen sheets and in the dead bee traps;
- Flight intensity in the field (mean number of forager bees / 5 x 2 m² / min);
- Observation of honeybees visiting sugar beet plants displaying guttation;
- Occurrence and proportion of guttation;
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date);
- Bee health (bee disease and bee virus analysis);
- Overwintering performance

Dates of work: 15 May 2013 to 26 May 2014

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1.2 **Findings**

	Treatment group	Control (C)	Test item (T)
Daily mean mortality (dead bees/colony) ± STD	15DBE to 11DBE (Pre-exposure)	22.4 ± 5.7	21.5 ± 7.6
	1DAE to 40DAE (Exposure)	13.1 ± 2.9	14.1 ± 3.0

DAE: days after start of exposure; DBE: days before start of exposure; STD: standard deviation

1.2.1 **Mortality**

During the pre-exposure period at the monitoring site (15DBE to 11DBE), the mean daily mortality, assessed by using dead bee traps, was on the same level in the control group and in the test item treatment group (22.4 and 21.5 dead bees/colony/day for the control group C and test item treatment group T, respectively).

Throughout the entire field exposure period of the colonies, no conspicuous differences regarding the mortality levels were observed on a daily basis between the test item treatment group and the control group. During the entire exposure period at the field sites (assessed from 1DAE to 40DAE), the mean daily mortality, assessed by dead bee traps, was 13.1 and 14.1 dead bees/colony/day for the control group C and test item treatment group T, respectively.

On the linen sheets, spread out in the test fields (mortality within the crop area), throughout the entire exposure period, a mean of 0.3 and 0.0 dead bees/day was found in C and T, respectively.

Thus, no notable difference in mortality was observed between the control group and the test item treatment group during the entire exposure period.

1.2.2 Flight Intensity in the Field and Observation of Honeybees Visiting Sugar Beet Plants

The assessments of flight intensity in the field and the observation of honeybees visiting sugar beet plants were conducted in the morning after flight activity at the hive entrances had started. The flight assessment areas were all located close to the colonies, with a distance of 10–15 m to the hives. During the entire assessment period from 1DAE to 40DAE, a total of 77 honeybees was observed in the observation areas in the control group, as well as in the test item treatment group. In the control group, 56 honeybees were flying over the crop, 14 honeybees were located on sugar beet plants and 7 honeybees were observed on the soil. In the test item treatment group, 53 honeybees were flying over the crop, 15 were located on sugar beet plants and 9 honeybees were observed on the soil. No honeybees taking up guttation liquid were observed in both the control and the test item treatment group during the entire observation period. Overall, the number of honeybees observed in the five in-crop assessment areas was on the same low level, in both the control and the test item treatment group. There were no notable differences between the test item treatment group and the control group.

1.2.3 Behaviour of the Bees

During the assessment period from 1DAE to 40DAE, small numbers of honeybees exhibiting abnormal behaviour were observed on 30 out of 40 days in both the test item treatment group and the control group. On the remaining days, only normal behaviour was recorded in both treatment groups.

On 1 out of 40 days, honeybees from one colony in the control group showed aggressiveness towards other honeybees (fighting at the hive entrance). In the test item treatment group, this behaviour was not observed during the entire assessment period.

Intensive cleaning was observed in a small number of honeybees on 16 out of 40 days in the test item treatment group (range: 1–10 bees/8 colonies and assessment date), and on 8 out of 40 days in the control group (range: 1–2 bees/8 colonies and assessment date).

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Clustering of large numbers of bees at the hive entrance was observed in a minor fraction of the colonies on 3 out of 40 days in the test item treatment group (range: 1–2 colonies/assessment date), and also on 3 out of 40 days in the control (range: 1–2 colonies/assessment date).
Cramping was observed in a small number of honeybees on 12 out of 40 days in the test item treatment group (range: 1–4 bees/8 colonies and assessment date), and on 11 out of 40 days in the control (range: 1–2 bees/8 colonies and assessment date).
Locomotion problems were observed in a small number of honeybees on 24 out of 40 days in the test item treatment group (range: 1–9 bees/8 colonies and assessment date), and on 19 out of 40 days in the control (range: 1–6 bees/8 colonies and assessment date).
Trembling was observed in a small number of honeybees on 10 out of 40 days in the test item treatment group (range: 1–5 bees/8 colonies and assessment date), and on 5 out of 40 days in the control (range: 1–3 bees/8 colonies and assessment date).
Small numbers of inactive honeybees were observed on 29 out of 40 days in the test item treatment group (range: 1–25 bees/8 colonies and assessment date), and on 27 out of 40 days in the control (range: 1–15 bees/8 colonies and assessment date). It has to be noted that the assessments were conducted early in the day and the numbers of inactive honeybees may as well include cold-impaired bees.
Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behaviour was observed between the test item treatment group and the control. If abnormal behaviour was observed, it was only observed in a small number of honeybees on all assessment dates in both, in the test item treatment group and in the control group.
Thus, no test-item related adverse effects on honeybee behaviour were observed.

1.2.4 Occurrence of Guttation and Percentage of Plants Displaying Guttation
In the control group, guttation of sugar beet plants in the assessment areas was observed on 3 out of 40 assessment days. In the concurrently assessed off-crop area, guttation occurred on 25 out of 40 assessment days. In the test item treatment group, guttation of sugar beet plants in the assessment areas was observed on 5 out of 40 assessment days. In the concurrently assessed off-crop area, guttation occurred on 20 out of 40 assessment days.
When guttation occurred in the in-crop assessment areas in the control group, the percentage of plants exhibiting guttation per assessment area varied from 2.9 % to 57.1 %. In the test item treatment group, the percentage of plants exhibiting guttation per assessment area varied from 3.0 % to 82.1 %, when guttation was detected.
Overall, guttation occurred only infrequently in sugar beets, and in the overall abundance of guttation droplets was rather low, particularly when compared to adjacent off-crop areas.

1.2.5 Condition of the Colonies
1.2.5.1 Strength of the Colonies
The mean number of bees per colony assessed during the first colony assessment on 12 Jun 2013 (2DBE) shortly before start of exposure revealed a mean colony strength of 15933 bees/colony in the control C (range: 8190 to 24635) and 15340 bees/colony in the test item treatment group T (range: 8580 to 24765).
At the second colony assessment on 04 Jul 2013 (20DAE) during exposure, the mean colony strength had increased in C (18428 bees/colony; range: 10530 to 23400) as well as in T (24651 bees/colony, range: 17335 to 29250). The increase of colony strength was more pronounced in the test item treatment group.
At the third colony assessment on 24 Jul 2013 (40DAE) at the end of the exposure period, the mean colony strength had moderately decreased in C (11724 bees/colony; range: 3510 to 17745) as well as in T (19419 bees/colony; range: 16575 to 24830). The decrease of colony strength was more pronounced in the control group.
At the fourth colony assessment on 13 Aug 2013 (60DAE), approximately three weeks after the end of the exposure period and relocation of the colonies to the monitoring site, the mean colony strength had again increased in C (22319 bees/colony; range: 13325 to 31005) as well as in T (24651 bees/colony, range: 18070 to 32175) back to the level of the second colony assessment.
During the subsequent colony assessments on 16 Sep 2013 (94DAE), 14 Oct 2013 (122DAE, start of overwintering) and 10 Mar 2014 (269DAE, end of overwintering), the mean colony strengths in the control and in the test item treatment group followed the natural course of colony strength development,

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with a decreasing tendency from late summer to autumn and spring of the following year. At the start of overwintering in autumn 2013, the mean colony strength was 11724 bees/colony in C (range: 8840 to 15145) and 11594 bees/colony in T (range: 7865 to 16965). At the end of overwintering in early spring 2014, the mean colony strength was 7670 bees/colony in C (range: 4745 to 10140) and 9815 bees/colony in T (range: 7215 to 12805).
Throughout the entire observation period, the mean colony strength in the test item treatment group T was on the same level as or slightly higher than in the control group C.
Thus, no test-item related adverse effects on colony strength were observed during the entire course of the study.

1.2.5.2 Brood Stages and Overwintering Performance
In the colonies of the control group C and the test item treatment group T the natural and typical changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred during the observation period. From 12 Jun 2013 (2DBE) up to and including 13 Aug 2013 (60DAE), all colonies in the control (except colony Ce on 04 Jul 2013 (20DAE), see below) and in the test item treatment group contained all brood stages during the brood assessments.
In colony Ce, no pupae were present on 04 Jul 2013. This was most probably due to a loss of the queen during or shortly after the first colony assessment. The absence of the queen in Ce was first noticed during a beekeeper check on 19 Jun 2013 and a new queen was added to colony Ce on 26 Jun 2013.
In early autumn, when the natural period of breeding activity of the colonies ended, the number of cells with brood had notably declined in both, the control and the test item treatment group on the day of the colony assessment on 16 Sep 2013 (94DAE). On the last colony assessment before start of overwintering, on 14 Oct 2013 (122DAE), no brood stages were observed in C and T (except residual amounts of pupae in the colonies Cg and Ch as well as Ta, Tc and Td).
The overwintering period lasted from 14 October 2013 until 10 Mar 2014. After overwintering, all colonies of the test item treatment group and the control were viable and all were found to have resumed breeding activity (except colony Ce).
Thus, no test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance.

1.2.5.3 Food Storage
In the colonies of the control group C and the test item treatment group T, respectively, the natural and typical changes and fluctuations in the relative amount of nectar and pollen storage cells occurred during the observation period. The control group C and the test item treatment group T showed approximately equal mean numbers of pollen and nectar storage cells throughout the entire observation period.
Thus, no test item-related adverse effects on the food storage of the exposed colonies were observed.

1.2.5.4 Colony Health
1.2.5.4.1 Evaluation of Varroa Infestation in the Colonies
Varroa mite occurrence in the colonies was assessed via a 'Varroa board' beneath the hives. The infestation level of a colony was monitored by counting dead mites on the board.
From the first assessment on 03 Sep 2013 (Varroa board was inserted on 13 Aug 2013) to 14 Oct 2013 only small numbers of mites were detected.
Both, control and test item treatment colonies showed approximately the same low Varroa infestation levels during the course of the study and at the end of the honeybee season. No test item-related adverse effects were detected.

1.2.5.4.2 Bee Diseases
Samples from three sampling dates in 2013 and one sampling date in 2014 were analysed for the pathogens Nosema sp., Malpighamoeba mellificae, Varroa destructor and Paenibacillus larvae.
In the bee samples taken from the control colonies before start of exposure, Nosema sp. spores were found in colonies Cb, Cc, Cf and Cg (medium infestation level). Control colonies Ca, Cd, Ce and Ch were free of analysable spores.

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In the bee samples taken at the end of exposure, no Nosema sp. spores were found in any sample taken from control colonies.
In the bee samples taken from the control colonies at the start of overwintering, the control colonies Ca, Cf and Cg had a low infestation level and the control colony Ce had a medium infestation level with Nosema sp. spores. The control colonies Cb, Cc, Cd and Ch were free of analysable spores.
In the control bee samples taken at end of overwintering, Nosema sp. spores were analysed in control colonies Cc and Cg (medium infestation level). All other control colonies were free of analysable spores.
The highest infestation rate with Varroa mites was 4.4 % in the bee sample taken from the control colony Ch at start of overwintering. In all other bee samples examined, the Varroa infestation rate was between 0.0 % and 1.1 %.
In the bee samples taken from the test item treatment colonies before the start of exposure, Nosema sp. spores were on a low level in test item treatment colony Th, on a medium level in colony Tg and on a high level in colony Tc. Test item treatment colonies Ta, Tb, Td, Te and Tf were free of analysable spores.
In the bee samples taken at the end of exposure, one test item treatment colony had a low infestation level (Ta) and one test item treatment colony had a high infestation level (Th), six test item treatment colonies were free of analysable spores (Tb, Tc, Td, Te, Tf and Tg).
In the samples taken at the start of overwintering, the test item treatment colonies Te and Th had a medium infestation level. No Nosema sp. spores were found in any of the other test item treatment colonies (Ta, Tb, Tc, Td, Tf and Tg).
In the bee samples taken at the end of overwintering, no Nosema sp. spores were found in any sample taken from test item treatment colonies.
The highest infestation rate with Varroa mites in samples taken from the test item treatment colonies was found in colony Tf with 1.3 % before overwintering. The infestation rate of all other test item treatment colonies varied between 0.0 % and 0.5 %.
No Malpighamoeba mellificae and no spores of Paenibacillus larvae were found in any of the samples taken in 2013 and 2014, neither in the control nor in the test item treatment colonies.
Overall, no distinct differences in the bee health status between the colonies of the control group and the test item treatment group could be observed.

1.2.5.4.3 Bee Viruses

The objective of the bee virus analysis was to determine the following bee viruses in bee samples collected at different time points of the year: DWV (deformed wing virus), SBV (sacbrood virus), ABPV (acute bee paralysis virus), CBPV (chronic bee paralysis virus), KBV (Kashmir bee virus), IAPV (Israeli acute paralysis virus), BQCV (black queen cell virus).
In this study the viruses CBPV, DWV, KBV, and IAPV were not detected in any of the samples taken between ‘before exposure’ in 2013 and ‘end of overwintering’ in 2014.
BQCV was detected in one samples taken from colonies of the control group (Cb), but not in the test item treatment group at the time point ‘before start of exposure’ on 16 Jun 2013. At the time point ‘end of exposure’ on 24 Jul 2013, BQCV was detected in five colonies of the test item treatment group (Tc, Td, Te, Tf, Tg), as well as in five colonies of the control group (Ca, Ce, Cf, Cg, Ch). At the start and at the end of overwintering, the colonies of both treatment groups were free of BQCV. The BQCV infestation level in the test item treatment group showed therefore no differences to the control group.
SBV was detected in two samples taken from colonies of the test item treatment group (Ta, Tb), but not in the control group at the time point ‘before start of exposure’ on 16 Jun 2013. The pre-exposure SBV infestation level was therefore slightly higher in the test item treatment group. At the time point ‘end of exposure’ on 24 Jul 2013, SBV was detected in five different colonies of the test item treatment group (Tc, Te, Tf, Tg, Th), but not in the control group.
ABPV was detected at the time point ‘end of exposure’ on 24 Jul 2013 in the samples of two colonies of the test item treatment group, but not in the control.
However, at the time point ‘start of overwintering’ on 14 Oct 2013, all test item treatment as well as all control colonies were free of SBV and ABPV, suggesting that the increased SBV and ABPV infestation levels in the test item treatment group were only a temporary phenomenon and of no notable

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consequences for the affected colonies. At the time point 'after overwintering' in spring 2014, all colonies of the control group and the test item treatment group were free of SBV and ABPV.
Overall, no distinct differences in the bee health status in terms of virus infestation between the colonies of the control group and the test item treatment group could be observed.

1.2.6 Residue Analysis

The determined clothianidin residues in guttation liquid, as analysed in the samples collected on each day where guttation droplets were actually present on the sugar beet plants in the test item treatment group T, were within the range of 17-64, 2.9-12 and 3.1-11 µg/kg for parent clothianidin and its metabolites TZNG and TZMU, respectively. The corresesponding imidacloprid residues were within the range of 2.9-10, 1.2-4.2 and < LOQ-1.3 µg/kg for parent imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, respectively. Residues of beta-cyfluthrin in all guttation liquid samples were virtually inexistent.

1.3 Conclusion

The objective of this study was to determine the potential effects of exposure of honeybees (Apis mellifera L.) to guttation liquid from sugar beet plants, grown from pills, commercially prepared with the insecticides clothianidin, imidacloprid and beta-cyfluthrin at a rate corresponding to nominally 0.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrin/pill during the first approximately 6 weeks after emergence under field conditions.
Guttation in the test fields was observed on 5 out of 40 days in the test item treatment group and on 3 out of 40 days in the control. During the entire assessment period at the exposure sites, a total of 77 honeybees was observed in the assessment areas in the control group as well as in the test item treatment group. The number of honeybees observed in the crop was therefore on the same level in both the control and the test item treatment group. Overall, guttation occurred only infrequently in sugar beets, and if, the overall abundance of guttation droplets was rather low, particularly when compared to adjacent off-crop areas.
No test item-related adverse effects were observed on mortality and behaviour of the honeybees.
No test item-related adverse effects were observed on colony health, colony development (including colony strength, brood development and food storage of the colonies) as well as on overall colony vitality throughout the entire field exposure period and throughout the entire monitoring period until the end of overwintering in spring 2014.
Moreover, the overwintering performance of the colonies in the test item treatment group was not adversely affected when compared to the performance of the control group.

Overall, it can be concluded that the exposure of honeybee colonies to guttation liquid from sugar beet plants, grown from pills, commercially prepared with the insecticides clothianidin, imidacloprid and beta-cyfluthrin at a rate corresponding to nominally 0.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrin/pill during the first approximately 6 weeks after emergence, did neither cause acute, short-term nor long-term adverse effects on mortality, honeybee behaviour, colony strength, colony health and vitality, brood and food development and overwintering performance in the exposed colonies.

>>M-507734-01-3

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Report:	02.02.05/06; [redacted]; 2015; M-503349-03-2
Title:	A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of potato plants, grown from seed tubers treated with Monceren G in southern Germany in 2014 and 2015 - Final report
Report No.:	S14-01385
Document No.:	M-503349-03-2
Guideline(s):	OEPP/EPPO Guideline No. 170(4) (2010) US EPA OCSPP Guideline # 850.3040 Field Testing for Pollinators
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-503349-03-2@S-602314-01-1

1.1 Material and methods:

Test item:

Monceren G, IMD+PCC FS 370 (120+250) G; Spec. No. 102000008024; TOX number: TOX10501-00; Batch: 2014-001766-01; content of a.i. (nominal): 120 g/L imidacloprid + 250 g/L pencycuron; content of a.i. analysed: 120.5 g/L imidacloprid + 251.2 g/L pencycuron

The potential effects of exposure of honeybees (Apis mellifera L.) to guttation fluid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: imidacloprid + pencycuron) during planting at a rate corresponding to nominally 1.5 L product/ha, were investigated under field conditions in Germany during the first 59 days after emergence by following the OEPP/EPPO Guideline No. 170(4), 2010.

The field study consisted of two treatment groups: The test item treatment group T (seed tubers treated with Monceren G) and the control group C (test item untreated seed tubers). Commercial bee colonies (8 per treatment) were placed at the field sites shortly after emergence of the plants (BBCH 10). The mortality of the honeybees was assessed over a period of 5 days shortly before start of exposure and daily after set-up of the colonies at the field sites from 1DAE to 58DAE. Flight intensity and behaviour as well as the number of honeybees visiting potato plants and the occurrence and proportion of guttation on potato plants was assessed daily after set-up of the bee colonies at the field sites from 0DAE to 58DAE. The condition of the colonies was assessed once before set-up of the colonies at the field sites and regularly thereafter after until end of overwintering. The Varroa infestation level was evaluated and samples of honeybees for bee disease and bee virus analysis as well as nectar for American foulbrood (AFB) analysis were collected to monitor colony health. Samples of guttation fluid from potato plants (test item treatment group T only) and dead worker bees from dead bee traps were collected for residue analysis.

The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control under consideration of the results of:

- Mean number of dead bees on the linen sheets and in the dead bee traps;
- Flight intensity in the field (mean number of honeybees per m2 and minute);
- Observation of honeybees visiting potato plants displaying guttation;
- Occurrence and proportion of guttation;
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date);
- Bee health (bee disease and bee virus analysis);
- Overwintering performance;
- Residue analysis.

Dates of work: 02 Apr 2014 until 23 Jul 2015

1.2 Findings

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Mortality of Honeybees

	Treatment group	Control (C)	Test item (T)
Daily mean mortality (dead bees/colony) ± STD	7DBE to 3DBE (Pre-exposure)	10.6 ± 5.4	10.5 ± 5.1
	1DAE to 58DAE (Exposure)	16.0 ± 2.8	13.8 ± 4.9

DAE: days after start of exposure; DBE: days before start of exposure; STD: standard deviation

1.2.1 Mortality

During the pre-exposure period at the monitoring site (7DBE to 3DBE), the mean daily mortality, assessed by using dead bee traps, was on the same level in the control group C and in the test item treatment group T (10.6 and 10.5 dead bees/colony/day for the treatment groups C and T, respectively). Throughout the entire field exposure period of the colonies, no conspicuous differences regarding the mortality levels were observed on a daily basis between the test item treatment group and the control group. During the entire exposure period at the field sites (assessed from 1DAE to 58DAE), the mean daily mortality, assessed by dead bee traps, was 16.0 and 13.8 dead bees/colony/day for the treatment groups C and T, respectively.

On the linen sheets, spread out in the test fields (mortality within the crop area), throughout the entire exposure period, no dead bees were found in either the control group C or the test item treatment group T. Thus, no notable difference was observed between the control and the test item treatment group concerning mortality during the exposure period.

1.2.2 Flight Intensity in the Field and Observation of Honeybees Visiting Potato Plants

The assessments of flight intensity in the field and the observation of honeybees visiting potato plants were conducted early in the morning when the occurrence of guttation droplets was expected. The concomitant flight activity of the colonies at the hive entrances was monitored at about the same time. The flight assessment areas were all located close to the colonies, with a distance of 10–15 m to the hives. During the entire assessment period from 0DAE to 58DAE, a total of 1124 honeybees was observed in the observation areas in the control group, whereas a total of 3025 honeybees was observed in the test item treatment group. In the control group, however, all 1124 honeybees were flying over the crop, whereas no honeybees were located on potato plants or were observed on the soil during the entire observation period. In the test item treatment group, 3023 honeybees were flying over the crop, whereas only 2 honeybees were located on potato plants and no honeybees were observed on the soil during the entire observation period. No honeybees taking up guttation fluid were observed in both the control and the test item treatment group during the entire observation period.

Overall, the vast majority of honeybees detected in the five in-crop assessment areas in both the control and the test item treatment group were observed flying in the air above the crop, presumably including a substantial fraction of honeybees that were only accidentally passing through the observation areas due to their close vicinity to the hives. However, virtually no honeybees were observed in direct contact with potato plants or soil in both treatment groups, with no notable differences between the test item treatment group and the control group. Moreover, uptake of guttation droplets by honeybees from potato plants (treated and untreated) did not occur during all assessments.

1.2.3 Behaviour of the Bees

During the assessment period from 0DAE to 58DAE, honeybees exhibiting abnormal behavior, mainly in small numbers, were observed on 29 out of 59 days in the test item treatment group and on 25 out of 59 days the control group. On the remaining days, only normal behavior was recorded.

Cramping was observed in a small number of honeybees on 22 out of 59 days in the test item treatment group (range: 1–20 bees/8 colonies and assessment date), and on 17 out of 59 days in the control (range: 1–56 bees/8 colonies and assessment date).
Locomotion problems were observed in a small number of honeybees on 14 out of 59 days in the test item treatment group (range: 1–15 bees/8 colonies and assessment date), and on 12 out of 59 days in the control (range: 1–69 bees/8 colonies and assessment date).
Small numbers of inactive honeybees were observed on 15 out of 59 days in the test item treatment group (range: 2–36 bees/8 colonies and assessment date), and on 13 out of 59 days in the control (range: 1–165 bees/8 colonies and assessment date). It should be noted that the assessments were conducted early in the day and the numbers of inactive honeybees may as well include cold-impaired bees.
Trembling was observed in a small number of honeybees on 2 out of 59 days in the test item treatment group (range: 1–2 bees/8 colonies and assessment date), and on 2 out of 59 days in the control (range: 1–2 bees/8 colonies and assessment date).
Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behavior was observed in the test item treatment group compared to the control.
Consequently, no test-item related adverse effects on honeybee behavior were observed.

1.2.4 Occurrence of Guttation and Percentage of Plants Displaying Guttation
In the control group, guttation of potato plants in the assessment areas was observed on 18 out of 59 assessment days. In the concurrently assessed off-crop area, guttation occurred on 29 out of 59 assessment days. In the test item treatment group, guttation of potato plants in the assessment areas was observed on 17 out of 59 assessment days. In the concurrently assessed off-crop area, guttation occurred on 33 out of 59 assessment days.
When guttation occurred in the in-crop assessment areas, the percentage of plants exhibiting guttation per assessment area varied from 6.7 % to 100 % in the control group as well as in the test item treatment group.

1.2.5 Condition of the Colonies
1.2.5.1 Strength of the Colonies
The mean number of bees per colony assessed during the first colony assessment on 30 Apr 2014 (3DBE) shortly before start of exposure revealed a mean colony strength of 13804 bees/colony in the control C (range: 9425 to 19305) and 13975 bees/colony in the test item treatment group T (range: 9945 to 18590).
During the following assessments during exposure and at the monitoring site after exposure, the colony strengths of both, control group C and test item treatment group T, followed the natural course of colony strength development, with an increasing tendency up to the assessment in midsummer on 23 Jul 2014 and a decreasing tendency thereafter until the start of overwintering on 13 Oct 2014 and the end of overwintering in spring of the following year (17 Mar 2015).
At the start of overwintering in autumn 2014, the mean colony strength was 13731 bees/colony in C (range: 10595 to 15470) and 9953 bees/colony in T (range: 7150 to 12675). At the end of overwintering in early spring 2015, the mean colony strength was 10498 bees/colony in C (range: 7345 to 13130) and 8523 bees/colony in T (range: 4160 to 12935).
Throughout the entire observation period, the mean colony strength in the test item treatment group T was approximately on the same level as in the control group C without any major differences.
Thus, no test-item related adverse effects on colony strength were observed during the course of the study.

1.2.5.2 Brood Stages and Overwintering Performance
In the colonies of the control group C and the test item treatment group T the natural and typical changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage (capped brood), occurred during the observation period. From 30 Apr 2014 (3DBE) up to and including 15 Sep 2014 (135DAE), all colonies in the control and in the test item treatment group (except colonies Tf and Th on 01 Jul 2014 (59DAE), see below) contained all brood stages during the brood assessments.

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In colony Tf, no larvae were present on 01 Jul 2014 (59DAE). During the previous beekeeper check on 24 Jun 2014 (52DAE), all brood stages were present. During the following beekeeper check on 07 Jul 2014 (65DAE), the colony had regained normal breeding activity by itself and all brood stages were present again.
In colony Th, for undetermined reasons, no queen, and therefore no eggs and larvae, were present during the beekeeper check conducted on 18 Jun 2014 (46DAE). A new queen was added on 24 Jun 2014, resulting in the restoration of breeding activity up to 01 Jul 2014 (59DAE).
In early autumn, when the natural period of breeding activity of the colonies ended, the number of cells with brood had declined in both the control and the test item treatment group on the day of the colony assessment on 15 Sep 2014 (135DAE). On the last colony assessment before start of overwintering on 13 Oct 2014 (163DAE), the breeding activity of the colonies of the study had almost ended. Virtually no eggs and larvae, but still residual amounts of pupae were observed in the control and in the test item treatment group, respectively.
The overwintering period lasted from 13 Oct 2014 until 17 Mar 2015. After overwintering, all colonies of the test item treatment group and the control were alive and all were found to have resumed breeding activity normally (with the exception of the control colony Cc, which showed an interruption of egg-laying activity for unknown reasons).
Thus, no test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance.

1.2.5.3 Food Storage
In the colonies of the control group C and the test item treatment group T, respectively, the natural and typical changes and fluctuations in the relative amount of nectar and pollen storage cells occurred during the observation period. The treatment groups C and T showed approximately equal mean numbers of pollen and nectar storage cells throughout the entire observation period.
Thus, no test item-related adverse effects on the food storage of the exposed colonies were observed.

1.2.5.4 Colony Health
1.2.5.4.1 Evaluation of Varroa Infestation in the Colonies
Varroa mite occurrence in the colonies was assessed via a 'Varroa board' beneath the hives. The infestation level of a colony was monitored by counting dead mites on the board.
During the assessments from 06 Aug 2014 to 15 Sep 2014, only relatively small mean numbers of mites were detected. Moderately elevated mean numbers of mites were observed on 01 Oct 2014 in both treatment groups. This was due to a previous Varroa treatment of the colonies with formic acid on 16 Sep 2014.
The Varroa infestation levels of the test item treatment colonies were approximately on the same level as or even lower than those of the control colonies during the course of the study and at the end of the honeybee season. No test item-related adverse effects were detected.

1.2.5.4.2 Bee Diseases
In the honeybee samples taken from the control colonies before exposure, Nosema sp. spores were found in control colonies Cf (low infestation level) as well as in Cc and Ch (medium infestation level). Control colonies Ca, Cb, Cd, Ce and Cg were free of analysable spores.
In the honeybee samples taken at end of exposure, no Nosema sp. spores were found in any of the samples taken from control colonies.
In the honeybee samples taken from the control colonies at start of overwintering, the control colony Ca had a medium infestation level with Nosema sp. spores. All other control colonies were free of analysable spores.
In the honeybee samples taken at end overwintering, no Nosema sp. spores were found in any of the sample taken from control colonies.
The highest infestation rate with Varroa mites was 3.5 % in the honeybee sample taken from the control colony Ch at start of overwintering. In all other honeybee samples examined, the Varroa infestation rate was between 0.0 % and 2.4 % based on all sampling points.

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In the honeybee samples taken from the test item treatment colonies before exposure, infestation with Nosema sp. spores were on a low level in the test item treatment colonies Ta and Tf, on a medium level in the colonies Tb and Th and on a high level in the colony Tc. Test item treatment colonies Td, Te and Tg were free of analysable spores.
In the honeybee samples taken at end of exposure, test item treatment colony Tf had a low infestation level with Nosema sp. spores. All other test item treatment colonies were free of analysable spores.
In the honeybee samples taken at start of overwintering, test item treatment colony Th had a low infestation level with Nosema sp. spores. No Nosema sp. spores were found in any of the other test item treatment colonies.
In the honeybee samples taken at end of overwintering, test item treatment colony Tb had a high infestation level with Nosema sp. spores. No Nosema sp. spores were found in the honeybee samples taken from the other test item treatment colonies.
The highest infestation rate with Varroa mites in samples taken from the test item treatment colonies was found in colony Tb with 3.3 % before overwintering. The infestation rate of all other test item treatment colonies varied between 0.0 % and 2.3 % based on all sampling time points.
No infestation with Malpighamoeba mellificae was found in any of the honeybee samples taken in 2014 and 2015, neither in samples taken from the control nor from the test item treatment colonies.
No spores of Paenibacillus larvae were found in any of the nectar/fresh honey samples taken in 2014 and 2015, neither in those taken from the control nor from the test item treatment colonies. The nectar/fresh honey sample from test item treatment colony Ta taken before exposure was not assessable due to contamination with other Bacillaceae.
Overall, no distinct differences in the health status between the honeybee colonies of the control group and the test item treatment group were observed.

1.2.5.4.3 Bee Viruses

The viruses CBPV, KBV, and IAPV were not detected in any of the samples at the time points ‘before exposure’, ‘end of exposure’ and ‘start of overwintering’ in 2014 and ‘after overwintering’ in 2015.
At the time point ‘start of overwintering’ in 2014, DWV was detected in the sample of one colony of the control group (Cc), and in the sample of one colony of test item group (Tb). DWV also was detected in the sample of one colony of the test item group at the time point ‘after overwintering’ in 2015 (Tf).
In 2014, SBV was detected in the samples of three colonies of the control group (Cb, Cd, Cg), and in the samples of seven colonies of the test item group (Tb-Th) at the time point ‘before exposure’, and in the sample of one colony of the test item group at the time point ‘end of exposure’ (Tb). From the start of overwintering on, all colonies were free of SBV. SBV was mainly present in the colonies before the start of exposure and its occurrence was therefore not test item-related.
ABPV was detected in the sample of one colony of the test item group (Tc) at the time point ‘start of overwintering’ in 2014.
In 2014, BQCV was detected in the samples of seven colonies of the control group (Cb-Ch), and in the samples of six colonies of the test item group (Ta-Td, Tf, Tg) at the time point ‘before exposure’, in the samples of all colonies of the control group and the test item group at the time point ‘end of exposure’, and in the sample of one colony of the test item group (Ta) at the time point ‘start of overwintering’. At the end of overwintering, all colonies were free of BQCV. Since BQCV was already present in seven out of eight control colonies and in six out of eight test item group colonies before the start of exposure, its presence in all colonies at the end of the exposure period was not considered to be test item-related.
Overall, no distinct differences in the bee health status in terms of virus infection between the colonies of the control group and the test item treatment group were observed.

1.2.6 Residue Analysis

Analysis of residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine in guttation fluid samples taken from 7DAE to 42DAE was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection. The Limit of Quantitation (LOQ) was 1 µg/L for imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, respectively. The Limit of Detection (LOD) was 0.3 µg/L for imidacloprid,

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imidacloprid-5-hydroxy and imidacloprid-olefine, respectively. (No guttation occurred before 7DAE and after 42DAE).
The residue levels of the parent imidacloprid in guttation fluid ranged from 32 µg a.i./L to 1958 µg a.i./L. The residue levels of the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine in guttation fluid ranged from 13 µg a.i./L to 583 µg a.i./L and from below the LOD (0.3 µg a.i./L) to 15 µg a.i./L, respectively. They were thus several orders of magnitude below the values measured for the parent. Maximum residues values were detected at the earliest samplings after emergence and residues declined over time. Particles of soils or dust have been observed in the specimen collected on 36DAE, which most likely have caused the high residue values in this sample.

1.3 Conclusion

The objective of this study was to determine the potential effects of exposure of honeybees (Apis mellifera L.) to guttation fluid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: 120 g imidacloprid/L + 250 g pencycuron/L) during planting at a rate corresponding to nominally 1.5 L product/ha, during the first 59 days after emergence under field conditions.
Guttation in the test fields was observed on 17 out of 59 days in the test item treatment group and on 18 out of 59 days in the control. During the entire assessment period of 59 days at the exposure sites, no honeybees with direct contact to the crop or the surrounding soil surface were observed in the assessment areas in the control group, whereas a total of 2 honeybees located on potato plants was observed in the test item treatment group. The number of honeybees observed in the crop was therefore on the same low level in both, the control and the test item treatment group.
Uptake of guttation droplets by honeybees from potato plants (treated and untreated) did not occur during all assessments.
No test item-related adverse effects were observed on mortality and behaviour of the honeybees.
No test item-related adverse effects were observed on colony health status, colony development (including colony strength, brood development and food storage of the colonies) as well as on overall colony vitality throughout the entire field exposure period and throughout the monitoring period until the end of overwintering in spring 2015.
Moreover, the overwintering performance of the colonies in the test item treatment group was not adversely affected when compared to the performance of the control group.
Overall, it can be concluded that the exposure of honeybee colonies to guttation fluid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: 120 g imidacloprid/L + 250 g pencycuron/L) during planting at a rate corresponding to nominally 1.5 L product/ha, during the first 59 days after emergence, did not cause acute, short-term or long-term adverse effects on mortality, honeybee behaviour, colony strength, colony health and vitality as well as brood and food development and overwintering performance in the exposed colonies.

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Report:	02.02.05/07; [redacted]; 2015; M-503344-03-2
Title:	A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of potato plants, grown from seed tubers treated with Monceren G in Southern Germany in 2014 and 2015 - Final report
Report No.:	S14-01392
Document No.:	M-503344-03-2
Guideline(s):	Regulation 1107/2009 (Europe) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3040
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-503344-03-2@S-602313-01-1

1.1 Material and methods:

Test item: Monceren G, IMD+PCC FS 370 (120+250) G; Spec.No. 102000008024; TOX number: TOX10501-00; Batch: 2014-001766-01; content of a.i. (nominal): 120g/L imidacloprid + 250g/L pencycuron; content of a.i. analysed: 120.5g/L imidacloprid + 251.2g/L pencycuron

The potential effects of exposure of honeybees (Apis mellifera L.) to guttation fluid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: imidacloprid + pencycuron) during planting at a rate corresponding to nominally 1.5 L product/ha, were investigated under field conditions in Germany during the first 58 days after emergence by following the OEPP/EPPO Guideline No. 170(4), 2010.

The field study consisted of two treatment groups: The test item treatment group T (seed tubers treated with Monceren G) and the control group C (test item untreated seed tubers). Commercial bee colonies (8 per treatment) were placed at the field sites shortly after emergence of the plants (BBCH 10). The mortality of the honeybees was assessed over a period of 5 days shortly before start of exposure and daily after set-up of the colonies at the field sites from 1DAE to 57DAE. Flight intensity and behaviour as well as the number of honeybees visiting potato plants and the occurrence and proportion of guttation on potato plants was assessed daily after set-up of the bee colonies at the field sites from 0DAE to 57DAE. The condition of the colonies was assessed once before set-up of the colonies at the field sites and regularly thereafter after until end of overwintering. The Varroa infestation level was evaluated and samples of honeybees for bee disease and bee virus analysis as well as nectar for American foulbrood (AFB) analysis were collected to monitor colony health. Samples of guttation fluid from potato plants (test item treatment group T only) and dead worker bees from dead bee traps were collected for residue analysis.

The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control under consideration of the results of:

- Mean number of dead bees on the liner sheets and in the dead bee traps;
- Flight intensity in the field (mean number of honeybees per m2 and minute);
- Observation of honeybees visiting potato plants displaying guttation;
- Occurrence and proportion of guttation;
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date);
- Bee health (bee disease and bee virus analysis);
- Overwintering performance;
- Residue analysis.

Dates of work: 02 Apr 2014 until 12 Aug 2015

1.2 Findings

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Mortality of Honeybees

	Treatment group	Control (C)	Test item (T)
Daily mean mortality (dead bees/colony) ± STD	5DBE to 1DBE (Pre-exposure)	45.9 ± 42.0	35.7 ± 20.6
	1DAE to 57DAE (Exposure)	20.7 ± 6.1	18.3 ± 3.8

DAE: days after start of exposure; DBE: days before start of exposure; STD: standard deviation

1.2.1 Mortality

During the pre-exposure period at the monitoring site (5DBE to 1DBE), the mean daily mortality, assessed by using dead bee traps, was moderately higher in the control group C compared to the test item treatment group T (45.9 and 35.7 dead bees/colony/day for the control group C and test item treatment group T, respectively).

Throughout the entire field exposure period of the colonies, no conspicuous differences regarding the mortality levels were observed on a daily basis between the test item treatment group and the control group. During the entire exposure period at the field sites (assessed from 1DAE to 57DAE), the mean daily mortality, assessed by dead bee traps, was 20.7 and 18.3 dead bees/colony/day for the control group C and test item treatment group T, respectively.

On the linen sheets, spread out in the test fields (mortality within the crop area), throughout the entire exposure period, a mean of 0.1 and 0.2 dead bees/day was found in the control group C and in the test item treatment group T, respectively.

Thus, no notable difference in mortality was observed between the control group and the test item treatment group during the entire exposure period.

1.2.2 Flight Intensity in the Field and Observation of Honeybees Visiting Potato Plants

The assessments of flight intensity in the field and the observation of honeybees visiting potato plants were conducted early in the morning when the occurrence of guttation droplets was expected. The concomitant flight activity of the colonies at the hive entrances was monitored at about the same time. The flight assessment areas were all located close to the colonies, with a distance of 10–15 m to the hives. During the entire assessment period from 0DAE to 57DAE, a total of 650 honeybees was observed in the observation areas in the control group, whereas a total of 1791 honeybees was observed in the test item treatment group. In the control group, however, 647 honeybees were flying over the crop, whereas only 2 honeybees were located on potato plants and 1 honeybee was observed on the soil during the entire observation period. In the test item treatment group, 1788 honeybees were flying over the crop, whereas only 3 honeybees were located on potato plants and no honeybees were observed on the soil during the entire observation period. No honeybees taking up guttation fluid were observed in both the control and the test item treatment group during the entire observation period.

Overall, the vast majority of honeybees detected in the five in-crop assessment areas in both the control and the test item treatment group were observed flying in the air above the crop, presumably including a substantial fraction of honeybees that were only accidentally passing through the observation areas due to their close vicinity to the hives. However, virtually no honeybees were observed in direct contact with potato plants or soil in both treatment groups, with no notable differences between the test item treatment group and the control group. Moreover, uptake of guttation droplets by honeybees from potato plants (treated and untreated) did not occur during all assessments.

1.2.3 Behaviour of the Bees

During the assessment period from 0DAE to 57DAE, small numbers honeybees exhibiting abnormal behaviour were observed on 37 out of 58 days in the test item treatment group and on 35 out of 58 days in the the control group. On the remaining days, only normal behaviour was recorded.

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Cramping was observed in a small number of honeybees on 37 out of 58 days in the test item treatment group (range: 2–31 bees/8 colonies and assessment date), and on 33 out of 58 days in the control (range: 1–21 bees/8 colonies and assessment date).
Locomotion problems were observed in a small number of honeybees on 16 out of 58 days in the test item treatment group (range: 1–11 bees/8 colonies and assessment date), and also on 16 out of 58 days in the control (range: 1–18 bees/8 colonies and assessment date).
Small numbers of inactive honeybees were observed on 14 out of 58 days in the test item treatment group (range: 1–20 bees/8 colonies and assessment date), and on 13 out of 58 days in the control (range: 1–14 bees/8 colonies and assessment date). It should be noted that the assessments were conducted early in the day and the numbers of inactive honeybees may as well include cold-impaired bees.
Trembling was observed in a small number of honeybees on 1 out of 58 days in the test item treatment group (2 bees/8 colonies), and also on 1 out of 58 days in the control (1 bee/8 colonies).
Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behavior was observed in the test item treatment group compared to the control.
Consequently, no test-item related adverse effects on honeybee behaviour were observed.

1.2.4 Occurrence of Guttation and Percentage of Plants Displaying Guttation
In the control group, guttation of potato plants in the assessment areas was observed on 33 out of 58 assessment days. In the concurrently assessed off-crop area, guttation occurred on 27 out of 58 assessment days. In the test item treatment group, guttation of potato plants in the assessment areas was observed on 37 out of 58 assessment days. In the concurrently assessed off-crop area, guttation occurred on 21 out of 58 assessment days.
When guttation occurred in the in-crop assessment areas, the percentage of plants exhibiting guttation per assessment area varied from 8.3 % to 100 % in the control group as well as in the test item treatment group.

1.2.5 Condition of the Colonies
1.2.5.1 Strength of the Colonies
The mean number of bees per colony assessed during the first colony assessment on 16 May 2014 (3DBE) shortly before start of exposure revealed a mean colony strength of 17184 bees/colony in the control C (range: 9685 to 23140) and 17704 bees/colony in the test item treatment group T (range: 9750 to 31135).
At the second colony assessment on 11 Jun 2014 (23DAE) during exposure, the mean colony strength had decreased in C (14365 bees/colony) as well as in T (14121 bees/colony). However, the decrease of colony strength was equally pronounced in both, the test item treatment group and the control.
During the following assessments during exposure and at the monitoring site after exposure, the colony strengths of both, control group C and test item treatment group T, followed the natural course of colony strength development, with an increasing tendency up to the assessment in midsummer on 05 Aug 2014 and a decreasing tendency thereafter until the start of overwintering on 14 Oct 2014 and the end of overwintering in spring of the following year on 18 Mar 2015.
At the start of overwintering in autumn 2014, the mean colony strength was 15633 bees/colony in C (range: 11700 to 19500) and 15836 bees/colony in T (range: 11440 to 24180). At the end of overwintering in early spring 2015, the mean colony strength was 8767 bees/colony in C (range: 4030 to 15600) and 7711 bees/colony in T (range: 5265 to 11505).
Throughout the entire observation period, the mean colony strength in the test item treatment group T was approximately on the same level as in the control group C without any remarkable differences.
Thus, no test-item related adverse effects on colony strength were observed during the course of the study.

1.2.5.2 Brood Stages and Overwintering Performance
In the colonies of the control group C and the test item treatment group T the natural and typical changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage (capped brood), occurred during the observation period. From 16 May 2014 (3DBE) up to and

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including 02 Sep 2014 (106DAE), all colonies in the control (except colony Cc on 16 May 2014 (3DBE)) and in the test item treatment group contained all brood stages during the brood assessments.
In colony Cc, no eggs and larvae were present on 16 May 2014 (3DBE). During the following beekeeper check on 26 May 2014 (7DAE), colony Cc was found to have regained normal breeding activity by itself and all brood stages were present again.
In early autumn, when the natural period of breeding activity of the colonies ended, the number of cells with brood had notably declined in both, the control and the test item treatment group on the day of the colony assessment on 01 Oct 2014 (135DAE). On the last colony assessment before start of overwintering, on 14 Oct 2014 (148DAE), the breeding activity of the colonies of the study had almost ended. Virtually no eggs and larvae, but still residual amounts of pupae were observed in the control and in the test item treatment group, respectively.
The overwintering period lasted from 14 Oct 2014 until 18 Mar 2015. After overwintering, all colonies of the test item treatment group and the control were alive. Seven out of eight colonies in the test item treatment group were found to have resumed breeding activity normally, whereas one colony (Th) did not contain any brood cells. This was most likely due to the presence of a virgin queen as a result of queen replacement by the colony itself during overwintering, which can be considered as a naturally occuring process. In the control group, seven out of eight colonies were found to have resumed breeding activity normally, whereas one colony (Cb) did not contain any brood cells. This was due to the absence of an egg-laying queen in the colony. Consequently, no differences in terms of overwintering success and the resumption of breeding activity in early spring were observed between the test item treatment group and the control.
Thus, no test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance.

1.2.5.3 Food Storage
In the colonies of the control group C and the test item treatment group T, respectively, the natural and typical changes and fluctuations in the relative amount of nectar and pollen storage cells occurred during the observation period. The treatment groups C and T showed approximately equal mean numbers of pollen and nectar storage cells throughout the entire observation period, except in the course of two assessments on 15 Jul 2014 and 05 Aug 2014, during which the mean number of nectar cells in the test item treatment colonies was considerably higer than in the control colonies.
Thus, no test item-related adverse effects on the food storage of the exposed colonies were observed.

1.2.5.4 Colony Health
1.2.5.4.1 Evaluation of Varroa Infestation in the Colonies
Varroa mite occurrence in the colonies was assessed via a 'Varroa board' beneath the hives. The infestation level of a colony was monitored by counting dead mites on the board.
During the assessments from 05 Aug 2014 to 15 Sep 2014, only relatively small mean numbers of mites were detected. Moderately elevated mean numbers of mites were observed on 01 Oct 2014 in both treatment groups. This was due to a previous Varroa treatment of the colonies with formic acid on 16 Sep 2014.
The Varroa infestation levels of the test item treatment colonies were approximately on the same level as or even lower than those of the control colonies during the course of the study and at the end of the honeybee season. No test item-related adverse effects were detected.

1.2.5.4.2 Bee Diseases
In the honeybee samples taken from the control colonies before exposure, Nosema sp. spores were found in control colonies Cc (low infestation level), in Cb (medium infestation level) as well as in Ca, Cc, Cd and Ch (high infestation level). The samples taken from control colonies Cf and Cg were free of analysable spores.
In the honeybee samples taken at end of exposure, no Nosema sp. spores were found in any sample taken from control colonies.

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In the honeybee samples taken from the control colonies at start of overwintering, the control colonies Ca and Cf had a low infestation level with Nosema sp. spores. All other samples taken from the control colonies were free of analysable spores (Cb, Cc, Cd, Ce, Cg and Ch).
In the honeybee samples taken at end of overwintering, the control colonies Cb, Cc and Ch had a high infestation level with Nosema sp. spores. All other samples taken from the control colonies were free of analysable Nosema sp. spores (Ca, Cd, Ce, Cf and Cg).
The highest infestation rates with Varroa mites were 7.7 % in the honeybee sample taken from the control colony Cg and 7.4 % in the honeybee sample taken from the control colony Ca at start of overwintering followed by 6.4 % in control colony Cb taken at the start of overwintering. In all other honeybee samples from control colonies examined, the Varroa infestation rate varied between 0.0 % and 4.6 % taken between start of exposure and end of overwintering.
In the honeybee samples taken from the test item treatment colonies before exposure, infestation with Nosema sp. spores were on a medium level in the test item treatment colonies Te, Tg and Th and on a high level in the colonies Ta, Td and Tf. The samples taken from test item treatment colonies Tb and Tc were free of analysable spores.
In the honeybee samples taken at end of exposure, at the start of overwintering and at end of overwintering, no infestation with Nosema sp. spores could be analysed in any of the test item treatment colonies.
The highest infestation rates with Varroa mites in samples taken from the test item treatment colonies were found in colony Th with 6.6 % and Te with 6.2 % before start of overwintering. The infestation rate of all other samples tested from test item treatment colonies varied between 0.0 % and 4.5 % taken between start of exposure and end of overwintering.
No infestation with Malpighamoeba mellificae was found in the honeybee samples taken in 2014 and 2015, neither in samples taken from the control nor from the test item treatment colonies.
No spores of Paenibacillus larvae were found in any of the nectar/fresh honey samples taken in 2014 and 2015, neither in samples taken from the control nor from the test item treatment colonies. The nectar/fresh honey samples from control colony Cd taken before exposure and from test item treatment colony Tf taken before start of overwintering were not assessable due to contamination with other Bacillaceae. No nectar/fresh honey samples were available from control colonies Cc, Ce and Cf as well as from test item treatment colony Ta before exposure.
Overall, no distinct differences in the health status compared between the honeybee colonies of the control group and the test item treatment group were observed.

1.2.5.4.3 Bee viruses

The viruses ABPV, CBPV, KBV, and IAPV were not detected in any of the samples at the time points ‘before exposure’, ‘end of exposure, and ‘start of overwintering’ in 2014 and ‘after overwintering’ in 2015.
At the time point ‘start of overwintering’ in 2014, DWV was detected in the samples of two colonies of the control group (Ce, Cg) and in the samples of two colonies of test item group (Te, Tf). DWV was also detected in the samples of two colonies of the control group (Cg, Ch) and in samples of three colonies of the test item group (Tc-Te) at the time point ‘after overwintering’ in 2015.
At the time point ‘before exposure’ 2014, SBV was detected in the sample of one colony of the control group (Ce) and in the samples of three colonies of the test item group (Tc, Td, Tf). SBV was also detected in the samples of three colonies of the control group (Cb, Ce, Cg) at the time point ‘end of exposure’ in 2014 but not in the samples from the test item group. SBV was only present in the test item group colonies before the start of exposure and its occurrence was therefore not test item-related.
At the time point ‘before exposure’ in 2014, BQCV was detected in the samples of four colonies of the control group (Ca, Cb, Ce, Cg) and in the sample of one colony of the test item group (Td). BQCV was also detected in the samples of five colonies of the control group (Ca-Cc, Cg, Ch) and in all eight samples of the test item group (Ta-Th) at the time point ‘end of exposure’ in 2014. Since both treatment groups were approximately equally affected, a test item-related effect seems unlikely. From the start of overwintering on, all colonies were free of BQCV.
Overall, no distinct differences in the bee health status in terms of virus infection between the colonies of the control group and the test item treatment group could be observed.

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1.2.6 Residue Analysis

Analysis of residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine in guttation fluid samples taken from 0DAE to 57DAE was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.
The Limit of Quantitation (LOQ) was 1 µg/L for imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, respectively. The Limit of Detection (LOD) was 0.3 µg/L for imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine, respectively.
The residue levels of the parent imidacloprid in guttation fluid ranged from the LOQ (1 µg a.i./L) to 4749 µg a.i./L.
The residue levels of the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine in guttation fluid ranged from the LOQ (1 µg a.i./L) and from below the LOD (0.3 µg a.i./L) to 19 µg a.i./L, respectively. They were thus several orders of magnitude below the values measured for the parent.
Maximum residues values were detected at the earliest samplings after emergence and residues declined over time. Particles of soils or dust have been observed in the specimen collected on 2DAE, 3DAE, 23DAE, 25DAE and 29DAE, which most likely have caused the high residue values in these samples.

1.3 Conclusion

The objective of this study was to determine the potential effects of exposure of honeybees (Apis mellifera L.) to guttation fluid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: 120 g imidacloprid/L + 250 g pencycuron/L) during planting at a rate corresponding to nominally 1.5 L product/ha, during the first 58 days after emergence under field conditions.
Guttation in the test fields was observed on 37 out of 58 days in the test item treatment group and on 33 out of 58 days in the control. During the entire assessment period at the exposure sites, a total of 3 honeybees with direct contact to the crop or the surrounding soil surface was observed in the assessment areas in the control group, whereas a total of 3 honeybees located on potato plants was observed in the test item treatment group. The number of honeybees observed in the crop was therefore on the same low level in both, the control and the test item treatment group.
Uptake of guttation droplets by honeybees from potato plants (treated and untreated) did not occur during all assessments.
No test item-related adverse effects were observed on mortality and behaviour of the honeybees.
No test item-related adverse effects were observed on colony health status, colony development (including colony strength, brood development and food storage of the colonies) as well as on overall colony vitality throughout the entire field exposure period and throughout the monitoring period until the end of overwintering in spring 2015.
Moreover, the overwintering performance of the colonies in the test item treatment group was not adversely affected when compared to the performance of the control group.
Overall, it can be concluded that the exposure of honeybee colonies to guttation fluid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: 120 g imidacloprid/L + 250 g pencycuron/L) during planting at a rate corresponding to nominally 1.5 L product/ha, during the first 58 days after emergence, did not cause acute, short-term or long-term adverse effects on mortality, honeybee behaviour, colony strength, colony health and vitality as well as brood and food development and overwintering performance in the exposed colonies.

>>M-503344-01-2@S-60213-01-1

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02.02.06 - Dust

Report:	02.02.06/01; [redacted]; 2010; M-366273-01-3
Title:	Monitoring of dust drift deposits during and after sowing of winter barley (W-BAR) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 149.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany
Report No.:	R09247-1
Document No.:	M-366273-01-3
Guideline(s):	US EPA OCSPP Guideline No. 850.SUPP 91/414/EEC of July 15, 1991, SANCO/3029/99 Rev. 4, 2000-07-11
Guideline deviation(s):	not specified
GLP/GEP:	**no**

<<M-366273-01-3@S-602225-01-1

Material and Methods

Test item

Two different W-BAR varieties (i.e. Lomerit and Highlight) were purchased untreated and commercially cleaned-up from a commercial seed distributor (Gut Peterhof, D-50127 Bergheim, Germany) and were thereafter seed-treated at Bayer CropScience's Seed Treatment Application Centre in D-40789 Monheim am Rhein, Germany (non-GLP):

- Manta® Plus FS 145.2 (TOX08744-00) treated winter barley seeds, dressed with 1000mL product/100 kg seeds (= nominally 70 g imidacloprid/100 kg seeds); identification of treated seeds: TOX08780-00 (variety Lomerit), TOX08779-00 (variety Highlight)

and

- Smaragd® forte FS 455 (TOX08741-00) treated winter barley seeds, dressed with 133mL product/100 kg seeds (= nominally 50 g clothianidin/100 kg seeds); identification of treated seeds: TOX08775-00 (variety Lomerit), TOX08774-00 (variety Highlight)

After seed-dressing, the seeds were subject to chemical analysis for the determination of the actual seed loading. Finally, the seed bags were unequivocally labelled and shipped via road transport to the respective study sites in Germany.

Study sites and sowing

The multiple site study was conducted at two different regions in Germany: one in Southern Germany in the federal state of Baden-Würtemberg in Renningen, southwest of Stuttgart at the experimental station Ihinger Hof of the University Hohenheim (in the following called Ihinger Hof) and the second in Northern Germany in the federal state of Lower Saxony near Celle northeast of Hannover (in the following called Celle) with two fields per location (see Figure 1). The sizes of the test fields sown with Manta® Plus-treated W-BAR seeds at Ihinger Hof and Celle were 4.8 ha and 8.0 ha, respectively. The fields drilled with Smaragd® forte treated W-BAR seeds at Ihinger Hof and Celle were 3.9 ha and 7.0 ha, respectively. The variety of W-BAR sown at Ihinger Hof was 'Highlight' and the variety drilled at Celle was 'Lomerit'. More detailed information about the study sites are given in chapter 3.4 and 3.5.
A total of 200 kg seeds/ha were sown at both test locations resulting in nominal application rates of 140 g imidacloprid a.s./ha on fields drilled with Manta® Plus and 100 g clothianidin a.s./ha on fields drilled with Smaragd® forte. The seeds were drilled using two different pneumatic sowing machines.

Sampling method during sowing

Shortly before sowing the wind direction at the site was determined and ten Petri-dishes were placed in groups of two at distances of 1, 3 and 5 m from the downwind border of the field to give a total of 30 Petri-dishes per field. The actual placement of the Petri-dishes on the field edges followed the actual wind direction, in order to collect as much dust as possible. The actual situation per monitoring field, including the exact position of the sampling areas in relation to the rest of the field, the study plot dimensions

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(length & width of the sown area), any adaptations to the prevailing local conditions as well as the wind direction and wind speed during the sowing operation was documented in the raw data. Each Petri-dish for sampling dust drift deposits (Ø 13.7 cm, 147.41 cm²) was filled with 70 to 80 ml of a 1:1 (v/v) glycerol/water mixture immediately before the start of the sowing. The Petri-dishes were arranged horizontally using metal racks approximately 1.5 to 2 cm above the soil or at the height of the ground vegetation surface, depending on the field boundary morphology. If necessary, the vegetation at the field border was removed to allow air to move freely across the open Petri-dishes. In order to allow any airborne dust to settle, the Petridishes remained open for 15 minutes following the cessation of sowing operations. The aqueous sampling medium of each Petri-dish was then individually transferred to a separate polyethylene flask. To ensure that all possible deposits of imidacloprid or respectively clothianidin from the inside of the Petri-dish were transferred to the corresponding polyethylene flask, each Petri-dish and its corresponding funnel was additionally rinsed with fresh tap water (≈ 20 mL) and the rinse was combined with the content of the respective Petri-dish within the corresponding polyethylene flask. After rinsing, each polyethylene flask was tightly closed. To avoid cross-contaminations the Petri-dishes were always approached from the downwind direction. Each polyethylene flask was labelled with the sampling date and an individual sample identification number consisting of the field number and the sampler number (see 0).

Sampling method after sowing
In order to monitor any potential dust drift during the 24h-period following sowing, a second set of ten Petri-dishes were placed in pairs at the approximate middle of each field side at a distance of 1 m to the field borders to give a total of 40 Petri-dishes per field. After 24 hours the sampling medium from each dish was individually transferred to a separate polyethylene flask following up the same workflow as described in the section above.

Residue analysis
Imidacloprid and clothianidin residues in the samples were subsequently determined by Bayer CropScience AG by High Performance Liquid Chromatography, coupled with Tandem Mass Spectrometry. Until shipment, the samples were stored at room temperature.

Results
A total number of 279 samples were collected from fields drilled with Manta® Plus or Smaragd® forte - treated seeds. One Petri-dish was inadvertently left closed. Of these 279 samples, 208 samples (74.5 %) were found to contain no quantifiable residues of imidacloprid or clothianidin, respectively (LOQ1); this included 194 samples (69.5 % of all 279 samples) with no detectable residues (LOD1). A total of 63 samples (22.6 %) were found to contain residues of imidacloprid or clothianidin above the limit of quantification (LOQ1). 55 of these samples were taken at the time of sowing, the remaining 8 were taken 24h after drilling was completed. The maximum observed residue level was 0.283 g a.s./ha (see Table S1).
For mathematical processing, the data sets obtained with imidacloprid and clothianidin were combined and any residue value below the limit of detection (LOD: 0.004 g a.s./ha) was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification (LOQ: 0.014 g a.s./ha) was conservatively set to equal the LOQ. The calculated average residue values for samples collected during the sowing operation were 0.019 g a.s./ha for samples at a nominal distance of 1 m to the sowing border, 0.039 g a.s./ha for samples at a nominal distance from of 3 m and 0.020 g a.s./ha for samples at a nominal distance of 5 m. For the samples collected during a 24h-period after sowing, the average residue value was below the LOQ. The 90th%ile residue values during the sowing operation were 0.037 g a.s./ha, 0.031 g a.s./ha and 0.027 g a.s./ha for the nominal distance of 1 m, 3 m and 5 m, respectively. For the samples collected during a 24h-period after sowing, the 90th%ile residue value was below the LOD (see Table S1).

These results indicate that the dust drift deposits, produced during and after the sowing of Manta® Plus or Smaragd® forte - treated W-BAR seeds with pneumatic sowing machines, are limited.

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The results of the imidacloprid and clothianidin residue analysis of the dust drift samples are summarised in the table below and are detailed in the Analytical Phase Report ([redacted], 2009; Report No.: MR-09/153).

Table S1: Summary of residues at respective distances to the sowing borders (imidacloprid and clothianidin, combined)

	During Sowing			24h-sampling	Total
Nominal distance (actual distance)°	**1 m (1 m)**	**3 m (3 m)**	**5 m (4.5 - 5 m)**	**1 m (0.8 - 1 m)**	
No. of samples analysed	40	40	40	159	279
No. of samples not recovered in the field *	0	0	0	1	1
Residue level	**Number of samples with residues levels [n]**				
< LOQ	22	21	22	151	216
0.014-0.050 g a.s./ha	18	16	17	8	59
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	3	1	0	4
	Residue levels [g a.s./ha]				
Average **	0.018	0.029	0.020	<LOD	n.a.
90th%ile **	0.037	0.091	0.027	<LOD	
Maximum **	0.045	0.283	0.272	0.026	

LOD = 0.004 g a.s./ha (imidacloprid, clothianidin); LOQ = 0.014 g a.s./ha (imidacloprid, clothianidin); n.a. = not applicable

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field

* In one case, due to an operator error, the lid of one single Petri-dish was inadvertently not removed during the 24h-period after sowing. As such, no potentially dislodgeable residues could be trapped with this particular Petri-dish and consequently this sample was not considered for the mathematical processing

** Calculated from the respective number of analysed samples (imidacloprid and clothianidin, combined; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ

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Report:	02.02.06/02; [redacted]; 2010; M-366277-01-3
Title:	Monitoring of dust drift deposits during and after sowing of winter wheat (W-WHT) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany
Report No.:	R09247-2
Document No.:	M-366277-01-3
Guideline(s):	91/414/EEC of July 15, 1991, SANCO/3029/99 Rev. 4, 2000-07-11 US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	not specified
GLP/GEP:	**no**

<<M-366277-01-3@S-602228-01-1

Material and Methods

Test item
Two different W-WHT varieties (i.e. Hermann and Manager) were purchased untreated and commercially cleaned-up from a commercial seed distributor (Gut Peterhof, D-50127 Bergheim, Germany) and were thereafter seed-treated at Bayer CropScience's Seed Treatment Application Centre in D-40789 Monheim am Rhein, Germany (non-GLP):

- Manta® Plus FS 145.2 (TOX08744-00) treated winter wheat seeds, dressed with 1000mL product/100 kg seeds (= nominally 70 g imidacloprid/100 kg seeds); identification of treated seeds: TOX08781-00 (variety Manager); TOX08782-00 (variety Hermann)

and

- Smaragd® forte FS 455 (TOX08741-00) treated winter wheat seeds, dressed with 133mL product/100 kg seeds (= nominally 50 g clothianidin/100 kg seeds); identification of treated seeds: TOX08776-00 (variety Manager); TOX08777-00 (variety Hermann)

After seed-dressing, the seeds were subject to chemical analysis for the determination of the actual seed loading. Finally, the seed bags were unequivocally labelled and shipped via road transport to the respective study sites in Germany.

Study sites and sowing
The multiple site study was conducted at two different regions in Germany: one in Southern Germany in the federal state of Baden-Württemberg in Renningen, southwest of Stuttgart at the experimental station Ihinger Hof of the University Hohenheim (in the following called Ihinger Hof) and the second in Northern Germany in the federal state of Lower Saxony near Celle northeast of Hannover (in the following called Celle) with two fields per location (see Figure 1). The sizes of the test fields sown with Manta® Plus-treated W-WHT seeds at Ihinger Hof and Celle were 6.0 ha and 16.21 ha, respectively. The fields drilled with Smaragd® forte treated W-WHT seeds at Ihinger Hof and Celle were 4.0 ha and 9.84 ha, respectively. The variety of W-WHT sown at both study sites was 'Manager'. More detailed information about the study sites are given in chapter 3.4 and 3.5.

A total of 200 kg seeds/ha were sown at both test locations resulting in nominal application rates of 140 g imidacloprid a.s./ha on fields drilled with Manta® Plus and 100 g clothianidin a.s./ha on fields drilled with Smaragd® forte. The seeds were drilled using two different pneumatic sowing machines.

Sampling method during sowing
Shortly before sowing the wind direction at the site was determined and ten Petri-dishes were placed in groups of two at distances of 1, 3 and 5 m from the downwind border of the field to give a total of 30 Petri-dishes per field. The actual placement of the Petri-dishes on the field edges followed the actual wind direction, in order to collect as much dust as possible. The actual situation per monitoring field, including the exact position of the sampling areas in relation to the rest of the field, the study plot dimensions

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(length & width of the sown area), any adaptations to the prevailing local conditions as well as the wind direction and wind speed during the sowing operation was documented in the raw data. Each Petri-dish for sampling dust drift deposits (Ø 13.7 cm, 147.41 cm²) was filled with 70 to 80 ml of a 1:1 (v/v) glycerol/water mixture immediately before the start of the sowing. The Petri-dishes were arranged horizontally using metal racks approximately 1.5 to 2 cm above the soil or at the height of the ground vegetation surface, depending on the field boundary morphology. If necessary, the vegetation at the field border was removed to allow air to move freely across the open Petri-dishes. In order to allow any airborne dust to settle, the Petridishes remained open for 15 minutes following the cessation of sowing operations. The aqueous sampling medium of each Petri-dish was then individually transferred to a separate polyethylene flask. To ensure that all possible deposits of imidacloprid or respectively clothianidin from the inside of the Petri-dish were transferred to the corresponding polyethylene flask, each Petri-dish and its corresponding funnel was additionally rinsed with fresh tap water (≈ 20 mL) and the rinse was combined with the content of the respective Petri-dish within the corresponding polyethylene flask. After rinsing, each polyethylene flask was tightly closed. To avoid cross-contaminations the Petri-dishes were always approached from the downwind direction.
Each polyethylene flask was labelled with the sampling date and an individual sample identification number consisting of the field number and the sampler number (see 0).

Sampling method after sowing
In order to monitor any potential dust drift during the 24h-period following sowing, a second set of ten Petri-dishes were placed in pairs at the approximate middle of each field side at a distance of 1 m to the field borders to give a total of 40 Petri-dishes per field (where necessary the distance of 1 m had to be adjusted to the field boundary morphology). After 24 hours, the sampling medium from each dish was individually transferred to a separate polyethylene flask following up the same workflow as described in the section above.

Residue analysis
Imidacloprid and clothianidin residues in the samples were subsequently determined by Bayer CropScience AG by High Performance Liquid Chromatography, coupled with Tandem Mass Spectrometry. Until shipment, the samples were stored at room temperature.

Results
A total number of 280 samples were collected from fields drilled with Manta® Plus or Smaragd® forte - treated seeds.
Of these 280 samples, 272 samples (97.1 %) were found to contain no quantifiable residues of imidacloprid or clothianidin, respectively (LOQ[1]); this included 228 samples (81.4% of all 280 samples) with no detectable residues (LOD[1]). A total of 8 samples (2.8 %) were found to contain residues of imidacloprid or clothianidin above the limit of quantification (LOQ[1]). 5 of these samples were taken at the time of sowing, the remaining 3 were taken 24h after drilling was completed. The maximum observed residue level was 0.258 g a.s./ha (see Table S1).

For mathematical processing, the data sets obtained with imidacloprid and clothianidin were combined and any residue value below the limit of detection (LOD: 0.004 g a.s./ha) was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification (LOQ: 0.014 g a.s./ha) was conservatively set to equal the LOQ. Both, the calculated average and 90th%ile residue values for all samples collected during the sowing operation at the nominal distances of 1 m, 3 m and 5 m were below LOQ. For the samples collected during a 24h-period after sowing, the average residue value was < LOQ and the 90th%ile residue value was < LOD (see Table S1).
These results indicate that the dust drift deposits, produced during and after the sowing of Manta® Plus or Smaragd® forte - treated W-WHT seeds with pneumatic sowing machines, is limited.

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The results of the imidacloprid and clothianidin residue analysis of the dust drift samples are summarised in the table below and are detailed in the Analytical Phase Report ([redacted], 2009; Report-No.: MR-09/159).

[1] LOD = Limit of Detection = 0.004 g a.s./ha, LOQ = Limit of Quantification = 0.014 g a.s./ha (for imidacloprid and clothianidin, respectively)

Table S1: Summary of residues at respective distances to the sowing borders (imidacloprid and clothianidin, combined)

	During Sowing			24h-sampling	Total
Nominal distance (actual distance)°	**1 m (1 - 2 m)**	**3 m (3 - 4 m)**	**5 m (5 - 6 m)**	**1 m (-1, 0 or 1m)**	
No. of samples analysed	40	40	40	160	280
No. of samples not recovered in the field	0	0	0	0	0
Residue level	**Number of samples with residues levels [n]**				
< LOQ	39	37	39	157	272
0.014-0.050 g a.s./ha	1	3	0	3	7
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	0	1	0	1
	Residue levels [g a.s./ha]				
Average*	< LOQ	< LOQ	< LOQ	< LOQ	n.a.
90th%ile *	< LOQ	< LOQ	< LOQ	< LOD	
Maximum*	0.034	0.030	0.258	0.027	

LOD = 0.004 g a.s./ha (imidacloprid, clothianidin); LOQ = 0.014 g a.s./ha (imidacloprid, clothianidin); n.a. = not applicable

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field.
During sowing: The close proximity of a drainage ditch to the downwind border of study field 18 prevented samplers from being deployed outside the study field as required for sampling. In order to circumvent this problem, the farmer sowed a 6 m strip parallel to the field's downwind margin. Samplers were then placed in the sown strip at distances of 2 m, 4 m and 6 m from a line marking the inner edge of the strip (see Figure A12).
24h-sampling: A hedge adjacent to one edge of study field 11 required the samplers along side C to be placed at a distance of 1 m inside the study field (see Figure A15). On study field 18, the samplers had to be placed directly along the border of the field at a distance of 0 m to the sown area because of a drainage ditch (see Figure A13).

* Calculated from the respective number of analysed samples, imidacloprid and clothianidin, combined; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ.

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Report:	02.02.06/03; [redacted]; 2014; M-502885-03-3
Title:	Investigation of dust drift deposits of clothianidin & imidacloprid treated winter barley seeds with pneumatic sowing machinery on fields in Germany in autumn 2011
Report No.:	R11129
Document No.:	M-502885-03-3
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GLP/GEP:	**yes**

<<M-502885-03-3@S-605130-01-1

Aim

This study was conducted in order to determine occurring aerial and ground dust drift deposits during the pneumatic sowing operation of dressed winter barely seeds on three study fields in the district of Gießen (Hesse) in Germany in autumn 2011.

Material and Methods

Test item
Dressed winter barely seeds (Clothianidin + Imidacloprid FS 100 + 1.75 G at a nominal seed- treatment rate of 200 mL product/100 kg; corresponding to nominally 20 g clothianidin and 35 g imidacloprid/100 kg).

Study site and drilling
The study was conducted in the district of Gießen (Hesse) in Germany on three commercial winter barley fields. The dimension of the drilled area on each individual study field was approximately 50 m x 200 m which corresponds to a treated area of approximately 1.0 ha. The target drilling rate was 200 kg/ha (**actual 183.1 to 194.9 kg/ha)** (corresponding to nominally 20 g clothianidin and 35 g imidacloprid/100 kg). Each pneumatic sowing machine was filled on the farm site. Sowing of the dressed seeds was exclusively performed by typical commercial pneumatic sowing machinery, provided by the respective cooperating farmer.

Sampling method
Shortly before sowing the wind direction was determined and two different sampling devices to measure aerial and ground dust drift deposits were set up at the downwind border on each study field or its boundary (depending on the actual field boundary morphology): Petri-dishes, horizontally arranged at a height of approximately 2 cm above the soil surface and vertically erected gauze-netting-samplers (effective sampling area: 2 m x 3.3 m). The sampling devices were set up rectangular to the prevailing wind direction. The drilling was only performed when the wind speed at the beginning of each row was between 2 and 5 m/s and the deviation to the prevailing wind direction was ≤ ± 30°. The border of the downwind study field side was described as "zero line".

Samples of dressed seeds were taken at the time of bagging and from the used seed bags shortly before filling of the drilling machine for Heubach analysis by the Seed Growth Center of Bayer CropScience AG (non-GLP).

Two lines of 3 x 10 Petri-dishes were set-up in pairs of two along a line of 5 m at a distance of 3 and 1 m to the zero line. The space between each row of ten Petri-dishes was approximately 9.3 m. Additionally one line of three gauze-netting-samplers were set-up in a distance of 3 m to the zero line. Sampling devices were arranged in an alternating order around the center of the zero line where wind breaking structures were lacking, in order to exclude any deflection of the wind. Shortly before beginning of the sowing the gauze-netting- samplers were wetted with a 1:1 (v/v) glycerol/water mixture and the Petri-

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dishes were filled with 80 mL of a 1:1 (v/v) glycerol/water mixture. Soil samples for the analysis of residues, water content (non-GLP) and soil characterisation (non-GLP) were taken shortly before sowing.

Additionally, field fortification samples (0 µg, 1 µg, 100 µg clothianidin + imidacloprid/fortified gauze sample and 0 µg, 0.1 µg, 10 µg clothianidin + imidacloprid/fortified Petri-dish sample) were established just before the start of sowing in order to investigate the stability of the samples during transport and storage.

Thirty minutes after sowing of the respective study field, the aqueous solutions of the Petri- dishes and the gauze samples (five 50 x 50 cm squares were cut-out of each individual netting) were gathered and immediately transferred into separate polyethylene flasks.

Weather conditions during sowing and sampling
Weather was always dry during and after sowing.

For drilling at study field 1 the target wind direction was 265°. The measured mean wind direction was 280° (± 19°). The mean wind speed was 3.3 m/s (± 0.9 m/s). For study field 2 the target wind direction was 120°. The measured mean wind direction was 129° (± 33°). The mean wind speed was 2.4 m/s (± 0.9 m/s). The target wind direction for study field 3 was 140°. The measured mean wind direction was 128° (± 14°). The mean wind speed was 3.8 m/s (± 0.9 m/s).

Residue analysis
Residues of clothianidin and imidacloprid in all Petri-dishes and gauze netting samples as well as all field fortification samples, filters used in the Heubach abrasion tests obtained from the seed samples taken shortly before drilling and in soil samples were analysed by laboratory of the Analytical Test Site (BCS-D-HS-RA, Bayer CropScience AG) ([redacted] R., Report # MR-12/006). Chromatography and detection by MS/MS in Heubach filters, gauze nettings and Petri-dish solutions was done according to method MR-338/00 (clothianidin) and MR-06/144 (imidacloprid). Analysis in soil samples was done according to method MR- 106/02 (clothianidin) and MR-106/03 (imidacloprid). The Limits of Quantitation (LOQ) for clothianidin and imidacloprid for the gauze samples were 0.04 g a.s./ha, respectively. The corresponding Limits of Detection (LOD) were 0.01 g a.s./ha. For the Petri-dish samples the LOQs for clothianidin and imidacloprid were 0.07 g a.s./ha, respectively, the corresponding LODs were 0.02 g a.s./ha. For the soil samples the LOQs were 5 µg a.s./kg soil for clothianidin and imidacloprid, respectively, the corresponding LODs were 2 µg a.s./kg soil.

Results

Residue level of all non-spiked control samples and the soil samples was < LOD.

The Heubach value determined shortly after seed treatment process was 0.045 g/100 kg (non-GLP). Additional Heubach values were determined after drilling from samples taken shortly before drilling. These measurements resulted in Heubach values of 0.097 g/100 kg, 0.022 g/100 kg and 0.144 g/100 kg for study field 1, study field 2, and study field 3, respectively (non-GLP).

The filter from the Heubach-test that were conducted after drilling were also analysed for their content of clothianidin and imidacloprid residues. For clothianidin the mean residue content of the filters were 0.97 mg/100 kg seeds, 0.72 mg/100 kg seeds, and 0.74 mg/100 kg seeds for study field 1, study field 2, and study field 3, respectively. For imidacloprid the mean residue content of the filters were 1.05 mg/100 kg seeds, 0.80 mg/100 kg seeds, and 0.82 mg/100 kg seeds for study field 1, study field 2, and study field 3, respectively.

A total of 180 Petri-dish samples (60 per study field) and 45 gauze samples (15 per study field) were collected at the study fields during the Field Phase of the study.

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In 44 Petri-dish samples from study field 1 the residue level of clothianidin was below the LOD and in eight Petri-dish samples below the LOQ. Eight Petri-dish samples had residue values above the LOQ (range 0.08 – 1.7 g a.s./ha). In 41 Petri-dish samples from study field 1 the residue level of imidacloprid was below the LOD and in eight samples below the LOQ. Eleven samples had residue values above the LOQ (range 0.08 – 2.4 g a.s./ha).

In all Petri-dish samples from study field 2 and study field 3 the residue level of clothianidin and imidacloprid was below the LOD and none of the 45 gauze samples from study field 1, 2 and 3 had residue levels above the LOQ of clothianidin or imidacloprid (see Table S1).

For calculation residue values below or equal the LOD were set conservatively 0.02 g a.s./ha in Petri-dish samples and 0.01 g a.s./ha in gauze netting samples; residue values below or equal the LOQ were set conservatively 0.07 g a.s./ha in Petri-dish samples and 0.04 g a.s./ha in gauze netting samples. If all residue values of one sample type of one study field were < LOD or < LOQ the mean value and the 90th%ile are reported as <LOD or <LOQ, respectively.

The average residue level of clothianidin found in the Petri-dishes placed in a distance of 1 m to the zero line was 0.10 g a.s./ha at study field 1 and <LOD at study field 2 and 3. At a distance of 3 m to the zero line the average residue level of clothianidin in the Petri-dishes was 0.05 g a.s./ha at study field 1 and <LOD at study field 2 and 3. For imidacloprid the average residue level in the Petri-dishes from study field 1 at 1 m distance to the zero line was 0.14 g a.s./ha and <LOD at study field 2 and 3. At a distance of 3 m to the zero line the average residue level of imidacloprid in the Petri-dishes was 0.07 g a.s./ha at study field 1 and <LOD at study field 2 and 3.

The mean residue level of clothianidin and imidacloprid in the gauze netting was 0.040 g a.s./ha for all three study fields, as values >LOD and ≤LOQ were set to LOQ for calculation.

The results of the residue analysis of all samples are summarised in the table below and are detailed in the Analytical Phase Report (Attachment 1).

Table S1: Summary of clothianidin + imidacloprid residues in Petri-dishes and gauze nettings

	Residue levels of clothianidin [g a.s./ha]								
	Study Field 1			Study Field 2			Study Field 3		
	Petri-dish		Gauze netting	Petri-dish		Gauze netting	Petri-dish		Gauze netting
	1m	3m		1m	3m		1m	3m	
Mean *	0.10	0.05	0.02	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
90th%ile *	0.12	0.07	0.04	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Max *	1.66	0.50	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Min *	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
	Residue levels of imidacloprid [g a.s./ha]								
	Study Field 1			Study Field 2			Study Field 3		
	Petri-dish		Gauze netting	Petri-dish		Gauze netting	Petri-dish		Gauze netting
	1m	3m		1m	3m		1m	3m	
Mean *	0.14	0.07	0.03	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
90th%ile *	0.20	0.11	0.04	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Max *	2.41	0.75	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Min *	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ

LOD Petri-dish = 0.02 g a.s./ha; LOQ Petri-dish = 0.07 g a.s./ha;
LOD gauze netting = 0.01 g a.s./ha; LOQ gauze netting = 0.04 g a.s./ha;
* calculated from the number of analysed samples per study field with rounded values: 30 Petri-dishes per distance, 15 gauze netting samples; residue values below or equal the LOD were conservatively set to equal the LOD, residue values above the LOD and below or equal the LOQ were conservatively set to equal the LOQ

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>>M-502885-03-3@S-605130-01-1

Report:	02.02.06/04; [redacted]; 2015; M-504522-02-2
Title:	Assessment of potential impacts on honeybee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of imidacloprid FS 350A G - Treated winter barley with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering Phacelia tanacetifolia in United Kingdom
Report No.:	R1440009
Document No.:	M-504522-02-2
Guideline(s):	US EPA OCSPP Guideline 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-504522-02-2@S-602343-01-1

Aim

According to the Regulation (EC) 1107/2009 (2009) the possible adverse effects of crop protection products on honeybees have to be assessed. Therefore this study aimed to assess potential effects on honeybee colonies during and after air sowing operation of winter barley seeds, sown in June 2014 directly adjacent to full-flowering Phacelia tanacetifolia. The employed winter barley seeds were commercially treated with Imidacloprid FS 350A G (nominal rate: 70.0 g imidacloprid/100 kg seeds). Moreover, dust drift deposits during the sowing operation of the treated winter barley seeds were concurrently monitored.

The study comprised in total four study fields, two treatment fields and two control fields, both of similar size. The Imidacloprid FS 350A G-treated winter barley seeds were sown on treatment fields, while untreated winter barley seeds dressed with the standard fungicide Prothioconazole FS 100 G were sown on the control fields.

All fields were sown with typical commercial available pneumatic sowing machines. Possible impacts on the colony development and their hibernation performance were investigated. All assessments made on bee colonies placed at the two treatment fields were compared to concurrent and equal assessments made on the two control fields.

Furthermore, concurrent dust drift measurements of the active substance of Imidacloprid FS 350A G (a.s. imidacloprid) were performed by placing vertical gauze-netting-covered construction fences directly adjacent to the sowing area on the two treatment fields.

Material and Methods

Test item

Conventional winter barley seeds dressed with Imidacloprid FS 350A G (nominal treatment rate of 70.0 g imidacloprid/100 kg seeds).

The test item was bagged at the Seed Treatment Application Centre of Bayer CropScience AG in D-40789 Monheim am Rhein, Germany (non-GLP), by employing typical seed-treatment and bagging practices.

The seeds were bagged into 50 kg paper bags, and were labelled with a unique label for conventional seed bags.

Study site and sowing

The study was conducted in the vicinity of Selby, North Yorkshire, United Kingdom, on four different study fields, each two control and treatment fields. To ensure exposition of the honeybees to the potential arising dust drift deposits, the winter barley sowing area was surrounded by flowering Phacelia tanacetifolia, a highly bee attractive crop. The dimension of the winter barley-sown area inside the Phacelia tanacetifolia fields on each study field was approximately 2.0 ha (effective 1.77 to 2.11 ha). The target sowing rate was 200 kg/ha for the control and 206.4 kg/ha on the treatment fields (due to the analysed degree of insecticide loading of 96.9%, effective 219.13 to 221.06 kg/ha) which corresponded to nominally 140 g imidacloprid/ha (effective 148.64 and 149.95 g imidacloprid/ha). In order to keep

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driving distances with filled sowing machines constant, the sowing machines were filled on previously designated filling points at an approximate distance of 1 km from the treatment fields. For the sowing of the treated winter barley seeds, two pneumatic sowing machines (one for the control, one for the treatment fields, manufacturer: Horsch) were used.

Set-up of honeybee hives
In total 32 honeybee colonies were monitored, eight on each study field. The honeybee colonies were placed in the assessment plots on 12 June 2014, with a distance of approximately 3 m between the edge of the winter barley sowing area and the hive entrance. When a queen died or showed significant reduced egg laying capacity, it was replaced by another sister queen. The entrance of each hive was straightened in the direction to the Phacelia to correspond to the apicultural practice. After the exposure period the honeybees were relocated to a monitoring site on 10 July 2014 in the region of York without intensive agricultural activities in the near vicinity.

Honeybee mortality and behaviour
The mortality of honeybees (e.g. workers, pupae, drones) was recorded at the study fields using dead bee traps. If there were ten or more dead bees in one colony after sowing, they were sampled for potential further residue analysis. Behavioural abnormalities of the honeybees at the entrance hole were recorded during the mortality assessments.

Population development and health assessment
Population strength and development (number of cells filled with eggs, larvae or capped brood) as well as food stores (i.e. pollen and nectar) were assessed every three weeks.
At each assessment the percentage coverage of bees, sealed brood, open brood, eggs and food stores (pollen and nectar) on each side of each frame was recorded. This was judged by eye by an experienced assessor who carried out all of the colony assessments. The percentage coverage was given to the closest 5%. For analysis, these percentages were converted to total numbers per hive equivalents per hive.
The quotient between honeybee numbers after and before hibernation was calculated as a value for the hibernation success of honeybee colonies.
During the Field Phase and the Bee Health Phase, bee colonies were kept according to Good Apicultural Practice and all typical apicultural measures were respected.

Dust drift sampling
Three days before the start of the sowing activities seed samples for Heubach analysis (non-GLP) and seed loading (non-GLP) were taken from five seed bags.
To measure aerial and ground dust drift deposits vertically erected gauze-netting-samplers were set up on each assessment plot at the treatment fields. The sowing was only performed when the wind speed at the beginning of the sowing was below 5 m/s.
A total of eight units of gauze-netting-samplers (each with an effective sampling area of approximately 2 m x 3.3 m) were set up at a distance of approximately 3 m from the zero line. Shortly before the beginning of the sowing the gauze-netting-samplers were wetted with a 1:1 (v/v) glycerol/water mixture.
Soil samples for water content and soil characterisation were taken shortly before sowing.
Additionally, field fortification samples (0 µg, 1 µg, 100 µg imidacloprid and clothianidin -fortified gauze sample) were established just before the start of sowing in order to investigate the stability of the samples during transport and storage.
30 minutes after the completion of sowing, the gauze samples (five 50 cm x 50 cm squares cut out of each individual netting unit) were gathered and immediately transferred into separate polyethylene wide mouth bottles.

Residue analysis
Imidacloprid residues in the gauze samples were determined by the Analytical Test Site Bayer CropScience AG.

Results

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Honeybee mortality
In the control and treatment groups, adult honeybee mortality was on the same, generally low level, mostly alternating around five dead bees per day in mean. After sowing statistically significant differences between control and treatment worker bee mortality were observed only on two single days. As the control showed also 2 times during this period an increase of the mortality and the mortality was in both groups on average on a low level (< 10 worker bees/colony) for colonies with on average approximately 11,000 to 20,000 worker bees, it can be concluded that there were no test item related effects regarding to the mortality.
The mortality of the worker bee brood, i.e. pupae or larvae was also on a very low level in almost all colonies. Here on most days, in both groups a mean of < one dead larva or pupa per colony was found in the dead bee traps. Therefore it can be assumed, that there was no test item related effect also regarding to the worker bee brood mortality.

Honeybee colony development
At the pre-sowing assessment, the number of worker bees was very similar in the control and treatment group. At both groups the colony strength increased in a similar way towards the first colony assessment after sowing, which resulted in still very similar numbers of adult worker bees. Also during the following assessments in 2014 and at the assessment after hibernation in April 2015, no significant differences could be detected. Due to the good food supply at the study fields, the amount of brood increased in the period from the pre-sowing assessment towards the first assessment after sowing and remained at this level until the second assessment. From the second assessment on; the colony strength decreased as bees started preparing for hibernation. During the whole Bee Health Phase, the total amount of worker brood was approximately on the same level in both groups.

No statistically significant differences were detected between the control group and the treatment group; neither for the number of worker bees nor for the total brood amount. Also the hibernation index indicates that there is no effect of the test item, as the colonies from the test item group hibernated even slightly better than those of the control group (hibernation index of 0.516 in test item group and 0.443 in control group). Altogether, it can be concluded that the test item did not affect the honey bee colonies in any manner.

During the Field Phase and the Bee Health Phase, the queens of three colonies were replaced by another sister queen according to Good Apicultural Practice due to different reasons. As the replacements had to be done also in the control colonies, there is no limit for a test item related effect on the health of the queens.

Varroa destructor infestation
Natural daily mite fall was recorded during all colony assessments. Though it was on a generally very low level, the Varroa infestation was slightly higher amongst the treatment colonies, at the second assessment even statistically significant. As the values were alternating around only approximately one dead mite per day in mean, it did not influence the honeybee colonies in any manner.

Residues
No residues were found in the control gauze samples. In the field spike samples, the mean recovery at study field T1 was 102 % ± 3.2 % and at study field T2 101 % ± 2.5 %. The Limit of Quantification (LOQ) referring to the determination of imidacloprid from gauze netting samples was 1 µg imidacloprid/L gauze extract, equivalent to 0.04 g a.s./ha. The corresponding Limit of Detection (LOD) was 0.1 µg imidacloprid/L gauze extract, equivalent to 0.004 g a.s./ha.
Due to changing wind conditions and low wind speed, the association of the assessment plots at study field T1 to upwind and downwind was not as clear as on study field T2. This was demonstrated by relatively low residue levels also on the downwind assessment plots (up to 0.086 g a.s./ha). Upwind assessment plot residue levels were below the LOQ beside of the samples from assessment plot A7, were two of five samples were below the LOQ and the other three approximately on the level of the LOQ.

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On study field T2, a clear wind-depending distribution of residues could be shown as the wind conditions were very stable. Downwind assessment plots residues were distinctly higher (0.18 - 0.32 g a.s./ha) compared to those determined on the upwind assessment plots, which were below the LOQ (<0.04 g a.s./ha) beside of assessment plot A3, were three of five samples were below the LOQ and the two other approximately on the level of the LOQ.

Conclusion

To assess the potential effects of Imidacloprid FS 350A G on the colony development of honeybees (Apis mellifera L.), Imidacloprid FS 350A G – treated winter barley seeds (nominal treatment rate 70.0 g imidacloprid/100 kg seeds) were sown during bee flight under field conditions in summer 2014. To increase the possible exposition of the bees, the winter barley was sown inside two fields of flowering Phacelia tanacetifolia, a highly bee attractive crop.

The dust drift measurements made during the sowing operation of imidacloprid-treated winter barley seeds on the treatment fields (nominal treatment rate 70.0 g imidacloprid/100 kg seeds) indicate that seed-treatment dust, abraded and released during the sowing operation with typical, commercial available, pneumatic sowing equipment, resulted in a measurable off-field exposure, which was distinctly higher at the downwind borders of the winter barley sowing areas as compared to the corresponding upwind borders. The maximum vertical dust deposition, as measured by vertically erected gauze-netting units, directly adjacent to the winter barley sowing areas, corresponded to a maximum drift rate of 0.32 g a.s./ha.

The application of Imidacloprid FS 350A G did not cause any effects on the survival of adult bees and bee pupae, foraging activity, behavior, also not on colony development, hibernation performance and colony strength as well as on the bee brood.

Thus this study demonstrated that Imidacloprid FS 350A G – treated winter barley seeds (nominal treatment rate 70.0 g imidacloprid/100 kg seeds), sown during bee flight, did not adversely affect honeybee colonies.

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Report:	02.02.06/05; [redacted]; 2014; M-504065-01-3
Title:	Assessment of potential impacts on honeybee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of Poncho Beta Plus - Treated sugar beet pills with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering Phacelia tanacetifolia in Germany
Report No.:	R12261A
Document No.:	M-504065-01-3
Guideline(s):	Regulation (EC) 1107/2009 BBA Drift Guideline Part VII, 2-1.1 (1992) SANCO/825/00/rev. 8.1 US EPA OCSPP Guideline 850.3040
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-504065-01-3@S-602329-01-1

Aim

According to the Regulation (EC) 1107/2009 (2009) the possible adverse effects of crop protection products on honeybees have to be assessed. Therefore this study aimed to assess potential effects on honeybee colonies during and after vacuum-pneumatic sowing operation of coated sugar beet pills, sown directly adjacent to full-flowering Phacelia tanacetifolia. The employed sugar beet pills were commercially treated with Poncho Beta Plus (nominal rate: 0.60 mg clothianidin a.s./pill, 0.08 mg a.s. beta-cyfluthrin/pill and 0.30 mg a.s. imidacloprid/pill). Moreover, dust drift deposits during the sowing operation of the treated sugar beet pills were concurrently monitored.

The study comprised in total three study fields, one treatment field and two control fields, all of similar size. The Poncho Beta Plus-treated sugar beet pills were drilled on the treatment field only, while maize seeds dressed with the standard fungicide Thiram SC 700 were drilled on the control fields.

Maize seeds at the control fields were sown with a typical deflected vacuum-pneumatic sowing machine, while the Poncho Beta Plus-treated sugar beet pills were drilled by the same machine, but with demounted deflector. Possible impacts on the colony development and their hibernation performance were determined. All assessments made on bee colonies placed at the treatment field were compared to concurrent and equal assessments made on the two control fields.
Furthermore, concurrent dust drift measurements of the active substances of Poncho Beta Plus (a.s. clothianidin and beta-cyfluthrin) were performed by placing vertical gauze covered construction fences directly adjacent to the sowing area on the treatment field.

Material and Methods
Test item
Commercially prepared sugar beet pills, treated with Poncho Beta Plus, at a nominal rate of 0.60 mg clothianidin a.s./pill, 0.08 mg beta-cyfluthrin a.s./pill and 0.30 mg imidacloprid a.s./pill.
The sugar beet pills were seed-coated and bagged at KWS SAAT AG (D-37555 Einbeck, Germany) (non-GLP), by employing typical seed-treatment and bagging practises. The pills received a conventional seed treatment and were dressed in addition to Poncho Beta Plus also with the two standard fungicides Thiram 65 ZR and Hymexazol WP 70.
The coated pills were bagged into 1 Unit (=100,000 pills) cardboxes, and were labelled with a unique label and the TOX-Number.
The maize control seeds have been dressed and bagged by the Seed Treatment Application Centre of Bayer CropScience AG in D-40789 Monheim am Rhein, Germany (non-GLP). The control seeds have received one standard fungicidal seed-treatment (Thiram SC 700, active substance: thiram).

Study sites and sowing

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The study was conducted in the vicinity of Nauen, Eastern Germany, on three study fields, two control and one treatment field. Maize seeds were sown on the control fields and sugar beet pills were sown on the treatment field. To expose the honeybees to the potential arising dust drift deposits, the sugar beet and the control maize sowing areas were surrounded by flowering Phacelia tanacetifolia, a highly bee attractive crop. The dimension of the sugar beet and the control maize-drilled areas inside the Phacelia tanacetifolia fields on each study field were approximately 2.6 ha. The target sowing rate was 130,000 sugar beet pills and 100,000 maize seeds/ha (actual 137,708 sugar beet pills/ha and 103,189 to 101,368 maize seeds/ha). This corresponded to nominally 78.0 g clothianidin a.s./ha, 10.4 g beta-cyfluthrin a.s./ha and 39.0 g imidacloprid a.s./ha. In order to keep driving distances with filled sowing machines constant, the vacuum pneumatic sowing machines were filled on previously designated filling points at an approximate distance of 1 km from the study fields. For the sowing, a vacuum-pneumatic sowing machine (with deflector technology for the control fields and dismounted deflector technology for the treatment field, manufacturer: Amazone) were used.

After the exposure the honeybees were relocated to three monitoring sites in a region of North-Rhine-Westphalia near Gummersbach, with no intensive agricultural activities in the near vicinity. The honeybee hives were set up on these three different locations to avoid potential impacts due to a high density of honeybee hives, like a lack of food due to food concurrence or Varroa destructor infestation. To avoid local factors influencing the results of this study, honeybee hives from each study field were relocated randomly to the monitoring sites (one third of the hives of each study field to each monitoring site).

Set-up of honeybee hives
In total 48 honeybee colonies were monitored in the study, 16 on each study field. The honeybee colonies were placed in the assessment plots on 27.06.2013 with a distance of approximately 3 m between the edge of the maize or sugar beet sowing area and the hive entrance. When a queen died or showed significant reduced egg laying capacity, it was replaced by another sister queen. The entrance of each hive was straightened in the direction to the Phacelia to correspond to the apicultural practise. They were relocated to the monitoring sites in the night of 23.07.2013 to 24.07.2013.

Honeybee mortality and behaviour
The mortality of honeybees (e.g. workers, pupae, drones) was recorded using dead bee traps while the honeybees were located at the study fields. If there were ten or more dead bees in one colony after sowing, they were placed in a sample bottle and labelled unmistakably for potential further residue analysis. Since there were no sampling periods with clearly increased bee mortality no analysis of bee samples have been conducted. Behavioural abnormalities of the honeybees at the entrance hole were recorded during the mortality assessments.

Honeybee colony strength and health assessment
Population strength and development (number of cells filled with eggs, larvae or capped brood) as well as food stores (i.e. pollen and nectar) were assessed using the estimation method developed by the Bee Institute Liebefeld (Imdorf, Buehlmann et al. 1987). The pre-colony assessment was done shortly after colony setup, but before sowing, for the definition of the starting conditions of the colonies. Further colony assessments were done every three weeks until mid of October. In March 2014, the last colony assessment took place to evaluate the overwintering success of the honeybee hives.

Sampling method
To measure aerial dust drift deposits, vertically erected gauze samplers were set up on each assessment plot at the treatment field. The sowing started when the wind speed was below
5 m/s.
Eight gauze samplers (each with an effective sampling area of 2 m x 3.3 m) were set up at a distance of approximately 3 m from the zero line on each assessment plot. Shortly before the beginning of the sowing the gauze samplers were wetted with a 1:1 (v/v) glycerol/water mixture. 30 minutes after the completion

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of sowing, the gauze samples (five 50 x 50 cm squares cut out of each gauze sampler) were gathered and immediately transferred into separate polyethylene flasks.
Additionally, field fortification samples (0 µg, 1 µg, 100 µg clothianidin/beta-cyfluthrin/imidacloprid/methiocarb fortified gauze sample) were established just before the start of sowing of the test item in order to investigate the stability of the samples during transport and storage.
Soil samples for water content analysis (non-GLP) and soil characterisation (non-GLP) were taken shortly before sowing on all study fields.

Residue analysis
Residues of clothianidin, imidacloprid and beta-cyfluthrin in gauze samples as well as all field fortification samples were analysed by Bayer CropScience AG ([redacted] R. & [redacted], Report: MR-14/074). Chromatography and detection by MS/MS in gauze was done according to the methods 00554/M001 (clothianidin), 00537/M002 (imidacloprid) and 00922 (beta-cyfluthrin).
The Limit of Quantitation (LOQ) of the gauze samples (0.25 m2) was 0.04 g a.s./ha for all analytes. The Limit of Detection (LOD) was 0.004 g a.s./ha for both clothianidin and imidacloprid and 0.012 g a.s./ha for beta-cyfluthrin.

Results
Honeybee mortality
In control and treatment group, worker bee mortality was on the same, generally low level, mostly around five to ten dead bees per day in mean. A statistical significant difference between control and treatment worker bee mortality could be seen on some days before the application, so that a test item related effect can be excluded. After sowing, the mean worker bee mortality in the treatment group was never significantly higher than in the control group. In contrast, on two days the worker bee mortality in the control group was significantly higher than in the treatment group. However, no test item related effect regarding to the worker bee mortality could be detected during the whole Field Phase. The mortality of the bee brood was on a very low level (mean control group: 0.52 ± 1.92; mean treatment group: 0.28 ± 0.67). On most days, no brood was found in the dead bee traps.

Honeybee colony development
Honeybee colony strength showed a similar development in the control and treatment group. It slightly increased during the first three weeks after setup of the bee colonies on the study fields. Due to the excellent food supply, the amount of brood increased in the same period. This led to a strong increase of the colony strength from the first to the second colony assessment, both in control and treatment colonies. From the second colony assessment (mid of August), the colony strength decreased towards winter and stagnated on a stable level. During winter, all colonies lost worker bees and due to the normal reduction or even stop of the breeding activity, the number of worker bees decreased towards spring. In the whole Field Phase, the mean colony strength of the control and treatment group was on the same level, no statistical significant differences were detectable.
The mean amount of honeybee brood was at the pre-colony assessment in the treatment group statistically significantly higher than in the control group. This is probably due to a slightly faster adaption of queens of the treatment group to the new colony size after assembling the colonies prior to the pre-colony assessment. This is a random factor that cannot be excluded, even if sister queens are used in this study. Also in the first colony assessment it was higher, but not statistically significantly anymore. However, this indicates that the test item had no adverse effect to honeybee brood. The honeybee brood increased even during sowing to the first colony assessment and decreased afterwards rapidly to a very low level at the fifth colony assessment. This is a normal development for honeybee colonies, which reduce their brood amount typically towards winter. With the beginning of the spring the honeybees started to breed again, approximately on the same level both in control and treatment group.

Varroa destructor infestation
While the infestation with Varroa mites was on approximately the same level in colonies of the control and the treatment group, there were significant differences between the three monitoring sites.

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Statistical analysis showed no significant differences between the locations Agger 1 and Agger 2, but between these two locations and the location Müller in some cases. After the second formic acid treatment, the number of dead Varroa mites was statistically significantly higher at the location Müller than at the location Agger 2. After the first oxalic acid treatment, the number was also higher than at both other locations, but not statistically significantly. In contrast to this, it was statistically significantly lower after the second oxalic treatment in winter. The main reason therefore is the reduced strength of the colonies at Müller compared to the colonies at Agger 1 and Agger 2.

Residues
The results of all field spiked fortification gauze samples showed that clothianidin, imidacloprid and beta-cyfluthrin were stable during storage and transport. Residues in control samples were always below the LOD.
No residues of clothianidin, imidacloprid and beta-cyfluthrin above the LOD (0.012 g a.s./ha for beta-cyfluthrin and 0.004 g a.s./ha for clothianidin and imidacloprid) were detected in any of the gauze samples obtained from the study field during sowing of the test item.

Conclusion
To assess the potential effects of Poncho Beta Plus on the colony development of honeybees (Apis mellifera L.), Poncho Beta Plus – treated sugar beet pills (0.60 mg clothianidin a.s./pill, 0.08 mg beta-cyfluthrin a.s./pill and 0.30 mg imidacloprid a.s./pill) were sown (138,500 sugar beet pills/ha) during bee flight in summer 2013. To increase the possible exposition of the bees, the sugar beet was sown inside a field of flowering Phacelia tanacetifolia, a highly bee attractive crop.
The application of Poncho Beta Plus did not cause any effects on the survival of adult bees and bee pupae, foraging activity, behaviour, colony development and colony strength as well as on the bee brood and the hibernation success.
The dust drift measurements made during the sowing operation of Poncho Beta Plus - treated sugar beet pills on the treatment field indicate that pill-treatment dust, abraded and released during the sowing operation with non-modified (not deflected) vacuum-pneumatic sowing equipment and dismounted chassis of the discharged air system, did not result in a measurable off-field exposure as all analysed samples were below their respective LOD (0.012 g a.s./ha for beta-cyfluthrin and 0.004 g a.s./ha for clothianidin and imidacloprid).

Thus this study demonstrated that Poncho Beta Plus – treated sugar beet pills (0.60 mg clothianidin a.s./pill, 0.08 mg beta-cyfluthrin a.s./pill and 0.30 mg imidacloprid a.s./pill), sown during bee flight did not adversely affect honeybee colonies.

>>M-504065-01-1@S-602328-01-1

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Report:	02.02.06/06; [redacted]; 2012; M-424386-01-2
Title:	Imidacloprid FS 350 - Investigating residues in dust deposits following vacuum-pneumatic drilling of imidacloprid treated cotton seeds in Greece during springtime 2011
Report No.:	S11-02083
Document No.:	M-424386-01-2
Guideline(s):	Working document 1607/VI/97 rev. 1 with the partial integration of the BBA Drift Guideline Part VII, 2-1.1 (1992) and 2010/21/EU
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-424386-01-2@S-603073-01-1

Test item: Pella site Name: Cotton seeds treated with Imidacloprid FS 350 Active ingredient: Imidacloprid Analyzed content of active ingredient: 484.67 g/100 kg seeds Batch: EA 385 11 10 23

Larissa site Name: Cotton seeds treated with Imidacloprid FS 350 Active ingredient: Imidacloprid Analyzed content of active ingredient: 555.07 g/100 kg seeds Batch: EA 385 11 10 27

The field study was conducted in Greece, one trial in the vicinity of Pella (trial S11-02083-01) and a second trial in the vicinity of Larissa (trial S11- 02083-02). Cotton seeds, pre-treated with Imidacloprid FS 350 (provided by Bayer CropScience AG), were sown in Giannitsa near Pella (S11- 02083-01) on 12 May 2011 and in Glafki near Larissa (S11-02083-02) on 13 May 2011 and 14 May 2011.

The purpose of the study was to determine the deposition of dust from the seed treatment emitted from a vacuum-pneumatic drilling machine during sowing of Imidacloprid FS 350 treated cotton seed. Dust (mechanical abrasion of the treated seed item) released during seeding of cotton seeds was collected using Petri dishes and cellulose air filters attached to the air fan of the driller (see FIGURE 10 and FIGURE 12). The size of the field plots where dust deposition was measured was 200 x 52.08 m for the trial at Pella and 176 m x 57 m for the trial at Larissa (dust collected in Petri dishes). The plots where dust emission was measured had a total size of 10490.4 m² for the trial at Pella and 10776.8 m² for trial at Larissa (dust collected in air filters which were fitted to a filter box connected to the fan exhaust outlet via tubing).

Before the filter trials started 20 m were drilled to prime the tube. The actual drilling rate for the Pella site (S11-02083-01) was 88.84 g a.i./ha, equivalent to 181,126 seeds/ha. A total area of 2.0906 ha was drilled.

For the Larissa site (trial S11-02083-02) the Petri dishes trial the actual applied drilling rate for the dust trial at 202,241 seeds/ha was equivalent to an application rate of 109.18 g a.i./ha. For the filter trial the actual applied drilling rate at 195,908 seeds/ha was equivalent to an application rate of 105.76 g a.i./ha. A total area of 1.0032 ha was drilled for the Petri dishes trial and 1.0777 ha for the air filter trial.

For Pella site (trial S11-02083-01) the average wind speed during drilling was 2.33 ± 0.89 m/s (0.39 m/s to 4.79 m/s) and the average deviation to the intended wind direction was 19.91° ± 23.85° (range -54.95° to 193.22°).

For Larissa site (trial S11-02083-02) the average wind speed during drilling was 2.44 ± 0.89 m/s (0.79 m/s to 6.77 m/s) and the average deviation to the intended wind direction was 9.60° ± 39.07° (range -79.11° to 173.31°).

70 Petri dishes, filled with glycerol/water (1/1, v/v) were placed at 1, 3, 5, 10, 20, 30 and 50 m distance from the zero line of sowing (first driller pass). The Petri dishes were placed horizontally on the ground. Soil samples from the upper 10 cm were taken before drilling for soil characterization and for analysis of potential residues of imidacloprid in the soil that might have originated from previous treatments. Soil samples from the upper 5 cm were taken for the moisture content determination.

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The sampling liquid in the Petri dishes and the air filters were analysed for residues of imidacloprid after drilling. Soil samples were not analysed for imidacloprid.

Dates of work: 12 May 2011 (Pella site) and 13 May 2011 (Larissa site) start of field work to 12 September 2011 (end of residue analysis).

Findings (Residue Analysis)

For the trial at the Pella site (S11-02083-01) the residue results of two of the dishes in 30 m distance and seven of the dishes in 50 m distance were below the LOQ. The average amount of imidacloprid was 33.115 mg a.i./ha in a distance of 1 m, 27.316 mg a.i./ha in a distance of 3 m, 21.028 mg a.i./ha in a distance of 5 m, 18.444 mg a.i./ha in a distance of 10 m, 15.090 mg a.i./ha in a distance of 20 m, 8.733 mg a.i./ha in a distance of 30 m and 6.986 mg a.i./ha in a distance of 50 m. The 90th percentile was 35.769 mg a.i./ha, equivalent to 0.040 % of the field rate for 1 m distance, 31.857 mg a.i./ha, equivalent to 0.036 % of the field rate for 3 m distance, 25.849 mg a.i./ha, equivalent to 0.029 % of the field rate for 5 m distance, 23.823 mg a.i./ha, equivalent to 0.027 % of the field rate for 10 m distance, 27.526 mg a.i./ha, equivalent to 0.031 % of the field rate for 20 m distance, 10.200 mg a.i./ha, equivalent to 0.011 % of the field rate for 30 m distance and 6.986 mg a.i./ha, equivalent to 0.008 % of the field rate for 50 m distance.

For trial at the Larissa site (S11-02083-02) the average amount of imidacloprid was 237.390 mg a.i./ha in a distance of 1 m, 151.810 mg a.i./ha in a distance of 3 m, 195.543 mg a.i./ha in a distance of 5 m, 96.549 mg a.i./ha in a distance of 10 m, 65.530 mg a.i./ha in a distance of 20 m, 36.887 mg a.i./ha in a distance of 30 m and 26.827 mg a.i./ha in a distance of 50 m. The 90th percentile was 370.616 mg a.i./ha, equivalent to 0.339 % of the field rate for 1 m distance, 249.756 mg a.i./ha, equivalent to 0.229 % of the field rate for 3 m distance, 249.546 mg a.i./ha, equivalent to 0.229 % of the field rate for 5 m distance, 136.510 mg a.i./ha, equivalent to 0.125 % of the field rate for 10 m distance, 92.218 mg a.i./ha, equivalent to 0.084 % of the field rate for 20 m distance, 51.278 mg a.i./ha, equivalent to 0.047 % of the field rate for 30 m distance and 44.921 mg a.i./ha, equivalent to 0.041 % of the field rate for 50 m distance. The air filters attached to the fan exhaust including the tube trapped a total of 1151.5 mg imidacloprid/ha, equivalent to 1.3 % of the field rate (Pella site) and 4415.8 mg imidacloprid/ha, equivalent to 4.2 % of the field rate (Larissa site).

Summary of deposition collected in petri dishes and air filters					
	Petri dishes			Air Filters and Tubes	
Distance from Zero Line [m]	Imidacloprid mean Deposition [mg a.i./ha]	90th percentile [mg a.i./ha]	% of field rate (90th percentile)	Imidacloprid mean Deposition [mg a.i./ha]	% of applied a.i./ha
S11-02803-01					
1	33.115	35.769	0.040	1151.5	1.3
3	27.316	31.857	0.036		
5	21.028	25.849	0.029		
10	18.444	23.823	0.027		
20	15.090	27.526	0.031		
30	8.733	10.200	0.011		
50	6.986	6.986	0.008		
S11-02803-02					
1	237.390	370.616	0.339	4415.8	4.2
3	151.810	249.756	0.229		
5	195.543	249.546	0.229		
10	96.549	136.510	0.125		
20	65.530	92.218	0.084		
30	36.887	51.278	0.047		
50	26.827	44.921	0.041		

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>>M-424386-01-2@S-603073-01-1

Report:	02.02.06/07; [redacted]; 2005; M-257837-01-2
Title:	Summary of particle size measurements of dust generated by six seed drilling macines
Report No.:	MEF-05/429
Document No.:	M-257837-01-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

<<M-257837-01-2@S-605935-01-1

Six seed drilling machines were tested at the Arvalis institute (Boigneville, 91720, France), in order to compare their operational characteristics with respect to the generation of dust from the seed drilling operation. The comparisons included observations of orientation of vented air from the blower or fan, measurements of blower outlet dimensions, maximum airspeed at the exit of the fan, and measurements of the particle size distribution of the dust emitted by the blower. The latter measurements are summarised here, with respect to their possible effects on the drift of dust from seed drilling operations.

The principal objective of the measurements was to determine if the machinery type had a large effect on the quality of the dust generated during the drilling process. Although the quantity of dust generated by different machinery does differ depending on the mechanism for attaching the seed to the distribution wheel, the age of the equipment, *etc.*, it is more likely that the quality of the dust is most strongly affected by formulation and formulation additives rather than equipment differences.

>>M-257837-01-2@S-605935-01-1

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03 - Bumble bees

03.01 – Effects

03.01.01 - Lab Studies

Report:	03.01.01/01; [redacted]; 1999; M-016786-01-3
Title:	Bumblebee (Bombus terrestris L.) oral toxicity study in the laboratory with imidacloprid techn.
Report No.:	AH99.4.22.2
Document No.:	M-016786-01-3
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

<<M-016786-01-3@S-602475-01-1

The purpose of the toxicity study was to examine the effects of imidacloprid techn. on bumblebees when applied in the laboratory.

Per concentration 30 bumblebees were fed individually with 10 µl sucrose solution 50%, containing a range of concentrations of imidacloprid techn.

A range finding test preceded the definitive test. It appeared that 1.1 µg imidacloprid techn. per bumblebee killed 97% of the bumblebees, within 24 hours. The oral intake of 0.1 µg imidacloprid techn. per bumblebee, affected 90% of the bumblebees within 24 hours. Mortality was 0% after 48 hours. No effects on behaviour or survival were observed for doses of 0.01 µg or less imidacloprid techn. Based on these data, 0.96 µg, 0.72 µg, 0.53 µg, 0.33 µg and 0.11 µg imidacloprid techn. per 10 µl was offered to the bumblebees.

All concentrations fed in the definitive test, resulted in effect on the bumblebees. The most significant effect was the "frozen behaviour" at which the bumblebees are motionless except for a little trembling of body parts like abdomen, antennae or tarsus. Beside that, spasms and paralysis were observed as well. These effects lasted at least during the observation period of 72 hours. Most of the affected bumblebees which had taken in amounts of imidacloprid techn. of 0.33 µg / bumblebee or more died within 24 hours.

Amounts of imidacloprid techn. higher than 0.11 µg per bumblebee, cause effect and mortality of the bumblebees.
The LD_{50} of imidacloprid techn. based on the linear regression is:
LD_{50} (24 hours): 0.33 µg imidacloprid techn. ($r^2 = 0.73$)
LD_{50} (48 hours): 0.22 µg imidacloprid techn. ($r^2 = 0.53$)
LD_{50} (72 hours): 0.15 µg imidacloprid techn. ($r^2 = 0.53$)
The effect of imidacloprid techn., in the concentrations higher than 0.1 µg / bumblebee is obvious. The ED_{50} is between 0.1 and 0.01 µg / bumblebee. The data provide no basis for an accurate ED_{50} calculation.

>>M-016786-01-3@S-602475-01-1

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Report:	03.01.01/02; [redacted]; 1999; M-017116-01-4
Title:	Bumblebee (Bombus terrestris L.) contact toxicity study in the laboratory with imidacloprid techn.
Report No.:	AH99.4.22.1
Document No.:	M-017116-01-4
Guideline(s):	US EPA OCSPP Guideline no 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-017116-01-4@S-602493-01-1

The purpose of the toxicity study was to examine the effects of imidacloprid techn. on bumblebees when applied in the laboratory.

Per concentration 30 bumblebees were exposed individually to imidacloprid techn. by way of administration on the ventral part of the thorax with 1 µl acetone, containing a range of concentrations of imidacloprid techn.

A range finding test preceded the definitive test. In order to determine a toxic concentration, based on the data, obtained in the range finding test and in consultation with the sponsor, six concentrations: 101 µg, 65 µg, 31 µg, 8 µg, 4 µg and 0.1 µg imidacloprid techn. per 1 µl acetone were administered to the bumblebees.

All concentrations tested in the definitive test, resulted in effect on the bumblebees. The most significant effect was the "frozen behaviour" at which the bumblebees are motionless except for a little trembling of body parts like abdomen, antennae or tarsus. Besides that, spasms and paralysis were observed. These effects lasted at least during the observation period of 72 hours.
There was no correlation between the amount of imidacloprid techn. and the number of dead and affected bumblebees, whether the bumblebees were treated with 4 µg imidacloprid techn. or with 101 µg imidacloprid techn. Within 72 hours these treatments resulted in 90% to 100% dead or affected bumblebees. Imidacloprid techn., administered to bumblebees in the amount of 0.1 µg resulted in 47% mortality and 60% effect. Concentrations imidacloprid techn. of 0.05 µg / 1 µl or less, administered per bumblebee did not cause effect and mortality (result range finding test). Mortality continued during the observation period. Bumblebees that were affected may have died of starvation.

The data provide no basis for an accurate LD_{50} and ED_{50} but it is obvious that the exposure of 0.1 µg imidacloprid techn. or more per bumblebees does seriously affect bumblebees

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Report:	03.01.01/03;[redacted]; 2014; M-494283-01-3
Title:	Clothianidin + imidacloprid FS 275 (100+175 g/L): Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No.:	S13-05151
Document No.:	M-494283-01-3
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001) US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

<<M-494283-01-3@S-602260-01-1

Materials and Methods:
Test item: Name: Clothianidin + Imidacloprid FS 275 (100+175 g/L)
TOX No.: 10068-00
Specification No.: 102000025006-01
Content of a.s.: 100.3 g/L clothianidin (analysed) 176.7 g/L imidacloprid (analysed)

The contact toxicity of Clothianidin + Imidacloprid FS 275 (100+175 g/L) to the bumble bee (*Bombus terrestris* L.) was determined in a dose-response test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).

In the laboratory, the bumble bees were exposed to 1.23, 3.70, 11.11, 33.33 and 100 µg total a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24, 48 and 72 hours after treatment. The control group was exposed for the same period of time under identical exposure conditions to tap water.

Dates of work: 27 November 2013 – 07 February 2014
Findings:
In the control group, treated with tap water, no mortality was observed during the 72 hour test period.
In the test item treatment group, a mortality of 63.33 % was observed at the highest dose level corresponding to 100 µg total a.s./bumble bee at the final assessment after 72 hours.
In the reference item group, mortality was ≥ 50 % at the end of the test. Thus, the test was considered to be valid.

Table 1: LD50 values in the bumble bee contact toxicity test with Clothianidin + Imidacloprid FS 275 (100+175 g/L)

Clothianidin + Imidacloprid FS 275 (100+175 g/L)	Contact toxicity test [µg total a.s./bumble bee]
LD_{50} (24 h)	>100
LD_{50} (48 h)	79.2
LD_{50} (72 h)	54.9

In the test item treatment group, moribund, affected and apathetic bumble bees were observed at all tested dose levels at the 24, 48 and 72 hour assessments.
The test item dose level corresponding to 3.70 µg total a.s./bumble bee was determined to be the NOED (No Observed Effect Dose) for mortality.
Conclusion:
The 72 hour contact LD50 value for Clothianidin + Imidacloprid FS 275 (100+175 g/L) was determined to be 54.9 µg total a.s./bumble bee.

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>>M-494283-01-3@S-602260-01-1

Report:	03.01.01/04; [redacted]; 2014; M-494307-01-3
Title:	Imidacloprid FS 350 (350 g/L) - Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No.:	S13-05153
Document No.:	M-494307-01-3
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001) U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

<<M-494307-01-3@S-602261-01-1

Materials and Methods:

Test item: Name: Imidacloprid FS 350 (350 g/L)
TOX No.: 10231-00
Specification No.: 102000007262
Content of a.s.: 355.2 g/L imidacloprid (analysed)

The contact toxicity of Imidacloprid FS 350 (350 g/L) to the bumble bee (Bombus terrestris L.) was determined in a dose-response test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).
In the laboratory, the bumble bees were exposed to 1.23, 3.70, 11.11, 33.33 and 100 µg imidacloprid a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24, 48, 72 and 96 hours after treatment. The control group was exposed for the same period of time under identical exposure conditions to tap water.

Dates of work: 17 December 2013 – 08 February 2014

Findings:

In the control group, treated with tap water, no mortality was observed during the 96 hour test period.
In the test item treatment group, a mortality of 46.7 % was observed at the highest dose level corresponding to 100 µg imidacloprid a.s./bumble bee at the final assessment after 96 hours.
At the dose level corresponding to 33.33 µg imidacloprid a.s./bumble bee, a mortality of 53.3% was observed after 96 hours.
In the reference item group, mortality was ≥ 50 % at the end of the test. Thus, the test was considered to be valid.

Table 1: LD50 values in the bumble bee contact toxicity test with Imidacloprid FS 350 (350 g/L)

Imidacloprid FS 350 (350 g/L)	Contact toxicity test [µg imidacloprid a.s./bumble bee]
LD_{50} (24 h)	>100
LD_{50} (48 h)	>100
LD_{50} (72 h)	>100
LD_{50} (96 h)	85.3*

* Due to a week dose-response, no meaningful confidence limits can be derived

Moribund, affected and apathetic bumble bees were observed at all tested dose levels during the entire test period of 96 hours.
The NOED (No Observed Effect Dose) was determined to be < 1.23 µg imidacloprid a.s./bumble bee.

Conclusion:

The 96 hour contact LD50 value for Imidacloprid FS 350 (350 g/L) was determined to be 85.3 µg imidacloprid a.s./bumble bee.

>>M-494307-01-3@S-602261-01-1

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Report:	03.01.01/05; [redacted]; 2014; M-494321-01-3
Title:	Imidacloprid + pencycuron FS 370 (120+250 g/L) - Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No.:	S13-05154
Document No.:	M-494321-01-3
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001) US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

<<M-494321-01-3@S-602263-01-1

Materials and Methods:
Test item: Name: Imidacloprid + Pencycuron FS 370 (120+250 g/L)
TOX No.: 09865-00
Specification No.: 102000008024-02
Content of a.s.: 119.8 g/L imidacloprid (analysed) 252 g/L pencycuron (analysed)

The contact toxicity of Imidacloprid + Pencycuron FS 370 (120+250 g/L) to the bumble bee (*Bombus terrestris* L.) was determined in a dose-response test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).
In the laboratory, the bumble bees were exposed to 1.23, 3.70, 11.11, 33.33 and 100 µg imidacloprid a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24, 48, 72 and 96 hours after treatment. The control group was exposed for the same period of time under identical exposure conditions to tap water.

Dates of work: 03 December 2014 – 08 February 2014

Findings:
In the control group, treated with tap water, no mortality was observed during the 96 h test period.
In the test item treatment group, a mortality of 80.0 % was observed at the highest dose level corresponding to 100 µg imidacloprid a.s./bumble bee at the final assessment after 96 hours.
In the reference item group, mortality was > 50 % at the end of the test. Thus, the test was considered to be valid.

Table 1: LD50 values in the bumble bee contact toxicity test with Imidacloprid + Pencycuron FS 370 (120+250 g/L)

Imidacloprid + Pencycuron FS 370 (120+250 g/L)	Contact toxicity test [µg a.s./bumble bee]
LD_{50} (24 h)	>100
LD_{50} (48 h)	>100
LD_{50} (72 h)	>100
LD_{50} (96 h)	28.1

In the test item treatment group, moribund, affected and apathetic bumble bees were observed at all tested dose levels during the entire 96 hour test period.
The test item dose level corresponding to 3.70 µg imidacloprid a.s./bumble bee was determined to be the NOED (No Observed Effect Dose) for mortality.

Conclusion:
The 96 hour contact LD50 value for Imidacloprid + Pencycuron FS 370 (120+250 g/L) was determined to be 28.1 µg imidacloprid a.s./bumble bee.

>>M-494321-01-3@S-602263-01-1

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03.01.02 - Field

Report:	03.01.02/01; [redacted]; 2001; M-081939-01-3
Title:	Evalution of the effects of a soil treatment of ornamental plants with imidacloprid WG 5 on nectar and pollen sampling bumblebees (Bombus terrestris) in the semifield
Report No.:	BT001
Document No.:	M-081939-01-3
Guideline(s):	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-081939-01-3@S-603226-01-1

Material and methods: Ornamental plants, *Lobelia erinus*, received soil treatment at a rate of 15 mg a.i./l soil substrate before flowering and/or at full blossom with Imidacloprid WG 5 (NTN 33893: article No. 0004897447, formulation No. 03584/0344(0285), a.i. content 4.95%, TOX No. 05672-00. Control plants received no treatment.
The following 5 treatments with two replicates for each treatment were defined by different modes of application and different proportions of treated and untreated plants:

K: control: no treatment

A: 15 mg a.i./l soil substrate, pre-flowering application, 50% treated and 50% untreated plants in the tent;

B: 15 mg a.i./l soil substrate, pre-flowering application plus application at full blossom, 50% treated and 50% untreated plants in the tent;

C: 15 mg a.i./l soil substrate, pre-flowering application plus application at full blossom, 10% treated and 90% untreated plants in the tent;

D: 15 mg a.i./l soil substrate, application at full blossom, 50% treated and 50% untreated plants in a tent

The plants were placed inside tents (floor space 4.5m x 4.5m) on the experimental farmland "Höfchen". In each tent one bumblebee colony (containing approx. 50 bumblebees) was allocated.
The bumblebees were observed for the parameters mortality, foraging activity and colony strength and condition.

Dates of biological work: 2001-06-19 to 2001-07-10

Findings: Findings for the treatments are presented in table 1.

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Treatment	K	A	B	C	D
Average mortality per treatment and day in front of the hive [n]	0.00	0.09	0.09	0.25	0.17
Average mortality per treatment and day inside tents [n]	0.15	1.80	2.42	0.42	2.75
Average foraging activity per treatment and day [n]	137.09	51.75	17.75	66.00	31.92
Weight decrease of the mini-hives during the study [%]	30.95	21.65	25.40	28.60	22.90
Average number of bumblebees alive at study termination in the mini-hives [n]	50.00	22.00	20.00	33.50	14.00
Average number of bumblebees dead at study termination in the mini-hives [n]	0.00	0.50	7.00	0.00	3.50
Food stores at study termination	yes	yes	yes/no	yes	no
Non-capped brood at study termination	yes	yes	no	yes	no

Conclusion:
Increased mortality was observed in the bumblebee colonies of treatment A, B and D. Foraging activity was higher in the control K than in each of the treatments. The hive weight development was comparable in all treatments. The highest number of alive bumblebees and the lowest number of dead bumblebees was found in the control and treatment C. Food was stored in all treatments except in treatment D and one replicate of treatment B. Capped brood was found in all treatments except B and D.

In general, the least effects observed were found in treatment C where only 10 % of the plants were treated prior to flowering and at full blossom. Furthermore in treatment A, where only pre-flowering treatment was performed, there were less effects observed than in treatment B and D where an application at full blossom was carried out.

>>M-081938-01-3@S-603226-01-1

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Report:	03.01.02/02; [redacted]; 2002; M-060086-01-3
Title:	Evaluation of the effects of a soil treatment of ornamental plants with Imidacloprid WG 5 on nectar and pollen sampling bumblebees (Bombus terrestris) in the semifield (test plants: Erica and Lobelia)
Report No.:	M-060086-01-3
Document No.:	M-060086-01-3
Guideline(s):	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-060086-01-3@S-604656-01-1

Material and methods: Ornamental plants, *Lobelia erinus* and *Erica gracilis,* received soil treatment at a rate of 15 mg a.i./l soil substrate at full blossom with lmidacloprid WG 5 (NTN 33893; article No. 0004897447, formulation No. 03584/0344(0285), a.i. content 4.93%, TOX No. 0502-00. Control plants received no treatment.

5 treatments with two replicates for each treatment were defined by different proportions of treated and untreated plants with a proportion of 50% of the ground covered with untreated and 50% of the ground covered with treated plants for the treatments A and B and a proportion of 10% of the ground covered with treated and 90% of the ground covered with untreated plants for the treatments C and D. When taking into account the different sizes of the two plant species used, the numbers of plants for the treatments and the control were as follows:

K: control: no treatment, 130 untreated Lobelia erinus and 90 untreated Erica gracilis in the tent

A: 15 mg a.i./I soil substrate, application at full blossom, 130 treated Lobelia erinus and 90 untreated Erica gracilis in the tent

B: 15 mg a.i./I soil substrate, application at full blossom, 90 treated Erica gracilis and 130 untreated Lobelia erinus in the tent

C: 15 mg ad 1 l soil substrate, application at full blossom, 22 treated Lobelia erinus and 160 untreated Erica gracilis in the tent

D: 15 mg a.i./1 soil substrate, application at full blossom, 22 treated Erica gracilis and 198 untreated Lobelia erinus in a tent

The plants were placed inside tents (floor space 4.5m x 4.5m) on the experimental farmland "Laacher Hof". In each tent one bumblebee (Bombus terrestris) colony (containing approx. 50 bumblebees) was allocated.

The bumblebees were observed for the parameters mortality, foraging activity and colony strength. Condition of the colonies (brood, food storage) were assessed according to an internal assessment scheme.

Dates of biological work: 2001-09-10 to 2001-09-27

Findings:

Findings for the treatments are presented in table 1.

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Bumblebee Semi Field Test

Table 1: Summary

Treatment	K	A	B	C	D
Average mortality per treatment and day inside tents [n]	0.07	1.69	1.32	0.69	0.94
Average foraging activity per treatment and day [n]	182.50	7.38	8.82	29.94	28.94
Weight development of the colonies during the study [% of initial weight]	-16.49	7.55	9.26	-7.70	-8.63
Average number of bumblebees alive at study termination in the colonies [n]	36	8.5	6	13	17
Average number of bumblebees dead at study termination in the colonies [n]	0	23.5	16.5	6.5	8.5
Nectar deposition in % cells designated for food stores visually assessed in the nest at study termination*	97.5-100	0-32.5	0-32.5	0-65	0-65
Effects on brood visually assessed in the nest at study termination*	No	Strong	Strong	Slight-medium	Strong

* for details on scale see description in paragraph 3.7

Conclusion:

The effects of the treatment to bumblebee colonies confined in tent cages with treated ornamental plants was clearly related to exposure intensity, i.e. the ratio of treated vs. untreated plants. Foraging activity, survival and brood development were clearly affected in all treatments with significantly greater effects in the treatment groups with a greater proportion of treated plants. The very strong decrease in foraging activity indicates a significant antifeedant response caused by the treatments. This antifeedant response may act protectively to pollinating hymenopterans under field conditions where alternative foraging sites are available.

It is very likely that the observed effects on brood development are caused by the reduced number of remaining live adult bumblebees at study termination.

2060086-01-3@S-604656-01-1

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Report:	03.01.02/03; [redacted]; 2003; M-109444-01-3
Title:	Assessment of the effects of a soil treatment of ornamentals with imidacloprid WG 5 on nectar and pollen collecting bumblebees (Bombus terrestris) in the field (test plant: Lobelia erinus)
Report No.:	M-109444-01-3
Document No.:	M-109444-01-3
Guideline(s):	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

<<M-109444-01-3@S-604664-01-1

Material and methods: Ornamental plants of the species *Lobelia erinus* received a soil treatment at a rate of 0.015 g a.i./l soil substrate at full blossom with Imidacloprid WG 5 (NTN 33893: article No. 00-05439280, formulation No. 03584/0460(0460), a.i. content 4.68%, TOX No. 06066-00). Control plants received no treatment.

The study was carried out in 34 gardens in the surroundings of Cologne/Düsseldorf and the "Bergisches Land" in Nordrhein-Westfalen, Germany. In each garden, 50 *Lobelia erinus* and a bumblebee *(Bombus terrestris)* colony (containing approx. 80-100 bumblebees) were placed. In 17 gardens, the plants were treated and in the other 17, the plants were untreated. Endpoints of the study were mortality, flight activity and foraging activity on *Lobelia* and on alternative plants growing in the gardens. As far as possible, the assessments were conducted every day. Six weeks after setting up the colonies in the gardens, a final monitoring of the bumblebee nests was conducted to examine the brood cells and the condition of the nests.

Dates of biological work: 2002-05-23 to 2002-07-26

Findings:

Findings for the treatment groups are presented in Table 1.

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Bumblebee Field Test Imidacloprid WG 5

Table 1: Summary results of the bumblebee monitoring

Treatment group	Control	Treatment
Total mortality of all bumblebee species per treatment group [n]	5	49*
Average mortality of all bumblebee species per garden [n]	0.29	2.88*
Average mortality of all bumblebee species per garden and day [n]	0.01	0.07*
Total mortality of *B. terrestris* per treatment group [n]	5	33*
Average mortality of individuals of *B. terrestris* per garden [n]	0.29	1.94*
Average foraging activity on *Lobelia* per garden and assessment [n]	0.39	0.17
Average foraging activity on alternative plants growing in the gardens per garden and assessment [n]	3.09	6.02
Average flight activity per garden and assessment [n]	4.82	4.25
Nest structure** [n] -	6	11
+-	1	1
+	3	0
++	6	2
+++	1	3
Average number of bumblebees found alive in the nest at the final assessment [n]	16.35	16.24
Average number of bumblebees found dead in the nest at the final assessment [n]	2.12	2.18
Average increase in weight during the study [%]	12.65	14.94
Average nest size at the final assessment [cm^2]	67.29	73.65

* Statistically significantly different from control (Mann-Whitney U Test, one-sided, p<0.01)
** Nest structure as a figure of quality of the brood cells; it was visually assessed and classified from "-" i.e. the nest was in a poor condition, to "+++" which means that the cells were very well developped

Observations: 9 nests of the control and 12 nest of the treatment were parasitized by the bee moth *Aphomia sociella* (Lepidoptera: Pyralidae) during the study. The larvae of A. *sociella* live on the wax cells and on the bumblebee's larvae and destroy the nest. Thus, the endpoint "colony condition" was severely influenced by this not treatment related parameter.

Conclusions: The mortality was higher in treatment than in control. However, the absolute mortality levels were low in control as well as in treatment, and far below a level where effects to the colonies would have to be expected.
The foraging activity on *Lobelia* and the flight activity did not differ significantly between the treatment groups, although the foraging activity on *Lobelia* was higher in the control compared to the treatment. The foraging activity on alternative plants was higher in the treatment than in the control.

A treatment of ornamentals in home gardens with lmidacloprid WG 5 at a rate of 15 mg a.i./I soil poses only a negligible risk to foraging bumblebees. Mortality was slightly increased in the treatment, but it remained on a rather low absolute level, so that the colonies were not at risk. In all other endpoints, no clear difference was found in treatment and control. There seemed to be a preference of the bumblebees for untreated plants over treated ones, which will further act protective to the bumblebees under field conditions.

>>M-109444-01-3@S-604664-01-1

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Report:	03.01.02/04; [redacted]; 2014; M-504174-01-3
Title:	A field study to evaluate effects of Monceren G on the bumble bee (Bombus terrestris L; Hymenoptera, Apidae) in potato in southern Germany in 2014
Report No.:	S14-03554
Document No.:	M-504174-01-3
Guideline(s):	No specific guidelines are available. The test design is based on: SETAC/ESCORT recommendations (BARRETT et al. 1994) OEPP/EPPO Guideline No. 170 (4), 2010 US EPA OCSPP Guideline No. 850.3040
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-504174-01-3@S-602337-01-1

1.1 Material and Methods

Test item: Monceren G; TOX number: TOX10501-00; Batch: 2014-001766-01; content of a.i. (nominal): 120 g/L imidacloprid + 250 g/L pencycuron

Test species: Bombus terrestris L. (Hymenoptera, Apidae)

Test design: The field study was carried out on agricultural fields in southern Germany (Heilbronn) following the SETAC/ESCORT recommendations and the OEPP/EPPO Guideline No. 170 (4), 2010. The field crop was potato; Solanum tuberosum L.
The study included 2 treatment groups (C = control / T = test-item) with six replicates (6 replicate bumble bee colonies) per treatment group for biological assessments.
Bumble bees were assessed for their flight activity within the crop, flight activity at the entrances of the hives. The weight of the hives and the sugar consumption were assessed. Moreover, the mortality of adult bees and larvae was observed at every assessment date during the field phase and at the monitoring site. Additionally, three samplings of pollen for residue analysis and palynological analysis at different dates were carried out by taking the pollen loads from forager bumble bees of additional colonies only used for residue sampling. Before set-up and after the field phase, brood assessments were done to document all stages of development and the vitality of the colonies.

Endpoints: Flight activity in the crop, flight activity at the entrance of the hives, mortality of adults and larvae, weight of hive and sugar consumption, initial and final brood assessment including the production of young queens and drones.

Application: The application was done at a separate study S14-01392. The insecticide Monceren G was applied as in-furrow application at planting at a rate corresponding to nominally 1.5 L product/ha (equivalent to 180 g imidacloprid/ha and 375 g pencycuron/ha) under field conditions on potato (Solanum tuberosum L.).

Test conditions: Exposure of the bumble bee colonies started at the beginning of potato flowering. After end of flowering, the colonies were transferred to a monitoring site were the assessments were followed until the colonies reached their peak of colony development and switched over to the reproduction phase i.e. young queen and male (drone) production.

Dates of work: 01 Jul 2014 to 09 Oct 2014

1.2 Findings

The mortality of adult bumble bees, bumble bee larvae within the hives, flight activity at the entrance of the hives, flight activity in the crop, the sugar consumption and the weight of the hives were assessed. No statistically significant differences were observed between treatment groups for the total mean mortality of adult bumble bees and larvae (Table 1).

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Table 1: Mean numbers of dead bumble bees (adults and larvae)

Mean number of dead bumble bees (adult and larvae) per day and per treatment					
		Treatment group			
Date	DAE	Control		Treatment	
		Mean	STD	Mean	STD
02 Jul 2014	0	0.0	0.0	0.5	0.8
03 Jul 2014	1	0.3	0.8	0.2	0.4
04 Jul 2014	2	0.8	1.6	0.0	0.0
07 Jul 2014	5	1.8	1.3	2.5	2.8
10 Jul 2014	8	2.3	2.3	2.5	2.9
13 Jul 2014	11	1.8	2.1	1.3	1.2
16 Jul 2014	14	4.7	3.6	3.2	3.1
18 Jul 2014	16	1.5	1.4	2.2	2.0
21 Jul 2014	19	3.3	2.1	3.0	1.1
24 Jul 2014	22	6.3	8.5	3.8	3.7
28 Jul 2014	26	7.2	5.6	7.8	8.9
31 Jul 2014	29	34.3	13.8	14.5	8.7
04 Aug 2014	33	7.8	5.0	5.7	3.4
07 Aug 2014	36	12.3	10.2	16.5	8.9
11 Aug 2014	40	4.5	2.1	6.3	3.8
14 Aug 2014	43	19.0	-	11.8	6.7
18 Aug 2014	47	20.0	-	11.0	7.1
21 Aug 2014	50	17.0	-	9.0	-
Mean exposure phase		1.7		1.5	
Total sum of means exposure phase		11.8		10.2	
Mean post-exposure phase		12.1		8.3	
Total sum of means post-exposure phase		133.2		91.6	
Total mean over all phases		8.1		5.7	
Total sum of means over all phases		145.1		101.8	

DAE = days after exposure (day indicates dates on monitoring site)
STD = standard deviation
Mean = mean values of all available replicates, mean values calculated with unrounded values
- = data not available as hives were already deep frozen
[a] mean values of 3 hives, [b] mean values of 4 hives, [c] mean values of 4 hives, [d] value for 1 hive

A slight increase in mortality for both treatment groups was observed at the end of the exposure phase. Peaks in mortality were observed at 29 DAE for the control and at 36 DAE for the test item treatment. Total mortality during exposure and post-exposure was lower for the test item treatment compared to the control.
The flight activity was observed to be slightly higher in the test item compared to the control. At one assessment date (14 DAE) the bumble bees in the test item treatment showed statistically significant higher flight activity in the crop (**Error! Reference source not found.**).

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Table 2: Mean numbers of foraging bumble bees in the crop (4 m^2 areas)

Mean numbers of foraging bumble bees in the crop (4m^2 areas / 10 min)					
		Treatment group			
Date	DAE	C		T	
		Mean	STD	Mean	STD
02 Jul 2014	0	0.7	0.6	2.7	0.6
03 Jul 2014	1	1.0	0.0	2.3	0.6
04 Jul 2014	2	0.3	0.6	1.3	0.6
07 Jul 2014	5	0.7	1.2	1.0	0.0
10 Jul 2014	8	2.0	1.0	1.7	1.2
13 Jul 2014	11	1.3	0.6	2.7	1.2
16 Jul 2014	14	0.0	0.0	2.7*	0.6
Mean flight activity		0.9		2.0*	

DAE = days after exposure
STD = standard deviation
Mean = mean values of all replicates, means calculated with unrounded values
* = statistically significant difference to control (t-test ($p \leq 0.05$))

Flight activity at the entrance of the hives was statistically significant higher at one assessment date (8 DAE) for the test item treatment (Table 3). As it started raining during the assessment at the treated field site, the bumble bees were entering the colonies at higher numbers than at the control field. In general, the flight activity was similar in both treatment groups.

Table 3: Mean numbers of bumble bees entering the colonies

Mean numbers of bumble bees entering the colonies / 15 minutes					
		Treatment group			
Date	DAE	C		T	
		Mean	STD	Mean	STD
02 Jul 2014	0	0.7	0.7	0.9	1.2
03 Jul 2014	1	2.3	0.6	1.8	1.5
04 Jul 2014	2	2.7	1.8	4.3	1.9
07 Jul 2014	5	3.8	3.1	5.4	1.7
10 Jul 2014	8	2.8	1.9	8.5*	2.7
13 Jul 2014	11	3.7	1.1	3.6	1.2
16 Jul 2014	14	10.8	3.5	8.8	2.8
Mean flight activity		3.8		4.8	

DAE = days after exposure
STD = standard deviation
Mean = mean values of all replicates, calculated with unrounded values
* statistically significant difference to control (t-test ($p \leq 0.05$))

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Regarding the sugar consumption and the weight development of the hives, no statistically significant treatment related effects were observed (Table 4 and Table 5). The consumption of sugar solution as well as the weight development of the bumble bee hives was similar for both treatment groups.

Table 4: Mean consumption of sugar solution

Mean consumption of the sugar solution (g)					
		Treatment groups			
Date	DAE	Control		Treatment	
		Mean	STD	Mean	STD
03 Jul 2014	1	31.7	9.8	20.0	6.3
04 Jul 2014	2	21.7	7.5	23.3	16.3
07 Jul 2014	5	106.7	25.8	96.7	16.6
10 Jul 2014	8	51.7	42.2	86.7	41.3
13 Jul 2014	11	151.7	48.8	95.0	83.4
16 Jul 2014	14	181.7	54.2	171.7	130.3
18 Jul 2014	16	173.3	43.7	175.0	48.1
21 Jul 2014	19	141.7	11.8	141.7	51.5
24 Jul 2014	22	165.0	48.9	140.0	67.5
28 Jul 2014	26	296.7	58.0	278.3	77.8
31 Jul 2014	29	193.3	126.4	153.3	126.8
04 Aug 2014	33	440.0 [a]	226.7	468.3	83.0
07 Aug 2014	36	430.0 [a]	84.1	425.0	97.1
11 Aug 2014	40	596.4 [a]	67.0	571.7	99.9
14 Aug 2014	43	670.0 [d]		412.5 [b]	95.4
18 Aug 2014	47	540.0 [d]		260.0 [c]	169.7
21 Aug 2014	50	230.0 [d]		359.0 [d]	
Consumption during exposure		545.0		493.3	
Consumption during post.exposure		3876.4		3384.8	
Total consumption [e]		4421.4		3878.2	

DAE = days after exposure (grey indicates dates on monitoring site)
STD = standard deviation
Mean = mean values of all replicates, calculated with unrounded values
- = data not available as hives were already deep-frozen
[a] mean values of 3 hives
[b] mean values of 4 hives
[c] mean values of 4 hives
[d] value for 1 hive
[e] total sum of mean consumption values

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Table 5: Mean weights of bumble bee hives

Mean weights of bumblebee hives (g)					
		Treatment groups			
Date	DAE	Control		Treatment	
		Mean	STD	Mean	STD
02 Jul 2014	0	554.8	16.3	562.0	11.8
03 Jul 2014	1	559.3	15.3	549.5	11.1
04 Jul 2014	2	560.7	17.4	561.2	16.0
07 Jul 2014	5	590.8	31.1	587.8	24.0
10 Jul 2014	8	601.2	39.6	594.2	29.3
13 Jul 2014	11	631.3	49.4	618.0	50.8
16 Jul 2014	14	650.8	58.6	635.3	89.0
18 Jul 2014	16	687.3	73.9	664.0	103.8
21 Jul 2014	19	743.3	88.0	695.5	116.5
24 Jul 2014	22	765.0	55.9	713.8	123.9
28 Jul 2014	26	859.0	40.8	775.2	135.4
31 Jul 2014	29	897.5	89.1	819.2	135.5
04 Aug 2014	33	1055.4 [a)]	93.5	913.5	162.4
07 Aug 2014	36	1051.6 [a)]	53.4	955.8	172.9
11 Aug 2014	40	1139.5 [a)]	88.7	1064.3	209.9
14 Aug 2014	43	1251.5 [d)]		1097.5 [b)]	235.6
18 Aug 2014	47	1254.5 [d)]	-	934.5 [c) f)]	344.4
21 Aug 2014	50	1227.5 [d) f)]		773.0 [d) f)]	-
Mean weight exposure		**592.7**		**585.1**	
Weight increase exposure [e)]		**96.0**		**83.3**	
Mean weight post-exposure		**993.8**		**855.1**	
Weight increase post-exposure [e)]		**435.3**		**455.8**	
Total mean weight		**837.8**		**750.1** [f)]	
Total weight increase		**567.8**		**567.8**	

DAE = days after exposure (grey indicates dates on monitoring site); STD = standard deviation
Mean = mean values of all replicates, calculated with unrounded values
- = data not available as hives were already deep-frozen
[a)] mean values of 3 hives, [b)] mean values of 4 hives, [c)] mean values of 4 hives, [d)] value for 1 hive, [e)] calculated as mean values of single replicate values, [f)] lower values due to the fact that remaining hives during these assessments were lower in weight compared to the ones that were already deep-frozen

The results of the final brood evaluation showed a statistically significant difference in one out of all parameters assessed, a lower number of alive young queen larvae. However, the number of alive young queens and alive queen pupae were higher in the test item treatment resulting in a total queen reproduction that was well above the reproduction in the control. For all other parameters of the final brood assessment (number of alive young queens, workers, males, eggs, larvae and pupae), no statistically significant treatment related adverse effects were observed.

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Palynological analysis showed that the bumble bees collected pollen from several different plant sources. Potato pollen was detected in varying amounts in most of the forager bumble bee pollen samples at the control and test item treatment field site at the given sampling dates (Table 6). It is assumed that the exposure to potato pollen was given in the treated field site.

Table 6: Results of the forager bumble bee pollen analysis

% of potato pollen in pollen samples of forager bumble bees		
Sampling date	**C**	**T**
5 DAE	47.4	1.6
12 DAE	2.2	23.5
16 DAE	0.0	29.2

Residue analysis was carried out on pollen samples collected from forager bumble bees at 5, 12 and 16 days after exposure (DAE). No residues of imidacloprid and its metabolites (imidacloprid-5-hydroxy and imidacloprid olefine) were detected in pollen from the control field. Residue levels of imidacloprid in samples from the treated field were below the limit of quantification at sampling date 5 DAE and below the limit of detection at 16 DAE. The maximum residue level of 0.71 µg/kg was found at the sampling date 12 DAE (Table 7). At all sampling dates, the residue levels of imidacloprid-5-hydroxy and imidacloprid olefin were below LOD.

Table 7: Residues of imidacloprid and its metabolites in potato pollen

Treatment group	Sampling date	Residues [µg/kg]		
		Imidacloprid	Imidacloprid-5-hydroxy	Imidacloprid olefine
C	5 DAE	< LOD	< LOD	< LOD
	12 DAE	< LOD	< LOD	< LOD
	16 DAE	< LOQ	< LOD	< LOD
T	5 DAE	< LOQ	< LOD	< LOD
	12 DAE	0.71	< LOD	< LOD
	16 DAE	< LOD	< LOD	< LOD

DAE = days after exposure
LOQ = limit of quantification = 0.6 µg/kg for imidacloprid, 1.0 µg/kg for imidacloprid metabolites
LOD = limit of detection = 0.2 µg/kg for imidacloprid, 0.3 µg/kg for imidacloprid metabolites

>>M-544174-01-1 - S.602337-01-1

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Report:	03.01.02/05; [redacted]; 2014; M-503597-01-3
Title:	A field study to evaluate effects of Monceren G on the bumble bee (Bombus terrestris L; Hymenoptera, Apidae) in potato in southern Germany in 2014
Report No.:	S14-03553
Document No.:	M-503597-01-3
Guideline(s):	No specific guidelines are available. The test design is based on: SETAC/ESCORT recommendations (BARRETT et al. 1994) OEPP/EPPO Guideline No. 170 (4), 2010 US EPA OCSPP Guideline No. 850.3040
Guideline deviation(s):	none
GLP/GEP:	**yes**

<<M-503597-01-3@S-602317-01-1

1.1 Material and Methods

Test item: Monceren G; TOX number: TOX 10501-00; Batch: 2014-001766-01; content of a.i. (nominal): 120 g/L imidacloprid + 250 g/L pencycuron

Test species: Bombus terrestris L. (Hymenoptera, Apidae)

Test design: The field study was carried out on agricultural fields in southern Germany (Karlsruhe) following the SETAC/ESCORT recommendations and the OEPP/EPPO Guideline No. 170 (4). The field crop was potato; Solanum tuberosum L.

The study included 2 treatment groups (C = control, T= test-item) with six replicates (6 replicate bumble bee colonies) per treatment group for biological assessments.

Bumble bees were assessed for their flight activity within the crop, flight activity at the entrances of the hives. The weight of the hives and the sugar consumption were assessed. Moreover, the mortality of adult bees and larvae was observed at every assessment date during the field phase and at the monitoring site. Additionally, three samplings of pollen for residue analysis and palynological analysis at different dates were carried out by taking the pollen loads from forager bumble bees of additional colonies only used for residue sampling. Before set-up and after the field phase, brood assessments were done to document all stages of development and the vitality of the colonies.

Endpoints: Flight activity in the crop, flight activity at the entrance of the hives, mortality of adults and larvae, weight of hive and sugar consumption, initial and final brood assessment including the production of young queens and drones.

Application: The application was done at a separate study (S14-01385). The insecticide Monceren G was applied as in-furrow application at plating at a rate corresponding to nominally 1.5 L product/ha (equivalent to 180 g imidacloprid/ha and 375 g pencycuron/ha) under field conditions on potato (Solanum tuberosum L.).

Test conditions: Exposure of the bumble bee colonies started at the beginning of potato flowering. After end of flowering, the colonies were transferred to a monitoring site were the assessments were followed until the colonies reached their peak of colony development and switched over to the reproduction phase i.e. young queen and male (drone) production.

Dates of work: 11 Jun 2014 to 08 Oct 2014

1.2 Findings

The effect of Monceren G was evaluated by assessing the mortality of adult bumble bees and bumble bee larvae within the hives, flight activity at the entrance of the hives, foraging activity in the crop, the sugar consumption and the weight of the hives.

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No statistically significant differences were observed between treatment groups for mortality of adult bumble bees and larvae within the hives (Table 1).

Table 1: Mean number of dead bumble bees (adults and larvae)

Mean number of dead bumble bees (adult and larvae)					
		Treatment groups			
Date	DAE	C		T	
		Mean	STD	Mean	STD
12 Jun 2014	0	4.7	3.6	2.3	1.4
13 Jun 2014	1	1.0	1.3	0.2	0.4
14 Jun 2014	2	0.2	0.4	0.7	0.8
17 Jun 2014	5	1.2	1.7	1.3	1.5
20 Jun 2014	8	1.3	1.5	2.2	0.4
23 Jun 2014	11	1.2	0.8	3.8	3.1
26 Jun 2014	14	5.0	3.4	6.1	4.6
30 Jun 2014	18	21.5	11.9	17.3	4.1
03 Jul 2014	21	21.0	12.5	22.3	7.1
07 Jul 2014	25	14.3	7.8	11.3	5.2
10 Jul 2014	28	25.2	16.3	24.2	11.2
14 Jul 2014	32	16.2	11.0	18.7	12.5
17 Jul 2014	35	8.0 [a]	5.6	7.3 [b]	4.8
21 Jul 2014	39	-	-	9.0 [c]	3.7
Mean exposure phase		2.1		2.5	
Total sum of means exposure phase		14.3		17.2	
Mean post-exposure phase		17.7		15.7	
Total sum of means post-exposure phase		106.2		110.2	
Total mean over all phases		9.3		9.1	
Total sum of means over all phases		120.7		127.3	

DAE = days after exposure (grey indicates dates on monitoring site)
STD = standard deviation
Mean = mean values of all replicates, mean values calculated with unrounded values
- = data not available as hives were already deep-frozen
a) mean values of 4 hives
b) mean values of 3 hives
c) value for 1 hive

At the first assessment date, the mortality of adult bumble bees was at a peak and decreased in time. At the last assessment during the exposure phase mortality increased again for both treatment groups. During the post exposure phase at the monitoring phase, mortality increased with a peak at 28 DAE. Total mortality during exposure and post-exposure was similar in both treatment groups.
At two assessment dates (1 DAE and 14 DAE) the flight activity in the crop was statistically significant lower compared to the control (Table 2).

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Table 2: Mean numbers of foraging bumble bees in the crop (4 m^2 areas)

Mean numbers of foraging bumble bees in the crop (4 m^2 areas / 10 min)					
		Treatment group			
Date	DAE	C		T	
		Mean	STD	Mean	STD
12 Jun 2014	0	3.3	0.6	0.3	0.6
13 Jun 2014	1	4.0	1.7	0.7* a)	0.6
14 Jun 2014	2	1.7	0.6	0.7	0.6
17 Jun 2014	5	5.0	1.7	2.3	2.3
20 Jun 2014	8	3.7	1.2	2.7	0.6
23 Jun 2014	11	4.0	1.0	2.7	1.2
26 Jun 2014	14	5.0	1.0	2.7* a)	0.6
Mean flight activity		3.8		1.7* a)	

DAE = days after exposure
STD = standard deviation
Mean = mean values of all replicates, mean values calculated with unrounded values
* = statistically significant difference to control
a) = t-test ($p \leq 0.05$)

Flight activity at the entrance of the hives was statistically significant lower compared to the control at two assessment dates (2, 14 DAE) (Table 3). For the other assessment days no significant differences were observed. The overall mean flight activity was slightly lower for the test item but no statistically significant difference was found.

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Table 3: Mean numbers of bumble bees entering the colonies

Mean numbers of bumble bees entering the colonies / 15 minutes					
		Treatment group			
Date	DAE	C		T	
		Mean	STD	Mean	STD
12 Jun 2014	0	4.2	2.7	3.5	2.0
13 Jun 2014	1	4.8	2.1	3.3	1.7
14 Jun 2014	2	5.6	1.9	2.5* a)	2.0
17 Jun 2014	5	10.6	3.1	11.4	5.3
20 Jun 2014	8	10.8	2.2	9.6	3.2
23 Jun 2014	11	15.1	6.1	11.9	4.8
26 Jun 2014	14	22.5	9.4	9.8* a)	4.1
Mean flight activity		10.5		7.4	

DAE = days after exposure
STD = standard deviation
Mean = mean values of all replicates, mean values calculated with unrounded values
* = statistically significant difference to control
a) = t-test ($p \leq 0.05$)

Regarding the sugar consumption two statistically significant differences were observed (Table 4). As a significant decrease was followed by a significant increase in sugar solution consumption in the test item treatment it is concluded that no treatment related adverse effects on sugar solution consumption were observed. Sugar consumption during exposure was slightly higher in the control whereas sugar consumption during post exposure and total sugar consumption were higher in the test item treatment.

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Table 4: Mean consumption of sugar solution

Mean consumption of sugar solution (g)					
		Treatment group			
Date	DAE	C		T	
		Mean	STD	Mean	STD
13 Jun 2014	1 DAE	23.3	12.1	23.3	5.2
14 Jun 2014	2 DAE	28.3	14.7	35.0	8.4
17 Jun 2014	5 DAE	115.0	40.9	103.2	24.4
20 Jun 2014	8 DAE	193.3	38.8	163.5	50.7
23 Jun 2014	11 DAE	191.7	69.7	138.3	36.6
26 Jun 2014	14 DAE	288.3	153.5	158.3	41.7
30 Jun 2014	18 DAE	306.7	18.6	208.3 * x)	86.2
03 Jul 2014	21 DAE	200.0	44.3	326.7	181.2
07 Jul 2014	25 DAE	476.7	241	323.3	148.0
10 Jul 2014	28 DAE	120.0	69.0	192.7	172.1
14 Jul 2014	32 DAE	331.7	99.1	240.0	87.9
17 Jul 2014	35 DAE	172.5 a)	39.5	290.0 b)	141.1
21 Jul 2014	39 DAE	-	-	370.0 c)	
Total consumption exposure		840.0		621.7	
Total consumption post-exposure		1607.5		1951.0	
Total consumption d)		2447.5		2672.7	

DAE = days after exposure (grey indicates dates on monitoring site)
STD = standard deviation
Mean = mean values of all replicates
* = statistically significant difference to control, total values calculated with unrounded values
- = data not available as hives were already deep frozen
a) mean values of 4 hives
b) mean values of 3 hives
c) value for 1 hive
d) total sum of mean consumption values
x) = t-test ($p \leq 0.05$)
y) = Mann Whitney Exact ($p \leq 0.05$)

The weight development of the hives showed no statistically significant treatment related adverse effects (Table 5). Mean weights during exposure phase, total mean weights and total weight increase of the bumble bee hives were slightly higher in the test item treatment.

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Table 5: Mean weights of bumble bee hives

Mean weights of bumble bee hives (g)					
		Treatment group			
Date	DAE	C		T	
		Mean	STD	Mean	STD
12 Jun 2014	0	637.2	15.6	647.7	39.0
13 Jun 2014	1	635.0	2.0	648.8	38.1
14 Jun 2014	2	568.7	101.4	647.5	39.5
17 Jun 2014	5	604.0	42.2	669.3 *)	48.6
20 Jun 2014	8	681.2	48.0	739.0	77.0
23 Jun 2014	11	746.8	80.4	790.3	103.5
26 Jun 2014	14	769.7	97.8	825.5	120.1
30 Jun 2014	18	838.5	104.7	900.8	140.2
03 Jul 2014	21	857.3	105.1	918.7	145.2
07 Jul 2014	25	949.3	113.3	1009.0	153.0
10 Jul 2014	28	952.2	122.6	1016.5	136.1
14 Jul 2014	32	942.0	109.3	982.0	142.5
17 Jul 2014	35	906.3 a) e)	59.7	983.0 b) e)	157.6
21 Jul 2014	39	-	-	791.0 c) e)	-
Mean weight during exposure		661.9		710.3	
Weight increase exposure d)		123.5		181.8	
Mean weight post-exposure		907.6		943.0	
Weight increase post-exposure d)		93.0		61.7	
Total mean weight		775.3		826.6	
Total weight increase d)		294.3		314.8	

DAE= days after exposure (grey indicates dates on monitoring site)
STD= standard deviation
Mean= mean values of all available replicates, mean values calculated with unrounded values
* = statistically significant difference to control
- = data not available as hives were already deep-frozen
a) mean values of 4 hives
b) mean values of 3 hives
c) value for 1 hive
*) = t-test ($p \leq 0.05$)

The results of the final brood evaluation did not show any statistically significant differences between the control and the test item treatment in the number of alive young queens, workers, males, eggs, larvae and pupae. Regarding the trend of the observations, the bumble bee colonies of the test item treatment seem to have not significant but more individuals in most investigated parameters. Also with regard to the queen production, the number of produced young queens (larvae, pupae and adults) was slightly higher in the test item treatment.
Palynological analysis showed that the bumble bees collected pollen from several different plant sources. Potato pollen was not detected in forager bumble bee pollen samples at the control field site at the given

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sampling dates (Table 6). At the treated field site the percentage of potato pollen was up to 56.3% and it is therefore assumed that the exposure to potato pollen was given in the treated field site.

Table 6: Results of the forager bumble bee pollen analysis

% of potato pollen in pollen samples of forager bumble bees		
Sampling date	**C**	**T**
5 DAE	0	24.8
12 DAE	0	56.3
15 DAE	0	54.8

Residue analysis was carried out on pollen samples collected from forager bumble bees at 5, 12 and 15 days after exposure (DAE). No residues of imidacloprid and its metabolites (imidacloprid-5-hydroxy and imidacloprid olefine) were detected in pollen from the control field. Residue levels in samples from the treated field were below the limit of quantification at the sampling dates 5 DAE and 12 DAE. The maximum residue level of imidacloprid of 1.4 µg/kg was found at the sampling date 15 DAE (Table 7).

Table 7: Residues of imidacloprid and its metabolites in potato pollen

Treatment group	Sampling date	Residues [µg/kg]		
		Imidacloprid	Imidacloprid-5-hydroxy	Imidacloprid olefine
C	5 DAE	< LOD	< LOD	< LOD
	12 DAE	< LOD	< LOD	< LOD
	15 DAE	< LOD	< LOD	< LOD
T	5 DAE	< LOQ	< LOD	< LOD
	12 DAE	< LOQ	< LOD	< LOD
	15 DAE	1.4	< LOQ	< LOD

DAE = days after exposure
LOQ = limit of quantification = 0.6 µg/kg for imidacloprid, 1.0 µg/kg for imidacloprid metabolites
LOD = limit of detection = 0.2 µg/kg for imidacloprid, 0.3 µg/kg for imidacloprid metabolites

1.3 Conclusion

No statistically significant treatment related adverse effects were observed with regard to mortality of adult bees and mortality of larvae. Statistically significant differences observed for the sugar consumption and weight development of the bumble bee colonies are likely not biologically meaningful.
At the beginning of the exposure phase, the mortality of adult bees was higher probably due to the stress caused by transport and initial brood assessment.
It can be recognized that the weight of the hives was increasing during the exposure phase, that the bumble bee colonies developed well and reached the “switchpoint” with reproduction of young queens and drones.
At two of seven assessment dates, statistically significant differences between the treatment groups were observed for both, flight activity in the crop and flight activity at the hive entrances.
Regarding the final brood assessment the observed parameters: number of young queens, workers, males, eggs, larvae (queen and worker) and pupae (queen and worker), filled nectar and pollen cells and mean weight of hives no treatment related differences were observed.

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Palynological analysis and residue analysis of forager pollen samples showed that exposure to potato pollen was given in the test item treated field site.
It can be concluded that the use of Monceren G (applied at rates of 180 g imidacloprid/ha and 375 g pencycuron/ha) at potato planting has no adverse effects on the behaviour and development of bumble bee colonies exposed during bloom.
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