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### **Imidacloprid Bee Studies Compilation of Study Summaries**

Issue date 2017-11-22

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### **Imidacloprid Bee Studies – Compilation of Study Summaries**

## 01 - Metabolism

### 01.01 - Plant

**Report:** Title: Report No.: Document No .: Guideline(s): Guideline deviation(s): **GLP/GEP:** 

01.01/01; 1988; M-024270-01-3 Absorption and translocation of 14C-NTN 33893 in eggplants and rice plants NR1273 M-024270-01-3 not specified no pcation of NTN 33893 {imitacloprid (under apprecation to ISO) minoimidazolidine} in young eggplants and rice in the second

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

Re

The absorption and translocation of NTN 33893 {im@taclopped (upder approximation), 1-(2\_chloro-5pyridinylmethyl)-2- nitroiminoimidazolidine} in young eggplantoand rice plants weroinvestigated over a period of 8 days following application of wridin M-14C-methyll NTN-33893 by painting to the actual parts and addition to the nutrient solution. The behavior of CONTN 33803 was similar between the two plants. Following application to the aerial parts, <sup>14</sup>C penetrated into the plants and exhibited significant acropetal translocation. The distribution of -<sup>14</sup>C applied to leaf blade, peticle (eggplants) and leaf sheath (rice plants) was almost restrictive to the applied leatespectfully to its marginal area, and was small in the other parts. In the case of stem application (eggplants) in which <sup>14</sup>Openetrated via the lower part of the plants than in the cases above, <sup>4</sup>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 photogegradation products were assumed to possess note leaf penetrability and volatility. In nutrient solution application, <sup>1</sup> was absorbed via pots and translocated rapidly to the aerial parts, and accumulated to the leaf matgins, Although NTN 33893 was metabolized in plant tissues after uptake via roots, the parent compound was still the main component of tabelled residue in plants. >>M-024270-01-3@**S-602974-01-1** Ŝ

Report:	01.0892; ; 1989; M- $024273-02-3$ , $0''$
Title: 👋	S Isolation and Identification for metadolities of NTN 53893 in fice by water culture
Report No.:	NK1282 ~ ~ ~
Document No .:	
Guideline(s):	
Guideline deviation	
GLP/GEP:	n(s) no sy fr g

<<M-024273-0 Ô Metabolites of NTN 3893 in rice plants were investigated by applying <sup>14</sup>C and <sup>13</sup>C labeled and nonlabeled 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 to se. The absorbed chemicals dominantly located in the aerial part (99% of adioactivity in the whole plants). The methanol extracts (85% of the dose) were fractionated into dichlotomethane (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 N/N 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, 1, 33519, V, 1%), olefinic metabolite (NTN 35884, VI, 0.4%) and 6chloronicotinic 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 <sup>13</sup>C-labeled NTN 33893 were analyzed by <sup>13</sup>C-NMR. Metabolites [I], [II], [IV] and [V] were detected on <sup>13</sup>C-NMR spectra. Especially compound [II] was known to be a dominant metabolite at the early stage of this study and the information contributed to develop the analytical procedure.

<b>Report:</b> Title:	01.01/03; 1991; M-024279-01-3 Metabolism of (pyridinyl-14C-methyl) NTN 33892pin rice plants mursery box	"Q
Thie.	application)	Ş
Report No.:	NR1284	<i>y</i>
Document No .:	<u>M-024279-01-3</u>	
Guideline(s):	EPA Guideline [Subdivision $O$ , Section 171-4(a) $O$ $O$ $O$	
Guideline deviation(s):	not specified	
GLP/GEP:	yes of the	
< <m-024279-01-3@s-602991-01-1< td=""><td></td><td></td></m-024279-01-3@s-602991-01-1<>		

The absorption, translocation and metabolism of pyridinyl-<sup>14</sup>C-methyl NTNS 3893 in rice plants vere 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 rates by nursery box treatment.

Approximately 4% of applied dose was translocated to immature rise show 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) <sup>14</sup>C-NTN33893 equivalents.

In the shoot and spaw, 7 compounds were identified including unchanged NTN33893. The metabolites were NTN38014, WAR3839, WAR4103, NTN35884, NTN35819 and CNA (6-chloronicotinic acid). NTN38014 was the pajor 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, WAR3839, WAR4103 and NTN35884 were less than 2% tespectively. 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 apartifact.

NTN33893 was the major component in the extractable fraction from grain, accounting for 12% of the total terminal esidues. Merobolites in the extractable fraction included WAK4103 (3.5%), NTN35884 (2.0%) and trace amounts of NTS38014. CNA and WAK3839. About 70% of the radioactivity in grain was unexpactable bound residues. The crude starch contained 67% (48% of total <sup>14</sup>C) of the bound residues. The glucose obtained by plycolysis of the starch was revealed to be radiolabeled with a constant specific radioactivity, suggesting that H6-carbon dioxide derived from <sup>14</sup>C-NTN33893 was incorporated into natural constituents.

The percentages of metabolices identified in shoot, straw and grain were 79.2%, 73.8% and 83.8%, respectively. The metabolic pathway of WTN33893 in rice plants was proposed on the basis of metabolices identified in this study.

991-01-1



Report:	01.01/04; ; 1992; <u>M-024334-01-2</u>		
Title:	Metabolism of NTN 33893 in eggplant by planting	hole application	0
Report No.:	NR1290		
Document No.:	<u>M-024334-01-2</u>		
Guideline(s):	EPA Guidelines Subdivision 0 Section 171-4(a)2	~	
Guideline deviation(s):	not specified	a a a a a a a a a a a a a a a a a a a	
GLP/GEP:	yes	O,	
	•	1	

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

Metabolism of NTN 33893 (pyridylmethy-<sup>14</sup>c-label) in eggslant was done under the GLP regulations. The objective of this study was to clear absorption, translocation and degradation of NTN 338930n the plant after 1 % granule (0.94 % a.i.) was applied to soil at a maximum conditionercial 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 drays 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 dound for the leaves, the absorbed radioactivities seemed to be transfocated 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 #103, WTN 35884, NTN 33519, WAK 3839, NTN 38014, RBN 1114 and CNA were found as notabolity in the foliage at 69 days after the application. Major metabolites in the foliage were NTN 38014 an avorage of 24.6% of the radioactivity found in the foliage), RBN 1114 (5.6%) and WAK 4403 (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 8.9% of the radioactivity found an average of 0.0081 mg/kg), NTN 38014 (14.0%, 0.0049 mg/kg) RBN ©114 (1.0%, 0.0066 mg/kg) CNA (13.4%, 0.0035 mg/kg), WAK 4103 (252 %, 6001540)  $\bigcirc$ 

g/kg), NTN 35884(2%, <0.000 mg/kg) and WAK 3839 (Q1 %, <0.0005 mg/kg) were found. Major metabolites in the dible parts were NTN 38014, RBN 1114 and CNA. The amount of nonextracts was accounted for 65% (02028 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

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



Report:		l; <u>M-026229-02-2</u>			° ^
Title:	NTN 33893 - Metabol	lism in tomatoes - Adde	endum to NT	N 33893 tomato	o report PF no 🔿
	3257 (study numbers 1	M 173 0 237-3 and M 1	173 0238-4) -	Investigation o	n thế 🖉
	metabolism of NTN 3	3893 after application t	to tomatoes	ð	, <sup>o</sup> <sup>o</sup> b
Report No.:	PF3257		(	A. Y	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Document No.:	<u>M-026229-02-2</u>		.4	Ş	
Guideline(s): Guideline deviation(s):			K,		
CI P/CFP.	 no	Ğ	Å	×,	
Report No.: Document No.: Guideline(s): GLP/GEP: M-026229-02-2@S-602558-01-1 The metabolism of the methyl] NTN 33893 to 14 days prior to the ma immature fruits until ru application. A total residue of 0.85	10	~~~~	Q		9° 20° 49°
< <m-026229-02-2@s-602558-01-1< td=""><td>:</td><td>2 (1)</td><td>1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td><td></td><td></td></m-026229-02-2@s-602558-01-1<>	:	2 (1)	1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		
I ne metabolism of the	insecticide NTN 3389	3 (1) was investigate	Gatter appli	cation of (pyri	anayi-"Co
methyl] NIN 33893 to	tomatoes.		, Oʻ	Q' O'	o Ú
14.1	. 1		N O		
14 days prior to the ma	in harvest, an 0.2% sp	ray solution of a 25 V	WP was app	lied to the sur	tace of
immature fruits until ru	n-off. The fruits were	harvested 4, 1/2 14 ar	oor 21 days (=	= postharwest s	sample) after
application.	S,		AÓ	Şara k	Ş, Q
			Å NY		
(I), more than 0.59 mg/	kg were located on the	e surface and could b	e washed of	With methan	10).
	Q <sup>*</sup>			/	
0.071 mg/kg·of the resi	due were shared by at	t heast 8 metabolites v	which result	ed fom hydro	xylation of the
parent compound and/c	or hydrolysis and conji	ugation of hydrolysis	products. O	of these, the fo	llowing
compounds were identi	fied by cockromatogra	aphy with reference's	standards:		
	0.022 mg/kg /guani	ane metabolite" 🖉		( IA) <sup>×</sup>	
, Q	0.0160mg/kg, "urea, "	meta <b>bolite</b> "			
(// )			" ~	(IV, V)	
, D	0.045 mg/kg "monohy	VULOXY AUE LADOL TLE			
	0,004 mg/kg≪"olefï	n metabolite	y w	(VI)	
ð s	0.004 mg/kg«"olef1 0.000 mg/kg "nitvo	simome metabolite'	"	(VIII)	
, Ö, <	0.006 mg/kg "nitro: 0.001 mg/kg "chlor 0.007 mg/kg &chlor	obicolv Daluzoside	eh	(X) and	
	.007 mg/kg Chlor	anicatul gentiati	"	(XI)	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1.001 9401 kg ~ cu 104	ohico Al activition	osiae	(/1)	
			1. 11	,	• 11
Structural elucidation o	INTN metabolites fro	protomato plants was	s achieved b	y spectroscop	ic methods
after isolation of the co	mpounds in a special	model experiment set	t up for this	purpose. In th	is case <sup>13</sup> C-
and <sup>14</sup> C-labelled parent	compound was used t	besides unlabelled N	IN 33893 to	) facilitate the	structure
investigations. The app	lication was made by	steph injection. Suffic	cient amoun	ts of the subst	ances could be
obtained because of the	good motabolization	$\sim$ 24% of the 14c-rac	dioactivity a	ccounted for p	olar
metabolites. The identi	fied compounds are pr	resented in a metabol	ic pathway (	(F1g. 40).	
				•, , , , ••	
In an additional translo					
into the fruit via the fol	lage. Thus, the level of	the total residue is	determined	by the spray d	eposit on the
tomatoes 🖑 , 🐧		•			

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

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tomatoes.

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-2@S-6@\$\$8-01-1



Report:	01.01/06; ; 1992; <u>M-024320-01-2</u>		
Title:	Metabolism of NTN 33893 in corn after seed dress	sing	0
Report No.:	PF3673		
Document No.:	<u>M-024320-01-2</u>		
Guideline(s):	171-4 Nature of Residue (Metabolism) - Plants	~	
Guideline deviation(s):	not specified		
GLP/GEP:	yes	O'	
	•	1	

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

The metabolism of the insecticide NTN 33893 (I)was investigated in corb 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 plants were separated into dry grain, fodder, husks and cobs. The total residue, expressed in active plants were separated into dry grain, fodder, husks and cobs. The total residue, expressed in active plants were separated into dry grain, fodder (day 134) 2,08 mg/kg, inclusts (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 certified the radioactivity and in mg/kg active ingredient equivalents in the respective plant parts).

•				
	1. Grain Unchanged parefit compound Olefine compound 5-Hydroxy compound Dihydroxy compound 6-Chloropicol@alcohol Guanidine compound 6-Chloropicol@alcohol Further components in lower concentrations 2. Fodder Unchanged Darent compound Guanidine compound 5-Hydroxy compound Olefine compound Nitrosimine compound Nitrosimine compound Fing opened guanidine compound 6-Chloropicolylatcohol 5-Hydroxy compound Conjugate Dihydroxy compound Urea compound Further components in lower concentrations	-Ca		
		6	,	
	Unchanged parent compound	/ (I) 🕜	25.2%	∑ 0.010 mg/kg
	Olefine compound	(YA)	3.1%	۩005 mg/4kg
	5-Hydroxy compound	Arý) 🔍 🤇	Ĵ <sup>™</sup> 9. <b>9</b> %	0.004 mg/kg
	Dthydrox@compound	» (VII) (*	′ 4. <b>4</b> %	0.002 mg/kg
	S-Chlocopicolojalconor	(XEĤ)	<b>@4.4%</b>	0,002 mg/kg
Ô	Guanidine compound	(M)	ా 2.0%	~00.001 mg/kg
~°	6-Onoronicotinic acid O	AXIIP	traces	traces
Ū.	Burther components in lower	Š	IS. 4% 🖑	ca.0.006 mg/kg
	concentrations 💭 Ö	-0 ~ ~		
je G <sup>i</sup>		ŠÝ V	× 0″	
* 2	Z Fodder S	' K	S	
		Oʻ,	~	
Q	Unchanged parent compound	(I) (	✓ 22.2%	0.68 mg/kg
	Ganidime compound 0	)՝ (II) ֎	10.9%	0.34 mg/kg
***	5-Hydroxy compound 2 2	(10)	5.0%	0.15 mg/kg
- A	Olefine compound	_≪(¥I)	2.2%	0.07 mg/kg
L.	Nitrosimine compound	<sup>≫</sup> (VIII)	1.8%	0.06 mg/kg
s the second sec	Ring opened goanidine compound	d (XV)	1.6%	0.05 mg/kg
· >	6-Chipronicotinic acid	(XII)	1.3%	0.04 mg/kg
¢`	6-Chloropicolylaticohol	(XIII)	1.1%	0.03 mg/kg
Õ,	5 Hydroxy compound conjugate	(IV )	ca.1.0%	ca.0.03 mg/kg
	Dihydroxy compound	(VII)	0.5%	0.02 mg/kg
	Urea compoind	(111)	traces	traces
	Further components in lower		20.3%	0.62 mg/kg
	Öčoncentrations			

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 radioactivity 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:** Title: Report No.: Document No .: Guideline(s): Guideline deviation(s): **GLP/GEP:** 

01.01/07; ; 1992; M-0243 Metabolism of (14C) NTN 33893 in apples PF3676 M-024315-01-2 171-4 Nature of Residue (Metabolism) - Want not specified ves

<<M-024315-01-2@S-603064-01-1

tardes after application 64 The metabolism of the insecticide NTN 3893 (1) wavinvestigated in potatoes after application of [pyridinyl-<sup>14</sup>C-methyl] NTN 33893. Ap 0.2% spray third 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 Onder practical conditions; in this case the total residue amounted to 1.35 mg/kg?  $\bigcirc$ 

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

(VIŴ

- 0.51 mg/kg "NTN \$3893" 0.17 mg/kg "Guanidine-metabolite 0.095 mg/kg "Hydroxy-metabol te"
- 0.034 mg/kg "01@ ine-metabolite"
- 0.036 mg/kg "Dihydroxy-metabolote
- 0.030 mg/kg "Nitresimine-metabolice
- 0.026 mg/kg "Chivoropicoly l⊱g]ucos

The identified composition and represented in a degradational pathway (Fig. 19).

The <sup>14</sup>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 <sup>14</sup>C-rapioactivity (\$0.001 mg/kg) which could be assigned chromatographically to NTN 33899 (I). Approx. 0.003 prg/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. 

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Report:	01.01/08; ; 1992	2; <u>M-024277</u>	7-02-2			
Title:	Investigation of the m	etabolism of	f NTN 338	393 in pot	atoes follow	ing granular
	application					
Report No.: Document No.:	PF3628 <u>M-024277-02-2</u>					N N
Guideline(s):	<u>IVI-024277-02-2</u> 				jo j	jũ b
Guideline deviation(s):					Ĩ	
GLP/GEP:	yes				A	Gration Of a Contract of the second s
< <m-024277-02-2@s-605095-01-1< td=""><td></td><td></td><td>Ĉs</td><td>Å</td><td></td><td></td></m-024277-02-2@s-605095-01-1<>			Ĉs	Å		
The metabolism of the in	nsecticide NTN 3389	93 was inve	strgated i	n potatoe	es after app	
[pyridinyl- <sup>14</sup> C-methyl] N	NTN 33893. An in-fu	rrow appli	cation of a	5% granı	ules at a <b>ka</b> t	e of 0.05 g active $\sqrt{0}$
ingredient per running n	neter was made at the	e time of pla	anting the	e potatoe	s. The wines	s and tubers wer
harvested 129 days after		ime or harv	est the vi	ines were	e with gred a	nd mostly dry as
would be under practica	l conditions.	k, õ	• 5	L.	17 D	
The total residues, expre	essed in a i equivaler	US wete 5	76 m/ko	wines	and 0 991	morko in tubers Of
the radioactivity applied						
	d		1° .0°	s, y	. U 🐇	
By chromatographic cor	nparison with referen	ice compou	inds and o	other phy	sical metho	ods the following
could be identified (amo	ounts given in per cer	n radioactiv	vity and i	n mg/kg	a.i. equival	ents in vines and
tubers respectively):	Ŷ,	\$ 6	, 2			y vy
		o S	, O		°r <sub>≫</sub> o`	х. К.
1. Vines	Å. ×	о <sup>2</sup>	A L	O <sup>V</sup> Q	Ča –	0″
	ى ئۇرىمى ۋۇرىچى	Ű,	The second secon			)
Unchanged parent co	ɔ`mp`oung 🤍 🔍	(N)	26Ç7%	ູ (ປີ.53 ະ	mg/kg)	
5-Hydroxy compound		<sup>⊃</sup> (IV) ⊂	_^^¥.6%	0.26	mg/kg)	
Dihydroxy compound		(ŴĨI)	× 0,3%	(0.02	mg/kg)	
Olefine compound			3:3%		ang/kg)	
Nitrosimine compour	nd o c «		گ¢۲.6%	Çr və	mg/kg)	
Guanidine compound			8.2%	,	mg/kg)	
6-Chloronicotinic	id S S		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	mg/kg)	
Glucoside of 6-chi			1 4		mg/kg)	
			, 1.72% ^	(0.00	mg/ Kg/	
alcohol						
Another 14 unknown	etabolites were detec	ted in Nowe	r Concent	rations w	which in tota	al amounted to
16.1%, 0.93 mg/kg. The	non-extractable resi	duecorrese	fonded to	26.4%, 1	1.52 mg/kg.	
	n 2 6 2	y . V				
<ol> <li>16.1%, 0.93 mg/kg. The</li> <li>2. Tubers</li> <li>Unchanged parent co</li> </ol>		, ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~				
Unchanged parent co	mpound of S		48.3%	(0.044	mg/kg)	
5-Hydroxy compound		QÎV)	8.0%	(0.007	mg/kg)	
0lefine compound		(VI)	3.1%	(0.003	mg/kg)	
Guaniding		(II)	11.3%	(0.010	mg/kg)	
6-Chlorenicovinic	rcid a	(XII)	9.4%	. (0.009		
NR A		(	- • • • •	(		
Another 5 unknow met	abolites occurred in	very low co	oncentrati	ons and	in total amo	ounted to 13.1%,
0.012  mg/g. The non-e						

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

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

Please click on the hyperlink to order a Study Report.



Donoute	01 01/00
Report:	01.01/09; <b>M-024289-01-2</b> 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
CI P/CFP.	vos
	01.01/09; 1992; M-024289-01-2 Study on the metabolism of NTN 33893 after spray application to potatoes PF3678 M-024289-01-2 171-4 Nature of Residue (Metabolism) - Plants not specified yes insecticide NTN 33893 (I) was investigated in potatoes after application of NTN 33893. An 0.2% spray fluid of a 25 WP was applied to the foliage of potato harvest. Vines and tubers were harvested 7, 28 and 64 days after application. At the the vines were withered and largely dry like under practical conditions; in this mounted to 1.35 mg/kg.
< <m-024289-01-2@s-603017-01-1< td=""><td></td></m-024289-01-2@s-603017-01-1<>	
The metabolism of the	insecticide NIN 33893 (1) was investigated in potatoes after application of
[pyridinyl- <sup>14</sup> C-methyl]	NTN 33893. An 0.2% spray fluid of a 25 WP was applied to the follage of potato
plants 64 days before h	arvest. Vines and tubers were harvested 7, 28 and 64 days after application. Age he
time of harvest (day 64	t) the vines were withered and largely dry like under practical conditions; in this
case the total residue an	mounted to 1.35 mg/kg.
0.90 mg/kg of this coul	ld be identified by chromatographic comparison with reference standards and by
other physical methods	
other physical methods	
0.51 mg/kg "NTN 33	893" & & & & & & & & & & & & & & & & & & &
0.17 mg/kg "Guanid	ine-metabolite" (II) X X X X
0 00E mg/kg "Wudno	
0.095 mg/kg Hyaro	xy-metabolites, a star of the
0.034 mg/kg "01efi	insecticide NTN 33893 (I) was investigated in potatoes after application of NTN 33893. An 0.2% spray fluid of a 25 WP was applied to the foliage of potato harvest. Vines and tubers were harvested 7, 28 and 64 days after application. After the vines were withered and largely drx like under practical conditions; in this mounted to 1.35 mg/kg. Id be identified by chromatographic comparison with reference standards and by set of the standards and by
0.036 mg/kg "Dihyd	roxy metabolites @ (VII) ~ ~ ~
0.030 ma/ka "Nitro	simine-metabolite" (VIST)
0.026 mg/kg "Chlor	$pp1co_{1}y1-gtucos1de$ $(x) = (x)$
Į.	
The identified compou	ndoare represented in a degradational pathway (Fig. 19).
$\cap$ (	
0.19 mg/kg, correspond	ding to 14.1% of the total esidue accounted for unextractable <sup>14</sup> C-radioactivity.
Hydrolysis experiment	s indicated a partial incorporation into lignin constituents of the potato vines.
The <sup>14</sup> C-radioactivity	the potato tuber corresponded to a total residue of 0.009 mg/kg. This is
organosoluble <sup>14</sup>	oactivity K 0 000 mg/kg) which could be assigned chromatographically to NTN
33893 (I) Approx 0.04	B my be of the notat portions consisted of 6-chloronicatinic acid
55075 (I). Reprox. 0.2	nextractable residue (0.001 mg/kg), potar portions (0.007 mg/kg) and oactivity $\langle 0.001 mg/kg \rangle$ which could be assigned chromatographically to NTN a mg/kg of the polar portions consisted of 6-chloronicotinic acid.
This study was conduc	tad from Dy 1007 to Knyary 2000 at the Institute for Matcheliam Descerab of
This study was conduc	AED C A A A A A A A A A A A A A A A A A A
Sayer Aco Leverkusen	Krku,
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
, ,	
L. 4	
j <sup>o</sup> v	
27 2 A	
4 <sup>73°</sup> 4 <sup>9°</sup> 10°	
× Å	inextractable residue (0.004 mg/sg), potar portions (0.007 mg/kg) and oactivity < 0.001 mg/kg) which could be assigned chromatographically to NTN of mg/kg of the polar portions consisted of 6-chloronicotinic acid. ted from fully, 1987 to January 1990 at the Institute for Metabolism Research of FRG
Ċ Ĕ	



Report:	01.01/10; ; 1	993; <u>M-024</u>	<u>1294-02-2</u>	1.		a s
Title:	Metabolism of NTN	33893 in o	cotton afte	er seed tr	reatment	
Report No.:	PF3675				*	S O
Guideline(s):	<u>M-024294-02-2</u>				Č,	Ű, Ó
Guideline deviation(s):	none				Ĩ,	× , \$
GLP/GEP:	ves				1	
	<i>j</i> 05		(Pro-		K <sup>*</sup>	
Intrinsic Study Summ	ary of report adda	ndum			jų č	
Intrinsic Study Summ	ary or report adde	nuum.	× A	C		N & .0
The extracts of cotton s	eeds of the soil drei	nch exneri	Went from	n then	ioinal NTN9380	93 metabolism
study report No 3675	were further invest	igated 0				
study, report 100. 2070,		Butter	0	S.		
Report: Title: Report No.: Document No.: Guideline(s): Guideline deviation(s): GLP/GEP: «M-024294-02-2@S-605105-01-1 Intrinsic Study Summ The extracts of cotton s study, report No. 3675, The major metabolite (n methanol/6N HCI reflu chromatography with th methylation. Metabolite 19.1 (0.7 %, 0.06 mg/kg corresponding authentic	metabolite 15, 27,1	% of the r	adioactiv	ity in th	ie seeds. 2.54 mg	y/kg) in the
methanol/6N HCI reflu	x extract was identi	fied as 64	vdroxvn	icotific	acid methyl este	toy co
chromatography with th	ne authentic referent	ce compou	indusing	two di	mensional TLC a	ind GG/MS after
methylation. Metabolite	e 16 (1.6 %, 0.15 m	g/kg) was	identified	Pas 6-b	droxynicotinic :	acid and metabolite
19.1 (0.7 %, 0.06 mg/kg	g) as 6-chloropeoti	nie acid m	ethyl este	er by co	-chromatography	with the
corresponding authentic	reference compou	nd using tv	wo-dimer	nsional '	THC. N	
		&	ð j	S' (		
Furthermore, the residu						
extract (44.5 %, 4.16 m	g/kg) were characte	rized as b	eing base	d næstl	y on 6-chloronic	Otinic acid, 91 %
and 87 % of the radioac	tivity, respectively	Ĵ Ĝ <sup>r</sup>	Ø	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
	×	S -	4 0	s s		
Intrinsic Study Summ	ary of original rep	ort: Se		×.	«. ».»	
The metabolism of the	incasticida MTN 2	602 . S	wationt.	u U in no	Otor annli	action of
The metabolism of the [pyridinyl- <sup>14</sup> C-methyl]		695 was II	Prostigat	of 50Å	ranges after appli	of 0.05 g active
ingredient per ranning	mater was mode at t	hotimo of	nlanting	the pot	atask The vines	and tubers were
harvested 129 days after	r appleation At the	time af h	arweet th	and por	acces. The vines	and mostly dry as
would be upder practica						ia mostry ary as
		O,	, <sub>~</sub> ~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
The total residues, exp	essed a i equival	ents were	5.76 mg		rines and 0 091 m	ng/kg in tubers Of
the radioactivity applied	d to the soil 2.2%	as taken u	p by the	whees ar	nd $0.3\%$ by the tu	ibers.
<sup>2</sup>		<i>4</i>	~ ~			
By chromatographic co	mparison with refer	ènce com	oounds a	nd other	r physical metho	ds the following
could be identified (an	ounts given in per s	ent rådioa	ctivity an	id in mg	g/kg a.i. equivale	nts in vines and
tubers respectively):		. 6 <sup>2</sup> .	Ø,			
Ö, v			J			
1. Vines 🔊		÷.,9				
unchanged parent co	moound 0	AV O	26.7%	(1.53	mg/kg)	
5-Hydroxy		YTVN	4 6%	(0.26	ma/ka)	
		(1)	0.2%	(0.20	mg/ (g)	
Dihydroxy compared		(VII)	0.3%	•	mg/kg)	
		(VI)	3.3%	-	mg/kg)	
Nitresimine compour	d v	(VIII)	2.6%	(0.15	mg/kg)	
Guanidine?compound		(11)	8.2%	(0.48	mg/kg)	
6-Chlo@nicotinic a		(XII)	8.3%	(0.48	mg/kg)	
Glucoside of 6-chlo		(X)	1.4%	-	mg/kg)	
alcohol			21.74	,		

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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. *a*,

		PP	<i></i>	88	
2. Tubers				≫.	
Unchanged parent compound	(1)	48.3%	(0.044 mg/kg)	N N N N N N N N N N N N N N N N N N N	
5-Hydroxy compound	(IV)	8.0%	(0.007 mg/kg)		
Olefine compound	(VI)	3.1%	(0.003 mg/kg)	2	
Guanidine compound	(11)	11,5%	(0.010 👩/kg)	Č,	
6-Chloronicotinic acid	(XII)	9.4%	(0.003 mg/kg)	×°	
		"O"	Å.	,O (	

Another 5 unknown metabolites occurred in very low concentrations and in total amounted to 13.1%

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

 >>M-024294-02-2@5-605105-01-1

 Report:
 01.01/11;

 Title:
 Admire (2.5 Giranular) - Residues in field rotational crops

 Report No.:
 105153

 Document No.:
 M-024356-01-2

 Guideline deviation(s):
 EPA Ref.: 165 2 Field Rotational Crops (Linsted)

 GLP/GEP:
 yc

Field rotational crop studies were conducted in Benoit, MS, Stanley, KS; and Fresno, CA to determine the residue levels of imidacloprid [APMIRE @TN33893, 12 (6-chloro-3-pyridiny1)methyl]-4,5-dihydro-Nnitro-lH-imidazol-Samine and metabelites in field cops at 4, 4, 8 and 1 month plant-back intervals following a single soil application of ADMIRE 2.5% Grantilar 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) accreal grain crop (wheat or sorghuro), (2) a root crop (turnips), and (3) a leafy vegetable crop (spinach of mustand green). All crops were harvested at normal maturity. In addition, immature wheat or sore hum green for age was collected for analysis at 45 days post-planting in each interval. Ċ L, A  $\bigcirc$ 

In cereal grain crops, residues i imidacloprid were 0.12 ppm in wheat forage and 0.19 ppm in wheat straw at the 8-month Plant-back interval in Presno, CA. When extrapolated, residues of imidacloprid in wheat forage and straw were <0.05 ppm of the 12 -month plant back interval in Fresno, CA. Residues of imidaclopted 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 imidaclopri@were \$.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 priorith plant-back interval in Fresno, CA. Based on having residues of 0.07 ppm in turn profit at 8 months in Fresno, CA, the residues at an 11- month plant-back interval were anticipated to  $b_{\infty} < 0.05$  ppm  $\sim$  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 leaf vegetable crops, residues of imidacloprid were 0.32 ppm in spinach leaves at the 8-month plantback interval in Fresno, CA. When extrapolated, residues of imidacloprid in spinach leaves were



mustard leaves at the 11-month plant-back interval in Benoit, MS and Stanley, KS.

<0.05 ppm at the 11-month plant-back interval in Fresno, CA.

Issue date 2017-11-22

Imidacloprid residues were <0.05 in

mustard leaves at the 11 >>M-024356-01-2@ <b>S-603096-01-1</b>	-month plant-back inte	rval in Benoit, MS	S and Stanley, F	S.	
Report:	01.01/12; ; 1996;	M-010590-01-2		) V	
Title:	Admire 2F - Magnitude		ld rotational co	bs 👋	S.
Report No.:	107133		1	A.	No Por
Document No.:	<u>M-010590-01-2</u>		s and a second s	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Guideline(s):		Ğ	â	$\sim$ $\sim$	<sup>y</sup> a <sup>S</sup> as
Guideline deviation(s):		~¥v	Q		\$ A
GLP/GEP:	yes	a second	40 °	N Q	
< <m-010590-01-2@s-603095-01-1< td=""><td></td><td>A</td><td></td><td>5 L (</td><td>Ĩ, Ĉ</td></m-010590-01-2@s-603095-01-1<>		A		5 L (	Ĩ, Ĉ
Section 2: Constant and Cons	ls were conducted in B	eport, MS; Stante	y, KS and Fres	no, CA to dete	rmine the
levels of imidacloprid [A	ADMIRE, NTN33893.	1-1(6-chloro-3-9)	vridinvl)me@vl	1-Nonitro 2-	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
imidazolidinimine] resid	lues in field crops at a	I-month plant ba	ck interval follo	wing a single a	soil
application of ADMIRE			mative rotationa	al crops weren	anted at
all three locations at the					
(sweet corn and corn gra	ain). (2) cereal erop for	age and straw (con	rn green forage.	orn green for	age with
ears, and corn dry fodde	r). (3) legume@egetabl	e crops (sovbeans	beans and pe	(4) toliage	of legumes
(soybean forage and hay	() and (5) sattlow as see	eds All crops wer	e harvested av	orma maturity	/
(so jocun roruge und nuj				$\tilde{c}$	•
All residues of imidaclo	prid well converted to	à common april vi	e and derivatize	Prior to injec	tion on a
gas chromatograph equi	nned with a mass selec	tive detector (gc/r	(A) The limit	of quantitation	(LOO)
was 0.05 ppm.				©	(LOQ)
				K.	
The highest residue valu	les were 0 05 nom får	ceres crondrain	40.26 ppm for d	real crop fora	ae and
straw 0.22 nnm for lan	ma Mantakka crop 21	nom for legume	Poliage and an	05 npm for saf	flower
seeds.				os ppin for sai	nower
>>M-010590-01-2@S-603095-04-1					
<b>Report:</b>					
Report: 🖒 🖧	01,09/13;	M&24331@1-4			
Title:	Photolysis of imidaclopy		leaf surfaces of	tomato plants	
	40F427 $6$ $10$		~ 107	1	
Document No.:	<u>M-023331-014</u>		0″		
Guideline(s):	US EPA OCSPP Guidel	ine Number: 860 S	<b>UPP</b>		
Guideline deviation	none is in	ñ o' 🔬			
GLP/GEP: 🖗 🦼	yes & o w				
< <m-024331-01-4@s-60.09-01-1< td=""><td>′<u>0</u>′<u>0</u>′0′</td><td></td><td></td><td></td><td></td></m-024331-01-4@s-60.09-01-1<>	′ <u>0</u> ′ <u>0</u> ′0′				
GLP/GEP: 	cansectionae with good	Mactivity as a cont	act and stomac	h poison. The a	ctive
ingredient was assigned	the proposed common	name imidaclopri	id.		
		ž M			
With methylene-14C9N	FN 33893 the photodeg	gradation on tomat	to leaves under	field condition	s was
investigated. The average	se total recovery of the	ndividual sample	s ranged from 9	94.7 to 105.8%	of the
applied radioactivity in t	the course of the study.	· · ·	-		
A A					
The DT-50 value of NT	N \$3893 on leaf Surfac	es depended very	much on the glo	obal radiation.	Since there
was only little degradati	on under dark control c	conditions, the glo	bal radiation of	cloudy and sur	nny days in
September and Sctober	in Monheim (51°4` lati	itude North, 45 m	above NN) led	to DT-50 value	es of 1.4
and 0,7 days The radiat	ion on leaf surfaces wa	s measured and th	e mean daily va	alues were 0.04	and 0.26
kJ/emi <sup>2</sup> .	1 Alexandre		2		
In totaÇup to ≥ 14metab	olites were detected in	the leaf extracts a	along with pare	nt compound. H	Besides
three well known plant r				-	
ring was metabolised ste		rriouuot miti		The minut	
	r				
Please click on the hype	rlink to order a Studv F	Report.			
//-	· · · · · · · · · · · · · · · · · · ·				



The proposed degradation pathway of NTN 33893 on tomato leaf surfaces is shown in Appendix 18, . 01.01/14; 1999; M-016760-02-4 Residues of 14C-NTN 33893 (imidacloprid) in blossoms of sunflower (Heffanthus annuus) after seed dressing MR-550/99 M-016760-02-4 US EPA OCSPP Guideline Nmber: 860.SUPP none yes es of the insecticide NTN 33893 (imidacloprid) as a set >M-024331-01-4@S-602559-01-1 **Report:** Title: Report No.: Document No.: Guideline(s): Guideline deviation(s): **GLP/GEP:** -<m-016760-02-4@S-602423-01-1</p>
The occurrence of residues of the insecticide NTN 33893 (imitacloprid) and/ots metabolites in nectar and pollen of sunflower was investigated after seed dressing in a greenkouse experiment. [Methylene- $^{14}$ C]imidacloprid was formulated as a WS 70 (equivalent to 'Gaucho''). The application conditions  $45^{\circ}$ 

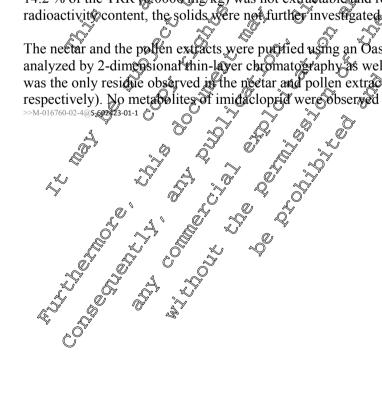
projected for this experiment simulated the practice conditions of 150 g WS (1) unit sunflower seeds (1) unit = 150,000 grains), equivalent to 105 g a.i. whit. In the experiment, each sunflower seed was coated with ca. 1.0 mg of formulation, equivalent to ca. 0.7 mg a i A total of 22 sunflower plants (variety "Fleurv") were separately grown in 34-L pors (ca: 40 cm diameter) in the greenhouse Subdi ded into two rows of 11 plants each. Ð

m

During flowering, nectar was collected every day with a capillar 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. Pollenowas collected with the aid of plastic boxes that were installed underneath the inflorescence. The poller freely trickled into the plastic boxes. In total, ca. 4.8 gpollep/row was collected.

The total radioactive residues (TRR) of both rows (negtar 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 PRR (20006 mg/kg) was not extractable and remained on the solids. Due to the very low radioactivit@content, the solids were not further investigated, m

The nector and the polien extracts were publied using an Vasis® 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 neetar 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.





Report:	01.01/15; 2008; <u>M-308</u>	<u>631-01-3</u>		e °
Title:	Imidacloprid residue movemen implications for potential bee e	t in plants following for	oliar application	is and the 🖉 🖉
	implications for potential bee e	xposure		
Report No.:	<u>M-308631-01-3</u>		ð	
Document No.:	<u>M-308631-01-3</u>		ð	
Guideline(s):	US EPA OCSPP Guideline Nu	mber: 158.400(e)	100	
Guideline deviation(s):			A	6 <sup>3</sup> 6 <sup>3</sup> 9
GLP/GEP:	no			
		, <sup>¥°</sup>	, Ű	

### **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 imidaclopted is known to exhibit systemic behavior following seed or soil treatments, questions have been faised about the potential for imidacloprid residues resulting from four sprays to move throughout aplant into nectar and /or pollen for some weeks after pre-flowering applications are made. In addition, potential exposure to bees by nectae and/or pollen of plants exposed by spray drift is also questioned.

- 1) to summarize available information concerning the systemicity and translocation of imidacloprid in plants to demonstrate that imidacloprid residues in frectar or pollen will

2) to summarize key studies that can be used in risk assessment to address potential bee exposure from off-orop drift.
 (1) to summarize key studies that can be used in risk assessment to address potential bee exposure from off-orop drift.



### 01.02 - Soil and water

Ô

<<M-024011-01-24 (S-602963-pl-1

01.02 - Soil and	water y a
Report:	01.02/01; 1991; <u>M-023983-01-2</u>
Title:	Terrestrial field dissipation for NTN 33893 in California soil
Report No.:	MR101989
Document No.:	<u>M-023983-01-2</u>
Guideline(s):	EPA Guideline Ref. No.: 164-1 Solf Field Dissipation
Guideline deviation(s):	none
GLP/GEP:	yes A Q o A Y C Q

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

NTN 33893 (a broad spectrum, systemic insecticide) was appled to a tomat@plot near Fresho, Canfornia, on June 19, 1990, to evaluate mobility and persisten of in soft 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 deptile 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 papent NEN 33893 by gradient high performance liquid chromatography. The half-life  $(t_{1/2})$  and first other rate constant (k) for the dissipation of NTN 33893 from Day 0 to Day 91 was 53 days (10 -0.96 and 4013, respectively, The tig 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 mit below the 0-6 inch defth. These data indicate that NTN 33893 does not leach.

Total accumulated ranfall for the study period through Qune 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 \$1.43 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.

~Q~ Ù	
Report:	01.02/02 1991 21-024 1-01-2
Title:	TerreGrial field dissignation for NTN 33893 in Georgia soil
Report No.:	MR101987 ~ ~
Document No.:	MC02401 201-2
Guideline(s):	PA Guideline Ref. NO. 164-1 Soil Field Dissipation
Guideline deviation(s):	none Q
GLP/GEP:	yes difference with the second s
< <m-024011-01-246-602963-04-5< td=""><td></td></m-024011-01-246-602963-04-5<>	

NTN 33893 (a broad spectrum systemic insecticide) was applied to a bare ground plot near Tifton, Georgia on April 16-1990, we 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 240 S formulated as a 23.3% active ingredient liquid suspension was applied broadcast to bare ground  $\Re$  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 reference of the parent NTN 33893 by gradient high performance liquid reference of the parent NTN 33893 from Day (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 phrough 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 Sear near 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 94.44 inches. Air and soft temperatures during the study did not differ significantly from a NOAA 10-year mean.

Report: Title: Report No.: Document No.: Guideline(s): Guideline deviation(s): GLP/GEP:

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

01.02/03; 1991; <u>M-023988-01-2</u> Terrestrial field dissipation for WTN 33893 in Minnesota soil MR101988 <u>M-023988-01-2</u> EPA Guideline Bef. No. 164-1 Soil Field Dissipation none **yes** Setrum, systemic insecticide) was applied in 4990 at a site near H-11 te mobility and persistence of soil. Soil at the site horizon. The site received one and 11 240 FS) was among 1

NTN 33893 (a broad spectrum, systemic insecticide) was applied in 1990 at a site near Hollandale, Minnesota to evaluate the mobility and persistence at 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 0 inchest immediately post-application (Day 0) and then to a depth of 48 inches for all remaining sample intervals. A total 0015 core samples was taken per sampling interval. Bach core was sectioned into 0.6-in. (A), 6-12-in. (B), 12-19-in. (C), 18-24-in. (D), 24-30-in. (E), 30-36-in. (F), and 36-48-in. (C) layers. The 15 core samples were composited to three pelicates 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 infigation 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.



<b>Report:</b> Title:	01.02/04; <b>M-02351</b> Metabolism of (pyridinyl-14C-me	4-01-2 thylene) NTN 33893 in sandy loam	under aerobic
	conditions	•	
Report No.:	PF3433	×	
Document No.:	<u>M-023514-01-2</u>	- A - A - A - A - A - A - A - A - A - A	
Guideline(s):	none	, v Or	
Guideline deviation(s):	none		
GLP/GEP:	yes		
< <m-023514-01-2@s-602954-01-1< td=""><td></td><td>R U U</td><td></td></m-023514-01-2@s-602954-01-1<>		R U U	
The metabolism of NTN	33893 in soil was investigated	in a laborator study according to	othe general 20
protocols of the respectiv	ve EPA and BBA Guidelines.		
[Pyridiny1-14c-methyle	neINTN 33893 was applied to a	sandy hoam from Kansas A SA	Tabe samoles
were incubated in the da	rk at $20 + 2 \cdot c$ and 75 % of 1/3?	var prodisture level under aerobic o	conditions The
application rate of $0.33$ r	ng/kg was based on the recomm	perded maximum use rate of 260	g/ha=Sampling
times were $0.1.3.7.14$	30, 59, 100, 120, 482, 370 and	366 days	g ng panging
	30, 39, 100, 120, 182, 24, and		
represented 66.9 % of th	e applied radioactivity after an i	incubation time of 366 days.	Pagent
compound accounted for	: 61.6 % @the applied radioact	ivity in the soil extracts 366 days	pösttreatment.
			0
The amount of radioactiv	vity bound to the soil increased	gradually with time and attained	a maximum of
25.6 % of the applied do	se 274 days posttieatment.	s along with parent compound. S	
**			
A total of 7 metabolites	was observed in the soil extract	s along with parent compound. S	ix metabolites
were identified by specifi	osconfic methods and comparise	with withen to reference subst	ances. One
additional metabolitewa	s detected by reverse isotope di	Jution analysis. The degradates re	epresented a total
of 3.4% of the applied r	adioactivity after 366 days No	single degradate accounted for m	ore than 1.7 %
of the applied dose (0.90	6 npm at a sty time.		
of the applied dose (0.6			
The degradation of WTN	[22802 jim coil progod at via	eavage and gridation of the dihyd	tra imidazala
		ocyclic ring to the key intermedia	
ablerer solutions of the	the second motor the intact rester	dioxide During the incubation time	$m_{2} \text{ of } 266 \text{ down}$
the activation of 7 40	the unitate metapointe capoon	transformed into early diavide	The of 500 days
the equivalent of 7.4.5% C	in the appred ratioactivity was		
TI (1)			C (1
The average total recover	ry ranged from 94,4%0 98.3*% (	of the applied radioactivity in the	course of the
study.		<sup>je</sup>	
- A.			· · · · · · ·
The composition of the	alf-life was based on the initial	100 days of incubation. Accordi	ng to statistical
interpretation of the data	a first order root function prov	ed to be the best fit. Extrapolation	n resulted in a
half $f(DT-50) > 4$ yea			
>>M-023%14-01-2@S-602954-01-1			
te de la companya de la	5 <sup>8</sup> x 4		
N Q A	, A S		
A A ·	Le la		
, ON			
$\lor$		dioxide. During the incubation the transformed into carbon dioxide. of the applied radioactivity in the 100 days of incubation. Accordi ed to be the best fit. Extrapolation	
Please click on the hype	rlink to order a Study Report.		



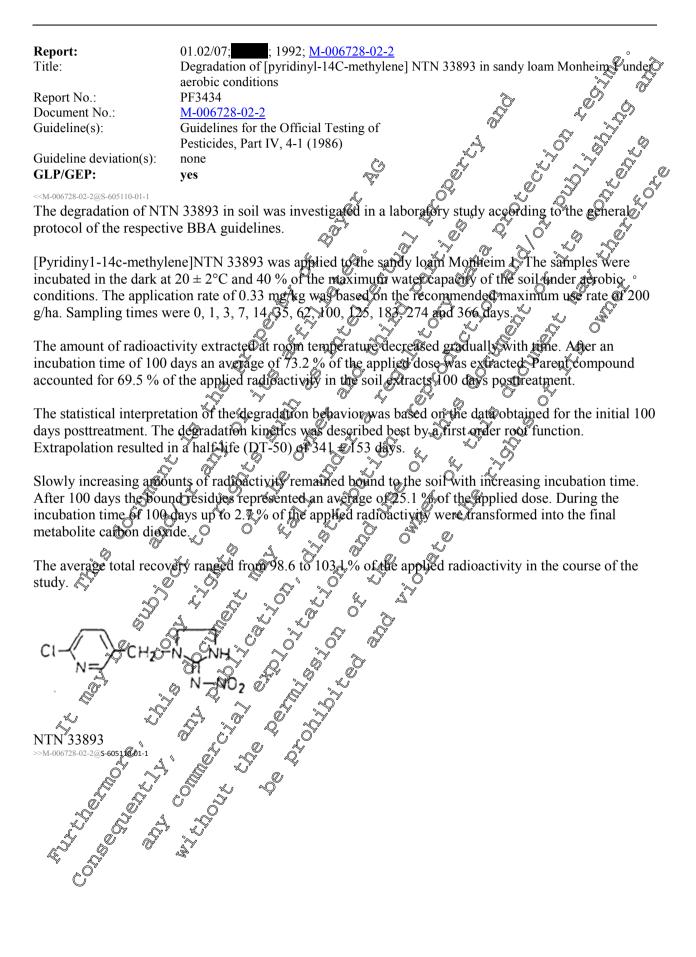
Report:		<u>M-006742-02-2</u>		
Title:	aerobic conditions	I-14C-methylene) N	TN 33893 in loamy soil BBA	A 2.2 under O
Report No.:	PF3321		∕⊗.	S O
Document No.:	M-006742-02-2			
Guideline(s):	EPA Pesticide Assessme BBA Ref.: Guideline IV		ivision N: § 162-1(1982)	A 2.2 under G
Guideline deviation(s):	none	, + 1 (1900) &	× ×	
GLP/GEP:	yes	- Contraction of the second se		Y O' O
< <m-006742-02-2@s-605115-01-1< td=""><td>-</td><td>۶ ۲</td><td></td><td></td></m-006742-02-2@s-605115-01-1<>	-	۶ ۲		
In a laboratory study [r	ovridinyl- <sup>14</sup> C-methylen	elNT 33893 wa	applied to the loamy sa	nd soil BASA
2.2 and maintained aer	obically in the dark at	$20 \approx 2^{\circ}$ C The and	plication rate of 0.23 mg/	kg was based
on the recommended m	aximum use rate of 20	00 p/ha Sampling	times were 0 1 7 14	35, <b>62</b> and
100 days.	×			, 20, <sub>10</sub> - and
-	0			4 00
The amount of radioact	tivity extracted at toor	n temperature dec	reased gradually with tin	and
represented 68.6% of t	the applied radioactivi	v after an incuba	tigh time of 100 days. Par	rent
compound accounted t	for 63.2 % of the applie	d radioactivity in	the soil extracts 100 days	s post
treatment. The degrada	tion kinetic could be	described best by	a reaction order of 2. Ex	tapolation
resulted in a half life (	DT-50) of $388 \pm 25$ da	ys. 🔊 🔊		) <sup>*</sup>
			A & A (	
The amount of radioact	tivity bound to the soil	increased gradua	By with time and reached	ł a maximum
of 21.6 % of the applie	d. One hundred days p	ost treatment 7.9	% of the applied radioact	ivity were
released from the soil b	ox teflux extraction, 7	\$% of which we	readentified as parent con	npound.
~		. Ý. Ý	×	
Six metabolites were it	lentified by spectrosco	pre-methods and	comparison with authenti	ic reference
substances. One adoution	onal metabolite was de	tected by reverse	isotope dilution analysis.	Neither of
them accounted for mo	ore than 2.2 % of the ap	plied radioactivit	ty at any time. The degrad	lation of
NTN 33893 in soil pro	ceeded via cleavage a	nd exidation of the	dihydro-imidazole-ring	and via loss
of the nitro group from	the untact heteroeyclic	c rung to the key n	termediate 6-chloronicot	tinic acid and
			e 8₽ 100 days the equival	ent of 10.0
% of the applied radio	guivity was ignisionine		xude.	
The total recovery con	rade from the 1 to 303	% of the applied	radioactivity in the course	ofthe
study.	200 1011 29.4 (0903.8		autoactivity in the course	
Not a single soil home	metholitesurnassed	the concentration	of 0.01 ppm in the soil: o	only the
parent compound rema	ained at a level above.	201 pan.	or otor ppin in the sont o	ing the
parent compound rema		201 ppm.		
		AN .		
	A. O ST			
×				
¢ ,		N-NO	2	
	St w v			
J & A	NI NI	IN 33893		
>>M-06042-02-202-505115-01-0	2			
	48			
č <sup>O</sup> <sup>v</sup>				
Not a single soll borne parent compound rema				
Please click on the hype	rlink to order a Studv R	eport.		



### Imidacloprid Bee Studies Compilation of Study Summaries

Report:	01.02/06; <u>M-006740-02-2</u>
Title:	Degradation of [pyridinyl-14C-methylene] NTN 33893 in silt soil Hoefchen under
	aerobic conditions
Report No.:	PF3322
Document No.:	<u>M-006740-02-2</u>
Guideline(s):	Guidelines for the Official Testing of
	Pesticides, Part IV, 4-1 (1986)
Guideline deviation(s):	none A OV AV
GLP/GEP:	yes
< <m-006740-02-2@s-605112-01-1< td=""><td>Degradation of [pyridinyl-14C-methylene] NTN 33893 in silt soil Hoefchen under aerobic conditions PF3322 <u>M-006740-02-2</u> Guidelines for the Official Testing of Pesticides, Part IV, 4-1 (1986) none yes</td></m-006740-02-2@s-605112-01-1<>	Degradation of [pyridinyl-14C-methylene] NTN 33893 in silt soil Hoefchen under aerobic conditions PF3322 <u>M-006740-02-2</u> Guidelines for the Official Testing of Pesticides, Part IV, 4-1 (1986) none yes
In a laboratory study	[pyridinyl-14C-methylene] NTN 33893 was applied to the silt soft Höfeben and
	by in the dark at $20 \pm 2 \cdot c$ . The application of a construction of $0.36 \text{ mg/kg}$ was based on the
mannanicu acrouicai	
	num use rate of 200 g/ha. Sampling times were $(0, 1, 30, 7, 14, 30, 59)$ and $(100)$
days.	
The amount of radioa	ctivity extracted at room temperature decreased gradually with time. After an
incubation time of 10	0 days 71.5% of the applied adioactivity were extracted. Parent compound
accounted for 66.8 %	of the applied radioactivity in the soilextracts 100 days post treatments
The degradation kine	tics was described best by a reaction order of 2 Extrapolation resulted in a half
life (DT-50) of $248 \pm$	50 dave
$(D1-50)01240 \pm$	
<u>S1</u> 1 :	ounts gradioactivity remained bound to me soil with Hicreasing incubation
Slowly increasing am	ounts our adioactivity remained bound to me soil with increasing incubation
time. After 100 days f	the bound residues represented an average of 21.6 % of the applied
radioactivity. Reflux	extraction with agetonity released 8.5% of the applied radioactivity 100 days
post treatment, 8.1%	of which were identified as parent sompound.
During the incubation	i time of 180 days up to 64 % of the applied radioactivity were transformed
into the final metaboli	ter carbon dioxide. ~ ~ ~
Č,	recarbon dioxide.
The total recovery rat	gred from 907 to \$03.6 % of the applied radioactivity in the course of the
study	
study.	
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Ň	A NEW NONO
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SM-006740-02-2@ 5-608112-01-1	A CHINE CHOM NH AND CHOME
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Report:	01.02/08;	; 1992	2; <u>M-023</u>	828-01	<u>-2</u>			e °
Title:	Soil/sedime	nt adsorpt	ion-deso	rption o	f 14C-imi	daclopr	id	
Report No.:	MR103816	1.2					*	N O
Document No.: Guideline(s):	<u>M-023828-0</u> EPA Ref.: 1		sorption/I	Desornti	on			Ũ D
Guideline deviation(s):	none	05 I, I <b>U</b>	,orption, i	Jesoipa	.011		O'	
GLP/GEP:	yes					, A	A Ö <sup>y</sup>	
Report:       01.02/08; 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         <								
Aqueous solutions of <sup>14</sup>	<sup>4</sup> C-imidaclop	rid were	equilibr	atedwi	th four so	oibtype	es and the adsorp	on and K
desorption coefficients	and constant	s were de	etermine	edLiqu	uid scinți	Pation	counting analysis	was w
employed to measure the	he test materi	al conce	ntration	in the	aqueo	phases	. Following desor	ption, 🦉
combustion-radioanaly			~~~		(Cost)	° Roman		
The definitive soil adso	orntion-desor	ntion stu	dy was o	° Anduca	ed at 25	, ¥1°¢≹	in the dark with 14	C-45
imidacloprid and four s	soils (sand $\#3$	96. loam	v sand	#398_le	Jam #398	sildic	am #307. and silt	toam #307
with sodium azide). Th	e nominal co	ncentrati	ons of 1	<sup>4</sup> C-imate	lacloprid	for all	sold types were	50, 189.5,
125, and 25 ppm. The	soil-to-wate	r ratio wa	as 1%.	ð.		€ <sup>ÿ</sup> ?		
		Å. 4		, , ~C	y. w	, Č		0
The mean percent of $cc$	Support ads $\frac{1}{2}$	orbed to	the test's	soils≪dù ⇔haanna	$\mu_2$ (mathe)	detuñit	$\mu$ $\alpha$ $\gamma$ $\alpha$	2, 16.8, 35.7,
33.5, and 29.5% for san loam #318, respectively	10 #390, 100	ny sana +	+398, SII	t peam :	#201, SIII	 om th∂	507 (With Socium	he definitive
study was 83.4% for #3	$396 \operatorname{san}^{44}$	3 <sup>°</sup> for #	398 loar	hy safe	1 41 8/%	for#3	07  silf loard  44  8	% for #307
silt loam (with sodium	azide), and 4	4.1% før	#31 <b>%</b> /cl	ay toar	n.			/01011/00/
		ŝ	<i>A</i>	d.	_ \ _ X			
Although only 3 of the	soil types tes	sted were	within	the des	red 20-8	0 % fð	r which the Freund	dlich model
is typically defined; the	Freugellich	adsorptio	n isothe	rms tøi	all @ the	e soul t	ypes were calculat	ted and
demonstrated a high de coefficients (r) of the a	egree of linear	r correlat	101 For a		1 bg (Ce)	versus	In (x/m). Correla	tion
(with sodium azore) at	asorption #318	were 0		870, 10 87 0 90	13 0.088	and 0	987 respectively	implying
(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. $\beta$								
	Ŭ Ŝ		. 4	×	, ~, ,			
High-performance liqu	id chromatog	raphy an	d thin 1a	iyer ch	romatogr	aphy w	vere used to measu	ire the
stability of the test on	npound under	the test	conditio	ns Gre	at <del>er</del> than	95 %	of the <sup>14</sup> C activity	found in the
aqueous supernatants w	0 .~	,°0° `≈	V C		)* 1			
The mean <sup>14</sup> mass ba	lance of the t	est comp	ound	om the s	sand #39	6. loan	ny sand #398. silt l	oam #307.
silt loam #307 (with so	dium azido),	and Poan	n#3∕18 w	as 99.9	9, 93.5, 9	6.7, 99	0.1, and 100%, resp	pectively.
		Ú Å		J				
The Freud	dlich constants	and mobili	ity class) w	ere dete	rmined as s	summari	zed below:	
~~ <u>~</u>	A A	Q-		rption	Desor	ntion		
<sup>©</sup>			- Auso		Desor	puon		
O A		∽ % ~∽ Organic						
Son Id	entification	Carbon	K <sub>d</sub>	_K <sub>oc</sub>	K <sub>d</sub>	K <sub>oc</sub>	Mobility Class	
Sar Sar	nd #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 L	oan #307	1.51	4.18	277	4.68	310	Medium Mobility	
	oam #307 odium azide)	1.51	4.76	315	3.38	224	Medium Mobility	
	m #318	1.16	3.45	296	4.40	378	Medium Mobility	
>>M-023828-01-2@ <b>S-602914-01-1</b>		1.10	J. TJ	<u> </u>	7.70	570		
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Please click on the hun	erlink to ord	ar a Stud	v Ronard	+				
r icase click off the hyp	Please click on the hyperlink to order a Study Report.							



Report:	01.02/09; 1992; <u>M-024377-01-2</u>
Title:	
Report No.:	MR103817
Document No.:	<u>M-024377-01-2</u>
Guideline(s):	EPA Ref.: 163-1, Adsorption/Desorption
Guideline deviation(s):	none
GLP/GEP:	yes A Ö Ö Y
< <m-024377-01-2@s-603090-01-1< th=""><th>Soil/sediment adsorption-desorption of 14C-NTN 33823 MR103817 <u>M-024377-01-2</u> EPA Ref.: 163-1, Adsorption/Desorption none yes</th></m-024377-01-2@s-603090-01-1<>	Soil/sediment adsorption-desorption of 14C-NTN 33823 MR103817 <u>M-024377-01-2</u> EPA Ref.: 163-1, Adsorption/Desorption none yes
$\mathbf{A} = \mathbf{a} \mathbf{a} \mathbf{b} \mathbf{a} \mathbf{b} \mathbf{c} \mathbf{a} \mathbf{c} \mathbf{b} \mathbf{c} \mathbf{c} \mathbf{c} \mathbf{c} \mathbf{c} \mathbf{c} \mathbf{c} c$	<sup>4</sup> c-NTN-33823 were equilibrated with four soil opes and the desorption and
Aqueous solutions of	<sup>4</sup> c-NTN-33823 were equilibrated with four soil opes and the disorption and s and constants were determined Liquid scintillation counting analysis was a second statement of the second statement of t
	the test material concentrations in the aqueous phases. Following desorption,
combustion-radioanaly	vsis was used to demonstrate C-mass balance.
The definitive soil ads	orption-desorption study was conducted at $23 \neq 1$ °C in the dark with <sup>14</sup> c-NTN-
33823 and four soils (s	sand #396, loamy sand #398 silt loam #307 and loam #318). The nominal
concentrations of <sup>14</sup> C-1	NTN-33823 for all son types were 250, 187.5, 125, and 25 ppm. Sand #396 Coamy
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 c	ompound accorbed to the test soils during the definitive study was 38.5, 57.8, 73.3,
and 63 8% for sand #3	96, loamy sand #398, site loam #307, and loan #318 respectively. The mean
nercent of compound of	desorbed from the test soils during the definitive study was 59.1 \$7.4, 20.3, and
	loamy sand #398, sit loam #307, and loam #318 respectively. The percent
$2 \pm .070$ 101 Salid #390, 1	to any sand #370, she to and #307, and to and #310 respectively. The percent

adsorbed for all soil types rested was within the desired 20-80 % for which the Freundlich model is typically defined.

The Freundlich adsorption isothern's for all of the soil types were calculated and demonstrated a high degree of linear correlation for a plot of in (Co) versus in (Am). 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, ospectively, implying that all of the soils adequately fit the model for this compound. Desorption isotherms were also calculated

High-performance light chromatography and thin layer chromatography were used to measure the stability of the test compound under the test conditions. Greater than 93 % of the <sup>14</sup>C activity found in the aqueous supernatants was identified as parent compound.

The mean <sup>14</sup>C mass balance of the test compound from the sand #396, loamy sand #398, silt loam #307, and loam #318 was 101, 94.2, 145, and 105% respectively.

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

	% Grganig		rption	Desor	otion	
Soil Identification	Carbon	~K_d	K	K	K <sub>oc</sub>	Mobility Class
Sand #396	0.233	0.761	327	0.456	196	Medium Mobility
Loamy Sand #398	َ رَبِّي 349	2.91	833	2.45	702	Low Mobility
Stit Loam #307	۵.54) ۱.51	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** 



Report:	01.02/10; ; 1998; <u>M-023911-01-3</u>		° .
Title:	Long-term soil dissipation study with Zelmone	e 350 FS in Great B	ritain following seed
	dressing of winter barley		
Report No.:	MR-196/98	ð	
Document No .:	<u>M-023911-01-3</u>		A A
Guideline(s):	US EPA OCSPP Guideline Number: 835.610	0 40%	
Guideline deviation(s):	none	A	
GLP/GEP:	yes 🖒	Å,	
< <m-023911-01-3@s-603800-01-1< td=""><td>- And And And And And And And And And And</td><td></td><td></td></m-023911-01-3@s-603800-01-1<>	- And		
Long-term field trials v	with repeated single annual application of Ze	lucone 350 FS in	winter barley were O
• • • • •		× . (	· · · · · · · · · · · · · · · · · · ·

Long-term field trials with repeated single annual application of Zehnone 350 FS in winter barles were a carried out to investigate the behaviour of imidaclop id in soil over several years. Winter barles was a 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 Zehnone 350 FS the application rates were 100 (=55 g.a.i.) and 200 (=70 g.a.i.) ml per 100 kg seed. Two first sites were selected. At each test site both application rates were investigated in parallel on adjacent plots. At each site one untrated plot served as control. The dressed seed was analysed for concentrations of imidacloprid. About 21 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 (0-20cm) and after harves bus before dialling for the next year (0-40 cm) (0-50 cm at the last sampling date) from the treated and intreated plots using a pushing sampling device ('Wacker Hammer'). The soft cores were segmented into 10 cm layers and carefully homogenized to ensure a representative laboratory sample.

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The residues of Imidacloperd in soil were determined according to Bayer residue method no. 2 70 (**1990**). Residues were extracted with boiling method of followed by column chromatography on silica gel. The quantitation was performed via HPDC with UV-detection. The fimit of quantitation (LOQ) was 6  $\mu$ g/kg, while the limit of detection was 2  $\mu$ g/kg.

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

Before the first application traces of imidacloprid  $P_6 \mu g/kg$ ) 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 ware due to an interference from the matrix. However, at later sampling intervals and also in all control samples from Weilesbourne no residues were detected.

At the test site in Bury St Edmonds the sidues were significantly higher than at Wellesbourne. This indicates a faster dissipation of mida@oprid 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 fail were observed in the upper 20 cm layer. This was expected, since the upper soil by provide by ploughing and harrowing.

In the 20 30 cm ayer residues below 6  $\mu$ g/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  $\mu$ g/kg occurred in the 20 4 30 cm samples after the third trial year. It is possible that cultivation activity also led to some mixer mixing of soil containing imidacloprid residues also into the 20-30 cm layer.

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



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 44 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 hat inful effects are to be expected from these residues to be expected from these residues. >M-023911-01-3@S-603800-01-1

 Report:
 01.02/11; 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, 835.6600

 Guideline deviation(s):
 none

 GLP/GEP:
 yes

 Yes</

were carried out to investigate the Behaviour of midacoprid 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 soft with midagloprid. 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 Ostandard deviation Dabout 10%.  $\bigcirc$ 

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The trials were performed at threefest sites in apple growing agions in Northern (Burscheid) and Southern (Bechtofsheim, Preinsheim) Germany in typical apple orohards. On the test plots the soil between the tree rows & covered with grass mulch band, about 1/2 to 2/9 of total area), while under the trees there is a strip of baresoil. 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 (0,50 cm), (0-50 cm since 1996) were taken at -several intervals after application using a pushing sampling device ("Wacker Hammery. The soil cores were segmented into 10 cm layers and carefolly homogenesed to ensure a representative laboratory sample.

The residues of imidae oprid in soil were determined according to Bayer residue method no. 270 ( , 1992) Residues are extracted with boiling methanol followed by column chromatography on silica gel. The quantitation is performed via LPLC with UV detection. The limit of quantitation (LOQ) was 6  $\mu$ g/kg while the limit of detection was 2  $\mu$ g/kg.

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

This method allows the determination of parent compound and total residues of imidacloprid from a single extrace The total restrict method is a common moiety method comprising the parent compound and all its toetabolites 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- cleanedup by XAD & 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.



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 silvlation 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 a 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 Q 50 % of the initial value within 3-4 months. The degradation was fastest in Höfcher and was slowest in Freinsheim.

In the first three years the residues remaining in the soil (0-30 cm) until the next appleation 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 Payer maximum residues were about the LOQ in a very few samples, but for most of these samples the residues In the 20-30 cm layer no residues were detected in nearly all the samples.

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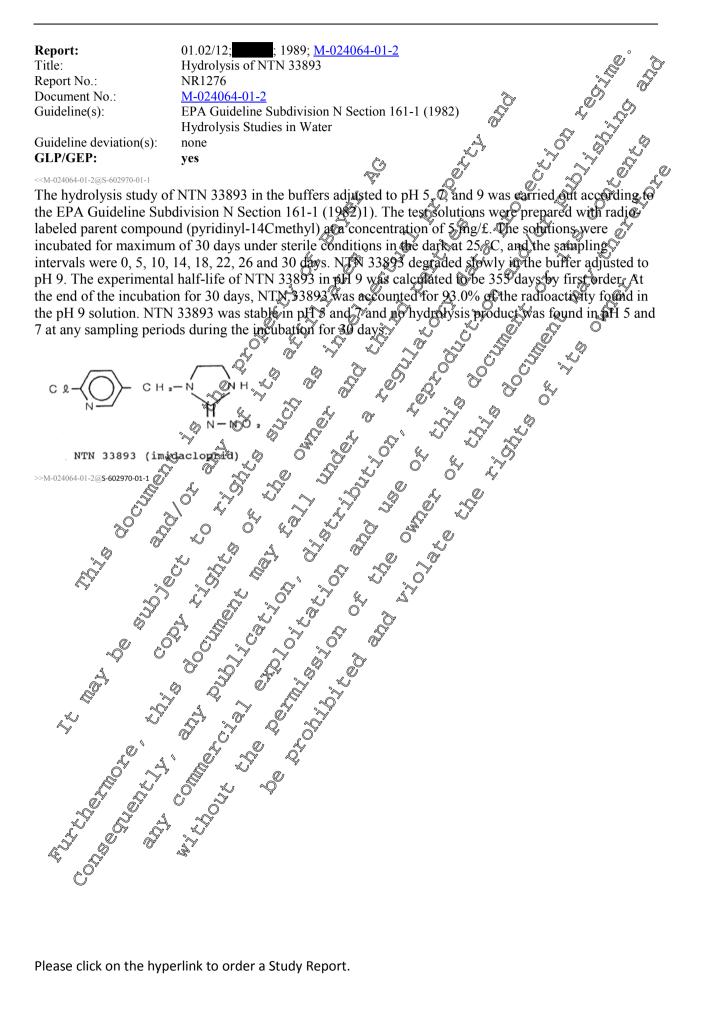
These results indicate very little movement of indaclopfid into deeper soil tayers doring the study. Considering the 2-3 X overdose in terms of soft exposure as compared to practical application conditions, this represents an absolute worst case scenario. Under practical use conditions probably no residues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

With respect to provement of inidacloprid into deeper soil by yers 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 Bechtolsneim some of the factors promoting preferential flow, e.g. soil cracking, worth holes, root holes, etc., were observed.

21 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 imidaeloprid into sofPlayerObelow 6-10 cm occurred, and this movement is considered attributable to







Donorte	01.02/13; 1990; M-024040-01-3
<b>Report:</b> Title:	
I IIIC. Depart No.:	MD 100240
Report No.:	MR100249
Cuideline(a):	$\frac{\text{M}-0.24040-01-5}{\text{EDA}}$
Guideline(s):	Photodegradation of NTN 33893 on soil MR100249 <u>M-024040-01-3</u> EPA Guidelines Subdivision N Section 161-3 (1982) Photodegradation Studies on Soil none yes NTN 33893 on soil was carried out under continuous irradiation for maximum of ificial light source at $25 \pm 2^{\circ}$ . The study was done according to the EPA
Guideline deviation(s):	none
GLP/GEP:	yes ch l y y y
< <m-024040-01-3@s-604673-01-1< td=""><td></td></m-024040-01-3@s-604673-01-1<>	
The photolysis study of	FNTN 33893 on soil was carried out under continuous irradiation for maximum of
15 days by using an art	ificial light source at $25 \pm 2^{\circ}$ The study was done according to the ERA
Cuidalinas Subdivision	N Section 161.2 (1022) 1) (File radio labeled per ent approximation $\frac{140}{1000}$
	N Section 161-3 (1982). <sup>1</sup> ) The radio-labeled parent compound (pyridinyl- <sup>14</sup> )
methyl) was applied on	to the soil layer at a concentration of 48.5 mg/kg. The sampling intervals were 0,6
	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 605 % 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	s (Rate constant $K = 1.78 \times 10^{-1}$ day <sup>-1</sup> ) 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 41	03 was identified as the major photoproduct at the end of the inadiation 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 radiosetivity at any time during the irradiation.
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NTN 30893 (	imidacloprid
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>>M-024040-0 565-604673-01-1	
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	of the radioactivity applied was referenced and 6b5 % of the radioactory applied was referenced and 6b5 % of the reference of the optimizer reference on the information of the optimizer reference on the information of the solution of the applied of the solution of the applied of the solution of the solution of the applied of the solution of the optimizer reference of the optimizer and of the reference of the optimizer and
Please click on the hype	erlink to order a Study Report.



Report:	01.02/14; 1988; M-024286-01-2 Photodegradation of NTN 33893 in water PF3517 M-024286-01-2 EPA Ref.: 161-2, Photodegradation Studies in Water none yes of NTN 33893 in buffer pH 7.0 was investigated with artificial sunfight under study was conducted according to the respective EPA- Gurdelines in compliance
Title:	Photodegradation of NTN 33893 in water
Report No.:	PF3517
Document No.:	<u>M-024286-01-2</u>
Guideline(s):	EPA Ref.: 161-2, Photodegradation
Guideline(s).	Studies in Water
Guideline deviation(s):	none A S 2 2
Guideline deviation(s). GLP/GEP:	
GLP/GEP:	yes & y y y y
< <m-024286-01-2@s-603010-01-1< td=""><td></td></m-024286-01-2@s-603010-01-1<>	
The photodegradation	of NTN 33893 in buffer pH 7.0 was investigated with artificial sunfight under
sterile conditions. The	study was conducted according to the respective EPA- Guidelines in compliance
with the current GLP r	requirements
[Duridinul 14a mathul]	INTN 33893 was used. Buring the irradiation period of 2 hours the radioactivity
	IN THE SSOSS was used. During the intrutiation period of 2 figure the faultoactivity
balance was $100.2 \pm 1$ .	.9% of the amount at zero time.
At a concentration of 5	5.4 mg/l and a temperature of 23-24.5. CNTN 33893 was degraded rapidly with a
nali-life of 57 min. The	e corresponding rate constant was 0,972 mbg-1. The environmental nait-me was
calculated to be 4.2 ho	urs.
Under natural sunlight	urs. 60 % of the compound were degraded after thours of the major photoproducts
Onder natural sunnght	
A longo usunh on of ah o	town hot of difference it is the state it to an a former of the two of the state of
A large number of pho	stoproducts of different light stability was formed. Two of the major photoproducts
were identical with the	reference substances NTN 33519 and NTN 38014. Both photoproducts were
stable under the condit	ions of the experiment After 220 mm of irradiation they represented 9.8 and 17.2
% of the radioactivity	according to KIPLC.
>>M-024286-01-2@S-603010-01-1	
S.	
Report:	$\sim 01.02/15$ $\sim 1000$ $\sim 100$
Title:	Join 22/13, 19, 19, 19, 19, 19, 19, 19, 19, 19, 19
	<ul> <li>01.02/15; 2002; M-039425-01-2</li> <li>Invitacloprid - Small scale prospective ground-water monitoring study, Montcalm County, Michigan, 1996</li> <li>M-009425-01-2</li> <li>M-009425-01-2</li> <li></li> </ul>
Report No	¥10772
Document No.:	$\mathcal{O}^{1107,05}_{2}$ $\mathcal{O}^{2}$ $\mathcal{O}^{2}$ $\mathcal{O}^{2}$ $\mathcal{O}^{2}$
Guideline(s):	$\frac{1}{\sqrt{2}} \frac{1}{\sqrt{2}} \frac{1}{\sqrt{2}$
Guideline deviation(3).	
CL D/CED:	
	yes of the
GLP/GEP:	tive pround-water pronitoring study was conducted approximately 3 miles north-
A small-scale prospect	live ground, water chonitoring survey was conducted approximately 3 miles north-
northwest of Vestaburg	g. Michigan, The Test Site consisted of an approximately 3-acre Test Plot and a
0.5-acre©ontrol Plot.	Surficial soils (0-6 inches) were consistent across the study site, and consisted of
loamy sand with appro	ximately 82% sand 12% silt, 6% clay and 1.0% organic matter, pH 5.8, cation
exchange capacity of 3	ximately 82% sand 12% silt, 6% clay and 1.0% organic matter, pH 5.8, cation 3.5 meq/100 g, and bulk density of 1.5 g/cc. Deeper soils consisted of sand with
lavers of loamvænd	and a few discontinuous layers of sandy loam. Individual soils from the deeper
denthe contained mark	than 1% send, less than 22% silt, less than 17% clay, and approximately 0.1%
	at 6.02 inches). The average pH across the site increased with soil depth and ranged
trom 6.5 to 8.6. Depth	towater was 16-18 feet below ground surface (bgs) at the time of well installation.
Imidacioprid was appl	ied to the Test Plot as an in-furrow application of Admire 2F on May31, 1996, at a
target rate of 0.34 lb in	nicacloprid per acre (110% of the label rate of 0.31 lb a.i. per acre for potatoes;
	.BLD, dated 12/05/95). Application verification containers containing soil
	on was made at 105% of the label rate. Potassium bromide was applied on the same
	50 lb/acre as a tracer of water movement

day as a 50% band at 50 lb/acre as a tracer of water movement.

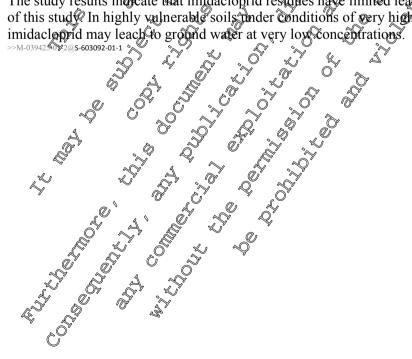


The Test Plot contained six instrument clusters, each containing suction lysimeters at 3.5, 6, 9, and 12 feet bgs, for monitoring soil-pore water. Each cluster also contained a shallow well screened to intercept the water table, and a deep well placed to allow sampling five feet below the shallow well, 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  $\mu$ g/L. The Method Detection Limits (MDLs) in softranged from 0.02  $\mu$ g/L to 0.04  $\mu$ g/L, depending on the analyte. In water, the MDLs ranged from 0.02  $\mu$ g/L to 0.04  $\mu$ g/L, depending on the analyte and matrix (soil-pore water or ground water). Browned was quantified in soil with an LOQ of 0.1 mg/kg, and in water with an LOQ of 0.2  $\mu$ g/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 3 k days of soil sampling with a half-life of 7 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 coll-port water samples, with maximum concentrations of 5  $\psi$  µg/L at 3.5 feet (474 DAT), 1.3 cp/L at 6 feet (1201 DAT), 0.5 µg/L at 9 feet (1201 DAT), and 1.3 µg/L at 12 feet (1201 DAT). The mean soil-pure 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. Groundwater concentrations 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.





Report:	01.02/16;	
Title:	Imidacloprid prospective groundwater monitoring study Monterey County, California	
Report No.:	110889	
	<u>M-107157-01-2</u>	
	EPA Ref.: 166-1, Small-Scale Prospective Ground Water Monitoring	
Guideline deviation(s):	none	
GLP/GEP:	Imidacloprid prospective groundwater monitoring study Monterey County, California 110889 <u>M-107157-01-2</u> EPA Ref.: 166-1, Small-Scale Prospective Ground Water Monitoring none yes	
< <m-107157-01-2@s-602557-01-1< td=""><td></td></m-107157-01-2@s-602557-01-1<>		
A small-scale prospectiv	re groundwater monitoring study was conducted approximately 11 miles	
southwest of Salinas in N	Monterey County, California. The test site consisted of a 4 acre test plot and $a^{O}$	
acre control plot. The su	rficial soils 0 to 6 inches below ground sugace (bgs) were relatively uniform	
	l consisted of sandy loam with approximately 58% sard, 29% silt, 13% day, and	
0.9% organic matter. De	eper soils also consisted of sandy loangand 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 por	rtion of the test site between 28-34 feet bes. Depth to water was 19-24 feet hes at	
the time of well installati	ion.	
Imidacloprid was applied	d to the test plot as an in-furtow application of Admire 20 on Lavy 9, 1996, at a	
target rate of 0.45 lb imi	dacloprid per acre 120% of the label rate of 0.375 lb an. per acre for broccoli).	
Potassium bromide was	applied on the same day in a 50% band at 5008/acre	
The treated plot containe	ed six Susters of suction lysimeters for monitoring soil-pore water. The suction	
lysimeters were installed	l at a depth of 3.5 6, 9, and 12 feet bgs. An additional slit trench lysimeter duster	
was installed on the treat	ecoplot with 5-toot screens which sampled at 1-4 80t, 4.8 8.6 ft, 8.6-12.4 ft, and	
12 4-16 2 ft bgs Twenty	groupdwater monitoring wells were installed on the treated plot. Eight well	
	ow well screened to intercept the water Table and a medium depth well placed	
	Four well dusters contained a shallow and medium depth well plus an additional	
	andwater level dropped severely due to regronal use. A single instrument duster	
	and wells (skallow and medium) was installed in the Control Plot (Cluster 7).	
Shallow soil somples	24 inches bgs) were collected a f 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 45-year period (1,654 days).	
Soil was analyzed for im	nidacloprid and it degradates, unidacloprid guanidine, imidacloprid guanidine	
olefin and imidaeloprid	threa using high performance liquid chromatography. The Limit of Quantitation	
	s 0.0 mg/kg. The Method Detection Limit (MDL) for imidacloprid,	
imidacloprid quanidine	imitaclonitid guardine thefin, and imidaclonrid urea in soil was 0.005, 0.003	
0.002 and $0.001$ mg/kg	imidaclopfid guan dine ofefin, and imidacloprid urea in soil was 0.005, 0.003, respectively. The average concentration of imidacloprid in top soil (0-24 inches)	
decreased from 0 945 m	g/kg to 0.248@ng/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-bore water was anal	Wzed for imidaclopricand its degradates, imidacloprid guanidine, imidacloprid	
	idacloprid area using high performance liquid chromatography and electrospray	
ionization tandem mass	spectrometry (LC-ESVMS/MS). The LOQ for the mass spectrometry method was	
0.05 ug/L. The method d	effection limit for imidacloprid and metabolites in soil-pore water ranged from	
0.02 µg/I@o 0.05 µg/L	Limited imidacloprid residues were detected in soil-pore water. A maximum	
concentration of 0.26 yg/L was found 666 days after treatment in a 9-foot lysimeter. Imidacloprid-		
guanidine, increquently detected in soil-pore water, had a maximum concentration of 0.77 $\mu$ g/L 182 days		
after treatment. Imidacloprid-olefin was also infrequently detected, with a maximum concentration of		
$0.05 \ \mu g/\Omega$ found 31 and 756 days after treatment. Imidacloprid-urea detections in the lysimeters were		
infrequent with a maximum concentration of 0.75 $\mu$ g/L found 182 days after treatment.		



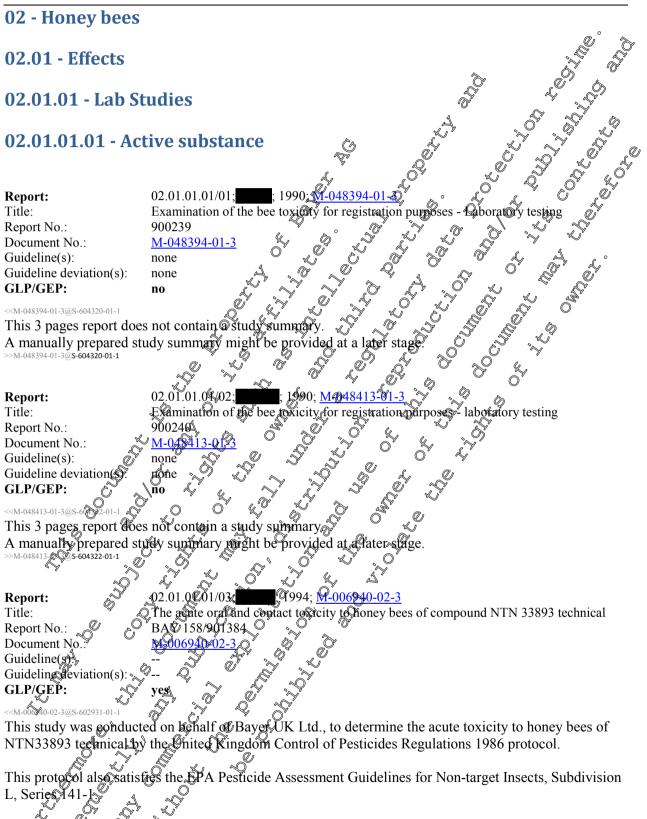
Groundwater was analyzed for imidacloprid and its degradates, imidacloprid guanidine, imidacloprid guanidine olefin, and imidacloprid urea using high performance liquid chromatography and electrospray ionization tandem mass spectrometry (LC-ESVMS/MS). The LOQ for the mass spectrometry method was 0.05 µg/L. The method detection limit for imidacloprid and metabolites in ground water ranged from 0.04 ug/L to 0.06 ug/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 electrole 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/k. The QIDL 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 (22).6 feely was

s be specified of the s A careful review of the water balance during the study shows that inidae loprid residues are that mobile. A total of 18 feet of excess water (total water applied minus crop evaportanspiration) was applied to the test plot following the chemical treatment. After the application of 18 feet of excess water initiaclosfid was not found in any of the 3.5-foot lysingers. The fact that be mide proved quickly through the soil profile while imidacloprid residues did not is a reflection of the tack of mobility of the insecticide in the sandy (vulnerable) soil profile. The study results indicate that midas oprid residues have dittle of no leaching







Prehiminary 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  $0.0015 - 0.025 \mu g$ /bee for the oral route and  $0.025 - 0.40 \mu g$ /bee for the contact route.



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The 48-hour LD<sub>50</sub>S with 95% confidence limits were found to be:

Oral LD<sub>50</sub> 0.0037  $\mu$ g/bee (limits 0.0026 - 0.0053) Contact LD<sub>50</sub> 0.081  $\mu$ g/bee (limits 0.055 - 0.12) It is concluded that NTN.33893 technical is highly toxic to bees by both oral and contact routes  $\mu$ 

Report:	02.01.01/04; 1999; <u>M-016792-01-4</u>
Title:	Honeybee (Apis mellifera L.) grat toxicity study in the laboratory with mida@prid
	techn.
Report No.:	$AH99.4.22.4$ $\sim$
Document No.:	<u>M-016792-01-4</u>
Guideline(s):	US EPA OCSPP Guide me Nor 850.SUPP
Guideline deviation(s):	
GLP/GEP:	yes y y y y y y y y y y

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

The purpose of the toxicity study was to examine the effects of intrdaclopfid techn. on honeybees when applied in the laboratory. Per concentration 10 honeybees were fed with 100 upsucrose 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 \$2050 was between 5 and 20 ng / howeybee, That is why the concentration range of about 4 ng to 20 mg Imidaclopud techn./ honeybee was tested.

Õ Per concentration 10 koneybers were fed with 100 µl sucrose solution 50% containing respectively: 21.22 ng imidacloprid techn., 1678 ng midacloprid techn., 1218 ng midacloprid techn, 8.63 ng imidacloprid techn. and 4.09 ng imida Oprid techn. per 10 pl. Ň 1 The treatment was compared to a 50% sucrose-solution (negative control) and a Dimethoate positive control. Õ

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The concentrations of initiactoprid 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 honeybess are motionless except for Mittle trembling of body parts like abdomen, antennae or tarsus. Some hone, bees, which had taken in about 20 ng, slowed spasms and were paralysed. As there are no data on mortality, the  $LD_{50}$  imige clopric techn. could not be determinated. The lethal concentration as more than at ng bee

The ED<sub>50</sub> of imidacloprid techn offer 24 hours calculated with the linear regression is 34 ng / honeybee.  $(r_2 = 0.500)$ 

The data on effect vary a lot but the effect is clean Amounts of 4.39 ng / honeybee or less do not result in

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 6.85 ng initial apprid techn. or higher result more or less in the described frozen behaviour.



Report:	02.01.01/05; <b>M-017133-01-4</b>
Title:	Honey bee (Apis mellifera L.) contact toxicity study in the laboratory with imide formation
1110.	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 A S S S
GLP/GEP:	ves
	Honey bee (Apis mellifera L.) contact toxicity study in the laboratory with imidacloprid technical AH99.4.22.3 <u>M-017133-01-4</u> US EPA OCSPP Guideline no. 850.SUPP none <b>yes</b> city study was to examine the effects of imidacloprid techn, on honeybees when y. Individual honeybees were exposed to imidacloprid techn, by way of cloprid techn., dissolved in actione, on the ventral part of the thorax that the contact LD <sub>50</sub> was between 40 and 200 ng / honeybee. That is why a about 40 ng to 200 ng imidacloprid honeybee was tested. //bees were treated with 1/µl actione containing respectively, 207 ng imidacloprid prid techn., 125 ng imidacloprid techn, 85 ng imidacloprid techn, and 42 ng 1 µl.
< <m-017133-01-4@s-602498-01-1< td=""><td></td></m-017133-01-4@s-602498-01-1<>	
The purpose of the toxic	city study was to examine the effects of imidagloprid technic on noneybees when
applied in the laborator	y. Individual honeybees were exposed to indidacloprid teem. by way or
administration of imida	cloprid techn., dissolved in acetone, on the ventral part of the borax
The sponsor indicated t	hat the contact LD <sub>50</sub> was between 40 and 200 ng / honeybee. That is why a
concentration range of a	about 40 ng to 200 ng imidacloprid@hone@ee was tested?
Per concentration honey	bees were treated with Vul acetone containing respectively 207 ng imidacloprid
techn 166 ng imidaclo	prid techn 125 ng ingidacloggid techn 85 ng imidacloprid techn and 49 ng
imidacloprid techn per	
The treatment was com	pared to an accetone treatment (negative control) and Dimethoate positive
control	pared to adjectione treadment (acguirvectonitio)) and PDimphoate positive
control.	
The concentrations init	lacloprid techn. administered to the honeybees in this test caused mortality of the
The concentrations imit	actoprid techn. additinistened to one noneybees on this test caused mortality of the
honeybees. Mortality w	as preceded by effect. The most significant effect was the "trozen behaviour" at
which the honeybees ar	e motionless except for a little trembling of body parts like abdomen, antennae or
	effect were observed within 30 minutes after administration of imidacloprid
	ied during the observation period.
The LD <sub>50</sub> of Imida lopr	i Diechn? based on the linear regression is:
LD <sub>50</sub> (72 hours)	129 ng imidacloprid techn $4r^2 = 0.42$
The ED <sub>50</sub> , of lonidaclop	rid techn. based on the linear regression is:
$FD_{co}$ (72 hours):	13 I ng imidaclanrid techn $(r \approx 0.38)$
The effect of imidacion	rid techn, administered in the concentrations from 40 to 200 ng / honeybee is
clear. The typical "from	en behaviour" is observed in all concentrations tested. Mortality continued during
	use honeybees that are immobilitised for several days eventually die, after 72 hours
the I Dro and the PDro a	re in the some songe .
>>M-017133-01-4@ <b>S-602498-01-1</b>	$re$ in the same songe $\sqrt{2}$
Û, U	
~Q^	
Report:	02.01.0001/06; (20099); M-016942-01-4
Title:	Laboratory testing for poxicity (acute oral LD50) of NTN 33893 on honey bees (Apis
	mellifera 🔊 (Hymenopter@Apidae)
Report No.:	6400036
Document No.:	$\frac{1}{2} \frac{1}{2} \frac{1}{2} \frac{1}{4} \frac{1}$
Guideline(s):	
Guideline deviation(s):	
GLP/GEP:	Kes KI NO
< <m-016942-01 0="" s-1<="" s-602486="" td=""><td></td></m-016942-01>	
Material and methods	test substance: NTN 33893, purity: 99.4%, batch number: M00680; under
laboratory conditions's	tarved honey bees (Apis mellifera, 3 groups of 10 bees per dose) received a single
	22.9, 12.2, 6.0, 3.1, 1.5, 0.8 or 0.1 ng per bee in ca. 20 mg sugar solution.

oral dose operatory of the factory bess (*Apis methylera*, 5 groups of 10 bees per dose ) received a single oral dose operatory of 20.9, 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  $\mu$ g dimethoate per bee) caused a 100 % mortality (the facility-specific LD<sub>50</sub> dose for dimethoate is typically between 0.10 and 0.14  $\mu$ g/bee).



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Issue date 2017-11-22

#### Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	NTN 33893	5
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 -		
LD <sub>50</sub> ng product/bee (48 and 96h)	approximately 40.9 %	° Ø

\* 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 of behavioural impacts were recorded at oral doses of 1.5 ng/bee and lower. Orak 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.

Report:
Title: Substance A Acute Contact toxicity to honey bees (Apis mellifera)
Report No.: Report No.: H H H H H H H H H H H H H H H H H H H
Document No: $M-068009-01-3$
Guideline S. OUS ERA OCSUP Guideline No 850 SCPP
Guideline deviation(s); on pecified
$\mathbf{GLP}/\mathbf{GEP}: \qquad \mathbf{O}  \mathbf{ne}  ne$

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, OPPAS 8502020 Honey Bee Acute Contact Toxicity) and OECD guideline 214 Honeybers, 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  $\mu$ l drops containing 140, 110, 78, 56, or 40 ng Substance A /been acetone. Mortality and sub-lethal effects were assessed at 4, 24 and 48 bours after dosing. Résults indicated that the 24-hour contact LD<sub>50</sub> of Substance A is greater than 140 ng bee boy 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



Report:	02.01.01.01/08;				
Title:	Substance A - Acute	oral toxicity to hon	ey bee Apis mellife	era	ş Ö
Report No.:	HT0400b			Ň	ð
Document No.:	<u>M-067996-01-3</u>			ð d'	\$
Guideline(s):	US EPA OCSPP Gui	deline no.: 850.SU	PP 🧳	Ş Ç.	-Q
Guideline deviation(s):	none		-U 4		1 <sup>°</sup> ka
GLP/GEP:	no		L.	, O' , QY	L.
< <m-067996-01-3@s-602702-01-1< td=""><td></td><td>Ś</td><td>, S</td><td>era</td><td>as a</td></m-067996-01-3@s-602702-01-1<>		Ś	, S	era	as a
Tests were carried out to	o determine the acute	e oral toxicity of S	Substance A to ac	luit norrey bees (Apr	S 🔬
<i>mellifera</i> L.). The proto	col followed the EPH	O guidelinges (19	92) and OECD gi	indefine 2 B Honeyt	bees
Acute Oral Toxicity Tes		All doses and to	xicitx data for the	test substance refer	to©
Substance A as the activ	ve ingredient.	DO '		Ŷ,ŎŸ Ø	Ű <sup>Y</sup>
			O N O		ÿ
Three batches of bees, i	n groups of 10 bees,	were offered the	equivalent doses	of <b>3</b> .6, 24.6, 8.2, 2.	8, 0.94
ng /bee Substance A in	50% w/v aqueous su	crose solution th	e test substance h	aving first been diss	otyed
in acetone. At the highe	st treatment level the	mean dose consi	umed was 45 ng	bee Substance A, 39	🅬 less
than the actual dose offe					
bees observed as knock	ed down at 4 bos, the	bees were on the	ir feet but immob	ile and therefore un	able to
feed.				F. S. Q	
	Q .				
Mortality was assessed	at 4 hours afterdosir	ngoGlass test feed	lers were theoren	noved and further	
assessments made at 24	and 48 hours after r	smoval of the glas	ss test feeders. Re	sults indicated that	the 24-
hour and 18 hour oral I	D. of Substance	is granter than 15	ng hee Signific	nt sub lathal affacts	s (50-
100% knockdown) were	e observed at 4 hos in	the highest two	doses but only 10	% Knockdown was	<b>`</b>
observed in the highest,	dose at 24 hrs		" (L. "		
			Ó & ž	¥	
>>M-067996-01-3@S-602702-01-1		N N	<i>a</i> , o ~~		
S.					
Report:	02.01.01.01.09;	; 2000; M-068023			
Title:	Ague toxicity of sub	stanceA to the hon	expee And mellife	ra L. under laboratory	
čo Čo	conditions A				
Report No	> 00 10 AS 0501				
Document No.:	<u>M-068023-01-3</u>	, , , Ox ~~	×O'		
Guideline(s):		/170(2) (1999); OE		ECD 214 (1998)	
S'	US EP COCSPP Gui	defone no \$30.SUP	Р		
Guideline deviation(s): GLP/GEP: CM-068023-01-3@S-602709-01-1 Results: The testendpoints were	Thot specified	Y & S			
GLP/GEP:	<sup>no</sup> v v v				
< <m-068023-01-3@s-602709-01-1< td=""><td>or a g</td><td></td><td></td><td></td><td></td></m-068023-01-3@s-602709-01-1<>	or a g				
Results:	x 2 0 1	× . V			
The test endpoints were	mortality and behave	four of the honey	bees in comparise	on with the control.	
Contact exposure to sub	stance A coused the	following mortal	ities:		
Contact exposure to sub	Nº Nº Nº	Õ			
		tality/Corrected mortal		(%)	
Substance A right	re S Ath	48	72	96	
E J					
Control	2 3.3/- 76.6/75.9	3.3/- 80.0/79.3	6.7/- 80.0/78.6	6.7/- 80.0/78.6	
109.80	60.0/58.6	73.3/72.4	73.3/71.4	80.0/78.6	
X 78 0 5 5	26.7/24.2	50.0/48.3	50.0/46.4	56.6/53.6	
	23.3/20.7	30.0/27.6	30.0/25.0	36.6/32.2	
CLD <sub>50</sub> (contact) ng/b	30.0/27.6 pee 97.7	33.3/31.1 74.9	33.3/28.6	36.6/32.2 69.0	
Confidence limits	511	140	/0.4		
lower	79.08	61.77	64.70	56.06	
upper Slope h	120.73	90.90	94.99	85.0	
Slope b	2.56	2.63	2.75	2.6	
Please click on the hype	erlink to order a Stud	ly Report.			



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<sub>50</sub> 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 ware observed after up to 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 %, 10 %, 20.0 %, 3.3 % and 6.7 % mortality after 48 h.

Therefore it is concluded that the LD<sub>50</sub> (48 h) is 74.9 ag substance A per bee in the contact toxicity test and slightly higher as the highest provided dose of \$1 (70% consomed) ng test substance A per bee in the oral toxicity test.

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

The validity criterion - mortality in the control  $\leq 10$  % - 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:** Title:

02.01.01.00 10; Report amendment no. 1 to study S11-04962 - Imidacloprid (tech.) - Assessment of chronic effects to the honey bee, Apis mellifeer L., in a 10 days continuous laboratory feeding test

Report No .: Sk1-01962 Document No: Guideline(s). (1)Guideline deviation(s); **GLP/GEP:** 

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

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

Over a period of 10 days, hopey bees were exposed to 50 % (w/v) aqueous sucrose feeding solution, containing noninally, 10, 26, 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 unweated 50 % ( v) aqueous sucrose feeding solution. Mortality, sublethal effects and behaviour of the low severation was 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

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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  $\mu$ g 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  $\mu$ g 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 (d)0), the bees in the highest test item treatment group of 100  $\mu$ g a.i.d. 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  $\mu$ ga.i./L during the entity test period, espectively (day-byday comparison). In the lowest test item treatment group of 10  $\mu$ g a.i.Q., 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 sucross feeding solution ( $\therefore$  average value over 10 days) was 47.1, 37.7, 39.8 and 33.3 mg/bee in the test itera treatment groups of 0, 2000 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/bec).

After 10 days of continuous exposure, the accumulated nominal intake of the test item imidacloprid (tech.) via imidacloprid  $\tau$  treated success solution vas 0.00997, 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 tear item accumulated over all test days and cumulative mortality at the final assessment of day 10

		' 0 <sup>7</sup>			
	Treatment Level	<u> </u>	∕∽ Test	Item	
4	Treatment Level Control	√ 10 <sup>∞</sup>	مْ <sup>م</sup> 20	50	100
			, [µg a	a.i./L]	
	Overall mean daily consumption of aqueous sucrose feeding polution [ntg/bee] <sup>2</sup> Mean intake accumulated over test days [µg-a i./bee]	<sup>2</sup> 7 27 27 27 27 27 27 27 27 27 2	37.7*	39.8*	33.3*
٤)	Mean intake accumulated over test days [µg-a i./bea]	90.00397	0.00638	0.01674	0.02820
K) Y	Cumulative of 2.67	4.00	0.00	1.00	4.00 <sup>3</sup>
	Corrected cumulative	1.37	-2.74	-1.72	1.37

<sup>1</sup> The control group was fed with untreated 50 % (w/v) aqueous sucrose feeding solution; the test item weatmend group was fed with imidacloprid-treated 50 % (w/v) aqueous sucrose feeding solution

The near values per sole 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

<sup>3</sup> Determined to be the NOEC based on mortality (not significantly different compared to the control;

Risher's Exact Test (Bonferroni-Holms corrected, one-sided, p ≤ 0.05))

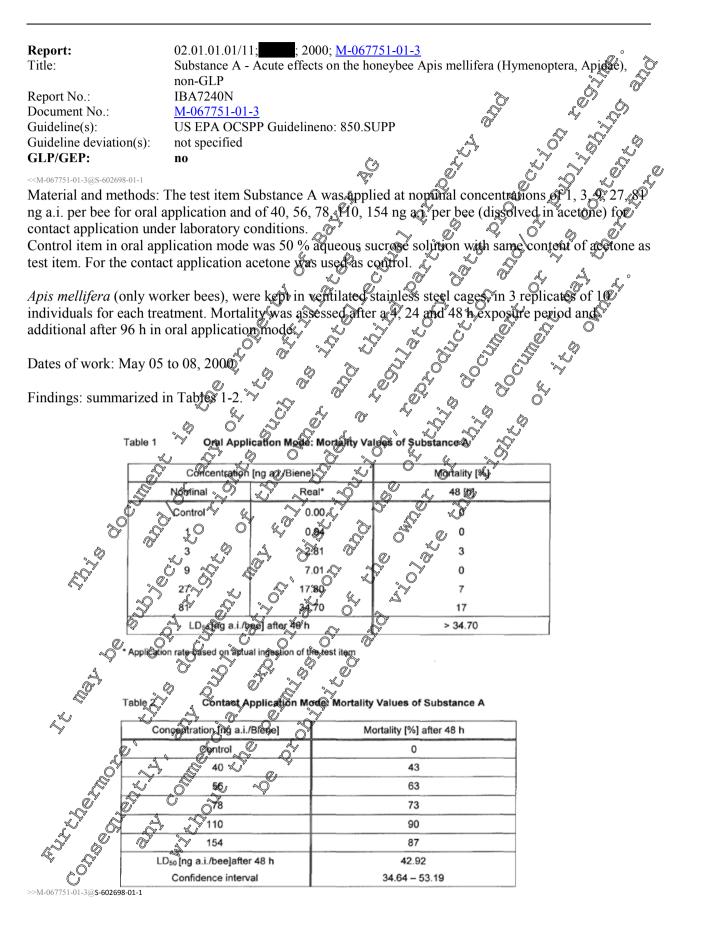
 $\propto \frac{1}{2}$  ood consumption significantly lower compared to the control group (Bonferroni-U test; one-sided, p  $\leq$  0.05)



#### Conclusions

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of  $10^{10}$ consecutive days with the test item imidacloprid (tech.) at the treatment levels of 10, 20, 50 and 100  $\mu g$ 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 verscalm 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 20230 and 100 we a.i./L resulted in a statistically In recently of madacioprid (tech.) at the dose rates of 20,500 and 100 ugai.) Lesslity in substitution of a substitution 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







Report:	02.01.01.01/12; 2011; M-41461 Imidacloprid tech.: Effects of exposure carnica) in an in vitro laboratory testing	9-02-4	<b>°</b>
Title:	Imidacloprid tech.: Effects of exposure	to spiked diet on honey	bee larvae (Apis mellifer
	carnica) in an in vitro laboratory testing	design	
Report No.:	E 318 4110-8	~	
Document No.:	<u>M-414619-02-4</u>	Ĩ	
Guideline(s):	US EPA OCSPP guideline # 850.SUPP	10,	
Guideline deviation(s):	none	A	
GLP/GEP:	yes (**)	Å.	
< <m-414619-02-4@8-604959-01-1< td=""><td>The second se</td><td>Ű</td><td></td></m-414619-02-4@8-604959-01-1<>	The second se	Ű	

Material and methods: Test item: Imidacloprid, tech. (Developmer Ocode: NTN 33893; FOX-No.: 09352-00; Specification No.: 102000006766; Batch Code: AE F106464-01-44; LAMS No.: 1109587; C content of a.s. (analysed): 99.4% w/w). Principle of the testing proceedure. At day +1 thirst instar bee larvae (Apis mellifera carnica) were transferred from their beconve into an artificial in vitro testing system. The bee larvae were fed with standardised amounts of untreated artificial thet at day +1 and day +3. On day +4, +5 and +6, the bee larvae in the test titem treatment groups were fed with standardised amounts of test item spiked artificial exposure die On day +4, the beg larvag in the reference item  $Q^{2}$ treatment group were fed with standardised amounts of reference iters spiked artificial exposure thet. Concurrently, the bee larvae in the control group (or day +47+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 pominal test concentrations of 5, 10, 20 and 40 µg as kg diet. The actual concentration of imidacloprid in the test item spiked exposure diet was determined according of Modification M002 to analytical method 00537 (MRby using High Performance Liquit Chromatography, coupled with 06/144, 2006-11-02, R. tandem mass spectrometry,

(\*) Day 0 was the anticipated day of arval hatching

Ó During the development of the honeybee larvae, the larvae were inertbated at about +35 °C. From day +1 to +8, the relative humber inside the incubator was on average about  $95\pm5\%$  and from day +8 to +22 the mean relative hypothity was about  $80 \pm 5\%$ . Mortality was determined on day +5, +6, +7, +8, +11, +13, +15 and +22. Dead test animals were disparded for sanitary reasons.

# Dates of experimental work: May 04, 2001 - Jul 27, 2011

Results: In total Bur 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 (AUPINELset 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. mortQity 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 mformation due to elevated mortality levels.

An In or An



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

Validity criteria	Origin of validity criteria	Validity threshold			d results		
			Test	Test	S Test	A Test v Tur v T	1
			run	i run	IUII .(		ิส
			No. 1	ر No. 2	No. 3	, MO. 4 , *	1
Mortality in the control group until day +7	INRA - method for testing pesticide toxicity to honeybee	≤ 15%	2,2%	No. 2 2 7	No. 3	× rìth Nộc. 4 - 2 2 3 2 3 3 4 2 3 4 4 5 4 5 4 5 4 5 4 5 4 5 4 5 4 5 4 5	
Mortality in the reference group until day +7 (Abbott)	brood in laboratory conditions (January 2008)	≥ 50%	er2.2%	\$3.8%	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
Mortality in the control group until day +22	Self-set						
<sup>‡</sup> Actual control pe	rformance at the end c	of the test has r	not met the self-	set validity criter	rion		
	Self-set			Set alidity criter			



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Issue date 2017-11-22

able 2 Control and test item performance and				
Test object	(/	Honeybee I Apis mellifera		a) j <sup>0</sup> 5
	Control	Test iten®	ŕ	<b>Reference</b> item
		idacloprid sp		dimethoate 🤣
	exposure	exposure diet	)	spiked exposure
	diet)			ydiet)
Test concentration		20	40	
[µg a.s./kg diet]				′∜µg a ©/larva∱∕
Те	storun No. 1 a 🖴			
Mortality until day +22 [%]	37 9 47.8	47.8 \$2.6	A1.3	95.7
Abbott-corrected mortality until day +22 [%]	J.0 0 17.20	17.2 -6.9	6.90	093.1 s
	st run No. 2			
Mortality until day +22 [%]	s, \$8.8 √ 16,7	20,80 33,3	8.3	Ø <mark>97.9</mark>
Abbott-corrected mortality until ay +22 [%]		205 10.9	-02.8	۶∛ 97.4
	st run No. 3		ð ö	¥
Mortality until day +22 🙀 O	<b>16,7</b> 46,7	20.0 20.0	<b>16.</b> 7	100.0
Abbott-corrected mortality until day +22 [%	Q.0 036.0	4.0 16.0	0.0	100.0
	st run No. 4		,	
Mortality until day +22.[%] 🞸 🌜 🚿	14.6 20.8	016.7 12.5	22.9	100.0
Abbott-corrected restality until day +22 [%]	Ø 0.0 7 7 8	2@ -2.4	9.8	100.0
Test ouns No.	2, 3 and 4 com	buried <sup>b</sup>		
Mortality until day 2 [%) 🖉	້≫16.7 25 🏈	19.0 24.6	16.7	99.2
Abbott-corrected mortality un day 22 [%]	0.0 30.5	2.9 9.5	0.0	99.0
Statistical comparison to the control o	0 <sup>°</sup> 0 <sup>°</sup> n.s.	n.s. n.s.	n.s.	
NOEC NOEC	¢ 2 40 µg	a.s./kg diet		
		j a.s./kg diet		

#### Table 2 Control and test item performance and associated statistical evaluation

<sup>a</sup> Although control performance metrice validity criteria as stated in the INRA - method for testing pesticide toxicity to hyperbee brood in labor fory conditions Danuary 2008), the self-set validity criterion for control performance at the end of the test (i.e. ≤ 30%) was for metric or distinct differences in larval mortality can be observed at concentrations of up to and including 40 µg midacioprid as 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. Wg diet; Chi<sup>2</sup> Test [Bonferroni-Holms corrected, one-sided, α = 0.05])

<sup>b</sup> 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

Childrest, (Bonferrout) Holms corrected, one-sided,  $\alpha = 0.05$ )

nst mear alue 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:

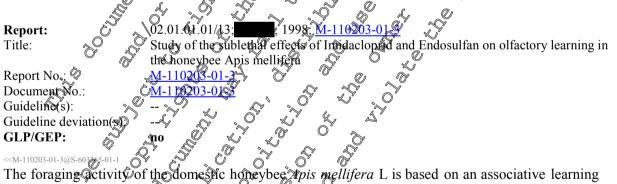


5 μg a.s./kg diet - treatment level: nominal	On average 98 - 105% [test runs 2, 3 and 4 98-105%] of
10 μg a.s./kg diet - treatment level: nominal	On average 103 -110% [test runs 2, 3 and 4 103-110%]
20 µg a.s./kg diet - treatment level: nominal	On average 101 -115% [test runs 2, 3 and 4 101-115%] of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

#### **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% mortably in the control group and > 50% mortality in the reference group), three independent test runs (fest 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 unit 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 or mortably 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, Bonferronf-Holps corrected, one-sided, a = 0.05). The outcome of this statistical evaluation is further support 9 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 arvae study is > 40µg imitacloprid a.s. Arg diet.



The foraging activity of the domestic honeybee *tpis 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 flord food sources. This individual learning process is linked to the gathering of fellow creatures at the beart 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 recorded 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 (22,3;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 onto an angle between the direction of the dance and gravity. The anti-cholinesterase activity of parathron [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.



Finally, apparists blame a new insecticide used to treat sunflower seeds (trade name Gaucho<sup>®</sup>) 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 ô data suggest that the neurotoxic substances may have sublethal effects and, in marticular, 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 an immobilized state. This experimental process was used in our study to evaluate the effects of weak doses of imitacloprid 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. (n

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], achemical similar to DT. Our analysis of behaviour was combined with electrophysiological, analysis aimed at evaluating the concomitant modifications of peripheral olfactory sonsitivity 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]. Noreover, among nucleorous arthropods, measuring receptor potentials has allowed evaluation of the neurobological impact of certain

pyrethroids [18:19]. M-110203-01-3@S-603265-01-1

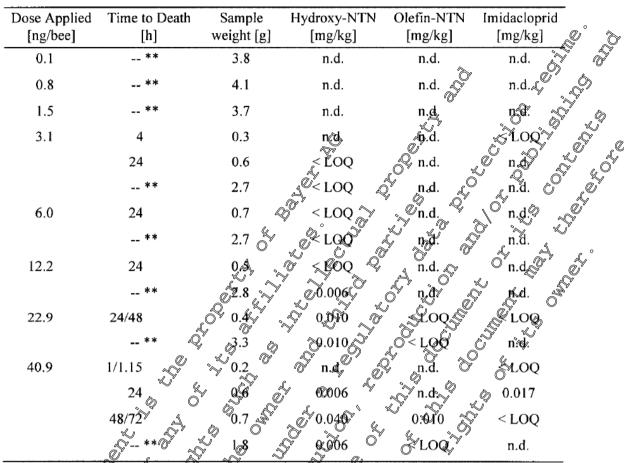
¥999; A-017409-01-4 02.01.01.01/14; **Report:** Residue leveloof imitacloping and initiacloprid metabolites in honeybees orally dosed Title: with mida oprid in standardized toxicity tests (EPPO 170)2 Report No .: SXR/AMO013 Q1-017079-01-4 Document No .: US EPA OPETS: NA (EPPO guideline 170) not specified Guideline deviation(s) Guideline(s): not specified <<M-017079-01655-602138-01-1

Material and methods: test substance specification: imidaclopsed techn., batch no. M00680, purity 99.4%. Adult honey Dees were orally dosed with either 9.0001, 0.0008, 0.0015, 0.0031, 0.006, 0.012, 0.023 or 0.041 µg honeybee imitaclopfied 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 asphy vation and retained also at 220°C till residue analysis. After shipping the honeybee samples to Bayer AG, the were analyzed for residues of imidacloprid and toxicologically relevant metabolites, i.e. olefin and hydroxy-midacloprid

Dates of biological work July 6-10,1999 (IBACON study 6400036). Dates of analysis of biological samples? September 15-17, 1999. Findings: Residues in hones bees orally dosed with imidacloprid techn.:

Please click on the hyperlink to order a Study Report.

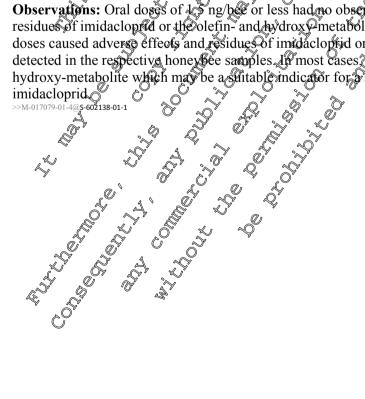




\* Limit of quantitation: 0,005 mg/g (imidacloprid & hydroxy-metabolite, 0.01 mg/kg (olefin-metabolite); n.d. = below limit of detection \$0.0015 mg/kg and 0.003 mg/kg, respectively

\*\* Honeybees were as hyxiated by CO2 at study termination

Ľ Observations: Oral doses of 15 ng/bee or less had no observable adverse effects on honeybees and no residues of imidaclon fd or the olefin- and hydrox metabolite could be detected in those bees. All other doses caused adverse effects and residue of imidacloptid or the olefin and hydroxymetabolite could be detected in the respective honeybee samples IP most cases the highest residue level was found for the hydroxy-metabolite which may be a ditable indicator for a significant exposure of honeybees to





Report:	02.01.01.01/15; 1992; M-008940-01-2 NTN 33893: Toxicity to honey bees on alfalfa treated foliage 103938 M-008940-01-2 FIFRA Guideline 141-2 Hazard Evaluation: Nontarget Insects none yes TN 33893 240FS E UNIVERSITY PROJECT NO; 92-004 oney Bees Toxicity of Residues of Foliago N 33893 240FS (0.045 lb(AI)/acre) era L., order Hymenoptera. ith 2 hour old tesidues was 5-6; with 8 hour old residues T2 and with 24 hour old N 33893 240FS (0.167 (b(AI)/acre) era L., order Hymenoptera.
Title:	NTN 33893: Toxicity to honey bees on alfalfa treated foliage
Report No.:	103938
Document No.:	<u>M-008940-01-2</u>
Guideline(s):	FIFRA Guideline 141-2
	Hazard Evaluation: Nontarget Insects
Guideline deviation(s):	none A O <sup>V</sup> A
GLP/GEP:	yes
< <m-008940-01-2@s-602467-01-1< td=""><td></td></m-008940-01-2@s-602467-01-1<>	
TEST SUBSTANCE: N	TN 33893 240FS
	TN 33893 240FS E UNIVERSITY PROJECT NO; 92-000 oney Bees Toxicity of Residues on Foliago N 33893 240PS (0,045 lb(Al)/acros) era L., ordof Hymenoptera.
WASHINGTON STATI	E UNIVERSITY PROJECT NO; 92-000 🖓 🔊 👦 🔬 🔊
STUDY NTN 33893/H	oney Bees Toxicity of Residues of Foliage
DESLUTS.	N 33893 240PS (0.045 lb(Al)/acres era L., order Hymenoptera. ith 2 hour old residues was 5.6, with 8 hour old residues T2 and with 24 hour old
RESULTS.	
Residue bioassay of NT	N 33893 2404 S (0,045 10, A1)/acres 2 5 5 5
Bioassay on Apis mellif	era L., order Hymenoptera.
The percent mortality w	ith 2 hour old residues was 5.6, with 8 hour old residues D2 and with 24 hour old
residues 11.9.	
Residue bioassay of NT	N \$3893 240FS (0.167 (b) (Al) (acre)
	era L., order Hymenoptera
	era L., order Hymenoptera. th 2 bour old residues was 11.7, with 8 bour old residues 16.1 and with 24 hour
	ith 2 pour oto residues was 11.7. With 8 bour of residues 16.1 and with 24 hour
old residues 15.9.	
Residue bioassay of NE	N 33893 240ES (0.5)6(AI) acre)
Bioassay on Apris menif	era, P., order Hymenoptera.
The percent mortality w	ith 2 hourold residues was 11, with 8 hour old residues 23.1 and with 24 hour
old residues 20.8.	
E <sup>7</sup> O	
CONCLUSION	
NITNI 22802 240EE (0 0	N 33893 240ES (0.5 b)(Al) acre) era D., order Hymenoptera. ith 2 hour old residues was 11%, with 8 hour old residues 23.1 and with 24 hour of 5 lb(31)/acre was non-hazardous to honey bees if applied in early morning or
	so to bai/acted was pon-inavaluous to noney bees in applied in early morning of
late evening when bees a	are not ioraging.
1	
NTN 33893*240FS (0.1	15 lb(Al)/acres was non-hazardous to honey bees if applied in early morning or are bot for aging. 67 lb(AP)/acres was non-hazardous to honey bees if applied in late evening when 10(Al)/acres was moderatly hazardous to honey bees if applied in late evening.
bees are not foraging >>	
NTŃ 33893 240FS (0.5	(Al)/acte) was moderatly hazardous to honey bees if applied in late evening.
TEST DATES	
First Test:	
Experimental Start - 9 S	abtember 1002
Experimental Termination	on Aug September 1992
Second Test	
Experimental Start - 14	
Experimental Termination	on - 16 September 1992
$\lor$	
STUDY COMPLETION	J: 16 September 1992
>>M-008940-01-2@ <b>S-602467-01-1</b>	*
Please click on the hype	rlink to order a Study Report.



Report:	02.01.01.01/16;	; 2000; <u>M-110229-01-3</u>		
Title:	Impact of imidaclopr	id and its main metabolite	es on the honeybe	e Apis mellifera
	effect of chronic expo	osure on mortality and lea	arning	ee Apis mellifera E.: O
Report No.:	<u>M-110229-01-3</u>		ð	
Document No.:	<u>M-110229-01-3</u>		<u>A</u>	
Guideline(s):	not specified			
Guideline deviation(s):	not specified		4	
GLP/GEP:	no	Ĉs	L.	
< <m-110229-01-3@s-604666-01-1< td=""><td></td><td>T</td><td></td><td></td></m-110229-01-3@s-604666-01-1<>		T		

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 toxic bogy tests before products are placed on the market, there is contently no objective way or detecting the sub-left al effects of pesticides on bee behaviour or of evaluating their chronic toxicity

× î During the national study programme carried out in 1998 to evaluate the ffects of Garcho® stufflower seed dressing on bees we studied the chronic toxicity of the active ingredient for 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 a concentrations of 8 and 40 ppb after 11 days ingestion of inidacloprid. Furthermore, after the 1 days administration of concentrations of 4, 8 and 40 pph we observed a significant decline in learning performance compared to untreated individuals when we performed a Pavlovian olfastory conditioning procedure. However, we did not find any concentration-response relationship or any go-effect concentration. It should be noted that the concentrations of imidaeloprid 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 unidacloprid 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 infidactoprid (olefin and hydroxy-infidacloprid) or fearning ability. The acute concentration crested 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.

# **Report:**

Title:

2015; M \$14897-01-3 02 01.01 01/17; Imethy the-14 Imidac oprid: Storage stability in honeybees (Apis mellifera) after oral expositre Ensa-15-0140 Report No.:

1051489701-3 Document No. US EPA OCSPI Guideline(s) Guideline deviation(s): Ô none 🖓 GLP/GEP: no Ľ S. <<M-514897-01-3@S-603093-01-1

The storage stability of parent (meth) fene-1457]Imidacloprid and related residues was investigated in dead honeybees (Apis mellifera) the test compound was orally administered in commercially available sugar syrup (Apiin vert, 50%) at a dose of 40.0 ng a.s. per 20 mg diet, which represents the amount of sugar necessary for one noneybee 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 start ation 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



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 extractive as 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-<sup>14</sup>C]Imidacloprid was found to be the main compound throughout the entre storage period (8 days) and amounted to 0.118 mg/kg at day 0, 0.093 mg/kg at day 1, 0.090 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 finidacloprid and the detected metabolites is given in the following table.

			Ľ,	<sup>1</sup>	$\sim$	ð	A.	0* 5	U	Ś
Storage duration	Da	у 0	Da	¥ 2	C Da	y <sup>2</sup> 2	D Da	y 4 🖉	Qay	y 8 Õ
Residues	% of TRR	mg/kg	0 % of TRR		% of IRR	mgykg	%%) TORR	ng kg	of √TRR.	ng/kg
Parent Imidacloprid	63.3	6,118	√39.4	<b>0</b> 9.093	56.6	0.099	🖌 51.ອີ	0.090	51.7	0.096
Unknown	n.d		n 🏷	n_d.	n.d.	"Na.	<b>©</b> 0.6	8,001	0 <sub>0.4</sub>	0.001
6-Chloronicotinic acid	\$ \$ \$ \$	0.@3	Q <sup>2.3</sup>	0004	S .	la v	2 <b>4.4</b>	~ ~~	r I	0.010
4/5 Hydroxy- Imidacloprid	‰ 10.3						&8.1 O	0.075 4	7.4	0.013
4,5 Dihydroxy-	0 <sup>5</sup> 2	<b>2,92</b> 9 √√ ¢ ) 0	274.3 7 7 7	0.021	~014.1 //	0.024	د 15.9 ک ر	0.030	13.7	0.025
Total analysed	<b>√</b> 91.1	<b>0</b> ,169		0.128	082.9	0.144	80.7	0.150	78.7	0.145
RR: Total Radioactive	Besidue	ST &		, Ö						

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 boneybees. 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 conviuted 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).



*a*,

Issue date 2017-11-22

### 02.01.01.02 - Metabolites

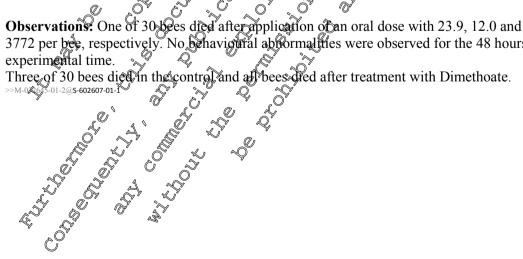
Report:	02.01.01.02/01; (1999; <u>M-032645-01-2</u>
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 3 22 on honey bees (Apis
	mellifera L.) (Hymenoptera, Apidae)
Report No.:	6330036
Document No.:	<u>M-032645-01-2</u>
Guideline(s):	EPPO No.170
Guideline deviation(s):	Temperatur: 29 C; relative humidity 68-70% instead of 25 C + 2 $\bigcirc$ and $\bigcirc$ ative $\bigcirc$
	humidity of 60-70 % as indicated in the guideling where the second secon
GLP/GEP:	yes a start when the
< <m-032645-01-2@s-602607-01-1< td=""><td></td></m-032645-01-2@s-602607-01-1<>	

Material and methods: test substance: WAK 3792, purity: 95%, batch number: MOO 136, under laboratory conditions, starved honey bees (*Aprimellifera*, 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 up per bee in 20 mg sugar solution. Subsequently, honey bees were observed over a period of 48 hrs for behavioutal impairments and survival rate. The reference treatment (0.9 µg dimethoate per bee) caused a 100 % prortality (the facilityspecific LD50 dose for dimethoate is topically between 0.10 and 0.74 µg/bee).

Ŵ	
Test substance	WAK 3772
Test object	6 6 L Apis mellitera
Application rates reg	48.5*, 23.9* 12.0*, 6.0* 3.1* 1.5*, 0, 7* and 0.1*
Application rates rep product/bee	Sugar Solution)
LD <sub>50</sub> ng product/bee (24 and 48h)	$\begin{cases} & & & & & & & \\ & & & & & & & \\ & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & & \\ & & & & & & & & & \\ & & & & & & & & & \\ & & & & & & & & & \\ & & & & & & & & & \\ & & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ & & & & & & $

\* values based on actual infake of the test substance Õ Ô  $\bigcirc$ 

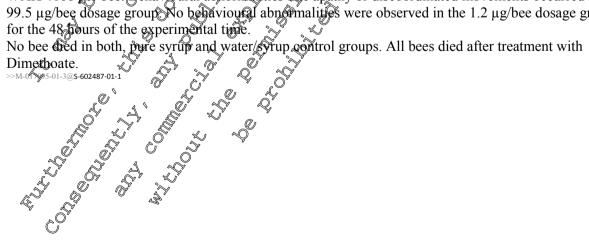
Observations: One of 30 bees died after application of an oral dose with 23.9, 12.0 and 3.1 ng WAK 3772 per bee, respectively. No behavioural abnormalities were observed for the 48 hours of the





Report:	02.01.01.02/02; 1999; M-017095-01-3
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 4168 on honey bees (Asis
	mellifera L.) (Hymenoptera, Apidae) - limit test -
Report No.:	6370036
Document No.:	<u>M-017095-01-3</u>
Guideline(s):	GLP compliant study based on EPPO 170 (1992)
Guideline deviation(s):	
GLP/GEP:	6370036 <u>M-017095-01-3</u> GLP compliant study based on EPPO 170 (1992) 
< <m-017095-01-3@s-602487-01-1< td=""><td></td></m-017095-01-3@s-602487-01-1<>	
Material and methods	: test substance: WAK 4168, purity: 99.0%, Datch number 960118ELBQC, under
laboratory conditions, s	tarved honey bees (Apis mellifera, 3 groups of 10 bees perdose) received a single
oral dose of either 99.5	or 1.2 µg per bee in 20 mg orgar solution. Subsequently, honey bees were
	of 48 hrs for behavioural impairments and survival rate. The reference treatment
	bee) caused a 100 % mortality the facility-specific LD50 dose for dimethoate is
typically between 0.10	and 0.14 µg/bee).
51 5	
Findings: Toxicity to H	Honey Bees, Laberatory Dests 7 2 4 5 2 2
8	and 0.14 µg/bee).
Test substance	WAK 4168 2 6 5 5
Test object	WAK 4168 Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y
Application rates µg	$3^{\circ}$ $3^{\circ}$ $3^{\circ}$ $99.5^{*}$ and $1^{\circ}2^{*}$ $3^{\circ}$ $3^{\circ}$ $3^{\circ}$
product/bee	
Exposure	y g gral y y g (sugar solution) y g
Exposure	(sugar solution)
L Q	
<u>Q'</u>	
$LD_{50} \mu g \text{ product/bee} (2)$	4@nd ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
(48n)	
* values based on actua	1 intake of the test substance
NO NO	
Observations: Obvior	sly the best substance appeared to have a repellent effect in the 99.5 μg/bee dosage
group indicated by the	long period of untake by the best in this dosage group, although bees were

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 (3697 %) bees died after application of an oral dose with 99.5 μg WAK 4168 per bee One of the 300(3.3 %) bees died after application of an oral dose with 1.2 μg WAK 4168 perbee. Behavioural abnormanties 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





Report:	02.01.01.02/03; 1999; <u>M-017098-01-3</u>
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 4140 on honey bees (or is mallifered L) (Humenontera Anidae) Limit test
	mellifera L.) (Hymenoptera, Apidae) - Limit test
Report No.:	6360036
Document No.:	<u>M-017098-01-3</u>
Guideline(s):	EPPO 1992: Guideline on test methods for evaluating the side-effects of plant projection
	products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215 1992, No 770
Guideline deviation(s):	Temperature: 29 °C; relative humidity: 64 - 70 % instead of 25 °C $\neq$ 2 °C $\sim$
GLP/GEP:	yes
< <m-017098-01-3@s-602491-01-1< td=""><td></td></m-017098-01-3@s-602491-01-1<>	

**Material and methods:** test substance: WAK 4146, purity: 97.9%, batch number: 966908ELB01; under laboratory conditions, starved honey bees (*Apis mellifera*, 3 groups of 10 bees per dose) received single oral dose of either 93.2 or 1.2  $\mu$ g per bee in 20 ong sugar solution. Subsequently, honey bees were observed over a period of 48 hrs for behavioural impairments and survival rate. The reference treatment (0.2  $\mu$ g dimethoate per bee) caused a 100 % mortality (the facility specific LD<sub>20</sub> dose for dimethoate is typically between 0.10 and 0.14  $\mu$ g/bee).

Findings: Toxicity to Honey Bees, Jaboratory Tests

Test substance	2 0 5 WAX 4140 0 0 4
Test object	Apis mellifera
Application rates µg 🎺	6 6 93.2* and 1.23 5 5
Exposure	<sup>4</sup> <sup>γ</sup>
LD <sub>50</sub> µg product/bee (24 and 48h)	approximately 93.2
* values based on actual intake of	f the test substance

**Observations:** Obviously the test substance appeared to have a repellent effect in the 93.2  $\mu$ g/bee dosage group indicated by the long period of uptake by the bees in this dosage group, although bees were previously starved for 60 mnutes 16 of 30 (53 3 %) bees died after application of an oral dose with 93.2  $\mu$ g WAK 4P40 per bee. None of the 30 bees died after application of an oral dose with 1.2  $\mu$ g WAK 4140 per bee. Behavioural approximatives (discontinated movement and apathy) of two bees during the 24 hours check occurred after ingestion of 93.2  $\mu$ g/bee. No behavioural abnormalities were observed in the 1.2  $\mu$ g/bee dosage group for the 48 hours of the experimental time.

No bee died in both, pure sympt and water/syrup control groups. All bees died after treatment with

Dimethoate.



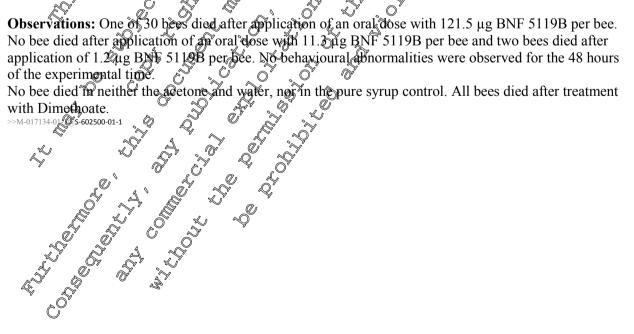
Report:	02.01.01.02/04; ; 1999; <u>M-017134-01-3</u>
Title:	Laboratory testing for toxicity (acute oral LD50) of BNF 5119B on honey bees (Apis
	mellifera L.) (Hymenoptera, Apidae) - Limit test
Report No.:	6380036
Document No.:	<u>M-017134-01-3</u>
Guideline(s):	EPPO 1992: Guideline on test methods for evaluating the side-effects of plant protection
	products on honey bees, Bulletin OEPP/EPPO Bulletin 22/203-215 1992, No. 140
Guideline deviation(s):	Temperature: 29 °C; relative humidity: 64 - 70 % instead of 25 °C $\pm 2^{\circ}$ C
	and relative humidity of 60 -70 % as indicated in the guideline
GLP/GEP:	
	yes a c c c c c c
< <m-017134-01-3@s-602500-01-1< td=""><td></td></m-017134-01-3@s-602500-01-1<>	

Material and methods: test substance: BNF 5119B @urity: 99.6% batch number 870922ELB96; under laboratory conditions, starved honey bees (Apis mellifera, 3 groups of 100 bees per dose) received a single oral dose of either 121.5, 11.3 or 1.2 µg per bee m20 mg sugar solution. Subsequently, honey bees were observed over a period of 48 hrs for behavioural impagements and survival tate. The reference treatment (0.2  $\mu$ g dimethoate per bee) caused a 100 % mortality (the facility pecific LD<sub>50</sub> dose for dimethoate is twoically between 0.10 and 0.14  $\mu$ g/bee). typically between 0.10 and 0.14 µg/bee). 

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	$\left[ Q^{2} \right] = BNES119B^{2}$
Test object	Apis mellifera
Application rates µg 🔊	$\circ$
Exposure LD <sub>50</sub> µg a.i./bee (24 and 48h) * values based on actual intake	Coral O (angar solution) O Congar solution) O
LD <sub>50</sub> µg a.i./bee (24 and 48h)	
* 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.





Report:	02.01.01.02/05; 1999; <u>M-018622-01-4</u>
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 3745 on honey bees (whis)
	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 State of the S
	side-effects of plant protection products on honey bees,
	Bulletin OEPP/EPPO Bulletin 22, 293-215 1992, No. 170
Guideline deviation(s):	none
GLP/GEP:	yes an yes a start with the second seco
< <m-018622-01-4@s-602525-01-1< td=""><td></td></m-018622-01-4@s-602525-01-1<>	
Material and method	s: test substance: WAK 3745, purity: 98%, batch number: M00804; under
	si test substance. Write 5/45, punty, 500, butta numper. Robot gunder
laboratory conditions,	starved honey bees (Apts mellifera, 3 groups of 10 bees per dose) received a single
oral dose of either 35.7	7, 17.9, 10.3, 5.6, 2.4, 19, 0.6 or 0.1 og per bee in så. 20 mg sugar solution.
	ees were observed over a period of 6 hrs for behavioural impartments and and
	was prolonged up to 96 hours because @increasing mortality between 24 and 48

hours. The reference treatment (0.2 µg methodate per bee) caused \$83.3% mortality (the facilityspecific LD<sub>50</sub> dose for dimethoate is optically between 0, 19 and 6 Findings: Toxicity to Honey Bees aboratory Pests Test substance WAK 3745 Test object .6\* and 0.1\* Application rates 5 product/bee Exposure oral gar solution) LD<sub>50</sub> ng product 96h) Ŕ

\* values based on actual intake of the test substance C

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. Qual 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 inspacts such as whathy 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% f the 0 bee (83.3%) died in the groups treated with the toxic standard.



Report:	02.01.01.02/06; 1999; M-018647-01-4
Title:	
	mellifera L.) (Hymenoptera, Apidae)
Report No.:	6340036
Document No.:	<u>M-018647-01-4</u>
Guideline(s):	US EPA OCSPP Guideline no. 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	yes
< <m-018647-01-4@s-602527-01-1< td=""><td>Laboratory testing for toxicity (acute oral LD50) of WAK 4103 on honey bees (Apris mellifera L.) (Hymenoptera, Apidae) 6340036 <u>M-018647-01-4</u> US EPA OCSPP Guideline no. 850.SUPP none yes : test substance: WAK 4103, purity: 99.4%, batch number: 930323ELB05, under tarved honey bees (Apis mellitera, 3 groups of 10 bees per dose) received a single</td></m-018647-01-4@s-602527-01-1<>	Laboratory testing for toxicity (acute oral LD50) of WAK 4103 on honey bees (Apris mellifera L.) (Hymenoptera, Apidae) 6340036 <u>M-018647-01-4</u> US EPA OCSPP Guideline no. 850.SUPP none yes : test substance: WAK 4103, purity: 99.4%, batch number: 930323ELB05, under tarved honey bees (Apis mellitera, 3 groups of 10 bees per dose) received a single
	: test substance: WAK 4103, purity: 99.4%, batch number 930323ELBOS, under
laboratory conditions s	tarved 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.
	es were observed over a period of 96 hps for behavioural inspairments and
	vas prolonged up to 96 bours because of increasing prortality between 24 and 48
survival fate. The test w	vas protonged up to 90 hours because of increasing mortawy between 24 and 48
	eatment (0.2 $\mu$ g dimethoate per bee Ceause Da 83.5% mortality (the facility-
specific $LD_{50}$ dose for c	limethoate is typically between 0,0 and 0.14 µg/bee)
Findings: Toxicity to F	Ioney Bees, Laboratory Tests 2 2 2 2 2 2
Test substance	<u> </u>
Test object	Apis mellifera
Application rates ng	\$59.2* \$1.9* \$9.1* \$9.0*. 10.4*. 4.6* and 2.2*
product/bee	
Exposure	
	<sup>ω</sup> <sub>α</sub> <sup>α</sup> (sugat solution) Ο <sup>2</sup> <sup>α</sup>
LD <sub>50</sub> ng product/bee	96h) approximately 159.2
S O	
* values beed on actu	a) intake of the lest substance
values pased on actu	
"× ">	

Observations: the observation period was extended for 48 hours because of delayed mortality in the highest dose groups. Not mortalities occurred after ingestion of 19.0 ng/bee. Oral doses of 1.2, 4.6, and 10.4 ng/bee caused 3.5% mortality. A mortality rate of 69, 40.0 and 53.3% was found for oral doses of 39.1, 81.9 and 159.2 ng/bec respectively.

doses of 6 ng and higher. The behavioural impacts lasted dose-related up to 24 hours. No behavioural

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/bec. In the control, none of the 30 bees died, whereas 25 of the 30 bees (83.3 %) died of the proups deated with the toxic standard.



Report:	02.01.01.02/07; 1999; <u>M-018470-01-3</u>
Title:	Laboratory testing for toxicity (acute oral LD50) of WAK 3839 on honey bees (or a mallifera L) (Hymenontera Apidae) Limit test
	mellifera L.) (Hymenoptera, Apidae) - Limit test
Report No.:	6390036
Document No.:	<u>M-018470-01-3</u>
Guideline(s):	EPPO 1992: Guideline on test methods for evaluating the side-effects of plant protection
	products on honey bees, Bulletin OEPP/EPPO Bulletin 2, 203-215 1992, No 70
Guideline deviation(s):	$T_{1} = 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1$
GLP/GEP:	yes

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

Material and methods: test substance: WAK 3839, purity: 99 %, batch number. 9504 1ELB02, (test substance was obtained in a 0.1 % ethanol solution; WAK 3839 was extracted by evaporating of the ethanol solution); under laboratory conditions starved noney Dees (Apis mellifera S groups of 10 bees per dose) received a single oral dose of either 21.8, 0.3 or 0.0 ug perfore in 4-19 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 portality between 24 and 48 hours at 0.08 µg/bee. The reference treatment (0.2 µg dimethoate per bee) cause a 100% mortality (the facility-specific LD50 dose for dimethoate is typically between 0.00 and 9.14 us bee)

Findings: Toxicity to Honey Be	es, Laboratory, Pests, WAIK 3839 Apris meltifiera, 200
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Test substance	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Test object	Aps meltiera 2 2
Application rates $\mu g$	
Application rates µg product/bee	(sugar solution)
LD <sub>50</sub> µg bee (48 and 96h)	2 A 67 07 07 5 Ca. 008 7 5 7 4 4 57
* 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 storved 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 gecurred 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 occurred until the end of the experiment.

No ber died in neither the acetone, nor in the pure syrup control. All bees died after treatment with Dimethoate.

>>M-018470-00 s-602521-01-1



Report:         02.01.01.02/08; 2000; M-019352-01-2           Title:         Laboratory testing for toxicity (acute oral LD50) of WAK 5074 on honey bees (pis )
mellifera L.) (Hymenoptera, Apidae) - Limit test
Report No.: 7150036
Document No.: <u>M-019352-01-2</u>
Guideline(s): EPPO No. 170
Guideline deviation(s): none
The: Laboratory testing for toxicity (acute oral LDS0) of WAK 50/4 on honey bees (tepts mellifera L.) (Hymenoptera, Apidae) - Limit test Report No.: 7150036 Document No.: M-019352-01-2 Guideline deviation(s): EPPO No. 170 Guideline deviation(s): none <b>GLP/GEP:</b> yes M-019352-01-2@S-602529-01-1 <b>Material and methods:</b> test substance: WAK 5074, purity: 98%, batch number: BU11377, under laboratory conditions, starved honey bees ( <i>Apis mellifera</i> , 3 groups of 10 bees per dose.) received a single oral dose of either 119.8 or 1.2 $\mu$ g 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 $\mu$ g dimethoate per bee) caused a 100 % mortality (the facility-specific LD <sub>50</sub> dose for dimethoate is typically between 0.10 and 0.14 $\mu$ g/bee).
Material and methods: test substance: WAK 5074, purity: 98%, bach number: BU11377, under
laboratory conditions, starved honey bees (Apis mellera, 3 groups of 10 bees per dose ) received a single
oral dose of either 119.8 or 1.2 µg per bee in ca. 250mg sugar solution. Subsequently, honey bees were
observed over a period of 48 hrs for behavioural impairments and survival rate. The reference treatment
$(0.2 \ \mu g \ dimethoate \ per \ bee)$ caused a 100 % mortality the tacility-specific $D_{50}$ dose for dimethoate is
typically between 0.10 and 0.14 $\mu$ g/bee).
Findings: Loxicity to Honey Bees, Laberatory Vests a v v v v v v v v
Test substance
Test object
Application rates $\mu g$ $\gamma \gamma \sim 119.8$ and $k_2^*$ $\gamma \sim \gamma$
Test substance     WAK 5074       Test object     Apis mellifera       Application rates μg     119.8® and ½*       product/bee     4       Exposure     4       Source     5
Exposure
(sugat solution) (
LD <sub>50</sub> $\mu$ g product/bes (24 and $\gamma \gamma \gamma$
$48h) \qquad \qquad$
* values based on actual intake of the test substance of the cost
Observations: None of 30 best 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@ <b>S-602529-0</b> 37
died in neither the water, nor in the pure syrup control All bees died after treatment with Dimethoate.
died in neither the stater, nor in the pure syrup control All bees died after treatment with Dimethoate.
Ũ
died in neither the weiter, nor in the pure syrup control. All bees died after treatment with Dimethoate.



Report:	02.01.01.02/09; ; 2000	0; <u>M-068030-0</u>	1-3	
Title:	Acute oral toxicity of substant conditions prolonged for 10	nce B to the ho days	neybee Apis mel	lifera L. under laboratory
Report No.:	00 10 48 0502b	2		
Document No.:	<u>M-068030-01-3</u>		212 (1008)	, y y
Guideline(s):	EPPO Standard PP 1/170(2)	(1999); OECD	213 (1998) ""	
	US EPA OCSPP Guideline n	io 850.SUPP	4	
Guideline deviation(s):	none	Ĉa	Å.	
GLP/GEP:	no	- Co - Co - Co - Co - Co - Co - Co - Co		
< <m-068030-01-3@s-602713-01-1< th=""><th></th><th>Å.</th><th>Ö¥</th><th></th></m-068030-01-3@s-602713-01-1<>		Å.	Ö¥	
Results:		40	Ó <sup>×</sup>	

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 supplied up for the whole test doration. 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 hopeyber mortably 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 Gaused 0.0 % 8 % and 12 % mortality after 10 days. Therefore it is concluded that providing the test substance sucross 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 conceptrations of 01, 1.0 and 10 Oppb Substance B per bee. The test substance at concentrations of 0.1, 1.0 and 10 ppb Substance per bee caused 200%, 36% and 96 % mortality after 10 days. The increasing mortality observed starting will day 2 was observed for all treatment groups including the control. The sensibility of field bees (including the control reatment) compared to house bees was significantly higher. Therefore a higher overall modality 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 behavior 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) \$0 % (day 9) and 44 \$ (day 10).

The validity criterion - mortality in the control  $\leq 10$  % - 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** 

m



Report: Title: Report No.: Document No.: Guideline(s): Guideline deviation(s): GLP/GEP: <pre> cm-068056-01-3@S-602719-01-1 Material and methods: A test concentrations of 0. ventilated stainless stee </pre>	02.01.01.02/10;	· 2000· M-068056 01-3	
Title:		$, 2000, \underline{W-008030-01-3}$	
	Substance B: feeding	test on the honey bees (Apis m	ellifera), non-GLP
Report No.:	IBA7241N		, Î Î
Document No.:	<u>M-068056-01-3</u>		ð "O" þ
Guideline(s):	US EPA OCSPP Guid	leline no. 850.SUPP	
	EPPO No. 170		
	according to the Guide	eline No. 170	
	of the European and N	Aediterranean Plant Protection	
	Organisation (EPPO)	A A	
Guideline deviation(s):	none	á. Ő <sup>y</sup>	
GLP/GEP:	no	Ú <sup>Y</sup> Á	
<		A Y Q	
Material and methods.	A feeding test was con	nducked over 10 days with th	he test item Substance Basili the
est concentrations of 0	1 10 10  pph Anis n	wallifora Boraging bees and	woung ware her her were kent in
contributed stainless staat	1, 1.0, 10 ppu. Apis ii	Of 10 individuals for asht	Hotmore Control itom was 50.9
chillated stalliess sice	reages, in 5 repredict.	sol luindividuals lobeacing	geatment. Control item was 50 %
aqueous sucrose solutio	n.		
	4 -		
Mortality was assessed	after 2, 4, 6, 8 and 10	days exposure period. The	control and test tem solutions
were exchanged every 2	2 days and thomges to	d test them anyount was cale	alated S
<b>c</b> <i>i</i>			8 . S . 4
Dates of work · May 11	to 21 2000 .		
cutos of work. May II			
Findings: summarized i	n Tablac 1 2		
munigs. summarized i			s o
			A A A
Table 1 Mortal	lity Values of Substan	ce B, Worker Bees	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			corrected mortality [%] after 10 days
Test item	Ingested test item	Mortality 1%] after 10 days	Corrected mortality [%] after 10
concentration [ppb]	amount [og a.i./bee]		days days
Control			
0.1	× 0.046 .	2 7 7 10 5 7 27 7 37 20 20 27 7 37 20	30
0.1 0.1	× 0.046	27 57 37 L	_
0,1 0,1 0,1	<sup>بل</sup> 0.046 ک رو:394	$\begin{array}{c} \begin{array}{c} & & & \\ & & & \\ & & & \\ \end{array} \end{array}  \\ \begin{array}{c} & & \\ & & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \\ \end{array}  \\ \end{array}  \\ \end{array}  \\ \end{array}  \\ \begin{array}{c} & & \\ & \\ \end{array}  \end{array}  \\  \\ \end{array}  \\ \end{array}  \\ \end{array}  \end{array}  \\ \end{array}  \\ \end{array}  \end{array}  \\ \end{array}  \end{array}  \\  \end{array}  \\ \end{array}  \end{array}  \end{array}  \\  \end{array}  \end{array}  \\  \end{array}  }  \end{array}  \end{array}  }  \end{array}  \end{array}  \end{array}  }  \end{array}  \end{array}  \end{array}  \end{array}  \end{array}  }  \end{array}  \end{array}  \end{array}  }  \end{array}  \end{array}  \end{array}  \end{array}  }  \end{array}  \end{array}  \end{array}  \end{array}  \end{array}  }   \end{array}   \end{array}  \end{array}   \end{array}   }    \end{array}    \end{array}    \end{array}   \end{array}          $	30 -7
0,1 , 1.0 10	0.046 0.394 3.672		_
0,1 ,71.0	0.046 0.394 3.672		-7
0,1 71.0	0.046 0.394 3.672 0 0.394	<sup>2</sup> <sup>3</sup> <sup>3</sup> <sup>3</sup> <sup>37</sup> <sup>37</sup> <sup>37</sup> <sup>37</sup> <sup>37</sup> <sup>37</sup> <sup>37</sup> <sup>3</sup>	-7
0,1 71.0 10 0,1 0 0 0 0 0 0 0 0 0 0 0 0 0	0.046 0.394 0.394 0.394 0.394 0.394 0.046 0.394 0.046 0.394 0.046 0.394 0.046 0.394 0.046 0.394 0.494 0.394 0.		-7
0,1 71.0 10 0,1 0 0,1 0 0 0 0 0 0 0 0 0 0 0 0 0	0.046 0.394 0.394 0.394 0.394 0.046 0.394 0.046 0.	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	-7
0,1 1.0 10	0.046 0.394 3.672 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		-7
A Table 2 Mortal		ce B Foraging Bees	-7 59
A 1 A 1.0 Table 2 Test item	Ingested test item	ce B Foraging Bees	-7 59 Corrected mortality [%] after 10
0,1 1.0 Table 2 Mortal		$\begin{array}{cccccccccccccccccccccccccccccccccccc$	-7 59
0,1 1.0 10 Table 2 Mortal Test item concentration [ppb]	Ingested test item	ce B Foraging Bees	-7 59 Corrected mortality [%] after 10
0,1 1.0 10 Table 2 Moreal Control	Pingested test item	ce B. Foraging Bees	-7 59 Corrected mortality [%] after 10
0,1 1.0 10 Table 2 Moreal Concentration [ppb]	Pingested test item	ce B Foraging Bees Mortality [%] after 10 days	-7 59 Corrected mortality [%] after 10
0,1 1.0 10 Table 2 Moreal Control	Pingested test item	Ce B Foraging Bees Mortality [%] after 10 days	-7 59 Corrected mortality [%] after 10 days 43
0,1 1.0 10 Table 2 Moreal Concentration [ppb]	Pingested test item	ce B Foraging Bees Mortality [%] after 10 days	-7 59 Corrected mortality [%] after 10 days
0,1 1.0 10 Table 2 Moreal Concentration [ppb] Control 0.1	Pingested test item	Ce B Foraging Bees Mortality [%] after 10 days 30 60 50	-7 59 Corrected mortality [%] after 10 days 43 29
0,1 1.0 10 Table 2 Moreal Concentration [ppb] Control 0.1	Pingested test item	Ce B Foraging Bees Mortality [%] after 10 days	-7 59 Corrected mortality [%] after 10 days 43
0,1 1.0 10 Table 2 Moreal Concentration [ppb] Control 0.1	Pingested test item	Ce B Foraging Bees Mortality [%] after 10 days 30 60 50	-7 59 Corrected mortality [%] after 10 days 43 29
Table 2 Test item concentration [ppb]	Pingested test item	Ce B Foraging Bees Mortality [%] after 10 days 30 60 50	-7 59 Corrected mortality [%] after 10 days 43 29
0,1 1.0 10 Table 2 Moreal Concentration [ppb] Control 0.1	Ingested test item	Ce B Foraging Bees Mortality [%] after 10 days 30 60 50	-7 59 Corrected mortality [%] after 10 days 43 29
0,1 1.0 10 Table 2 Moreal Concentration [ppb] Control 0.1	Pingested test item	Ce B Foraging Bees Mortality [%] after 10 days 30 60 50	-7 59 Corrected mortality [%] after 10 days 43 29
0,1 1.0 10 Table 2 Moreal Control	Pingested test item	Ce B Foraging Bees Mortality [%] after 10 days 30 60 50	-7 59 Corrected mortality [%] after 10 days 43 29
0,1 1.0 10 Table 2 Moreal Concentration [ppb] Control 0.1	Pingested test item	Ce B Foraging Bees Mortality [%] after 10 days 30 60 50	-7 59 Corrected mortality [%] after 10 days 43 29



Report:	02.01.01.02/11; ; 2000; <u>M-068043-01-3</u>
Title:	Substance B: feeding study with honey bees (Apis mellifera)
Report No.:	HT0400c
Document No.:	<u>M-068043-01-3</u>
Guideline(s):	US EPA OCSPP Guideline No.: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	no A O A
< <m-068043-01-3@s-602715-01-1< td=""><td>Substance B: feeding study with honey bees (Apis mellifera) HT0400c <u>M-068043-01-3</u> US EPA OCSPP Guideline No.: 850.SUPP none <b>no</b> determine the effect of feeding Substance B on mortality of adult poney bees</td></m-068043-01-3@s-602715-01-1<>	Substance B: feeding study with honey bees (Apis mellifera) HT0400c <u>M-068043-01-3</u> US EPA OCSPP Guideline No.: 850.SUPP none <b>no</b> determine the effect of feeding Substance B on mortality of adult poney bees
Tests were carried out to	o determine the effect of feeding Substance B of mortality of adult honey bees
(Anis mallifara I) over	a 10 day period. All doses and toxicity data for the test substance effer to $\sqrt{2}$
Substance B as the activ	
	groups of 10 bees, were offered 10, 1 @and 0.1 ng/ ml Substance B in 50% w/v
Five batches of bees, in	groups of 10 bees, were offered 10, 1 (pand 0.4 ng/ not Substance B/in 50% w/v
aqueous sucrose solution	
	daily after dosing. Olassatest feeders were removed and weighed and replaced
Mortality was assessed of	daily after dosing, Olasstest feeders were removed and weighed and replaced
with fresh feed each day	
2	
Results indicated that th	e Substance B had no significant of fect of montality.
>>M-068043-01-3@S-602715-01-1	
Report:	02.0101.02/12; 2000; M-068120-023
Title:	Substance B. Assessment of side effects in a ten days feeding testion the honey bee, Apis
	mellifera L. in the laboratory - trive bees (< 5 days)
Report No.:	20001448/01-BLEU 2 0 0 0 0 0
Document No.:	$\frac{M-06}{2} \frac{120-40}{2} \frac{3}{2}$
Guideline(s):	US EPA GESPP Childeline No.: \$50.SUPP
Guideline deviation(S).	By specified with a spe
GLP/GEP:	
< <m-068120-01-3@s-60022-01-1< td=""><td></td></m-068120-01-3@s-60022-01-1<>	
Young honey bees (9-5	days old) were fed over a ten days period with sucrose solution mixed with
Substance B. The feeding	g test was carfied out with three different concentrations of the test substance and
with five replicates.	
To obtain bees of approx	the same age competition bee brood, deriving from a healthy colony, were
incubated in the laborat	ry for five days. The bees which hatched within five days were used for this
	bees only fed the honey which was found in the combs, until the test started.
leeding test. Are young	bees only red the toney winch was found in the comos, until the test started.
The monte the Sul	stance b treatment groups rose up to 8 %, observed in the treatment fed with the
The mortaney in the Sur	stance by treatment groups rose up to 8 %, observed in the treatment red with the
lowest concentrated test	substance solution of 0.1 mg/L which corresponded to an actual intake of 0.04458
ng/bee after ten days	
7	
No mortality occurred in	n the treatment group fed with the highest concentrated test substance solution (10
$\mu$ g/L) of Substance <b>B</b> (a	ctude intake 4.316 ng/bee)
No mortabily was observe	and in the control group after the ten days exposure period.
>>M-0681204 @S-602792-01-1	
>>M-068120 \$ @ \$ 60278 01-1	
õ	



Report:	02.01.01.02/13; 2000; <u>M-068060-01-2</u>
Title:	Substance B: Assessment of side effects in a ten days feeding test on the honey bee, Apio
	mellifera L. in the laboratory - Foraging bees (= 22-32 days)
Report No.:	20001148/01-BLEU
Document No.:	<u>M-068060-01-2</u>
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	no la
< <m-068060-01-2@8-602721-01-1< td=""><td>mellifera L. in the laboratory - Foraging bees (= 22-32 days) 20001148/01-BLEU M-068060-01-2 none no c approx. 22 - 32 days) were fed over a four days period with success solution of the test</td></m-068060-01-2@8-602721-01-1<>	mellifera L. in the laboratory - Foraging bees (= 22-32 days) 20001148/01-BLEU M-068060-01-2 none no c approx. 22 - 32 days) were fed over a four days period with success solution of the test
Worker honey bees (age	$\cdot$ approx 22 - 32 days) were find over a four five period with success solution.
mixed with Substance B	. The feeding test was carried out with three different concentrations of the test
	replicates. Due to a high nortality which occurred in the control group the test
was terminated after fou	r days instead of a ten days exposure period in the second s
The mortality in the Sub	stance B treatment group rese up to 34 % observed in the treatment fed with the
	substance solution of 0.1 ftg/L which corresponded to an actual intere of @02873
ng/bee after four days.	
A 16% mortality occurred	ed in the treatment group ted with the bighest concentrated test substance solution B (actual intake. 2.881 ng/bee)
$(10 \mu g/L)$ of Substance 1	B (actual infake. 2.881 ng/bee)
In the control group a 20	) % mortality was observed after the fourdays exposure period
>>M-068060-01-2@S-602721-01-1	) % mortality was observed after the four days exposure period
Report:	02.01 02/14; 2000 41-068027-0143
Title:	Acuto oral toxicity of substance C to the honeybee Apis mellifera L. under laboratory
	conditions prolonged for 10 days
Report No.:	00 10 48 0502 c ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Document No.:	$M - 068 \frac{927 - 02 - 3}{3}$
Guideline(s):	EPPO Standard PP (17170(2) (19990) OEC (1998)
	USEPA OCSPP Guidefine no 859.SUPP
	Apone to the total and the total and t
GLP/GER	
< <m-068127-01-3@s-602725-01-1< td=""><td></td></m-068127-01-3@s-602725-01-1<>	
Results:	
Q L	
During a 10- day test	riod the bees consumed subrose solution containing 0.1, 1 and 10 ppb Substance C.
The amount of consume	M-068 27-04-3 EPRO Standard PK (170(2) (1999) OECD 213 (1998) USEPA OCSPP, Guideline no 859. SUPP no no riod the bees consumed sucrose solution containing 0.1, 1 and 10 ppb Substance C. d sucrose solution was summed up for the whole test duration. The total amount
	aining the test substance was used to determine the total amount of test substance
	est enopoints were mortality and behaviour of the honeybees in comparison with
consumer per bee. The	is chapters were mortany and benaviour of the noneybees in comparison with

Ŋ House bees:

the control.

N N N No statisticate significant effects on honeybee mortality were observed after oral exposure to Substance C at conceptrations of 0, 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 daysbad 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 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 ber caused 30 %, 40 % and 32 % mortality after 1 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 Cup to 10 ppb (equivalent to 8.056 ng Substance C/bee) over the protonged 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 sublethat effects) were observed in comparison with the control bees.

#### **Control bees:**

The mortality in the control was 4% for the house bees and 4% 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?) up to 16 % (day 8), 30 % (day 9) and 44 % (day 10)?

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

>>M-068127-01-3@S-602725-04

 Report:
 02.01.01.02/15;
 2000; M. 06813P-01-3.

 Title:
 Substance C: feeding study with hone Obees (Apis mellifera)

 Report No.:
 M. 068131-01-3.

 Guideline(s):
 US EPA OCSPP Guideline No.:

 Guideline deviation(s):
 Jone

 GLP/GEP:
 no

Tests were carried out to determine the effect of feeding Substance C on mortality of adult honey bees (*Apis methfera* 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 doily 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.



<b>Report:</b> Title:	02.01.01.02/16; 2000; M-068147-01-3 Substance C: Assessment of side effects in a ten days feeding test on the honey see, Apie
Report No.: Document No.:	substance C. Assessment of side effects in a ten days reeding test on the honey <b>pee</b> , App mellifera L. in the laboratory - Hive bees (< 5 days) 20001149/01-BLEU <u>M-068147-01-3</u> US EPA OCSPP Guideline No.: 850.SUPP none <b>no</b> days old) were fed over a ten days period with sucrose solution mixed with
Guideline(s): Guideline deviation(s): GLP/GEP:	US EPA OCSPP Guideline No.: 850.SUPP none no
< <u>M-068147-01-3@S-602783-01-1</u> Young honey bees (1-5 of Substance C. The feedin with five replicates.	days old) were fed over a ten days period with sucrose solution mixed with g test was carried out with three different concentrations of the test substance and
To obtain bees of approximetated in the laborate	x. the same age, combo with boe brood, deriving from a healthy colony, were bry for five days. The bees which bached within five days were used for this bees only fed the honey which was found in the combo, until the test started
	ubstance C the mortality rose up to 4% observed at a test substance concentration 0.4585 ng bee) after 10 days.
No mortality occurred ir $\mu g/L$ ) of Substance C (a	n the treatment group fod with the highest concentrated teo substance solution (10 ctual in take: 4,6769 ng/bee)
No mortality was observ >>M-068147-01-3@S-602783-01-1	redan the control group after the ten days exposure period
Report: Title:	02,01.01 0/17; 2000; M2968153201-3 Substance C: Assessment of side effects in a ten days beeding test on the honey bee, Apis mellifera L. in the laboratory Foraging bees (= 22*32 days)
Report No.: Document No.: Guideline(s) Guideline deviation(s):	$\frac{M_{-0}@8155-91-3}{M-0681525-01-3}$ $\frac{WS}{OCSPP}$ Guideline No.: 850 SCPP
GLP/GÈP:	$\mathbf{n}_{\mathbf{v}}$ $\mathbf{v}$ $\mathbf{o}^{\mathbf{v}}$ $\mathbf{v}$ $\mathbf{v}$ $\mathbf{v}$
substance and with five	approx. 22, 32 days) were fed over a four days period with sucrose solution . The feeding test was carried out with three different concentrations of the test replicates. Due to a high mortality which occurred in the control group the test r days instead of a ten days exposure period.
In the treatment with Su of 1 $\mu$ g/L after four days	bstance Cane mortality rose up to 10 % observed at a test substance concentration
A 6 % mortality occurre (10 μg/L) of Substance	d in the treatment group fed with the highest concentrated test substance solution Factual intake 2.731 ng/bee).
In the control group as 20	) whortality was observed after the four days exposure period.
$\checkmark$	



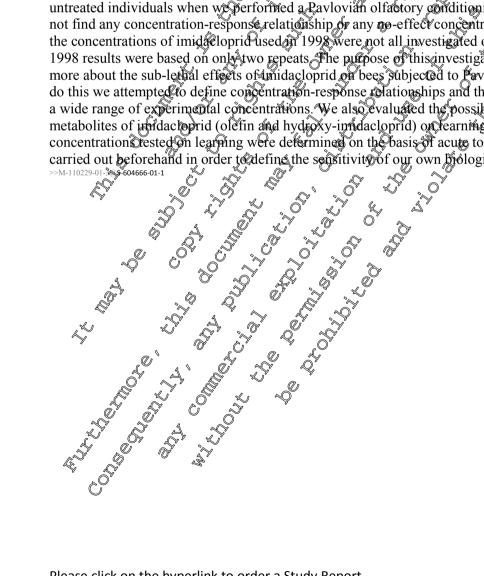
Report:	02.01.01.02/18; 2000; <u>M-068134-03-3</u>	@.° ^
Title:	Repeat test: Substance C: feeding test on the honeyb	ee Apis mellifera L. (Hymenoptera 🛇
	Apidae), non-GLP	ee Apis mellifera L. (Hymenoptera O
Report No.:	IBA7242N	
Document No.:	<u>M-068134-03-3</u>	
Guideline(s):	US EPA OCSPP Guideline 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	
	A Q'	
< <m-068134-03-3@8-602774-01-1 Matarial and mathad</m-068134-03-3@8-602774-01-1 	s: A feeding test was conducted over 10 days with of 0.1, 1.0, 10 ppb. Young worker bees (Apis mell	the test item Substance Quith
the test concentrations	of 0.1.1.0.10 ppb. Voltag worker bees <i>Anistral</i>	lifting) ware kent in ventilited
stainless steel cages in	a 3 replicates of 10 individuals for each treatment.	
	b aqueous sucrose solution. $\mathcal{D} = \mathcal{D}$	
Mortality was assessed	lafter 2 4 6 Sand 10 days & possing period The	Control and talk item solutions
wore evolution and every	2 days and the ingo and test item amount was all	vloted
were exchanged every	2 days and the ingested lest nem amount was care	
Dates of work. July 1		To so k
Dates of work: July 1		
Findings: gummorized	Lin Tenhlo 10 A A A	
rinuings. summarized		
		~~
Table 1: Mortality	after 2, 4, 6, 8 and 10 days exposure period. The 2 days and the ingested test item amount was care 1 to 21,2000 in Table 10 Values of Sopstance C, Worker Bees	
		Ű,
Test item	Ingested test item / Montality [%] after 10 days	Corrected mortality [%] after 10 days
concentration [ppb]	amoOnt [ng Oi./be4]	days
Congol		-
É 0.1		3
1.0		0
10 👰	A SB01 W A	0
>>M-068134-03-3@ <b>\$402974-01-1</b> ()		
A		
4 4	× Q <sup>Y</sup> ~ Q <sup>Y</sup>	
Q' ^		
të që	L.B.	
¢°″		
$\lor$		



<b>Report:</b> Title:	Impact of imidaclopri	; 2000; <u>M-110229-01-</u> d and its main metabolit osure on mortality and lea	es on the honeybe	ee Apis mellifera
Report No.:	<u>M-110229-01-3</u>		~	
Document No.:	<u>M-110229-01-3</u>		Å	A A
Guideline(s):	not specified		10%	
Guideline deviation(s):	not specified		4	
GLP/GEP:	no	Ĉs	Å.	
< <m-110229-01-3@s-604666-01-1< td=""><td></td><td></td><td></td><td></td></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 toxic bogy tests before products are placed on the market, there is contently no objective way or detecting the sub-leftal effects of pesticides on bee behaviour or of evaluating their chronic toxicity

 $\sqrt{2}$ During the national study programme carried out in 1998 to evaluate the offects of Garcho® signflower seed dressing on bees we studied the chronic toxicity of the active ingredient of this produce (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 flowests as they forage. At that time we observed significant mortality compared to the control group a concentrations of 8 and 40 ppb after 11 days ingestion of ionidacloprid. Furthermore, after the 11 days administration of concentrations of 4, 8 and 40 pph we observed a significant decline in leafong performance compared to untreated individuals when we performed a Pavlovian olfactory conditioning procedure. However, we did not find any concentration-response relationship or any go-effect concentration. It should be noted that the concentrations of imid a loprid 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 unidacloprid 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 infidactoprid (olefin and hydroxy-infidacloprid) or fearning ability. The acute concentration rested on learning were determined on the basis of acute toxicity test results which we carried out beforehand in order todefine the sensitivitg of our own biological material.





Issue date 2017-11-22

## 02.01.01.03 - Formulations

Report:	02.01.01.03/01;			
Title:	Laboratory testing for	toxicity (acute conta	act and oral LD5	50 of Confidor S@200 to
	honey bees (Apis mell		tera, Apidae)	\$ 4 .4
Report No.:	790036		n	
Document No.:	<u>M-032525-01-2</u>			
Guideline(s):	EPPO 170 (1992)	Ĉħ	L	
Guideline deviation(s):	none			
GLP/GEP:	yes	A .	Č <sup>%</sup>	
< <m-032525-01-2@s-602595-01-1< td=""><td>•</td><td>Á</td><td>Q &amp;</td><td></td></m-032525-01-2@s-602595-01-1<>	•	Á	Q &	

The contact and oral LD50 (24 h and 48 h) of Confidor SC 200 to honey Bees were texted 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  $\mu$ g a.i./bee). The following decages of the test substance were tested in three replicates often bees each:

1		- A C				
	Contact t	oxicity test			icity test?	S.
	Test Substance Dosage		rtalitêg	Prest Substance Dosage	O Mor	Glity ^
	(nominal)	°~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	N O	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
	0.8	24 h (%)	48 0 (%)	est Substance Desage μg/bee	23 (%)	<b>@3</b> h (%)
	20 2 1		86.2		40,00)	80.0
		6076	86.7	5 <b>G</b> /083	20.0	23.3
ð	, <i>"</i> Q", , O	\$\$0.0 \$	~~~ e 4	0.015 4	6.7	10.0
		164 20.0	ð.7 ()		0.0	0.0
Ş,			20.0	× 0.00018 × 1	0.0	0.0
	calculated BD50 µg/ace	6478	0.29	calculed LD <sub>50</sub> µg/bee	> 0.169	0.103
~	(95 % Oldence pare)	20.0 ( 0476 ((C.R to 1.39)	(0.19 to 45)	(O% cofidence range)		(0.073 to 0.144)
A A	Controls 29	Mor	talify	Controls	Mor	tality
	JA A	24 h (%)	48°b (%)		24 h (%)	48 h (%)
	(Nř 20.1		0.0	-	-	-
	salvent control	×J <sup>¥</sup> 0.0	0.0	negative control	0.0	3.3
ő, Í	Spositive controp	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 est. The acute contact LD<sub>50</sub> (48h) was calculated to be 0.29 µg/bee and the acute oral LD<sub>50</sub> (48h) was calculated to be  $0.103 \mu g/bee$ .

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



Report:	02.01.01.03/02; ; ; 19	995; <u>M-032532-01-</u>	2	٥
Title:	Laboratory testing for toxic	city (acute contact a	and oral LD50) of (	Confidor WG 70 0
	honey bees (Apis mellifera	L.) (Hymenoptera	, Apidae)	Confidor WG 7046
Report No.:	780036		ð	
Document No.:	<u>M-032532-01-2</u>		Ą	L'AND
Guideline(s):	EPPO 170 (1992)		10,	
Guideline deviation(s):	none		A	
GLP/GEP:	yes	Ĉ	Ľ,	
< <m-032532-01-2@s-602599-01-1< th=""><th></th><th>A Provide A ProvideA ProvideA ProvideA Provide A Provide A Provide A Provide A Provide</th><th></th><th></th></m-032532-01-2@s-602599-01-1<>		A Provide A ProvideA ProvideA ProvideA Provide A Provide A Provide A Provide A Provide		
		A A		
The contact and oral LD	0 <sub>50</sub> (24 h and 48 h) of Con	fidor WG 70 to b	oney bees were to	ested according to

EPPO 170 (1992) and GLP regulations. Confider WG 70 was applied in five desages frontact and fal toxicity test), one solvent control, one untreated negative control (contact test) and one positive control with toxic standard (Dimethoate 0.2  $\mu$ g a.i./beey. The following docages of the text substance were tested in three replicates often bees each: Õ Â 4

		×.		$\sim$ $\sim$ $\rightarrow$ $\rightarrow$	. 6 <sup>57</sup> 4	
	Contact t	coxicity est	Cality	τest Substance Dosage	foity test	48 h (%)
	Test Substance Dosage	С <sup>У</sup> моя	Mality 🔊	Test Substance Dosage	Mor	Norty (
	(nominal)				\$°~~°	, ~
	(nominal) μg/bee	24 h (%)*	(48 h (%)	μg gee 2	24.h (%)	48h (%)
	1.0 7	30.0 <sup>2</sup>	576.7 6059	μg / bee	5 36.7 53.3	76.7
		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	60.9		53.3	56.7
		20.0	~23.3 °	℃ 0.008 °C	40.0	40.0
, Ĉ	0.05	Q 3.3		9 0.0017 v	0.0	6.7
	0.00°, 55°	5% ×	0.00 <sup>4</sup>	× 0.0009	0.0	0.0
	calculated LD <sub>40</sub> µg/bee	~ × ×	0:35	Calculated LD <sub>50</sub> µg/bee	≥ 0.085	0.0167
	(95 % cofidence limits)	°~~~~~	(0.25 to 631)	(%% cofidence limits)		(0.0105 to 0.0264)
A R	Controls	Mor	talito	Controls	Mor	tality
Mar	Controls		talify $48 \text{ h}(90)$		24 h (%)	48 h (%)
	untreated Control		£ 6.7	-	-	-
	untiveated Control	0.0	3.3	negative control	0.0	3.3
	sositive 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 LD50 (48h) was calculated to be 0.35 µg/bee and the acute oral (48h) was calculated to be 0.0167  $\mu$ g/bee. >>M-032532-01-2@S-602599-01-1



Report:	02.01.01.03/03; ; 2001; <u>M-060864-01-</u>	<u>2</u>
Title:	Acute effects of imidacloprid AE 0.025 to Ap	ois mellifera (Hymenoptera, Apidage tested
	as imidacloprid-AE VL 0.0625	
Report No.:	IBA73871	
Document No.:	<u>M-060864-01-2</u>	
Guideline(s):		
Guideline deviation(s):		
GLP/GEP:	yes Ca	
< <m-060864-01-2@s-602153-01-1< td=""><td>The second s</td><td></td></m-060864-01-2@s-602153-01-1<>	The second s	
Material and methods	The insecticide Imidacloprid A VL 0.00	62 Opresolution of Imida Cloprid AF.
0.025 (purity: 0.062 g/I	, specification: article no.: 00004434447,	formulation ne $0.07553/00060001$
was applied at nominal	doses of $0.00032 - 0.001 - 0.0032 - 0.01 - 0.0132 - 0.01 - 0.0132 - 0.01 - 0.0132 - 0.01 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0132 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - 0.0032 - $	$0.032 \ge 0.1$ u $0.3$ i per bee for oral and
topical application unde	r laboratory conditions	
As a set that 50 % a support	is sucrose solution in oral mode and $OO_2$ -p	
+ acetone in contact mo	(Dimethoate: $0.046 + 0.1 = 0.22 - 0.46 \text{ µg}$	
ADIMETHOAT 40 EC	(Dimethoate: $0.046 \times 0.1 - 0.22 \times 0.46 \mu g$ ?	a.1./bee (oral and contact mode)) was
used as reference treatm	ient.	
Apis mellifera (only wo	rker bees), were kept in stair less steel cage	s, in 3 groups of 10 individuals for
each treatment. Mortalit	y was assessed after 4, 24, 48, 76 and 96	i exposure period. The LDG-value after
24 h in the reference tre	atment was 0.11 µg a.i./bee (oral) and 0.14	4 μga.i./bee (contact).
Dates of work: August	23 to 27, 2000 (oral) August 29 to Septem	ber 02, 2000 contact
E. 1.		Ber 02, 2000 (Contact)
r munigs.		
×		× 4. ~~
Table 1: Toxicity to	Honeybees Laboratory Tests	ON A
	Honeybees, Caboratory Tests	
Test item	(presorbijon of midacloprid Al	0.025 article no. 04434447)
Test object	O & Apism	
Exposure	là A cotal or	contact
	24 b 2 2 0.056t	0.021
		(0.018 - 0.024)
	48 <sup>°</sup> h 5 <sup>°</sup> 0.041 <sup>*</sup> 5 <sup>°</sup>	0.010
LD <sub>50</sub> [µg a.i. / beer]	$\sqrt{100}$ $\sqrt{100}$ $\sqrt{100}$ $\sqrt{100}$ $\sqrt{100}$	(0.006 - 0.017)
(95 % confidence interval)	72 0 0.025	0.003
	<sup>2</sup> 72 h <sup>3</sup> <sup>2</sup>	(0.002 - 0.005)
	96 h ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	0.002
A		(0.0016 - 0.0022)
* = Dose based on actual	ngestion of the test item	
Observations:		
In the oral test mode su	blethal effects were beserved in the follow	ving actual consumptions:
<ul> <li>0.00030 μg/a.i./</li> </ul>	bee. after 4 h nosublethal effects were obs	served. After 24 h and 48 h 1 bee
	otions and had coordination problems. Aft	
Sobserved.		

• 0.0009 µg 37 bee after 4 h 13 bees showed slow motions and had coordination problems. After 249 10 bees showed slow motions and had coordination problems and 1 bee had problems in

- founding 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.



- 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 66 h 10 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 45 bees showed slow motions and had coordination problems and 9 bees had problems in standing up. After 72 h 45 bees showed slow motions and coordination problems and 9 bees had problems in standing up. After 72 h 45 bees showed slow motions and coordination problems and 9 bees had problems in standing up. After 72 h 45 bees showed slow motions and coordination problems and 9 bees had problems in standing up. After 72 h 45 bees showed slow motions and coordination problems and 9 bees had problems in standing up. After 72 h 45 bees showed slow motions and coordination problems and 9 bees had problems in standing up. After 72 h 45 bees showed slow motions and coordination problems and 9 bees had problems in standing up. After 72 h 45 bees showed slow motions and coordination problems and 9 bees had problems in standing up. After 96 h 9 bees showed slow motions and coordination problems.
- 0.056 µg a.i./bee: after 4 h 9 bees had problems in standing µp and 19 bees showed slow motions and had coordination problems. After 24 h 41 bees had problems in standing up and 7 bees had problems in standing up and 3 bees showed slow motions and had coordination problems. After 48 h 7 bees had 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  $0.12\mu$ g a.i dree.

#### **Contact test mode:**

In the **contact test mode** sublethal effects were observed at the test item doses  $\ge 0.00032 \ \mu g$ 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 & bees showed slow motions and had coordination problems. After 24 h 3 bees, after 48 k 4 bees and after 72 h and 96 h 2 bees showed slow motions and coordination problems.
- 0.0032 Qg a.i Dee: 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 problems have showed slow motions and coordination problems, but nortality increased from 10 % (24 h) to 67 %. After 72 h 3 bees and after 96 h 1 bee showed slow motions and coordination problems and coordination problems.
- 0.01 µg a, bee: after 4 b2 bees had problems in standing up and 28 bees showed slow motions and coordination problems. After 24 4/26 bees showed slow motions and coordination problems. After 48 h 25 bees showed slow motions and coordination problems. After 48 h 25 bees showed slow motions and coordination problems. After 48 h 25 bees showed slow motions and coordination problems. After 48 h 25 bees showed slow motions and coordination problems. After 48 h 25 bees showed slow motions and coordination problems but motions and coordination problems but slow motions and coordination problems but motality increased from 13 % (48 h) to 67 % After 96 h only 1 bees showed slow motions and coordination problems but 90 % of the bees were dead.
- 0.032 μg a. bee: after 4 h y bees had problems in standing up and 26 bees showed slow motions and coordinating problems. After 24 h y 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 notions and coordination problems. After 72 h only 1 bee had problems in standing up. After 96 h all bees were dead. Que
- 0. Kug 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 5. s@2153-01





In the or 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.



- 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 rad problems concerning coordination and 1 bee had problems in standing up. After 34 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 flow 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  $\mu$ g 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 24 011 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. After 72 h 6 bees had problems in standing up. After 96 h 9 bee had problems in standing up.
- 0.506 μg a. Bee: after 4 b 97 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 aft bees were dead.

For details see Table 4.

In the contact mode, soblethad effects were observed in the following nominal doses:

«

- 0.001 μg at /bee; after th 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 Spees and after 96 h Poes proved slow motions and problems concerning coordination.
- 0.01 µg@a.i./bee: after # 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.
- 0.030 µg a bees after 4 h 5 bees had problems in standing up. After 24 h 1 bee had problems in standing up and 43 bees showed slow motions and problems concerning coordination. After 48 h 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).



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

Issue date 2017-11-22

- 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 9 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 mortaney was 100 %. For details see Table 6.

	02.01.01.03/05; 2001; M-08192501-3 Acute toxicity of imidac loprid & 200 to the honeybeo Apis mellifera L. under laboratory conditions 01 10 48 048 M-081923-01-3 OECD 213 (1998), OECD 214 (1998) US EPA OCSPP Gurdeline for 850 SUPP none yes Apis mellifera carnica b oral toxicity and conract toxicity test of Imidacloprid SL 200 on honeybees
Report:	02.01.01.03/05; 2001; M-08192201-3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Title:	Acute toxicity of imida oprid & 200 to the honeybee Apis mellifera L. under
	laboratory conditions
Report No.:	01 10 48 048
Document No.:	<u>M-081923-01-3</u>
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
	US EPA OC SPP Gurdeline no 850 SVPP 0 5 5 6
Guideline deviation(s):	none of the state
GLP/GEP:	yes y a a a a a a a a a a a a a a a a a a
< <m-081923-01-3@s-602211-01-1< th=""><th></th></m-081923-01-3@s-602211-01-1<>	
Material and methods:	
Test species:	Apis mellifera capica by Sin V Si
× ×	
Test system:	oral toxicity and contact toxicity test of Imidacloprid SL 200 on honeybees
Treatments:	oral for item and to be standard (Dumethouse EC 400)
Treatments.	
Tast itam trackmont 1	Is: The test tem was applied at the following doses: oral toxicity test: 0.0064, $0.0128, 0.0256, 0.0512$ and $0.1025 \ \mu g \ x^{1}$ bee contact toxicity test: 0.0029, $0.0057 \ 0.00114 \ 0.0229, 0.0457 \ and 0.0914 \ \mu g \ a \ i / bee$
	Is. The test term was applied at the forewing doses. Oral toxicity test, $0.0004$ ,
	$0.0128, 0.0250, 0.0512$ and $0.1025 \ \mu g a M./bee contact toxicity test. 0.0029, 0.0457 0.0057 0.00457 0.0057$
	0.0057, 00114, 0.0229, 0.0457 and 0.0914 μg a.i./bee
Toxic standard:	Dimethoate DC 400 was applied at the following doses: oral toxicity test:
	$0.004, 0.089, 0.104, 0.120, 0.149 \ \mu g a.i./bee contact toxicity test: 0.012,$
	Dimethoate OC 400 was applied at the following doses: oral toxicity test: 0.024, 0.089, 0.104, 0.120, 0.149 µg a.i./bee contact toxicity test: 0.012, 0.023, 0.046, 0.093, 0.986 µg a.i./bee August 28 September 10, 2001
Dates of work:	August 28 September 10, 2001
in <sup>v</sup>	
The insecticide Imidack	prid SL 200 (purity: 200 g/l; specification: Development No.: 3000249869,
TOX No.: 05752-00 For	rmthation No.: 0\$833/0818 (0753) was tested under laboratory conditions on the
honevbee A. mellifera at	for oral and contact exposure. Endpoints were mortality and behaviour of the
bees compared @ contro	l up to 96 h after application. Mortality values were used to provide a regression
line and calculate the me	edian lethal dose value (LD <sub>50</sub> ) expressed in $\mu$ g of active ingredient or product per
bee.	
D' AS (	

Findings: A S

				-			
Test item			Imid	acloprid SL 20	)0		. 6
Test object				ee Apis mellife	era L.		N 0
Exposure			c	ral / contact		ð	
Treatment				LI	D <sub>50</sub> &	()	
Test item	time		ral toxicity t	est		ftact toxicity	test 🔊
Imidacloprid SL 200		μg a.i./bee	slope b	μg product/bee	μg a.i./hee	slope b	produce de
	24 h 95 %-cl lower upper	n.d.		ိတ္ n.d. ∛	nd.		~n,d. → 0.306 ↓ ↓ ↓
	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		0.404
	72 h 95 %-cl lower upper	0.056 0.040 0.077	Q.89	0.306 0.299 0.421	• <b>9.048</b> 0.036 0.065	* 2.03 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	<b>9.262</b> 9.197 9.197 9.355
	96 h 95 %-cl lower upper	0.053 0.038 0.044		© 0.290 © © 0.208 > 0.404	0.034 0.034 0.060	© 2.09 Ky	0,246 0,186 0.328
cl: confidence limits n.d.: not defined a second se							
Treatment			di di		<u>* 0</u> *	8 4	1
Reference item	time		ral toxicity	test L	D <sub>50</sub>	ntact toxicity	v test
Dimethoate EC 400		µg a tybee	Slope b	μg product/bee	grg a.i./bee	slepe b	μg product/bee
	24 h 95 %-cl lower	0.133 0.123 0.1¢4		0.358 0.33 0.388	0.113	2.22	0.304 0.226 0.409
C. C. M.	48 h 95 Oct lower upper	<b>0.129</b> <b>0.120</b> 0.139	8.17	0.347 0.323 0.374	0.102 0.078 0.134	2.37	0.275 0.210 0.361
l: confidence junits		O KO		d st			

#### Table: Oral and contact toxicity LD<sub>50</sub> values of bees treated with Imidacloprid SL 200

No statistically significant effects on survival were observed at doses of 0.0064 and 0.0128  $\mu$ g a.i. per bee in the oral toxicity test (0 and 3.3 % mortality, respectively) during 48 hours. Statistically significant effects on survival were observed at doses of 0.0256, 0.0512 ard 0.1025  $\mu$ g a.i. per bee in the oral toxicity test (23.3, 36.7 and 66.7 % mortality, respectively) during 48 hours. The calculated LD<sub>50</sub> (48 h) is 0.066  $\mu$ g a.i. per bee in the oral toxicity test (20.361)  $\mu$ g 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  $\mu$ gQ i.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  $\mu$ g a.i. per bee in the contact toxicity test (33.3 and 5.0 % mortality, respectively) during 48 hours. Therefore the calculated LD<sub>50</sub> (48 h) is 0.056 rg a.i. per bee in the contact toxicity test (equivalent to 0.306  $\mu$ g product/bee based on analysed content of a.?.).

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  $\mu$ g a.i./bee after 96 h. The calculated LD<sub>50</sub> (96 h) are 0.053 and 0.045 $\mu$ g a.i. per bee in the oral and the contact toxicity tests (equivalent to 0.290 and 0.246  $\mu$ g product/bee, respectively, based on analysed content of a.i.).



The LD<sub>50</sub> of the reference item Dimethoate was 0.133  $\mu$ g a.i./bee in the oral toxicity test after 24 hours. This value was also within the preferred range of 0.10-0.35  $\mu$ g a.i./bee cited in the OECD Guideline 213. The LD<sub>50</sub> of the reference item was 0.113  $\mu$ g a.i./bee in the contact toxicity test after 24 hours. This value corresponds to the expected range for the oral 24h - LD<sub>50</sub> (0.10-0.30 µg a.i./bee published in the OFCD Guideline 214.

In the reference treatments apathy, discoordinated movements and immobility were obse died.

The study was performed in compliance with the GLP principles. The validity criterion - mortality in the control  $\leq 10^{10}$  - was accomplished (being 0 % in the oral and m the contact toxicity tests after 48 hours). The LD<sub>50</sub>-24 h values for the toxic standard of 0.1-0.35 µg a jobee (oral) and 0.1-0.30 µg a.i./bee (contact) were accomplished (being 0.133 µg@.i./be@and 0.413 µg a.i./be@in the oral and the contact toxicity test, respectively). >>M-081923-01-3@\$-602211-01-1

**Report:** 02.01.01.03/06 Effects of invide lob and SL 200 (acute contact and ral LDS0) on boney toes (Apis Title: mellifera (1) in the laboratory 99810**36**/ Report No.: <u>M-08</u>4112-01-2 Document No.: GLP compliant study based on OECD 213 and 219 (1998) Guideline(s): and the recent recommendations of the LCPBR group, Heid in Avienon, France, 1999 non Guideline deviation(s): **GLP/GEP:** <<M-084112-01-2@S-602212 Material and methods; Imidacloprid SL 200 (NTN 33893 200 SE), purity: NTN 33893: 194 g/L; (specification Article No.: 0004958608; Batch No.: 232925888; Tox No.: 5428-00); under laboratory conditions Apis mellifera (30 worker bees per reatment) were exposed for 96 hours to doses of 98.7, 38.5, 11, 1, 5.6 and 1.2 mg a.i. per beeder feeding (oral, value based on the actual intake of the test item) and to coses of 800, 400, 200, 100 and 50 ng a.i. per bee for topical application (contact). The oral and the compact test were prolonged up to 96 hours because of increasing mortality between 24 and 48 hours. The LD50 of the Greener item was 0.19 µg dimethoate per bee in the oral and 0.18 µg Dimethoate per bee in the contract exposure after 24 hours

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

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



#### **Imidacloprid Bee Studies Compilation of Study Summaries**

Issue date 2017-11-22

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*	nellifera 800, 400, 200, 100 and 56		
Exposure	oral (50% sugar solution)	(solution in water + 1 %, wetting agent)		
LD <sub>50</sub> ng a.i/bee after 48 h and 96 h (95 % Confidence Limits)	48 h: 5.6* (3.3 to 99) 96 h: 5.3* (3.4 to 8.4)	A8 h: 42.2 (20 y to 85.9) 6 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 expended for 48 bours because of delayed Ì mortality. m

Behavioural impairments (e.g. apathy or discoordinated movements) were observed for the first thours in the 98.7, 38.5, 11.1 and 5.6 ng a.i./b& 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 pours wathy 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 howed apath and moving coordination problems in the 98.7 and 38.5 mg a.i. dose group, respectively. After 72 hours two bees were apathetic or showed a discoordinated movement in the group dosed with 98.4 and 38.5 ngg, i./bee. In the 98.7 ng a.i. dose group two bees were cound apathetic at the 96 hours check.

In the contact test behavioural impairments of the supplying bees like apathy, von ting and discoordinated movements occurred in all groups dosed with Imidaelopric SL 200 during the whole experimental time of 96 hours.

3.3 % control mortality was found after the hours in the contast test and or attest, respectively

Conclusions: The LD<sub>50</sub> (contact) 48 hrs was determined to be 42.2 nga i./bee. The LD<sub>50</sub> (oral) was determined to be 5.6 ng a i./bee after 48 hrs and 5.3 fg a i./be after 96 hrs. Sectored 12 + Conclusions: Co



Report:	02.01.01.03/07; ; 2004	; <u>M-121776-01</u>	<u>-2</u>	
Title:	Laboratory bioassays to dete	rmine acute ora	al and contact toxici	ty of Confidor SL 200 to
	the honeybee, Apis mellifera	L		
Report No.:	BAY-03-9		ð	
Document No .:	<u>M-121776-01-2</u>		- S	A A
Guideline(s):	OECD 213 (1998), OECD 2	214 (1998)	10%	
Guideline deviation(s):	none		A	. 6 <sup>4</sup> . 6 <sup>4</sup> . 9
GLP/GEP:	yes	Ò	Å.	
< <m-121776-01-2@s-602879-01-1< td=""><td></td><td></td><td>Q,</td><td></td></m-121776-01-2@s-602879-01-1<>			Q,	

Materials and methods:

Confidor 200 SL (Development No 30-00325832; Batch No 038350914(0912); POX No 06339-00) nominally containing 200 g/L imidacloprid (NTN \$893), was provided both cally and topically to honeybees (Apis mellifera L.). Following preliminary range-finding tests, for the contact texicity, fest, 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 rades, equivalent to 0.451, 0.270, 0.114, 0.101, 0.029 and 0.0 Sµg a (NTA) 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 Bare, a wetting agent. For oral dosing, the test item was dispersed in a 50% w/v surges solution Control treatments of deionised water and an untreated solution of Farmon Blue (0.05/20/v) (both topically applied) and untreated sucrose (administered or ally), 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 timethoate. This was applied at nominal rates of 0.200, 0.175, 0.150, 0.125, and 0 100 µg a.i./bec O

Worker bees (approximately 2 weeks old) were obtained from a high of a commercial bee keeper. In preparation for the tests, the bees were lightly anaesthetised with four diffed CO<sub>2</sub> 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 from deep by 40 mm in diameter, and were closed at both ends with bungs of polyurethane form. Feeding tubes containing 50% w/v sucrose solution were provided for the bees interided to receive the contact dose. The begoused in the oral bioassays were deprived of food prior to dosing.

Ľ For the topical (contactor application of treatments, the bees were lightly anaesthetised using humidified CO2 gas and but oftest 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 nd of a 50% w/@sucross solution containing the appropriate treatment. The bees took the reated 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.storose 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  $\mathcal{Q}$  at the dose rate of  $\mathcal{Q}$  2  $\mu$ g a.i./20 $\mu$ l), the tubes were removed at this time and reweighed on a four decimal place balance, that the precise amount of treated food consumed could be calculated for each replicate.

Ŀ, 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,



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<sub>50</sub> values derived for the contact and oral methods of  $\sqrt[3]{2}$  application were 0.129 and 0.139 ug a i /bee respectively and these results in the second application were 0.129 and 0.139  $\mu$ g 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	n 28 August ar	nd 5 September 2003	
Findings:			
Test item		Corfidor SL 200	
Test object		Apis mellifera 📎	
Exposure		Contact and Oral	A A A
	Mortality (	%) at 96 h 🙏 🔗 /	
Contact exposure	C C C	Súcrose control	re Ö
Water control	O N	Sucrose control	<u>گ</u> ر ک
FB control	508	g er gr & gr	4
0.030 µg a.i./bee 🔬 🕵	43	0,015 µg a.i./bee 🌣 💡	<b>10</b>
0.063 µg a.i./bee 😽 🔬	<sup>2</sup> 30 5 <sup>4</sup>	0.029 µg a.i./bee	10
0.132 μg a.i./bee		0,101 µg a.i∂bee√	80
0.278 µg а ійрее	<sup>√</sup> 97∕> √		67
0.583 µga.i./bee 🖉 🔿	90	0,270 👸 a.i/bee	100
1.225 µg a.i./bee	90 5 2100 <sup>0</sup>	0.459 µg a.i./bee	97
LD <sub>50</sub> (µg a.i./bee)	0∕¶61.√ <sup>™</sup>	kD₅₀ (µg a.i./bee)	0.060
95% confidence limits ( (µg a.i./bee)		95% confidence limits	0.040 – 0.086

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



200 OD to O
2000D to 🔿
) )

Materials and methods:

NTN 33893 200 OD (Development No 30-00284249, Batch No 059/0060(0036), TOX N9 06445-00), nominally containing 200 g/L imidacloprid (NTN 33893), was provided both early 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 tesponse 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.ic), 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 Bule, a wetting agent. For oral dosing, the test item was dispersed in 50% v/v sources solution control treatments of deionised water and an untreated solution of Farmon Blue (0.05%) v) (both topically opplied) 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 timethoate. This was applied at nominal rates of 0.200, 0.175, 0.150 (0.125 and 0.400 µg a.1./bec

Worker bees (approximately 2 weeks old) were obtained from a histor of a commercial bee keeper. In preparation for the tests, the bees were lightly anaesthetised with dumidified CO<sub>2</sub> 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 toam. Feeding tubes containing 50% w/v sucrose solution were provided for the bees interded to receive the contact dose. The bee 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 buL of test solution was placed on the dorsal thorax of each bee using a Rainin EDP-2 motorised micropipette. For the oral application of doces, cages of bees were presented with glass feeding tubes containing 0.22 ml of a 50% w/0 sucrose solution containing the appropriate treatment. The bees took the freated 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 subrose 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? and b 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,



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<sub>50</sub> values derived for the contact and oral methods of application were 0.119 and 0.144 ug a i /bee respectively and these rest is in the second sec application were 0.119 and 0.144  $\mu$ g 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.

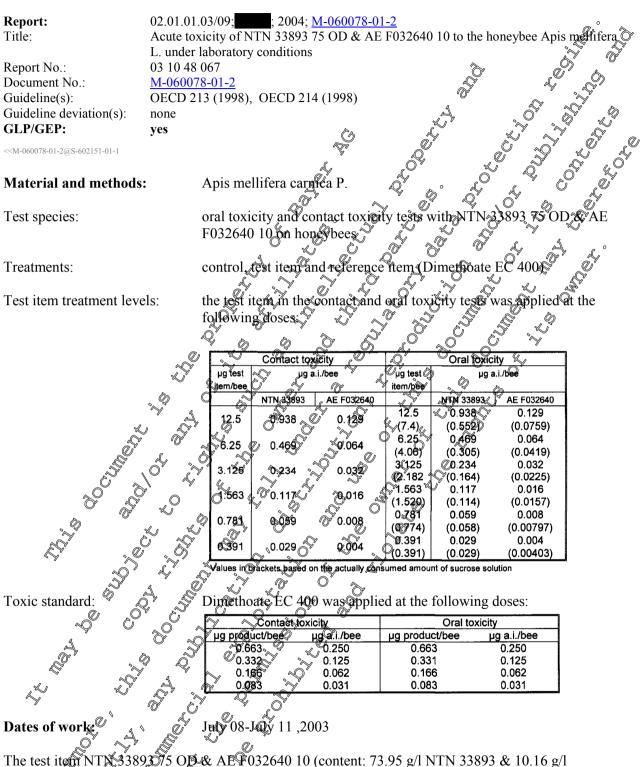
Findings:

- ,	28 August and 8 Santambar 2002	5 5 <u>1</u> 2	
idings:	28 August and 8 September 2003		
Test item	NTN 33893 200 OD		
Test object	🖉 🖉 Apis mellifera 🖉		
Exposure	Contact and Oral		
	Antality (%) at 96 h A O		
Contact exposure		G O	
Water control	O Successe control	. * 3	
EB control		· · · · · · · · · · · · · · · · · · ·	
Corrected montality (%) at 96 th			
0.020 ug a.i/bee		7	
0.043 μg a thee δ		28	
0.092 µgoa.i./boee 🖂		31	
0.199 ug a //beeo 0	93 🖉 0.013 µg a.i./bee	93	
0.427 µg a.i./bee	93 0.013 μg a.i./bee 83 0 0.145 μg a.i./bee 97 0.343 μg a.i./bee	79	
б, 919 µg а, ℓ/bee )	97` 0.343 µg a.i./bee	79	
LD <sub>so</sub> (µg)a.i./bee)	0.078 <sup>0°</sup> μ <sup>O°</sup> L <b>D</b> <sub>50</sub> (μg a.i./bee)	0.057	
95% confidence limits	97 0.343 μg a.i./bee 0.078 LD <sub>50</sub> (μg a.i./bee) 044 - 95% confidence limits 0.123 (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





The test item NTN 33893075 OD & AE F032640 10 (content: 73.95 g/l NTN 33893 & 10.16 g/l Deltameterin, specification: Development No.:30-00317155, Batch: 08137/0023(0019), TOX No.: 06314 00, density: 0.986 g/cm<sup>3</sup>) was tested under laboratory conditions on the honeybee *A. mellifera* after oral and confact 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<sub>50</sub>) expressed in  $\mu$ g of active ingredient or product per bee.



#### **Findings:**

Test item		NTN 33	893 75 OD & AE F	032640 10	
Test object		Но	neybee Apis mellif	era L. 🔗	×
Exposure			contact / oral		
Treatment			LD <sub>50</sub>		
	time		xicity test	oral toxicity fe	
Test item	unic	µg test item/bee	slope b	µg test item/bee	slope b
NTN33893 75 OD & AE F032640	<b>24 h</b> 95 %-cl lower upper	2.554 2.111 3.091	3.326	2.504 4 1.991 2 3.151 4	slope to
10	<b>48 h</b> 95 %-cl lower upper	2.218 1.795 2.741	2:798 J	2.401 2.068 2.788	, <b>₿</b> , <b>6</b> 66 , ĵ, <sup>6</sup>
	time	µg a.i./bee	Slope b	μg@a.i./bee	slope
Reference item Dimethoate EC 400	<b>24 h</b> 95 %-cl lower upper	0.164 0.158 0.489	<b>5</b> .521	0.148 0.126 0.173 0.138 0.109	-6,049 - <sup>6</sup>
EC 400	<b>48 h</b> 95 %-cl lower upper	0.159 0.136 0.185	××× 5.468	0.138 0.109 0.109	3:125

No statistically significant effects of the test item NTN 33893 75 OD & ARF032640 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 test doses of 0.563, 3.125, 6.25 and 12.5 µg test item per bee statistically significant effects of the test item or survival were observed (33.3, 66.7, 90.0 and 100 % mortality, respectively) during 48 hours. The calculated L $D_{50}$  (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 surfival were observed at consumed doses of 0.391, 0.774 and 1.520 µg test item per bee (0.07 and 353 % mortality, respectively) during 48 hours. For the tested oral exposure doses of 2.18274.063 and 7.365 µg test item per bee statistically significant effects of the test item on surfival were observed (96.7, 100 and 900 % mortality, respectively) during 48 hours. Therefore, the calculated  $1D_{50}$  (48%) was 2.401 µg test item per bee in the oral toxicity test. Before bees died in the test item freatments, anathy and immobility were observed shortly after application until the 24 pour assessment.

The LD<sub>50</sub> of the reference tem Drinetheate EC 400 was 0.161  $\mu$ g a.i. per bee in the contact toxicity test after 24 hours. This value was within the preferred range of 0.10- 0.30  $\mu$ g a.i./bee cited in the OECD Guideline 214.

The  $J_{2050}$  of the reference item Dimethoace 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<sub>50</sub> (0.10-0.35 µg a.i./bee) published in the OEC Guideline  $J_{3}$ .

In the reference treatments mathy 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  $\leq 10$  % - was accomplished (being 0 % in the contact and or at toxicity tests after 48 hours).

The LD $\infty^2$  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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<b>Report:</b> Title:	02.01.01.03/10; 2011; <u>M-411260-0</u>	$\frac{1-2}{1}$ S 200 (175+25) G (acute contact and gral) $\hat{0}$
Report No.:	63941035	
Document No.:	<u>M-411260-01-2</u>	A A A
Guideline(s):	OECD 213 and 214 (1998)	
Guideline deviation(s):	none	
GLP/GEP:	yes 🖉	
< <m-411260-01-2@s-602249-01-1< td=""><td></td><td>doNTN 33893); 95.8 % W/w 076.8 gL), s analytical): Batch ID:: 2011-001084,</td></m-411260-01-2@s-602249-01-1<>		doNTN 33893); 95.8 % W/w 076.8 gL), s analytical): Batch ID:: 2011-001084,
Material and Methods		
Imidacioprid + protnioc	onazole FS 200 ( $1/5+25$ ) G midaclopri	$(0) \times 10^{-1} $
Sample Description: TC	476): 2.26 % w/w (25.29 geV), (all value 0X09319-00; Material No.: 80184883; &	s analysical). Salich $10^{\circ}$ . 2051-001694,
density: 1.119 g/mL (20	(0, 1)	
	ions Apis mellifera 30 worker beetwere	Amosether 96 hours to doset of 5.0 °
2 5 1 3 0 63 0 31 and	0 16 ug product per bee by topical applic	ation (contact dose response test) and 30
worker bees per treatme	ent were exposed for 72 hours to doses of	0.49 0.34 0.14 0 12. 0.058. 0.027 and
$0.017 \mu g$ product per be	e by feeding (of al dose response test, val	ue based on the actual aptake of the test
item). The contact toxic	ity test was prolonged for A8 hours due to	Pincreasing portality between 24 and 72
hours, up to a maximum	of 96 hours. The oral toxicity test wasp	rolonged for 24 hours due to increasing
mortality between 24 ar	nd 48 hours, up to a maximum of 72 your	s. A & Q (.
Findings:		
Table 1. Toxicity to Hor	ney Bees; laboratory texts	
~		& . S
Test Item	Imidacloprid + prothioco	nazol <b>O</b> FS 206 (175+25) G
Test object	Or in the second	nelffera Q
Exposure 0		oral
	(solution in Adhäsif (0.5 %)/water	$\mathcal{Q}$ (sugar solution)
Application rate	5.0, 2, 5.1.3, 0.83, 0.31 and 6.6	49, 0.34, 0.14, 0.12, 0.058, 0.027
μg product/bee	5.0, 2, 571.3, 0.03, 0.31 and 0.46	and 0.017
Equivalent to:	0 00 0 40 0 21 \$ 10 0 10 and	0.077, 0.054, 0.022, 0.019, 0.009,
Application rate		0.0043 and 0.0027
μg a.i. imidacloprid/be		
LD50 µg product/bee	24 hours: 45 67 67	24 hours: 0.42
	48 nour 8.2	48 hours: 0.21
	<b>Q2</b> hours: 0.318	72 hours: 0.19
	A 96 hours: 0 Y	
Equivalent to:	24 hours: 0.71 0	24 hours: 0.066
LD <sub>50</sub> µg a.i.	48 hour 0.516	48 hours: 0.033
imidacloprid Dee	72 Insurs: 0.05	72 hours: 0.029
	96 hours 005	
NOEC by product/bee:	04 hours: n.d.	24 hours: 0.017
N & A	48 hours: n.d.	48 hours: 0.017
	72 hours: n.d.	72 hours: 0.017
	48° 96 hours: n.d.	
A LD and a LD <sub>20</sub> coul		
The context and crol I	$D_{\rm c}$ (24 h) values of the reference item (di	matheasta) were calculated to be 0.24

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 magner 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 x and/or apathy) were observed amongst the dose levels. There was a dose and time related pattern discernible.

#### Oral Test:

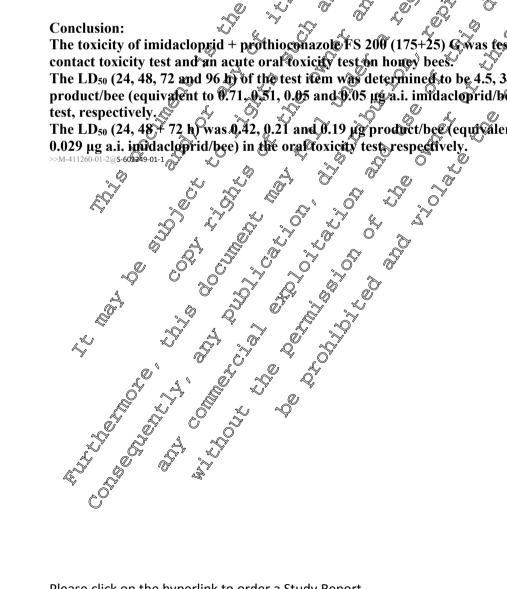
The oral test was also prolonged for a further 24 bours up to 72 hours due to increasing mortality between 24/48 hours. In the oral test, the maximum nominal dose level of the test item (1.0 0.5 and 0.25 ug product/bee) could not be achieved, because the bee did not ingest the fully volume of treated sugar solution even when offered over a period of 6 hours. Oral doses of 0.49, 0.34, 0.14, 002, 0.058 and 0.027 µg product per bee resulted in mortality ranging from \$0.0 % to 16.7 A at the end of the test (72 hours after application). No mortality occurred in the 0.017 dg per bee - dose group and in the control group (50% sugar solution), respectively.  $\Im$ X, 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 imidaclopyid + prothioconazole FS 200 (175+25) Gwas tested in both, an acute

The LD<sub>50</sub> (24, 48, 72 and 96 b) 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

The LD50 (24, 487 72 h was 0, 42, 0.21 and 0.19 pg product/be (equivalent to 0.066, 0.033 and





Report:	02.01.01.03/11; ; ; 2014; <u>M-500305-0</u>	)1-3	o
Title:	Effects of imidacloprid FS 350A G (acute	contact and oral) on l	honey bees (Apis
	mellifera L.) in the laboratory		inoliey bees (Apise 5
Report No.:	89281035	ð	
Document No.:	<u>M-500305-01-3</u>	- A A A A A A A A A A A A A A A A A A A	A A
Guideline(s):	OECD 213 and 214 (1998)	10%	
	US EPA OCSPP Guideline No. 850.3080	A	
Guideline deviation(s):	none 🔊		
GLP/GEP:	yes v	<u> </u>	
< <m-500305-01-3@s-602282-01-1< th=""><th>L.</th><th>,Õ¥ ×</th><th></th></m-500305-01-3@s-602282-01-1<>	L.	,Õ¥ ×	
Material and Methods	: " <sup>©</sup>		

Imidacloprid FS 350A G: imidacloprid (NTN 33895) 30.4 % w/w, 355 29/L avalytical, Batch ID:EDFL020681; Sample Description: TOX10239-00; Workoder: 13011454; Material Noc 0481 «

Under laboratory conditions Apis melliferation worker bees per treatment level were exposed for 9@hours to doses of 500.0, 250.0, 125.0, 62.5, 31.3, 15.6 and 7.8 ng a j per bee by topical application (contact dose response test) and 30 worker bees per treatment level were exposed also for 36 hours to doses of 91.7, 72.5, 37.8, 17.7, 10.0, 7.2 and 35 ng pr. 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

	A & S & S	4
Test Item	A S S Inidaclop	n AFS SOA G
Test Species		mellifera O
Exposure	mantact ~	A V oral
	(solution in Adhäsit) (solution in Adhäsit)	w w/v sucrose solution)
Application rate ng a.j. Bee	500.0, 50.0, 125.0, 2.5, 31, 3,	01.7, 72.5, 37.8, 17.7, 10.0, 7.2 and
	2 15% and 78 8	3.5
LD <sub>50</sub> ng a.i./bee	24 hours 154.0, O	24 hours: n.d.**;
	5 048 howrs: 60 07, 5	48 hours: 53.7
	72 hours: 49.5;	72 hours: 29.3;
<u> </u>	20 nourse 47.6	96 hours: 26.5
LD <sub>20</sub> ng G1./bee	24 hours: 39 8,	24 hours: n.d.**;
	48 Kours; 29.7;	48 hours: 6.9;
	76 hours 23.9;	72 hours: 7.6;
	96 house: 24.9	96 hours: 9.0
LD <sub>10</sub> ng a.i./be	24 hours: 19.6;	24 hours: n.d.**;
	4@hours: 14.6;	48 hours: 2.4;
	2 hours: 16.3;	72 hours: 3.8;
	96 hours: 17.7	96 hours: 5.1
NOED ng a Bee* 5	24 hours: 31.0;	24 hours: < 3.5;
	48 hours: 16.0;	48 hours: 7.2;
	72 hours: 16.0;	72 hours: 7.2;
Ŭ	96 hours: 16.0	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.



The contact and oral LD<sub>50</sub> (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, 2500, 125.0, 62 5, 31.3 and 15 6 ng a between 24/48 and 48/72 hours. Dose levels of 500.0, 2500, 125.0, 62 5, 31.3 and 15 6 ng a between 24/48 mortality of 100.0, 96.7, 90.0, 73.3, 16.7 and 13.3 % at test termination (96 hours). Bo 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 (7.9 ng a bee). During the 48 hours assessment some bees in the four highes 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 my more. All ther subviving bees appeared normal.

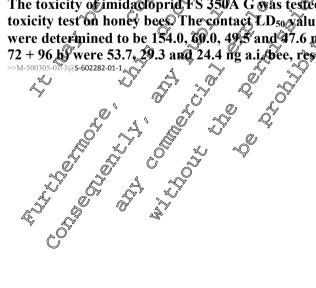
¥,

#### Oral Test:

The oral toxicity test was also prolonged for a torther to hous up to 96 hous 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 \$25 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 coses of 91.7, 42.5, 37.8, 17.4, 10.0, 7.2 and 3.5 ng a.i./bee resulted in mortality ranging from 9000 % to 00.0 % at the ord of the test (96 hours after application). There was 6.7 % morfality in the control group (sperose 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, 1,77, 10.0, 7.2 and 3.5 bg a.i./bee). After 24 hours discoordinated movements, moribuperity 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 94.7, 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 2.5 ng a.i./beg and after 96 hours only one single bee in the highest (91.7 ng a.i./bee) showed moving coordination problems. K, A

#### **Conclusion:**

The toxicity of imidactopric FS 350A G was tested in both, an acute contact and an acute oral toxicity test on honey been The contact LD50 alues (24 + 48 + 72 + 96 h) of imidacloprid FS 350A G were determined to be 154.0, 69.0, 49.5 and 47.6 pg a.i./bee, respectively. The oral LD<sub>50</sub> values (48 + 72 + 96 by were 53.7, 29.3 and 24.4 ng a.i. bee, respectively.





Report:	02.01.01.03/12; 2014; M-503109-01-3 Effects of imidacloprid + pencycuron FS 370 (120+250) G (acute contact and ord) on honey bees (Apis mellifera L.) in the laboratory 89661035 M-503109-01-3 GLP compliant study based on OECD 213 and 214 (1998) US EPA OCSPP Guideline No. 850.3020 none <b>yes</b> s: rron FS 370 (120+250) G: initial coloprid (NTN 33893): 40.4 % W(119.8 gA), 01): 21.9 % w/w (252.0 g/L), (all values malytical); Batch HD: ECE9101025; DX09865-00; Material No.: 05866316 Specification No.: 40200008024 - 02; 0°C). tions <i>Apis mellifera</i> 30 wolker bees were exposed for 96 hours to doses of 40, 2.0, µg product per bee by topical application (contact dose response test) and 30 ent were exposed for 96 hours to doses of 0.75, 0.39, 0.2320.14 and 0.07µg ing (oral dose response test, value based on the actual inftake of the test item). prolonged for 48 hrs due to increasing nortality between 24 and 72 hours, up to a
Title:	Effects of imidacloprid + pencycuron FS 370 (120+250) G (acute contact and ord) on $\bigcirc$
Damant Mari	honey bees (Apis mellifera L.) in the laboratory
Report No.:	89661035 M 502100 01 2
Guideline(s):	GLP compliant study based on OECD 213 and 214 (1009
Guideline(s).	US EPA OCSPP Guideline No. 850 3020
Guideline deviation(s).	none
GLP/GEP:	ves an a c c c c c c c c c c c c c c c c c
OLI/OLI:	
< <m-503109-01-3@s-602312-01-1 Matavial and Mathada</m-503109-01-3@s-602312-01-1 	
Inide alarrid   nonavou	1.100 = 1.00 + 1.00 + 1.00 + 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 = 1.00 =
minuaciopita + pencycu	1011 FS 570 (120+250) G. Mardaciopila (N11N 55895). $49.4%$ (119.8 g. 2),
pencycuron (NTN 1970	1): 21.9 % W/W (252.0 g/B), (all values analytical); Balch $\mu$ X EC E9101023;
Sample Description: TC	JX09805-00; Material No.: 058005 (6, Specification No.: 02000008024 - 02;
density: 1.151 g/mL (20	$f(\mathbf{C})$
Under laboratory condition	tions Apis melliferation worker bees were exposed for the nours to doses of 400, 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 treatme	ent were exposed for $96$ hours to doses of $0.75$ , $0.39$ , $0.2420.14$ and $0.09 \mu g$
product per bee by feed	ing (oral dose response test value based on the actual uptake of the test item).
Both toxicity tests were	prolonged for 48 his due to increasing mortality between 24 and 22 hours, up to a
maximum of 96 hours.	
<b>T</b> 1 <b>1</b>	
Findings:	
Table 1. Toxicity to Hereit	oney Bees, laboratory tests
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	rron FS 370 (120+250) G: imidaeloprid (NTN 33893): 40.4 % V/w (119.8 gH), 1)): 21.9 % w/w (252.0 gH), (all values inalytical); Batch ID: ECEd9101025: DX09865-00; Material No: 0566316 Specification No: A0200008024 = 02; (-), tions <i>Apis mellifere</i> 30 wolker bes were exposed for 96 hours to dose of 40, 2.0, µg product per be by topical application (contact ubse response test) and 30 ent were exposed for 96 hours to doses of 0, 75, 0, 39, 0.220, 14 and 0.00 µg ing (oral dose response test) value based on the artual inflake of the test prolonged for 48 hrs due to increasing montality between 24 and 72 hours, up to a one? Bees Plaborator vests
õ	



### **Imidacloprid Bee Studies Compilation of Study Summaries**

Issue date 2017-11-22

Test Item	Imidacloprid + pencycu	ron FS 370 (120+250) G	
Test Species	Apis mellifera		
Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (50 % w/ySucrose solution)	F
Application rate μg product/bee	4.0, 2.0, 1.0, 0.50, 0.25 and 0.13	0.75, 0.39, 0.23, 0.14 and 0.07	Ņ
Equivalent to: Application rate μg a.i. imidacloprid/bee	0.416, 0.208, 0.104, 0.052, 6026 and 0.014	0.0790, 0.0406, 0.0239, 0.0146 and 1 0.0003 5 4 0.0005 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	
LD <sub>50</sub> μg product/bee	24 hours: 2.50 48 hours: 0.54 72 hours: 0.42 96 hours: 0.38		
LD <sub>20</sub> μg product/bee	24 hours: 0.54 72 hours: 0.42 96 hours: 0.38 24 hours: 0.33 48 hours: 0.33 48 hours: 0.42 96 hours: 0.33 48 hours: 0.42 48 hours: 0.45 48 hours: 0.45	24 hours: < 607 & 0 48 hours: < 607 & 0 27 hours: 0.0620 & 0 96 hours: 0.089 & 0	
LD <sub>10</sub> μg product/bee	4@hours 0.11 0 5 0 4@hours 0.15	45 hours 0.07 0 22 hours 0.07 0 22 hours 0.07 0	
NOED µg product/bee* 📎	24, 48, 72, 96 hours: 0.25	24, 48, 72, 96 honrs: < 0.07	
Equivalent to: LD <sub>50</sub> µg a.i. imidacloprid/bee	24 hours: 0.260 48 fours: 0.956 72 hours: 0.044 96 hours: 0.040	24 hours: > 0.078 48 hours: > 0.078 72 hours @.108 56 hours 0.100	
Equivalent to 5 2 LD <sub>20</sub> µg a 6 imidacloprid/bee	96 hours: 0.44 24, 48, 72,96 hours: 0.25 24 hours: 0.260 48 hours: 0.260 48 hours: 0.056 72 hours: 0.044 96 hours: 0.044 24 hours: 0.034 48 hours: 4.019 24 hours: 0.023 96 hours: 0.024 24 hours: 0.024 24 hours: 0.024	24 1&urs: < 0.0073 48 hours: 0.004 % hours: 0.006 96 hours: 0.009	
Equivalent to: LD <sub>10</sub> µg a.i. imidacloprid/bee	12 hours: 0.001 96 hours: 0.000 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. anidacloprid/best	29, 48, 77, 96 hours: 0.026	24, 48, 72, 96 hours: < 0.0073	

\* The NOED was estimated as ang Eister Exact Test (a minuse comparison, one-sided greater,  $\alpha = 0.05$ ).  $\bigcirc$ 

The contact and oral LD<sub>50</sub> (26h) values of the reference item (dimethoate) were calculated to be 0.22 and 0.23  $\mu$ g a.i./bee respectively. 0.23 µg a.i./bee, respectively

### **Observations:**

# Contact Tes

Ì The contact test was prolonged for a further 48 hours up to 96 hours due to increasing mortality between 24 and 2 hours. Application of 4.0, 2.0, 1.0, 0.50, 0.25 and 0.13 µg/bee of imidacloprid + pencycuron 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,



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 45 and 2 0.50 µg/bee dose groups, respectively. At the last assessment (96 hours following application) and or two bees were still affected in the 2.0, 1.0 and 0.50 ug/bee dosing groups. No behavoural 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 ducto increasing portality between 24 and 72 hours. In the oral test, the maximum nominal dose level of the test item (1, 0, 0.50 and 0.2) 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 \$3.3 % to 16.7% at the end of the test (i.e. 96 hours after application). 6 2% mortality decurred 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 bserved in all dose . groups during the 4-hours assessment. 24 and 48 hours for wing reatment 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 apathetic in the 0.75, 0.39 and 0.25  $\mu$ g/bee dose levels, put include the point of the bee was found to be affected in the 0.75 and 0.23  $\mu$ g/bee dose levels, respectively.

Conclusion: The toxicity of imidacloprid + pencycurof FS 370 (120+250) & was tested in both, an acute contact toxicity test and an acute oral toxicity test on honey bees. The LD50 (24, 48, 72 and 96 h) of the test item was betermined to be 2,50, 0.54, 0.42 and 0.38 µg product/bee (equivalent to 0,260, 0,456, 0.044 and 0.040 µg a, Kimidaclopric bee) in the contact toxicity test, respectively.  $\bigcirc$ 

toxicity test, respectively. The LD<sub>50</sub> (72 + 969) was 1.04. and 0.96 µg product/bee (equivalent to imidacloprid/bee) in the oral toxicity test. The LD<sub>50</sub> (72 + 96 a) was 1.04 and 0.96 µg product/bee (equivalent to 0-108 and 0.10 µg a.i.



<b>Report:</b> Title:	02.01.01.03/13; 2014; <u>M-501653-01-3</u> Effects of clothianidin + imidacloprid FS 275	$(100+175)$ G (acute contact and or $\overset{\circ}{\otimes}$ on $\overset{\circ}{\otimes}$
The.	honey bees (Apis mellifera L.) in the laborator	v
Report No.:	89691035	
Document No.:	<u>M-501653-01-3</u>	
Guideline(s):	Effects of clothianidin + imidacloprid FS 275 honey bees (Apis mellifera L.) in the laborator 89691035 <u>M-501653-01-3</u> GLP compliant study based on OECD 213 and US EPA OCSPP Guideline No. 850.3020 none yes prid FS 275 (100+175) G: of othianidin (TI- 03): 15.8 % w/w (176.7 gL) (all analysical ; Sample Description: TOX10068-00, Spec °C). ions <i>Apis mellifera</i> 30 worker bees per trea 5, 0.13, 0.063 and 0.031/µg product per be orker bees per treatment level were exposed ig product per bee by feeding (oral dose res	
Guideline deviation(s):	none	
GLP/GEP:	yes 🖓	
< <m-501653-01-3@s-604671-01-1< td=""><td>Ly</td><td></td></m-501653-01-3@s-604671-01-1<>	Ly	
Material and Methods:		
Clothianidin + imidacloj	prid FS $2/5$ (100+1/5) G: <b>G</b> othianidin (11-	435) * 8.95 % W/W (100.3 g/L),
Imidacioprid (NTN 3389	93): 15.8 % W/W (1/6./ g/L) (all analytical	Values); Batch-UX: 2013-001547,
$\frac{1}{20}$	Sample Description. AOX 10008-00, Spec	nication 100
Under Jahoratory conditi	C).	tment level were exposed for 18 hours
to doses of 1.0.0.50.0.2	15, 0.13, 0.063 and $0.03$ up product for the	eby topical application (contact dose
response test) and 30 wo	orker bees per treatment level were exposed	for 48 hours of doses of 0 0 0 11
0.053, 0.027 and 0.013 i	ig product per bee by feeding (oral dose re	sponse test. Value based on the actual
intake of the test item).	ig product per bee by feeding (oral dose res	
Findings:		
T munigs.	V & B L & Y	
Table 1. Toxicity to Ho	nev Bees, laboratory rests	
Test Item	Clothoniding midaelo	priorS 276 (100+175) G
Test species	Clothending imidaelo	ellifer O
Exposure	(solution in Adh: At (0.2%)/water)	v oral v (sugar solution)
Application rate µg product/bee	5 1.0, 050, 0.25, 0.13, 0.062 and 0 0.030	© 0.17, 0.11, 0.053, 0.027 and 0.013
LD50 µg product/be	4 24 Dourse 0.39 2 2 2	24 hours: 0.062
	A shouty 0.2%	48 hours: 0.058
LD20 µg product/bee	24 hours: 0901 0	24 hours: 0.034
	~ 4 Nours 0.093 9	48 hours: 0.030
LD10 µg product/bee	2 0.24 hours: 0.050	24 hours: 0.025
	48 hours: 0.951	48 hours: 0.021
NOED µg product/bee*		
NOZD µg product/bee	2 /hours 0.06 / /	24 hours: 0.027 48 hours: 0.027
* The NOED as estimate	ed using Fisher Exact Test (pairwise comparisor	n, one-sided greater, $\alpha = 0.05$ ).
The control and Sol I I	30 (2/H) values of the reference item (dim	othests) were calculated to be 0.28
and 0.1 Aug a jobee, resp		lemoate) were calculated to be 0.28
Observations:		
s O		
Contact Test:		
	rlink to order a Ctudu Danast	
Please click on the hype	rlink to order a Study Report.	



Test item dose levels of 1.0, 0.50, 0.25, 0.13, 0.063 and 0.031 ug 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). ð 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 pours 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 xctual oral doses of 0.17, 0, 1, 0.03, 0.037 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 (steerose 90% w/v solution = 900 g %sucrose/L tap water).

Behavioural abnormalities (e.g. moribund becs or affected bees) were found during the 4-hours assessment in the 0.17, 0.11, 0.053 and 0.027 µg product/bee treament groups. A few bees were behaving abnormal 24 hours following treatment in the 0.17, 0.11 and 0.053@g/bee dose levels and one and 6 bees were found to be affected during the 48-hours assessment in the 0.17 and 0.1 Lug/bee treatment group, respectively. No behavioural abnormalities were found in the 0013 µc product/bee dosing group during the test. 

#### **Conclusion:**

The toxicity of clothianidin + imidacloppid FS275 (100+175) G was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD50 values 724 and 48 h) of clothianidin + imidacloprid FS 275 (100+175) Gavere determined to be 0.39 and 0.29 µg product/bee,

and an acute oral toxicity test on honey bees. The contact LD50 values (24 ard 48 h) of clothianidi + imidacloprid FS 275 (100+075) (Govere determined to be 0.39 and 0.29 µg product/bee, respectively. respectively. The oral LD50 value (24 hr) 48 h) was (002 and 0.058 µg product/bee, respectively.



# 02.01.02 - Semi-field

	02.01.02/01; [1988; M-038201-01-4 Tolerability of seed treatments to bees (bee tunnel I) VAZ 4/88 M-038201-01-4 US EPA OCSPP Guideline No. 850.SUPP none no for the root systemic active ingredients NCN 33893 and KU 0337 is the seed irst of these two active ingredients was tested last year. Ten weeks after the there was an unmistable reduction in flower visits and an increased number of 13/87). We have now epeated this trial with winter rape. There were 230 days wing (= seed treatment) and Howering. associated with this more realistic trial design. Winter rape can be seed treated 93 and JKU 0337 without any risk to bees 02.01.02/02; [197; M-005376-01-3]
Renort.	02.01.02/01·
Title:	Tolerability of seed treatments to bees (bee tunnel I)
Report No.:	VAZ 4/88
Document No.:	<u>M-038201-01-4</u>
Guideline(s):	US EPA OCSPP Guideline No. 850.SUPP
Guideline deviation(s):	none & A A A
GLP/GEP:	no V Q Q Q Y
< <m-038201-01-4@s-604650-01-1< td=""><td></td></m-038201-01-4@s-604650-01-1<>	
One possible indication	for the root systemic active ingredients NTN 33893 and KU 0337 is the seed
treatment of rape. The f	irst of these two active ingredients was tested last year. Sen weeks after the
summer rape was sown,	, there was an unmistakable reduction for 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 sow	ving (= seed treatment) and Howering.
No danger to bees was a	associated with this more realistic triat design. Winter rape can be seed treated
with oftanol, NTN 33 8	93 and JKU 0207 without any risk to bees in the second secon
>>M-038201-01-4@ <b>S-604650-01-1</b>	
	02.01.02/02; Bees Systemic nature of insecticidal seed freatments DVG 10/97
Report:	$02.01.02/02;$ $1997;$ $M_{2}005376001-3$
Title:	Bees V systemic nations of insecticidal seed freatment O
Report No.:	DVG 10/9%
Document No.:	DVG 10/94
Guideline(s):	
Guideline deviation(s):	
GLP/GEP:	or the root systemic derive ingredients that very subscripts under the sector of 13/87). We have now repeated this trial with winter rape. There were 230 days ving (= seed treatment) and Howering. associated with this more realistic trial design. Winter rape can be seed treated 93 and JKU 0337 without any risk to bees 02.01.02/02; 1997; M-005376-01-3 Bees I systemic nature of insecticidal seed freatment DVG 10/9 M-005376-01-3 possibility that systemic active ingredients may appear in nectar. The active
<	possibility that systemic active ingredients may appear in nectar. The active
I nere is the theoretical	, which is dangerous to bees, is used as a seed greatment for winter rape. The
ingredient imidacioptid	, which is dangerous to bees, is used as a seed meatment for whiter rape. The
question to be investiga	ted was whether there is a risk to bees at the time of flowering, i.e. 7 to 8 months
after sowing. In this tria	h, the seed-treated winter rape was sown on 10.9.96 and started to flower on er sowing. The bee colonies (three comb nucleus colonies) were placed in the
5.5.9/, «Ise. 25/ days and	$50 \text{ fp}^2$ of ground on 6.5 $\text{sf}$ . The bee contained were removed from the tunnels after
24 davia on 20.5 m wh	5044° Of ground on 0.5.30. The see conomies were removed from the tunners after
24 days, on 50.5.9 P, will	en flow Fing ended. In addition to an untreated control, the following seed
ireatinents were used.	
No. 2) Pote ovfluthrin	2 + 2 + 1 + 2 + 2 + 2 + 2 + 2 + 2 + 2 +
No. 2) Deta cyfluthrin 6 No. 2) Deta cyfluthrin 6	z  fundation for a single f
No. 3) $\Delta \mathcal{C}$	$\int \frac{1}{2} \int $
No. 54 TL 425	$a_{\rm M} = \frac{1000}{2}$ g/dt = 1050 g a.1./dt
>>M-0057/6-01-3@\$-602104-01-2	$\sqrt{3}$
a, <sup>\</sup>	
A A	
Ũ	which is dangerous to bees, is used as a seed treatment for winter rape. The ted was valether there is a risk to bees at the time of flowering, i.e. 7 to 8 months is the seed-treated winter rape was sown on 10.9.96 and started to flower on er sowing. The bee colonics (three comb nucleus colonies) were placed in the 50 m² of ground on 6.5.97. The bee colonies were removed from the tunnels after en flowering ended. In addition to an untreated control, the following seed the time of 900 k 420 k 2500 g/dt = 200 & 1050 g a.i./dt imidaeloprid (080 k 420 k 2500 g/dt = 400 & 2100 g a.i./dt 1022 70 WS 1500 g/dt = 1050 g a.i./dt 1022 70 WS 1500 g/dt = 1050 g a.i./dt



Report:       02.01.02.03;       1999; M438651-01-4         Title:       Observations in a tunnel trial with bees following seed treatment of summer rate;         Report No:       M438651-01-4         Guideline(s):       US EPA OCSPP Guideline no. 850 SUPP         Guideline(s):       US EPA OCSPP Guideline no. 850 SUPP         Guideline(s):       no         CLP/GEP:       no         Clease of KKO 3334, the suspicion arcse, data they way fraction by the bees is the fire of flowfing of summer rate; way strength; global mathematical in time (stagered soving of scel). Poichon 2.5 and 50 (single and flowfing of summer rate; way strength; global mathematical and opposed strength; global mathematical and sceles of KKO 3334, the suspicion arcse, data they way fraction by the bees is the fire weight and colony strength; decreased; the nectar supply could be be gaintaided and datages its to fires.         In the case of KKO 3334, the suspicion arcse, data they way fraction by the bees is the fire weight and colony strength; decreased; the nectar supply could be be gaintaided and strengt be weight and colony strength; decreased; the nectar supply could be be gaintaided and when cells were more minor, they observe optimized in a strengt be weight and colone strengt be represented with the sector of the by 50.0169. <sup>3</sup> Title:       1999 Fagluagotoff Gradeho spid forgeting applied to another proper the they be explored and strengt be more and when the sector be strengt be weight and colone strengt be represented with the sector be strengt be they be proper to the book sector be strengt be represented by the sector be more flow book sore the strengt be weight and the sector be pr		
Tite:       Observations in a tunnel trial with bees following seed treatment of summer raps         Report No:       M486651.00.4         Guideline deviation(s):       -         GLP/GEP:       no         Construction of seed treatments on bees at the time of flowFing of sumper raps was injectinged in an funnel trial with two series separated in time (staggered sowing of seed). Postho 2.5 and 5.0 (single and doubd quantities). It 435 and fipronil had no effect on mogfinity. Hower Apils, hire weight, colony strength decreased, the nectar supply could bot be not dangerous to bees.         In the case of KKO 3334, the suspicion arose that their wass? fraction by the bees has the fire weight and colony strength decreased, the nectar supply could bot be daintaifed and egg laying wis reduced. At resulting in a reduction in the size of the bood mest and with long for reduced. At resulting in a reduction in the size of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of the bood mest and with long for reduced at the strength of th	Donart.	02 01 02/02: 1000: M 086651 01 4
Report No:       DVG 7/98         Document No:       ML86651-01-4         Guideline deviation(s):		
trial with two series separated in time (staggered sowing of seed). Portho 2.5 and 50 (single and double quantities), TI 435 and fipronil had no effect on morganity, flower zeits, hive weight, colony strength food supply and brood. They can therefore be assumed to be not dangegoins to bees. In the case of KKO 3334, the suspicion arose that their was freaction by the bees as the five weight and colony strength decreased, the nectar supply could for be available and seg laying was reduced, a 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 a strength of the series and a striking increase in the proportion of empty cells. Although these effects were minor they occurred in both series a strength of the series and a striking increase in the proportion of empty cells. Although these effects were minor they occurred in both series a strength of the series and the		DVG 7/98
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cells. Although these effects were minor they occurred in both series         Preport:       02.01.02/04         1999 Evaluation of:       Gatcho scid dressing applied to canola of the loney bec (Apis mellifer Limitacus) and infead. Staktactowan (budian head research station site)         Report:       Multipsoul-133         Document No:       Multipsoul-133         Guideline(s):       US EPA OCSPP Guideline 850 SUPP.         Guideline(s):       No:         Multipsoul-133       none.         GL/GGP:       no         Onclusions:       Gatcho set affects on colony development. Brood rearing was with inste considered to be normal, worker bee survival was within expected limits, worker bee populations, increased while the colonic syster confined to the colonic syster sources, dead bees were not observed in front of the colonic syster confined to the colonic syster sources, dead bees were not observed in front of the colonic syster source affects of Confidor SL 200 on the honey bee (Apis nething Science 14).         Report:       02.01/92/05       2001; W2084030-01-2         Title:       10.02/04/04-2       2001; W2084030-01-2         Title:       02.01/92/05       2001; W2084030-01-2         Title:       02.01/92/05       2001; W2084030-01-2         Title:       02.01/92/01-2       2001; W2084030-01-2         Total best Assessment of side effects of Confidor SL 200 on the honey bee (Apis neffice 20)         O	colony strength decreas	in the size of the head at a day of the training in a second signal and the size of the head at a size of the head at a size of the size of the head at a size of the size of
Conversion       02.01.02/04       1999       M-005504-0023         Title:       1999       Evaluation of: Catcho sed dressing applied to canola or the loney bee (Apis mellifera Linnäeus) at Indian head, Saskatchowan (Indian head research station site)         Report:       M-00750401-3         Document No:       M-00750401-3         Guideline (s):       VS EPA OCSPP Guidenne SasSUPP         Guideline deviation(s):       none         GLP/GEP:       no	resulting in a reduction	In the size of the prood mest and a striking increase in the proportion of empty
Report:       02.01.02/04       1999; M-02504-023         Title:       1999 Equivation C Grache speed applied to enhole op the kopey bee (Apis melliphra Linnáeus) at Indian head, Saskatclówan (Indian head research station site)         Report No:       M-075504-01-3         Guideline(s):       US EPA OCSPP Guideline 850 SUPP         Guideline(s):       Inon         GLP/GEP:       no         M-075504-01-3       Inon         Guideline(s):       US EPA OCSPP Guideline 850 SUPP         Guideline(s):       Inon         Guideline(s):       Inon         Guideline(s):       no         Guideline(s):       Inon         Gaucho treated ranolactid not Show day obvieus or pressured 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 colonice over confined to the follmation cages, dead bees were not observed in front of the colonice and forging activity was simplar in both colonies.         Subtraction for cage deviation(s):       Turget lest, Assessment et pide effects of Confidor SL 200 on the honey bee (Apis in effect activity)         Propert       02.0149/09/01-12         Title:       M-08403/021-2         Opcument No:       M-08403/021-2         Opcument No:       M-08403/021-2         Opuidelindeviation(s):       Fost substation </td <td>cells. Although these ef</td> <td>rects were minor they occurred in both series in so in the series in the</td>	cells. Although these ef	rects were minor they occurred in both series in so in the series in the
Title:       1999 Ecaluation of: Gate ho seed dressing applied to can all on the honey bee. (Apis mellifera Limitaeus) at Indian head, Saskatel@wan (Judian head research station site)         Report No:       Mo175501401-3         Guideline(s):       US EPA OCSPP Guideline 850 SUPP         Guideline deviation(s):       none         GLP/GEP:       no         Guideline deviation(s):       no         Gaucho treated can alardin do show alay obvious oppleasured adverse affects on colony development. Brood rearing was with limfs considered to be normal, worker bee survival was within expected limits, worker bee populations increased what the obtaine system of the colonies.         Report:       02.0192/05         Title:       Tubel test. Assessment of side effects of Confidor SL 200 on the honey bee (Apis mellifera Limite test. Assessment of side effects of Confidor SL 200 on the honey bee (Apis mellifera Limite and the colonies.         Provide Limite test.       2001/09/01-BZEU         Poent No:       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Guideline (s):       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Guideline (s):       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Guideline (eviation(s):       fone	~~wi-000031-01-4@ <b>S-004059-01-1</b>	
Title:       1999 Ecaluation of: Gate ho seed dressing applied to can all on the honey bee. (Apis mellifera Limitaeus) at Indian head, Saskatel@wan (Judian head research station site)         Report No:       Mo175501401-3         Guideline(s):       US EPA OCSPP Guideline 850 SUPP         Guideline deviation(s):       none         GLP/GEP:       no         Guideline deviation(s):       no         Gaucho treated can alardin do show alay obvious oppleasured adverse affects on colony development. Brood rearing was with limfs considered to be normal, worker bee survival was within expected limits, worker bee populations increased what the obtaine system of the colonies.         Report:       02.0192/05         Title:       Tubel test. Assessment of side effects of Confidor SL 200 on the honey bee (Apis mellifera Limite test. Assessment of side effects of Confidor SL 200 on the honey bee (Apis mellifera Limite and the colonies.         Provide Limite test.       2001/09/01-BZEU         Poent No:       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Guideline (s):       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Guideline (s):       Mo8403(201-2)         Cuideline (s):       Mo8403(201-2)         Guideline (eviation(s):       fone		
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Report No.:       M.07550401.3         Guideline(s):       US EPA OCSPP Guideline & Stassure & Stassu		1999 Evaluation of: Gaucho seed dressing applied to canola of the honey bee, (Apis
Report No.: Me07550401-3 Document No.: Me07550401-3 Document No.: Me07550401-3 Guideline(s): US EPA OCSPP Guideline Sto SUPP Guideline deviation(s): none GLP/GEP: no Conclusions: Gaucho treated canolactid not show any obvious or preasured 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 colonie swere confined to the follination cages, dead bees were not observed in front cfthe colonies and foreing activity was similar in both colonies. Materials and Methods Report No. GLP/GEP: yes Could D99/01-BZEU Document No. GLP/GEP: yes Could D99/01-BZEU Document No. GLP/GEP: yes Could D99/01-BZEU Document No. Materials and Methods Test solution(s): fione GLP/GEP: yes Could D99/01-BZEU Materials and Methods Test solution(s): fione GLP/GEP: yes Could D99/01-BZEU Materials and Methods Test solution(s): fione CLP/GEP: yes Could D99/01-BZEU Materials and Methods Test solution(s): fione CLP/GEP: yes CLP/GEP: ye		mellifera Linnaeus) at Indian head, Saskatchewan (Indian head research station site)
Guideline(s): US EPA OCSPP Guideline & Sty SUPP Guideline deviation(s): non- GLP/GEP: no Conclusions: Gaucho treated ranolactif not show any obvious or measured adverse affects on colony development. Brood rearing was with limfs considered to be normal, worker bee survival was within expected limits, worker bee populations, increased while the golonies over confined to the folling of the cases, dead bees were not observed in front of the colonies and forgeing activity was similar in both colonies. ************************************	Report No.:	<u>M-075504401-3</u>
GLP/GEP:       no         Second status       no         Conclusions:       Gaucho treated canolacid 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 over confined to the pollination cages, dead bees were not observed in front of the colonies, and foroging activity was similar in both colonies.         Prepert:       02.0192/05         Turnel test:       Assessment of side effects of Confidor SL 200 on the honey bee (Apis mellifer U) in apple or pard following application before flowering (mouse-ear stage) of the colon.         Preport No:       2001099/01-BZEU         Document No:       M48403(201-2)         GLP/GEP:       yes         yes       Yes         Materials and Methods       Test splostance         Test splostance       Signed on ZPO yes         Name? Confidor SI/200;       yes         Purity: 1947g/L (nominal: 200 g/L).       The following study was designed to determine the effects of Confidor SL 200 on the honey bee (Apis	Document No.:	M207550 P01-3 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
GLP/GEP:       no         Second status       no         Conclusions:       Gaucho treated canolacid 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 over confined to the pollination cages, dead bees were not observed in front of the colonies, and foroging activity was similar in both colonies.         Prepert:       02.0192/05         Turnel test:       Assessment of side effects of Confidor SL 200 on the honey bee (Apis mellifer U) in apple or pard following application before flowering (mouse-ear stage) of the colon.         Preport No:       2001099/01-BZEU         Document No:       M48403(201-2)         GLP/GEP:       yes         yes       Yes         Materials and Methods       Test splostance         Test splostance       Signed on ZPO yes         Name? Confidor SI/200;       yes         Purity: 1947g/L (nominal: 200 g/L).       The following study was designed to determine the effects of Confidor SL 200 on the honey bee (Apis		US EPA OCSPP Guidenne 850 SUPP 4 S
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Gaucho treated anolatid not show any obvious or measured adverse affects on colony development. Brood rearing was with limits considered to be normal, worker be survival was within expected limits, worker bee populations increased while the follone over confined to the follination cages, dead bees were not observed in front of the colonies and forging activity was similar in both colonies. ************************************	Conclusions.	
rearing was with limits considered to be normal, worker be survival was within expected limits, worker bee populations increased while the colonies were confined to the follination cages, dead bees were not observed in front of the colonies and foreging activity was similar in both colonies. MOTSOULD AGES 60432401-1 Report: 02.0192/05 02.0192/05 001; 0c084032-01-2 Turnel test, Assessment of olde effects of Confidor SL 200 on the honey bee (Apis mellifera L/) in apple or bard following application before flowering (mouse-ear stage) of the colo Report No: 02.0014099/01-BZEU Document No.: 04.08403(201-2) GLP/GEP: 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.0000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10.0000 10.000 10.000 10.000 10.000 10.000 10.000 10.00	Gaucho treated canolacti	d not how any obvious or measured adverse affects on colony development. Brood
populations increased while the colonies over confined to the collination cages, dead bees were not observed in front of the colonies and foraging activity was similar in both colonies. M-07504-01-3655604324-01-1 Report: Turbel test, Assessment of ide effects of Confidor SL 200 on the honey bee (Apis mellifera L) in apple or bard following application before flowering (mouse-ear stage) of the coop Report No: Quideline (s): Guideline deviation(s): Materials and Methods Test substance Name: Confidor SL 200 g/L) The following study was designed to determine the effects of Confidor SL 200 on the honey bee (Apis	rearing was with limits c	onsidered/to be normal worker bee survival was within expected limits, worker bee
in front of the colonies and foreing activity was similar in both colonies. Metorssol 01365 604324 01-1 Report: Title: Report No: Document No: Guideline deviation(s): GLP/GEP: Yes Materials and Methods Test substance Name: Confidor SL 200 on the honey bee ( <i>Apis</i> Materials and Methods Test substance Name: Confidor SL 200 g/L) The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	populations increased wi	the colonie were confined to the colling tion cages dead bees were not observed
<b>Report:</b> 02.0192/05;       2001; Mc084029-01-2         Title:       Turbel test, Assessment of idde effects of Confidor SL 200 on the honey bee (Apis methifera L.) in apple or chard following application before flowering (mouse-ear stage) of the gop         Report No:       20014099/01-BZEU         Document No.:       M-084030-01-2         Guideline deviation(s):       Based on EPPOQuide hie No. 170         Guideline deviation(s):       from         GLP/GEP:       yes         Verous State       Verous State         Test substance       Name: Confidor SL 200 g/L)         The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	in front of the colonies	ind foraging activity was signifar in both colonies
Title: Turnel test Assessment of side effects of Confidor SL 200 on the honey bee (Apis methifers [2]) in apple ordeard following application before flowering (mouse-ear stage) of the erop Report No: 20014099/01-BZEU Document No.: M-084030-01-2 Guideline deviation(s): Hone GLP/GEP: yes M-084030-01-2 Substance Name: Confidor SL 200 g/L) The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>		
Title: Turnel test Assessment of side effects of Confidor SL 200 on the honey bee (Apis methifera L.) in apple ordeard following application before flowering (mouse-ear stage) of the erop Report No: 20014099/01-BZEU Document No.: M-084030-01-2 Guideline deviation(s): Hone GLP/GEP: yes M-084030-01-2 Substance Name: Confidor SL 200 g/L) The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	Q 1	
Title: Turnel test Assessment of side effects of Confidor SL 200 on the honey bee (Apis methifera L.) in apple ordeard following application before flowering (mouse-ear stage) of the erop Report No: 20014099/01-BZEU Document No.: M-084030-01-2 Guideline deviation(s): Hone GLP/GEP: yes M-084030-01-2 Substance Name: Confidor SL 200 g/L) The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>		
Report No. Report No. Courgent No.: M_084030_01-2 Guideline(s): Based on EPPO Quideline No. 170 Guideline deviation(s): GLP/GEP: Yes M-084030_01-2 Guideline State 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 ( <i>Apis</i>	•	
Report No: 200140999/01-BZEU Document No.: M4084030-01-2 Guideline deviation(s): from GLP/GEP: yes 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 ( <i>Apis</i>	Title:	Turbel test: Assessment of side effects of Confidor SL 200 on the honey bee (Apis
Report No.: 20014099/01-BZEU Document No.: M-084030-01-2 Guideline(s): Based on EPPO Guideline No. 170 Guideline deviation(s): none GLP/GEP: yes M-084030-01-200 ess23-01- 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 ( <i>Apis</i>	*	methifera D.) in apple orchard following application before flowering (mouse-ear stage)
Document No.: M4084030-01-2 Guideline(s): Based on EPPO Guideline No. 170 Guideline deviation(s): frone GLP/GEP: yes M-084030-01-2@5 823-01-1 Materials and Methods Test substance Name: Confidor SI2 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 ( <i>Apis</i>	Dana No	
Guideline (s): Guideline deviation(s): Hone GLP/GEP: yes Worker Waterials and Methods Test substance Name: Confidor SI2200; 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 ( <i>Apis</i>		
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	Document No.:	$\frac{M-084030-01-2}{100} \Rightarrow \qquad $
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	Guideline deviation(a):	Based OR EFFO. Ulidemue INO. 1/U
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	CL D/CED.	
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	ULI/UEI:	y y cs w w w w
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	< <m-084030-01-2@s< td=""><td></td></m-084030-01-2@s<>	
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	Materials and Method	
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>		
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	Test substance	
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i>	Name Confidor SI 200	); 🏷
The following study was designed to determine the effects of Confidor SL 200 on the honey bee ( <i>Apis</i> )	purity: 19@ g/L (nomina	af: 200 g/L)
	The following study wa	s designed to determine the effects of Confidor SL 200 on the honev bee (Apis

Please click on the hyperlink to order a Study Report.

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 Mediteranears 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 tupnels 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 apploplots the untreated apple plots for the control.

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

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

- Ŵ
- Flight intensity in the crop (number of Dying bees/tree minute). Behaviour of the bees on the crop and around the hive. •

Dates of work: 30MAB2001 30APR2001 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 8 dead bees/tent in the test substance treatment and 7.3 dead bees/tent in the control. During evaluation day  $\Psi$  - 7 the daily average of dead bees recorded on the linen was 7.9 dead bees/tent in the test substance treatment compared to Q.4 dead bees/tent in the control. The total daily average of dead bees per tent was 12, or 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 dails average flight intensity in the crop ranged from 0.04 - 20.89 forager bees/tree/minute/pent 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 borager bees leaving/entering the hive per minute was 10.31 bees/tent in the test substance freatment and 10.47 bees/tent in the control during the period of assessments.

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

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.s./ha in 500 L water/ha did not cause adverse effects to honey bee mortality, flightentensity in the crop or the brood development of the colonies in this semi-field study.

Report:	02.01.02/06: 2091: M-089338-00-3 × ×
Title:	Confidor SL 200: a multiple rate cage study to determine efforts on honeybees, Apis.
	mellifera L, when applied to flowering Phacelia tanacetifolia
Report No.:	B074AMS
Document No.:	B074AMS
Guideline(s):	US EPA OCSEP Guideline No 850 SUPP
Guideline deviation(s):	US EPA OCSEP Guideline No 850 SUPP
GLP/GEP:	
< <m_089338_01_3@8_603080_01_1< td=""><td></td></m_089338_01_3@8_603080_01_1<>	

<<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 semifield cage test design for honeybees *Apis fiellifera* 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./ba, 9 g a.i./ba, 4 g a.i./ba, 2 g a.i./ba, 1.2 g a.i./ha and 0.6 g a.i./ha. The overall test design was integret with OFEP/EPPO-guidelines (EPPQ, 1992) for cage studies with honeybees.

Small, standardised horevbee colonies were placed in meshed cases of 4 x 5 meter and 2 meter high. Each cage contained approximately 108 untreated flowering *Phicelige* 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 to 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 vater treated control and a reference item (PennCap M, a 240 g/I CS formulation of methylparchion, at 1000 g a.i./ba). 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



-	
Report:	02.01.02/07; 2003; <u>M-090327-01-3</u>
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.:	<u>M-090327-01-3</u>
Guideline(s):	OEPP/EPPO 1992: Guideline on Test Methods for Evaluating the Side Effects of
	Plant Protection Products on Honeybees. Bulletin OFPP-EPPO Bulletin 22, 203-215 US EPA OCSPP Guideline Number 250 SUPP
Guideline deviation(s):	
GLP/GEP:	yes at a so the so
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Materials and method	$\mathbf{A}_{\mathcal{O}}^{(\mathcal{O})} \longrightarrow \mathcal{O}^{\mathcal{O}} \longrightarrow \mathcal{O}^{\mathcal{O}} \longrightarrow \mathcal{O}^{\mathcal{O}}$
vialerials and method	

#### Materials and methods:

The insecticide Confidor SL 200 (active ingredient NPN 33898, content: 196 g/l, JOX nov. 603700, Art. no: 0004958808, Batch no.: 233026473) was applied to flowering Phacella tandoetifolia planta (Fiddleneck), approximately 24, 48 and 96 hours before bee exposure at two mominal Pates, 24 and 25 g a.i./ha at an application volume equivalent to 200 1/ha. The control was treated with deionised water. PennCap M at a rate of 5 g product per uter (a. 2. 1000 g product/ha Was psed as pxic reference For each treatment there were four replicate goups. Kive 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 fents. Before reatment, these plants had been growing under identical conditions. The timing of freatments 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. Treatment's were compared to the detenised 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 days before exposure was used as a covariate. Treatments were compared to be dejonised water control using linear contrasts.

# Dates of work (biological part): 27 Julo 2002 5 August 2002.

#### Finding

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 hat 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 toraging 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 Confider SL 260 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. 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 to significant effection mortality was observed. Ñ 

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

	2		A.	, 6 <sup>Q</sup>		$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
	Pre-exposure Exposu	Ire	Ş	$\varphi^{*}$	di di	
Treatment			2 (see note) Q	day 3 🛴	) da	ay 40 .0
Deionised water		± 8.5 05	.3 ± 1.8	69 ± 12 Q	03.3	± 5.3
Confidor SL 200	(hours before application)	~~	.3 ± 1.8	×99.5 ± 8.9 63.0 ± 1.1 77.0± 18.7		5 ~ C
21 g a.i./ha (24)	88.1 ± 8.5 58.8	± 14.6 50	≫)± 2,3>°	≪99.5 ± 8.9	ల్లో 28.3	y± 1.1√√
21 g a.i./ha (48)	97.9 ± 10.2 54.0	± 14.6 50 ± 6.0 0	5 ± 10/6	¥63.0 € 1.1 77 € 18 7	30.0 32.3	± 4.3
21 g a.i./ha (96)		± 47.6 . 0 1			ð2.3	
35 g a.i./ha (24)	82.5 ± 18.5 38.3		0.7	32.0 ± \$9	26.0	£ 2.4 Q
35 g a.i./ha (48)	64.5 ± 4.3 51.8	10.2 1	≫± 0,0°	A6.3 5 9.6	\$ 29.3	± 2.8
35 g a.i./ha (96)	90.5 ± 6.6 85.0 60.9 ± 7.1 34	+ 0/8/	2 . 20	)50.8 (12.4 13.50 4.6	265	
PennCap M	60.9 ± 7.1 348 0.01 (Difference with water contributions) timal weather conditions) as biologically meaningful.			045.3 ± 7.6 50.8 12.4 13.5 4		$ \begin{array}{c}                                     $
= F < 0.00, = F < 0	timel unethor condition			ð ö	S.	K,
should not be taker	timal weather conditions Oragin as biologically meaningful.	activity on this o			are sus	ected and
			, Ū L			
T-11-12	Summary of findings m		N O		0″	
Table 1.2	Summary of Andings m	organty data	Ø <sup>4</sup>		Ĉ	
				Contraction of the second s	Ĵ.	
Y	Pre-e	xposues 0		* Expos	¢.	
Deiopised water	(V' AV6099	e day 2 -1	$\rightarrow$	cumul 90	ve 3.0	
Confidor SL 200	(hour the fore application)	Q S	No.	0 18.5	3.0	
21 g a.i./ha (24)	4.04	× 0.8 .~		() 2008 ±	2.1	
21 g a.i./ha (48)	× × × 2.8	± ~0,4	° S' C	\$6.0 ±	3.5 *	
21 g a.i./ha (96)		* 01.1 ×		36.8 ±	4.0 •	
35 g a.i./ha (24)		£√ 1.2 Q		28.3 ±	5.5	
35 g a.i./ha (48)	Q 3.5	± 137	ð í	27.5 ±	5.0	
35 g a.i./ha (96)	5.50	± 0.9		40.0 ±	8.8 *	
PennCap M		± 1.2	_~~```````````````````````````````````	216.3 ± 3	4.8 ***	
*= P<0.05; *** = P<	0.001 Ofference with water cor	itros ANCOVA foll	owed by linear of	ontrasts)		
		y m (				
>>M-090327-01-3@S-60466		. K ~	Q°			
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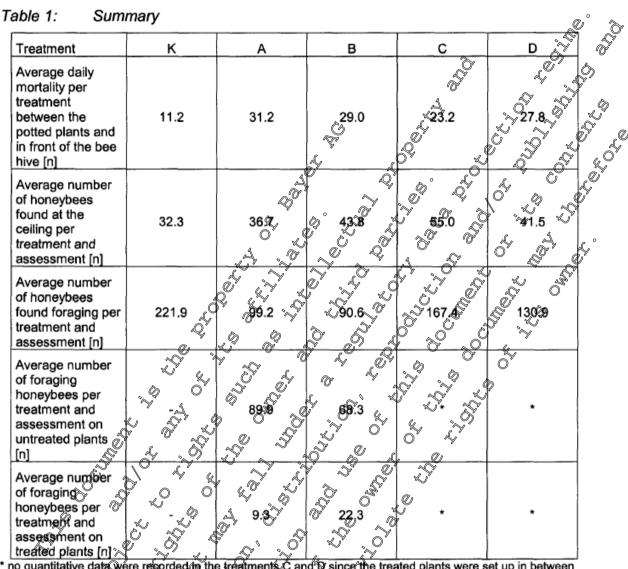


Report:	02.01.02/08; 2003; <u>M-116136-01-3</u>
Title:	Evaluation of the effects of a soil treatment of ornamental plants with Imidacloped $WGO^{\circ}$
The.	5 on nectar and pollen sampling honeybees (Apis mellifera) in the semifield (jest plans)
	Erica and Lobelia)
Report No.:	Erica and Lobelia) M-116136-01-3 U.S. EPA OCSPP 850.SUPP not applicable yes : In a tunnel test ornamental plants, <i>Lobelia erinus</i> and <i>Erica gracilis</i> , received of 0.015 g a.i./l soil substrate at full blossom with midacloprid WG 5 (NTN)
Document No.:	<u>M-116136-01-3</u>
Guideline(s):	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	not applicable
GLP/GEP:	yes a c c c c
< <m-116136-01-3@s-602223-01-1< td=""><td></td></m-116136-01-3@s-602223-01-1<>	
	: In a tunnel test ornamental mants, Lobelin erinus and Ereca gracilis, received
iviater la allu methous	of 0.015 g a.i./l soil substrate at full blossom with and great WG 5 (NTN)
	)5439280, Batch-No.: PFOOOOOREC content of a . 5.5% TOX No. 6455-00).
Control plants received	no treatment.
5 treatments with two re	eplicates for each treatment were defined by different proportions of treated and
untreated plants with a j	proportion of 50% of the ground covered with untreated and 50% covered with
treated plants for the tre	atments A and B and a proportion of 19% of the ground covered with treated and
90% covered with untre	eated plants for the treatments c and D. The numbers of prants for the treatments
and the control were as	follows: O' the start of the st
K: control: no treatment	t, 300 untreated <i>Bobelig erinus</i> (equivalent to 50%) and 300 untreated <i>Erica</i>
gracilis (equivalent to 5	0%) in the tunnel of the second secon
A: 15 mg a.i./l soil subs	strate, 300 Preated Lobelin erinus (equivalent to 50%) and 300 untreated Erica
gracilis (equivalent to 5	10%) in the tunnel $3%$ $0%$ $5%$ $4%$
	trate 05 treved Labelia articles (Application of the topological and 535 untreated Erica
B: 15 mg a.i./l soil sobs	trate, 300 freated Efica gracilis requivalent to 50%) and 300 untreated Lobelia
erinus (equivalent to 50	Sin the tunnet of the the the
C: 15 mg a.i./Dooil subs	trate 05 treated Lebelia ernus (equivalent to 10%)* and 535 untreated Erica
gracilis (equivalent to 9	10%) in the tunnel
D: 15 mg a.i./l soil subs	trate of treated Erica gradis (equivalent to 10%)* and 535 untreated Lobelia
erinus (equivalent to 90	%) sin a turnel O z z & A
~ <i>y</i>	
* for an easier arranger	acht of the treated plants between the untreated plants, the number of treated plants
was increased to 65 inst	iead of 60 ments a second second prime in the second prime is a second prime in the se
, as mereuser of the dist	tead of 60 plants
The plants were placed	inside tunnels floor space, 10 m x 5 m) on the experimental farmland "Höfchen".
In each winnel one how	by bee ( <i>Apis mellifered</i> ) colory (containing approx. 3000 honeybees) was allocated.
The boneybees were on	ce this deserved for the parameters mortality and foraging and flight activity
during a period of 17 da	
	$\frac{1}{4}$
Dates of biological	$\frac{1}{100}$ $\frac{1}{100}$ $\frac{1}{100}$ $\frac{1}{100}$ $\frac{1}{100}$ $\frac{1}{100}$
Findings	
Findings for the freetme	O V V Ots granted in table 1
	by bee ( <i>Apis mellifered</i> ) colony (containing approx. 3000 honeybees) was allocated. ce daily observed for the parameters mortality and foraging and flight activity ave. <b>rk:</b> 2002-09-02 to 2002-09-19 fors are presented in table 1.
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#### Imidacloprid Bee Studies Compilation of Study Summaries

Issue date 2017-11-22



\* no quantitative date 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 treatment than in the control; however, absolute mortality was not high, neither incontrol 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 : freated) group the foraging activity was slightly higher than in the 50:50 (untreated : freated) group.

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

>>M-11613 0 @S-602223-01-1



<b>D</b> (	02.01.02/00	200(25.01.2		
Report:		<u>-308625-01-2</u>		
Title:	On the relevant endpoint of th	e study of Bakker (	2001) Confidor S	SL 200: a multiple rate
	cage study to determine effect	s on honeybees, A	ois mellifera L., v	when applied to $\heartsuit$
	flowering Phacelia tanacetifol	ia		
Report No.:	<u>M-308625-01-2</u>		~	
Document No.:	<u>M-308625-01-2</u>		<i>S</i>	
Guideline(s):			O,	
Guideline deviation(s):			A	
GLP/GEP:	no	Ò		
			N'	
1. Introduction		, C	s	
For the honey bee of	ff-crop risk assessment on	sprav applicatio	ns of products	s containing the

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

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#### 2 Summary of the study results

In order to determine the effects of an imidacloprid spray application on horeybees, 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 oaged conditions (Bakker 2001), *Phacelia* being chosen because it ensures a high oraging 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, PennCan 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 motality was observed in any imidacloprio 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 fates 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	<u>+</u> @12 (II-249) 0		s fer uay			
Treatment 🔊	Pre-treament		Post-treatment			
g a.s./ha 🔟	11-jun-01	12-jun-01	∑ 13- <b>∫</b> un-01	14-jun-01	15-jun-01	16-jun-01
De-ionized water	∿.59.0 <u>+</u> 6.₽	74.3 <u>+</u> 110	98 0 <u>+</u> 19.3	87.3 <u>+</u> 21.2	87.8 <u>+</u> 22.0	74.0 <u>+</u> 17.8
Imidacloprid 0.6	\$47.3 <b>≠</b> 3.6	₩7.0 <u>+</u> Ø₿.4	°∕∕ø9.3 <u>+</u> 12.2	77.5 <u>+</u> 8.6	76.5 <u>+</u> 16.1	61.8 <u>+</u> 12.4
Imidacloprid 1.2	38.7 4 16.8	54.0 <u>+</u> 22.5	75.8 <u>+</u> 10.0	85.0 <u>+</u> 16.1	76.0 <u>+</u> 11.4	67.8 <u>+</u> 13.5
Imidacloprid 2.0	56.5 <u>+</u> 9,5	64 3 <u>+</u> 12	57.5 <u>+</u> 3.0* <sup>a</sup>	89.5 <u>+</u> 12.6	74.0 <u>+</u> 7.7	74.0 <u>+</u> 16.7
Imidacloprid 🔊 🖉	50.0 9.2	*65.5 <u>+</u> 12.5	50.5 <u>+</u> 9.4*	59.0 <u>+</u> 6.7	64.0 <u>+</u> 16.8	48.5 <u>+</u> 16.6
Imidacloprid 9.0	46 <u>,®+</u> 6.6 ×	/ 72.0 <del>9</del> 9.4	43.8 <u>+</u> 6.0*	58.5 <u>+</u> 6.8	64.0 <u>+</u> 5.5	51.3 <u>+</u> 8.9
Imidacloprid 14	52.3 <u>+</u> 63	61.0 <u>+</u> 10.4	40.8 <u>+</u> 3.0*	48.0 <u>+</u> 12.8*	56.5 <u>+</u> 12.5*	49.5 <u>+</u> 12.4
Penncap 5.00	65.3 <u>+</u> 17.4	84.3 <u>+</u> 16.6	31.0 <u>+</u> 3.1*	2.5 <u>+</u> 1.0* <sup>b</sup>	10.8 <u>+</u> 4.2*	11.0 <u>+</u> 5.2*

# Average number + 🖉 (n=4) of for aging bees per day

\* Starstical significantly different from control (P<0.05 ANCOVA followed by Fisher's LSD test)

<sup>a</sup> Exclusion of colonies with reduced foraging activity in the pre-exposure period, identified as outliers in the startistical analysis ed to statistically significant conclusion <sup>b</sup> The very high mortality observed from day of treatment onward is considered to contribute to conspicuous reduction

<sup>b</sup> The we'ry high mortality observed from day of treatment onward is considered to contribute to conspicuous reduction in foraging days 2 to 4 post-application



Report:	02.01.02/10; ; 2	2001; <u>M-0526</u>	37-01-3		0	
Report: Title: Report No.: Document No.: Guideline(s): Guideline deviation(s): GLP/GEP: <<	Effects of residues of	imidacloprid	in maize poller	n from dressed	seeds on honey bees	Ô,
Demont Maria	(Apis mellifera)			*	N O	n"
Report No.: Document No.:	<u>M-052637-01-3</u> M-052637-01-3			, Or	SU D	
Guideline(s):	US EPA OPPTS 850.	3040		10 m		
Guideline deviation(s):	not specified					
GLP/GEP:	yes		Ò	Å.		<i></i>
< <m-052637-01-3@s-602655-01-1< td=""><td></td><td>li li</td><td>V<sup>r</sup></td><td><math>\mathcal{Q}</math></td><td></td><td>S</td></m-052637-01-3@s-602655-01-1<>		li li	V <sup>r</sup>	$\mathcal{Q}$		S
Material and methods:	test substance: Gauch	o WS 70, te	sidues in ma	ze pollen from	dressed seeds, 🔬	)
dressing rate: 49 g/unit	a.1 Residues of imida	cloprid an th	e pollen were	found to be b	elow limit of	
dressing rate: 49 g/unit quantitation (LOQ = 0.) detection: 0.003 mg/kg	005  mg/kg. No oleffi and 0.0015 mg/kg, re	ne and rydro	xy metadolite			
detection. 0.005 mg/kg	and 0.0015 mg/kg, re	spectively).	5 2			
Small bee colonies (app	or. 700 honevbees) we	ere contined	in tent cares	$(ca)^2 20 \text{ m}^2$ or	n short græss	
meadows and exclusive	ly fed with maize po	llen which w	as harvested	from plants, th	ne seeds of which	
were dressed with Gau	cho WS 70 or which v	were untreat	ed (gontrol)	Sunflower han	ey was provided as	
carbohydrate source. The	ne small bee cotonies	wyere exami	ned for treatm	ent-related in	pacts over a period	
of 38 days. In particular	r, the following endpo	vints were ev	aluated mor	ality, comb ce	Production, food	
consumption, storage b			g laying active	ty, breeding s	access, colony	
strength, foraging inten	sity and penavioral ai	nogenalies	ju g	, <sub>0</sub> , 9	×	
Dates of biological wor	k. 2000-08 \$21 to 200	0-09-28			, O	
-					2	
Findings: Effects of Ga	ucho WS 70 residues	th maize po	llen on small	honeybee	onies	
			N ON			
		N N	J (	). <i>A</i>		
Testing Endpoint		Countrol AQ	J _ (	Treatment A	Treatment B	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			Control B			
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			Control B		Treatment B 30	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			Control B			
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			Control B		30	
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~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			Control B		30 151 2	
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~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			Control B		30 151 2 255 185 26 877 664 399 16.6	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			Control B		30 151 2 255 185 26 877 664 399	
Mortality (no of dead bee hives) Mortality (no of dead edges) Foraging intensity (no. of bees at the foll Foraging intensity (no. of bees at the foll Foraging intensity (no. of bees at the foll Bee activity (no. of bee Pollen collected [g] Honey collected [g] Honey collected [g] Honey storage area at a [cm <sup>2</sup> ] Hive weight increase [% of the initial occight Egg lacing activity[cm containing eggs at stud	en feeder) 5 y fe	32 <sup>4</sup> 346 346 346 346 346 346 340 340 340 340 340 340 340 340	Control B() 27 141 27 27 27 253 203 58 853 618 254 6.6 63	20 139 29 274 196 43 819 660 417 12.4 15	30 151 2 255 185 26 877 664 399 16.6 18	
Mortality (no of dead bee hives) Mortality (no of dead edges) Foraging intensity (no. of bees at the foll Foraging intensity (no. of bees at the foll Foraging intensity (no. of bees at the foll Bee activity (no. of bee Pollen collected [g] Honey collected [g] Honey collected [g] Honey storage area at s [cm <sup>2</sup> ] Hive weight increase [% of the initial occiden Egg lacing activity[cm contaming edgs at stud Colory strongth [cm <sup>2</sup> c	en feeder)		Control B		30 151 2 255 185 26 877 664 399 16.6	
Mortality (no of dead bee hives) Mortality (no of dead edges) Foraging intensity (no. of bees at the foll Foraging intensity (no. of bees at the foll Foraging intensity (no. of bees at the foll Bee activity (no. of bee Pollen collected [g] Honey collected [g] Honey collected [g] Honey storage area at a [cm <sup>2</sup> ] Hive weight increase [% of the initial occight Egg lacing activity[cm containing eggs at stud	en feeder)	32 <sup>4</sup> 346 346 346 346 346 346 340 340 340 340 340 340 340 340	Control B() 27 141 27 27 27 253 203 58 853 618 254 6.6 63	20 139 29 274 196 43 819 660 417 12.4 15	30 151 2 255 185 26 877 664 399 16.6 18	

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,



ð

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; ; 2002; <u>M-052238-01-3</u>	
Title:	Evaluation of the effects of residues of imidacloprid	I FS_600 in maize pollen from
	dressed seeds on honeybees (Apis mellifera) in the s	sexprifield
Report No.:	<u>M-052238-01-3</u>	
Document No.:	<u>M-052238-01-3</u>	
Guideline(s):	not applicable	
Guideline deviation(s):	The following procedures were not carried out unde	r GLP: seed dressing, solving of the
	seeds, analysis of soil contents of the field where se	eds were sown Harvesting of the
	maize panicles, sieving and drying of the follen.	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
GLP/GEP:	yes & Q X	

#### <<M-052238-01-3@S-602148-01-1

Material and methods: Test substance: marze pollen with grown residues of midacloprid \$\$ 600 (seeds dressed with commercially available product at a rate of 1 g a ?./1000 seeds). Small honeybee colonies (approx. 500 honeybees) were confined on out plots (50 m<sup>2</sup> arilled on 2001-05-@) in turnels and fed with maize pollen containing grown residues of Initaclopfid or Untreated control pollen. For treatment and control, three replicates were set up each. Sunflower hone was provided as carbohydrate source. The small bee colonies were examined for treatment-related effects over a period of 50 days. In particular, the endpoints mortality and foraging intensity were evaluated. Likewise the endpoints comb cell production, food consumption, pollen and honey store geg bying activity, breeding success, colony strength and hive weight development were assessed and statistically analysed using a topest. Behavioural anomalies were also assessed.

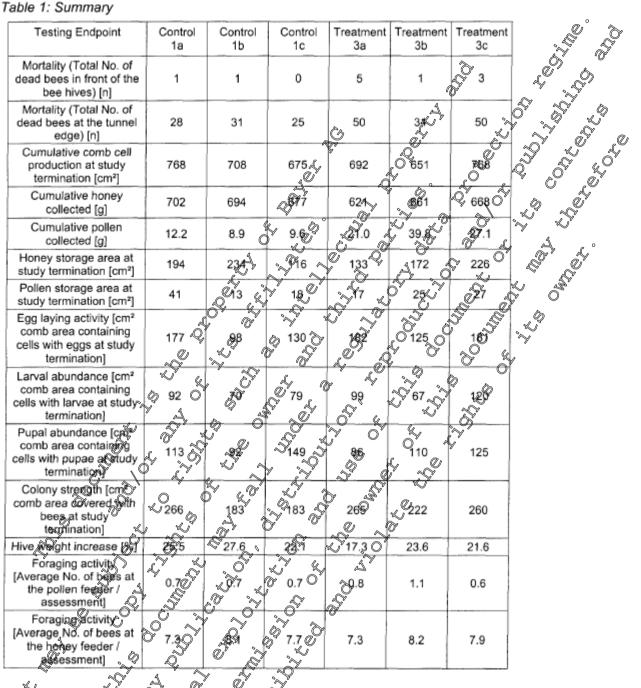
Ŵ

Dates of analytical work 2001-08-21 to 2001-08-12 Dates of analytical work 2001-08-14 to 2001-06-05 Findings: Effects of residues of Imdacloped FS 500 in coller on small honeybee colonies



#### Imidacloprid Bee Studies Compilation of Study Summaries

Issue date 2017-11-22



**Observations:** There were no significant differences between control and treatment in comb cell production (t=-0.478, p=0.641), hone consumption (t=2.530, p=0.065), hive weight increase (t=1.720, p=0.161), pollen stores (t=-0.260, p=0.725) and honey stores (t=0.086, p=0.933), egg deposition (t=-0.176, p=0.863), larval abordance (t=-0.228, 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

Ø1



## **02.01.03 - Field and Monitoring**

Report:	02.01.03/01; 1991; <u>M-048426-01-2</u>
Title:	Integrated pest and pollinators investigations 1991 (including hony bee toxing of NTN
	33893)
Report No.:	103815
Document No.:	<u>M-048426-01-2</u>
Guideline(s):	Ecological Effects Requirements: Studivision E
	40 CFK 136.143
	Supplemental to Guidelines 141,1 and 141-2
Guideline deviation(s):	none $\sqrt{2^{\vee}}$ $\sqrt{2^{\vee}}$ $\sqrt{2^{\vee}}$
GLP/GEP:	no the second se

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

Pollinating bees were exposed to NTN treated alfalfa toliage to evaluate poisoning risk. NTN 33893 240 FS was sprayed onto second growth alfalfa at 0.025, 0.05, and 0.10b. At A. Folfage 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 (ARISME), alfalfa leafontting bees (MPGCRO), and alkali bees (Nomia melanderi). Bee repetiency was no revaluated in this study.

RESLUTS: There appeared to be no separation of % mortality by rate. The alkalidee 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 to 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 200 FS Batch No. = 1093004

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	õ	NO N		0	£	~~~	$\searrow$
Report	<u>ر</u> 02ء	09203/02		A 995;	<u> <b>A-0085</b></u>	<u>17-01-3</u>	© ≯
Title:	~⊙ <sup>°</sup> B&	VII: Bu	d bur®	spraytre	atment	, upper	Ítaly
Report No.:	NA NA	Z 35095	J.		$\bigcirc$	ð	-
Document No .:	<u><u> </u></u>	<u>00\$\$17-0</u>	108	°	Q.	S.	
Guideline(s):	,0×	õ v	$\mathcal{O}$	D´ 、C	) (	0	
Guideline deviation	on(s): 🔶	) _ ^'		, O	ð		
GLP/GEP	no	Å.					
		Ŵ,	Ŵ		Ś		
< <m-008517-03@s-60464< td=""><td>9-01-1</td><td>~~</td><td>ć</td><td></td><td>, y</td><td></td><td></td></m-008517-03@s-60464<>	9-01-1	~~	ć		, y		

At Upper Italy four trials were conducted in appre with bud burst spray treatments at stage 54 (mouse-ear) with Confider 0.01% + 6 hocin 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 brd 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.



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 foreging cannot be excluded at Confidor treatment 8 days prior to blossom.

Report:	02.01.03/03: : 1	1998: M-006826-01-4				
Title:	The impact of Gauche	o 70 WS seed treated sunflower seeds on hone bees				
Report No.:	BF 1/98	1998; <u>M-006826-01-4</u> o 70 WS seed treated sunflower seeds on honey bees 76 WS (0/7 mg an. per seed) and sover on 8.5.98. Four bee field 75 days fater when the plants were in flower. The same				
Document No.:	M-006826-01-4					
Guideline(s):						
Guideline deviation(s):						
GLP/GEP:	no					
<< <u>M-006826-01-4@S-602891-01-1</u>	dragged with Couche "	76 VI/S (67 more in a strength and any in a 500 Four has				
Sumower seeds were t						
colonies were introduce	ed to the 1.25 ha triag	field 75 days ater when the plants were in flower. The same				
process was carried out	⊢using unaressea se∉a	1 on a control field of the same size 4 kilometres away, where				
the same parameters we	ere measured.	d to increased bee mortality.				
	A &	d to încreased bee mortaling.				
The use of Gaucho see	d dressing did not lead	i to invreased bee mortaling.				
	Q' &					
		ing visits to surgiowers of so k				
		d to increased bee mortality.				
Bees collected large an	nounts of noten from	both sunflower fields. at both sites. This is not unusual as weight depends on the mine the number of bees returning to the hive from the				
Bees concerca harge an						
Colony weights remain	almeet unchanged	at bath une This is not unusual advaight depends on the				
site veriety and weath	vu annose unougangeu	at obtastics, mills is doit unusual as weight depends on the				
site, variety and weather						
A bee counter allowed	us to accurately deten	mine the number of bees returning to the hive from the				
treated field. Noevider	ice of bee disorientation	on was found,				
No residues of imidacle	oprid or its main metal	bontes were found in the honey bladders after preparation or				
in the remaining bees.						
Our final conclusion	that at the time when	supplowers are in flower no relevant residues of the				
treatment producerema	in the nectar that co	ould affect bee				
treatment produce remain in the nectar that could affect bee						
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~						
Barranta 🔊		2009; <u>MAG1852-02-3</u>				
Report:	02.01.03/04, 201, 2	al doses of imidacloprid, dihydroxy-imidacloprid and olefine-				
	imidacion tokon the	an doses of himdacrophia, anydroxy-innuacrophia and orenne-				
Repart No.:		ehaviour of honeybees				
Document No.:	Q1-031@52-02-3					
- *	US EPA ORRIS: NO	$\mathbf{i}$				
Guideline deviation(s):	note ~					
GLP/GEP:						
< <m-031852-02 05-60493="" 1-1<="" td=""><td>, O</td><td></td></m-031852-02>	, O					
		its metabolites, olefine-imidacloprid and dihydroxy-				
imidate lopride 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						
		ion behaviour analyzed. The behavioural effects of olefine-				
imidacloprid are found	to be similar to those	of imidacloprid itself. However, the effects are much less				
		nificant in the range of concentrations tested, was an increase				
	11 1 1					

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 100ppk but no at 50ppb, 20 ppb or 10 ppb. Both of the metabolites, olefine-imidacloprid and dovdroxy-imidacloprid. did not significantly affect the learning performance at 100ppb. However, with olefine-imidaeloprid 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 the 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 Bager. It are on picoting acetylcholine receptors. Previous studies indicated that imidacloprid has sublethal effects on leading 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  $\mathcal{Q}$ imidacloprid to two of its metabolites in treated plants, dihydroxy-imidacloprid and olefine-imidacloprid, and to investigate effects of imidacloprid and is metabolites on the tearning performance of honeybees. >>M-031852-02-3@S-604935-01-1

02.01.03/05; 1999; Mr 32344-01-3 Field test of Gancho 3 0 FS seeddressed sundowers on honeybee caronies. 3103/99 M-032341-01-3 US EP 4 OCSPP Guideline no 850.3040 none yes slianftas annuus) **Report:** Title: Report No.: Document No .: Guideline(s): Guideline deviation(s): **GLP/GEP:** <<M-032341-01-3@S-602554-01-1 Test item. Gaucho 350 FS Test crop: sunflower (Meliantus anarus) Q Seedressing dose. 9 1/150,000 weds Test species: Honey bee Apis mellifera carnica) Colony number 30, 10 on the treated field and 15 on the control field. Placing: The colonies were allocated in multiple store loves of 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. Ô 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. March uly 1999 Experimental phase of the stud 16 December 1999 Issue of the

Results

Forgsing activity and beliavior of the bees

Except the fast 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.



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 Jack

On both fields in average 1.9 bees per minutes were observed entering the hives with oranger pollenloads.

No abnormal behavior of the bees was observed during foraging and around the hives.

#### Weight gain of the hives

% on the control freeds. The weight gain of the hives on the treated field was  $\frac{1}{2}$ .5 % and 23(

#### *Strength of the colonies*

Initially, the strength of the colonies has been slightly higher if 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 $\mathcal{K}$

The number of the combs with brood and the total area of all brood stages increased at the bee solonies placed on the treated field. In contrast the brood of the beer colonies placed on the control field decreased. The number of the combs with broad in the control bee colonies also decreased. Some empty cells were found at the endosf the experiment in size 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 unharged brood development was normal. In the experimental period 3 natural requeening was observed in case of the colonies on the treated field and 1 in case of the colomes on control field. The behavior and eggs laying of the queens were normal in case of the other be colonies.

#### Mortality

Except 2 cases in the control becolonies the Bee mortality and novexcept the accepted natural mortality level which is less that 100 dead bees colory/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 44-36 dead bees per day and 15-972 dead bees per day on the control feld were found. The portality of the drones was not significant during the whole period of the experiment.

Weather conditions and soil mosture with a solution of the weather conditions. Most of the During the experiment sunny days and no winds characterised the weather conditions. Most of the rainfalls were registered during the hight, which was 90 mm on the treated and 109 mm on the control field.

The soil poisture of the treated field was 17 2 - 32,53 % and 16.26 - 34.08 % on the control field.

## Evaluation made by the beckeeper

At the initiation of the experiment lot of rainfalls and high relative humidity were registered. This why the sunflower produced thin nector. That was one of the reasons for slight weight gain of the hives. As the weather changed for the better, greater not ar 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 hone 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 1/150,000 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



produced substantially more brood. The seeddressing product had no adverse effect on the forager bees, the queens and the brood.

<b>Report:</b> Title:	02.01.03/06; 1998; <u>M-038</u> Flower visits to sunflowers seed-tu		
Report No.:	Bees 1/98	4	
Document No.:	<u>M-038723-01-4</u>	S N	
Guideline(s):	US EPA OCSPP Guideline no. 85	0.0040	
Guideline deviation(s):	none	V Q	
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 unreated 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 at 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 trias. Weakening of the colony strength as a result of disordentation cannot be offerred. Without exception, the polyen-carrying bees came from the sunflower field. Any other source of neural flow could be ruled out because of the orangey-red colour of the polyen.

>>M-038723-01-4@**S-604651-01-1** 

		<i>′</i>
Report:	$\times 102.0103/07;$ ; 2015; NO038733-01-4 $\times$ (	
Title:	Bee9VI: Flower visits after seed reatment	
Report No.:		
Document No.:	$\sqrt{\underline{M} - 038 \overline{n} 33 - 01 - 4}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$	
Guideline(s):	US EPA OCSPP Guideline Stamber: 850.StopP	
Guideline deviation(	US EPA OC SPP Guideline Stümber: 850.Stopp	
GLP/GEP: 🔊	no O A AY AY	
< <m-038733-040-01< td=""><td></td><td></td></m-038733-040-01<>		

Two fields in the Sologne area of Erance, each of 1.5 ha, were sown with sunflowers on 22 May 1995, the seeds of one field having been treated with Gaucho 0 fg a.i./grain. A nearby field, which had also been treated with Gaucho 49 g.a.i./ha was also included in the treat. At the start of flowering on 22 July 1995 (= 61 days after sowing), 6 bee colones 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 of the second on the landing board was even higher on the Gaucho field than on the

The progress of bowering 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.



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 ke obtained in the control. The bees in the Gaucho field obtained their pollen main from mustand and maize, but collected nectar from sunflowers.

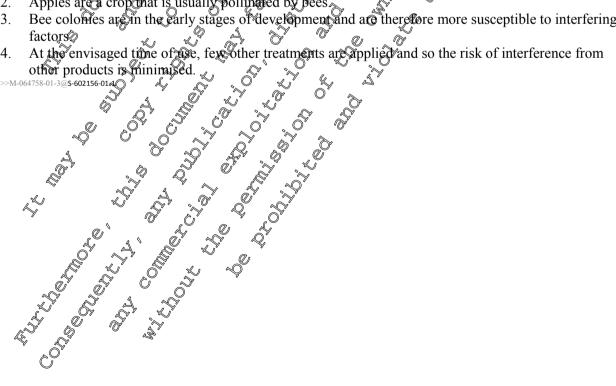
The yield from the sunflower harvest was one and a half times as high in the Gaucho fred as in the control field, which is only possible following perfect pollination by been, together with good fertilization @ and watering of the crop.

Following this extensive field trial, it is inconceivable that treatment of the sunflower seeds with Gaucho can affect the bees and their honey production.

Report:	02.01.03/08; 01-064768-01-30
Title:	Side effects of Confider SI 200 on bees following one application to apple trees at the
	mouse-ear stage
Report No.:	ITA-98-901
Document No.:	$M-064758$ $M \sim 2$ $M \sim 2$ $M \sim 2$
Guideline(s):	
Guideline deviation(s):	
GLP/GEP:	no the
< <m-064758-01-3@s-602156-01-1< th=""><th></th></m-064758-01-3@s-602156-01-1<>	

In 1998, a field trial on apple trees was performed to assess the risks to hopey bees associated with use of Confidor 200 SL. Apple-growing represents a realistic worst-case scenario for soch a risk assessment for Ő the following reasons  $\bigcirc$ O L,

- Apple trees blossom shortly after the point at which it is @commended that treatment takes place, i.e. 1. the mouse-ear stage.
- Apples area cropohat is usually pollinged by bees 2.
- Bee colonies are in the early stages of development and an therefore more susceptible to interfering 3.
- 4





Report:	02.01.03/09; 2002; <u>M-066846-01-3</u>
Title:	02.01.03/09; 2002; <u>M-066846-01-3</u> Field test: Side effects of oil-seed rape grown from seeds dressed with imidacloged and beta cyfluthrin ES 500 on the honey bee (Apis mellifera L)
The.	Field test. Side effects of on-seed tape grown nom seeds dressed with initiactopy and
	beta-cyfluthrin FS 500 on the honey bee (Apis mellifera L.)
Report No.:	99398/01-BFEU
Document No.:	M-066846-01-3 US EDA OCSED Cuidalina Number: 850 204
Guideline(s):	US EPA OCSPP Guideline Number: 850.304
Guideline deviation(s):	not specified
GLP/GEP:	yes a final state of the second state of the s
< <m-066846-01-3@s-602206-01-1< th=""><th></th></m-066846-01-3@s-602206-01-1<>	
Procedures	

#### Materials and methods:

Fields with oil-seed rape (Brassica napus, variety Dirajet) dressed with 951.1 Dg a.i & 187,31 g a 187,31 g kg seeds Imidacloprid & Beta- Cyfluthrin FS 500 (dressed seeds: article number 02,00944819 A, product used for dressing: development number 0195939, for sulation number 0055, tox number 4867-00) and the fungicide Thiram were used as test substance treatment group. Plots withoil-seed rape dresset 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. The study was cattied out with one replicate (she 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 27/04/2009 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 prortality 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 laves of the first group was recorded continuously. Samples of pollen, neetar and honey were collected during the sody, for analysis of residues of the test

substance and metabolites of the test substance.

The influence of the test Substance Imidacloprid & Bera-Cyfaithrin VS 500 was evaluated by comparing the bees of the test field to the bees of the copirol field. Dates of work: 23/08/1999 - 13/06/2000 Biological Findings:

			¥	
Test substance				
Test substance	õ	Appis r	nellifer	а
			ed rap	e
Endpoints	Ø		Τe	est substance field
Dead bees in the bee traps	, Q	594		350
Dead bees in the field		2 2		11
Meaoflightactivite		2,3 bees/m²/min		3.3 bees/m <sup>2</sup> /min
	hivé 76	+ 31.7 kg (54.7 %)	hive 35	+ 24.3 kg (44.6 %)
Colony strength described by the weight of the test colonies	hive 90	+ 26.3 kg (49.0 %)	hive 124	+ 27.7 kg (52.5 %)
Č <sup>×</sup>	hive 15	+ 24.8 kg (44.5 %)	hive 19	+ 24.4 kg (47.6 %)



#### **Analytical Findings:**

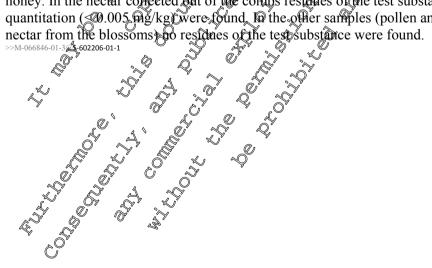
Test substance		Imidaclo	prid & Bet	a-Cyfluthrin	FS 500	
Test organism			Apis m	nellifera	- A	
Exposure			Oil-see	ed rape		- A A A
Sample material	С	ontrol field	d (ČA	Test	ubstance	field
Analysed for [mg/kg]	Hydroxy- ímida- cloprid	Olefin- ímída- cloprid	Imida- cloprid	Hydrox Imida cloorid	Olefin- Imida√ clopri©	Field $(1, 1, 2, 2, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3,$
Nectar from the comb	n.d.	n.d. 🤻	n.d.	n.d	.d.	(4).d. top < LQC
Pollen from the comb	n.d.	n.a.	ي الأربي n.d. الأ	0:d. 8	n.dØ	Ag.d.
Honey from the comb	n.d.	x.n.d. ^>	n.ď.	n.d.A	0:d.	n.d.
Nectar from the blossoms	n.d. Q	R.8.	<sup>2</sup> n.d.		n.d	J.d.

Limit of quantitation: 0.005 mg/kg for Imidacloprid and Hydroxy-Metabolite, 0.07 mg/kg for the Olefin-Metabolite O

Limit of detection: 0.0015 mg/kg for Imidacloprid and Hydroxy-Metabolite, 0.008 mg/kg Limit of detection: 0.0015 mg/kg/kg/kg/minoreaction 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, nector and pollen on the test substance field compared to the control the development of bee brood waonot 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 metabolite of the test substance were found in pollen, nectar or honey. In the nector 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





Report:	02.01.03/10; ; 2009;	; <u>M-074400-01-4</u>		
Title:	02.01.03/10; 2009; The effects of sublethal do	ses of imidacloprid	on the foraging be	haviour and origination
	ability of honeybees			
Report No.:	<u>M-074400-01-4</u>		ð	
Document No.:	<u>M-074400-01-4</u>		Â.	A A
Guideline(s):	US EPA OPPTS: N/A		10%	
Guideline deviation(s):	none		A	
GLP/GEP:	no	Č4	L'	
~M 074400 01 4@5 602208 01 1			<u>v</u>	

<<M-074400-01-4@S-602208-01-1

This paper examines the possible effects of sublethal doses of the insecticide imidad oprice on the behaviour and orientation performance of foraging honeybees. Sugrose solutions containing indacloprid was fed to bees, and changes in behaviour were found for imidacloprid concervations of 20 ppb to 100 ppb after comparison with the control groups. No effect was observed at 10 ppb. In the suffect hal concentration range indicated above, imidacloprid causes a reduction in the foraging activity of the treated bees and induces trembling dances by which the foraging fees discourage other worker bees from foraging, which in turn reduces the foraging activity of the bees in the nest. In addition, the affectiveness 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 prease. Although these effects on the behaviour of the bees were observed to start at imidaeloprid concentrations of 20 ppb, nordamage 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 with affect the population development, such effects appear not very likely unless bee hives without any food stopes 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 houry 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 Erench beekeepers of disordented 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 undertaker on various aspects of the foraging behaviour of honeybees. In contrast to many other inect 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 wagging dances used to attract conspecifics in the hive to food sources are reduced.

Indications of such complex offect 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), regulate 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 neetar 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 Scheck (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 as to characterise any impairment of orientation more accurately. When hopeybees find a good source of food they learn its smell, colour and visual appearance, and also its position relative to the nive (von Frisch 1965, Seefey 1995). They do not only return on a direct route from the food source to the five 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 of danging. 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 (Kirchne and Braun 1994).

The purpose of our study was therefore to quantity the possible effects of imidaclopite on the behaviour and orientation ability of individual bees and in particular the behaviour of individually marked bees returning to the hive from a toog source. The limited to a range from 10 ppb to 100 ppb to Materials and methods returning to the hive from a food source. The concentrations of the active mgredient examined were

The experiments were performed on two poneybes populations of the strain Apis mellifera carnica. Each population contained about 5,000 bees. The test hives were placed in twincomb 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 coloures were given the opportunity to forage out-doors. All bees returning to the Rive were directed to one side of the como so that all individually marked foraging bees could be observed.

, ô<sup>g</sup>

The tests in the flight form were performed between April and Dane 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 midactoprid (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 be met a nive be 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 for daclogrid was used at concentrations ranging from 10 ppb to 100 ppb derived from Confider 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 Kifehner et al. 1988).

67 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



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 rank and temporal trends were analyzed. However, the relative low longevity of fourager bees restrict the possibility to monitor chronic effects. In the field, the average longevity of fourager bees is about 8 to 10 days. Fouragier bees which were marked on the food source will, therefore, five 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 inclucion 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 53893 70 WS) or 101 7 mg initiacloprid tech. (98.3%) was pre-solved in 1 L A. dest. and stirred for 4 hrs (results in 60 pp.). 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 p to 500 ml into a 2 molar sucrose solution fresulting in 1020, 50 and 100 ppb (W/v) initiacloprid 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)

 Report:
 02.0103/11;
 2001; 008472 01-3

 Title:
 The impact of Gaucho and 21-435 seed-treated Casola or honey bees, Apis mellifera L

 Report No.:
 140403

 Document No.:
 140403

 Guideline(s):
 US EPA OC SPP Guideline Sumber: 850.StePP

 Guideline deviation(s)
 not specified

 GLP/GEP:
 yes

GAUCHO® (Bayer forp.) 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 (Acicht, 1993). Since us 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 aphid leafhoppers, planthoppers, thrips and whiteflies (Elbert et al., 1991;

1999). In addition, this compound has been found to be active against some species in the orders Coleoptera, Diptera and Lepidoptera (Elbert et al., 4991). Today, imidacloprid is registered for use in many countries, having considerable agricultural importance as a broad spectrum multi formulation insecucide that can be used on a wide variety of crops.

Imidacloprid is highly water coluble 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;

as carola, 1999). The offer, in idacloprid as a seed treatment can be used with confidence on crops, such as carola, that block >60 days after planting and are pollinated by insects such as the honey bee (*Apis meltifera*). Further more, the systemic nature and residual activity of imidacloprid make it a valuable tool in the grated pest management programs for many agricultural insect pests.



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 how honey production all leading to a severe decrease in colony strength and in some instances coloring 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 2000), 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 chloronicotin 1, seed Treated canola grown in Ontario, Canada and Minnesota USA bad 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 blossom contained residues of imidteloprid plus two metabolites, olefin-imidicloprid and hydrox 4 imidicloprid or TI 435 above the "no observable adverse effect concentration" (NOAECO of 20 ppb (0, 2 ppn)) (Schnudt and 1000, 2000).

02.01.03/12; 2009, M-09752-093 Evaluation of effects on the foraging activity of bee population in the sunflower field of **Report:** Title: Western France - Is Saucho seed dressing (active ingredient: imidacloprid) responsible for the effects? Report No.: ₩0635 Document No .: M-08475 US BPA OCSPP Guideline(s): Guideline deviation nøne **GLP/GEP:** ണ് <<M-084752-01-3@S-6046

Since 1996, beckeepers in the West of France have been observing massive depopulation of their apiaries during the sanflower honey harvest, accompanied by characteristic symptoms. The beckeepers 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 varioa, a parasite which is spreading following the development of resistance to varioacides (acaricides) in these regions since 1996, a infections with spiroplasms, which caused such problems in the South-Vest 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 oche1998 trials evaluated in accordance with the methods accepted and practiced in bee ecotoxicology, do bot indicate that Gaucho, used on sunflower seeds, presents a risk to bees.



02.01.03/13; ; 2001; M-088167-01-2 **Report:** Assessment of side effectsc of Confidor SL 200 on the honey bee (Apis melliferal.) in Title: apple orchard following application before flowering (mouse-ear stage) of the soop Report No.: 20011099/01-BFEU M-088167-01-2 Document No .: 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 St 200 on the boney 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 30X/AR2001. Apuntreated orchard of apple trees from the same variety served as control. At the start of full flowering 29AP 2001) 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 operiod of 7 days.

1 The influence of the dest substance Confider SL 200 was evaluated by comparing the bees in the pesticide-treated field to dose in the control field regarding the following observations:

- Mortanty in the bee graps
- Flight intensity in the crop (number of flying bees/tree/minute)

L)

- Fight intensity in from of the hives (number of fees feaving/ 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

#### **Findings**

Effect on honey bee mortality:

In the Confider 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 beesper colony/day in the Confider SL 200 treated group compared to a mean maximum of 43.5 dead be colony day of 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.



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 entite test period (33.62 bees leaving/entering the hive per minute in the treated group and 37.32 bees leaving/entering the hive per minute in the treated group and 37.32 bees leaving/entering the hive per minute in the treated group and 37.32 bees leaving/entering the hive per minute in the treated group and 37.32 bees leaving/entering the hive per minute in the treated group and 37.32 bees leaving/entering the hive per minute in the treated group and 37.32 bees leaving/entering the hive per minute in the treated group and 37.32 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 tost 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 \$1, 200 treated hives compared to the control lives.

Conclusion:

>>M-088167-01-2@**S-602488-01-1** 

The treatment of apple trees at the mouse car stage with Confider SL200 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.

Report: Title: Report No.: Document No.: Guideline(s): GLP/GEP: Month State St

<sup><<M-090324-0</sup> 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 towers 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 dicot denotes 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.

Flowsfing and star of the trial in days after sowing:

Field beans 83 = 12 weeks Summer rape 99 = 14 weeks Sunflowers 80 = 11.5 weeks >>M-990324-01-4/05-604660-01-1



Report:	02.01.03/15;	; 2000; <u>M-090720-01-2</u>		
	02.01.03/13,	, 2000, 101-090720-01-2		
Title:	Field evaluation in	Argentina of possible risk for	or honey bees from	m the product Gaucho
	on sunflowes		5	
Report No.:	LPE-41/00		ð	
Document No.:	<u>M-090720-01-2</u>		<i>S</i>	
Guideline(s):			10%	
Guideline deviation(s):			A	
GLP/GEP:	yes	ĈĄ	Å.	
<->> 000720 01 2@E (02221 01 1		457	<u> </u>	

<<M-090720-01-2@S-602221-01-1

The possible risk that the product Gaucho may present to honey bee Os assessed in this paper through a field test. The evolution of hives that were exposed to flowering similower from seeds treated with Gaucho was qualitatively and quantitatively evaluated. Variables sensitive to factors that have an inpract 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 revolution of Imidaeloprid for possible negative effects on bees*" (SENASA) at the 01/10/2000 freeting. As required by the *Good Laboratory Practices* (GLP), *Standardized Operation Proceedines* (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 Argentia as well as institutes and researchers of the Consejo Nacional de Investigaciones Científicas y Tecnicas (CONICET), to proceed to analytical chemical, statistical, palifologic, 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 Gaucia, and PE - 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, communent follow up of the sunflower crop and the plant health treatments was made, a well as the tollow up of wild florg 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 varioa and nosema diseases.

The samples during the test were taken in triplicate and immediately distributed to SENASA, BAYER S.A. and LPE MACN - CONICEP, 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 Imidaclopid 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.



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 proughout the flowering period a higher proportion of plants without pollen was observed in the control site at the compared with the treated site.

As for bees activity and mortality in test sites between dates T2 and T3, 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. Mortably measured in front of hives of both test sites was not statistically different.

In pollen counts made on honey samples taken in 73 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; Riceiardella d'Albere, 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 winform; 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 Dives amount of honey and nectar to 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 hiver 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 Imidaeloprid/seed was determined. That is in agreement with the treatment of seed that was applied. As for the Imidaeloprid residue tests no quantifiable Imidaeloprid residues were found in samples of soil and supflower heads at date 12. No quantifiable Imidaeloprid residues were found in samples of either polien, heavy of yax at lates 33 and 54.

It can be considered that, during the stay of bives 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

...-190720-01 0/5-60222001-1 flowering.



Report:	02.01.03/16; 1998; <u>M-105190-01-4</u>
Title:	02.01.03/16; 1998; M-105190-01-4 Feeding test with bees in field conditions MO-03-010457 M-105190-01-4 US EPA OCSPP Guideline # 850.SUPP 
Report No.:	MO-03-010457
Document No.:	<u>M-105190-01-4</u>
Guideline(s):	US EPA OCSPP Guideline # 850.SUPP
Guideline deviation(s):	
GLP/GEP:	
< <m-105190-01-4@s-604662-01-1< td=""><td></td></m-105190-01-4@s-604662-01-1<>	
FEEDING TEST WITH	BEES IN FIELD CONDITIONS
This test consists in com	paring the behaviour of two beehives, one was fed with sugar syrup, the other
with the same syrup con	taining 20 ppb of imidacloprid. The trial was conducted from June 23rd, to July
8th, 1998. Two hives we	ere placed in two sites, about 5 km apatr. The bees were used to feeding in a
feeder containing sugare	ed water and at a 150 prodistance of the hive. After contamination of the sugar
syrup in one of the two s	sites, the feeder attendance was noted, as well as the quantity of consumed scrup
and the return activity to	the beehive.
>>M-105190-01-4@S-604662-01-1	02.01.03/17 Effects of erop protection products on bees, effects of Goucho seed dressing on losses of foraging bees with comments on the summary eport from Gable Curé and Bernard
Report:	02.01.03/17
Title:	Effects of cop protection products on bees, effects of Goucho seed dressing on losses of
	Torn Bridge and State and Stat
	Ambolic 16 $121998$
Report No.:	$\frac{M-110240602-3}{M-110240602-3}$
Document No.:	$\underbrace{M_{2}^{2}1024}_{0} \underbrace{02-3}_{0} \underbrace{0}_{1} \underbrace{0}$
Guideline(s):	none A A A A A A A A A A A A A A A A A A A
Guideline deviation(s):	$\frac{M-110240602-3}{M@110240602-3}$ $\frac{M}{100}$ $\frac{M}{1$
< <m-110240-02-3@s-604948-0< td=""><td>beekeepers observed increasingly large fails in their sunflower honey yields; the</td></m-110240-02-3@s-604948-0<>	beekeepers observed increasingly large fails in their sunflower honey yields; the
Between 1993 and 1997	beekeepers observed increasingly large parts in their sunflower noney yields; the
central and western-cent	tral 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
	the a rise in the area of land given over to sunflower cultivation using seed dressed
with Gaueno (active ing	redient imidacloprid). Field surveys carfied out by CNEVA and ACTA, field
trials carried out by say	er and the observations and questions taised by beekeepers highlighted the need
>>M-110240-02-3@s-604948-02-1	iscover whether sunflower seed dressing was affecting bee populations.
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4	
Q <sup>°</sup>	
	liscover whether sunflower seed dressing was affecting bee populations.
£ .1 <sup>\</sup>	
for concerted action to do	redience initiacion (initiacion (initiacio
õ	



<b>Report:</b> Title:	02.01.03/18; 2003; M-114 Assessment of side effects of imic (Apis mellifera L.) in the field fol	lacloprid & deltamethrin C	DD 85 on the honey bee
Report No.:	20031216/01-BFEU		T P A
Document No.:	<u>M-116169-01-2</u>	S	, <u> </u>
Guideline(s):	OEPP/EPPO Guideline No. 170 (	3) and BBA Guideline 🕅,	23-1
Guideline deviation(s):	Yes, but acceptable	, <b>A</b>	
GLP/GEP:	yes	Ö Á	
< <m-116169-01-2@s-602224-01-1< td=""><td></td><td>T I</td><td></td></m-116169-01-2@s-602224-01-1<>		T I	
Material and methods	Â	, Ö <sup>v</sup>	

Test substance: Name: Imidacloprid & Deltamethrin OD 85; Development No.: P-00317155; Batch 08137/0023(0019); Tox-No.: TOX06314-00; purity: NJN 33893 (imidacloprid): 73,95

g/L (75 g/L nominal), AE F032640 (deltamethorn): 1016 g/L (10 g/L nominal). The effects of Imidacloprid & Deltamethrin QD'85 were tested on the honey bee (Apis mellifera L.) under field conditions following the guideline of the European and Medderranean 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 Bological Research Centre for Agriculture and Forestry, Federal

Republic of Germany (BBA), part VI 3-1 (STUTE et al. 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 product/ha in 400 L water/ha. The application was carried only in the evening after daily fight agrivity of the bees anobefore full flowering (before BBCH stage 65) of the M-seed spring rape (Brassicanapus) field, A field of untreated oilseed spring rape was used as control treatment. According to the OEPP/CPPO guideline No. 170 (3) the use of a toxic standard for field studies is option a 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 braging activity was chosen. Four Commercial bee colonies were placed near each ten field 2 days before the application? To insure that the bees are exposed to the test field detailed assessments of for aging activity were done before as well as after the application. Mortality and oraging 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 Y1 days (test substance) and weeks after the application.

The influence of the results of the test substance on the hone were 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
- Mortality in front of the nives and in the crop
  Foraging activity (number of foraging bees/m<sup>2</sup> flowering oil-seed spring rape crop)
- Condition of the colonies and development of the bee brood

• Behaviour of the bees in front of the drives and in the crop

Dates of work: 13JUL 2003 to 10JUL2003

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Findings: Toxic	ity to He	brey Bee	s, field	test O
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#### **Imidacloprid Bee Studies Compilation of Study Summaries**

Test substance		Imidacloprid & Delta		
Test object		Apis melli	fera	v° 🏷
Exposure			Spray treatment in the evening after foraging activity of the bees in flowering oil-seed spring rape	
Treatme	nt group	Test substance (Imidacloprid & Deltamethrin OD 85)	Control (unifeated)	
Applicat [in 400 L		1 L (0.986 kg@oduct)		
		Ly c <sup>(</sup>	Ô <sup>v</sup> v ô	
Average	pre:	4 <sup>Q</sup> 40.2	9,30	
Mortality rate	post [1]:	43.8		
[dead bees/	post [1-10]:	22.5 <sup>3</sup>		
hive/day]	Q <sub>M(average)</sub> :		🖌 🖉 0.7 🔿	
Average	pre:	22.5 22.5 22.5 202 202 202 202 202 202 202 20	☆ 10.5 🖌	
Flight intensity [foraging	post [1]:		8.2	
bees/ m²/dav1	post [1-10]	<u>4</u> 9.9 5 0	4.6 <sup>9</sup> Q	
pre = post [1] =	average value dav after appli	s for day $T_1$ and $T_2$ cation ( $T_1$ ), for day $T_1 - T_0$ after application ality per day before application	2 4.67 2 2 4.67 2 3 4.67 2 4.67 2 5 4.67 2 5 7 5 7 5 7 5 7 5 7 5 7 5 7 5 7	
post [1-10] =	average value	for dagT1 - Taafter application	\$F; _0 _0 `^	Ý
Q <sub>M(average)</sub> =	Average morta mortality per d	ality per day before application	<i>b</i> . 0	
	~ (/ .		Se Co .	

#### **Observations**

Effect on honey bee mortality: The application of the test substance in the evening after daily be flight activity of the bees caused a Ô significant increase of the average honey bee mortality on the dirst two assessment days after application  $(T_1 43. 8 \text{ dead bees hive } \mathbb{Q}_2: 100.5 \text{ dead bees hive})$ . 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 been hive in the test substance treatment compared to 0.7 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 T1 to T<sub>4</sub> compared to the values recorded in the control treatment (test substance: 0.7 - 5.4 bees/m<sup>2</sup>/day, control: 8.2 - 11.0 bees  $n^2/day$ . From T<sub>5</sub> 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/m<sup>2</sup> in the test substance treatment and 4.7 bees/m<sup>2</sup> in the control treatment.

#### Effects on honey bee brood development: "C

1 P

Regarding the solony strength and the bee bood development no differences attributable to the influence of the test substance were deserved 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 a soli-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



Report:	02.01.03/19; 2005; <u>M-428629-01-3</u>
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.:	<u>M-428629-01-3</u>
Document No.:	<u>M-428629-01-3</u>
Guideline(s):	not specified
Guideline deviation(s):	
GLP/GEP:	yes & y y y g
< <m-428629-01-3@s-605921-01-1< td=""><td>Monitoring of depopulation and mortality events of bees in beehives with different agricultural destinatuions in the region Emilia Romagna - Final report 2005 <u>M-428629-01-3</u> not specified  yes bees mortality observed in the last years in several EU Countries including Italy uld hypothetically be the use of different agricultural practices, it has been</td></m-428629-01-3@s-605921-01-1<>	Monitoring of depopulation and mortality events of bees in beehives with different agricultural destinatuions in the region Emilia Romagna - Final report 2005 <u>M-428629-01-3</u> not specified  yes bees mortality observed in the last years in several EU Countries including Italy uld hypothetically be the use of different agricultural practices, it has been
Taking into account the	bees mortality observed in the last years in several EU Countries including Italy
the reason of which show	uld hypothetically be the use of different agricultural practices, it has been
considered suitable to ch	leck in open nero the mechanism of this phenomenon and particularly its possible
causes. The attention ha	s 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 comin	ng from different combinations. According to the indications of bee-losses from
the bee-keepers, it has b	een supposed a possible relationship between bee-hives depopulation and corn
sowing Therefore a fiel	d protocol has been processed to be used in a corn-area where corn was
considered the most imr	portant crop: as contral another area without corn belds has been selected
Furthermore in this stuc	ly a third are has been included with mixed crons and without a preponderance
of maize	ly a third area has been included with mixed crops and without a proponderance
>>M-428629-01-3@ <b>S-605921-01-1</b>	
Report:	02.01.03/20 2005 ×1-428@2-01-3
Title:	Abonitoring about possible events of decline of bee populations and mortality in
Demont New 🐇	different cultivated areas in the Region Veneto - Report 2005 <u>M-428632-91-3</u> mo no bees mortality observed in the last sears in Several EU Countries including Italy,
Report No.:	$\frac{M-4a^{8}632-40^{-3}}{M-408(22)}$
Document No.:	$\frac{W-42803}{80}$
Guideline(s): Guideline deviation(s):	An spearied to the second seco
GLP/GEP:	
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< <m-428632-01-3@2605933-01-1< td=""><td></td></m-428632-01-3@2605933-01-1<>	
Taking into account the	bees mortality observed in the last cears in Several EU Countries including Italy,
the reason of which sho	uld hopothetically be the use of different agricultural practices, it has been
considered suitable to cl	neck in open field the mechanism of this phenomenon and particularly its possible
causes. The attention ha	s been for used on different possible factors related both to agricultural and
environmental practices	weather conditions, bee-practices, etc., with the aim to point out the synergy
coming from their differ	ent combinations
In particular according t	othe indication of becoosses from the bee-keepers, it has been supposed a
possible retationship bet	ween bee-hives depopulation and corn sowing, taking into account that these two
events occur at the same	rent combinations of becaosses from the bee-keepers, it has been supposed a ween bee-hive depopulation and corn sowing, taking into account that these two time. Therefore a held protocol has been processed to be used in a corn-area
where corn was conside	red the most important crop; as control an area without corn has been selected.
Furthermore, in this stud	the third area has been included, with mixed crops and without a preponderance
of mais.	
>>M-428632-01-3@S-605933-01-1	
Y A	
Ĉ	red the most important crop; as control an area without corn has been selected. Is a third area has been included, with mixed crops and without a preponderance



<b>Report:</b> Title:	Monitoring of depopu	2006; <u>M-428630-01-3</u> llation and mortality eve ns in the region Emilia	nts of bees in bee Romagna - Final	hives with different of
Report No.:	M-428630-01-3	ins in the region Dimina	Normagna i mar	
Document No.:	M-428630-01-3		<i>S</i>	y p
Guideline(s):	not specified		°.	
Guideline deviation(s):			4	
<b>GLP/GEP:</b>	no	ĈA		
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#### Bees and plant protection products

The insecticide applications, particularly frequent or the last decades, car 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 prostigate 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 arbammates and pyrethroids

Chloro-derivates, phosphorganic, Oarbammates and pyrethroids Generally phosphorganic and carbammate products show a prong brock-down activity, while chlorderivates 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 for aging bees themselves) that normally are contaminated by residues of products introduced in the beehive

1 The effects of some PPPs can be afferent and may depend, for example, on the age of bees. Young bees are more sensitive fowards carbaryl, while adult bees are more sensitive towards malathion and methylparathion (Johansen, 1979). Following the exposure to phosphore mics and pyretroids the bees seem to be more aggressive and regurgifate 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 for aging thes 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

Ĉ It has been demonstrated that many systemic products (dimethoate, acephate, methomyl, methamidophos, monocrotophos, etc.) applied in pre-flowering an later contaminate the nectar provoking, depending on the imported quantity, serious damages to be hive and particularly to the brood, and, in some cases, even the death of the whole family (Fiedler, 1987)

### Microincapsulated

Microincapsula@d methylparathion is one of the product provoking devastating effects on bees because the microcape fe 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 slow release the inside active ingredient in the environment - when the water film wrapping the capsules drigs 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 de 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



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 the 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 age due to very bad and insidious effects on useful entomofauna (particularly on silkwoffi), has been tecognised 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 Ruijtere van der Steen, 1987). The observed anomalies are different: eyes without pigments or with a typical half lumar streak, short and small thorax more or less pigmented, wings wrapped up in the pupal exovia, deformed and not suitable to 19, teguments with uncompleted skeleton and abdomen differently pigmented (Gerig, 1991; Marletto *et al.*, 1992). Colonies treated with fenoxycarb (insegare) showed a sapid decline the molecule has an activity on colonies both on short and long terms. On the contrary, Diffubenzirfon (Bimiline) demonstrated a negative effect on the strength of colony (number of adults and barvae) in the short time, but a minor impact on long terms and no effection survivals of queens (Thompson e Withins, 2002).

#### Neonicotinoids (imidacloprid)

Among the active ingredients recently introdued in the market, imidacloprid (Gauch@®, 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. Inidacloprid 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 poticed only for some time after the treatment (30-60 minutes) and disappeared after some bours. Imidactoprid 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 formal, thiamethoxan, clothianidin.

#### Synergic effects

Another thifty bee into action mechanism is the sphergic 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 nurgen 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 delemethrin's pharmacokinetic in presence on not of prochloraz (Chalvet-Monfray *et al.*, 1996).

Treatments gainst arroa (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.



#### 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 came for flux anate that is 4 times more toxic at 20°C in comparison to 32°C (Niijima et al., 1985). On the contrary Malathian is often dangerous for bees in the hot climatic conditions of California, but not in the fresh comatic conditions of Washington State (Johansen, 1979). Treatments should not be done if a sepsible 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 (Ichanser) 1979).

>>M-428630-01-3@**S-605927-01-1** 

#### **Report:**

Title:

Report No.: Document No .: Guideline(s): Guideline deviation(s): **GLP/GEP:** 

<<M-428631-01-3@S-605930-01-1 Bees and PPPs

02.01.03/22; 2006; M-428631-40-3 Monitoring of depopulation and mortality events of bees in behives with different agricultural destinations in the region Veneo - Report 2006 M-428631-01-3 M-428631-01-3 not specified The insecticide applications, particularly frequent in the last decades can provoke out-and-out hecatomb of bees and wild pollinators. The use of poorty selective active incredients together with a long lasting toxic activity and the tack of expertise showed by farmers in different occasions, are some of the causes of the bee-intoxication that every dear occurs in our cutivate tields. There are numerous experimental trials carried out in laboratory and field, to investigate on the activity of RPPs towards bees, but the market introduction of the modern molecules requires continuously checking activities about their possible side effects

Chloro-derivates, phosphorganic, carbammates and pyrethroids Generally phosphorganic and earbammate products show a strong knock-down activity while chlorderivates 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 be broods and "home bees" (i.e. those that remain in the beenive before becoming foraging bees then selves) that normally are contaminated by residues of products introducted in the behive. 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 malation 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 (Pohansen, 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 er Stephan, 1900; Schricker 1974).

## System products

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System 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  $\mu$ ) as pollen collected by foraging bees and then transported into beehive (Selkirk, 1976; Burgett e Fisher, 1977; Atkins e Kalum 🖉 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 s 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 beeking prolong the toxicity and therefore the brood and home bees mortality for very long time. till 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 or als indicate that there are no differences between the different microincapsulated products and between these products and the traditional formulations, while after 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 per inatory. In any case considering also the recent serious bee deaths, in which microincapsulated products were concerned, it has to be orderlined 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 tamous some dears ago ducto very bad and insidious

effects on useful entomofauna (particularly on silkwarm), 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 Ruijtere van der Steen, 1987). The observed anomalies are different: eyes without pigments or with a typical half Junar streak, short and small thorax more or less pigmented, wings wrapped up on the pupal eQuvia, deformed and not suitable to Dy, teguments with uncompleted skeletor and abdomen differently promented (Gerig, 1996), Marlotto et al., 1992; Nitsch, 1992). Colonies treated with fenoxycarb (Insegar®) showed a rapid decline during the season and a reduction of surviving of queers in next spring, confirming therefore that the molecule has an activity on colonies both of short and long terms? On the contrary, Difluber our (Dimilin®) demonstrated a negative effect on the strength of colony (number of addits and arvae in the short time, but a minor impact on long terms and no effect on survivals of queens (Thompson e Wilkins, 2002).

### Neonicotinoids

Among the active ageredients recently introduced in the market, imidacloprid (Gaucho®, Confidor®, etc.), a systemic insectivide used for seed dressing of different crops and to control sucking pests, has provoked stark stress between beekeepers and the theoroducer 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 sublet and 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 wegative effect was porticed 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 tone down which the insects behaviour is alterated could be fatal to foraging bees (Medrzweki et al., 2005, Bortolotti @al., 2003). Similar active ingredients which are nowadays on the market and for which Similar effects are expected, are fipronil, thiamethoxan, clothianidin.

### Synergie effects

Another shifty bee intoxication mechanism is the synergic effect of two or more active ingredients which, if used segarately, 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 servible. 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.

#### *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 subset of during the night. Nevertheless mevinphos increases is toxicity at few nightly temperatures, so that it is recommended to apply it in summer and not in spring (Beneder 1975) wit is the same for flugaling 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). Freatments should not be done if a sensible decrease of temperature is expected because, in addition to a Mower product degradation the following dew building makes the active ingredient sprayed the day before available for a longer number of bees Hohansen, 1979).

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US **OCSPP** Guideline 8

#### **Report:**

Title:

Report No.: Document No .: Guideline(s): Guideline deviation **GLP/GEP:** 

**GLP/GEP:** 

<<M-429243-01-3@S-6052 A series of field investigations were conducted in 2010 and 2001 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** 

2012; M@

Summary of key findings and conclusions of investigations to evaluate bee exposure

levels at Southern California citrus groves previously treated with imidacloprid

Report:	$\delta^{\gamma}$ $\epsilon^{\gamma}$ $s^{\Box}$ $\delta'$ $\delta'$
Report: 🔊	02001.03424; 2013; <u>M-468556-01-2</u>
Title:	Bee montoring task force: Survey study on pollination practices and their impact on bee
Q <sup>°</sup>	health the Hemish region Study 2012 -2013
Report No.:	$\frac{1}{\sqrt{2}} M - 463556 - 94 - 2 \sqrt{2} \sqrt{2}$
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Guideline(s):	WS EPA OCSPP 870.SOPP
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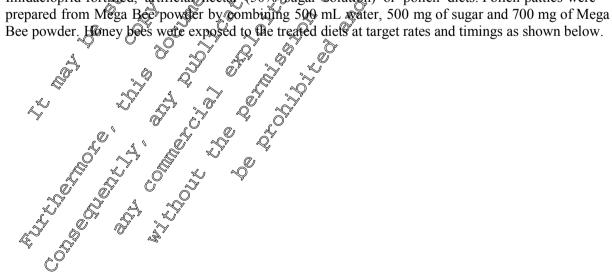
<<M-463556-01-2@ The purp & of this study was to evaluate if crop protection agents (neonicotinoids) used for insect control of fruit growing do have an impact on the colony development/health of honeybees that are used to polyinate truit crops. Therefore we examined if there is a difference in honeybee decline/winter merality 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



Report:	02.01.03/25; (2012; <u>M-442872-01-2</u> )
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Title:	Assessment of exposure of honey bees (Apis mellifera) to imidacloprid in controlled
	feeding study, interim report
Report No.:	<u>M-442872-01-2</u>
Document No.:	<u>M-442872-01-2</u>
Guideline(s):	US EPA OCSPP Guideline Number: 850.3030 (Ecological Effects
Guideline deviation(s):	M-442872-01-2 US EPA OCSPP Guideline Number: 850.3030 (Ecological Effects none no perseeded by the final report (M-442868-02-20 below.
GLP/GEP:	no
This interim report is su	perseeded by the final report ( $M_{42868-02-20}$ below.
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Report:	02.01.03/26; 2013; <u>1-442868-02</u>
Title:	02.01.03/26; 2013; <u>MI-442868-02</u> Pilot study: Honey bee brood and colory level effects following imidactoprid intake via
	treated artificial diet in a field study in North Sarolips of A
Report No.:	S12-01341 $A$ $\phi$ $\psi$ $\varphi$
Document No.:	$\underline{M}_{442868-02-2} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} \sim$
Guideline(s):	US EPA Ref.: OPPTS 850.304@(Ecological Effects)
Guideline deviation(s):	none Q ( , , , , , , , , , , , , , , , , , ,
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< <m-442868-02-2@s-605065-01-1< td=""><td>advatade datering acibility of Kalvating the actor al for holony level</td></m-442868-02-2@s-605065-01-1<>	advatade datering acibility of Kalvating the actor al for holony level
A full field trial was not	aduated the determine Reginitize of Reducting the Retented for heleny level

A full-field trial was conducted to determine feasibility of evaluating the potential for colony- level effects on honey bees (Apis wellifera L.) During and after forced dietary consumption of Imidacloprid. Thirty hives were chosen for the study based of Colorly Condition essessments, (CCA's) prior to study initiation. Parameters for choosing a prive included having all stages of brood, a laying queen, 7 frames of "colony components" consisting of diawn comb with food and brood, and 3 frames of empty or drawn frames for expansion. Hores were randomly assigned to a treatment group. The hives were placed in a postly non-agricultural county in central North Caroling, in an area with only scattered patches of croptand, hone of which were bee-attoactive 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 netabolites confirmed lack of exposure of these hives to any agrochemicals. Ô

The exposure part of the study was conducted by exposing honey bee colonies under field conditions to Imidacloprid-fortified, artificial rectar (50% sugar Solution) or pollen diets. Pollen patties were prepared from Mega Bee power by combining 500 mL water, 500 mg of sugar and 700 mg of Mega



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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 mg2		
Treatment 2 : UTC Pollen patty	Т2	Twice a week(12 total)	0 control)	y W0 g		
Treatment 3 : Low rate Sugar syrup	тз	4	ي 50 ppb (٢ گ	000 m2		
Treatment 4 : Low rate Pollen patty	Т4	Twice a weet 12 y	59 ppb			
Treatment 5: High rate Sugar syrup	T5 Q	Ywice a week(12 togal)	50 ppb			
Treatment 6: High Rate Pollen patty	T6 5 6	wesk(1207 togal)	200 ppb 🔪		бу О	
	× ×	\$ 0 \$		«. »»		

Treatment groups 1, 2 and 5 were fed artificial nextar 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 lave enhance, 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 11,70 mL. Pollen-fed coordinates 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 17.6.1g for the 6-week period. Treatment 2 (control) consumed an average of 1,370.3g. Treatment 4 (50 ppb) consumed 1,364.1g and Treatment 6 (200 ppb) consumed 821.4g.

Colony strength and general hearth 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, CCAs were conducted during weeks -1, 2, 4, 6, 7 and 8. An assessment on week 7 was conducted in addition to the tegular schedule of CCA's made every other week in order to obtain colony measurement/simmediately 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<sup>2</sup>) occupied by adult bees, open biood (eggs and larvae), capped brood, stored pollen, and stored honey and the starts 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.



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 store compound was not evident in these groups. On the basis of this study, a definitive test in which replicate test colonies are feed artificial nectar diets spiked with the test substance appears to be a Casible and sensitive method for determining colony-level responses of honey bees to dietary exposures.

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**Report:** Title:

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02.01.03/27;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ;  $30^{4}$ ; 3nectar diet on long term colony health in field study in Sorth Carolina: Colong condition assessment data & statistics Interingeport M-478404-02Q

M-478404-02 US EPA @CSPP none no

This interim report is superseeded by the final eport <u>M-501299-01</u> S.

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<<M-478404-02-2@S-602258-01-1 A colony-feeding study was conducted with honey bee zolonies (Apis melliferra 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 unidacloprid exposure to result in adverse effects on the bong-term headth of honey bee colonies. As treatment levels of 50 ppb and above, numerous endpoints over repeatedly affected, with pollon stores and capped brood initially being affected. The no observable adverse effect level (NQAEL) for this study is 25 ppb. M-478404-0205-602258-01-1

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Report:	02.01.0628; <b>100</b> ; 2004; <u>M-501299-01-2</u>
Title:	
	o artificial die (in a fiQd stud Qin North Carolina
Report No.: 🔊	$S_{A}$ $S_{A$
Document No.:	$M-501299-01-24^{3}$
Guideling(9):	US EPA OCSPP 850 SUPP
Guideline deviation	$n(s) \sim none \sim s \sim s$
GLP/GEP:	
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< <m-501299-01-2@8-602885-< th=""><th></th></m-501299-01-2@8-602885-<>	

A colony-feeding study was conducted with honey bees (Apis mellifera L.) in a field setting with freeforaging colories, exposed through sucress solution dosed with imidacloprid at nominal rates of 12.5 ppb, 25 ppb, 50 ppb, 100 ppb of 200 ppb. The purpose of this study was to evaluate the potential of imidaclopfid 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 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 opendpoints 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 pro, 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 30 ppb and note pronounced at the 100 and 200 ppb treatment level.

Increased overwintering losses (i.e. coloring 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 colories 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 SOAEL was 50 ppb. There are no indications that exposure to imidacloprid resulted in a higher susceptibility of colonies to V arroa and Nosema infestation.

 Report:
 02:01.03/29;
 2016;
 M-553526-02-3

 Title:
 Beport amendment 01
 Bayer CropScience sectionel hive study-Eastern Canada - Final

 Report No.:
 CEADN005
 CEADN005

 Document No.:
 M-55352602-3

 Guideline(s):
 US EPA OCSPP Guideline Number: @50.SUPP

 Guideline deviation(s):
 no

 Report
 no

A monitoring study wasset 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-sochean-wheat crop rotation is common, and all were close to corn and soybean field. Mapping of the land use in the area around the apiaries showed mainly corn-soybean wheat agricultural rotation but a great theresity 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 (Peason's A = 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 corp, dandelion of soybean pollen and a preference for between 1 and 3 dominant food sources at a time during the growing season.

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. Imidacloprice (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 given loss. In the latter case, aggressive robbing and wasp attacks were seen, which may have ledge to the given loss.

The in-hive temperature results showed that the bees maintained the temperature in the broot 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 a 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 Vational 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 diagnostic. Quantizene virology used for the 2014 and 2015 samples showed that deformed wine 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 Quantigen method. The pattern of occurrence of individual viruses was quite different front 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 NGEL for acute tethality, which was the main concern when the study was initiated. A value of 1.0 for the aggregate TU corresponds to the NGEL for acute lethality. The aggregate TU can be considered equivalent to a first quotient.

The results showed that a significant amount of exposure occurred at the pre-plant time, indicating that planting must have adready started in the area around the apiaries at the time the samples were taken. The maximum aggregate TU values were 0.255, 0 301 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  $\mu$ g/kg (0.428 ng/bee).



Beewatch<sup>®</sup> 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 and 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 foragets, no statistically significant correlation was found (brood area bees: Pearson's R 0.4959, p=0.0713, n=14; foragers? Pearson's R@.4919 p=0.074, n=14). Therefore the effect of loss of foragers in the field on the colonies was not significant anothere 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 rees in spring these residues are likely the result of abraded seed dist generated during planting, and drifting into trees which are in bloom. Nectar samples at this time do not contain significant concentrations of peonicationids Mitigation of this route of exposure can be achieved through use of improvements in dust control from planting treated seeds. An improved fluency agent had been developed and modifications to the sir exhaust system for air seeders have been developed to accomplish this. >M-553526-02-3@S-605078-01-1 02.01,03/30; ; 2016; <u>M 655888-01-2</u> **Report:** The importance of the green industry in reliably and sustainably protecting the natural beauty of our landscapes against destructive pests Title: Report No.: **UŠO564** Document No .: M-55588 Guideling or 850. SUPP Guideline(s): US RPA OCSPP Guideline deviation **GLP/GEP:** Under real-world environmental conditions, not every plant in a sindscape 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 imidaeloprid 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 colory. 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 imidaclopit are reviewed by the U.S. Environmental Protection Agency, its benefits must also be taken into consideration. ; 2017; M<u>581863-01-2</u> Report: Pollingtor field study Evaluating chronic effects of seed, in-furrow at planting and a pre-Title: folder application of imidac oprid to cotton, Gossypium barbadense L. - Final report Report No .: <u>\$581863-01-2</u>Q M-581863-01 Document No .: \ US BPA O€SPP 85QSUPP Guideline(s): Guideline deviation(s) none **GLP/GEP:** A field study was conducted to evaluate the potential long-term effects of imidacloprid exposure to honey

A field study of as 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 for 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 weeks. Thereafter, fives 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 kowl traps containing soapy water. Colony condition assessments (CCA) were conducted with digital photography at crifts a 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. Sample's of soil, crop matrices, included matrices, monitoring hives, and bees were collected at critical time perforts to characterize exposure to imidacloprid and other pesticides, floral resources and overall health status of the foves 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 survivabin addition, the report also includes results from the non-Apis bee surveys, residues of imidacloprid and other performed as and identification of floral resources. The results indicate that there were no significant differences in capped brood, pollen counts, and over vinter survival between the rives 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 anidacoprid-freated 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 inidacloprid-treated and unfroated cotton fields. The overall conclusion from this study is that hones 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:**

Title:

## -408@4-01-

02.03.03/32 2011 <u>408024-01</u> Determination of exposure levels of honey bees for aging on flowers of citrus trees previously treated with midacloprid DEBNI 2056-2

Report No Document No .: Guideline(s): Guideline deviation

### **GLP/GEP:**

<<M-408424-01-3@S-5Q221-01-1 A series of field investigations were undertaken to determine to what extent honey bees foraging on citrus blossoms may be exposed to initiacloprid when citrus trees are treated with systemic applications (soil treatments) of this insecticide.

#### Tunnel Cage Study (Section 29

- The objective of this composent of the study was to examine citrus groves that were treated with a soj application of imidacloprid systemic insecticide, to understand the levels of imidacloprid that occurred in (@) nectar extracted by hand from citrus flowers, (b) nectar collected by forager here bees and transported back to the hive, and (c) nectar or "uncapped honey" deposited by Bees in cells of the brood comb
- <sup>7</sup> Concentrations of imidacloprid, 5-hydroxy imidacloprid and imidacloprid olefin in nectar confected by hand from citrus flowers were similar to those in stomachs of bees foraging on the resame 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 refractometry measurements.

#### **Open Field Study (Section 3)**

- The objective of this component of the study was to examine a true groves that were treated with a soil application of imidacloprid systemic insecticide, to understand the levels of midacloprid that occurred in (a) nectar extracted by hand from citrus flowers (b) nectar collected by forager honey bees and transported back to the honey, (c) nectar or "uncopped 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 stom who of the ranging bees were somewhat to wer than for samples collected directly from flowers of nearby trees. This may reflect a "dilution effect" from bees foraging on other (untreated) flower type Mean inidas forid residues in nectar sampled from the trees were less than 7 ppl n
- Residue concentrations in stored nectar samples were somewhat greater that in flower nectar. This may be because combinection has lower water content and higher sugar content compared with unprocessed nectar, although our results are not conclusive based on refractometry measurements. S
- The imidacloprid concentrations measured in the limited potten available for analysis were equal to those in the stored nectar samples filected from the same hives

### Citrus Nectar Collections from Field Sites Treated on One Year with 10% and 2X Label Rates of Imidacloprid (Section 4)

- The objective of this composition of the start was to determine to what extent increasing the imidaclopri@application gate would impact resulues in the nectar
- Concentrations in flower nector samples appear to be linearly related to application rate, based on ca. 2-fold increases in residue levels with Odoub ing of application rate. L1

#### **%**\_/ Ô ×. Citrus Nectar Collections from Field Sites Treated in Successive Years with Imidacloprid (Section , Ĉ 5)

- The offective of this component of the study was to determine to what extent imidacloprid residues might persist and/or acomulate in circus trees from year-to- year following multi-year applications. Őı
- Based on experiments at the Hernet Site, invidacloprid residues in spring flower nectar appear to be a function of the rate applied of the post recent application only, and appear to be independent
- of applications made in prior years. To's 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 Findcove 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.
- <sup>©</sup> 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

02.01.03/33; 2015; M-534355-01-2 Weight of evidence assessment of higher tier studies on the toxicity and risks of imidacloprid in honeybees M-534355-01-2 none none yes thodology was used to assess a number of higher tier stretcher and the stretc **Report:** Title: Report No.: Document No.: Guideline(s): Guideline deviation(s): **GLP/GEP:** 

<<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 theid studies and some ecoepidemiology studies. °~

M

Assessment endpoints were population size and stability of continer fally managed bees; and, for the latter, quantity and quality of hige products. The field exposures were compared to the results of a higher-tier field toxicology study that used on umber of hive-relevant responses of horeybees. This study reported a NOAEC of 25 µg IMD/2, equivalent of an oral NOAED (73 ng/bee/d) for all responses measured. The LOAEC was 50, µg IMIØL, equivalent to an oral LOAED of 14,6 pg/bee/d. These toxicity values were expressed in doses per be to allow normalization from different sources of 0 exposure.  $\cap$ 

Reports provided by Bayer CropScience and papers from the openditerature 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 strongth of the studies and their relevance.

Potential risks from exposures of poneybees to MD various matrices sampled in the field were characterized. Some studies were stronger than others and the overall mean for strength of methods (SoM) was  $2.82 \pm SE_{\odot} = 0.10$ . The mean and SE for releasince was  $0.17 \pm 0.12$  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 IMD2 rom its use as a seed treatment. For exposures via treatments other than seeds (soil drench and coliar applications), some studies were stronger than others and the overall mean for SoM was  $274 \pm SE$  of 0.96. The mean and SE for relevance was  $1.17 \pm 0.29$ , suggesting greater variance in relevant offects in studies that were generally strong. These data suggest that some soil and foliar freatments 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 sectar for horeybees.

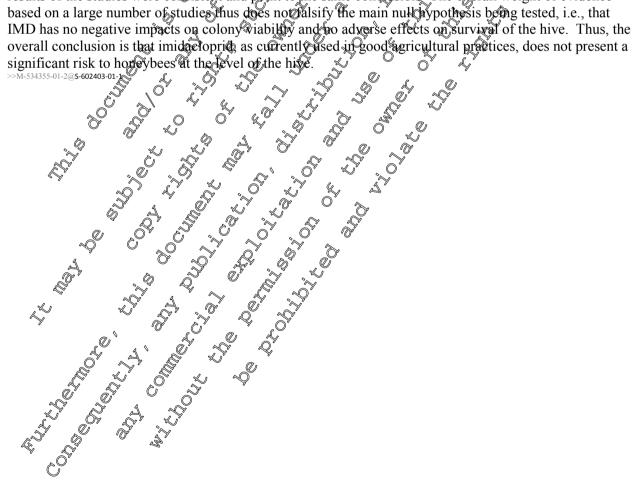
The Wop analysis of field studies of the effects on seed treatments with IMD on honeybees showed no relevant effects in a range of studies of variable strength. The WoE clearly demonstrates that environmentally realistic concentrations of IMD result in no adverse effects to honey bees at the colony level 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 occu 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 honeybees in adjacent fields with flowering plants indicated that there was minimal impact to honey bees. The SoM was  $1.48 \pm SE$  of 0.10 which was similar to that of the second dressing application. Low relevance was associated with the responses associated with the other types of applications.

With respect to honeybees, there were fewer higher ther observational (ecoepidebniological) studies conducted with IMD. As for other responses, some studies were stronger than others, the overall nean for SoM was  $1.88 \pm SE$  of 0.19. The mean and SE for relevance to adverse effects was  $0.11 \pm 0.01$ . In general, weaknesses were related to lack of full consideration of potential conformeders, 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. Ab the stronger studies and not identify adverse effects and the results of all but one of the weaker oudies were consistent. The fack of effects in these studies is likely due to combination of low exposures and label directions to minimize exposures to bees. 1 °

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 alsify the main null hypothesis being tested, i.e., that IMD has no negative impacts on colony viability and to adverse effects on survival of the hive. Thus, the





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Report:	02.01.03/34;	; 1999; <u>M-006815-0</u>				
Title:				nectar, blossoms, poller and		
	foraging honeybees		ner rape field and	effects of these residues on		
Report No.:	SXR/AM 001	5				
Document No.:	M-006815-01-3		9			
Guideline(s):			4	6 <sup>4</sup> 29 . 9		
Guideline deviation(s):		Ča	\$, "			
GLP/GEP:	yes	- A	Ū <sup>¥</sup>			
< <m-006815-01-3@s-602053-01-1< td=""><td></td><td>L,</td><td>Ő</td><td>&amp; 428.2 g/l fmidaclopria</td></m-006815-01-3@s-602053-01-1<>		L,	Ő	& 428.2 g/l fmidaclopria		
Material and methods:	Poncho FS 500, a.	i. content: <b>%</b> .3 g/L	Beta Syfluthrin	& 428.2 g/l Imidacioprid		
specification (formulatio	on No.: 030 based o	on 06200,0029, deve	elopmental % o.: (			
conditions small beehive						
rate: 5 kg/ha) as a sampl	ing device for rape	nectar and rape pot	len. Nectar was a	also directly sampled from		
flowers via micropipette	s. In addition, flow	vers were sampled b	y hand. The hon	eybees used as sample °		
collectors were observed	for signs of behav	toral impacts. All sa	mples including	the honeybees were		
subjected to a residue an	alysis for imidacio	prid and its refevant	metabolites.			
Dates of biological wor	<b>k:</b> June 15 - 18, 19					
subjected to a residue an Dates of biological wor Dates of analytical wor	<b>k:</b> June 30 Quly	22, 1998, <sub>1</sub> , <sup>y</sup>		Š Š J		
	<i>∞</i>		~~ () ~~			
Findings: Residues in ra	ipe plant matrices a	and mothe topaging	røneybees	$\sim^{\circ}$ $\ll$		
				) )		
Type of Sample			Residue Lever r	ng/kg/*		
		Onidacapprid . O	Qefin-NTN	Hydroxy-NTN		
Q						
Control Samples	L 6 2			ý		
HoneybeesDefor		$\sim < 0.0$	0.0↓	< 0.01		
Rape nectar sand	sled by bees 🖉	, 0 <sup>7</sup> - <del>(</del> )	\$ <sup>7</sup> <sup>*</sup>			
Rape nectar am	oled with micro-	× \$0.01 \$	Õ <i>≶</i> Ø.01	< 0.01		
capidaries from t	be flowers?	D' 'O O	, Ô			
Rope blossoms		< <b>0</b> ,	0.01	< 0.01		
Rape pollen samr	oled by bees		<u>s</u>			
Treatment Sampl	es O J	A O O				
Honeybees befor	exposire	× <6:01 ×	< 0.01	< 0.01		
Rape Octar sunp	oled by beas 🔪 🔿	x _x∞0.01∞	< 0.01	< 0.01		
Rape nectar sam	oled with micro-	_ @ < 0.0€	< 0.01	< 0.01		
Rape nectar sampled with micro- Concerned and Concerned an						
, Rape blossoms		Ś∽ <u>\$</u> 0.01	< 0.01	< 0.01		
Rape pollen samp	olew by bees Q	<b>0.01</b>	< 0.01	< 0.01		
* Limit of quantitatic	·0·					
	m. U. a mgagg					
		/	1 1.	1		

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.

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Report:		99; <u>M-006811-01-</u>		
Title:				ctar, blossoms, pollen and
				d effects of these residues on
Report No.:	SXR/AM 002		R.	
Document No.:	<u>M-006811-01-3</u>		O,	
Guideline(s):	Internal Testing Method			Ô <sup>4</sup> Ô <sup>4</sup> . ?
Guideline deviation(s):	not applicable	Ĉs	L.	
GLP/GEP:	yes	T.	, OU'	428.2 g/1 Imitacloped; 0195939); under field
< <m-006811-01-3@s-601942-01-1< td=""><td></td><td>4</td><td>,Õ<sup>%</sup></td><td></td></m-006811-01-3@s-601942-01-1<>		4	,Õ <sup>%</sup>	
Material and methods	Poncho FS 500, a.i. co	ontent. 98.3 g/L E	Beta-Cyfluthrin	& 428.2 g/1 Imidacloppid;
specification (formulation	on No.: 030 based on 06	520 <b>6/</b> 0029, devel	opmental No.: (	0195839); under field
conditions small beehiv	es (appr. 5,000 honeybe	ess were caged	on flowering sur	nmer rape plots (dolling
				ilso directly sampled from
flowers via micropipette				
				bees were subjected to a
residue analysis for imic	actoprid and its relevan	nt/metapolites		
Dates of biological wor				ž Čí "
Dates of analytical wo				
	* 0, .	\$ \$ \$		Č V
Findings: Residues in r	ane nation and i	in the foraging h	whees	8 K
Thungs, Residues in I			Sugyocca S ~ . Ø	
		$\mathcal{Q}^{*}$		Q
Type of Sample	Y A Y B	× <sup>×</sup> <sup>×</sup> <sup>×</sup> <sup>×</sup>	esidue Level [mg/	19 *
L. L	່ ຈີ່ 🖉 🕺 Imi	idactoprid	Defin-NWN	/ Hydroxy-NTN
Control Samples		<u>, , , , , , , , , , , , , , , , , , , </u>	<u> </u>	
Honeybees befor	e expositre &	< 0.01	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	< 0.01
Hone brees after		£0.01 D	\$ < 0,01	< 0.01
Rape nectar sam	*X /	× 0.01	\$0.01	< 0.01
Rape nectar sam	Jed with micro	< 0.01 ~~	<>> 0.01	< 0.01
Capillaries from	he flowers		y Y	
Rape bloss		ية 0.01 <i>لاي</i>	< 0.01	< 0.01
Rape pollen sam	pled by bees ** 🖉 🧹	· ò		÷
Treatment San	es of a of			
Honeybees befor	e exposure Q	Ø 0.01 Ø	< 0.01	< 0.01
Honeybees after	exposure of the	<sup>9</sup> < 0.20	< 0.01	< 0.01
Rape nectar same	pled by bees	≲.01	< 0.01	< 0.01
🦧 Rape nectar/sam		گَ <sup>©</sup> ∕0.01	< 0.01	< 0.01
capillaries from t	be flowers 🔧 👸	)Y'		
Rape blossoms		< 0.01	< 0.01	< 0.01
Rape follen sam	pled by bees *			
* Limit of quantitation: (	0 mg/kg/ ** Amount in	sufficient for resid	ue analysis	
Observatione No kena	vicital impacts (e.g. ana	thy exaggerated	motility discor	ordinated movements) or
suspicious mortality wa				
	hids were observed on		neeting rape ne	etal and tape policil. At
>>M-00681 (-0)-3@S-601942-01-1		ine rupe plants.		
Please click on the hype	rlink to order a Study R	leport.		



Report:	02.01.03/36; 1999; <u>M-0</u>			
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 fora			
Report No.:	SXR/AM 006	igning noneybees. Hoeld		Ŭ D
Document No.:	<u>M-016820-01-3</u>		O <sup>3</sup> <sup>(7</sup>	
Guideline(s):		.4	A 64	29° . Q
Guideline deviation(s):				
GLP/GEP:	yes			Y O' O
< <m-016820-01-3@s-602058-01-1< td=""><td></td><td></td><td></td><td></td></m-016820-01-3@s-602058-01-1<>				
Material and methods: s	unflower seed (variety "Flour	v") either dressed wit	th 150 g/U <sup>i</sup> Taucho	WS ZW(a i
content: 72.5% imidaclo	prid; batch no. 233 614 749,	developmental in 04	175 778 or imida	clopcid-free
were drilled on 10 May	1999 in soils with different in	idacloprid residue le	vels. Soil samples	for an
analytical determination	of the imidacloprid residued	evel were taken imme	diately beføre drill	ing. Drilling
rate was 0.5 U/ha. Durin	g peak flowering of the sumfl	owers (end of July) si	mall bee colonies	2,00000
	caged on these plots (appr. 50			
pollen. In addition, some	e pollen and flowers were san	pled by hand. The h	oneybees used as s	amplers
were observed for signs	of behavioraDimpacts. All sai	mples and a small san	nples of hopeybees	were
subjected to a residue an	alysis for onidacloprid and it	srelevantmetabolites		
		S 12 15 8		
Dates of biological wor	k: ~~ Luly 25 -	August 3, 1999. 🖉	, or or	
Dates of soil analysis:	کر ک	- 13, 1999.		
Dates of analysis of bio	logical samples. September	r 25 − 29, 199 <b>9</b> .	y y y y y y y y y y y y y y y y y y y	
		/		
Findings: Residues in Se	bil and in sunflower plant that	trices planted as succe	eeding crop (detect	s above the
LOQ are highlighted.	A B S A	à là chiến c	V Mn	
	bil and in sunflower plant that		· · · · · · · · · · · · · · · · · · ·	
Type of Sample		Residue Level mg/k	(g] *	
, Q .	Almidaeloprid	O Olefin-NTS	Hydroxy-NTN	
Control Plot (south of fig	d number 502) imidacloprid-free	e seed in imidacloprid-fre	e soil	
Soil sample (0-30 cm)	L'A L'A DA MAY	& 5 <sup>4</sup>		
Leaves (produced latest)	A R A A A.	<b>n.d.</b>	n.d.	
Flowers (male/ female)		n.d.	n.d.	
Nectar sampled from the	hive combs night.	n.d.	n.d.	
Pollen sampled from the	hive combs number of the second secon	n.d.	n.d.	
Pollen sampled from the	plants n.đ.	n.d.	n.d.	
Honeybees exposed to th	e sunflowers Q hd.	n.d.	n.d.	
* Limit of quantitation for so			t of detection (0.002 mg/	(kg)
Limit of quantitation for br	blogical samples 0.005 mg/kg for imid	below limit of detection (0.)		olefin-
		below minit of detection (0.	0015 und 0.005 mg/kg/.	
$\frac{1}{1}$ U ( $\frac{1}{100}$ = 150.0	00-seed			
	N. N			
	L.			
Č <sup>O.</sup>				
-				



#### Imidacloprid Bee Studies Compilation of Study Summaries

Type of Sample	Residue Level [mg/kg] *			
- , pe or campie	Imidacloprid	Olefin-NTN	Hydroxy-NTN	
Variant "1997" (field number 502) - imid		1 imidacloprid-contam	1 1	
Soil sample (0-30 cm)	0.018		\$ <sup>7</sup>	4 .4
Leaves (produced latest)	n.d.	n.d.	A n.d. 5	
Flowers (male / female flowers)	n.d.	🖒 n.d.	y n.d.	
Nectar sampled from the hive combs	n.d.	🔊 n.d. 🖉	n.@ ~	
Pollen sampled from the hive combs	n.d.	n.d	Gi.d.	
Pollen sampled from the plants	n.d.	n.d.	Q n.d.O	o ú
Honeybees exposed to the sunflowers	p.d.	° S <sup>n.d.</sup>		
Variant "1998" (field number 507) - imid	acloprid-free seed f	n imidacloprid contars	Grated soor	A d.°
Soil sample (0-30 cm)	LOQ		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Leaves (produced latest)	of the c	, ~ n.d.	in the second se	A CONTRACTOR
Flowers (male / female flowers)	9 & n.d. , S	S not s	, , n.d. ,	6
Nectar sampled from the hive combs	n.d.	Sn.d.	Önd 🖓	y <sup>*</sup>
Pollen sampled from the hive com	x 10d. S	n.do	P.d.	
Pollen sampled from the plants $\checkmark$	\$ n.d.	A	n.d. Oʻ	
Honeybees exposed to the shaflower		n.d. 2	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Variant "1999" (south of field number 507	) – Gaucho-dressed	seed imidacloprid-	free soil	
Soil sample (0-20 cm	n.d.S		~~	
Leaves (produced tarest)	× 0.007 ~	) ,	© <loq< td=""><td></td></loq<>	
Flowers (male Gemale Bowers)	V C <sup>n.d.</sup>	and a second sec	ی <sup>۷</sup> n.d.	
Nectar sampled from the hive combs	n <sub>s</sub> d.	Gr On.d.	n.d.	
Pollen sampled from the have comps	A Add	n.d.	n.d.	
Fonce sampled from the plants	n.ds	ingd.	n.d.	
Honeybees exposed to the sunflowers	in more c	n.d.	n.d.	
* Limit of quantitation for soil samples of Limit of quantitation for brogical samples of Observations: No behavioral empacts	0.005 may/kg for imida	cloprid n.d. = below lim clop and hydroxy-imi- low limit of detection (0	dacloprid, 0.01 mg/kg fo	or olefin-
				,
<b>Observations:</b> No behavioral impacts	s (e.g. apathy exa	aggerated motility,	discoordinated mo	ovements) or
>>M-04-52-0-01-3@S-602058-01-1		sed for concerning s	sunnower neetar an	la policii.
Observations: No behavioral impacts suspicious mortality was observed on M-04-570-01-3@S-602058-01-1 Gradient Construction of the second secon				



Report:	02.01.03/37; ; 1999; <u>M-0</u>			
Title:	Residue levels of imidacloprid a			, Or
	pollen of sunflowers cultivated		idacloprid residue levels and	d'
	effects on these residues on fora	ging honeybees. 'Laache	r Hoff 1999	-
Report No.:	SXR/AM 007			
Document No.:	<u>M-016827-01-3</u>	A		<u>م</u>
Guideline(s):				Q 1
Guideline deviation(s):		ò ý	LA LA S	
<b>GLP/GEP:</b>	yes	T d'	r Hog 1999	Ľ
< <m-016827-01-3@s-602071-01-1< td=""><td></td><td>í, jů</td><td></td><td>,0″</td></m-016827-01-3@s-602071-01-1<>		í, jů		,0″
Material and methods: s	unflower seed (variety "Fleur	(= va	rian <sup>®</sup> 1999) with AS0 g/6	,×
Gaucho WS 70 (a.i. con	tent: 72.5% imidacloprid: batc	h no. 233 614 749 de	velopmental no.04 175 77	8)
or imidacloprid-free (co	ntrol, variants "1997, 1998 and	d 1998 (2x)" wese drill	ed on 1 May 99 in sorts	- )
with different imidacion	rid residue levels and treatme	history Soil sample	s for an analytical	
determination of the imi	dacloprid residue level were t	akenûmmedûtely befo	re delling Drilling rate we	26
0.58 U/ba During peak	dacloprid residue level were t flowering of the supflowers (2	(1) and 26 (1) which amount h	and animal for the second s	13 ]
bonowhood) were and	on these plots (appr. 50 m2) a	and 20 July) Small U	Sunflyture poster of poll	) on
lioneybees) were caged	on these plots (appl. 30 m2) a	s a sampring device ha	sumpower bectar and poin	en.
In addition, some pollen	and flowers were sampled by	nang.yl ne togneybees	use was samplers were	
observed for signs of be	havioral impacts. All samples	and a small sample of	honeybees were subjected	to
a residue analysis for im	idaclopridand its relevant me	tabolites		
Dates of biological work	د: 🔊 🕉 🕺 🖓 Lyly 23 - 🖗	ugust 3, 1999.		
Dates of soil analysis:	∼ ( Aumustro_1	1_1000 🔧 🛸	R D	
Dates of analysis of biol	ogreal samples: August 25	- September 21, 1999		
Findings: Residues in Se	il, in sunflower plant matrices	planted as succeeding	t crop and in honeybees us	ed
as sampling device Wet	ects above the LOQ are fighli	ghted).		
~~ _`		5 2 5		
Type of Sample		Residue Level [mg	σ/kσ] *	
Type organiples.				
		VU0	Hydroxy-NTN	
Control Plot (field	umber (1) – in dacloprid-free see	d in invidacloprid-free soil		
Soil sample (0-30)cl				
~O .				
Leaves (produced la	utest) $O' < O' n.d.$	n.d.	n.d.	
Flowers (male / fem	ale flowers)	n.d.	n.d.	
Nectar sampled from		A nd	n d	
	name nive combs	n.d.	n.d.	
Pollen sampled from	n the hive combe 🕺 🔨 n 🕸	n.d.	n.d.	
Pollen sampled from	n the plants	n.d.	n.d.	
A		n.u.		
Honeybees exposed		n.d.	n.d.	
*Limit of quantitation (I		nidacloprid); n.d.=below limit	of detection (0.002 mg/kg)	
LOQ for biological sa	mples 0 005 mg kg (imidacloprid & hy	droxy-metabolite), 0.01 mg/k	g (olefin-metabolite);	
	n.d. = below limit of detection (	0.0015 mg/kg and 0.003 mg/k	(g, respectively)	
	Ő			
یر ۲ ' ۱۸۳ (Unit) + ۲	150,000 seed			
LOQ for prological sa				
	S.			
~O×				
$\lor$				



Issue date 2017-11-22

Type of Sample		Residue Level [mg/	kg] *	
	Imidacloprid	Olefin-NTN	Hydroxy-NTN	
Variant "1997" (field number 710) - imig		imidacloprid-contan		N O
Soil sample (0-30 cm)	0.016		 	
Leaves (produced latest)	n.d.	n.d.	A n.d. 🛠	
Flowers (male / female flowers)	n.d.	ارم n.d. ا	n.d	
Nectar sampled from the hive combs	n.d.	🕅 n.d. 🖉		
Pollen sampled from the hive combs	n.d. 🖉	n.d.	Ön.d. 🖓	
Pollen sampled from the plants	n.d	n.d. 9	° Q n.4	
Honeybees exposed to the sunflowers	e.d.	• n.d		
Variant "1998" (field number 702) - imid		imatic longial contant	Insted and c	1
Soil sample (0-30 cm)	A0.013		$\cap$	
Leaves (produced latest)	A A O	n.d	y and w	A A A A A A A A A A A A A A A A A A A
Flowers (male / female flowers)		n.d. y n.d. y	A - A A n.d. 4 B n.d. 4 B n.d. 4 B n.d. 4 B n.d. 4 C n.d.	0 Å
Northern and a difference that he have a second and		Sn.d.		
Pollen sampled from the hive contes	La add a	On the C	> ~ n.d. &	
Pollen sampled from the plants	7 ~~ n.d.	A Lad .	on.d.	
Honeybees exposed to the surflower		n.d.	n.d. y n.d.	
Variant 1008 (2x)" (field number A VIE	- imid Sopri A Vec	seeOin im dacloprid-	contant ated soil	
Soil sample (0-30 cm Leaves (produced safest)	0.0145 2 0 0.0145 2		~~	
Leaves (produced Rest)	w ngd.	n.d.,	Ø n.d.	
Flowers (male Gemale Gowers)	n.d.	A AN W	n.d.	
	ind a	S On.d.	n.d.	
Pollen sampled from the five cares			n.d.	
Pollen sampled from the plants	nd.	x, <sup>y</sup> O , x, yn.d.	n.d.	
Honeybees exposed the sunflowers	N N O	n.d.	n.d.	
Variant "1999" (field nober 75) - Gad	tho-dressed secor in i	misacloprid-free soil		
Soil sample 30 cm				
Leaves (produced latest)	-Q.906 V	n.d.	< LOQ	
Flowers (male / female Nowers)	n.d.	n.d.	n.d.	
Nector sampled from the hige combs		n.d.	n.d.	
Pollen sampled from the hive combs	y Lyn.d.	n.d.	n.d.	
Variant "1999" (field mober 75) – Gan Soil sample @ 30 cm) Leaves (produced latest) Flowers (male / female tlowers) Nactar sampled from the hive combs Pollen sampled from the hive combs Pollen sampled from the hive combs	n.d.	n.d.	n.d.	
Honeybeer exposed to the millowers	~Ç n.d.	n.a.	n.d. f detection (0.002 mg/k	

\*Limit of Cantitati@ (LOQ) for soil imples: 0.006 mg/kg (imidacloprid); n.d.=below limit of detection (0.002 mg/kg) LOQ to biological samples: 0.000 mg/kg (imidacloprid & hydroxy-metabolite), 0.01 mg/kg (olefin-metabolite); a 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.



Report:	02.01.03/38;	99; <u>M-016832-01-5</u>		
Title:	Effects of imidacloprid	residues in sunflower l	honey on the dev	elopment of small bee
	colonies under field exp	osure conditions		N R
Report No.:	SXR/Am 004		ð	
Document No.:	<u>M-016832-01-5</u>		J.	
Guideline(s):				
Guideline deviation(s):			A	
GLP/GEP:	yes	Ì	Å.	
< <m-016832-01-5@s-602121-01-1< td=""><td></td><td>- And - And</td><td></td><td></td></m-016832-01-5@s-602121-01-1<>		- And		

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 09. Under field exposure conditions small bee colonies (appr. 500 honeybees) were confined on pat plots 50 no, drilled on JApril 1999) and exclusively fed with sunflower honey which was for fied with either 0,3,5, 10 ev 20 µg/kg imidacloprid. One colony received comb cells produced by koneybees during a provious feeding experiment with a 10  $\mu$ g/kg sucrose solution. Pollen of the Meditebranean bush was provided as a protein source. The small bee colonies were examined for freatment related impacts over a period of 39 days. In particular, the following endpoints were evaluated: morality comb cell production, food consumption, storage behavior, hive weight increase egg laving activity, breeding success, colony strength, foraging S. intensity and behavioral anomalies  $\int_{a}^{O}$ 

# Dates of biological work: May 28 - July 7, 1998.

Findings: Effects of imidaclopfid residues in sunflower honey opermall boneybee colonies 4. L 

	Ĉo	Ň	<u>s</u>	y o	, v		, ¢
Testing Endpoint		Control	<sup>Q</sup> 2 μg/kg	5 mg/kg	Φ0 µg/kg	10 µg/kg*	20 µg/kg
Mortality (no. of dead bee			) 0 <sub>8</sub>	5 %	<u> </u>	<u>لار</u> 7 ک	→ 5
front of bee hives)	-	, S		Y NY	<i>a</i> ,	0 4	
Mortality (no of dead bees	at the	24	2V 2Q	. ∼Qĭ	6 18 2	18	26
tent margin)		× k	, N	la n	y Q	<i>S</i> <sup>*</sup>	
Foraging intensits	, , ,	10	<u>د</u> ۱۹	× 114	138	143	121
(no. of bees at the Hongy I	eeder)		A ~~			U 1	
Foraging intensity	K)	ي 26 چ		5 <sup>22</sup>		31	36
(no. of bees at the pollen f	eeders)		× .	Nº N	<sup>y</sup> 0 <sup>y</sup>		
Honey consumption [g]		546	* 546	581	566	616	546
Pollen consumption	~~	546 573 559 1999	مَّنَّ 76 مَ <sup>(</sup>	େଛମ	ີ 53	63	65
Comb cell production at st	yay	S 559 n	568 109	603 7 2752	<b>610</b>	583	576
termination [cm <sup>2</sup> ]	ð ð		0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,	Ó V	, ,		
Honey storage area at stud	у 🔊	~199	290 290 290 290 290 290 290 290	¥ 2°83,	201	313	165
termination [cm <sup>2</sup> ]	0°	240 C		Ű			
Hive weight increase at stu	illo ,	õ 240 🖗	200	205	235	270	220
termination	.4	· 🔊	Q 115	Ŷ.			
Egg/aying activity[cnicco	mb	× 120	0 115	143	208	60	148
area containing eggs] at st	udý©	Ô,	, × , O`				
termination	۰ ۵		, Q				
Colony strength Ccm <sup>2</sup> com	b area	MJ 1	252	231	213	210	351
area containing eggs] at structure termination	y 6 <sup>5</sup>	Ś	~Q~				
	<u> </u>	<u>~</u>					

\* Fed with comb coils from a prexious feeding experiment. N S ×1

Observations: There were no differences between the control and the treatment groups in any of the evaluate 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** 



Report:	02.01.03/39; ; 1999;	<u>M-016845-01-4</u>		
Title:	Effects of imidacloprid rest	idues in maize polle	en on the develop	ment of small bee
	colonies under field exposu	ire conditions		
Report No.:	SXR/AM 005		ð	
Document No.:	<u>M-016845-01-4</u>		<u>A</u>	A A
Guideline(s):			104	
Guideline deviation(s):			A	
GLP/GEP:	yes	Ĉ	S.	
< <m-016845-01-4@s-602132-01-1< td=""><td></td><td>- A</td><td><u>"</u></td><td></td></m-016845-01-4@s-602132-01-1<>		- A	<u>"</u>	

**Material and methods:** *test substance:* imidacloprid techn., *purity:* 98.6%, *identify:* 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 out ploto (50 m2, drilled on 1/ April 1999) and exclusively fed with maize pollen which was fortified with either 0.2, 5, 10 or 20 ug/kg imidacloprid. Sunflower honey was provided as carbofydrate 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, tood consumption, storage behavior, hive weight increase egg laying activity, breeding success, colony prength, foraging intensity and behavioral anomalies.

# Dates of biological work: May 20, July 6, 1

			, Q	
Testing Endpoint	ol 2 4 g/kg	5 μ <b>g</b> ¢kg	¢0∕µg/kg	20 µg/kg
Mortality (no. of dead bees in front of	10 × 50		∀ 8	7
bee hives) Mortality (no of dead bees at the tent margin)		Ç <sup>I</sup> Z	21	30
Foraging intensity	22 \$ 19 04 - <sup>25</sup> 124	23	37	24
Foraghing intensity a grad		<sup>≫</sup> 123	130	128
(no. of bees at the doney feeder) Pollen consumption [g], Honey consumption [g],	35 29	32	39	34
Honey consumption (g) 2 4	9 <sup>7</sup> 541	521	500	543
Comb cell production $[c_0r^2]$ $\mathcal{A}$	28 551	579	584	563
Comb cell production [cop <sup>2</sup> ] Honey torage area at study termination 7 1 [cm <sup>2</sup> ] Hive weight increase	77 201	186	147	174
Hive weight increase	80 230	215	200	200
	44 153	181	205	153
Colony strength form <sup>2</sup> comb area, 2 covered with bees at study termination)	17 258	305	314	221

Findings: Effects of imidaclopfid residues in maize pollen on small honeybee colonies

**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.



Report:	02.01.03/40; 1999; M-0	040023-01-3		0
Title:	Residues of imidacloprid and ir	nidacloprid metabolites	s in nectar, blossoms, polle	and O
	honey bees sampled from a Bri	tish summer rape field	and effects of these residue	s on
Papart No :	foraging honeybees SXR/AM 003 <u>M-040023-01-3</u> US EPA OCSPP Guideline Nun none yes S: Poncho FS 500, a.i. contents			Ô
Document No.:	SAR/AM 003 M_040023_01_3			Ş
Guideline(s):	US EPA OCSPP Guideline Nu	nber: 850.SUPP	A. 6 <sup>9</sup> 29	l do
Guideline deviation(s):	none	Ča d.		
GLP/GEP:	yes			Ø se
< <m-040023-01-3@s-602143-01-1< td=""><td></td><td>á, Ô<sup>v</sup></td><td></td><td></td></m-040023-01-3@s-602143-01-1<>		á, Ô <sup>v</sup>		
Material and methods	: Poncho FS 500, a.i. content	78.3 g/L Beta Cyflu	thrin & 428.2 g/1 Imidac	loprid;
specification (formulat	ion No.: 050 based on 0620000	029, developmentar	NO. 200195859), lest pro	ayet.
rape seed dressed with	2.5 1/dt Poncho FS 500; drillir	ng rate: 5/kg/ha, Und	er field conditions small	, ,
	oneybees) were caged on flow			
March 98) as a samplin	ng device for rape nectar and ra	pe pollen. Noctar 🗞	s also directly sampled f	rom
flowers via micropipet	es. In addition, flowers were s	ampled by hand. The	hopeybees used as same	Øers
were observed for sign	s of behavioral impacts. All say	mples including the l	mueybees were subjected	d to a
residue analysis for imi	dacloprid and its relevant meta	abolites alysis: September 25		
			S. S. P	
	rk: June 22-24, 1998 (soil an	alysis: September 25	- 29, 1998).	
	ork: June 30° - July 28, 1998.		Š <sub>S</sub> Č <sub>K</sub>	
Findings: Residues in	rape plant matrices and in the	toraging honeybees	× .0	
			ý <sub>k</sub>	
Type of Sample		Residue Level [mg/k	g] * %	
Type of Sample				
	Anndacloped		Hydroxy-NTN	
Control Samples			Ž.	
Honeybees before	exposure of a contract	~ < QQ1	< 0.01	
Honeybees aftere	kpostifie < 0.0 P eduby bees < 0.0 P	\$ \0.01 L	< 0.01	
Rage nectar sampl	ectby bees K	°°<<0.01℃	< 0.01	
Rape nectar sampl		$\sqrt{2} < 001$	< 0.01	
°capillaries from/h	e floweys	& S		
Rape blossom		$\bigcirc^{\nu} \approx < 0.01$	< 0.01	
Rape pollet ampl		0.01	< 0.01	
Treatment Sample		<i></i>		
Honeybees before		<ul><li>⊘ &lt; 0.01</li></ul>	< 0.01	
Horeybees after ex		< 0.01	< 0.01	
Rape nectar sampl		< 0.01	< 0.01	
Rape nectar sansi		< 0.01	< 0.01	
capillaries from the	e flowers a log	< 0.01	< 0.01	
Rape blossoms		< 0.01	< 0.01	
Rape pollen sampl		< 0.01	< 0.01	
* Limit of quantitation: 0.0	)1 mg/kg.			
	$\mathcal{O}$ $\mathcal{D}$ '			
Observations No bet	aviord impacts (e.g. apathy, ex	aggerated motility, a	liscoordinated movemen	ts) or
	as observed on the honeybees u	used for collecting ra	pe nectar and rape poller	1.
>>M-04923-01-3(05602143-01-1				
Ŭ,				
$\lor$				
Please click on the hyp	erlink to order a Study Report.			



Report:	02.01.03/41; ; 2	2006; <u>M-451677-01-3</u>	
Title:	Assessment of effects	of imidacloprid WG 70 on	foraging activity and mortality of °
	honey bees and bumb	lebees after drenching appli	cation under field conditions on thrubs
			florum surrounded by other ornamental
	plant species	e	
Report No.:	M-451677-01-3		
Document No.:	M-451677-01-3		On the state
Guideline(s):	none		
Guideline deviation(s):	none	*	
GLP/GEP:	no	G	
		, W	
< <m-451677-01-3@s-604680-01-1< td=""><td></td><td></td><td></td></m-451677-01-3@s-604680-01-1<>			
Material and method			
Shrubs of the species F	chododendron catawbi	iense grandiflorum locate	d at the experimental farmland
"Laacher Hof" near M	onheim (40789 Monhe	eim, Nordrhein Westfaler	Germany) received soil treatment
with lmidacloprid WG	70 dissolved in water	akan application volume	of 1 1 per strub in winter of 2005
(2005-01-13) at the approximation (20	olication rates given ir	Pable Control shrabs	(treatment@roup()) received no.
treatment Each treatm	ent group consisted of	3 parallel rows of 18 Rh	ododenation nlarts
treatment. Each treatm			
Table 1: Summar	y: Treatment Group	s and Rates	
			A A A A A A A A A A A A A A A A A A A
Treatment group		$\beta$ $\gamma$ $2$ $\gamma$	
		4.3 gals./m plant size*	2.15 g aus /m plant size*
		×	

	-		
Treatment group			
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		4.3 gas./m.phant size* 2.15 g as /m plant size*
Application ates		Contrôl	2.58 ga.s./shrub 129 g as / shrub
	A V	4	3.68 g product/shrub
Water volume rate	per plant		ې ۲ L tap water, ۲
* plants were 0 gr	n high/wide	5 .9	

Between the rows of Riododendron catawbiense grandiflorum, a mixture of bee attractive. potted ornamentals in watering travs was set up on the timen skeets 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., Cantana camara and Cobelia sp. In the near surroundings of the study site no other flowering crops were located

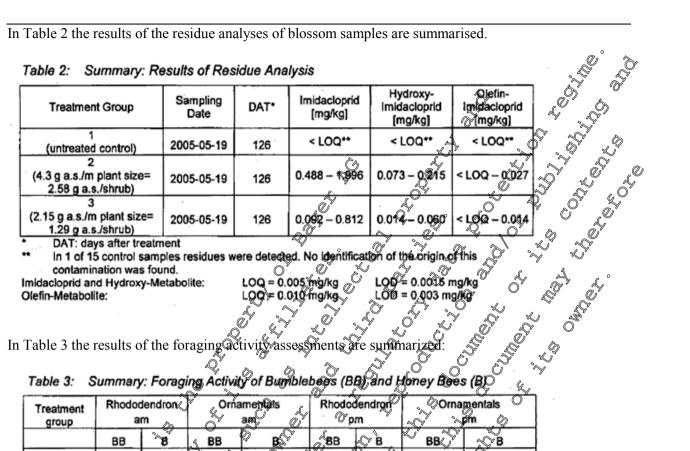
One hive colony of honey bees Apis mellifera and 3 colonies of bumblebees Bombus terrestris were placed next to the Rhododendron catawbiense grandiflorun shrubs on 2005-05-20 {honey bees} and 2005-05-21 (bumblebees). Assessments on foraging activity of the honey bees and bumblebees were conducted on 10 days during flowering of the Rhododendron catawbiense grandiflorum shrubs from 2005-05-24 to -25 (5 consecutive days) and 2005-05-24 to -25 (5 consecutive days) and 2005-05-24 to -25 (5 consecutive days) 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 the sheets lad 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 bridactoprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. Extraction sample clean up and determination of lmidacloprid, 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:



In Table 2 the results of the residue analyses of blossom samples are summarised.



In Table 3 the results of the foraging@ctivity/assessments are summarized

Table 3: Summary: Foraging Activity of Burnblebees (BB) and Honey Bees (B)

		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			10°	-	<	
Treatment group	Rhodoo	iendron m	×		Rhodoo		~~~ ~~	mentals O pm 🏷
	BB	¢%	A BB	B B	<b>8</b> B	N B	ВВС	~⊖B
Control	120		3 (Fuensia)	B Grawberg			(i conoici)	(strawberry, Lobelia sp.)
2.15 g a.s./m plant size	de la companya de la comp		✓ 1 (Fuch\$ia)		¢¥59 ⊧		(Fucturia)	1 (strawberry)
4.3 g a.s./m	702	060		× 0,~	ES V	1. 1		1 (Lobelia sp.)
N.				r, 0	∧ √		°.	

The foraging activity of burghebees on the Rhodorendron 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 bumble bees 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,

Ô Throughout the study only one honey we observed foraging on a Rhododendron plant (control). In none of the other treatment groups visits of this pant species occurred. Also the ornamental plants were only searcely visited by the honey bees, stoney 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 orgamentates set in this study. However, in the near surrounding of the study site no other dowering crops were located,

No dead the worker bees or bumblebees were found throughout the study on the individually labelled finen sheets had out between the Rhododendron catawbiense grandiflorum rows and the rows of the subrounding ported or momental plants and the linen sheets placed in front of the bee hive and the bumblebee colonies. õ

# 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 thestudy with lmidacloprid WG 70 at either 4.3 g a.s./m plant size (2.58 g a.s./shrub = 3.68 g produceshrub) resulting in residues in blossoms up to 1.996 mg imidacloprid/kg or at 2.15 g a.s./m plant size (129 g a.s./ shrub = 1.84 product/shrub) resulting in residues in blossoms up to 0.812 mg imidaclopric kg.

Untreated Rhododendron catawbiense grandiflorum plants were visited more frequently bothe 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

	(x, y) $(x, y)$ $(x, y)$ $(x, y)$
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	Rhododendron sp and to Hibiscus syriaces on foraging activity and mortality of money
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Material and methods	

The study was carried out in 2 parts: the first part was condocted 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 Hibisens syriacus located at the area of Bayer CropScience AG @0789 Monhem, Nordrheim-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@hrubs (treatment groups 1 and 3), located in acdistance of 200 m received no treatment. @

Treatment group			. 3	4
Treatment nome	Rhododendran,		Hibiscus, untreated	Hibiscus, treated
Application rates		4.3 g a.s./m.average	-	4.3 g a.s./m average plant height*
	\$ A . P	5.2 g a s./shrub ©7.37 sproduct/shrub	-	4.3 g a.s./shrub = 6.14 g product/shrub

Summary: Treatment Groups and Rates Table 1:

To describe the size of the Rhodedendron 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.  $\sim$ L,

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 be attractive potted ornamentals was planted or sown in flower beds. The composition of orpamental 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 Lafkspur (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 burblebees (Borrbus terrestris) per study part were placed next to each plot at the beginning of shrub flowering. Honoybees 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 Hiblecus) Assessments of foraging activity of the honeybees and bumblebees were conducted ones in the morning and once in the afternoon on 10 days during flowering of the Rhododendron sharbs, each ting on the Rhododendron shrubs and the surrounding ornamentals separately from 2006-05-21 to 2006-05-24 (4 consecutive dos) anothom 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 syriagus shrubs and the surrounding ornamental were separately conducted once in the morning and once in the afternoon from 2006-07-25 to 2006-07-27 (2) consecutive days), from 2006-07-31 to 2006-08-04 (5 consecutive days) and from 2006 08-07 & 2006 08-09 (2 consecutive days). The mortality of honeybees and humblebees 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 Hubiscus 106-117 days after the application. Samples were stored at -18°C until the sample preparation and even hally residue analysis for Imidacloprid and its Olefin- and Hydroxy Metabolites were capried out on the blossoms. Extraction, sample clean-up and determination of Inordacloprid, Hodroxy and Olefin-Metabolites by HPLC-MS/MS were performed according to method 01010 (MR-06/127). Dead hopeybees and burnblebees found on the linen sheets between the plants and in front of the bee haves and bumblebee colonies were also subjected to residue analysis for residues of Imidacloprid and its Oleffn- and Hydroxy-Merabolites. Extraction and determination of Imidacloprid, Hydrox and Olefin-Metabolites boHPLC-MS/MS was performed according to method 00\$37/MD02 (MR-6/144).

# Findings:

 $\bigcirc$ ging activity assessments in Rhododendron and Hibiscus are In the Tables 2 and 3 summarised. Ø)

Honeybees Table 2 Summary Bumblebees and on Rhododendron

	NY ∾ UNNALI	Q Total number per species observed per plot [n]				
	V Moneybees Bumblebees					
Treatment group	Rhododendron	Omamentals	Rhododendron	Ornamentals		
1: Control X	~\$23	64	608	238		
~ 2: Veatment	10	104	107	87		

On the 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 Hibisco

1	Total	number per spec	ies observed pe	r plot [n] 🗸 🔊
	Hone	ybees		bleber S
Treatment group	Hibiscus	Ornamentals	Hibiscus	Comamentals
3: Control	10	<sup>©</sup> 192	Q 233	\$37 2 5
4: Treatment	5	້ 108 🔨	9 0	<b>∞ 623</b> 0 <sup>×</sup>

Again only few honeybees were observed foraging on Hibiscurshrubs 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 bunblebees 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 Rhododention 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 eplicates, neither in the Rhododendron part. In the treatment replicates, in total 2 Gead bumblebees were found in the Rhododendron part, and 4 dead bumblebees in the the the biscus part.

Table 4: Summary Mortality of Honeybees

		K AHIDI	scus
		imber (n)	
Treatment group			in front of hive
Control	0 0 0	<i>©</i> , 0	0 .
َ ِ ۞ Treatment ِ , ،		°~ 0	0

Ô

Table 3: Summary: Mortality of Bumblebees

	Rhodod	endron 🏷	Hib	iscus
		o otal nu	mber (n)	
Treatment group	n the plot	in front of hive	on the plot	in front of hive
Control 6		X O	0	0
Treatment		ຸ 1	12	2
Y V ST	N Q N			

Colony health and condition of the herebee colonies was not different before and after the study, neither in the control for in the treatment. Colony health and condition of the bumblebee colonies after the Hibiscus parts of the study were not different between treatment and control.<sup>1</sup>

In Table 6 the residue analysis of the Rhododendron and Hibiscus blossom samples and the residues in horeybees and bumblebees are summarised.

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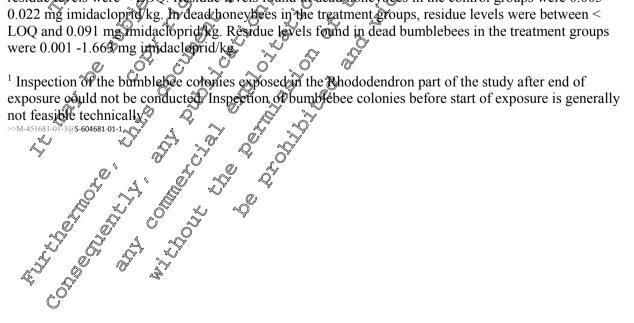


Table 6:	Summary:	Results	of Residue A	nalysis			0
Treatment Group	Sample description	Study part	Sampling Date	DAT*	Imidacloprid [mg/kg]	Hydroxy- Imidacloprid @mg/kg]	Oletin- Imidacloprig mg/kg]
1: Control	blossoms	Rh	2006-05-17	35	< LOQ	<sup>™</sup> < LOQ	~ <b>200</b>
2: Treatment	blossoms	Rh	2006-05-17	35	0.09 - 0.79		< 100 - 0.01
3: Control	blossoms	н	2006-07-27	106	< 600	حياقم	
4: Treatment	blossoms	н	2006-07-27 to 2006-08-07	106 - 117	0.78 - 5001	< 00 - 0.45	< LOQ -0.33
1: Control	2 honey- bees (colony)	Rh	2006-05-29	A7		< LOQ	0.001 -
	25 honey- bees (colony)	Rh	2006-05-21 to	39 - 649	0.016	<2LOO ↓ 0.001	CLOQ - 0.001
2:	2 honey- bees (plot)	Rh	2006-05-21 to 2006-05-31	<b>89 - 49</b>	0.002 - ` .0.091	< 100 0.018 \?	< 100 - 0.001
Treatment	1 bumble- bee (colony)	Rh	< <b>2006-05-29</b> ^		~ 0.063°	5 0.05 ·	0.005
	1 bumble- bee (plot)	Rh®	2006-05-01	27 49 U	6.005	~ <b>0</b> .003	0.003
4:	2 bumble- bees (colony)	v (	2006-07-26	1@5	√0.003,¥ 0;©©4	0.001 – 0.003	0.004 – 0.009
Treatment	12 bumble-	× H	2006-07-25 to 2006-08-08	¢04 - 168	0.077- 1.663	0.009 - 0.196	0.031 – 0.405

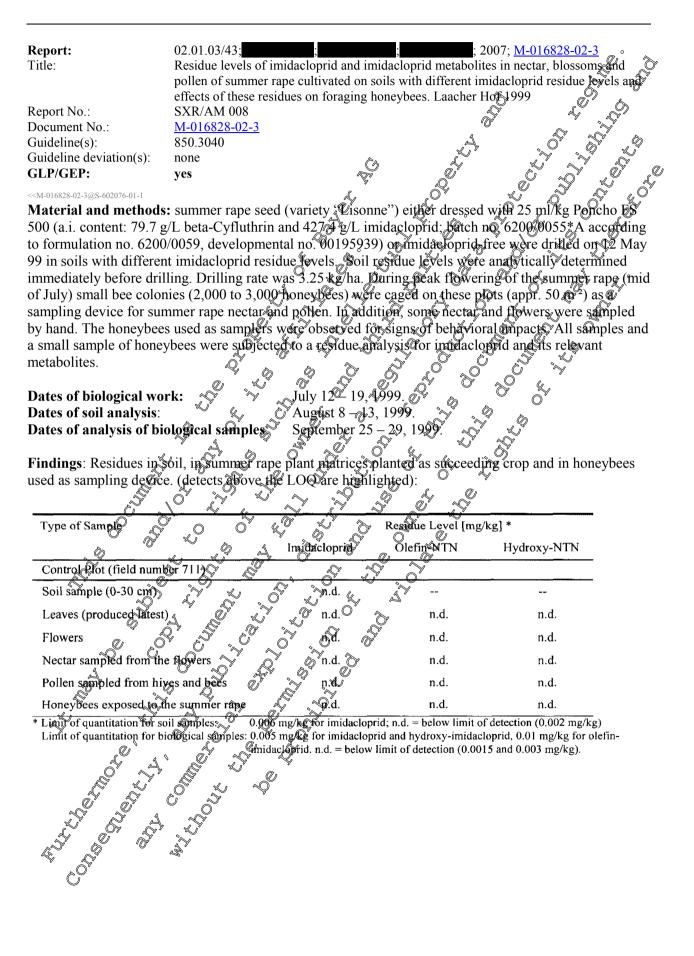
. . . .

DAT: days after treatment OH Rh Rhododendron *√* Hibiscus Blossom samples: Imidaclopric Hydroxy-Metabolite, Olefin Metabolite: LOQ = 0.010 mg/kg Insect samples: Imidacloprid, Hydroxy Metabolite, Oletin-Metabolite LOQ = 0.001 mg/kg

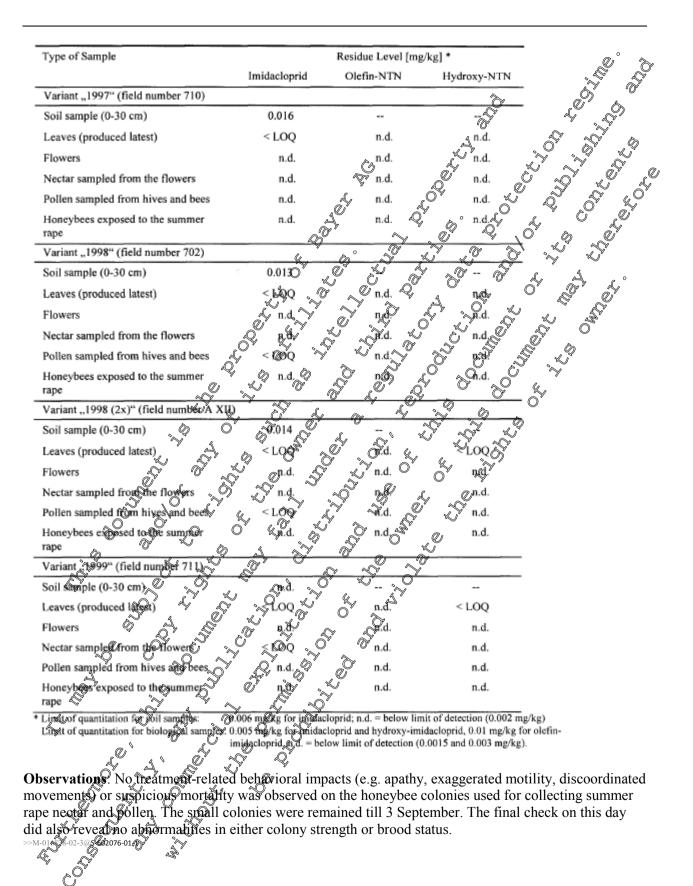
Residue levels in the Rhododendron blossoms were 0.09 - 0.79 mg in dacloprid/kg and in Hibiscus blossoms Q 76 - 5.01 mg/midaet prid g in the treated replicates ho the blossoms of the untreated plants, residue tevels were < LOQ. Residue tevels found in dead høneybees in the control groups were 0.005 -0.022 mg imidacloprid kg. In dead honeybees in the treatment groups, residue levels were between <













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<b>Report:</b> Fitle:	02.01.03/44; ; ; Residue levels of imidacloprid and imida		2007; <u>M-016842-02-3</u> • in nectar, blossoms and
inte.	pollen of summer rape cultivated on soils	with different imid	acloprid residue levels ar
	effects of these residue on foraging hone	ybees. 'Hoefchen' 1§	99 <sup>1</sup> 0 <sup>1</sup>
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Document No.:	<u>M-016842-02-3</u>		ST AN
Guideline(s): Guideline deviation(s):	US EPA OCSPP Giuideline Number: 850 none	J.SUPP	
GLP/GEP:	yes	Ŵ	
<	у С	í Se	99 4 25 mi/kg Percho F 62000055* A accord
Material and method	s: summer rape seed (variety "Disonne"	) either dressed wi	P 25 ml/kg Pencho F
500 (a.i. content: 79.7)	g/L beta-Cyfluthrin and 427 a g/L imida	igiopina, gaton no.	0200000000000000000000000000000000000
o formulation no. 620	0/0059, developmental no. 00195939) o	n imidacioprid fre	e were drikled on M
99 in soils with differe	nt imidacloprid residue weels Soil same	bles for an avalytic	cal determination of th
midacloprid residue le	evel were taken immediately before drill her rape (mid of July) small bee colonies	ing@Drilling rate	was 7 kg/ha. During pe
lowering of the summ	er rape (mid of July) small bee colonies	(2,000 to 3,000 h	oneybees) were caged
hese plots (appr. 50 m	n <sup>2</sup> ) as a sampling device for summer rap	e nector and poller	n in addition, some
nectar and flowers we	re sampled by hand. The hone bees used	as samplers were	Cobserved for signs of
benavioral impacts. A	Il samples and small sample of honeyb	ees were subjected	to agresidue analysis i
imiagaionfia ana lis fe	levant metabolites.		
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*	$rk = 104 $ $m^2 2 - 10 $ $m^2 1000$		
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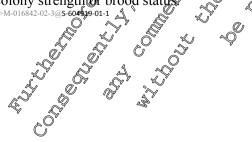


Issue date 2017-11-22

Type of Sample		Residue Level [r	ng/kg] *
	Imidacloprid	Olefin-NTN	Hydroxy-NTN 🖉 🧴
Variant "1997" (field number 502)			The second secon
Soil sample (0-30 cm)	0.018		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Leaves (produced latest)	< LOQ	n.d. 🔬	6 2 2
Flowers	n.d.	n.d. n.d. n.d.	The set of
Nectar sampled from the flowers	n.d. 💎	rQ.	
Pollen sampled from hives and bees	n.d	An.d.	nd provide
Honeybees exposed to the summer	र्जुव.		Q Ondo O
rape	<u> </u>		
Variant "1998" (field number 507)		j or or	The second second
Soil sample (0-30 cm)	$\mathcal{A} \sim \mathcal{O}_{\mathcal{A}} \sim$		
Leaves (produced latest)	n.d. 0	A Ond.	
Flowers			S and.g
Nectar sampled from the flowers $Q^{\vee}$		St sod.	
Nectar sampled from the flowers $\mathbb{Q}^{2}$ Pollen sampled from hives and bees $\mathbb{Q}^{2}$		D n.d. D n.d.	The state of the second
Honeybees exposed to the summer &			y y n.d.
rape			
Variant "1999" (south of field oumber 302		× 4.	
	y A.d. 5		Å/
Soil sample (0-30 cm) Leaves (produce) latest		5n.d.~~~	n.d.
Flowers O O		and.	n.d.
Nectar sampled from the flowers	~LOQ O	n.d.	n.d.
Pollen sampled from wves and bees	~ <1.00 ×	<b>n.d.</b>	n.d.
rape a la l	COO'0 < L60 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	n.d.	n.d.

Limit of quaddation for biological samples: 0.895 mg/kg/ for initiacloprid and hydroxy-imidacloprid, 0.01 mg/kg for olefin-pidacloprid. 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 ar study termination did also not reveal any abnormality in either colony strength or brood status





Report:	02.01.03/45; 2017; <u>M-</u>	<u>542796-03-2</u>		
Title:	Pollinator full field study evalu	uating chronic e	ffects of a post se	eding application of O
	imidacloprid in pumpkins (Cu	rcubita pepo per	oo) - Final report	eding application of
Report No.:	13798.4145		ð	
Document No.:	<u>M-542796-03-2</u>		Â	
Guideline(s):	US EPA OCSPP 850.SUPP		10,	
Guideline deviation(s):	none		A	
GLP/GEP:	no	ĈĄ	L.	
< <m-542796-03-2@s-605072-01-1< td=""><td></td><td>- T</td><td></td><td></td></m-542796-03-2@s-605072-01-1<>		- T		

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 field 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 by 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 4-16 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 fives were selected and randomly assigned in a stratified manner to other infidacloprid-treated (n=5) or antreated reference (n=5) pumpkin fields. Note study honey bee hives and one monitoring hive were assigned to each pumpkin field. Nine bumble bee nexts and two monitoring bumble bee nexts were randomly assigned to each pumpkin field. Honey bee hives and bumble bee nexts were moved into the fields once sufficient blooming of the pumpkins had occurred. The bives remained in the pumpkin fields for 6 weeks. Thereafter, hives were relocated to a post-expressive apiary near Durand, We

Samples for residue analysis were collected from field soils pre-Deatment and indicated very low, background levels (019 ppb) of invide loopid, clothianton, 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 imidacloopid in treated fields were detected; however, levels were very low (0.8, 2.1, and 1.2 opb 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 invidacloopid in reference pollen samples. In treated fields, however, imid cloprid 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 imitacloprid. 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 imitacloprid 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 cetts, or bee bread cells for any assessment. Overall colony survival, including overwintering was 66% for reference fields and 56% for treated fields. There were no significant differences in *Novema* 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



Issue date 2017-11-22

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 be colonies and non-Apis the surveys between reference and imidaeloprid treated fields. There were no statistical difference and numbers of dault the exponential differenc to the state of the second to the second tot without the second the Overall, no adverse effects were observed in honey bee colonies and non-Apis bee surveys between or reference and imidacloprid treated fields. There were no statistical differences in numbers of adult bees,



ð

# **02.02 – Exposure**

# 02.02.01 - Nectar and Pollen

Report:	02.02.01/01; ; 2012; M-0184	436-01-4	ý <sup>4</sup> ý
Title:	Residues of imidacloprid and imida	cloprid metabolites in s	unflower blossoms Simplet
The.	in Argentinia		
Report No.:	SXR/AM 002a		
Document No.:	M-018436-01-4	\$ Q	
Guideline(s):	US EPA OCSPP Guideline Number	r: 850.SUP	
Guideline deviation(s):	none	Q' p°	
GLP/GEP:	no		V O B O
	*		

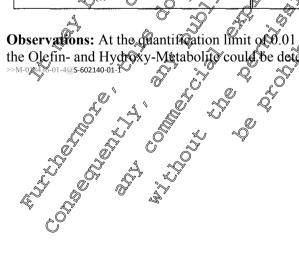
<<M-018436-01-4@S-602140-01-1

Material and methods: Gaucho WS 70 (700 gO imidacloprid) was tressed on supplement varieties (i.e. Rigasol, Albena, Tournesol c. Rigasol, Jaguar) at Grate of 0.7 mg a.i./sod. The target rate was verified by an analytical check of treated seed samples. The actual seed oressing rates ranged between 79 and 119% of the nominal value. The treated sunflower weds were drifted in 56 m<sup>2</sup> flots established within a conventionally managed sunflower field. The sunflower field was located in the vicinity of San Gregorio, Argentinia, Whet the sunflowers were in full blosson flowers were harvested from different zones of the sunflower heads (i.e. "early" and Bate" 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.  $\bigcirc$ 

Dates of work: February 6 to 7, 1998 Findings: Residues of Implacloped and Imidacloprid Stetabolites in Sunflower Blossoms

	e løvel [mg/kg	]
No the Infidacloprid Q	ofin-NTN	Hydroxy-NTN
Inner zone (= "eår y" flowers)	< 0.01	< 0.01
Central zone	< 0.01	< 0.01
Central zone Outer zone (,,late Plowers)	< 0.01	< 0.01

Observations: At the grantification 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.





Danauti	02.02.01/02.	1000- M 00/915 01	2	
<b>Report:</b> Title:		1999; <u>M-006815-01-</u> prid and imidaclopri		nectar, blossoms, poller and O
				l effects of these residues on
	foraging honeybees			$\sim$ $\sim$
Report No.:	SXR/AM 001			F F F
Document No.: Guideline(s):	<u>M-006815-01-3</u>		.1	
Guideline deviation(s):		۵.	s de la companya de l	
GLP/GEP:	yes	- Co	Ő	& A28.2 g/l limidactoprie
< <m-006815-01-3@s-602053-01-1< td=""><td></td><td>Å.</td><td>- Ô<sup>9</sup></td><td></td></m-006815-01-3@s-602053-01-1<>		Å.	- Ô <sup>9</sup>	
Material and methods:	Poncho FS 500, a.i.	content: 98.3 g/L E	Beta Syfluthrin	& 28.2 g/l Imidacioprid
specification (formulatio	on No.: 030 based on	. 0620079029, devel	opmental No.:	0@ 95939; under field /
conditions small beehive	es (appr. 5,000 honey	/bees) were caged a	m flowering su	primer rape plots (drifting
rate: 5 kg/ha) as a sample	ing device for rape n	ectar and fape poth	en. Nectar was	also directly sampled from
				neybees used as sample °
collectors were observed	for signs of behavit	ral impacts. All sai	nples including	The honeybees were <i>Q</i>
subjected to a residue an	alysis for imidacion	rid and its refevants	metabolites.	
Dates of biological wor				
Dates of analytical wor	<b>K:</b> June 30 July 2	2, 1998 y w	> 8 <sup>3</sup>	
Findings: Residues in ra	ne nlant matrice@an	d in the forging		
Findings. Residues in 18				S &
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		× ~	<u>م</u>
Type of Sample	CA O ST		Residue Level	mg/kg/*
4	A O	Onidactoprid . O	Quefin-NTN	, Hydroxy-NTN
Control Sam			- <del>0' %</del>	ý · · · · · · · · · · · · · · · · · · ·
	<u> </u>			- 0.01
HoneybeesDefor		$\gamma^{4} < 0.00$		< 0.01
Rape nociar sand			- Alan	
Rape nectar Sing captaries from t			٥ چ <b>ه</b> (01	< 0.01
Rope blossoms	i a		~< 0.01	< 0.01
Rape pollen same			∞ < 0.01	< 0.01
<u>~</u>			<u> </u>	
Treatment Sampl	<u>es</u> <u>v</u>	K D	······	
Honeybees before	expositre	~ <6:01 ~	< 0.01	< 0.01
Rape Dectar sump	led y beas	×≫0.01~	< 0.01	< 0.01
Rape nectar samp	oled with micro-	ر (¢ < 0.00 ) (¢	< 0.01	< 0.01
cupillaries from a	he flowers			
Rape blossom	AZÓ	′ <u>م</u> ي 0.01	< 0.01	< 0.01
🦾 Rape pollen samp	oled by bees Q	<u> </u>	< 0.01	< 0.01
* Limit of quantitation	n: 0 gr mg/kg			
it an		S.		
Observations: No beha	voral impacts (e.g.	apathy, exaggerate	d motility, dise	coordinated movements) or

**Observations:** No behavioral inspacts (S.g. apathy, exaggerated motility, discoordinated movements) or suspicious mortanty 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.

Solo Solo



Report:	02.02.01/03; ; 1999; <u>M-00681</u>	1-01-3	° .
Title:	Residues of imidacloprid and imidac	loprid metabolites in nec	tar, blossoms, poller and
	honey bees sampled from a summer		
	foraging honeybees	â	y a s
Report No.:	SXR/AM 002	Ş	
Document No.:	<u>M-006811-01-3</u>	0° 4	
Guideline(s):	Internal Testing Method		
Guideline deviation(s):	not applicable	3 L	
GLP/GEP:	yes 📎		
< <m-006811-01-3@s-601942-01-1< td=""><td>foraging honeybees SXR/AM 002 <u>M-006811-01-3</u> Internal Testing Method not applicable yes Poncho FS 500, a.i. content 78.3 on No.: 030 based on 06206/0029, yes (appr. 5 000 honeybees) were content for the formation of th</td><td></td><td></td></m-006811-01-3@s-601942-01-1<>	foraging honeybees SXR/AM 002 <u>M-006811-01-3</u> Internal Testing Method not applicable yes Poncho FS 500, a.i. content 78.3 on No.: 030 based on 06206/0029, yes (appr. 5 000 honeybees) were content for the formation of th		
Material and methods	: Poncho FS 500, a.i. content 78.3	g/L Beta-Cyfluthrin &	428.2 g/1 Imadacloperid;
specification (formulati	on No.: 030 based on 06206/0029,	developmental No.: Q	0195839); under field
conditions small beeniv	cs (appr. 5,000 noneybees) were e		ing supe poors (usining
	ling device for rape nectar and ap		
flowers via micropipett	es. In addition, flowers were samp	ed by hand. The honey	bees used as samplers
were observed for signs	s of behavioral impacts. All sample	s including the honeyb	ees were sabjected to a
residue analysis for imi	dacloprid and its felevant metaboli	tes <sub>t</sub> or sy	
Dates of biological wo	rk: July 2 - 601998 🐄 🖓	y <sub>n</sub> <sub>n</sub> y	
Dates of analytical wo	rk: July 9, 29, 1998		S 'r
		s including the hone of	
Findings: Residues in 1	rape plant matrices and in the forag		
			Ô
Truce of Coursels		Desides Levelle a	
Type of Sample		Residue Level [mg/]	2
Ş	The second second	🗸 Defin-NYN 🏑	<sup>*</sup> Hydroxy-NTN
Control Samples			
X		N N N N N	< 0.01
Honeybers befo		2 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	< 0.01
Hone brees after	exposure a goor a	< 001 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	< 0.01
Rape nectar sam	pled by been a control of the contro	0.01	< 0.01
Rape nectar sam	pled with micro < 0.01	S S 0.01	< 0.01
			< 0.01
Rape blossens Rape pollen sam		ڪَ <sup>`</sup> < 0.01	< 0.01
			₩ <del></del>
Treatment Sam	Nes of . O . O' . O' .	y"	
Honeybees befo	re prosure Q 0.01	< 0.01	< 0.01
Honeybees after	exposure of contract of the co	< 0.01	< 0.01
Rape nectar san	pled by bees States	< 0.01	< 0.01
🔬 Rape nectar	pled with moero- 2 20.01	< 0.01	< 0.01
capillaries from	the flowers &		
Rape blossoms	م من	< 0.01	< 0.01
Rape Sollen sam	pled by bees *		
* Limit of Quantitation:		r residue analysis	
		a residue analysis	
AQ	~~~		
	avioral impacts (e.g. apathy, exagge		
suspicious mortality wa	sobserved on the honeybees used	for collecting rape nec	tar and rape pollen. At
	phids were observed on the rape pla	ants.	
>>M-00681 0-3@S-601942-01-1			
Please click on the hype	erlink to order a Study Report.		



Dara arti		1(020 01 2	
Report:		16820-01-3	
Title:	Residue levels of imidacloprid a		
	pollen of sunflowers cultivated of		imidacloprid residue levels and
	effects of these residues on forag	ging honeybees. 'Hoefd	chen' 1899
Report No.:	SXR/AM 006		
Document No.:	<u>M-016820-01-3</u>		
Guideline(s):			
Guideline deviation(s):		à là	
GLP/GEP:	yes	AT O	
	•		
< <m-016820-01-3@s-602058-01-1< td=""><td>, n</td><td>X L</td><td>chen' (999)</td></m-016820-01-3@s-602058-01-1<>	, n	X L	chen' (999)
Material and methods:	sunflower seed (variety "Floary	() either dressed wi	th ISO g/U Graucho WS JO (a.i.
content: /2.5% imidac	loprid; batch no. 233 614 /49, d	evelopmental no. 04	1 1645 / 180 or immaclopfid-free
were drilled on 10 May	v 1999 in soils with different in	dacloprid residue le	vels. Soil samples for an
analytical determinatio	n of the imidacloprid residue	vel were taken imm	ediately before drilling. Drilling
rate was 0.5 U/ha Dur	ing neak flowering of the sunflo	wers (end of July) s	mathee colonies 2 0000
3 000 honeybees) were	caged on these plots (appr. 50	$m^{2}$ ) as a sampling d	whice for sunflower nextar and
nollon In addition son	a pollon and flowers were some	nlod by hand That	where the sum of a complete
ponen. In audition, son	ne pollen and flowers were sam	pice by name The	as samples
were observed for sign	s of behaviora Pimpacts. All sam	ipres and a small sar	npusoi noneybees/were
subjected to a residue a	analysis for midacloprid and its	relevantmetabolite	So & S
	Q , 4 0 5		Š 20 (k.
Dates of biological wo	ork: 🔗 🦄 🖓 July 25 🖉 A	ugust 3, 1099.	0,
Dates of soil analysis:	August 8 –	12, 1999.	
Dates of analysis of bi	iological samples September	25 - 29, 1999	
2		/ 4.7 %	J AG
Findings: Desidues in	soil and in surflower plantmatr		adit aron (detects above the
I non and highlights $\mathcal{R}$	son and in subrower plantmati	ice's planted as succ	certaing crop (detects above the
LOQ are highlighte			Ø1
, Di			× ×
	<u> </u>		<u> </u>
Type of Sampe		Residue Level mg/l	kg] *
Č)	Jmidachoprid	Olefin-NFN	Hydroxy-NTN
Control Dat (couth of fi	H number 502) imidacloprid free		
X		seed in mindacroprid-ind	
Soil sample (0-30 cm)		& A'	
Leaves (produced lates	the way was a construction of the construction	<b>n</b> .d.	n.d.
~ ~		A Indi	
Flowers (mal@/ female	t) the second s	0 n.d.	n.d.
Nectar sampled from the	ie have composed of the	n.d.	n.d.
	e hive combs of full.		
Pollen sompled from th	t) the hove compared of the sunflowers of the s	n.d.	n.d.
Pollen sampled from the	e plants y y n.a.	n.d.	n.d.
Honeybees exposed to			n.d.
* Limit of quantitation for s			it of detection (0.002 mg/kg)
Limit of quantitation for	plological samples 0.005 mg/kg for imid		
		elow limit of detection (0.	0015 and 0.003 mg/kg).
$\mathcal{L}^{1}$ U ( $\mathbf{D}$ it) = $\mathcal{L}$	.000 seed		
	Le la		
$\frac{1}{4} \frac{1}{\sqrt{2}} \frac$			
$\cup$			
Please click on the hun	erlink to order a Study Report.		
i lease click off the hyp	chink to order a study report.		



Type of Sample		Residue Level [mg	/kg] *	0
- , F	Imidacloprid	Olefin-NTN	Hydroxy-NTN	
Variant "1997" (field number 502) - imid	-	•		
Soil sample (0-30 cm)	0.018		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Leaves (produced latest)	n.d.	n.d.	A, n.d. 5	
Flowers (male / female flowers)	n.d.	🖒 n.d.	y n.d. y	
Nectar sampled from the hive combs	n.d.	🔊 n.d. 🖓	n, C 🕺	9 × 4
Pollen sampled from the hive combs	n.d. "(	ΰ n.d.S	Gi.d. Q	
Pollen sampled from the plants	n.d.	n.d.	? Q n.d.O	o d
Honeybees exposed to the sunflowers	p.d.	6° Sn.d. S		
Variant "1998" (field number 507) - imid	acloprid-fee seed	n imidacloprid contam	wated soft	A C
Soil sample (0-30 cm)	LOQ	N A A	\$ <sup>4</sup> \$	
Leaves (produced latest)	of mg.	n.d.	27	AN INCOMENT
Flowers (male / female flowers)	9 & n.d. &		Ç n.d	6
Nectar sampled from the hive combs $Q^{\vee}$	n.d.	a and	Önd v	J.
Pollen sampled from the hive com	x 10d.	§ @ n.d	Q.d.	
Pollen sampled from the plants	~~~ n.d.	and a	n.d.	
Honeybees exposed to the staflower	o na	, n.d. ,	N NA.	
Variant "1999" (south of field number 502	) – Gaucho-dresse	d seed i imidacloprid-		
Soil sample (0-20 cm	n.d.S		~~	
Leaves (produced thest)	₩ 0≈007	Q Q n.d. 4	© <loq< td=""><td></td></loq<>	
Flowers (male Semale Bowers)	n.d.		) <sup>×</sup> n.d.	
Nectar sampled from the hive combs	n.d.	Gr.d.	n.d.	
Pollen sanypled from the have comps	A A.	n.d.	n.d.	
Tonen sampled nom die planes	* S n.d		n.d.	
Honeybees exposed to the sunflowers	in the	n.d.	n.d.	
Limit of quantitation for biological samples 0	0.005 mg/kg for imid	acloprid, n.d. = below lim acloprid and hydroxy-imi	dacloprid, 0.01 mg/kg fo	or olefin-
		Ŵ,		
Observations: No behavioral impacts	s (e.g. apathy e	xaggerated motility,	discoordinated mo	ovements) or
>>M-04220-01-3@S-602058-01-1	the money bees u	used for collecting s	sunflower nectar an	d pollen.
	ų žy			
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
Observations: No behavioral impacts suspicious mortality was observed on M-04-20-01-3@5-602058-01-1				
Ċ				



<b>Report:</b> Title:	02.02.01/05; 1999; M-016827-01-3 Residue levels of imidacloprid and imidacloprid r pollen of sunflowers cultivated on soils with diffe effects on these residues on foraging honeybees.	rent imidacloprid residue levels and
Report No.:	SXR/AM 007	Laacher Hat 1999
Document No.:	<u>M-016827-01-3</u>	Or a star
Guideline(s):		
Guideline deviation(s):	&	
GLP/GEP:	yes the second s	
	yes "y"	
< <m-016827-01-3@s-602071-01-1< td=""><td></td><td></td></m-016827-01-3@s-602071-01-1<>		
Material and methods: s	unflower seed (variety "Fleug") either dressed	$d (= variant (1999) \text{ with } 30 \text{ g/g}^{\prime}$
Gaucho WS 70 (a.i. con	tent: 72.5% imidacloprid: batch no. 233 614 7	499 developmental no. 04 175 778)
	ntrol, variants "1997, 1998 and 1998 (🔊 )" we	
with different imidaclop	rid residue levels and meatment history. Soil's	amples for an analytical
determination of the imi	dacloprid residue level were taken immediatel	y before drilling Drilling rate was
0.58 U/ha. During peak	flowering of the sumflowers (21 and 26 July) s	mall bee colonies (2,000 to 2000
honeybees) were caged	on these plots (appr. 50 m2) as a sampling dev	tice for sunflower pectar and pollen.
In addition some pollen	and flowers were sampled by hand. The hone	whees used as saronlers were
observed for signs of be	havioral impacts. All samples and a small sam	nte of have vere subjected to
a residue analysis for im	idaclopridand its relevant metabolites	
a residue analysis for mi	reactopring and its relevant meadoontes.	
Deter efficiencies la service	k: 🖓 😽 July 23 - August 3, 1999.	° ~ 4
Dates of biological work	. • • • • • • • • • • • • • • • • • • •	
Dates of soil analysis:	August 9-11_1999.	
Dates of analysis of biol	ogical samples: August 25 - September 21,	<sup>7</sup> 1999 3 4 4
	il, in sunflower plant matthees planted assuce	eeding crop and in honeybees used
as sampling device,		
(detects above the DOQ	and highlighted to the second	
Type of Sample	O O & Resider Leve	
b b	A Imida Joprid D Defin-	9
Control Plot (field num		
Soil sample (0-30 cm)		
		-
Leaves (produced latest	n.d. of n.d.	n.d.
Flowers (male/ femate	flowers $(n, 0)$ $(n, 0)$ $(n, 0)$ $(n, 0)$ $(n, 0)$	n.d.
Nectar sampled from the	e hive combs Q gad. O n.d.	n.d.
Pollen ampled from the	flowers , , , n.d. , n.d. flowers , , , , , , , , , , , , , , , , , , ,	n.d.
Pollen sampled from the		n.d.
Honeybees exposed to t	hossunflowers On.d. n.d.	n.d.
*Limit of quantitation (LOQ	for samples: 0.000 mg/kg (initiactopha), n.ubelow	v limit of detection (0.002 mg/kg)
LOQ for biological sample	s: 0,005 mg/kg (imidacloprid & hydroxy-metabolite), 0.01	
	Ad. = below limit of detection (0.0015 mg/kg and 0.00)	3 mg/kg, respectively)
$\mathcal{L}$ 1 U ( $\mathcal{Q}$ nit) = $\mathcal{Q}$ 30,	000 seed	
$\frac{1}{\sqrt{2}} \frac{1}{\sqrt{2}} \frac{1}{\sqrt{2}$	~	
õ		



Issue date 2017-11-22

Type of Sample		Residue Level [mg/	kg] *	
	Imidacloprid	Olefin-NTN	Hydroxy-NTN	
Variant "1997" (field number 710) - imi	•			S P
Soil sample (0-30 cm)	0.016		 	
Leaves (produced latest)	n.d.	n.d.	л. п.d. 🔗	
Flowers (male / female flowers)	n.d.	ارم n.d. 🛴	n.dx	
Nectar sampled from the hive combs	n.d.	7 n.d. Q		
Pollen sampled from the hive combs	n.d.	n.dy	Ön.d. 🖓	
Pollen sampled from the plants	n.d.	n.d. 🖓	° Q n. 6	
Honeybees exposed to the sunflowers	g.d. o°	n.d.		
Variant "1998" (field number 702) - imid		michelopric contant	mated and c	4
Soil sample (0-30 cm)	4			
Leaves (produced latest)	A A O	n.d	A Cond. to	
Flowers (male / female flowers)	y and y y and y nd		n.d. & of n.d. & of n.d. & of n.d. & of n.d. &	ð Å
Nectar sampled from the hive combs	n,d. 💫	Sn.d.		
Pollen sampled from the hive control		On of	> ~ n.d. &	
Pollen sampled from the plants	y S <sup>g</sup> n.d.		n.d.	
Honeybees exposed to the stanflower	ñ R s.	n.d		
Variant "1998 (2x)" (field number A XI	Sonri A Via	eOin im dacloprid-	contaronated soil	
Soil sample (0-30 cm Leaves (produced sitest)	© 0.0145 ×		~~	
Leaves (produced Stest)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	2 n.d	Ø n.d.	
Flowers (male Gemale Gowers)		y SA. W	n.d.	
		O <sub>n.d.</sub>	n.d.	
Pollen sampled from the five carries	A A A A A A A A A A A A A A A A A A A		n.d.	
Pollen sampled from the plants	n.d.	Sond.	n.d.	
Honeybees exposed to the sunflow	A MAL O	n.d.	n.d.	
Variant "1999" (field n@ber 7.5) - Ga	tho-dressed sect in im	Acloprid-free soil		
Soil sample 30 cm	S nin S			
Leaves (produced latest)	£ .0.906	n.d.	< LOQ	
Flowers (male / female Nowers)	n.d.	n.d.	n.d.	
Nectar sampled from the hice combs		n.d.	n.d.	
Pollen sampled from the hive comos	z Zn.d.	n.d.	n.d.	
Variant "1999" (field monber 7 5) – Ga Soil sample (0-30 cm) Leaves (produced latest) Flowers (male / female flowers) Nector sampled from the hive combs Pollen sampled from the hive combs Pollen sampled from the plan	n.d.	n.d.	n.d.	
Honeybeer exposes to the mflowers	~Ç n.d.	n.d.	n.d.	

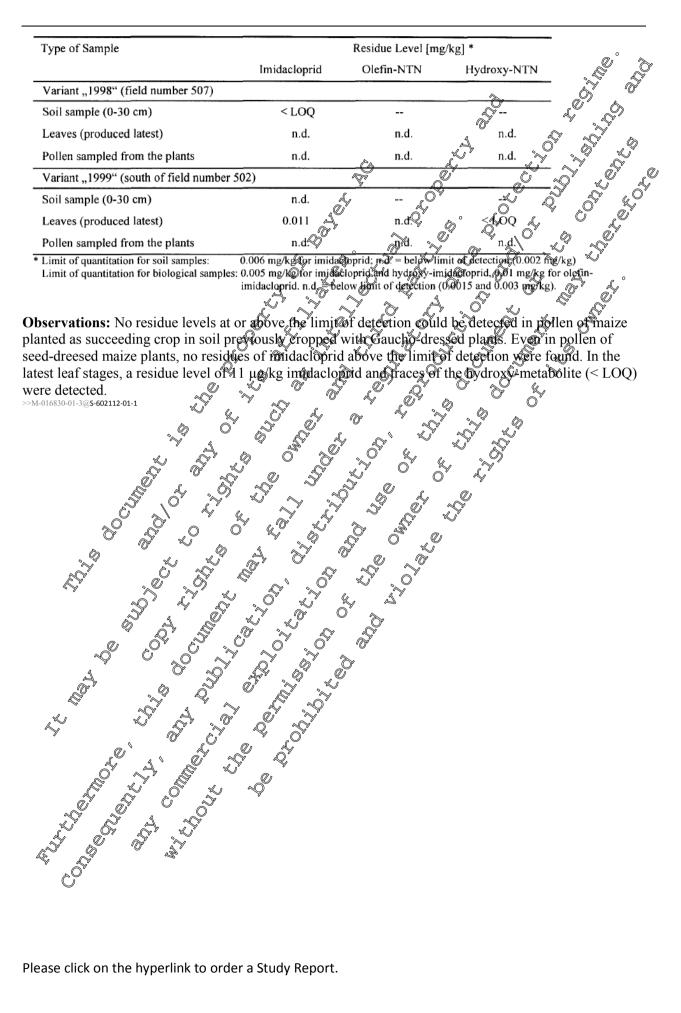
\*Limit of cantitati@ (LOQ) for soil imples: 0.006 mg/kg (imidacloprid); n.d.=below limit of detection (0.002 mg/kg) LOQ to biological samples: 0.060 mg/kg (imidacloprid & hydroxy-metabolite), 0.01 mg/kg (olefin-metabolite); a d= below limit of detection (0.0015 mg/kg and 0.003 mg/kg, respectively)

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.



<b>Report:</b> Title:	02.02.01/06; 1999; 1 Residue levels of imidaclopic cultivated on soils with diffe	M-016830-01-3 id and imidacloprid rent imidacloprid re	metabolites in pollen of r sidue levels Test location	naize plants 🖉
	'Hoefchen' - 1999	-	A	
Report No.:	SXR/AM 011		Å.	
Document No.:	<u>M-016830-01-3</u>		« \$	
Guideline(s): Guideline deviation(s):				
GLP/GEP:	yes	B		Y 67 .O
	yes	·¥'	\$	
< <m-016830-01-3@s-602112-01-1 Matorial and mathaday</m-016830-01-3@s-602112-01-1 	maize seed (variety "Ilias"	') Wither drassed	ith 70 g/UI Quucho	S ZQUA L
content: 72 5% imidadle	prid; batch no. 233 614 74	developmental	10170 g/01 eauch0 w	acloprid free
were drilled on 10 May	99 in soils with different h	nidacloprid	Nevels Soil samples for	r an
analytical determination	of the imidacloprid residu	e level were taken	immediately before dri	lling Drilling
rate was 2 U/ha During	peak flowering of the main	e plant end of	ilv pollen was haweste	ed from the
male flowers. These pol	len samples were subjected	to a residue avaly	sis for ingidacloprid and	its refevant
metabolites.	بې بې د بې			<u> </u>
				Ő
Dates of biological wor	k: Ö 🖉 July	92 - 29, 19990 19990		Ò
Dates of soil analysis:	A A	ugust 9-11, 1999.		) <sup>°</sup>
Dates of analysis of bio	logical samples Rug	ust 31 - Septembe	r 22, 1999.	
		The second se		
	oil, and in pollen of maize	planted as succeed	ling crop (detects abov	e the LOQ
are highlighted):				
~/				
Type of Sample		* Residue Lev	etime/kel*	-
	4 . 8 . 5 . ~ ~	NO .		
	o <sup>™</sup> i <sup>™</sup> Minidaclopr	id Øefin-N	N 🧶 Hydroxy-NTN	
				-
Control Plot (south of for	d field number 502) 7		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	_
Control Plot (south of the Soil sample (0-30 cm)	ld field number 502) 7	27 - 4 7 - 4 7 - 4	хў У	_
	K Q A R.		الله من المراجع	_
Soil sample (0-30 cm)	the second secon	S L MAR	≪∑	_
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	plantis			_
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant "1997" (field nur	plants n.d.			- - -
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant "1997" (field nur	plants n.d.		n.d. 	-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant "1997" (field nur	plants n.d.		n.d.  n.d.	-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d.	
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d.	mg/kg)
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d. low limit of detection (0.002 oxy-imidacloprid, 0.01 mg/kg	g for olefin-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d.	g for olefin-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d. low limit of detection (0.002 oxy-imidacloprid, 0.01 mg/kg	g for olefin-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d. low limit of detection (0.002 oxy-imidacloprid, 0.01 mg/kg	g for olefin-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d. low limit of detection (0.002 oxy-imidacloprid, 0.01 mg/kg	g for olefin-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d. low limit of detection (0.002 oxy-imidacloprid, 0.01 mg/kg	g for olefin-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d. low limit of detection (0.002 oxy-imidacloprid, 0.01 mg/kg	g for olefin-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	nber 502		n.d.  n.d. n.d. low limit of detection (0.002 oxy-imidacloprid, 0.01 mg/kg	g for olefin-
Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the Variant ,,1997" (field nur Soil sample (0-30 cm) Leaves (produced latest) Pollen sampled from the	plants 0.006 mg/kg for logičal samples: 0.005 mg/kg for		n.d.  n.d. n.d. low limit of detection (0.002 oxy-imidacloprid, 0.01 mg/kg	g for olefin-







Report:	02.02.01/07;			
Title:	Effects of imidacloprid		noney on the dev	elopment of small bee
	colonies under field exp	osure conditions		N R
Report No.:	SXR/Am 004		ð	
Document No.:	<u>M-016832-01-5</u>		- A	
Guideline(s):			10%	
Guideline deviation(s):			A	
GLP/GEP:	yes	Ò	Å,	
< <m-016832-01-5@s-602121-01-1< td=""><td></td><td>- The second sec</td><td></td><td></td></m-016832-01-5@s-602121-01-1<>		- The second sec		

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 out plots (50 no<sup>-4</sup>, drilled on f April 1999) and exclusively fed with sunflower honey which was for field with either 0.2.5, 10 ev 20 using imidacloprid. One colony received comb cells produced by honeybees during a previous feeding experiment with a 10  $\mu$ g/kg sucrose solution. Pollen of the Mediterranear bush was provided as a protein source. The small bee colonies were examined for freatment 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 reg laying activity, breeding success, colony strength, foraging intensity and behavioral anomalies

# Dates of biological work: May 28 - July 7, 1998.

Findings: Effects of imidacloperid residues in sunflower honey or small honeybee colonies

&	S Ó	<u>y</u> w	. %		Ş İQ	
Testing Endpoint 🔊 🔬	Control	2µg%kg	Spug/kg	10 μ <b>g/k</b> g	100prg/kg*	20 µg/kg
Mortality (no. of dead best in front of bee higes)	۶ <u>1</u> 4		y 6 <sup>y</sup>	× 8	× 7	5
front of bee highs)		y <sub>S</sub>	(A)	0 ~	1	
Mortality (no of dead bees at the	لان∛ 24م	. ZO	5 <sup>21</sup>		18	26
tent margin	4	S.		45 <sup>v</sup>		
Foraging intensity O C	\$4,7	26 546 7546	' Lan	@ <sup>135</sup>	143	121
(no. of bees at the Honey feeder)	A 26 0	- P	<i>a</i> . <i>b</i>	ý.		
Forseging intensity The of bees at the pollen (reeders)'	26 <sup>y</sup> 26	26	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	24	31	36
(the of bees at the pollen feeders)		Or K	ງ້ <sub>ເ</sub> ວົ			
Honey consumption [g]	<u></u> 🐴 🔬	× 546	581	566	616	546
Pollen consumption [g]	546 73 0 5594 73 0	<b>1</b> 6	⊘~ 80	53	63	65
Comb cell production at study	240 240 240 240 240 240 240 240	\$68	\$ 603	610	583	576
termination [cm]		у . У	y .			
termination [cm] Honey storage area actudy		100	252	201	313	165
termination [cm <sup>2</sup> ]	0° N	, KŬ				
Agrive weight increase at study	240 🕺	<u>(</u> )200	205	235	270	220
termination		1 <sup>v</sup>				
	~~120 °	115	143	208	60	148
area containing eggs] at study	246 246 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~					
termination A Q	ý ¥					
Cology strength [cm <sup>2</sup> @omb area	Q177	252	231	213	210	351
covered with bees at study	¥					
termination			i			
Fed with come cells from a previ	ous feeding ex	periment.				

**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** 

K

Please click on the hyperlink to order a Study Report.

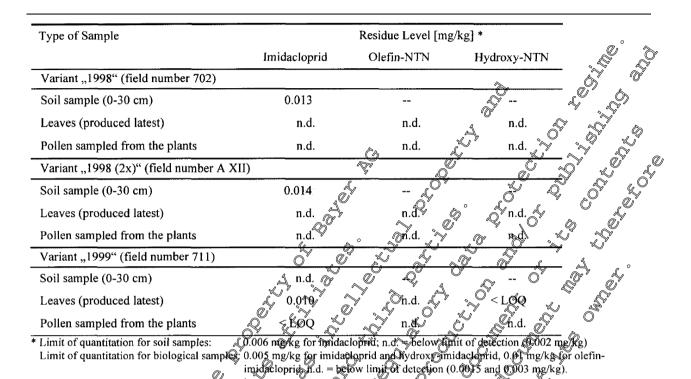


Report:		1999; <u>M-016836-</u>			
Title:	Residue levels of imi	dacloprid and imi	dacloprid metabo	olites in pollen of maiz	e plants
	'Laacher Hof' - 1999	th different imida	ciopria residue le	g/U <sup>i</sup> Gaucho WS 76 175778) of imidacio	$\mathcal{D}^{r}$
Report No.:	SXR/AM 009				í þ
Document No.:	<u>M-016836-01-3</u>			O'	
Guideline(s):			, A		Ş' İQ
Guideline deviation(s):		Ĉa	L.		
GLP/GEP:	yes	The second second second second second second second second second second second second second second second se	<u> </u>		<u> </u>
<-M-016836-01-3@S-602125-01-1		L,	Ő¥		Q, Q
M-016836-01-3@S-602125-01-1 Material and methods	: maize seed (variety	"Ilias") wither d	ressed with 70	g/U <sup>i</sup> Saucho WS 78	a.i. 🖉
content: 72.5% imidacl	oprid; batch no. 233 6	614 749, develo	omental no 94	1750778) or imidacle	oprictfree
were drilled on 12 May	99 in soils with diffe	rent midaclopr	d gesidue Jevel	Soil samples tor a	n∕≪S
analytical determination					
rate was 2 U/ha. During	g peak flowering of th	ie maize plant	end Of July po	llen was hatvested	rom the
male flowers. These po	llen samples were su	ajected to a resu	lue amalysis for	indicacloprid and its	s refevant
metabolites.	a de la companya de			Y Q & Q	S. S. S. S. S. S. S. S. S. S. S. S. S. S
	. 84				)
Dates of biological wo		uiy 22-29, 1999 August 9-11 19			
Dates of soil analysis:	P	14945t / 14, 1/			
Dates of analysis of bi	ological samples. A	August 345- Sep	tember 22, 195	19. <sub>2</sub> 0 <u>k</u>	
		×			1.00
Findings: Residues in a are highlighted):	soil, and in pollen of	maize planted as	succeeding cro	op, (detects above th	e LOQ
are highlighted):	N Q				
*		o 🎸 🖓	· ~ ~ ~	~	
Type of Sample		N NRe:	idue Level mg/k	eĵ¥	
			efin-NGN	Hydroxy-NTN	
		¥ 4.			
Control Plot field num					
Soil sample (0-30 cm)	X, X A	ma.	7, %)		
Leaves produced latest	Ŭ S &	n.d. S	n.d.	n.d.	
Pollen sampled from the	e plants 🔬 🍣	n.et	گُ∕≫n.d.	n.d.	
Variant "1997" (field nu			4		
<u>()</u>			¥		
Soil sample (0-30 cm)		\$0.016 ST ST			
Leaves (produced latest		< LOQ	n.d.	n.d.	
Pollen sampled from the	plants S	~n.d. «	n.d.	n.d.	
		Mrs. foribidaelanni		of datastian (0.002 mo/le	2)
* Limit of guantitation for s	$m$ samples. $\land$ 0.000 m	2/Kg LON AMMUACIODITC	; n.d. = below limit	of uclection $(0.002 \text{ mg/k})$	
* Limit of quantitation for s	iological samples: 0.000 m	g/kg for imidaclopric	; n.d. = below limit and hydroxy-imid	acloprid, 0.01 mg/kg for o	lefin-
* Limit of quantitation for a Limit of quantitation for b	iological samples: 0.000 m iological samples: 0.005 m initiaclo	g/kg for imidaciopric pris, n.d. = below lin	; n.d. = below limit and hydroxy-imid nit of detection (0.0	acloprid, 0.01 mg/kg for o 0015 and 0.003 mg/kg).	lefin-
* Limit of quantitation for b	iological samples: 0.000 m imiliaria interaction interaction	g/kg for imidaclopric prict n.d. = below lin	; n.d. = below limit and hydroxy-imid nit of detection (0.0	acloprid, 0.01 mg/kg for o 0015 and 0.003 mg/kg).	lefin-
* Limit of quantitation for b	iological samples: 0.000 m initial samples: 0.0000 m initial samples: 0.000 m initial samples: 0	g/kg tor imidaclopid prite n.d. = below li	; n.d. = below limit and hydroxy-imid: nit of detection (0.0	acloprid, 0.01 mg/kg for o 0015 and 0.003 mg/kg).	lefin-
* Limit of quantitation for b	iological samples: 0.000 m joing a samples: 0.000 m joing a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples: 0.000 m image a samples:	g/kg to midaclopin g/kg to imidaclopin prit h.d. = below li	; n.d. = below limit and hydroxy-imid: nit of detection (0.0	acloprid, 0.01 mg/kg for o 0015 and 0.003 mg/kg).	lefin-
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* Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limi		g/kg tor imidaclopid prit n.d. = below lin	; n.d. = below limit and hydroxy-imid: nit of detection (0.0	acloprid, 0.01 mg/kg for o 0015 and 0.003 mg/kg).	lefin-
* Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limit of quantitation for the Limi		g/kg top imidaclopin g/kg top imidaclopin prit h.d. = below lin	; n.d. = below limit and hydroxy-imid: nit of detection (0.0	acloprid, 0.01 mg/kg for o 1015 and 0.003 mg/kg).	lefin-
* Limit of quantitation for sy Limit of quantitation for b		g/kg toy imidaclopin g/kg toy imidaclopin production in the second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second	; n.d. = below limit and hydroxy-imid: nit of detection (0.0	acloprid, 0.01 mg/kg for o 1015 and 0.003 mg/kg).	lefin-
* Limit of quantitation for sy Limit of quantitation for b		g/kg toy imidaclopin g/kg toy imidaclopin provindent in the second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second	; n.d. = below limit l and hydroxy-imid: nit of detection (0.0	acloprid, 0.01 mg/kg for o 1015 and 0.003 mg/kg).	lefin-

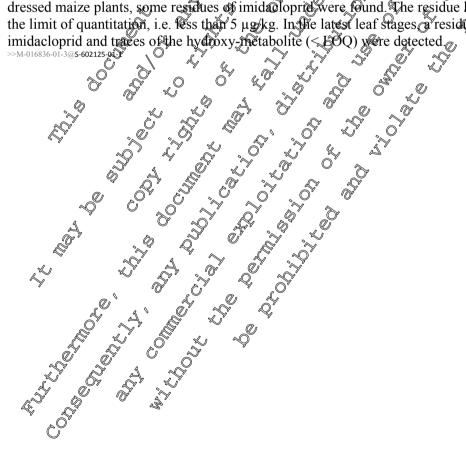


Issue date 2017-11-22

 $\cap$ 



**Observations:** No residue levels at or above the armit of detection could be detected in pollen of maize planted as succeeding crops in soil previously copped with Gaucho-dressed plants. In pollen of seed-dressed maize plants, some residues of imidaolopric were found. The residue level, however, was below the limit of quantitation, i.e. less than 5  $\mu$ g/kg. In the latest leaf stages a residue level of 10  $\mu$ g/kg imidacloprid and traces of the hydroxy-metabolite (<EOQ) were detected.





<b>Report:</b> Title:	02.02.01/09; ; 1999; M	<u>4-016845-01-4</u>	on on the develor	ment of small bea
The.	Effects of imidacloprid residu colonies under field exposure	e conditions	in on the develop	ment of small bee O
Report No.:	SXR/AM 005		~	
Document No.:	<u>M-016845-01-4</u>		Ő.	
Guideline(s):			10.	
Guideline deviation(s):				
GLP/GEP:	yes	Ò	Å.	
< <m-016845-01-4@s-602132-01-1< td=""><td></td><td>- An</td><td></td><td></td></m-016845-01-4@s-602132-01-1<>		- An		

Material and methods: *test substance*: imidacloprid techn., *purity*: 98.6%, *identify*: 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 ploto (50 m2, drilled on 1/ April 1999) and exclusively fed with maize pollen which was fortified with either 0.2, 5, 10 or 20 ug/kg imidacloprid. Sunflower honey was provided as carbofydrate 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, tood consumption, storage behavior, hive weight increase egg laying activity, breeding success, colony prength, foraging intensity and behavioral anomalies.

# Dates of biological work: May 28 July 6, 1

	0		, Òg	
Testing Endpoint Control	2 @ kg	5 µg@kg	₩µg/kg	20 µg/kg
Mortality (no. of dead bees on front of	<u>50</u>	° 6 ,	8	7
bee hives) Mortality (no of dead bees at the tent 22 margin)	Ž 521	ý z	21	30
Foraging intensity 2	5 19	یں کل	37	24
Foraging intensity , , , , , , , , , , , , , , , , , , ,	<sup>7</sup> <sup>2</sup> 124 <sup>0</sup> <sup>4</sup> <sup>1</sup> 124 <sup>0</sup>	123	130	128
(no. of bees at the poney feeder) Pollen consumption [gf, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5,	29	32	39	34
Honey consumption $\widehat{\text{yg}}$	® 541	521	500	543
Comb cell production [copr2]	551	579	584	563
Comb cell production [cm²] Honey torage area at study termination 177 [cm²] Hive weight increase	201	186	147	174
Hive weight increase	230	215	200	200
Hive weight increase Egg laying activity[cm <sup>2</sup> comb area containing eggs at study termination)	153	181	205	153
Colony strength form <sup>2</sup> comb area 217 covered with bees at study termination)	258	305	314	221

Findings: Effects of imidacloperid residues in maize pollen on small honeybee colonies

**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.



Report:	02.02.01/10; ; 1999; M-(	040023-01-3		0
Title:	Residues of imidacloprid and im		s in nectar, blossoms, pollen a	and 🔊
	honey bees sampled from a Briti	sh summer rape field	and effects of these residues of	on
	foraging honeybees SXR/AM 003 <u>M-040023-01-3</u> US EPA OCSPP Guideline Num none yes Poncho FS 500, a.i. content on No.: 030 based on 0620000			55
Report No.:	SXR/AM 003			
Document No.:	M-040023-01-3 US EDA OCSDD Cuidalina Num	how 950 SLIDD	A & &	Ô
Guideline(s):	US EPA OCSPP Guideline Num	iber: 850.SUPP		K)
GLP/GEP:	ves			Y (
	<i></i>			. 8
«M-040023-01-3@8-602143-01-1 Material and methods	Poncho FS 500 a i content	83 g/L Beta Cyflu	thrin & 28 2 gr Imaidaclo	and .
specification (formulation	$\sim 1000000000000000000000000000000000000$	129 developmental	$N_0 : 00195039$ ) test produ	kt∙
rane seed dressed with 2	2.5 1/dt Poncho FS 500; drilling	p rate: 5/2 p/ha Mind	erstield conditions small	ict.
beehives (appr 5 000 hc	oneybees) were caged on flow	ring summer rape p	Jots (600m <sup>2</sup> drilled on 20	
March 98) as a sampling	g device for rape nectar and rap	be pollen. Nectar wa	is also directly sampled fro	m
flowers via micropipette	es. In addition, flowers were sa	mpled by hand. The	hopeybees used as sample	ers
were observed for signs	of behavioral impacts. All san	ples including the l	toneybees were subjected t	to a
residue analysis for imic	lacloprid and its relevant metal			
·				
Dates of biological wor	•k: June 22 24, 1998 (soil and	lysis: September 25	5 - 99, 1998). · · · · ·	
Dates of analytical wor	•k: June 30 - July 28, 1998.		ð <sub>s</sub> ð <sub>k</sub> í	
		A ON O		
Findings: Residues in ra	ape plant matrices and in the fo	oraging honeybees	× 0	
		Residue Devel [mg/k		
Type of Sample			gj+y	
	Amidacloprid	Olego-NTN	Hydroxy-NTN	
Control Samples			× ·	
Honeybers before e	xpostore O & C 0.01	~ < <b>49</b> 01	< 0.01	
Honeybees after exp		\$ <0.01	< 0.01	
Rape nectar sample		Q< 0.Q10	< 0.01	
Rape nectar sample		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	< 0.01	
°capillaries from the	flowers &	k, A <sup>Y</sup>		
Rape blossoms		>″	< 0.01	
Rape polle Rample	at by bees 200.01	< 0.01	< 0.01	
Treatment Samp		*0°		
Honeybees before e		○ < 0.01	< 0.01	
Honeybees after exp		< 0.01	< 0.01	
Sape nectar sample		< 0.01	< 0.01	
$\ll$ Rape nectar samples $\swarrow$ capillaries from the		< 0.01	< 0.01	
Rape blossoms		< 0.01	< 0.01	
Rape pollen sample	d by bees 9 90.01	< 0.01	< 0.01	
* Limit of quantitation; 0.01		< 0.01	< 0.01	
			<b>1. 1 1</b>	
	vioral impacts (e.g. apathy, exa			) or
suspicyous mortality was	s observed on the honeybees us	sed for collecting ra	pe nectar and rape pollen.	
~~M/440923-01-3/85/602143-01-1	45			
ĉ				
<u> </u>				



Report:	02.02.01/11; ; 20	01; <u>M-05263</u>	<u>7-01-3</u>			
Title:	Effects of residues of in	nidacloprid ir	n maize polle	n from dresse	ed seeds on ho	ney bees O
<b>D</b>	(Apis mellifera)					ji di
Report No.:	<u>M-052637-01-3</u>			ð		
Document No.:	$\frac{M-052637-01-3}{M-052637-01-3}$	140		Ĩ	~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Guideline(s): Cuideline deviction(a):	US EPA OPP15 850.30	040		.1	A.	
Guideline deviation(s):	not specified			×,	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
GLP/GEP:	yes		Ś		$\sim$	
< <m-052637-01-3@8-602655-01-1< td=""><td></td><td>- V</td><td>7</td><td>Q,</td><td></td><td>~ ~</td></m-052637-01-3@8-602655-01-1<>		- V	7	Q,		~ ~
Material and methods:	test substance: Gaucho	WS 70 resi	dues in ma	Ze pollen fro	von dressed so	ecas, <sub>k</sub> o
dressing rate: 49 g/unit	a.i Residues of imidacl	oprid in the	pollen wer	e found to be	below limit	ĵof <sub>e</sub> o″
quantitation (LOQ = $0.0$	005 mg/kg). No olefine	andhydrox	y metabólit	es could be	det cited (lim	it of
Report No.: Title: Report No.: Document No.: Guideline(s): Guideline deviation(s): GLP/GEP: CM-052637-01-3@S-602655-01-1 Material and methods: 1 dressing rate: 49 g/unit quantitation (LOQ = 0.4 detection: 0.003 mg/kg Small bee colonies (app meadows and exclusive were dressed with Gauc carbohydrate source. Th of 38 days. In particular	and 0.0015 mg/kg, resp	pectively).	S X	, C		1 S
Small bee colonies (apr	or. 700 honeybees) were	e contined in	tent cases	$(ca)^{20} m^{20}$	on short gra	<b>5</b> 8.r°
meadows and exclusive	elv fed with maize polle	en which wa	s harvested	from plants.	the seeds of	which
were dressed with Gau	the WS 70 or which we	ere/untreated	(control)	Sunflower h	anev was pro	wated as
carbohydrate source. Th	he small bee colonies	ere examine	Afor treat	nent-relate	impacts over	9 neriod
of 38 days. In particular	the following endrou	its where eva	histed mo	tality could	celloroduet	ion food
consumption, storage b	ehavior hive weight in	crease and	aving activ	he bredding		ony
strength, foraging inten					Success con	lony
strength, foraging men				, <sup>o</sup> õ		
Dates of biological wor	k: 2000-08 \$1 to 2000-	02-28.			ð	
-					S.	
Findings: Effects of Ga	ucho WS, 70 residues fi	n maize poll	m <sup>o</sup> on small	honeybee	olonies	
Ś						
	- &(\$ ())			O' 😽		
TestingEndp		Control	Control B	Treatment A		
TestingEndp	oint	ControlOA	Control B	Treatment A		
TestingEndp	oint	ControlOA	Control B	Treatment A	Treatment B	
TestingEndp	oint	ControlOA	Control B	Treatment A	Treatment B	
TestingEndp	oint	ControlOA	Control B	Treatment A	Treatment B 30	
TestingEndp	oint	ControlOA	Control B	Treatment A	Treatment B 30	
TestingEndp	oint	ControlOA	Control B	Treatment A 20 139 29	Treatment B           30           151           2	
TestingEndp	oint	ControlOA	Control B	Treatment A	Treatment B 30 151	
TestingEndp	oint	ControlOA	Control B	Treatment A 20 139 29 274	Treatment B 30 151 2 255	
Testing Sndp Mortality (no becaives) Mortality (no odges) Foraging inte (no. of bees a Foraging Onle (no. of bees a Bee activity	oint of dead bees incront of of dead bees at the tent usity the potten feeder) nsity the hone deeder) of of best at the fint root	ControlOA	Gritrol B 27 Q41 4 15 7 253 203	Treatment A 20 139 29 274 196	Treatment B 30 151 2 255 185	
Testing Sidp Mortality (no becaives) Mortality (no edges) Foraging into (no. of bees a Bee activity Porten collect	oint of dead bees infront of of dead bees at the tent wity the poffen feeder) nsity the hone; deeder) of of best at the fent roof ed [g	ControlOA	Critical BC 27 Q41 4 5 253 203 58	Treatment A 20 139 29 274 196 43	Treatment B 30 151 2 255 185 26	
Testing Sndp Mortality (no becaives) Mortality (no odges) Foraging inte (no. of bees a Bee activity Porten colfect Honey collect	oint of dead bees incront of of dead bees at the tent usity the porten feeder) nsity the hone deeder) of of best at the fint root ed [g]	Contreto 	Control B 27 Q41 253 203 58 853	Treatment A 20 20 29 274 196 43 819	Treatment B 30 151 2 255 185 26 877	
Testing Sndp Mortality (no becaives) Mortality (no odges) Foraging inte (no. of bees a Bee activity Porten colfect Honey collect	oint of dead bees incront of of dead bees at the tent usity the porten feeder) nsity the hone deeder) of of best at the fint root ed [g]	Contreto 	Control B 27 41 27 41 253 203 58 853 618	Treatment A 20 20 29 274 196 43 819 660	Treatment B 30 151 2 255 185 26 877 664	
Testing Sndp Mortality (no becaives) Mortality (no odges) Foraging inte (no. of bees a Bee activity Porten colfect Honey collect	oint of dead bees incront of of dead bees at the tent usity the porten feeder) nsity the hone deeder) of of best at the fint root ed [g]	Contreto 	Control B 27 Q41 253 203 58 853	Treatment A 20 20 29 274 196 43 819	Treatment B 30 151 2 255 185 26 877	
Testing Sndp Mortality (no becaives) Mortality (no odges) Foraging inte (no. of bees a Bee activity Porten colfect Honey collect	oint of dead bees incront of of dead bees at the tent usity the porten feeder) nsity the hone deeder) of of best at the fint root ed [g]	Contreto 	Critrol B 27 Q41 253 203 58 853 618 254	Treatment A 20 20 29 274 196 43 819 660 417	Treatment B 30 151 2 255 185 26 877 664 399	
Testing Sndp Mortality (no becaives) Mortality (no odges) Foraging inte (no. of bees a Bee activity Porten colfect Honey collect	oint of dead bees incront of of dead bees at the tent usity the potten feeder) be of best at the ent roof ed [g] ed [g] ed [g] ed [g] ed [g] ed [g] ed [g]	Contreto 	Control B 27 41 27 41 253 203 58 853 618	Treatment A 20 20 29 274 196 43 819 660	Treatment B 30 151 2 255 185 26 877 664	
Testing Cridp         Mortality (no         becauses)         Mortality (no         odges)         Foraging inter         (no. of bees a         Bee activity         Potten collect         Honey collect         Honey storaging         Icm²l         Hive weight i         [% of the initial	oint of dead bees incront of of dead bees at the tent usity the potten feeder) of of best at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g]	Contreto 	Critrol B 27 Q41 253 203 58 853 618 254	Treatment A 20 20 29 274 196 43 819 660 417	Treatment B 30 151 2 255 185 26 877 664 399	
Testing Endp         Mortany (no bechives)         Mortany (no bechives)         Mortany (no obechives)         Foraging interview         (no. of bees a         Bee activity (no obechives)         Potten collect         Honey collect         Honey storay         [cm²]         Hive weight i         [% of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the initities of the inities of the initities of the inities of the	oint of dead bees incront of of dead bees at the tent usity the potten feeder) of of best at the fint roof ed [g] ed below at the fint roof ed [g] ed below at the fint roof ed [g] ed below at the fint roof ed [g] ed below at the fint roof ed [g] ed below at the fint roof ed [g]	Contree	Control B 27 41 27 41 253 203 58 853 618 254 6.6	Treatment A 20 20 29 274 196 43 819 660 417 12.4	Treatment B 30 151 2 255 185 26 877 664 399 16.6 18	
Testing Sidp         Mortality (no         becaives)         Mortality (no         becaives)         Mortality (no         odges)         Foraging inter         (no. of bees a         Bee activity (         Potten collect         Honey collect         Honey storms         [cm²]         Hive weight i         [% of the initi         Ege aying ac         containing eg	oint of dead bees incront of of dead bees at the tent usity the potten feeder) nsity the hone deeder) of of best at the fent roor ed [g] eduction Em <sup>2</sup> ] aduction Em <sup>2</sup> ] e area at study termination necesse ial weight tivity (m <sup>2</sup> comt area	Contreto 146 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 267 5 273 6 273 6 274 5 273 6 274 5 273 6 274 5 273 6 274 5 273 6 274 5 274 5 274 5 273 6 274 5 274 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5 275 5	Control B 27 41 27 41 253 203 58 853 618 254 6.6	Treatment A 20 20 29 274 196 43 819 660 417 12.4	Treatment B 30 151 2 255 185 26 877 664 399 16.6	
Testing Sidp         Mortality (no         becaives)         Mortality (no         becaives)         Mortality (no         odges)         Foraging inter         (no. of bees a         Bee activity (         Potten collect         Honey collect         Honey storms         [cm²]         Hive weight i         [% of the initi         Ege aying ac         containing eg	oint of dead bees incront of of dead bees at the tent isity the porten feeder) nsity the hone deeder) of best at the fint roor ed [2] duction [201 <sup>2</sup> ] e area at study termination necesse at weight tivity [30 <sup>2</sup> configrea gs at study termination]	Contree	Critrol B 27 Q41 253 203 58 853 618 254 6.6 63	Treatment A 20 20 29 274 196 43 819 660 417 12.4 15	Treatment B 30 151 2 255 185 26 877 664 399 16.6 18	
Testing Sidp         Mortality (no         becaives)         Mortality (no         becaives)         Mortality (no         odges)         Foraging inter         (no. of bees a         Bee activity (         Potten collect         Honey collect         Honey storms         [cm²]         Hive weight i         [% of the initi         Ege aying ac         containing eg	oint of dead bees incront of of dead bees at the tent usity the potten feeder) nsity the hone deeder) of of best at the fent roor ed [g] eduction Em <sup>2</sup> ] aduction Em <sup>2</sup> ] e area at study termination necesse ial weight tivity (m <sup>2</sup> comt area	Contree	Critrol B 27 Q41 253 203 58 853 618 254 6.6 63	Treatment A 20 20 29 274 196 43 819 660 417 12.4 15	Treatment B 30 151 2 255 185 26 877 664 399 16.6 18	
Testing Sidp         Mortality (no         becaives)         Mortality (no         becaives)         Mortality (no         odges)         Foraging inter         (no. of bees a         Bee activity (         Potten collect         Honey collect         Honey storms         [cm²]         Hive weight i         [% of the initi         Ege aying ac         containing eg	oint of dead bees incront of of dead bees at the tent isity the potten feeder) of of best at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed below at the ent roof ed [g] ed [g	Contreto 146 146 267 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 4 267 267 267 267 267 267 267 267	Critrol B 27 Q41 253 203 58 853 618 254 6.6 63 249	Treatment A 20 20 29 274 196 43 819 660 417 12.4 15 253	Treatment B 30 151 2 255 185 26 877 664 399 16.6 18 263	Γ,
Testing Ordp         Mortality (no         becauses)         Mortality (no         becauses)         Mortality (no         odges)         Foraging inter         (no. of bees a         Bee activity (Potter collect         Honey collect         Honey storms         [cm²]         Hive weight i         [% of the initi         Ege aying ac         containing eg         Colony streng         coverse with	oint of dead bees incront of of dead bees at the tent isity the potten feeder) nsity the hone deeder) of best at the fint root ed [g] eduction Fin <sup>2</sup> ] aduction Fin <sup>2</sup> ] e area at study termination necesse ial weight tivity (m) <sup>2</sup> configrea gs at study termination) there are study termination) there are study termination	Contret 146 146 146 146 146 146 146 146	Critrol B 27 Q41 41 57 253 203 58 853 618 254 6.6 63 249 me testing en	Treatment A 20 20 29 274 196 43 819 660 417 12.4 15 253 adpoints fora	Treatment B 30 151 2 255 185 26 877 664 399 16.6 18 263 aging activity	
Testing Endp         Mortality (no         becauses)         Mortality (no         odges)         Foraging inter         (no. of bees a         Bee activity (poten collect         Honey collect         Honey storas         [cm²]         Hive weight i         [% of the initi         Ege aying ac         containing ege         Colony streng         coveret with	oint of dead bees incront of of dead bees at the tent usity the potten feeder) nsity the honey deeder, ab of best at the fint roory ed [a bed below a rea at study termination nectorse at weight tivity [ab 2 comborea gs at study termination) the first at study termination at weight the first at study termination the first at study termination, co	Contreto 146 146 146 146 146 146 146 146	Critrol B 27 Q41 23 203 58 853 618 254 6.6 63 249 The testing enduction, ho	Treatment A 20 21 29 274 196 43 819 660 417 12.4 15 253 adpoints fora ney storage,	Treatment B 30 151 2 255 185 26 877 664 399 16.6 18 263 aging activity hive weight	increase,
Testing Sidp         Mortivity (no         bec0ives)         Mortality/no         edges)         Foraging inter         Foraging inter         (no. of bees a         Bee activity         Doften collect         Honey storm         Comb cell pro-         Hive weight i         1% of the initi         Egg laying ac         coverat with         Obset/vation@? There we         orientation, honey and p         population developmen	oint of dead bees infront of of dead bees at the tent wity t the pollen feeder) nsity t the hone; deder) ab, of best at the fent roor ed [a ed [a] ed	Contreto $146$ $3^{4}$ 267 $4267$ $4736749.819279effects in the mb cell, pro-ctivity, and the first second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second s$	Crittrol B 27 41 27 27 27 203 58 853 618 254 6.6 63 249 me testing enduction, hobreeding su	Treatment A 20 29 274 196 43 819 660 417 12.4 15 253 adpoints fora ney storage, ccess. There	Treatment B 30 151 2 255 185 26 877 664 399 16.6 18 263 aging activity hive weight are no hints	increase, that
Testing Sidp         Mortality (no         beQuives)         Mortality (no         beQuives)         Mortality (no         odges)         Foraging inter         (no. of bees a         Bee activity (Doffen collect         Honey collect         Honey storm         [cm²]         Hive weight i         [% of the initi         Ege faying ac         containing eg         Colony streng         coverst with         Obsect ations? There we         orientation, honey and p	oint of dead bees infront of of dead bees at the tent wity t the potten feeder) ab. of best at the fent root ed [ab ed [a] ed [a] ed [ab] e area at study termination area at study termination tivity[ab comtorea gs at study termination) et construction billen consumption, co t, mortality, breeding a n pollen from maize see	Contreto $146$ $3^{4}$ 267 $4267$ $4736736749.819279effects in themb cell, pro-ctivity, and eds treated we$	Crittrol B 27 41 27 27 27 203 58 853 618 254 6.6 63 249 me testing enduction, hobreeding su	Treatment A 20 29 274 196 43 819 660 417 12.4 15 253 adpoints fora ney storage, ccess. There	Treatment B 30 151 2 255 185 26 877 664 399 16.6 18 263 aging activity hive weight are no hints	increase, that

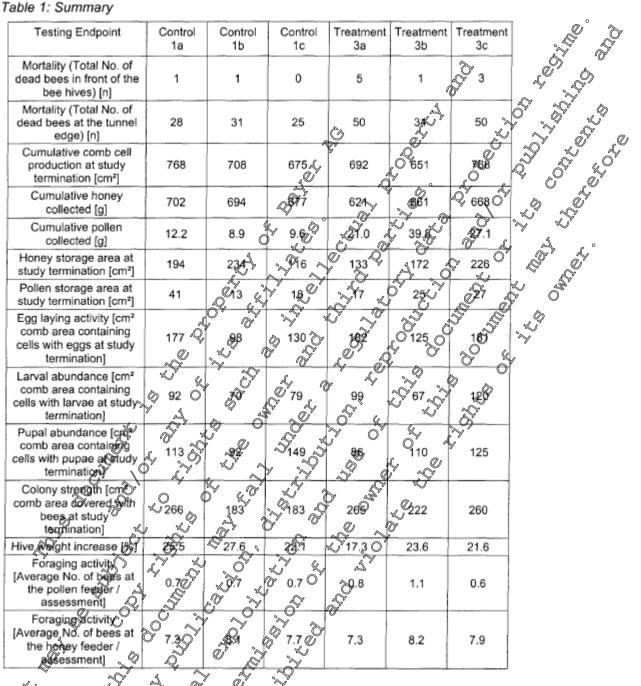
>>M-052637-01-3@S-602655-01-1



Donoute	02.02.01/12; (2002; M-052238-01-3)
<b>Report:</b> Title:	02.02.01/12; 2002; <u>M-052238-01-3</u> Evaluation of the effects of residues of imidacloprid FS 600 in maize pollen from dressed seeds on honeybees (Apis mellifera) in the semifield <u>M-052238-01-3</u> <u>M-052238-01-3</u> not applicable The following procedures were not carried out under GAP: seed dressing, sowing of the
The.	drossed souds on honourboos (Anis mallifere) in the samifield
Report No.:	M 052228 01 2
Document No.:	<u>M-052238-01-5</u> M-052238-01-2
	M-052236-01-5
Guideline(s):	The following procedures were not corried out under CND, and dressing source of the
Guideline deviation(s):	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:	ves
< <m-052238-01-3@s-602148-01-1< td=""><td></td></m-052238-01-3@s-602148-01-1<>	
Material and method	s: Test substance: maize policen with grown residues of Iondacloprid FS 600 seeds
dressed with commerci	ally available product at a vate of 1 g a m/1000 seeds). Small honeybee colonies
(approx. 500 honeybee	s) were confined on oat plots (\$0 m <sup>2</sup> , drilled on 200405-03) in tunnels and fed
with maize pollen cont	aining grown residues of Imidaclopita or upreated control pollep. For the atment
and control, three repli	cates were set up each. SumPower honey was provided as carbohydrate source. The
small bee colonies wer	e examined for treatment related effects over a period of 52 days. In particular, the
and a sinta mantality and	d fans ain a inter the man and start of the second start of the single and a second start on
food consumption nol	len and honey stores egg laving activity, breeding success, colory strength and
hive weight developme	and none stores, egg availy analyzed using a t Tast
Dehavioural anomalia	were also account and statistically analysis using a t-rest.
Benavioural anomalies	were also assessed.
Dates of biological wo	and honey stores egg laying activity, breeding success, coloring certification, en and honey stores egg laying activity, breeding success, coloring strength and ent were assessed and statistically analysed using a t-Test. were also assessed. ork: 2001-06-21 to 2001-08-12. ork: 2001-03-14 to 2001-06-05 stdues of Imidacloprid FS 600 in pollen on small honeybee colonies
Dates of analytical wo	ork: 2001-02-14 to 2001 06-05
Findings: Effects of re	sidues of Imidacloprid FS 600 in pollen on small honeybee colonies
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Issue date 2017-11-22



**Observations:** There were no significant differences between control and treatment in comb cell production (t=-0.478, p=0.641), hone construction (t=2.530, p=0.065), hive weight increase (t=1.720, p=0.161), pollen stores (t=-0.260, p=0.725) and honey stores (t=0.086, p=0.933), egg deposition (t=-0.176, p=0.863), larval abordance (t=-0.228, 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





Departs	02 02 01/12 · · · · · · · · · · · · · · · · · · ·						
<b>Report:</b> Title:	02.02.01/13; 2001; M-052524-02-3 Determination of residues of imidacloprid and relevant metabolites in nectar, pollen and						
The.	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						
	honey of winter rape MR-147/01 <u>M-052524-02-3</u> Equivalent to US EPA OPPTS 850.3040 not specified <b>yes</b> ectar and honey samples obtained from a German trial station were analysed for and its olefin- and Hydroxy metabolites. The results are summarized in the table						
< <m-052524-02-3@s-604946-01-1< td=""><td></td></m-052524-02-3@s-604946-01-1<>							
Rape flowers, pollen, n	ectar and honey samples obtained from a German trial station were analysed for $^{\bigcirc}$						
	d and its olefin- and Hydroxy metabolites. The results are summarized in the table						
below. Extraction, samp	bic clean-up and determination of mindaciophic, sydroxy- and preme inclausine						
by HPLC-MS/MS were	performed according to method 00537/E001 (MR-568/99) The linuit of						
	mg/kg for lmidaclopric and the Hydroxy-metabolite and 001 mg/kg for the						
	limit of detection was 0.0015 mg/kg for Indidacloprid and the Hydroxy-metabolite						
and 0.003 mg/kg for the	e Olefin-metabolite						
>>M-052524-02-3@S-604946-01-1	02.02.01/14						
Report:	02.02.01/14 2003; <u>AP-0756 0-01-5</u> Residue levels of imidacloprid and imidacloprid metabolites in sinflower pollen,						
Title:	Residue levels of imidacloprid and imidaeloprid metabolites in sinflower pollen,						
	sunflower honey and best from Gauche treated sunflowers in the field						
Report No.:	MR-700/99 *						
Document No.:	M-075630601-3						
Guideline(s):	M-075630601-3 USEPA OCSPP Quideline Number: 850.SUPP none yes yes nond bee samples obtained from the German trial station "Ahrweiler/Mayen"						
Guideline deviation(s):	none A & B O A & C A						
GLP/GEP:	yes fr i o o in o in in						
< <m-075630-01-3@s-602816-01-1< td=""><td></td></m-075630-01-3@s-602816-01-1<>							
Sunflower honey, polle	n and bee samples obtained from the German trial station "Ahrweiler/Mayen"						
were analysed for resid	es of inidactoprid and its defin- and hydroxy metabolites. The results are						
	below. Extraction, sample clean up and determination of imidacloprid, hydroxy-						
and olefin-metabolite h	y HPLC-MS/MS were performed according to method 00537/E001 (MR-						
568/00) The limit of au	antitation was 2005 mg/kg for imidaclopric and the hydroxy-metabolite and 0.01						
madra the alofin madra	tabolito. The limit of detection was 0.00 9 mg/kg for imidacloprid and the						
hudrowy motobalite	haborug. The minit producted on was 0.00 m mg/kg for minuacrophic and the						
>>M-075630-01-3@S-602816-01	10.003 mg/leg for the olefin-metabolite						
>>M1075050-01-5@3-002810-01-5							
Report: 🔊 🖒	$02_{4}92_{1}01_{1}15_{2}$ 2004, <u>M-451697-01-3</u>						
Title:	Residues of imight loprid WG 5 h blossom samples of Rhododendron sp. (variety Nova						
A .	Zembla) after soil treatment in the field - 2003,						
Report No.: 🗞	<u><sup>7</sup>M-459697-01-3</u>						
Document No.:	<u>M-451697-31-3</u>						
Guidekine(s):	pone y Q Q						
Guideline deviation(s):	none $\mathcal{L}$ $\mathcal{Q}$ $\mathcal{L}$						
GLP/GEP:	$\sim \operatorname{no} \mathbb{Q}^{v}$ , $\mathfrak{S}^{v}$						
< <m-451697-01-3@204694-01.1< td=""><td></td></m-451697-01-3@204694-01.1<>							
	Bight Sear old Rhododendron plants (variety "Nova Zembla") growing at the						
totatel lar and incusious	(Light on the Nuovoucius on plants (variety Nova Zeniola ) growing at the						

experimental familand "Hörchen" near Burscheid, Germany received pre and post-flowering soil treatment with Imidaeloprid WG 5 in two replicates (A and B: 8 plants each) per treatment group. Soil application with Imidaeloprid WG 5 (article No.: 0005439280, Batch No.: PF00000REC, TOX No. 6135-00, purity 5.5%) dissolved in water at an application volume of 2 I 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.



Issue date 2017-11-22

Treatment- No.	Application rate / 50 cm plant height	Sampled material
1	Control	Biossoms: 2003-05-20 and 26 (2 <sup>1</sup> time only replicate A)
2	2500 mg a.i. pre- flowering	Blossoms: 2003-05-20 and 26
3	2500 mg a.i. post- flowering	
4	1250 mg a.i. pre- flowering	Leaves: 2003-05-20 and 26, 2003-07-22, 2003-09-02 * Blossoms: 2003-05-20 and 26 Leaves 2003-05-20 and 26, 2003-07-22, 2003-09-02 * Leaves: 2003-05-20 and 26, 2003-07-22, 2003-09-02 *
5	1250 mg a.i. post- flowering	
6	100 pre- plus 200 mg a.i. post-flowering	Biossonis: 2002-05-20 Leaves: 2003-05-20, 2003-07-22, 2003-09-92 *

*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 midaeloprid and its Olefin and Hydroxy-Metabolites. Extraction, sample clean up and determination of Indeacloprid, Hydroxy and Olefin-Metabolites by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99) by R.

# 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 preflowering treatment are summarised.

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Pre-flowerin	gʻtçeatment		Br i	Omidactoprid in	Hydroxy- Imidacloprid in µg/kg	Olefin- Imidacloprid in µg/kg
	trol		/ 11 🔬	<lod -="" 02.5<="" th=""><th><lod -="" <loq<="" th=""><th><lod< th=""></lod<></th></lod></th></lod>	<lod -="" <loq<="" th=""><th><lod< th=""></lod<></th></lod>	<lod< th=""></lod<>
			17*~>	✓LOD ⇒12.7	<lod -="" <loq<="" th=""><th><lod -="" <loq<="" th=""></lod></th></lod>	<lod -="" <loq<="" th=""></lod>
Treatment 2 (250	)0 mg a.i.(50	) cm 📎	AP %	<lod -="" 20.0<="" th=""><th>&lt; LOD - 6.3</th><th>&lt; LOD</th></lod>	< LOD - 6.3	< LOD
plant h			Ø17 Ø	<loq -="" 23.2<="" th=""><th>&lt; LOQ - 8.7</th><th>&lt; LOD</th></loq>	< LOQ - 8.7	< LOD
Treatment 4 (12)	50 mg a.i./50		711.9	SEOD - 11.4	<lod -="" <loq<="" th=""><th>&lt; LOD</th></lod>	< LOD
				°∧,≮LOD – 13.6	<lod -="" <loq<="" th=""><th>&lt; LOD</th></lod>	< LOD
Treatment 6 (10	eight) 🔿	. <b>P</b>	ar va	<lod -="" 16.8<="" th=""><th><lod -="" <loq<="" th=""><th>&lt; LOD</th></lod></th></lod>	<lod -="" <loq<="" th=""><th>&lt; LOD</th></lod>	< LOD
midacloprid and Hydroxy-Metabolite: LOQ = 5 µg/kg					LOD = 1.5 µg/k	g
Olefin-Metabolite		(~ 0)	4.0Q =	= 10 µg/kg	LOD = 3 µg/kg	-
<ul> <li>only replicate A</li> </ul>	analysed	, . , . , . , . , . , . , . , . , . , .	$\sim$			
• only replicate A	y g		<u>v</u>			

# Conclusion:

(Ca

Imidactorid 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** 



Report:	02.02.01/16; 2004; <u>M-451701-01-3</u>
Title:	Determination of the residue levels of imidacloprid and its relevant metabolites in hectary pollen and other plant material of chestnut trees (Aesculus hippocastanum) after soil
	treatment application and sampling 2001
Report No.:	pollen and other plant material of chestnut trees (Aesculus hippocastanum) after soll of treatment application and sampling 2001 AM021 <u>M-451701-01-3</u> none no Four Horse Chestnut trees (Aesculus hippocastanum) (T1-T4) received soil of the product: Afticle No. 0004211898, Batch No.
Document No.:	<u>M-451701-01-3</u>
Guideline(s):	none
Guideline deviation(s):	none
GLF/GEF:	
< <m-451701-01-3@8-604670-01-1 Material and methods:</m-451701-01-3@8-604670-01-1 	Four Horse Chestnut trees (A Boulus hinned tanum) (T1 OT4) received Oril
treatment with Imidacle	oprid WG 70 (commercially available product: Article X0. 0004211898, Bater No.
233914158*0. 1. No. of	f sample: FAR00802-00) on 2001-03-13 at an application rate of 0.28 g a 3 cm
stem diameter (=0.4 g p	product/cm stem diameter at a freight of 1.3 m) at a water application rate of
2L/tree. The 4 control t	rees (C1 - C4) received no treatment of the Area and the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company of the company o
During flowering of the	e trees, blossoms were collected Nectar was sampled from the control group. Leaf times throughout the vegetation period and fruits were sampled once all he end of
samples wire taken five	times throughout the vegetation period and fruits were sampled once at the end of
metabolites	All samples were subjected to a residue analysis for Imidacloped and as relevant
metabonites.	
Residue analysis was ca	arried out on leaves and blossoms using the analytical method BA 00537 (1999, R.
). Fruits	were analysed using the method BA 00300 (PF Nachriehten 1993/2,
, E. ).	
Ş	
Dates of biological wo	rk: (2001-65-13 + 62001-09-30)
Dates of analytical wo	
, <sup>0</sup> )	
Q d	
- ¥ U	
<u>A</u>	5 5 6 2 L
. <sup>6</sup>	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
P & A	
Ö	Four Horse Chestnut trees (Asiculus hippoenstanum) (TJ-T4) received soil porti WG 70 (commercially available product: Afficle No. 000211898, Bator No. f sample: FAR00802-00) of 2001-03-12 At an application rate of 028 g a 30 cm product/cm stem diameter at a feight of 1.3 m) at a stater application rate of recs (C1 - C4) received no treatment.



### Findings: Summary of residues in leaves, blossom and nectar samples of 4 treatment and 4 control trees:

Treatment	Sample material	DAT	lmidacloprid in µg/kg	Hydroxy- Imidacloprid in µg/kg	Olefin- Imidacloprid in g/kg	
		58 (2001-05-10)	n.d <loq< td=""><td>n.d.</td><td><sup>0</sup> n.d.</td><td></td></loq<>	n.d.	<sup>0</sup> n.d.	
		90 (2001-06-11)	n.d <loq< td=""><td>n.d. 🔏</td><td>n.d. n.d. 0d. 0d. 0 n.d.</td><td></td></loq<>	n.d. 🔏	n.d. n.d. 0d. 0d. 0 n.d.	
Control (C1-C4)	leaves	118 (2001-07-09)	n.d <loq< td=""><td>n.d. n.d. n.d. n.d. n.d. n.d. n.d. 10<sup>2</sup> 53</td><td>n.d</td><td></td></loq<>	n.d. n.d. n.d. n.d. n.d. n.d. n.d. 10 <sup>2</sup> 53	n.d	
		153 (2001-08-13)	n.d.	n.d.	Od Q	
		181 (2001-09-10)	n.d	~_n.d. 0		
		58 (2001-05-10)	لاي n.d. يُ	~ ~ ~ ~ ~	i N° '	r ≪'
		90 (2001-06-11)	33 121	10-53	n.d 15	
Treatment (T1-T4)	leaves	118 (2001-07-09)	×3 - 330	029 - 105	<sup>0</sup> 5 <i>±</i> √2 √2	
	•	153 (2001-08-13)	126, 282	29 - 195 57, 98 Q	<b>4</b> – 17 <b>V</b>	0 Ø
		(2009-09-10)	59 - 250	J40-118		5 <sup>8</sup> 1
Control	blossoms	0 58 √ √2001-05⁄10)	n.d 4000	57,998 C 	On.d. &	
(C1-C4)	Ĉa	(2001 05 16				
Treatment (T1-T4)	blossoms	58 +2001-05-10) 62	4 < 00 4. d < 00	A n.d X	S and.	
Control (C2)*	nectar	(2090-05-14)		Ch.d.	ر <sup>ک</sup> n.d.	

Imidacloprid and Hydroxy-Metabolite: LOQ = 5 µg/kg Olefin-Metabolite: LOQ = 10 µg/kg I twas technically del feasible to sample the necessary amount of pectar for a residue analysis from all trees.

Treatment     Sample material     DA     Total residue of londacloped in µg/kg       Control (C1-C4)     Tuits     181 (2501-09-10)     181 (2001-09-10)       Treatment (T1-T4)     fruits     181 (2001-09-10)     4 <1.00	n
Treatment         Sample material         DAS         Total residue of Midaclobed           Control (C1-C4)         Outs         V81         Val	9
Control (C1-C4) 2011s (2601-09-16) 4. (2601-09-16)	
Transferrant	
(T1-T4) (Truits) (T1-T4) (T1-T4) (T1-T4)	
Total residue: COQ = 50 µg/kg OD = 15 µg/kg	
>>M-451701-01-1 	
Residues in fruit samples: Treatment Sample Control (C1-C4) Pults (2901-09-10) Treatment fruits (2901-09-10) Total residue of lendacidored (1-C4) Pults (2901-09-10) Treatment fruits (2001-06-10) Total residue: COQ = 50 µg/kg LOD = 15 µg/kg M-451701-01-2 4604670-01-1	



Donoute	02.02.01/17; 2004; <u>M-451703-01-3</u>
Report:	
Title:	Determination of the residue levels of imidacloprid and its relevant metabolites in nectao
	pollen and other plant material of horse chestnut trees (Aesculus hippocastanun) after
Demont Max	polien and other plant material of norse chestnut trees (Aesculus hippocastanum) arter, trunk injection application and sampling 2001 AM023 <u>M-451703-01-3</u> none noe no no no no no no no no no no no no no
Report No.:	
Document No.:	<u>M-451703-01-3</u>
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	
< <m-451703-01-3@s-604699-01-1< td=""><td></td></m-451703-01-3@s-604699-01-1<>	
Material and methods	: Four Horse Chestnut trees (Wesculus hinner astanum) (T9 - T4) were reated by
trunk injection with Im	idaclonrid SL 200 (commercially available product: Article No 2000/958608
Datah No. 0504*0.25	Na of complex EA D00201 00) on 2001 05 00 of on application rate of 0.000
Batch No. 0394 0.23, 1	No. of sample. $r A (0.001-00) on 2001703-09 at an application rate of 0.00 g$
a.1./cm stem diameter (	=0.3 mL product/cm steen diameter ur 42.6 mL water/cm stem diameter at a height
of 1.3 m). The 4 contro	I trees (C1 - C4) received not reatment. $\beta$ $\beta$ $\beta$ $\beta$ $\beta$ $\beta$
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 an official
were sampled once at f	ne end of the depetation period All samples were subjected to a residue analysis
for Imidacloprid and its	relevant metabolites
for mindactoprid and its	
D 1 1 1	
Residue analysis was c	arried out on leaves and blossoms using the analytical method RA 00537 (1999, R.
). Fruits were	e analysed using the method RA 00300 (PF-Nachrichten 1993/2,, E.
).	
Dates of biological wa	<b>FU:</b> 2001_05-00 to 2001_09-30 37 67 6 37
Dates of biological via	
Dates of analyticar wo	
Findings: Suiomary of	residues in leaves, plosson and pectar simples of 4 treatment and 4 control trees:
Č)	
i a c	
. Star	
Q,	
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~1	
10 M	
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N D A	
õ	
~	
	idacloprid SL 200 (commercially available product: Article NS'00049580(8) No. of sample: FAR00801-00) on 2001-05-09 at an application rate of 0.06g =0.3 mL product/cm stem diameter inf 2.6 mL water/cm stem diameter at a height I trees (C1 - C4) received no treatment. e trees, blossoms were collected from all treatment groups and nectar was sampled Leaf samples were taken sixtimes throughout the vegetation period and fruits he end of the vegetation period. Albsamples were subjected too residue analysis relevant metabolites. arried for on feaves and blossoms using the analytical method RA 00537 (1999, R. e analysed using the method RA 00300 (PF-Nachrichten 1993/2,, E. et 2001-05-09 to 2001-09-30 residues in feaves, blossom and dectar simples of 4 treatment and 4 control trees:



# Imidacloprid Bee Studies Compilation of Study Summaries

Treatment	Sample	DAT	lmidacloprid in µg/kg	Hydroxy- Imidacloprid in µg/kg	Olefin- Imidacloprid in µg/kg
		1 2001-05-10)	n.d <loq< td=""><td>n.d.~~</td><td>A. B</td></loq<>	n.d.~~	A. B
		33 (2001-06-11)	n.d <loq< td=""><td>n.d.</td><td>n.d.y</td></loq<>	n.d.	n.d.y
Control (C1-C4)	leaves	61 (2001-07-09)	n.d. <loq< td=""><td>n.d.</td><td>7</td></loq<>	n.d.	7
		96 (2001-08-13)	n.d <loq< td=""><td>8 n.d. 2</td><td></td></loq<>	8 n.d. 2	
		124 (2001-09-10)			
		1 🔗 2001-05-10)	n.d <1,00	X 2nd. 2	
		(2001- <u>0</u> 5-11)	140300	7 On.d49	√ n.d, → <loq°< td=""></loq°<>
Treatment	lanuar	(2001-06-11)	1205 - 1996	302 1106	54-243
(T1-T4)	leaves	Q 61 (2001-07-09)	155 2200	J18 - 2513	14 - 259
	a M	Q <sup>7</sup> 96	07 - 1871	10 791	°≁LOQ - 139
		ົ∼⁄ 124 ແ (2001-09-10) ແ	0 173 419 C	ັ້ 0164 - 611 🤇	17 - 56
Control	blossoms	(2001-05-10)	.n.d <loq< td=""><td>4 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~</td><td>n.d.</td></loq<>	4 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	n.d.
(C1-C4)	S South	0_7_0_ 2001_05-16).5	n 8 0	/ & n.ds	n.d.
Treatment	blossoms	2 (2001-05-14)			n.d.
Treatment (T1-T4)		2001-05-16	5-283 5 n.¢,	n.d. 7	nļ.d <loq< td=""></loq<>
0	I°0/ ♥ &		0 <sup>7</sup> n.ø,	n.d.	n.d.
midacioprid and Diefin-Metabolite It was technically	Hydroxy-Metabo	blite LOQ	5 µg/kg 0 10 µg/kg amount of nectar t	LOD = 1.5 µg/kg LOD = 3 µg/kg for a residue analysis fr	om all trees.
esidues in frout s	samples:		5 <i>5</i> 7		
Treatmen		ple 2		o n.d. LOD = 1.5 μg/kg LOD = 3 μg/kg for a residue analysis fr otal residue of In in μg/kg	nidacloprid
(C1-C4)		fal 7 . 9 ts 7 . 1200	124 1-09-10)	n.d.	
Treatmen (T1 4)	t A G fruit	<u>کې د د د د د د د د د د د د د د د د د د د</u>	124 1-09-10)	n.d <lo< td=""><td>a j</td></lo<>	a j
		LOQ = 5		LOD = 15 µg	/kg
Total cosidue					



n.d.

	Compliant	n or study sun	iiiai ies		15sue uate 2	017-11-22
Report:	02.02.01/18;	· 2004· M	-451700-01- <u>2</u>			0
Title:		on of the residue le		oprid and its me	tabolites hydro:	xy ô
		and olefin-imidad				
		ppocastanum) afte		A	001	Diana
Report No.:	<u>M-451700-0</u>				L. L.	
Document No.:	<u>M-451700-0</u>	<u>1-2</u>				
Guideline(s):	none				Ő,	
Guideline deviation(s):	none		Ĉ	L.		× ~
GLP/GEP:	no		A.	<u>"</u>	PI - T4) received	
< <m-451700-01-2@s-603148-01-1< td=""><td></td><td></td><td>å</td><td>,Ô¥</td><td></td><td></td></m-451700-01-2@s-603148-01-1<>			å	,Ô¥		
Material and methods	s: Four Horse	Chestnut Trees	Mesculus hipp	Scastanum) (E	P - T4) recein	ed soid
treatment with Imidacl	oprid WG 70	(Article No. 00)	4211898, Bat	th No 233904	158×0,1, No.	of sample:
FAR00802-00) on 200				em stem diame	eter =0.4@pro	ochuet/cm
stem diameter at a heig						
received no treatment.	, , .	O <sup>r 1</sup>		7 ~ 7 T	Y L A	~ ~ ~
Sampling was carried of	out in 2002 o	ne veat after the	application ha	d been carfued	out During f	owering
of the trees, blossoms a	ind leaves we	re collected Ad	litional leaf sa	moles were tal	ken four times	
throughout the vegetati	on period Al	l samples were s	whiected to an	esidue shalvsi	or Imidacio	Brid and
its metabolites Hydrox	vlmidaclopric	and Obstin-knig	factored Ra	due malución	vac repried Aut	on leaves
and blossoms using the	analytical	thad DA 00527	(1000 P *	Cuchendry Siss, V		on leaves
and biossoms using the	anarytical		(19999, K.	J.	Č 'N	
D.4				Ž <sup>y</sup> O <sup>y</sup> Č	× ×	
Dates of biological wo	ork: 2009 -03	-13 to 2002-09-3			0	
Dates of analytical wo				nles of 4 treat	L. G	
Findings: Summary	residates in k	aves, blossor a	nd neetar sam	ples of 4 treatr	ment and 4 cor	trol trees:
		d'a		L O		1
	Sample	k. X i	Imidacloprid	Hydroxy- Imidacloprid	Olefin- Imidacloprid	
Treament	material (		(byg/kg]	/µg/kg]	[µg/kg]	
	N ON	407 - 412		×1		
	K K	(2002-04-24 - 29)	• n.	🖉 n.d.	n.d.	
		<pre>     441     (2002-05-28)</pre>	n.d. <loq< td=""><td>n.d.</td><td>n.d.</td><td></td></loq<>	n.d.	n.d.	
Contreio (C1 - cor)		(2002-0702)	○ <sup>4</sup> n.d.	n.d.	n.d.	
		(200 <del>3</del> -07-30)	A.d.	n.d.	n.d.	
	So S	(2002-09-03)	@.d <loq< td=""><td>n.d.</td><td>n.d.</td><td></td></loq<>	n.d.	n.d.	
		2002-00-25 - 29	26 - 40	13 - 30	<loq< td=""><td></td></loq<>	
Treatment		(2002-05-28)	115 - 308	n.d 115	29 – 101	
	O leaves	476 (2002-07-02)	176 - 492	n.d161	119 - 114	
2 <sup>5</sup> ~ ~		(2003-07-30)	161 - 532	n.d 177	34 – 229	
Treatment (T1, F4) Control (C2-C4)		539 (2002-09-03)	80 - 185	63 - 107	19 - 52	
Control	blossoms	407 - 412 (2002-04-24 - 29)	n.d.	n.d.	n.d.	

n.d. - <LOQ blossoms n.d. (2002-04-25 - 29) (T1 - T4) LOQ = 5 µg/kg LOQ = 10 µg/kg LOD = 1.5 µg/kg Inidacloprid and Hydroxy-Metabolite: LOD = 3 µg/kg Olefin-Metabolite:

408 - 412

Treatment

n.d. = not detected

÷

<sup>&</sup>gt;>M-451700-01-2@S-603148-01-1



Report:	02.02.01/19;	: 2004 <sup>.</sup> M	-451696-01-2			
Title:	,	on of the residue le		oprid and its me	tabolites hydro	XV-
THE.		and olefin-imidad				
	(Aesculus hi	ppocastanum) afte	er trunk injection	n - Application	2001 and sampl	ina 2002
Report No.:	<u>M-451696-0</u>	1-2	i trunk injection			5 2004 C
Document No.:	M-451696-0			Č	Funk injection	, Ô
Guideline(s):	none	<u>12</u>		Ť	~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Guideline deviation(s):	none			.4	L.	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
GLP/GEP:	none			s and a second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second s	~ ~	
GLI/GLI.	110		Ò	Š	\$1 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1 S
< <m-451696-01-2@s-603097-01-1< td=""><td></td><td></td><td>- And And And And And And And And And And</td><td></td><td></td><td>x, X</td></m-451696-01-2@s-603097-01-1<>			- And And And And And And And And And And			x, X
Material and methods	5:		L	,Õ¥	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~ v <sup>0</sup>
Material and methods Four Horse Chestnut T Imidacloprid SL 200 (2 2001-05-09 at an appli	rees (Aesculu	s hippocastanun	🖗 (T1 - T4) we	treated by t	Punk injection	with of
Imidacloprid SL 200 (A	Article No. 00	04958608. Bate	No. 0594*0.	25. No. of son	ple: FAR008	01-001 on
2001-05-09 at an appli	cation rate of	0.06 g a s/cm/st	em diameter (=	=0.3 ml prod	ict cm stem di	iameter in
42.6 mL water/cm sten	n diameter at a	a height of 13 m	The Asontr	atrees 101 - (	R received n	N. The second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second se
treatment. Sampling wa						
d · · · · · · · · · · · ·		$111 \ 2002, \text{ one yc}$				. During
flowering of the trees,	biossoms and	leaves were con	ecteor. Additio	nai lear sampi	es were taken	Tournes
throughout the vegetati	on period. Al	l samples were s	ubjected to a r	esudue analysi	stor Inudacio	poid and
its metabolites Hydrox	y- Imidaclopr	id and Odefin-In	udaclopyid. 🧹			)
	ſ	0* <u>*</u> .5		anafytical conet	hod RA.09537	
Residue analysis was c	arried out on	leaves and bloss	oms using the	analytical	hod RA 0053	7 (1999, R.
Ď	. A			~~~~~.		
<i>)</i> ·		~ ~ ~		Ç, Ç	ž 🖌	
Datas of biological wa	-1- 2001 0¢				0	
Dates of biological wo	ork: 2001-035	09 10 2002-09430	0 % *		, Ôg	
		Q Q	s a	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	N N	
Dates of analytical wo	ork: 2002-03-	25 to 2005-03-1	o so k	, <sup>n</sup> õ	×	
	a (020 %	. / (>>	. ( )			
Findings: Summary of	residues in the	eaves, blossom a	ndrectarsam	ples of 4 treatr	nent and 4 cor	trol trees:
, D			Y N (			
			Inortaclopric	Hydroxy-	Olefin-	]
Toratment	Somple (	DAT		idacloprid	Imidacloprid	
°°		AN	O Chawab	🎸 [µg/kg]	[µg/kg]	
		350 - 355	, and a	n.d.	n.d.	
Å .	7.0	2002-04-24 - 28	L <sup>y</sup> O			4
		(2002-05-28)	(n.d <l@@< td=""><td>n.d.</td><td>n.d.</td><td></td></l@@<>	n.d.	n.d.	
Control (C1 (C4)	leaves	4190 (2002-07-02)	n n n n n n n n n n n n n n n n n n n	n.d.	n.d.	
l l l l			On.d.	n.d.	n.d.	1
		(2903-07-39)	On.d <loq< td=""><td>n.d.</td><td>n.d.</td><td></td></loq<>	n.d.	n.d.	
		2002-09-03) ( 350 355 ~	4			-
		(2002 04-24 - 29)	<loq -="" 29<="" td=""><td>n.d. – 14</td><td>n.d <loq< td=""><td></td></loq<></td></loq>	n.d. – 14	n.d <loq< td=""><td></td></loq<>	
		384 (2002-05-28)	15 - 190	<loq -="" 58<="" td=""><td>n.d. – 11</td><td></td></loq>	n.d. – 11	
Treatment (F <sup>22</sup> T4)	leaves	419 (2002,07-02)	7 - 92	<loq -="" 47<="" td=""><td>n.d <loq< td=""><td>]</td></loq<></td></loq>	n.d <loq< td=""><td>]</td></loq<>	]
		447	16 - 53	7 - 49	n.d. – 10	1
		482				1
	٣	(2002-09-03)	12 - 20	<loq -="" 19<="" td=""><td>n.d. – 10</td><td></td></loq>	n.d. – 10	
Control A CALL CALL Treatment CT1 - T4	bossoms	350 - 355 (2002-04-24 - 29)	n.d.	n.d.	n.d.	
Greatment (T1 - T4)	blossoms	350 - 355 (2002-04-24 - 29)	n.d <loq< td=""><td>n.d.</td><td>n.d.</td><td></td></loq<>	n.d.	n.d.	
Nimidacloprid an	d Hydroxy-Meta		ua/ka LOD	) = 1.5 µg/kg		1
Olefin-Metaboli		LOQ = 10		$= 3 \mu g/kg$		
		LOQ - 10	/µg/kg LOD	- 5 µg/kg		
n.d. = not detecte		200 - 10		- 5 µg/kg		

>>M-451696-01-2@**S-603097-01-1** 

Please click on the hyperlink to order a Study Report.



Report:		004; <u>M-451667-01-3</u>		
Title:	Residues of imidaclop	rid WG 5 in blossom s	amples of Rhodod	endron sp. (variets Nova
	Zembla) after soil treat	tment in the field - App	olication: Spring 2	endron sp. (variete Nova ) 003, sampling: 2003 and
	2004		ð	
Report No.:	<u>M-451667-01-3</u>		Ş	A A
Document No.:	<u>M-451667-01-3</u>		O <sub>A</sub>	
Guideline(s):	none			
Guideline deviation(s):	none	Ča		
GLP/GEP:	no	- A	<u></u>	
< <m-451667-01-3@s-604678-01-1< td=""><td></td><td></td><td>Ő¥ .</td><td></td></m-451667-01-3@s-604678-01-1<>			Ő¥ .	

**Material and methods:** Eight year old *Rhododendrou* plants (variety "Nova Zephola") growing at the experimental farmland "Höfchen" near Burscheid (Nordrhein-Westfaler, 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 Vo.: 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) seeeived no treatment of the application rates shown below.

Treatment Group	Application Rate per 50 cm Plant Beight	Application	Sampling Date of Blossoms
1	Control 5		2003-0\$-20 and 26 (2 <sup>50</sup> time only replicate A), 2004-05-25 (only replicate A)
2	2500 mg a.s pre-flowering	2003-05-09	O <sup>V</sup> ≪ 2003°95-20 and 26
3	\$500 mg a.s. oost-	2003-0005	© © 2004-05-26
4 ~	9250 og a.s. pre-flowering	2003-05-09	2003-05-20 and 26
	\$250 mg a.s. post-	2003-06-05	
5. Q 	Dowering 100 per- plus 200 mg a.s. postritowering	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 preflowering treatment groups 2,4 and 6, eleven and 17 days after application (except for treatment group 6, with only 9 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 NPLC/MS/MS were performed according to method 00537/E001 (MR-568/99) by R.

In the year 2004 samples of blossoms and leaves of *Rhododendron* sp. were collected from the control and the post-the wering treatment groups 3, 5 and 6, 356 days after application. Samples were stored as in 2003 and blossoms analyzed 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

Findings: In the following table the results of the residue analyses of blossom samples summarised.

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			Hydroxy-	Olefin-
Treatment group	DAT	Imidacloprid [mg/kg]	Imidacloprid [mg/kg]	Olefin- Imidacloprid [mg/kg]
	11	<lod -="" 0.0125<="" td=""><td><lod -="" <loq<="" td=""><td></td></lod></td></lod>	<lod -="" <loq<="" td=""><td></td></lod>	
1: Control	17*	<lod -="" 0.0127<="" td=""><td><lod -="" <lo@<="" td=""><td></td></lod></td></lod>	<lod -="" <lo@<="" td=""><td></td></lod>	
	356*	<lod -="" 0.0188<="" td=""><td><lod -="" <loq<="" td=""><td>&lt; OF S</td></lod></td></lod>	<lod -="" <loq<="" td=""><td>&lt; OF S</td></lod>	< OF S
2: (2500 mg a.s. per 50 cm plant	11	<lod -0,0200<="" td=""><td><lod -0.0063<="" td=""><td>STOD Y S</td></lod></td></lod>	<lod -0.0063<="" td=""><td>STOD Y S</td></lod>	STOD Y S
height, pre-flowering)	17	<loq 0.0232<="" td=""><td><loo -="" 0.0087<="" td=""><td></td></loo></td></loq>	<loo -="" 0.0087<="" td=""><td></td></loo>	
3: (2500 mg a.s. per 50 cm plant height, post-flowering)	356	0.001 - 1.4396	0 234 - 0.1575	<lod (2)="" (2)<="" td=""></lod>
4: (1250 mg a.s. per 50 cm plant	11 🖉	CLOD - 0.0114	<loo- <loo<="" td=""><td></td></loo->	
height, pre-flowering)	17 🌾	<lod -="" 0.0="" 56<="" td=""><td>&lt;€0D - &lt;⊧00</td><td>O &lt; LOD</td></lod>	<€0D - <⊧00	O < LOD
5: (1250 mg a.s. per 50 cm plant height, post-flowering)	356	0.0164 - 0.5430	0.0070 0.0682	S
6: (100 mg a.s. per 50 cm pre-	×11 ~	<lod -="" 0.0108<="" td=""><td>&lt;1,00 - &lt;1.000</td><td><lod +="" ,="" ,<="" 0.0122="" td=""></lod></td></lod>	<1,00 - <1.000	<lod +="" ,="" ,<="" 0.0122="" td=""></lod>
flowering, 200 mg a.s. per 50 cm plant height, post-flowering)	356 X	0.0518 - 0.1804	0,0082 - 0.0291	-LODG-LOQ
DAT: day after application		0.005 00/40		00thmaked
		0.005 mg/kg = 0.0 0 mg/kg		0.003 mg/kg
* only replicate A analysed aponly t	this replicate wa	is sampled	N N ?	
ion 👋	Y .~	"0" L		

### **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 between 1997- 2000 which included drenching treatment before planting out in the field. The possibility obcontamination 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 and obviously not significantly contribute to the residue levels detected.

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In the high cose pre-flowering treatment group (application rate: 2500 mg a.s./50 cm plant height) residues up to 0.0232 mg lmidaelopric kg, 0.0087 mg Hydroxy-Invdacloprid/ kg and 0.003 mg Olefin-Imidacloprid/kg were found 17 days after treatment.

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× 1

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-Imidacloprid/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-Imidacloprid/kg were found 356 days after treatment.

For flowers from the low dose post-dowering treatment group (application rate: 1250 mg a.s./50 cm plant height) residues up to 0.542 mg lmitacloprid/kg, 0.068 mg Hydroxy-Imidacloprid/kg and 0.012 mg Olefin-Imidacloprid/kg were formed 356 days after treatment.

In the group that had received pre-flowering treatment at 100 mg a.s. plus postflowering treatment 200 mg a \$//50 cm plan height, residues up to

0.180 mg/midacloprid/kg, 0.029 mg Hydroxy-Imidacloprid/kg and below

0.010 mgOlefin-Imidacloprid/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

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Report:	02.02.01/21; 2004; <u>M-451691-01-3</u>
Title:	Residues of imidacloprid WG 5 in blossom samples of lime trees (Tilia europaea after or soil treetment in the field Application: 2003, sampling: 2004
	soil treatment in the field - Application: 2003, sampling: 2004
Report No.:	P672034513
Document No.:	<u>M-451691-01-3</u>
Guideline(s):	
Guideline deviation(s):	none
GLP/GEP:	
< <m-451691-01-3@s-604682-01-1< th=""><th></th></m-451691-01-3@s-604682-01-1<>	
Dates of biological wo	k: 2006-05-21 to 2006-08-08 $O^{5}$ $\sqrt{2}$ $\sqrt{2}$
Dates of analytical wo	k: 2006-07-05 to 2006 40-19

### 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 Baser CropScience AG (40789 Monheim, Nordrhein-Westfalen, Germany received soil treatment with Imidacloprid WG 70 dissolved in water at an application votame of 2 L per shrub on 2006-04-12 at the application rates given In Table 1 (treatment groups 2 and 4). Control shrubs (freatment groups 1 and 3), located in a distance of 200 m received no treatment.

Treatment group	× 1,4	\$ 2 \$ 5		<b>4</b>
Treatment name	Rhodocendron, untreated	Kingdodeneron, ucaroo	unnearen	Hibiscus, treated
Application rates		د a.s./m.average) plant width* ని		4.3 g a.s./m average plant height*
		= 7.37 g product/shrub		4.3 g a.s./shrub = 6.14 g product/shrub

Table 1: Summary: Treatment Groups and Rates

\* To describe the size of the Rhodorendron shrubs the parameter shrub width was used for fixing the application rate. For Hibisous the parameter shrub height was used for fixing the application rate.

Each treatment group consisted of 3 parallel rows of Oshrubs each, Rhododendron and Hibiscus respectively. At the exterior sides of the 2 outer rows with Rhododendron sp. and Hibiscus syriacus a mixture of begrattractive 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 ornangental plants (Relargonium 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 erings 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 pointana, Phacena tanacetifona, 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 Dosson's, 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 terrestrist) 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)

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Assessments on foraging activity of the honevbees 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 2006-05-2444 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: or 2006-06-02 the last afternoon assessment was made. Foraging assessments on the Hibiscus syriacity 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 garub 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 106-11/ 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 capied out on the blossoms. Extraction, sample clean-up and determination of midacloprid Hydroxy and Olefin-Metabolites by HPLC-MS/MS were performed according to method 01010 (MR-06/107). 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 asidue analysis for residues of Imidaclopfied and its Olefin- and Hydroxy-Metabolites. Extraction and determination of Imidaelopric 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 Moneybees, and Bumblebees on Rhododendron

	Total	number per speci	of observed per p	lot [n]
		yobes 🐎 🔬	Bumbl	ebees
Treatmentgroup	Rhododendron	Omementals	Rhododendron	Ornamentals
1: Control	× 23	~ 64	608	238
2: Treatment	× 10 × ×	104	107	87

Only few honeybees were observed for aging on *Rhododendon* shrubs on the control and treatment plot respectively, but more on the control than on the treatment plots.

Foraging activity of honeybees of the surrounding orgamentals 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 reated plants. The optimisental plants on the treated plot were likewise significantly less visited than these on the control plot.

Table 3: Summary: Foraging Activity of Honeybees and Bumblebees on Hibiscus

	Total	number per speci	es observed pe	r plot [n]
	Hone	eybees	Buml	blebees
Treatment group	Hibiscus	Ornamentals	Hibiscus	Ornamentals
C 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 prot compared with the treated plot. The number of foraging bumblebees on the surrounding ornarientals 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 & 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 reatment replicates in total 2 dead bumblebees were found in the Rhododendron part, and 14 dead bumblebees in the Mbiscus part

Table 4:	Summary:	Mortality	of Hone	ybees

	Rhodødendn	wa° 5° ×	Sus 😽 🖓
		Total number	A A
Treatment group	on the plot in fr		in front of hive
Control	e in a	@ <sup>2</sup> , 4 0	
Treatment	82 44 . A		Ø 0 Ø



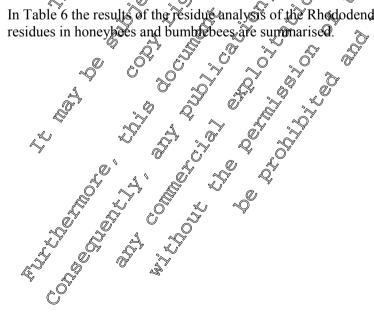
le 5: Summary: Mon	tality of Bumble	bees		
	<ul> <li>Rhodod</li> </ul>			scus
, ĝ	Or pr	🖉 🖉 Total nu	mpe©[n]	Ś.
Treatment group	on the plots	in front of live	on the plot	in front of hive
Control				. 0 .
Treatment	5 19 A		ر 12 <sub>0</sub>	2
	4° 4 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	A N	Q 19	

Colony health and condition of the boneyiee colordes was not entrent 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

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Ó Ô In Table 6 the results of the residue analysis of the Rhododendron and Hibiscus blossom samples and the





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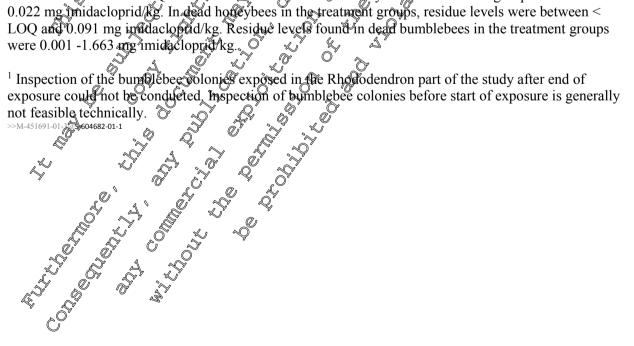
<u>
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Issue date 2017-11-22

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	κ Loα	~100~S	>
2: Treatment	blossoms	Rh	2006-05-17	35			QLOQ 0.01	
3: Control	blossoms	н	2006-07-27	106	< L00	<100	Sloo	
4: Treatment	blossoms	н	2006-07-27 to 2006-08-07	108-117	0.76 5.01	< LOQ 0.45	< LOUG- 0.33	Ű,
1: Control	2 honey- bees (colony)	Rh	2006-05-29	<b>47</b> °	0,005,-0 0.022	< LOQ + 0	0.019	
	25 honey- bees (colony)	Rh	2006-05-21 0 2006-05-31	19 کچ	6016 0	< LOQ -	<loq -<br="">0,001 (</loq>	0
2:	2 honey- bees (plot)	Rh	2006-05-21 to 2006-05-31	39~49	0.002	2.00 - >> 0.018	<200 -0 ∠, 0.00 €	
Treatment	1 bumble- bee (colony)	Rh	2906-05-29	47	0,001	0.018	0.005	
	1 bumble- bee (plot)	Rh 4	Q 2006-05-31	48,	20.005 20.005	0.002	∞0.003	
4:	2 bumble- bees (colony)	<b>H</b> C	2006-07-26	9105 ×	0.003 -	0.001 - 0.003	0.004 – 0.009	
Treatment	12 bumble- bees (plot)	ĢΗ (	2006-07-25 to 2006-08-08	104 - 118	0,077- 1.663	0.019 - 0.996	0.031 – 0.405	
<ul> <li>DAT</li> </ul>	days after tre	atment	Rh Rh	ododendron	Х <sub>(k</sub> , H <sub>)</sub> `	Hibiscus		

Amidacloprid, Hydroxy-Metabolite, Oletin-Metabolite LOQ = 0.010 mg/kg Blossom samples: Imidacloprid Hydrox Metabolite, Olefin-Metabolite LOQ = 0.001 mg/kg Insect samples: 

Residue levels in the Rhododendron blossom's were 0.09 - 0.79 mg imidaeloprid/kg and in Hibiscus blossoms 0.76 5.01 gr Im Paclop rd/kg w the treated replicates. In the blossoms of the untreated plants, residue levels were «LOQ. Residue levels found in dood honeybees in the control groups were 0.005 -0.022 mg inidacloprid/kg. In dead hourybees in the treatment groups, residue levels were between <





Report:	02.02.01/22; 2004; <u>M-451699-01-3</u>
Title:	Residues of imidacloprid WG 5 in blossom and leaf samples of apple trees after for treatment in the field - Application: 2003, Sampling: 2004
Report No.:	P672034511
Document No.:	<u>M-451699-01-3</u>
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	no la la la la la la la la la la la la la
02295087 batch No.: PF carried out on 2003-10-3	Residues of imidacloprid WG 5 in blossom and leaf samples of apple trees after spil treatment in the field - Application: 2003, Sampling: 2004 P672034511 <u>M-451699-01-3</u> none none noe No Apple trees (variety James Grieve) growing at the Bayer CropScience AG Hörchen", in the vicinity of Burscheid (Germany, Nordrhein-Westfalen), n autumn 2003 with Imidacloprid WG 5 in two replicates (A and B: 8 trees each) idacloprid WG 5 (active substance NTN 38893 purity: 5.3% inateriat No. 000006PD) dissolved in water at an application volume of 2 L per free water at the application rates given in the table below 3 ontrol trees received no
treatment. In treatment g	group 2, 0.28 ga.s./cm/stem/diameter/were applied and intreatment group 3, 0.14
g a.s./cm stem diameter.	
-	
The average stem diame	ter at a tree height of 1/3 m was 11 cm.
Treatment (	Skotip 6 4 Application Rate per Tree 6
1.4	a b b untreated control
·	0 3.08 g a.s. tree = 58.08 g p@duct/tree
3	0 7 1.54 g a 8./tree 29.04 product/tree
Blossom and leaf sample •blossoms of flowering (	es were collected once in spring 2004:
<ul> <li>leaves after flowering of</li> </ul>	in 2004-05-14 (197 days after application)
The samples of blossom	s and leaves were stored at -1 C until the sample preparation and eventually
residue analysis for Imic	acloped and its Oletin- and Hydroxy-Metabolites were carried out. Extraction,
sample clean-up and det	ermutation of Imidacloppin, Hydroxy- and Olefin-Metabolites by HPLC-MS/MS
	ng to method $00537/E001$ (MR 568/99) by R.
Dates of biological work	k: 2003×10,30 to 2004-05,44
Dates of analytical work	:: 2004-05204 to 2004-08-15
Findings: In the following summarised.	r: 2004-05704 to 2004-03715 ng table the results of the residue analyses of blossom and leave samples are
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# **Imidacloprid Bee Studies Compilation of Study Summaries**

Issue date 2017-11-22

Sampled MaterialTreatment GroupDAT*Imidacloprid (mg/kg)Hydroxy- Imidacloprid (mg/kg)Olefin- Imidacloprid (mg/kg)Imidacloprid (mg/kg)1 (untreated control)181 $Blossoms2 (3.08 g a.s./tree =58.08 g product/tree)1813 (1.54 g a.s./tree =29.04 g product/tree)181Leaves2 (3.08 g a.s./tree =58.08 g product/tree)181Leaves2 (3.08 g a.s./tree =58.08 g product/tree)197Imidacloprid and Hydroxy-Metabolite:Olefin-Metabolite:LOQ = 0.005 mg/kgLOQ = 0.0015 mg/kgLOD = 0.0015 mg/kgLOD = 0.003 mg/kgLOD = 0.003 mg/kgLOD = 0.003 mg/kgImidacloprid and Hydroxy-Metabolite:Olefin-Metabolite:LOQ = 0.005 mg/kgLOQ = 0.010 mg/kgLOD = 0.003 mg/kgLOD = 0.003 mg/kg** DAT: days after treatment**Unit of 16 control samples residuesLOD were detected No ideatification of the origin of thisContamination was found.**/**********************************$								
Blossoms2 (3.08 g a.s./tree = 58.08 g product/tree)181 <lod< th=""><math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LOD3 (1.54 g a.s./tree = 29.04 g product/tree)181<math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LODLeaves2 (3.08 g a.s./tree = 58.08 g product/tree)197<math>\leq</math>LOD - <math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LODLeaves2 (3.08 g a.s./tree = 58.08 g product/tree)197<math>\leq</math>LOD - <math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LODImidacloprid and Hydroxy-Metabolite: Olefin-Metabolite:LOQ = 0.005 mg/kg LOQ = 0.005 mg/kg LOD = 0.003 mg/kg LOD = 0.003 mg/kg LOD = 0.003 mg/kgLOD = 0.003 mg/kg LOD = 0.003 mg/kg LOD = 0.003 mg/kg• DAT: days after treatment • In 1 of 16 control samples residuesLOD were detected. No identification of the origin of this contamination was found.• M451699-01-3@5 604696-01-102.02.01/23 Residues of imidacloprid WG 5 in blossom samples of Mododendron sp. after soil</lod<>		Treatment Group	DAT		Imidacloprid [mo/ko]	Imidacloced (mg/kg)		
Blossoms2 (3.08 g a.s./tree = 58.08 g product/tree)181 <lod< th=""><math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LOD3 (1.54 g a.s./tree = 29.04 g product/tree)181<math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LODLeaves2 (3.08 g a.s./tree = 58.08 g product/tree)197<math>\leq</math>LOD - <math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LODLeaves2 (3.08 g a.s./tree = 58.08 g product/tree)197<math>\leq</math>LOD - <math>\leq</math>LOD<math>\leq</math>LOD<math>\leq</math>LODImidacloprid and Hydroxy-Metabolite: Olefin-Metabolite:LOQ = 0.005 mg/kg LOQ = 0.005 mg/kg LOD = 0.003 mg/kg LOD = 0.003 mg/kg LOD = 0.003 mg/kgLOD = 0.003 mg/kg LOD = 0.003 mg/kg LOD = 0.003 mg/kg• DAT: days after treatment • In 1 of 16 control samples residuesLOD were detected. No identification of the origin of this contamination was found.• M451699-01-3@5 604696-01-102.02.01/23 Residues of imidacloprid WG 5 in blossom samples of Mododendron sp. after soil</br></lod<>		1 (untreated control) 181 <lod -="" <lod<="" <loq**="" td=""></lod>						
Leaves       1 (untreated control)       197       COQ - <lod< th=""> <lod< th=""> <lod< th=""> <cod< th=""> <cod< th="">         Leaves       2 (3.08 g a.s./tree = 58.08 g product/tree)       197       <lod -="" 9.012<="" td=""> <lod -="" 0.013<="" td=""> <loq< td=""> <lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></loq<></lod></lod></cod<></cod<></lod<></lod<></lod<>	Blossoms	2 (3.08 g a.s./tree =         181 <lod< th=""> <lod< th="">           Blossoms         58.08 g product/tree)         181         <lod< td="">         57         <lod< td=""></lod<></lod<></lod<></lod<>						
Leaves       1 (untreated control)       197       COQ - <lod< td=""> <loq< td=""> <cod< td=""></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></cod<></loq<></lod<>	-	3 (1.54 g a.s./tree = 29.04 g product/tree) 181						
Leaves       2 (3.08 g a.s./tree = 58.08 g product/tree)       197 <lod -="" 0.012<="" td=""> <lod -="" 0.013<="" td=""> <lod -="" 0.013<="" td="">         Imidacloprid and Hydroxy-Metabolite:       LOQ = 0.005 mg/kg       LOO = 0.0015 mg/kg       LOD = 0.0015 mg/kg         Olefin-Metabolite:       LOQ = 0.010 mig/kg       LOD = 0.003 mg/kg       LOD = 0.003 mg/kg         • DAT: days after treatment       +       LOQ = 0.010 mig/kg       LOD = 0.003 mg/kg         • M-451699-01-3@5-604696-01-1       -       -       -         • M-451699-01-3@5-604696-01-1       02.02.01/23       2004       M-451694-01-3         • M-451699-01-3@5-604696-01-1       -       -       2004       M-451694-01-3         • M-451699-01-3       -       -       -       2004       M-451694-01-3         • M-451699-01-3       -       -       -       -       -       -         • M-451699-01-3       -       -       -       -       -       -       -         • M-451699-01-3       -       -       -</lod></lod></lod>		1 (untreated control)	197	1400 - <lodo< td=""><td></td><td></td></lodo<>				
Imidacloprid and Hydroxy-Metabolite:       LOQ =0.005 mg/kg       LOO = 0.0015 mg/kg         Olefin-Metabolite:       LOQ =0.010 mg/kg       LOD = 0.003 mg/kg         • DAT: days after treatment       LOQ =0.010 mg/kg       LOD = 0.003 mg/kg         • In 1 of 16 control samples residues       LOD were detected. No identification of the origin of this contamination was found.         • M-451699-01-3@5-604696-01-1       02.02.01/23       2004         Report:       02.02.01/23       2004         Title:       Residues of imidacloprid WG 5 in blossom samples of Chododeendron sp. after soil	Leaves 2 (3.08 g a.s./tree = 197 < OD - 0.012 < LOD 0.00 < DO - 200							
$\begin{array}{c} \text{contamination was round.} \\ \Rightarrow M-451699-01-3@5-604696-01-1 \\ \text{Report:} \\ \text{Title:} \\ \end{array} \begin{array}{c} 02.02.01/23 \\ \text{Residues of imidacloprid WG 5 in blossom samples of Shodockendron sp. after soil} \\ \end{array}$	Olefin-Metab DAT: da In 1 of 1	oolite: ays after treatment		e deflected No idea	LOD = 0.0015 m LOD = 0.003 mg	the frequency of the second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second se		
Report:       02.02.01/23:2004:2004:2004:2004:2004:2004:2004:20	>>M-451699-01-3@5-604696-01-1							
treatment in the field - Application: Autumn, 2003, sampling 2004								
Report No.: $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169401-3$ $M-45169$	Document No.	: <u>M-451694071-</u>	and a second					

Guideline(s): Guideline deviation(s): **GLP/GEP:** 

<<M-451694-01-3@8

### 604692-01-1 Material and methods:

Shrubs of the species *Bhodododron* 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 shrups each) per treatment group.

Soil application with Imidacloprid W@ 5 (active substance NTN 33893, purity: 5.3%, material No.: 02295087, baten No. PF000006PD dissoped in water at an application volume of 1.5 L per shrub was carried out on 2003-11-26 at the application rates give in the table below. Control shrubs received no treatment in treatment group 23.3 g 35./m shrub height were applied and in treatment group 3, 2.15 g a.s./m shoub height.

1 The average shrub height was 0,4 m.

\none 🛛

no

Application Data par Chruh
Application Rate per Shrub
untreated control
1.72 g a.s./shrub = 32.5 g product/shrub
0.86 g a.s./shrub = 16.2 g product/shrub

Blossof and leaf samples were collected once at flowering on 2004-05-19 (175 days after application). The sampled Rhododendron leaves were not analysed.



residue analy	of blossoms and leaves w rsis for Imidacloprid and i	ts Olefin- an	d Hydroxy-Metab	olites were carried	out on the
	traction, sample clean-up by HPLC-MS/MS were pe				
	logical work: 2003-11-26 lytical work: 2004-06-03			N N N	
	·		C5	d d	
Findings: In	the following table the re	sults of the r	esidue analyses of	blossom samples	are summarised.
Sampled Material	Treatment Group	DAT*	Imidaclop)d	Hydroxy- Midacloprid frig/kgt	Olefin Arnidaçî (mg/kg)
-	1: untreated control	175	ELOD Q		TOD S
Blossoms	2: 1.72 g a.s./shrub = 32.5 g product/shrub	Q <sup>1</sup> 17,5 <sup>7</sup>	0.027 - 0.85	<body>          &lt;</body>	Č×LOD→0.0082
	3: 0.86 g a.s./shrub 16.2 g product/shrub	0 175 0	012-037	< LOG - 0.003	LOD - <loq< td=""></loq<>
	after treatment		= 0.005 mg/kg = 0.010 mg/kg	. COD = 0.0015 m LOD = 0.0032mg	
>>M-451694-01-3@ <b>S-6</b>	504692-01-1 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5				
<b>Report:</b> Title:		idacloprid W			fter soil treatment in
Report No.: Document No	<sup>™</sup> <u>M<sup>*</sup></u> 451662-01-	$\frac{3}{3}$	samiong: 2005		
Guideline (5): Guideline dev	Unone S &				
GLP/GEP:	S nov S				
<-M-451662-01-3@S-0 Material and	d methods Shrabs of the	snemes Con	Nis magerowing a	t the market garde	n "Selders"
with Imidael	P. 217,42781 (Baan) in Go oprid WG 5 In two peplica	ermany (Sor nes (A and E	drhgn-Westfalen) B: Shrubs each) p	er treatment group	tment in autumn ).
Soil applicati	on with Omidaeloprid WC atch No.: PF@0006RD) d	3 5 factive su	<sup>≫</sup> bstance: NTN 338 vater at an applicat	893, purity: 5.3%, 1 tion volume of 1.5	material No.: L per shrub was
carried out of	n 2003-10-31 at the applic bixed no treatment. In frea	ation tates g	iven in the table b	elow. Control shru	ibs (treatment
treatment gr	up 3,2.15 g a s m shrub l	neight. The a	verage shrub heig	ht was 1.2 m.	

No. of Restment Group	Application Rate per Shrub
A & 18 X	untreated control
5 9 10 N 4 5 2	5.16 g a.s./shrub = 97.4 g product/shrub
3	2.58 g a.s./shrub = 48.7 g product/shrub

Blossom and leaf samples were collected once in spring 2005:

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- 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  $\hat{a}$ 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 2005-04-24 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.

				Hydroxy-		- A c °
Sampled Material	Treatment Group	DAT* 4	Imidiacioprid	Hydroxy- Initiaclopfid	<ul> <li>Olefin-</li> <li>Inidacloprid</li> </ul>	
	1: untreated control**	506	4 <100		Stog 2	
Blossoms	2: 5.16 g a.s./shrub = 97.4 g product/shrub***	9505 G	1.036 - 2.80	µ@daclopfd √ [mg/kg] √ < @OQ 0:029 - 0.088 √ 0.067 - 0.155	[mg/kg] \$COQ 0009 - 6030 0.014 - 0.063	
	3: 2.58 g a.s./shrub ** 48.7 g product/shrub***	505	€ <sup>71.537</sup> , 3.374	0.067 - 0.155	0.014 - 0.063	
* DAT: days ** num *** num **** num Imidacloprid	after treatment ber of samples in total: 3 ber of samples in total: 5 ber of samples in total and Hydro @Metapolite:	27 LOQ	1.036 - 2.80 (1.537 - 3.374 0.005 or g/kg		0.0015/mg/kg	
Olefin-Metab	polite: S O	~ LQQ	= 0.010 mg/kgQ		).0003 mg/kg	
The sample	ed leaves avere not an	alysed.			J <sup>V</sup>	
××××××××××××××××××××××××××××××××××××××						
				ý		
	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*** after treatment ber of samples in total: 3 ber of samples in total: 3 ber of samples in total: 3 ber of samples in total: 4 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total: 5 ber of samples in total					



		_		
Report:	02.02.01/25;	; 2005; <u>M-451656-01-3</u>		e °
Title:	Residues of imidac	loprid WG 5 in blossom a	nd leaf samples o	of Amelanchier spatter
	soil treatment in the	e field - Application: 2003	, sampling: 2004	and 2005
Report No.:	<u>M-451656-01-3</u>		8	
Document No.:	<u>M-451656-01-3</u>		Ą	
Guideline(s):	none		O,	
Guideline deviation(s):	none		A	
GLP/GEP:	no	Č,	Å.	
< <m-451656-01-3@s-604676-01-1< td=""><td></td><td></td><td>Q.</td><td></td></m-451656-01-3@s-604676-01-1<>			Q.	

**Material and methods:** Shrubs of the species Amelanchier sp. growing at the market garden "Solders" (Elberfelderstr. 217, 42781 Haan) in Germany (Nord Hein-Westfalon), received soil treatment in autumn with Imidacloprid WG 5 in two replicates {A and B: 8 shrubs each) per freatment group.

Soil application with Imidacloprid WG 5 (active substance: NTN 33893, pority: 5,3%, material No.: 02295087, batch No.: PF000006PD) dissolved in water at an application volume of 1.5L 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 keight was 1.6 m.

No. of Treatment Group	Application Rate per Shrub
1	U untreated phtrol U Q O O Q
2	6.88 g a.s. Shrub = 129.8 g product/shrub
3 . Ø	3.44 g as /shrut 64.9 g product/shrub
$\sim$	

Blossom and leaf samples were collected once in pring 2004:

- blossoms af flowering an 2004 4-14 (166 days after application)
- leaves after forwering an 2004-05-04 (186 days after application)

The samples of blossons and caves vere stored at 18° Contil the sample preparation and eventually residue analysis for Initiaclopiid and its Otefin- and Hydroxy-Metabolites were carried out. Extraction, sample clean-up and determination of Imidaclopiid, Hydroxy- and Olefin-Metabolites were performed by HPLC-MS/MS.

Blossom and teaf samples were collected once in spring 2005:

• bossoms at flowering in 2008-04-212 (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 imid oloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms the sampled feaves 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

	mmarized.			Ő	
Sampled Material	Treatment Group	DAT*	lmidacloprid [mg/kg]	Hydroxy- Imidacloprid [mg/kg]	Olefin Omidac oprid
	1: untreated control	166	₹.od	Q <lod< td=""><td>SKLOB</td></lod<>	SKLOB
Blossoms	2: 6.88 g a.s./shrub = 129.8 g product/shrub	- 166	LOD Q		
	3: 3.44 g a.s./shrub = 64.9 g product/shrub	166%		<lob v LOD v LOD v LOD v LOD v LOD</lob 	٠ ٣ ٢ ٢
	1: untreated control	186		<lod c<="" td=""><td>PLOD /</td></lod>	PLOD /
Leaves	2: 6.88 g a.s./shrub = 129.8 g product/shrub	1186 0 <sup>9</sup> 186 9	<b>20.56</b> - <b>3.2</b>	0.086 - 0.07	0.018 0.15
	3: 3.44 g a.s./shrub ≠ 64.9 g product/shru€	ر 186 م		0.1100.45	0,014 - 0.16
	1: untreated control	540	2 400 00		<rp><rp>LOQ</rp></rp>
Blossoms	2: 6.88 g a.s./shrub 129.8 g product/shrub	2540 Q	0.70 - 4.56	9 0.22 1.34 	0.12 - 0.79
	3: 3.44 g/a.s./shrub = 64.9 g/product/shrub	5 <sup>40</sup>	0.66 - 2.84	0.19 - 0.60	0.15 - 0.53
midacloprid Diefin-Metab		analysed.	0.005 mg/kg 0.010 mg/kg	LOD = 0	.0015 mg/kg .003 mg/kg
vi-451656€¥90 <b>5-6</b>	leaves from 2005 were p				



Report:		; 2005; <u>M-451673-01-3</u>		@ °
Title:	Residues of imidacl	oprid WG 5 in blossom	samples of shrubs of	of different sizes of the 🖉
	species Rhododendr	on sp after drenching a	pplication in he field	of different sizes of the O d - Application 2004,
	Sampling: 2005		ð	
Report No.:	<u>M-451673-01-3</u>		Š	Le la la la la la la la la la la la la la
Document No.:	<u>M-451673-01-3</u>		102	
Guideline(s):	none			
Guideline deviation(s):	none	Ča		
GLP/GEP:	no	T.	<u> </u>	
< <m-451673-01-3@s-604679-01-1< th=""><th></th><th>L.</th><th>Ő¥ s</th><th></th></m-451673-01-3@s-604679-01-1<>		L.	Ő¥ s	
Material and methods				
Shrubs of the species R	hododendron sp. gr	owing may the area of	a markel@ard@n`ir	Rastede pear Bad

Shrubs of the species *Rhododendron* sp. growing on the area of a marke 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. Nice shrubs were used per replicate.

defined by application rate in two replicates per sub-freatment group. Nine shrubs were used per replicate. Soil application with Imidacloprid WG 5 (active substance: NPN 33893, purity: 5%, material No. 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 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-Treament	Application Rate per Shrub
		Suntreated controp
1/0.5 m	5 20 ×	2.15 g a.s./shob = 43 product/shrub
		د 1.075 g a.s./shrub = 21.5 g product/shrub
Ŏ,	\$ <sup>4</sup> 4	0.338 g.a@/shrub 10.75 @produc@shrub
		4 S a a s@back =\$86 a pro@ct/shack
201	, 0 <sup>2</sup> , 5 <sup>3</sup>	
2711		2.15 ga/s./shruts = 43 g product/shrub
		1.075 g a.s./shrub = 205 g product/shrub
~Q		
3/1.5	20	6.45 g a.s./shrub = 129 g product/shrub
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	3.225 g a.s.shrub = 64.5 g product/shrub
, «	29 4 A	1011 g as shrub = 32.25 g product/shrub
×		Y Q Q

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 4 and flow all plants of treatment group 3 no sampling of blossoms was possible as due to the cord winter all flower bads 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.



# **Imidacloprid Bee Studies Compilation of Study Summaries**

Issue date 2017-11-22

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).

00537/E001	(MR-568/99).			_	a a a a a a a a a a a a a a a a a a a	~ .~~
				L.	ć	S 5 0
The sample	d Rhododendron leaves an	nd the young	g shoots were	not analysed.		
		1 10 20 / 20		Ű	õ	
Dates of Di Dates of an	ological work: 2004 alytical work: 2005	+-10-28 to 20 5-06-22 to 20	)05-06-21 )05-67-13	, Ô¥	× ź	
Jates of an	alytical work. 2001	-00-22 10 20	103-94-13			¥ 6° s <sup>q</sup> ¥
<b>indings:</b> I	(MR-568/99). d <i>Rhododendron</i> leaves at blogical work: 2004 alytical work: 2005 n the following table the r ts are summarised. Sampl	results of the	wesidue analy	ses of blossor	n samples an	d sample of
oung shoo	ts are summarised. Sampl	ed leavesewe	ere not analys			
		0 <sup>v</sup>			L	
Treatment	Sub-Treatment	× Ann >	Imidacioprid	WHydroxy-	Olefin Imidacloprid	
Group/ Plant Size			[mg/kg]	(mg/kg)	[r0g/kg]	
	2 (4.3 g a.s./m shrub size)	5 23 16	0,108-0,512		Se < LOQ	.0
1 / 0.5 m, blossoms	3 (2.15 g a.s./m shrub size)*	- 216		0.007 0.032	< 003 >	
	4 (1.075 g a.s./m shruf size)*	210	0.017-0.24	< LOQ - 0.018		1
	1 (Control) 🦏	216	, <rboa< td=""><td>√ &lt; L002 ,</td><td>\$ <lqq< td=""><td>]</td></lqq<></td></rboa<>	√ < L002 ,	\$ <lqq< td=""><td>]</td></lqq<>	]
2/1 m,	2 (4.3 g a.s./m shrub size)	\$ 216 \$	0.017 - 0.121	< LOQ - 0.014	~ LOO	
blossoms	3 (2.15 g a.s./m shoub size)	P 296	0031-0,205	≤b0Q -<0.022		
	4 (1.075 g a.s./m shrub size	) ~ 216 ~	0.013 0.105	< LOQ - 0.013	× 100	
	1 (Control) 2 (4.3 g a.s./m shrub size) 3 (2.15 g a.s./m shrub size) 4 (1.07 g a.s./m shrub size) 4 (1.07 g a.s./m shrub size) and Hydroxy-Metabolite: and Hydroxy-Metabolite: 5 604679-01:1 5 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7					
$\bigcirc$	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~					
	on the hyperlink to order					



<b>Report:</b> Title:	Assessment of effe	mblebees after drench	G 70 on foraging ing application un	activity and mortality of or der field conditions on shrups
	of the species Rhoo	dodendron catawbiens	se grandiflorum su	rrounded by other prnamental
	plant species		-	S A S
Report No.:	M-451677-01-3		la la la la la la la la la la la la la l	
Document No.:	M-451677-01-3		A	Ô <sup>y</sup> 2 <sup>st</sup> 2
Guideline(s):	none	(Pa	"Ś"	
Guideline deviation(s):	none		Ű <sup>Ŷ</sup>	
GLP/GEP:	no	Ŷ	R	
< <m-451677-01-3@s-604680-01-1< th=""><th></th><th>Ű,</th><th>L'</th><th></th></m-451677-01-3@s-604680-01-1<>		Ű,	L'	
Material and methods		A	R &	
Material and methods	8.			

Shrubs of the species Rhododendron catawbiense grandiflorum located at the experimental farmland "Laacher Hof" near Monheim (40789 Monheim, Nordchein, Westfalen, Gesnany) received soil freatment with Imidacloprid WG 70 dissolved in water at an application volume of L per shrubs 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 (P10 Rhododendron plants.

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Table 1:	Summary:	Treatment Groups	and Rates
	••••••		

	Ň		~		Ŭ.	õ S	, K
Treatment group		L &		200 5			× v
	NY &	×- 28 4	.3 g a.s./	n plant(size)	• 2.15	5 ga.s./m pla	ant size*
Application rates	× 1	control	2.58,9	a.s.(shrub «		1.29 g s.s./ s	
*		ç o :	= 3668 g p	roduct/shrul		1.84 produc	t/shrub
Water volume rate p	er plant		5 5	1 L tap w	ater	<i>∽</i>	
* plants were 0.6 m	ligh/wide			S S	1	1	

Ň

Between the rows of Rhododendron catawbiense grand florund a mixfure 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: Bichsia sp.: variety "Beacon", strawberry plant: variety "Fragoo", Alyssum sp., Lantana camara and Loberta sp. In the near surroundings of the study site no other powering crops were located.

One hive colony of honey bees Apis mellifera and 2 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 of for aging 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 ornarhentals. The nortality of honey bees and bumblebees was assessed in front of the hives/colonies and on liner sheets and out between the Rhododendron rows.

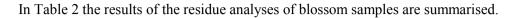
Blossom samples were collected from 15 Rhododendron plants per treatment group on 2005-05-19 (126 days after the application) and stored at 48°C until the sample preparation and eventually residue analysis for imidaeloprid and its Olefin- and Hydroxy-Metabolites were carried out on the blossoms. Extraction, sample clean-up and determination of Imidaeloprid, Hydroxy- and Olefin-Metabolites by HPLC MS/MS were performed according to method 00537/E001 (MR-568/99).

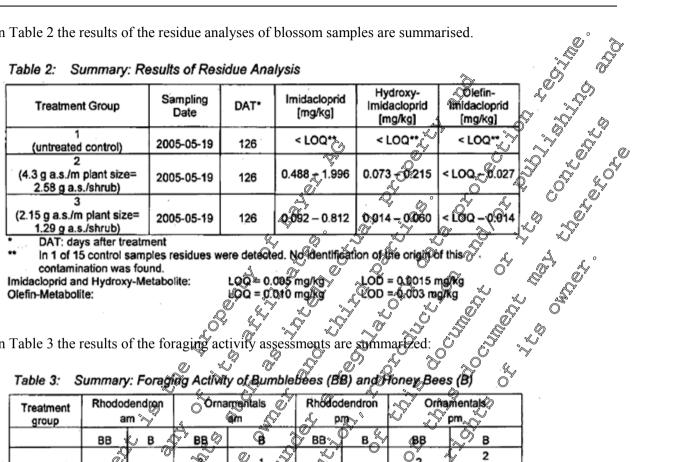
 Dates of biological work:
 2005-01-13 to 2005-06-02

 Dates of analytical work:
 2005-06-21 to 2005-07-13

**Findings:** 







In Table 3 the results of the foraging activity assessments are

Table 3: S			ag Activit	y of Bumble			foney Bees	(B) O
Treatment group	Rhododendron am ~~		am 😽 🖌 🐲 🐼		S pm V			mentals2 pm
	BB	⇔в⋌	<sup>→</sup> в <u>в</u> 🍳	<b>Q</b> (	Ø BB∿~	Р′в 🖑	<b>Ø</b> ₿₿	⊳У в
Control	1200 1200			0 1 5 (strawberry)		Q Q	2 ((Fuchsig)	2 (strawberry, Lobelia sp.)
2.15 g a.s./m plant size	0 72		1 (Fuchela)	400 ×	59		(Fuchsia)	1 (strawberry)
4.3 g a,s.lon plant size	70			) <b>0</b> 0	63	@0.	Ø O	1 ( <i>Lobelia</i> sp.)
	a		v - 🖓 -	§ 6		° O		

Ø1

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 around bees 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 afternoor ő

Throughout the study only one honey bee was observed foraging on a Rhododendron plant (control). In none of the other treatment group 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 crurning to the hive carried yellow pollen, which probably originated from plants other than the genamentals set, up in this study. However, in the near surrounding of the study site no other flowering crops were located.

No deachoneg bees worker bees or bumblebees were found throughout the study on the individually labelled linger sheets laid but between the Rhododendron catawbiense grandiflorum rows and the rows of the surrounding potted of namental 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 • plants (Fuchsia sp., strawberry plant, Alyssum sp., Lantana camara and Lobelia sp. The Rhododens on 🖉 catawbiense grandiflorum plants had received a soil drench treatment 126 days before the start of the m 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 gas. /m plant size (1.29 g a.s./ shrub = 1.84 product/shrub) resulting in residues in blossoms up to 0.842 mg imidacloprid/kg.

Untreated Rhododendron catawbiense grandiflorum plants vere visited profer frequently by the bumblebees than the treated ones, but frequency of visits was within a comparable offer or magnified between the sets of Rhododendron treated at differendrites. Alternative ornamental plants vere existed only every scarcely. Ś



Report:	02.02.01/28; ; ; 20	007: M-451681-01-3		٥
Title:	Assessment of effects	of a drench application	of imidacloprid	WG 70 to shrubs
	Rhododendron sp and	to Hibiscus syriacus or	n foraging activity	and mortality of honey
	bees and bumblebees u	inder field conditions		
Report No.:	<u>M-451681-01-3</u>		- C	
Document No.:	M-451681-01-3		Ø.	
Guideline(s):	none		A	
Guideline deviation(s):	none	Ča	s, '	
GLP/GEP:	no		Û <sup>Y</sup>	
< <m-451681-01-3@s-604681-01-1< th=""><th></th><th>,ſ</th><th>Å.</th><th></th></m-451681-01-3@s-604681-01-1<>		,ſ	Å.	
Material and methods		a O Y	Å .	

### ial 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 Hibjseus syrfacus located at the area of Bayer CropScience AG (40789 Monheim, Nordrhein-Westralen, Germany) received soft treatment with Imidacloprid WG 70 dissolved in water at an application volume of 2 L per skrub on 2006-04-12 at the application rates given In Table 1 (treatment groups 2 and 4). Control shrubs (treatment groups 1 and 3), Table 1: Summary: Treatment Groups and Rates

	-			~
Tabla 1.	Summon	Troatmont	Groupsand	Potoc
rable i.	Summary.	rieaunem	VCN UUUS allu	Nalas

Treatment group	A <sup>A</sup> (,		20 <sup>4</sup> . 3 Q	0 4 0 4
Treatment name	Rhododendron, ontreated	Binododendron, treated	Hibiscus, untreated	Hibiscus,
Application rates			0 <sup>4</sup> - <sup>4</sup> / <sub>2</sub>	. 04.3 g a.s./m average
		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 Rhododeration strubs the parameter shrub width was used for fixing the application rate For Hibiscus the parameter shrub height was used for fixing the application rate. à ď ×, O

Each treatment group consister of 3 parallel rows of 6 shrubs each, Rhododendron and Hibiscus respectively. At the exterior sides of the 2-suter rows with Rhododendron sp. and Hibiscus syriacus a mixture of bee-attractive potted smamentals 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 should rows further ornamental plants Pelargonium sp. and Surfinia sp.) were set up in flower boxes on the linen speets with which the ground around the rows was covered. Ornamental species composition for the Rhododen for part Fraga a sp. fulmonaria officinalis, Fuchsia sp. hybrids, Centaures montana, Lobelia equius 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 Hibiscos part: Lavendula augustifolia, Calluna vulgaris, Centaurea montana, Phacelia tanacetifolia, Lobelia erinus, Helianthus sp. And Fragaria sp. Near the control plot (treatment group) Marweed was growing on a field and next to the treatment plot (treatment group 4) Nowering Gladiolus (not attractive for honeybees), Snapdragons and Larkspur (approx. 20% open blossoms, minimally bee attractive) were present during the study period.

In approx. 2025 m distance to each plot 1 beehive (consisting of 11 combs at the start of the study and containing approx. T0,000 honeybees and a queen) was located. Two colonies of bumblebees (Bombus terrestrist per study part were placed next to each plot at the beginning of shrub flowering. Honeybees and build below 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. n 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 sheab rows. Blossom samples were collected from Q 18 treated and 9 untreated plants during flowering of the respective should species. For Rhododendron this was conducted 35 days after the application and for Hipsiscus 106-117 days after the appleation Samples were stored at -18°C until the sample preparation and eventually residue analysis for Imidaclopfid and its Olefin- and Hydroxy-Metabolites were carried on on the blossoms. Extraction, sample clean up and determination of Imidacloprid, Hydroxy-and Qlefin-Metabolites by HPLC-NPS/MSwere 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 bumble bee colonies were also subjected to residue analysis for residues of Imidacloprid and its Olefin- and Aydroxy-Metabolites. Extraction and determination of Imidacloprid, Hydrox and Olefin-Metabolites by HPLC-MS/MS was performed according to method 00537/M002 (MR-6/144).

### **Findings:**

in Rhodod@dron and Hibiscus are In the Tables 2 and 3 the results of the foraging activity asses smen summarised.

Table 2:	Summary:	Foraging	Activity	of Hoi	neybees ×	and Bumblebees	on
Rhododer	ndron				o y		

		er species observed per t	olot [n]
	Koneybees	సి 🖉 🖓 Bumb	lebees
	Rhododendron Omer	entats Rhododendron	Ornamentals
ې 1: Control	23 ~ 0 6	4 608	238
2: Treatment	\$ 10 \$ 1	4 107	87
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	

Only few honeybees were observed foraging of Rhododencon shrubs on the control and treatment plot respectively, but more on the sontrol man on the treatment plots.

Ô Foraging activity of hone bees on the surrounding or mentals 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 ornaption tal plants on the treated plot were likewise significantly less visited than those on the control plot.

#### Summary: Foraging Activity of Honeybees and Bumblebees on Hibiscus Table 3:

	Total	number per speci	es observed pe	er plot [n]
	Hone	eybees	Bum	blebees
Totalment group	Hibiscus	Ornamentais	Hibiscus	Ornamentals
Control	10	192	233	837
60 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 with the m surrounding ornamentals was slightly higher on the control than on the treated post. 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, nother its 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 Hibiseus part.

a.

Table 4: Summary: Mortality of Honeybee
-----------------------------------------

	Rhodo	dendron S		Bous 📈 🗸
	. 0*	K Fotal na	mber 🕅 🔗	4 A
Treatment group	on the plot	In front of hive	on the glot	in from of hive
Control		× 0 2 4	A A A	
Treatment	8 2 4 V	\$ <b>35</b>	, Ç0 §	<u></u>

		Å	1 0	~~~~~
Table Fr	Summary:			
l adie 5:	Summary:	мопашу		ndiedees

	0		$Q' \qquad S'$	<u> </u>
Ś.	- Roodod			scus
. Q		Total nu		? ?
Treatment group		indront of hive	on the plot	in front of hive
Control			0 x 1	0
Treatment		<u></u>	L 12	2
	Y & Y	A N L	ý <sub>s</sub> ý	

Colony health and condition of the honeybee colonies was not different before and after the study, neither

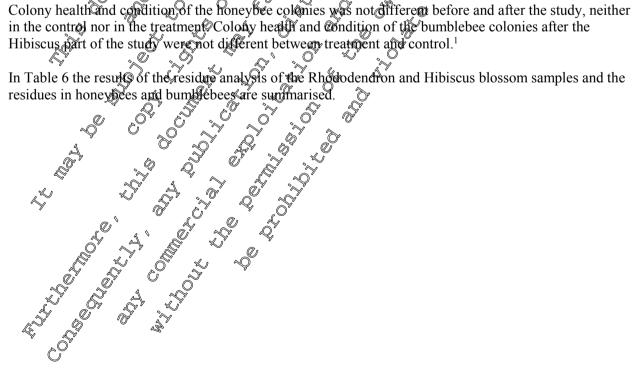




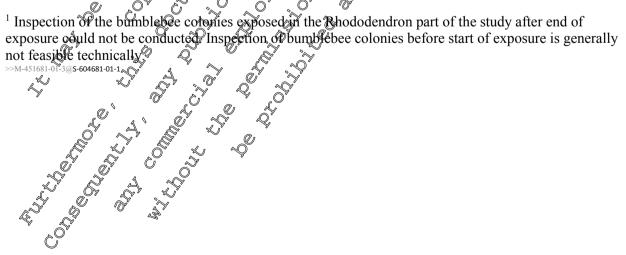
Table 6:	Summary:	Results	of Residue A	nalysis			0
Treatment Group	Sample description	Study part	Sampling Date	DAT*	Imidacloprid [mg/kg]	Hydroxy- Imidacloprid @mg/kg]	Oletin- Imidaclopric (mg/kg)
1: Control	blossoms	Rh	2006-05-17	35	<loq< td=""><td>~LOQ</td><td>&lt; 1000</td></loq<>	~LOQ	< 1000
2: Treatment	blossoms	Rh	2006-05-17	35	0.09 - 0,79	0.01 - 0.04	< LOQ - 6.01
3: Control	blossoms	н	2006-07-27	106	< 1000	<1,00	< 00 0
4: Treatment	blossoms	н	2006-07-27 to 2006-08-07	106 - 117	0.78 - 5001	< 00 - 0.45	<loq -0.33<="" td=""></loq>
1: Control	2 honey- bees (colony)	Rh	2006-05-29	A7	0,022	< LOQ	0.001 -
	25 honey- bees (colony)	Rh	2006-05-21 to 2006-05-31	39 - 649	0.016	<2LOO	€ LOQ - 0.001
2:	2 honey- bees (plot)	Rh	2006-05-21 to 2006-05-31	<b>39</b> - 49	0.002 - `~	< 100 - 2 0.018 5	< 1000 - 0.001
Treatment	bee (colony)	Rh	<, 2006-05-29 °		~ 0.000 <sup>0</sup>	5 <sup>50</sup> 0.059	0.005
	1 bumble- bee (plot)	Rh©	2006-05-01	27 49 U	6.005	~ <del>0</del> .003 %	0.003
4:	2 bumble- bees (colony)	H C	2006-07-26	1@5	√0.003,¥ 0;©©4	0.001 – 0.003	0.004 – 0.009
Treatment	12 bumble- bees (plot)	× H	2006-07-25 to 2006-08-08	¢04 - 168	0.077- 1.663	0.009 - 0.196	0.031 - 0.405

#### --------

DAT: days after reatment Rhododendron Rh OH *√* Hibiscus Blossom samples: Imidaclopric Hydroxy-Metabolite Olefin Metabolite: LOQ = 0.010 mg/kg Insect samples: Imidacloprid, Hydroxy-Metabolite, Oletin-MetaboliteC LOQ = 0.001 mg/kg

Residue levels in the Rhode dendron blossoms were 0.09 - 0.79 mg in dacloprid/kg and in Hibiscus blossoms Q 76 - 5.01 mg/midaet prid g in the treated replicates ho the blossoms of the untreated plants, residue tevels were < LOQ. Residue tevels found in dead høneybees 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 ms midacloprid kg. Residue levels found in dead bumblebees in the treatment groups were 0.001 -1.669 mg inidacloprid/kg Ś No No Ô

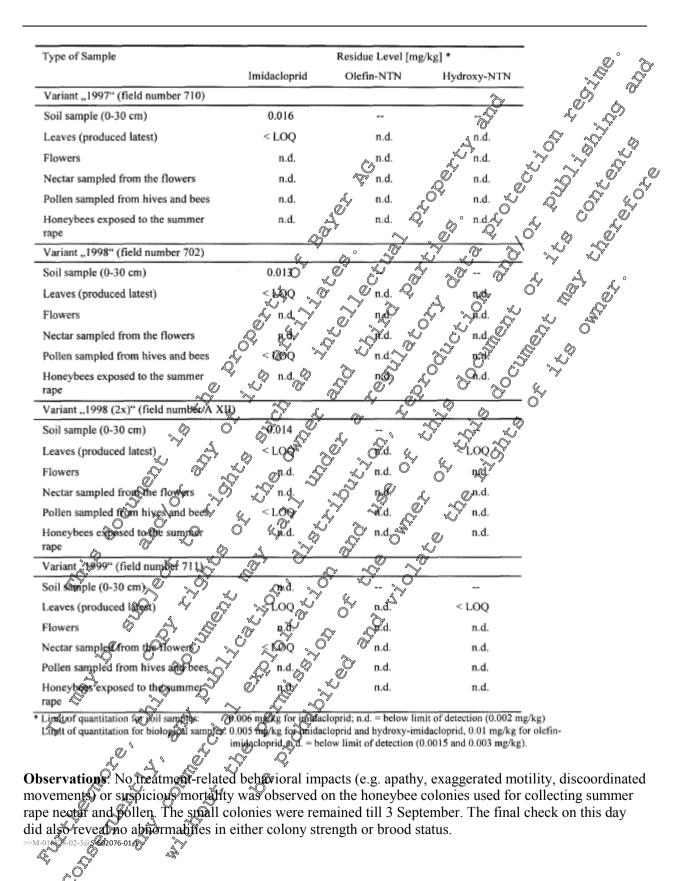
<sup>1</sup> Inspection of the bumblebee cotonies exposed in the Bhododendron part of the study after end of



Report:	02.02.01/29;		007; <u>M-016828-02-3</u>
Title:	Residue levels of imidacloprid and im		
	pollen of summer rape cultivated on s effects of these residues on foraging h		
Report No.:	SXR/AM 008		
Document No.:	<u>M-016828-02-3</u>	O <sup>v</sup>	
Guideline(s):	850.3040	4	
Guideline deviation(s):	none	, "Ľ	
<b>GLP/GEP:</b>	yes 🖓		1999
< <m-016828-02-3@s-602076-01-1< td=""><td>L</td><td>, Ô¥</td><td></td></m-016828-02-3@s-602076-01-1<>	L	, Ô¥	
Material and methods	: summer rape seed (variety	ne") either dressed w	
500 (a.i. content: 79.7 §	/L beta-Cyfluthrin and 427 A g/L in	idacloprid; batch no	62000055*A according
to formulation no. 6200	0/0059, developmental no. 00195939	) or imidae loprid-fre	e were drikled on 2 May
99 in soils with differen	nt imidacloprid residue vels. Soil i	esidue levels were ar	adjutically determined
immediately before dri	lling. Drilling rate was 3.25 kg/ha. K	uring peak flowering	of the summer rape (mid
of July) small bee color	nies (2,000 to 3,000 honeybees) wer	e caged on these plot	s (appr. 50 $\mathfrak{A}^{2}$ ) as $\mathfrak{A}^{2}$
	nmer rape nectar and pollen. In addi		
	s used as samplers were observed fo	rysigns of behavioral	Onpacts, All samples and
	ybees were subjected to a residue an	alysis for inneaclopr	id and its relevant
metabolites.			õ v
Datas of hislasiaal wa	rk: July 122 19,4 August 8 – 13 ological samples September 25 oil, in summer rape plant matrices		and and its relevant
Dates of poil analysis:	rk: July 12= 19,4	1000 °	° °
Dates of soil allalysis.	ological samples September 25	20 100	
Dates of allarysis of Di	September 23		ġ.
Findings. Residues in	oil, in summer rape plant matrices	) Jant <i>e</i> d as succeeding	
used as sampling de	e. (detects above the LOQ are highli	ohted).	erop and in noneybees
used us sampling deter		$\mathbf{D}^{1}$	
Type of Sample		Residue Level [mg/kg	] *
la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di			] * Hydroxy-NTN
la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di la di		Residue Level [mg/kg	
Control Plot (field num)	or 71128	Residue Level [mg/kg	
Control Plot (field num Soil sample (0-30 cm)	<u>کور ۲۱۱) کې کې کې کې کې کې کې کې کې کې کې کې کې </u>	Residue Level [mg/kg Olefin ATN	Hydroxy-NTN
Control Plot (field num Soil sample (0-30 cm) Leaves (produced latest	Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top <thtop< th=""> <thtop< th=""> <thtop< th=""></thtop<></thtop<></thtop<>	Residue Level [mg/kg Olefin MTN  n.d.	Hydroxy-NTN  n.d.
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Control Plot (field num Soil sample (0-30 cm) Leaves (produced latest	Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top <thtop< th=""> <thtop< th=""> <thtop< th=""></thtop<></thtop<></thtop<>	Residue Level [mg/kg Olefin MTN  n.d.	Hydroxy-NTN  n.d.
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Control Plot (field num Soil sample (0-30 cm) Leaves (produced latest	Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top <thtop< th=""> <thtop< th=""> <thtop< th=""></thtop<></thtop<></thtop<>	Residue Level [mg/kg Olefin MTN  n.d.	Hydroxy-NTN  n.d.
Control Plot (field num Soil sample (0-30 cm) Leaves (produced latest	Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top <thtop< th=""> <thtop< th=""> <thtop< th=""></thtop<></thtop<></thtop<>	Residue Level [mg/kg Olefin MTN  n.d.	Hydroxy-NTN  n.d.
Control Plot (field num Soil sample (0-30 cm) Leaves (produced latest	Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top <thtop< th=""> <thtop< th=""> <thtop< th=""></thtop<></thtop<></thtop<>	Residue Level [mg/kg Olefin MTN  n.d.	Hydroxy-NTN  n.d.
Control Plot (field num Soil sample (0-30 cm) Leaves (produced latest	Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top <thtop< th=""> <thtop< th=""> <thtop< th=""></thtop<></thtop<></thtop<>	Residue Level [mg/kg Olefin MTN  n.d.	Hydroxy-NTN  n.d.
Control Plot (field num Soil sample (0-30 cm) Leaves (produced latest	Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top <thtop< th=""> <thtop< th=""> <thtop< th=""></thtop<></thtop<></thtop<>	Residue Level [mg/kg Olefin MTN  n.d.	Hydroxy-NTN  n.d.
Control Plot (field num Soil sample (0-30 cm) Leaves (produced latest	e flowers fride from the summer rape	Residue Level [mg/kg Olefin MTN  n.d.	Hydroxy-NTN  n.d.
Control Plot (field num Soil sample (0-30 cm) Leaves (produced latest	Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top         Top <thtop< th=""> <thtop< th=""> <thtop< th=""></thtop<></thtop<></thtop<>	Residue Level [mg/kg Olefin MTN  n.d.	Hydroxy-NTN  n.d.



### Imidacloprid Bee Studies Compilation of Study Summaries





Report:	02.02.01/30;		: 2007	7; <u>M-016842-02-3</u> _ ∘
Title:	Residue levels of i	midacloprid and imida	cloprid metabolites i	n nectar, blossoms and 🖉
				cloprid residue levels and
Report No.:	effects of these res SXR/AM 010	idue on foraging honey	bees. 'Hoefchen' 19	<sup>19</sup> 5
Document No.:	M-016842-02-3		Ĩ.	
Guideline(s):		Giuideline Number: 850	).SUPP	
Guideline deviation(s):	none	Ô	L.	
GLP/GEP:	yes	- The second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second sec	iQ,	
< <m-016842-02-3@s-604919-01-1< td=""><td></td><td></td><td></td><td></td></m-016842-02-3@s-604919-01-1<>				
Material and methods 500 (a.j. content: 70.7 g	: summer rape see	a (variety veisonne)	) either dressed wh	62060055* A according
to formulation no 6200	/0059 development	$\alpha$ and $42 \%$ g/L milda ntal no $90195939$ $\alpha$	rimidar loprid-free	were drilled on May
99 in soils with differen	t imidacloprid resi	due fevels. Soil sam	bles for an analytic	a determination of the
imidacloprid residue lev	vel were taken imn	nediately before drill	ing@Drilling rate	as 7 kg/ha. During peak neybees) were caged on
flowering of the summe	r rape (mid of July	fsmall bee colonies	(2,000 to 3,000 ho	neybees) were caged on
these plots (appr. 50 m <sup>2</sup>	<sup>2</sup> ) as a sampling <b>d</b> e	vice for summer rap	e nector and pollen	In addition, some
nectar and flowers were	e sampled by hand.	The honeybees used	l as samplers were	observed for signs of
imidacloprid and its rele		all sample of noneyo	ees were subjected	to aresidue analysis for
initiaciopita ana its tere				
Dates of biological wor	rk: Jut 12-19,19	99. 8		to accesidue analysis for
Dates of soil analysis:	August 9-1& 1999		4 : 4 . 9	
Dates of analysis of bio	ological samples	August 27 - Septemb	per 21, 1999	L.
			6 × 6	<i>v</i>
Findings: Residues in S	soil, in summer rap	e plant matrices plan	ited as succeeding	crop and in honeybees
used as sampling de de de de de de de de de de de de de	e. (detects above a			
	V 4 4	N N N		
Type of Sample			Residue Level [mg	/kg] *
		midacloprid	Olefin-NTN	Hydroxy-NTN
Control Plot (south of	field namber 502)	~	<sup>°</sup> O <sub>A</sub>	
Soil sample (0-30 gm)		) ( n.& +	<u>з</u> у	
Leaves (produce) lates		, 🖉 _ n.d. 🖉	n.d.	n.d.
Flowers		O Ond O	n.d.	n.d.
		Y AY AY		
Nactor compled from t			nd	nd
Nectar sampled from the	he Otower of A		n.d.	n.d.
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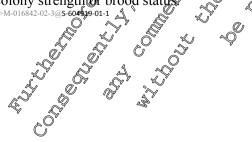
## **Imidacloprid Bee Studies Compilation of Study Summaries**

Issue date 2017-11-22

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Limit of quastration for biological samples: 0.995 mg/ky for initiation and hydroxy-imidacloprid, 0.01 mg/kg for olefin-pridacloprid, 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 ar study termination did also not reveal any abnormality in either colony strength or brood status





Report:		02.02.01/31; 2011; <u>M-401652-01-2</u>	
Title:		02.02.01/31; 2011; M-401652-01-2 Determination of residues of imidacloprid OD 200 and its metabolites applied viewfirip irrigation in watermelon in the semi-field in Spain in 2009 S09-00075 M-401652-01-2 IVA (1992), EU (1999) none yes imidacloprid OD 200 A G analyzed content of active incredient: 19.6 % w/s ngredients: Imidacloprid (NTN 33893) Batch: 2008-009969 s designed to determine the tesidues of Infodacloprid OD 200 in bee-refevant following an application by drip urigation in the semi-field in Spain dy encompassed the objectives of Commission Directive 96/68/EC amending 4/EEC concerning the placing of plant protection products on the market, Oct.21, Working Document 1607/VI/97 Rev. 2. General Recommendations for the Realisation of Residue Trials July 22 (1999) and the TVAS Leithine – üfungen an Pflanzen, Teil 1 A and 1B, Industrieverband Agrar.e. V.,	
Report No.:		S09-00075	
Document No.:		M-401652-01-2	
Guideline(s):		IVA (1992), EU (1999)	
Guideline deviati	ion(s):	none A O A Q	
GLP/GEP:		yes	
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Material and m	ethods		
Test item:	Name: I	midacloprid OD 200 A G malyzed content of active ingredient: 19.6 % w/	
	Active i	ngredients: Imidacloprid (NTN 33893) Batch 2008-009969	
The following s	study was	s designed to determine the residues of Infidacloprid OD 200 in bee-refevant, °	
matrices of wat	ermelon	following an application by drip urigation in the semt field in Spain	
This GLP comp	oliant stu	dy encompassed the objectives of Commission Directive 26/68/EC amending	
Council Directi	ve 91/41	4/EEC concerning the placing of plant protection products on the market, Oct.21,	
1996 and "Com	mission	Working Document 1607/91/97 Rev. 2. General Recommendations for the	
Design, Prepara	ation and	Realisation of Residue Trials July 22 1999 and the IVA Leithine -	
Rückstandsvers	suche, Pr	üfungen an Pflanzen, Teil 1 Agind 1 B?, Indostrieverband Ograr e. V.,	
Frankfurt/Main	, 1992.		
Particular attent	tion was	directed to the restaues in young plants, flowers, pollen and nectar.	
The study comp	orised on	e tral which was carried out in watermelon in Spain Commercially grown young	
plants were pur	chased a	nd then transplanted into the field, of 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 pe	erformed	once via drip invigation at the growth stage of BBCH 15 (after transplanting),	
corresponding t	to <b>20</b> 0 g i	indidacloprid a. S./ha. The control group remained infidacloprid-untreated.	
6	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
Before the wat	rmelons	approached their Nowering period, gapecovered tunnels of approx. 5 m x 50 m	
surface area we	re set-up	in the respective water melon fields. The test tem treatment group comprised 3	
replicates (trunn	els), the	control group comprised 1 replicate (tunnel).	
Thereafter, sma	ıll honey	bee colonies were placed in the tunnels as soon as enough flowers were present	
to allow foragir	ng of the	bees. The boney bees were used as a sampling device for nectar and pollen.	
Samples of you	ing water	melon plants were taken at the time of transplanting from the greenhouse to the	
field. Additiona	ılly, flow	ers from the plants as well as freshly collected nectar/honey and freshly collected	
pollen from cor	nbs was	sampled at several dates, starting at beginning of flowering and continuing during	
flowering perío	d of the	crop. The colleged samples were immediately stored in dry-ice in the field and	
kept deep froze	en thereas	fter, to be analysed for potential residues of the test item.	
Dates of work:	: 30 Apri	1 2009 to 26 December 2009	
¥	<b>\</b>		
Findings (Resi	due Ana	1 2009 to 26 December 2009	
$\bigcirc$ <sup>2</sup>			
		den TN 33893 and its metabolites imidacloprid-5-hydroxy and imidacloprid-	
		High Performance Liquid Chromatography coupled with tandem mass	
		S/MS). The Limit of Quantification (LOQ) for imidacloprid, imidacloprid-5-	
hydroxy and m	nidactopr	id offin, defined as the lowest validated fortification level, was 0.001 mg/kg and	
		EOD) was 0.0003 mg/kg, respectively.	
		s of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefin were found in	
	ig watern	nelon plant, pollen and honey/nectar samples (i.e. residues were always below the	
LOQ).			
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Please Click on	те пуре	rlink to order a Study Report.	



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-5hydroxy ranged between <LOQ and 0.0108 mg/kg. Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefin in control samples always below the LOO. Conclusions No quantifiable residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-oletin have revealed in young watermelon plants immediately before pansplanting to the designated treatment for before test item application) and control field plots. Residues of imidacloprid in flowers ranged between \$20017 mg/kg to 0.0460 mg/kg. Residues of imidacloprid-olefin in flowers ranged between <LOQ to 0.0041 mg/kg and residues of unidacloprid hydroxy ranged between <LOQ and 0.0108 mg/kg The study revealed no quantifiable residues of imidacloprid, indicatorid-5-flydrow and imidaclopridolefin in pollen and honey/ nectar, collected from howey bee colonies, exposed under confined conditions to flowering watermelon plants, which have been meated with Ingdacloprid OD 200 vor drip origation at BBCH 15, at a rate corresponding to 1 x 200 g invidacloprid as/ha. >>M-401652-01-2@S-602564-01-1 02.02.01/32 2011; <u>M-404577-01</u> Determination of the residues of Omidadoprid and its metabolities 5-hydroxy **Report:** Title: imidacloprid and imidacloprid plefin if bee recevant matrices collected from cotton, grown at locations related with imidaclopric at leastonce per year during two successive vears EBNTL056-01 Report No.: M-404577-01-3 Document No .: US A Rec OPPTS 850 SUPP (Èrologia Effects) Guideline(s): The field and sampling phase of this study were not conducted to meet the requirements Guideline deviation(s of EPA Good Laboratory Practice Standards (40 SFR part 160; FR, August 17, 1989). The antivical phase of this study was conducted to meet GLP standards. The preparation of the field Ortification samples was not conducted under GLP but their analyses met GEP standards. GLP/GEP: 🖏 ves ) DS-604667-01-1 <<M-40457 Five trials were conducted in clay soils classified as 'heavy' to determine the residues of imidacloprid and its metabolites (5-b) droxy imidacioprid and invidacloprid olefin) in nectar and leaves collected from

cotton plants grown at locations treated with imidacloprid or 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 or ay application of imidacloprid (Provado 1.6 F, 17.4% imitacloprid by weight) in 2010 during flowering (BBCH61, beginning of flowering to BBCH67, flowering finished, majority of flowers faded).

Composite samples of control nector and cotton reaves 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 indidacloprid, 5 hydroxy imidacloprid, and imidacloprid olefin were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable isotopically beled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

The linkits of quantitation (LOQs) are shown below.



# Imidacloprid Bee Studies Compilation of Study Summaries

Issue date 2017-11-22

Matrix	Analyte	LOQ (ppm)		
Cotton Nectar	Imidacloprid	0.001		<i>a</i> .°
	5-hydroxy imidacloprid	0.001		
	Imidacloprid olefin	0.001		N N
	Total Imidacloprid	0.001	ð	
Cotton Leaves	Imidacloprid	0.002	<sup>A</sup>	
	5-hydroxy imidacloprid	0.002	.4	
	Imidacloprid olefin	0.002	s de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de la companya de l	
	Total Imidacloprid	0.002	<i>Ó</i> <sup>4</sup>	

Transit stability samples (control nectar and leaves for fifed with initiacloprid, 5-bydrox amidaoloprid, and imidacloprid olefin) monitored the stability of the analytes during sampling transit, and storage. The average recovery of all analytes in these samples anged from 72% to 95%, demonstrating that residues were stable under the practices used in this study. The maximum storage period of prozen 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 Implacion of in Nectar and Leaves from Cotton Grown in Heavy Soils.

			Ś	° C	4		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Ř	S.	K)
				6		Imidacion	orid Resid	lu@_evel:	s (ppm) d	$\sim$
Commodity	Heavy Heavy Heavy Heavy Heavy Heavy Heavy	(pplication Rate b aitĂ (oz FP/A)					st ge/Site		l de la	Standard
Cotton Nectar	Heavy Soil	Ø NA	Pre-App (-7 Ø)-2 DA	01 10	0.0012	0.004,3	0.0042	0.0027	0.0028	0.0010
Cotton Nectar	Heavy	0.083 (5.0)	(Post-App (6 DAT)		0.013	0.066	9.056 (	90.021	0.029	0.018
Cotton Leaves	HQivy Soil			T) 10	0.0035	0.025	0.093	0.014	0.014	0.0078
Cotton Leaver	Hoavy	0,063	ost-A00 (6 DAT)	10	6015 	2 <sup>91.9</sup>	17	0.36	0.77	0.67

a Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natura Resources Conservation Service. "Pleavy" class represents soil with slow draininage capacity.

draininge capacity. b Although all plots had received applications of midicloprid 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 postapplication sample intervals.

d Abbreviations used are as follows: Maris 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 freated residue values; Standard Deviation is the standard deviation for a small population of n samples.

✓ Nor Applicable.



flui         gro         Report No.:       S11         Document No.:       M-         Guideline(s):       EU         Guideline(s):       EU         Guideline deviation(s):       nor         GLP/GEP:       yes          SA         Material and methods       Test item: Pella site Name:         Ingredient:       I         Batch:       EA 3         Larissa site Name:       Cotton p         Imidaclopri       10 27         The field study was conduct       01) and a second trial in Gla         The purpose of the study was       floral nectar in cotton plants         Carmen) were pre-treated w       site) and on 13MA\$ 2011 Jf         10416m²       Pella site) and 100         After emergence of the crop       Sampling of pollen, dectar a         BBCH stages       (Meier 2001),         Dates of work:       01ADG20I         21OCT2011 (end of residue         Findings (Residue Analysi         Residues of imidacloprid vares         residues vere <loq (i.e.="" <="" td="">         &lt;1 - 5 µc/kg).         Residues of imidacloprid vares         mean residue level of all neu         In extrafloral rectar sample     <th>dacloprid - Determination of residues of imidacloprid in pollen, extrafloral netar ds and nectar of cotton plants grown from imidacloprid-treated seeds in two cotton -02885 <u>124399-01-3</u> 1999: 1607/VI/97 NCO/3029/99 rev. 4 ective 2010/21/EU EPA OCSPP Guideline Number: 850.SUPP e Cotton plants grown from seeds treated with bridacloprid FS 350 Active midacloprid Adalyzed content of active ingredient: 484.67 g/100 kg seeds 85 11 10 23 ants grown from seeds treated with Imfacloprid FS 350 Active ingredient: d Analyzed content of active ingredient: 555 97 g/400 kg Batch: EA 385 11 ed of Greece, one trial in Giannitsa in the vicinity of Pella trial S11-02885- thi in the vicinity of Jearissa (trial SLI-02885-02) s the determination of residues of imidacloprid in poten, nectar and extra</th></loq>	dacloprid - Determination of residues of imidacloprid in pollen, extrafloral netar ds and nectar of cotton plants grown from imidacloprid-treated seeds in two cotton -02885 <u>124399-01-3</u> 1999: 1607/VI/97 NCO/3029/99 rev. 4 ective 2010/21/EU EPA OCSPP Guideline Number: 850.SUPP e Cotton plants grown from seeds treated with bridacloprid FS 350 Active midacloprid Adalyzed content of active ingredient: 484.67 g/100 kg seeds 85 11 10 23 ants grown from seeds treated with Imfacloprid FS 350 Active ingredient: d Analyzed content of active ingredient: 555 97 g/400 kg Batch: EA 385 11 ed of Greece, one trial in Giannitsa in the vicinity of Pella trial S11-02885- thi in the vicinity of Jearissa (trial SLI-02885-02) s the determination of residues of imidacloprid in poten, nectar and extra
10 27 The field study was conduct 01) and a second trial in Gla The purpose of the study wa floral nectar in cotton plants Carmen) were pre-treated w site) and on 13MA\$ 2011 [I 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, flectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01ADG201 210CT2011 (end of residue <b>Findings (Residue Apalysi</b> Residues of imidacloprid was residues were <loq (i.e.="" <1<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid was (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral pectar sample sample (BBCH 61, 04), low</loq>	ed fr Greece, one trial in Giannitsa in the vicinity of Pella (trial S11-02885- fki in the vicinity of karissa (trial SL)-02883-02) s the determination of residues of imidacloprid in potten, nectar and extra
10 27 The field study was conduct 01) and a second trial in Gla The purpose of the study wa floral nectar in cotton plants Carmen) were pre-treated w site) and on 13MA\$ 2011 [I 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, nectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01ADG201 210CT2011 (end of residue <b>Findings (Residue Apalysi</b> Residues of imidacloprid was residues were <loq (i.e.="" <1<br="">&lt;1 - 5 μg/kg). Residues of imidacloprid was (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral rectar sample sample (BBCH 61-04), low</loq>	ed fr Greece, one trial in Giannitsa in the vicinity of Pella (trial S11-02885- fki in the vicinity of Larissa (trial SL)-02883-02) s the determination of residues of imidacloprid in poten, nectar and extra
10 27 The field study was conduct 01) and a second trial in Gla The purpose of the study was floral nectar in cotton plants Carmen) were pre-treated was site) and on 13MA\$ 2011 [H 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, frectar as BBCH stages (Meier 2001), <b>Dates of work</b> : 01AD G201 210CT2011 (end of residue <b>Findings (Residue Analysi</b> Residues of imidacloprid was residues were <loq (i.e.="" <1<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid was (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral pectar sample sample (BBCH 61,04), los</loq>	ed fr Greece, one trial in Giannitsa in the vicinity of Pella (trial S11-02885- fki in the vicinity of Larissa (trial SL)-02883-02) s the determination of residues of imidaclopric in poten, nectar and extra
10 27 The field study was conduct 01) and a second trial in Gla The purpose of the study was floral nectar in cotton plants Carmen) were pre-treated was site) and on 13MA\$ 2011 [H 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, frectar as BBCH stages (Meier 2001), <b>Dates of work</b> : 01AD G201 210CT2011 (end of residue <b>Findings (Residue Analysi</b> Residues of imidacloprid was residues were <loq (i.e.="" <1<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid was (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral pectar sample sample (BBCH 61,04), los</loq>	ed fr Greece, one trial in Giannitsa in the vicinity of Pella (trial S11-02885- fki in the vicinity of Larissa (trial SL)-02883-02) s the determination of residues of imidaclopric in poten, nectar and extra
10 27 The field study was conduct D1) and a second trial in Gla The purpose of the study was floral nectar in cotton plants Carmen) were pre-treated was site) and on 13MA\$ 2011 [H 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, actar as BBCH stages (Meier 2001), Dates of work: 01ADG201 210CT2011 (end of residue Findings (Residue Analysis Residues of imidacloprid was residues were <loq (i.e.="" <1<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid was (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral pectar sample sample (BBCH 61,64), los</loq>	ed fr Greece, one trial in Giannitsa in the vicinity of Pella (trial S11-02885- fki in the vicinity of Larissa (trial SL)-02883-02) s the determination of residues of imidaclopric in poten, nectar and extra
10 27 The field study was conduct 01) and a second trial in Gla The purpose of the study was floral nectar in cotton plants Carmen) were pre-treated was site) and on 13MA\$ 2011 [H 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, frectar as BBCH stages (Meier 2001), <b>Dates of work</b> : 01AD G201 210CT2011 (end of residue <b>Findings (Residue Analysi</b> Residues of imidacloprid was residues were <loq (i.e.="" <1<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid was (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral pectar sample sample (BBCH 61,04), los</loq>	ed fr Greece, one trial in Giannitsa in the vicinity of Pella (trial S11-02885- fki in the vicinity of Larissa (trial SL)-02883-02) s the determination of residues of imidaclopric in poten, nectar and extra
10 27 The field study was conduct D1) and a second trial in Gla The purpose of the study was floral nectar in cotton plants Carmen) were pre-treated was site) and on 13MA\$ 2011 [H 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, actar as BBCH stages (Meier 2001), Dates of work: 01ADG201 210CT2011 (end of residue Findings (Residue Analysis Residues of imidacloprid was residues were <loq (i.e.="" <1<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid was (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral pectar sample sample (BBCH 61,64), los</loq>	ed in Greece, one trial in Giannusa in the vicinity of Pella (trial S11-02885- fki in the vicinity of Icarissa (trial StJ-02883-02) s the determination of residues of imidacloprid in poten, nectar and extra
10 27 The field study was conduct 01) and a second trial in Gla The purpose of the study wa floral nectar in cotton plants Carmen) were pre-treated w site) and on 13MA\$ 2011 [I 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, flectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01ADG201 210CT2011 (end of residue <b>Findings (Residue Apalysi</b> Residues of imidacloprid was residues were <loq (i.e.="" <1<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid was (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral pectar sample sample (BBCH 61, 04), low</loq>	ed in Greece, one trial in Giannusa in the vicinity of Pella (trial S11-02885- fki in the vicinity of Icarissa (trial StJ-02883-02) s the determination of residues of imidacloprid in poten, nectar and extra
11) and a second trial in Gla The purpose of the study wa floral nectar in cotton plants Carmen) were pre-treated w site) and on 13MA\$ 2011 [41 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, frectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01ADG201 210CT2011 (end of residue <b>Findings (Residue Analysi</b> Residues of imidacloprid wa residues were <loq (i.e.="" <<br="">&lt;1 - 5 µgskg). Residues of imidacloprid wa (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral nectar sample sample (BBCH 61, 64), low</loq>	s the determination of residues of imidacloprid in poten, nectar and extra
The purpose of the study wa floral nectar in cotton plants Carmen) were pre-treated w site) and on 13MA\$20114 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, frectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01ÅDG20Å 21OCT2011 (end of residue <b>Findings (Residue Analysi</b> Residues of imidacloprid wa (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral rectar sample sample (BBCH 61, 64), los	s the determination of residues of imidaclopric in poten, nectar and extra
floral nectar in cotton plants Carmen) were pre-treated w site) and on 13MA 2011 (H 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, dectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01AO G201 21OCT2011 (end of residue <b>Findings (Residue Analysi</b> Residues of imidacloprid w residues of imidacloprid w (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral pectar sample sample (BBCH 61-64), low	s the determination of residues of imidaclopric in potten, nectar and extra
floral nectar in cotton plants Carmen) were pre-treated w site) and on 13MA 2011 (H 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, dectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01AO G201 21OCT2011 (end of residue <b>Findings (Residue Analysi</b> Residues of imidacloprid w residues of imidacloprid w (BBCH 65-69) of the Lariss mean residue level of all nec In extrafloral pectar sample sample (BBCH 61-64), low	
Carmen) were pre-treated w site) and on 13MA 2011 [1 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, frectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01AD G201 21OCT2011 (end of residue Findings (Residue Apalysi Residues of imidacloprid w residues were <loq (i.e.="" <<br="">&lt;1 - 5 <math>\mu</math> skg). Residues of imidacloprid w (BBCH 65-69) of the Larsis mean residue level of all new In extrafloral mectar sample sample (BBCH 61,64), low</loq>	grown from imidacloperd-treated seeds. Cotton seeds (variety Flora and
site) and on 13MA\$2011 (1 10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, actar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01AD G201 21OCT2011 (end of residue Findings (Residue Analysi Residues of imidacloprid as residues were <loq (i.e.="" <1<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid wi (BBCH 65-69) of the Lariss mean residue level of all neo In extrafloral pectar sample sample (BBCH 61-04), los</loq>	th Imidaclopfed FS 350. The sowing had taken place on 12MAY2011 (Pella
10416m <sup>2</sup> (Pella site) and 10 After emergence of the crop Sampling of pollen, dectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01AO G201 21OCT2011 (end of residue Findings (Residue Analysi Residues of imidacloprid va residues were $<$ LOQ (i.e. $<$ $<1 - 5 \mu$ kg). Residues of imidacloprid va (BBCH 65-69) of the Lariss mean residue level of all new In extrafloral mectar sample sample (BBCH 61,64), low	arissa site). The sizes of the plots on which the cotton was grown were
After emergence of the crop Sampling of pollen, dectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01AO G201 21OCT2011 (end of residue <b>Findings (Residue Apalysi</b> Residues of imidacloprid wa residues were $<$ LOQ (i.e. $<$ $<1 - 5 \mu$ gkg). Residues of imidacloprid wa (BBCH 65-69) of the Lariss mean residue level of all new In extrafloral nectar sample sample (BBCH 61.04), low	)32m² (Icarissa site).
Sampling of pollen, frectar a BBCH stages (Meier 2001), <b>Dates of work</b> : 01AD G201 21OCT2011 (end of residue Findings (Residue Apalysi Residues of imidacloprid w residues were $<$ LOQ (i.e. $<$ $<1 - 5 \mu$ kg). Residues of imidacloprid w (BBCH 65-69) of the Larss mean residue level of all new In extrafloral nectar sample sample (BBCH 61,64), low	samples were laken for pollon, needer and extrafloral nectar.
BBCH stages (Meier 2001), <b>Dates of work</b> : 01AD G201 21OCT2011 (end of residue <b>Findings (Residue Apalysi</b> Residues of imidacloprid a residues were $<$ LOQ (i.e. $<$ 1 <1 - 5 µg/kg). Residues of imidacloprid with (BBCH 65-69) of the Larssis mean residue level of all new In extrafloral nectar sample sample (BBCH 61, 64), low	hd extrafloral nectar was carried out consecutively three times, at different
21OCT2011 (end of residue Findings (Residue Analysi Residues of imidacloprid of residues were $<$ LOQ (i.e. $<$ 1 <1 - 5 µg/kg). Residues of imidacloprid with (BBCH 65-69) of the Larses mean residue level of all new In extrafloral nectar sample sample (BBCH 61,64), low	on both stady locations, respectively.
Residues of imidacloprid a residues were <loq (i.e.="" <<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid w (BBCH 65-69) of the Larss mean residue level of all nee In extrafloral nectar sample sample (BBCH 61,04), low</loq>	P(Pella site) and 04 CUG2011 (Latissa site) (start of field work) to analysis).
Residues of imidacloprid a residues were <loq (i.e.="" <<br="">&lt;1 - 5 µg/kg). Residues of imidacloprid w (BBCH 65-69) of the Larss mean residue level of all nee In extrafloral nectar sample sample (BBCH 61,04), low</loq>	
<1 - 5 µgkg). Residues of imidacloprid wa (BBCH 65-69) of the Lariss mean residue level of all neo In extrafloral nectar samples sample (BBCH 61204), low	
Residues of imidacloprid wa (BBCH 65-69) of the Lariss mean residue level of all new In extrafloral nectar sample sample (BBCH 61264), low	The detected in four of the six cotton pollen samples. In two samples the $\mu g$ kg. The mean residue level of the six pollen samples was $2\mu g/kg$ (range:
In extrafloral nectar samples sample (BBCH 61,64), los	re detected in five of the six cotton nectar samples, except of the last sample a site where the residue level of imidacloprid was $<$ LOQ (i.e. $<1 \mu g/kg$ ). The
sample (BBCH 61-64), los	tat samptos was $2\mu g/kg$ (range: $<1 - 4\mu g/kg$ ).
	fesidies of imidacloprid were found in five of the six samples. In one
extrational mectar camples w	fesidues were detected ( <loq, <1="" <math="" i.e.="">\mug/kg). The mean residue level of all as <math>3\mu</math>g/kg (range: &lt;1 – 5<math>\mu</math>g/kg).</loq,>
extrafloral prectar samples w Results of Analysis of Polle	a Samples
Õ	



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 4							
L11-02885-02-004A	BBCH 63-66	<1							
L11-02885-02-007A	BBCH 65-69		i di di a						
Mean			S & A						
LOQ Imidacloprid: 1 µg	i/kg								
For calculation of the a	rithmetic mean, values bei	ow LOQ were set to the LOQ	¥ . 4						
(LOQ = 1 µg/kg) Results of Analysis of Net	(LOQ = 1 $\mu$ g/kg) Results of Analysis of Nectar Samples								
Sample	Growth Stage								
L11-02885-01-003A	BBCH 69-64		, Kur						
L11-02885-01-006A	BBCH263-66		* ¥ ¢_						
L11-02885-01-009A	- → BB€H 65-69 0°								
L11-02885-02-003A	BBCH @ -64 🗸		r						
L11-02885-02-006A									
L11-0288502-009A	🔨 🛁 BBCH 65-89 🔍 🖉	<u></u>							
Mean 🔊		y or the y'							
LOQ Imidacloprid ug	Kg & ~								
For calculation of the a	nthmètrc méan, values bêrc	w LOQ were set to the LOQ							
$(LOQ = 1 \mu g/kg)$									
(LOQ = 1 µg/kg) Results of Analysis of Extra Floral Neerar Samples Sample Sirowth Stage [µg/kg]									
Sample	Growth Stage	/ Imiđácloprid Residues گ- [µg/kg]							
L11-02885-01-002	BBCH 64264	<del>گ</del> <1							
L11-0288\$01-0\$5A	o ~BBCH_63-66~	¥ 4							
L11-02885-01-008A	©	1							
L11-02885-02-002	Q BBCH 6,264	5							
L11-02885-02-005A	A BBCH 68-66	3	-						
L1 -02885-02-008A	BBCH 65-69	3							
Mean		3							
LOQ Imidackoprid: 1 ug/kg . A ug/kg									
For calculation of the administrative mean, values below LOQ were set to the LOQ									
(LOQ = fug/kg)									

Imidat loprior residue levels in plant matrices grown from imidat lopridtreated cotton seeds ranged from <146 4  $\mu$ g kg in nectar, from <1 to 5  $\mu$ g/kg in pollen and from <1 to 5  $\mu$ g/kg in extrafloral nectar.



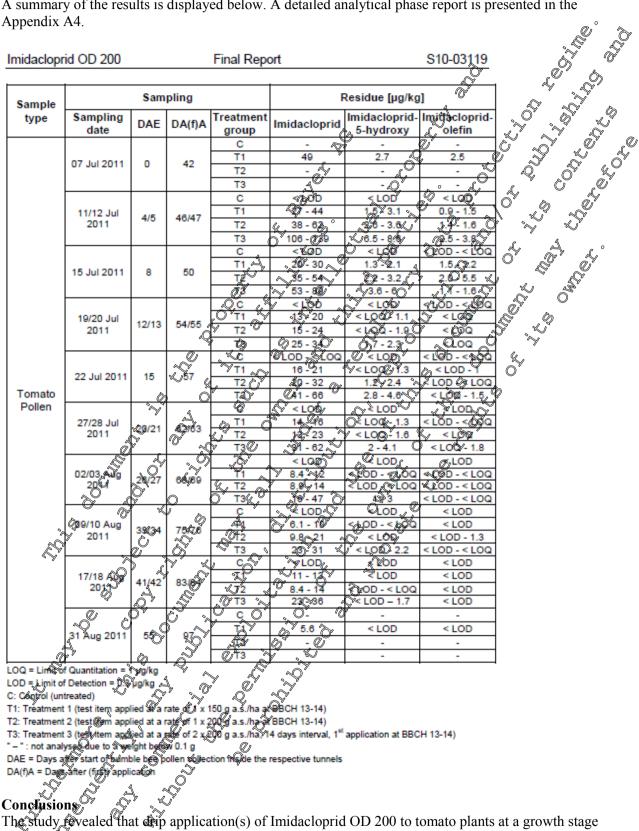
<b>Report:</b> Title:	02.02.01/34; 2012; M-428259-01-2 Imidacloprid OD 200: A semi-field study in Spain 2011 to determine residues informato pollen collected by forager bumble bees following drip application S10-03119 M-428259-01-2 IVA (1992), 7029/VI/95 (EU, 1997), 1607/VI/97 (EU, 1999), 1107/2009 (EU, 2009), 544/2011 (EU, 2011a) and 545/2011 (EU, 2011b) none yes Imidacloprid OD 200 Analyse econtent of active interedient. 203. Pg/L Active ents: Imidacloprid Batch: EQE5101280 y was to determine the residues of imidacloptid and its metholites imidacloprid- prid-olefin in tomato ( <i>Lycopersicon esculanum</i> ) follen, collected by bomble L., under confined semi-field (turnel tents) conditions following single or ns of Imidacloprid OD 200.
Report No.:	s10-03119
Document No.:	<u>M-428259-01-2</u>
Guideline(s):	IVA (1992), 7029/VI/95 (EU, 1997), 1607/VI/97 (EU,
	and 545/2011 (EU, 2011b)
Guideline deviation(s):	none
GLP/GEP:	yes
<pre>&lt;<m-428259-01-2@s-603000-01-1 and="" material="" methods<="" pre=""></m-428259-01-2@s-603000-01-1></pre>	
Test item: Name:	Imidacloprid OD 200 Analyset content of active ingredient. 203. Mg/L Active
ingredie	
The purpose of the stud	y was to determine the residues of imidaclopfud and its metabolites imidacioprid-
5-hydroxy and imidacle	pprid-olefin in tomator (Evcopersicon Sculentum) pollen, collected by bomble
bees, <i>Bombus terrestris</i>	L., under commed semi-field (tunnel tents) conditions following single or ns of Imida toprid OD 200.
repeated unp applicatio	
Before start of the test,	commercially grown, young comate plants were transplanted from pots to open,
natural soil as usual for	commercial open field tomato cultivation. The planting density was 25,000 northy before onset of flowering, gauze tunnels were set up.
tomato plants per na. Si	ionay before onservor movering, gauze junners were set up.
The study comprised for	ar treatment groups. There were three test item groups (T1, T2, T3) and one
untreated control group	(C). In T1: One application corresponding to 190 g a.s. ha was carried out at
at BBCH 13-14, 1.e. pust a	after transplanting. In T2, there was one application corresponding to 200 g a.s./ha 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	n, at BBC4 21-22.
The applications in The	T3 vore performed by using a water rate of 1.0 L/m2 via drip irrigation,
respectively. The control	bl group remained on treated. Set up of the bumble bee hives was at start of
flowering at BBCID 62-	63, just when mough nowers were present to allow foraging of the bumble bees.
BBCH 62-63	Then samples for residue analysis were taken 10 times after start of flowering at
BBCII 02-020	
Dates of work: 2 <sup>nd</sup> run;	25 Mao 2011 (start of field work) to 13 Dec 2011 (end of residue analysis)
Findings:	A A A A
Residue Analysis:	
	AE & i.e. the first day of bumble bee pollen collection inside the tunnels
At the first sampling () (exposure) dust when the	The first flowers opened at BBCH 62 - 63), only one single sample from one of the
three replicate tunnels (	$\mathbb{C}$ ) barely met the required minimum amount of 0.1 g pollen for residue analysis,
	rised only tiny amounts.
	mount of total imidacloprid residues was determined on DAE 4/5 (i.e. 4/5 days
	e pollen collection) in one replicate of treatment group T3. Total imidacloprid
residues in tomato polle	en declined over time in all treatment groups under investigation.



### **Imidacloprid Bee Studies Compilation of Study Summaries**

Issue date 2017-11-22

A summary of the results is displayed below. A detailed analytical phase report is presented in the Appendix A4.



typical for early transplanting (BBCH 13-14) resulted in total imidacloprid residues (i.e. imidacloprid + imidacleprid-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

Please click on the hyperlink to order a Study Report.



Report:	02.02.01/35; ; 20	012; <u>M-429087-01-2</u>		° .
Title:		sure levels of honey bee	s foraging on flor	wers of citrus trees
	previously treated with	n imidacloprid		N W
Report No.:	EBNTL056-7a		ð	
Document No.:	<u>M-429087-01-2</u>		S.	
Guideline(s):	none		10	
Guideline deviation(s):	none		A	
GLP/GEP:	no	Ĉ	L'	
< <m-429087-01-2@8-605225-01-1< td=""><td></td><td></td><td><u>v</u></td><td></td></m-429087-01-2@8-605225-01-1<>			<u>v</u>	

The objective of this study was to determine if residues of imidaclopid and its important thetabelites 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 cequested additional data for citrus growing in heavier clay soils. In response to this request, and also to supplement the residue data from form form and imidacloprid, imidacloprid olefin and 5 OH imidacloprid were quantified by LC/MS/MS. The full methodorogy for the collection and analyses are described in the April 2011 report.

- I report.
  We collected nectar from six citrus groves in Tulate 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 prove.
  We collected nectar from 6 proves in the temecula Valley (Riverside County) where the trees
- We collected nectar from 6 proves in the Aremecula 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 2
- 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 midacloprid in 2010, we had collected nectar from these same blocks to determine imidacloprid levels after 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 nectad from a lemon grove in Ventura County where the trees had been treated with the full label rate of impactopria at different omings during the season. The treatment timings were in May, July and September 2000. 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 circus 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 lown. Sixteen composite samples were collected from the trees at this site.





#### Summary of Residue Data for Total Imidacloprid in Strawberry Blossoms, Strawberry Anthers, Strawberry Pollen and Strawberry Leaves. $a^{\circ}$

		L	То	tal Imidad	loprid Resid	ue Levels	ppm) d	X ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Commodity	Soil Type	L	Min	Max	Highest Average Site Residue	Median	Mean Cr	0.082 0.082 0.082 0.095
Strawberry Blossoms	Light	6	0.21	0.52	0.50	0 0.38	C Mea	Q.13 5 40
Strawberry Anthers	Light	6	0.081	\$30	0.25	@20	0.18	y 0.082
Strawberry Pollen	Light	6	0.078 1.7 O	0.32 .	0.08	°∕70.21⊘	0.79	.0%995
Strawberry Leaves	Light	6	1.7 O°	29	2.4 Å	20	æ.2	0.41
Strawberry Blossoms	Medium	8	<0,0050	0.031	2.4 0.018	0.0064		0.0091
Strawberry Anthers	Medium	8	©0.011 ~	0.093	<ul> <li>0.023 €</li> </ul>	0.043	6918	0.00790
Strawberry Pollen	Medium	Q,Ö	<0.0010	°×0.010×		0.043 ~9.010	0.010 0011	<0,090
Strawberry Leaves	Medium	8	£0.010	0.0	<b>\$</b> 17 4	0.0	0011	<0.010

 <sup>a</sup> Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service.
 <sup>b</sup> Abbreviations used are as follows: Min is the lowest treated residue value; Max is the highest treated residue value; Mediafois the geometric median of the treated residue value; Mean is the mathematical average of the treated residue values; Standard Deviation is the standard deviation for a small population of n samples. Ĵ, С



Report:	02.02.01/37; ; 2013;	<u>M-404588-02-2</u>					
Title:		es of imidacloprid and its metabolite					
	imidacloprid and imidaclop	rid olefin in bee relevant matrices co					
	during two successive years	locations treated with imidaclopid	at least once per year				
Report No.:	EBNTL056-05-1		to meet the requirements				
Document No.:	M-404588-02-2	A					
Guideline(s):	US EPA Ref · OPPTS 850 3	SUPP (Ecological Effects)					
Guideline deviation(s):	The field and sampling phase of this study were not conducted to meet the require						
	of EPA Good Laboratory Pr	ractice Standards (40 SFR part 160;	F&, August 17, (1989).				
	The anlytical phase of this s	study was conducted to meet GLP of	andards The Deparation				
	of the field fortification sam	ples was not conducted under GLP	but their analyses met				
CI P/CFP·	OLF standards.						
ULI/ULI.	yes 🖉	assified as "heavy" or medium"					
< <m-404588-02-2@s-604953-01-1< td=""><td>U</td><td></td><td>, to a stand a stand of a</td></m-404588-02-2@s-604953-01-1<>	U		, to a stand a stand of a				
Nine thats were cond	rid and its matchalithd (5 h)	froxy inidaeloprid and implacio	not a late in the second				
residues of minuaciop	The and its inclabolities (3-40%	royar at locations greated with is	Rifu Olemi) in authers				
		ious cheorigation applications of					
rates ranging from 0	18 to 0.25 lb $\mathcal{A}$ (5 @ro 7.0°	If oz formulated product/A) in 20					
rates ranging from 0.	10 10 0.25 10 div (5.040 7.0						
Each trial received ar	polication (Q of Admire Pro in	n 2010 at the same rates as in 200	9 The six sites located				
in Kings County rece	ived two applications of Ada	nire Pro at 3 5 ft oz FP2A/applica	tion (0.13 lb				
imidacloprid/A/appli	cation for a total seasonal of	te of 7.0 fl oz FP/Ac0.25 lb/ai/A	The first applications				
were made at or close	elv following transmanting w	ith the second applications 5240	57 days following the				
		ounty received a single applicat					
	🗚 (5.0 fPoz EŘA) 2 to 25 da						
The growth stages of	the plants at the times of app	lications were not dochmented b	out likely occurred at				
growth stages BBC	2 21 to 51 (first primary she	soft to first inflorescence visible)	for the first				
applications and BB	H61 to BBCH69 (flowering	but perfor to Quiting for the seco	ond applications. All				
applications were ma	de through drip chemigation	(buried lines).					
A CA							
Composite samples of	n tomator anthers (pollen) and	tomato leaves were collected fro	om tomato plants 72 to				
102 days following a	he last treatment (DALT) at in	ndetorminate flowering and fruit	ing growth stages				
(BBCH6X to BBCH7	7X and analyzed for residues	s of imidaeloprid.					
		$\mathcal{O}^{v} = \mathcal{O}^{v}$					
The residue(\$) of im	daclogrid, 5-bydroxy imidaci	opride and imidacloprid olefin w	ere quantitated by high				
performance liquid cl	hromatography/trrpie stage qu	uadfupole mass spectrometry (LC ual analyte residues were summ	J/MS/MS) using stable				
isotopically labeled in	nternal standards. The abdivic	analyte residues were summ	ed to give a total				
imidacloprid residue		Ϋ́					
The limits of quantita							
The mints of quarkita	ation (LOOS) are shown below	w.					
Matrix	nalyte 2		7				
		LOQ (ppm) 0.002	_				
	-hydrox@midacloprid	0.002	-				
	ndacloprid olefin	0.002	-				
	otal	0.002	-				
	midacloprid	0.002	-				
	-hydroxy imidacloprid	0.002	-				
	midacloprid olefin	0.002	-				
	otal Imidacloprid	0.002	-				
		1					

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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.

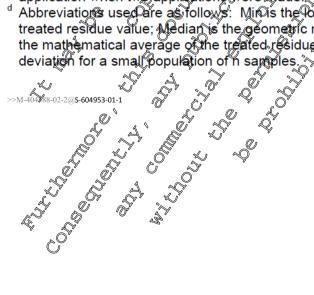
A summary of the residues is shown in the table below.

#### Summary of Residue Data for Total Imidacloprid in Anthers and Reaves from Fomato JO , Č Heavy and Medium Soils.

						~~~	Ô	Ň.	S S	~ ~
				Q	Total	Imidaclo	ricResic	lue Level	s (ppm)	
Commodity	Plot Type <sup>a</sup>	Application Rate Ib ai/A (oz FP/A) <sup>b</sup>	Dogs After Last Treatmont (DALT) <sup>c</sup>		D = 0 Min 2 2, 4 0 2, 0 0	Max x	Highest Averai		an 22	a l
Tomato Anthers	Heavy Soil	0.25 (7.0)	0 <sup>7</sup> 72 - 79	8	0.014	0030	9.027	<b>0.02</b> €	0.021	0.005
Tomato Anthers	Medium Soil	0.18 – 0.29 (5.0 – 7.9)	79 102	0 10	<b>@</b> .016~Ç	0.05	0.046		<b>0.034</b>	0.012
Tomato Leaves	Heavy Soil	0.25 (Z,0)	0 72 - <b>79</b>	<u></u>	0,057	0.14	\$0.12 \$	0.089	0.093	0.026
Tomato Leaves	Medium Soil	0,18 – 0.25 (5.0 – 7)	<b>7</b> 9-102	0 10 0	0.038	0.23	0.20	. 9.10	0.11	0.061

<sup>a</sup> A total of nine torsato trials were conducted. Your it heavy soils and five in "medium" soils. Ten trials were sched ded; 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 Natura Resources Conservation Service. "Heavy" class represents soil with slow dramage apacity and "medium" class represents soil with moderate draining capacity.

- <sup>b</sup> Although all plots had received applications of midicloprid in the previous year (2009) at rates ranging from 0.18 to 0.38 b ai A, the opplication rate cited refers to applications in 2010 only.
- <sup>c</sup> All trials received one or two applications of Admile Pro, DALT are the days following the last application when two applications were made. Abbreviations use are as follows: Min is the powest reated 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 h samples.





Report:	02.02.01/38:	; 2013; <u>M-444526-02-2</u>	2			0
Title:		of the residues of imidaclop		abolites 5-h	vdroxy	
	imidacloprid ar	nd imidacloprid olefin in be	e relevant matr	ices collect	ed from me	lons 🔊
	grown at locati	ons treated with imidaclopr	id at least once	per-vear du	ring two su	iccessive
	vears				L.	۵
Report No.:	EBNTL056-02	-1		° v	2	
Document No.:	M-444526-02-2	2	A.		\$ \$	, Ś
Guideline(s):	US EPA Ref.:	OPPTS 850.SUPP (Esologi	cal Effects	2	× ×	, s
Guideline deviation(s):	ves, see report	ATT I	Ū,	Č,	' . N	Ô, C
GLP/GEP:	ves	¥.	Q	, Ø		
	2		Å,	Ň	Q.	
< <m-444526-02-2@s-605035-01-1< td=""><td>4. 1 in California</td><td></td><td></td><td>A A</td><td>"», "Ö</td><td>1 day</td></m-444526-02-2@s-605035-01-1<>	4. 1 in California			A A	"», "Ö	1 day
Ten trials were conduc	ted in California	a in soils classified as eitr	ier neavy (fir	esture	i), or me	alam
(medium-textured)" to	determine the re	esidues of imidacloprid a	nd its metabo	utes (2-\hy	drøxy «	Ş
imidacloprid and imida	acloprid olefin)	in bee collected nectar (h	ive deposited)	, bec colle	cted polle	h
(pollen traps), and leav	es (hand collect	a in soils classified as eith sidues of midacloprid a in bee collected nectar (h ed) from melon plants (c feation methods for the t ver communications (see id/A application (0.26 to	ucorbits) grov	vn at locat	ions treate	d
previously with imidad	loprid.		~	S U	, S	Ů,
					d i	Y.
Imidacloprid application	on rates and app	feation methods for the t	rial Pocations	during the	gears of 2	008
through 2011 were col	lected from grow	Ver communications see	bolow), Indi	vietual app	lication rat	tes
ranged from 0.23 to 0.	38 lb imidaefoor	id/A/application (0.26 to	0.43 ke imid	clopricha	a application	on).
		pear transplant of the mel		- T C		- )-
			Q U	ð s	8	
Application History	a 🗸 🔬		4°~?	Ġ, Ö	)	
	ő ő					
lon be			. °			
	ente Q' a		tio 🖉	$\sim$		°
N N	a s tot			Ç <sup>y</sup>		te a.i./A)⁰
Field Number			eth "	Year	Date	Rate (Ib a.
	ĩ Đã ẵ 🏹	<u>~~~~~~~~~</u>	<u> </u>	۲e	Ď	r R
1 NT209 mper	County, CA	holtville Silty Clay	(S <sup>I</sup> Injected	2011	Jan 10	0.36

Field	Fielc	R R C C		App	Year	Date	Rate (Ib a
1	NT209	Oimperar County, CA		S Injected	2011	Jan 10	0.36
		NASTA Region 10		None	2010	NA <sup>d</sup>	NA
				hjected	2009	Oct. 30	0.36
				Injected	2008	Oct 5	0.31
2	NT210	Imperial County, CA,	Piolitville Silty Clay	✓ Injected	2011	Jan 10	0.36
		NAFTA Region 10	Heavy	None	2010	NA	NA
		NOFTA Region O		Injected	2009	Oct. 30	0.36
				Injected	2008	Oct 5	0.31
3	NT201	Imperial County, CA,	Holtvalle Siltvoclay	Injected	2011	Jan 7	0.29
	À	NAFTA Region 10	(Heavy)	Injected	2010	Jan 25	0.29
	Å.			None	2009	NA	NA
4≪	NT202	Imperial County, Co,	Øvieloland Very Fine Sandy Loam	Injected	2011	Jan 10	0.29
		NĂFTA Bégion 10	Sandy Loam	None	2010	NA	NA
			/ 《 (Medium)	Injected	2009	Jan 24	0.29
5	NT202	Imperial County, CA,	,Holtville Silty Clay	Injected	2011	Jan 10	0.29
		NAFTA Region 10	(Heavy) <sup>e</sup>	None	2010	NA	NA
	Q <sup>y</sup>			Injected	2009	Jan 24	0.29
6 >	NT208	Imperial Conaty, CA,	Imperial-Glenbar Silty	Seed Line	2011	Jan 3	0.36
		NAFTA Region 10	Clay (Madium)	None	2010	NA	NA
<u>k</u>		<u> </u>	(Medium)	Seed Line	2009	Oct 4	0.30
(	0						



# Imidacloprid Bee Studies Compilation of Study Summaries

Issue date 2017-11-22

Field Number	Field Identification	Location (City, State, NAFTA Region)	Soil Type <sup>b</sup>	Application Method <sup>b</sup>	ମୁକୁ Year	Bate	Rote (Ib a), A)°
7	NT206	Imperial County, CA, NAFTA Region 10	Imperial-Glenbar Silty Clay (Medium)	None Seed Line Seed Libe Seed Libe	2011 2010 2009 x 2009	NÁ ODct 21 Nox 21 March 2	NA 0,228 0,23 0,28
8	NT205	Imperial County, CA, NAFTA Region 10	Meloland Verg Fine Sandy Loam (Medum)	See Line	204) 2010 2009 0	Pan 9 (P NAC	0.30 0.4 2,1NA
9	NT204	Imperial County, CA, NAFTA Region 10	Holtville Silty Clay	Seed/Line ( None ( None)	2075 2010 2009	NA,	″ 0.36 NA ≪^NA
10	NT207	Imperial County, CA, NAFTA Region 10	Meloland Very Pine Sandy Loam (Medition)	Injected	20 <u>11</u> 2000 2009	Jan 20 ✓ NA ✓ Jan 20	0.36 NA 0.36

- <sup>a</sup> All rates, methods and dates were collected from verbal communications with the growers and could not be confirmed.
- <sup>b</sup> 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.
- slow drainage capacity
   Applications were made either by injection to a drip inogation system or with a drench application over the seed line.
- <sup>d</sup> NA Per grower information, an imidacloprid application was not made during the year.
- <sup>e</sup> One tent was in Holt/Me Silty Clay. The other tent in this site was divided between Holtville Silty Clay and Imperial-Glenbar Silty Clay Loan. 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 fight, ventilated mesh tent (24 ft x 100 ft x 10 ft tall) for sample collection. One formally developed, apparently healthy and queen-right honey bee colony was placed in each tended 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 abrood 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 hiveentrance 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 imidaelopro, 5-hydroxy midaeloprid, and imidaeloprid 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 imidaeloprid 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	
	Imidacloprid olefin	S 0.001 S
	Total imidacloprid	0.001
	Imidacloprid	<u>, 2</u> 0.0,10 , 2 , 2
Melon pollen and leaves	5-hydroxy imidacloopid	
	Imidacloprid olefin	Q Q010 X X
	Total imidaclop	0.016
	A.	

Transit stability samples (control nectar and poller fortified with imidatioprid, 3-hydroxy indacloprid, 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 Bis study. The maximum storage period of frozen samples in this study was 598 days (20 months).

A summary of the residues is shown or the table below. m

# Summary of Residue Data for Total Imidaclopud

	Total Imidacle	prid Resid	uedrevel	s (ppm) <sup>b,</sup>	c
ample Name	Max 20 20 20 20 20 20 20 20 20 20 20 20 20	Highest Average Site	Mediate 2	Mean	Standard Deviation
Bee collected (hive deposited) Beavy Soil 0	0.0012 0.00053	0.003	0.0024	0.0030	0.0015
Bee collected (bive deposited) Medium (10 10 10 10 10 10 10 10 10 10 10 10 10 1	0.00	0,0049	0.0030	0.0039	0.0025
Bee collected (pollen traps)	0.010 0.012	0.011	<0.010	<0.010	0.0028
Bee collected (pollectraps) Medium 10 melon pollen	0.010 0.032	0.019	<0.010	0.013	0.0086
	0.010 0.028	0.027	0.013	0.016	0.0067
	<0.040 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 <sup>b</sup> Total imidacloprid is the sum of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in parent equivalents.
- <sup>c</sup> Abbreviations used are as follows: Min is the lowest residue value; Max is the highest residue value; 'Highest average site residue as 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. 05035-01*4* 



Donoute	02 02 01/20
Report:	02.02.01/39; 2013; <u>M-260729-01-3</u>
Title:	Determination of the residues of imidacloprid, NTN33893-5-hydroxy and NTN28893- Or olefin metabolites in field sample of rape (blossom, nectar, dailey honey, bee bread,
	pollen, and soil)
Report No.:	MR-128/05
Document No.:	<u>M-260729-01-3</u>
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1994,
Guideline(5):	Annex II, part A, point 6 and Annex III, part A, point 8
	Residues in or on Treated Products Food and Feed 2
	US EPA OCSPP Guideline Number: 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	yes A Q & A U
< <m-260729-01-3@s-604674-01-1< td=""><td></td></m-260729-01-3@s-604674-01-1<>	
	Determination of the residues of imidacloprid, NTN33893-5-hydroxy and NTN33893- olefin metabolites in field sample of rape (blossom, nectar, dailey honey, bee bread, pollen, and soil) MR-128/05 <u>M-260729-01-3</u> EU-Ref: Council Directive 91/414/EEC of July 15, 1994 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 none yes lossoms, honey (nectar) bee bread and pollen of seed-treated winter rape were locations in a "Chemeal Monitoring Program" refluested by the German The purpose of the study is to determine the residues of similar oprid, and its 5 hydroxy and NPN32893 olefin in the residues of similar oprid, and its
collected from different	clocations in a "Chemeal Monitoring Program" requested by the German
Regulatory Authority 7	The purpose of the study is to determine the residues of imidacoprid and its
metabolites NTN33893	-5-hydroxy and NTN33893-olefin in rape (blossom, nectar daily honey, bee
bread pollen and soil)	after seed treatment with imide longit
bread, ponen, and son)	after seed treatment with imidacloprid.
Residues of imidaclour	id NTN33892 5 horrow and NTN33897 ale for in hear alevant matrices were
determined according to	p method 00537/4001 $2$ $2$ $3$ $2$ $3$
uctorninicu according u	
The individual recover	values for imidac oprid with method 00\$37/MO01 ranged from 90 to 106% with
averall recoveries betw	een297 and 99% and with relative standard deviations (RSDS) between 2.7 and
$\frac{9.90}{(n-4 \text{ to } 9)}$ For N	VIN33893-5-hydroxy scovestes ranged from 85 to 113% with overall recoveries
0.070 (II - 410.0). FOI T	and with RSD between 6.4 and 11 $\beta$ ( $\eta \approx 4 \text{ to } 8$ ). Recoveries for NTN33893-
olofin ware between	and $0.6\%$ grand $0.4\%$ and $0.4\%$ and $0.4\%$ and $0.4\%$ with DSDs between 1.8 and
5 10/ $(n - 4 to 8)$	and 106% overal precoveries between 88 and 004% with RSDs between 1.8 and
5.1% (n = 4 to 8). As 1 re	esults of the method validation were in accordance with the general requirements
for residue analytical m	ethods, therefore the method was validated successfully.
T1 1:	
The limit of quantitation	n (LOQ) was 0.001 mg/kg for indiacloprid, NTN33893-5-hydroxy and
NIN33893-jolefin for a	Ikbee refevant matrices.
Residues of imidaclopri	id in soil were determined according to irrethod 00790/M001.
The individual recovery	values for impract off with method 00790/M001 ranged from 88 to 110% with
an overall recovery of	01% and with a relative standard deviation (RSDs) of 10.6% ( $n = 4$ ).
All results of the metho	d validation were in accordance with the general requirements for residue
analytical methods, the	efore the method was validated successfully. The limit of quantitation (LOQ) of
	ng/kg for imidaclopfid. The limit of detection (LOD) of the method was 0.002
mg/kg.	
	cloped were all below the LOQ (0.001 mg/kg) in all bee relevant sample
materials with expect to	vo of 17 bee bread samples from Celle where the residues were 0.001 mg/kg for
	es of NJN33893-5-hydroxy and NTN33893-olefin above the LOQ were found in
any sample.	
	n soll samples from Kirchhain and Münster had positive detects ( $\leq 0.005$ and
0.007 mg/kg), confirmi	ng a seed treatment of sampled winter rape plants with Imidacloprid
>>M-26072 -3@S-604674-01-1	
Dloaco click on the hund	arlink to order a Study Poport

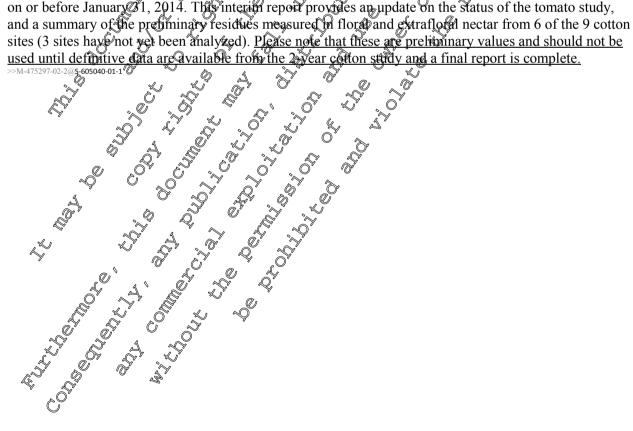


<b>Report:</b> Title:	02.02.01/40; 2014; M-475 Amended report - Interim progres tomato. Preliminary residue result	s report for imidacloprid rea	sidue studies in cotton and or collected from 6 of trials in
	year-1 of the 2-year cotton study	ĉ	
Report No.:	US0401-1	Ĩ	
Document No.:	M-475297-02-2	ſŎ.	
Guideline(s):	OCSPP 850.SUPP	1	
Guideline deviation(s):	none		
GLP/GEP:	yes		
< <m-475297-02-2@s-605040-01-1< td=""><td>Ą</td><td>, <sup>°</sup></td><td></td></m-475297-02-2@s-605040-01-1<>	Ą	, <sup>°</sup>	

Bayer CropScience (BCS) is conducting residue studies to measure potential residues in poller and neotar to support a pollinator risk assessment for imidacloprid. These required studies over designed to measure the magnitude of residues of imidacloprid and its metabolites 5 Hydroxy imidacloprid and inidacloprid Olefin. The study sites include fields at multiple locations with varying soft ypeson California. Crops include cotton, tomato, pome fruit and stone fruit. Matrice Cinclude polley, nectar and baves of cotton; stone fruit and pome fruit, and pollen and leaves of tomato. All target grops 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 insidacloppid applications overwo consecutive seasons. For cotton and tomato, year 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, fectar and leaf sample collection will occur during bloom in 2014. The porte and stone four studies will continue with additional applications in 2014 and sampling on 2015 Ò

As per a letter from the California Department of Perfectice Regulation (CDPR) (D# 254696) dated September 20, 2012, interim reports are to be submitted for the first year sampling on cotton and tomato on or before January 21, 2014. This interim report provides an update on the status of the tomato study, and a summary of the prefiminary residues measured in florad and extrafloral nectar from 6 of the 9 cotton

2





Report:	02.02.01/41; ; 2014; <u>M-500863-01-2</u>
Title:	Determination of the residues of imidacloprid and its metabolites 5-hydroxy
	imidacloprid and imidacloprid olefin in bee relevant matrices collected from seed treased
	field corn during two successive years and in white clover planted after seed the ated field
	corn
Report No.:	EBNTY009
Document No.:	<u>M-500863-01-2</u>
Guideline(s):	US EPA OPPTS/OCSPP 850.SUPP_Ecological Effects
Guideline deviation(s):	
GLP/GEP:	yes
< <m-500863-01-2@s-602294-01-1< th=""><th></th></m-500863-01-2@s-602294-01-1<>	
Executive Summary, Pa	

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 poller samples and in on leaves, tassels, and soil from corn plants grown from seed treated with Gauche, 600 For two years consecutively, and to measure the magnitude of the same residues in/or bee-relevant white cover 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. Gauche 600 Powable was applied to field corn seeds at target rates as shown below.

Target Application Summary

					í e		, ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
		0	Y A	, Tange	t Rate/App	cation	<i>&amp;</i> '	- S	Soil Lo	ading
			Formulated	roduct (fp)	Active In	gredien	it (ai)	Seeds	Ra	te
		Test Sups.	for fpreed	2		<sub>@</sub> "mg	lb (	¥		kg
Plot ID <sup>a</sup>	Year <sup>b</sup>	Subs.	floz fp/seed	mil fp/seed	Name of ai	ai/seed	ai/seed	seeds/A	lb ai/A	ai/ha
UTCA	1, 2		NA K	NAY	"Հ∕NA <sup>∾</sup>	NA	₹	40,250	NA	NA
UTCB	1, 2		_ONA O		NÃO	<b>ANA</b>	<mark>⊘</mark> NA	40,250	NA	NA
TRTSTA	<b>,19∕2</b>	Treated seeds		10	Imidaeloprid		2.95E-6	40,250	0.119	0.133
TRTST®	× 1	Treated seeds	1:22-02	0.0022	Inidacloprid	7 <b>4</b> ,34	2.95E-6	40,250	0.119	0.133
TRTSTB	2	NA A	NA	YNA @	NA A	NA	NA	NA	NA	NA

Plot ID: UTC R = Untreated control plot receiving untreated field corn seed in years 1 and 2.

UTGB = Unfreated control flot receiving Oftreat of field corn seed in year 1 and untreated for ge crop (white clover) in year 2.

2 and 2. 1

TRTSTB = Treated plot receiving field com seed treated with Gaucho 600 Flowable in year 1 and untreated for age crop (white clover) in year 2.

b Ayfresh batch of treated seed was used for the second year's planting of plot TRTSTA.

NA = Not applicable.

Plot TRTST Preceived field corn seed treated with Gaucho 600 Flowable in years 1 and 2 of the study (2012 and 2013, respectively). Pot TRYSTB received field corn seed treated with Gaucho 600 Flowable in year 1 and unweated forage grop (white clover) seed in year 2. All plots were tilled or disked at least once prior to the year's planting of corn or clover. For plot TRTSTA, the seed planting rate ranged from 36,449 to 4,480 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 9.121 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 no 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 whited 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 shoulding; 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 cloves 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 assel emergence, tassels fully emerged and separated; corn BBCH 71: beginning of grain development, kernels at blyster stage, about 16 dry matter) tolover BBCH 59: first petals visible, flowers/buds stol closed; closer BBCH 71; 30% of pods have reached typical length). Nine soil samples were collected using a soil sampling device prior to seed manting and at the end of the growing season per year from treated plots TRTSTA and TRTSTB.

Two composite samples of all field corn and dover matrices and the composite samples of soil were collected from the control plots UTCA and UTCBY at the same sampling periods as used for the treated samples of that sample type.

A

S In some trials, not enough matrix material was present to allow for the fall number of larget samples to be collected (see Appendix 1)  $\bigcirc$ 

The residues of Gaucho 600 Etowable (imidal oprid, 5-hydroxy midacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and Ke/high resolution mass spectrometry (LC/MRMS) using stable isotopically labeled internal standards, The Umit of detection (LQD) for the total residue is the highest LOD value for an individual analore 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

ummary of L	ODS and LOQS     Image: Constraint of the constraint of th	LOD <sup>a</sup> (ppm)
Maurix	Analyte LOQ (ppm)	0.0009
<b>_</b>	5-b@roxy@nidaeloorid. 0 . 0 0.010	
Field corn		0.0015
tassels/anthers	Imidacloprid olefin 0 0 0010	0.0019
, A	Imidacloprid olefin TotabResidge 0.010	0.0019
Å √y	5-hydroxy (midaeloprid O O O O O O O O O O O O O O O O O O O	
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# Imidacloprid Bee Studies Compilation of Study Summaries

Issue date 2017-11-22

Matrix	Analyte	LOQ <sup>ª</sup> (ppm)	LOD <sup>a</sup> (ppm)
	Imidacloprid	0.010	0.0016
	5-hydroxy Imidacloprid	0.010	0.0017
Field corn leaves	Imidacloprid olefin	0.010	0.0022
	Total Residue	0.010	0.0022
	Imidacloprid	0.001	0.0004
Field corn pollen	5-hydroxy Imidacloprid	0.001	A 0.0005 A
(hand-collected)	Imidacloprid olefin	0.001	0.0003
	Total Residue	0.00	@ <u>00005</u>
	Imidacloprid	Q.010	y .0030
Clover flowers	5-hydroxy Imidacloprid		0.0024
Clover nowers	Imidacloprid olefin	0.010	Q 0.0016
	Total Residue	🛇 0.010 🔊 🗞	0,0030
	Imidacloprid	<u>لار 20</u> 010 کې کې	2 (0.00177 <sup>2</sup>
Clover leaves	5-hydroxy Imidacloprid	0.010°	0.0 <b>0</b> 0.0027
Clover leaves	Imidacloprid olefin	A . 0 0.016 Q	0.9020
	Total Residue	ັ້ 🔨 🖍 🖓 🕺	
	Imidacloprid		<i>√ 0</i> ,000 0 0
Clover pollen	5-hydroxy Imidacloorid		0,005
(hive-collected)	Imidacloprid olefin	<sup>0</sup> 0.001 5 <sup>7</sup>	C
	Total Residue		0.0005
	Imidacloprid	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	0.003
Clover nectar	5-hydroxy Imidadoprid		\$ <u>0</u> .0007
(hive-collected)	Imidacloprid oleun	<del>ک</del> <u>0.001 کی ک</u>	<b>0.0006</b>
	Total Residue		📣 🖉 0.0007
	Imidacloprid	0.005	م محمد 0.0012
Soil	5 ydroxy Imidacioprid		0.0015
	🕅 idacloprid defin 🛛 🗸	م م 2005 م	0.0012

<sup>a</sup> Soil LODs and LOOs are reported in individual analyte equivalents, and no total imidacloprid residue is calculated. All ther matrix analyte LODs and LOOs are reported in parent equivalents.

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Storage stability studie, and transit spikes indicate that the imidacloprid residues would have been stable during frozen storage for at reast 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 014 days for clover leaves, 210 days for clover nectar (hive-collected), 156 days for clover pollen (hive-collected) @26 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 midacloprid residues in complexes tasses, 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 nectars described in Section 3.6.

An analysis of the total initial oprid 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 (TRTSPA), and low concentrations in the corn/clover plot (TRTSPA), indicating residues were available for potential uptake by the clover.

Executive Summary, Part B



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 seplanting 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 were residues detected. There were do 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 ppb, respectively. The predian and 90th percentile values for clover nectar were less than the COD (< LOD) and OOQ, respectively.

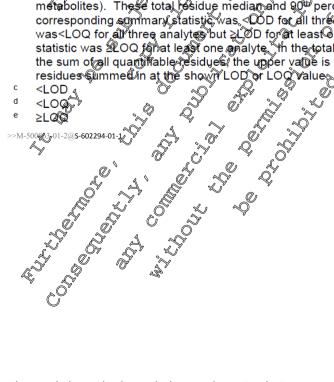
For the statistical analysis summarized in the table below the LOO/LOO for the total midactoprid residue (sum of imidacloprid, imidacloprid olefin) and 5-hydroxy imidacloprid) is taken to be the sum of the individual analyte LODs/LOQs. the individual analyte LODs/LOQs.

# Total Imidacloprid Residues in Corn Pollen, Clover Pollen, and Clover Nectar

	a the second sec	<sup>v</sup>
	Selected Summary 🥎 Imidacloprid   Imidacloprid   Imidacloprid	Total Residue
Matrix	Statistic (Source) 🖉 🖉 🖓 efin (ppb) 🔐 (ppb) 💦 (ppb)	(ppb) <sup>b</sup>
Corn	Median Q O O <0 <0 <0.5° A 6,5°	6.5 – 7.3 <sup>e</sup>
Pollen	90th Percentile	16 – 17 <sup>e</sup>
Clover	Median 🖉 🖉 🖉 <0.3 🖓 / 20.5 🖉 🎉 <1.0 🖉	<1.8 <sup>d</sup>
Pollen	90th Pe@entile 2.1 2.1 2.1 2.1 2.1 2.1 2.1 2.1 2.1 2.1	2.1 – 2.9 <sup>e</sup>
Clover	Mediato 0 .7	<1.6 <sup>b</sup>
Nectar	90th Percentile % & 30.6 ° & ~0.7 ° & 3 <	<2.3 d

a Corn poller statistical values are the highest values from any trail. Clover pollen and nectar statistical values are the values calculated across all trials.

values are the values calculated across all trials. Median and 90<sup>th</sup> 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 90<sup>th</sup> percentile values were considered <LOD if the corresponding sommary statistic was <00D for all three analytes, <LOQ if that summary statistic was<LOQ for all three analytes but >L0D for at least one analyte, and quantifiable if that summary statistic was 2LOQ for at least one analyte in the total residue ranges of values, the lower value is the sum of all quantifiable esidues; the upper value is the sum of all residues, with non-quantifiable residues summed in at the show'r LOD or LOO value b





Report:		4; <u>M-501306-01-2</u>		
Title:	Determination of the res	idues of imidaclop	rid and its metab	olites 5-hydroxy 🖉 🖉
	imidacloprid and imidac	loprid olefin in bee	e relevant matrice	es collected from treated
	cotton during two succes	ssive years and in v	white clover plan	ted after treated porton
Report No.:	EBNTY010		4	ž 4 A
Document No.:	<u>M-501306-01-2</u>			
Guideline(s):	US EPA OPPTS/OCSPI	P 850.SUPP, Ecolo	gical Effects	
Guideline deviation(s):	none	Ĉa	L.	
GLP/GEP:	yes	- C	<u>v</u>	E A A B L
< <m-501306-01-2@s-602302-01-1< th=""><th></th><th>J.</th><th><u>é</u></th><th></th></m-501306-01-2@s-602302-01-1<>		J.	<u>é</u>	
Executive Summary P	art A	.Ő	A	

Executive Summary, Part A

A total of three field trials were conducted each year for two specessive years to measure the magnitude of imidacloprid residues in bee-relevant cottor pollen and nectar samples and in/or leaves, blossoms, and soil from cotton plants grown from seed treated with Gaucho 600 Plowable 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 negtar samples and in blossoms deaves, 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 Dowable concentrate seed treatment formulation containing 600 g/L imidacloprid. Admire Pro Systemic Protectant is a suspension concentrate formulation for foliar spray rise containing 550 g/L imidacloprid. Admire Pro Systemic Protectant and Gaucho 600 Flowable were applied to cottob seeds at target rates as shown below. × ×

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Target /	Appli	cation S	umm	ary	Q A	<u> </u>				
		Te&t Subs.	· ·		) Ö	Target Rate	Opplication	<u> </u>	Target App.	Target
Plot ID <sup>a</sup>	Year <sup>ь</sup>	Subs.	of Apps	Doodu	ر(fp)	Name Of ai	6 lb ai/A	@kg ai/ha	Interval (days)	PBI⁰ (days)
UTCA	1, 2	ÔNA4	NA	Ś <mark>NA</mark> ∢	NAY	ŇĂ	N NA V	NA	NA	NA
UTCB	1, 🏖	, NA	NA	NAO <sup>v</sup>	<b>MA</b>	NA O	ANA .	NA	NA	NA
	0 7 1, 2	Treated seeds (Gauctor)		2.9E-5 fl oz fp/seed		Imidacloprid		0.054 (0.375 mg ai/seed, 58,000 seeds/A)	NA	NA
		Adquire	3	A/A	€∕πp/naχ;	midacloprid	0.061	0.068	5–8	14
TRTDB		Treated seeds (Gaucho)			6E ml		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
4	Ç.	Admire Pro	Ã.	1.7 fl oz fp/A	124 Mil fo na		0.061	0.068	5–8	14
TRUDB	2	ŇĂ	NĂ	°	INA 🕾	NA 🕅	NA d cotton seed i	NA	NA	NA

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

cop (white cloved in year 2. RTDA – Treated plot receiving cotton seed treated with Gaucho 600 Flowable and 5 foliar applications of Admice Pro Systemic Protectant in years 1 and 2.

TRX9B = Treated pot receiving cotton seed treated with Gaucho 600 Flowable and 5 foliar application of Admire Pro Systemic Protectant in year 1 and untreated forage crop (white over) in year &



- <sup>b</sup> 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.
- 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 \$25% ). Plot seeding rates ranged from 57,088 to 60,002 seeds/A. All plots were tilled prior to year 2 seed planting

Actual	Abb	incation Sun	IIIIa	y ioi ii				9	Ň		. Š	, 📞
					Individua		Individual	Rates per (	Рт	tal	Ο	¢"
				BBCH	Spray V	olonies	Appli	cation Q	Ra	ites	App.	1
		Application	App.	Growth		S (		les and	All	kg∕	Interval	
Plot ID	Year	Туре	No.	Stage <sup>a</sup>	GPA	LPK	Ib Ai/A		A	ai/ħa	(days) <sup>b</sup>	
UTCA	1, 2	NA°	NA	NA	NA	,NA	C NA O	NA (		<b>(NA</b>	ANA	
UTCB	1, 2	NA	NA	NA		(NA 🔊		A NA C	NA	NA	NA 🕡	r
TRTDA	1	Treated seeds	1	00	NA ^		0.04700.048	0.059	AJE	0:39	NA 6-8	]
INIDA	· ·	Foliar Spray	1–5	19–60	9.9-107	93,-94	0.060-0.069	0.067+0.069	0255		6-8	
TRTDA	2	Treated seeds	1	00%	<b>MA</b>	ANA	0.948-0.050	0.054-0.055	0.26	9.39-	0→8 NA 5-6	
INIDA	2	Foliar Spray	1–5	<b>24</b> ¥59	9.8-10.2		0.060-0.062	0067-0069	5	0.40	v <sup>™</sup> 5–6	
TRTDB	4	Treated seeds	1	00	NA 🤇	NA	0.0400.048	0%93				
IKIDB	· ·	Foliar Spray	1.5	19-60	9.9–10.1	93 <del>0</del> 94	0.040>0.048 0.060-0.081	0.067-0.069	0.55	239 0	5–8	
TRTDB	2	NA	ŇÅ	MA	AMA	χ, <mark>NA</mark>	NAS	🔊 🕺 🔊	NA	NA	NA	]

## Actual Application Summary for Imidacloprid

BBCH 19: nine or more reaves Onfolded, BBC 24: four side shoots detectable; BBCH 59: first petals visible, many individual flower buds still closed; BBCH 60: tost flowers open.

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First foliar spray applications were made 38-61 days after planting.

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NA = Not applicable.

Composite samples (separate rans 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 EC/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 LOD value is the highest LOQ value for an individual analyte in a particular matrix.

	ow.	Q, ,	»	, Ű	Q' O	
DQs and LODs	<u> </u>	<u>`</u> ```````````````````````````````````	<u> </u>			y v
Analyte	O <sup>v</sup>	LOQ (ppr	¥ 🖉	<u></u>		• • •
Imidacloprid	1	0.0100		0*	0.00	
		≫ 0.0100	ð	A (	0,0019	
		<u>0@100</u>	ý ó	Y Y	0,0020	j <u>i</u>
Total Residue	4×	0.0100	, v , v	<u>Č</u>	0.0020	, U
Imidacloprid	Ĩ	`∼∕`0.01¢0	$\sim$	S?	ఎ 0.00 9	, L
5-hydroxy Imida@oprid	ta (1	0.0400	R (	q o	0,0029	~~~~~~~~~~~~
Imidacloprid 🏟 fin 🛒 失	i o'	0.0100	ĩ â	' °ô~	~9.001₹	
Total Residue		້ ບໍ່.0100		<i>Q</i>	<u>0.00</u>	
Imidacloprid 🛛 🖇		_% U.UU7000	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	<u> </u>	<u> </u>	
5-hydroxy Imidacioprid	d de la constante de la consta	0,0010		<u>7</u> _9	0005	
Imidacloprid olefin 📎	- A	<b>0100</b>	) <sup>×</sup> (k,	×		
Total Residue		0.0010	0"	×	0.0005 🔨 🖉	
Inudacloprid	$\sim$ '	ິດທາງ	Q _		0.0003	
S-hydroxy Imidacloprid	ľ _~	0.0010		, ~~~	0.0007	
Imidacloprid olefin 🔍		%0.0010^		K) <sup>®</sup>	0.0006	
🖉 Total Residue	S.	്ര 0.000		æ	0.0007	
Imidacloprid 🖉 🛓		0.0000				
	<i>*</i> 0	NUTUW(			0.0015	
lmi@clopri@olefin 🚿					0.0027	
Total Residue	Ô <sup>v</sup> 4	) 0.0100	A		0.0027	
Y A Q L	' «,	<b>e</b>	<i>S</i> r			
Q Anatyte	N.	LOQ <sup>a</sup> (pp)	ĥ)			n)
		> 0.0100				
5-hydroxy Imidacloprid		00100			0.0025	
		্≪0.0100			0.0025	
َ الْمَعْ Total Residue	L.C.	<u>سُ<sup>×</sup>0.0100 م</u>			0.0025	
Imidacloptid		°∑ 0.0050			0.0006	
5-hydroxy Imidacloprid	× _0×	0.0050			0.0026	
	Å	0.0050			0.0016	
	DQs and LODs Analyte Imidacloprid 5-hydroxy Imidacloprid Imidacloprid olefin Total Residue Imidacloprid offin 5-hydroxy Imidacloprid Imidacloprid offin 5-hydroxy Imidacloprid Imidacloprid olefin Total Residue Imidacloprid olefin Total Residue	Analyte         Imidacloprid         5-hydroxy Imidacloprid         Imidacloprid olefin         Total Residue         Imidacloprid         5-hydroxy Imidacloprid         5-hydroxy Imidacloprid         Imidacloprid olefin         Total Residue         Imidacloprid olefin         Total Residue         Imidacloprid olefin         Total Residue         Imidacloprid         Imidacloprid         Total Residue         Imidacloprid         Imidacloprid         Fatal Residue         Imidacloprid         Imidacloprid         Total Residue         Imidacloprid         Imidacloprid         Imidacloprid         S-hydroxy Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid         Imidacloprid	Analyte       LOG/ (ppt)         Imidacloprid       0.0100         5-hydroxy Imidacloprid       0.0100         Imidacloprid olefin       0.0100         Imidacloprid olefin       0.0100         Imidacloprid       0.0100         Imidacloprid olefin       0.0100         Imidacloprid       0.00100         Total Residue       0.00100         Imidacloprid       0.00100         S-hydroxy Imidacloprid       0.0010         Imidacloprid       0.0010         Imidacloprid       0.0010         Imidacloprid       0.0010         Imidacloprid       0.0010         Imidacloprid       0.0010         Imidacloprid       0.0010         Imidacloprid       0.0010         Imidacloprid       0.0010         Imidacloprid       0.00100         Imidacloprid       0.00100         Imidacloprid       0.00100         Imidacloprid       0.0100 </td <td>OQs and LODs       LO@ (ppm)         Imidacloprid       0.0100         5-hydroxy Imidacloprid       0.0100         Imidacloprid olefin       0.0100         Imidacloprid olefin       0.0100         Imidacloprid       0.0100         Imidacloprid olefin       0.0100         Imidacloprid       0.0100         Imidacloprid       0.0100         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0010         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0100         Imidacloprid       0.0100</td> <td>Analyte       LOG/ (ppm)         Imidacloprid       0.0100         5-hydroxy Imidacloprid       0.0100         Imidacloprid olefin       0.0100         Total Residue       0.0100         5-hydroxy Imidacloprid       0.0100         Imidacloprid olefin       0.0010         Imidacloprid olefin       0.0010         Imidacloprid       0.0010         Imidacloprid olefin       0.0010         Imidacloprid olefin       0.0010         Imidacloprid olefin       0.0010         Imidacloprid       0.00100</td> <td>Analyte         LO@' (ppm)         LO@' (ppm)           Imidacloprid         0.0100         0.0007           5-hydroxy Imidacloprid         0.0100         0.0007           Imidacloprid olefin         0.0100         0.00020           Total Residue         0.0100         0.0029           Imidacloprid         0.0100         0.0009           Imidacloprid         0.0100         0.0009           Imidacloprid         0.0010         0.0009           Imidacloprid         0.0010         0.0000           Imidacloprid         0.0010         0.0000&lt;</td>	OQs and LODs       LO@ (ppm)         Imidacloprid       0.0100         5-hydroxy Imidacloprid       0.0100         Imidacloprid olefin       0.0100         Imidacloprid olefin       0.0100         Imidacloprid       0.0100         Imidacloprid olefin       0.0100         Imidacloprid       0.0100         Imidacloprid       0.0100         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0010         Imidacloprid       0.0000         Imidacloprid       0.0000         Imidacloprid       0.0100         Imidacloprid       0.0100	Analyte       LOG/ (ppm)         Imidacloprid       0.0100         5-hydroxy Imidacloprid       0.0100         Imidacloprid olefin       0.0100         Total Residue       0.0100         5-hydroxy Imidacloprid       0.0100         Imidacloprid olefin       0.0010         Imidacloprid olefin       0.0010         Imidacloprid       0.0010         Imidacloprid olefin       0.0010         Imidacloprid olefin       0.0010         Imidacloprid olefin       0.0010         Imidacloprid       0.00100	Analyte         LO@' (ppm)         LO@' (ppm)           Imidacloprid         0.0100         0.0007           5-hydroxy Imidacloprid         0.0100         0.0007           Imidacloprid olefin         0.0100         0.00020           Total Residue         0.0100         0.0029           Imidacloprid         0.0100         0.0009           Imidacloprid         0.0100         0.0009           Imidacloprid         0.0010         0.0009           Imidacloprid         0.0010         0.0000           Imidacloprid         0.0010         0.0000<

The LOQs and LODs are shown below.

Soil LODS and LOQs are reported in individual analyte equivalents, and no total imidacloprid residue is calculated. All other matrix analyte CODs and LOQs are reported in parent equivalents.

Storage stability studies and pansit spikes indicate that the imidacloprid residues would have been stable during frozer 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



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 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 midacloprid residues in soil showed higher concentrations in the second year of the study for the cotton plot (TRTDA) and lower residues in the second year of the study for the cotton/clover plot (TRTDBowhick received no year 2 application), indicating residues were available for potential aptake by the clover

Executive Summary, Part B

The total imidacloprid residues in/on cotton extrational nectar, floral nectar, and polleo 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 of oliar spray treatment, and residues will decrease rapidly with time. Ľ

The total imidacloprid residues in on closer nector and clover pollen were very low near the LOD of the analytical methods. No pattern & 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 clower pollen and pectar were compile by trial or by trial and year. The highest suppriary 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 LOLULOQ for the total imidacloprid

recommended for exposure risk assessment. For the statistical analysis summarized in the table below, the LOD/LOQ for the total imidacloprid residue (sum of midacloprid) imidacloprid/define and 5 shydroxy finidacloprid) is taken to be the sum of the individual analyse LOD/LOQS.



Overall Results of Summary Statistics									
			5-Hydroxy		Total				
	Selected Summary Statistic	Imidacloprid	Imidacloprid	Imidacloprid	Residuce				
Matrix	(Source)	Olefin (ppb)	(ppb)	(ppb)	(ppb)» 🔗				
Cotton Extrafloral	Median (NT013-12ZA, year 2)	<0.64 <sup>b</sup>	<0.65 b	<sup>©</sup> 10 ⁰	10 <sup>©</sup> 11 ¢				
Nectar	90 <sup>th</sup> Percentile (NT013-12ZA, year 2)	<0.64 b	1.7 d	<sup>18 d</sup>	20 - 21				
Cotton	Median (NT015-12ZA, year 2)	<0.64 <sup>b</sup>	<0.65	11 🖏	1192 4				
Nectar	90 <sup>th</sup> Percentile (NT015-12ZA, year 2)	1.	1.30	28 0	2.4 - 32 <sup>-1</sup>				
Cotton	Median (NT013-12ZA, year 2)	×0.33 <sup>b</sup>	<©48 ⁰	<b>24</b> <sup>d</sup>	2.4 - 320				
Pollen	90 <sup>th</sup> Percentile (NT014-12HA, year 2)	∠<0.33 <sup>▶</sup>	- <b>Q</b> €0.48.♭ °	لم 19⊄ م	19 <sup>©</sup> 20ª				
Clover	Median (NT015-12ZA)	<0.64 b	<0,65 <sup>b</sup>	<0.33°	<u>≤1.6</u>				
Nectar	90 <sup>th</sup> Percentile (NT015-12ZA)	0.64 b	£0.65 °	0.0 d	1.0 - 2.3 d				
Clover	Median (NT015-12ZA)	~0.36 <sup>5</sup>	Q <0.48	<1.00	€ <b>1.8</b> °				
Pollen	90 <sup>th</sup> Percentile (NT015-12ZA	<1.0 ° Õ	<0.48 b . (	200	2.2 - 3				

Median and 90th percentile summary statistics for each analyte were summed to estimate the value a weuen and out out percentile summary statisticy for each analyte were/summary statistic (or possible range of values) for ther summary statistic fot, btat residue (bridaclepind pLP) metabolites). These total residue/media/band 90° percentile (a) leas were considered \$LOD\_fthe corresponding summary statistic/was 4\_DD for all there analyses, <LOD of that summary statistic was<LOQ for all three analyses but a\DD for all there analyses, <LOD if that summary statistic was 2\_LOQ for at least one analyte. In the fotal residue, and quadrifiable fit that summary statistic was 2\_LOQ for at least one analyte. In the fotal residue, with non-quantifiable residues summed in at the shown LOD or LOQ value.

 <</li>
  <</li>
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 (or possible range of values) for that summary statistic for total residue (infidacles rid plus) metabolites). These total residue mediaritand 90% percentile values vere considered LOD if the



<b>Report:</b> Title:	02.02.01/43; 2014 Admire Pro - Magnitude imidacloprid and imidacle following foliar application	; <u>M-503101-01-2</u> of the residues of i oprid olefin in bee	midacloprid and relevant matrice	its metabolites 5-hydroxy or scollected from city trees
Report No.:	EBNTY007			
Document No.:	<u>M-503101-01-2</u>		Q.	
Guideline(s):	US EPA OPPTS/OCSPP	850.SUPP, Ecolog	gical Effects	. 6 <sup>4</sup> 6 <sup>4</sup> . 9
Guideline deviation(s):	none	Ĉa		
GLP/GEP:	yes		Ű	
< <m-503101-01-2@s-602311-01-1< td=""><td></td><td>L,</td><td>ŐÝ</td><td></td></m-503101-01-2@s-602311-01-1<>		L,	ŐÝ	

(1) n

Executive Summary, Part A

A total of three field trials were conducted each year for two successive gears to measure the magnified 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 500 g/L inidactoprid. ADMIRE PRO was ?? applied to citrus trees at target rates and timings as shown below

#### **Target Application Summary**

					$\sim$ $\vee$	•						
				Ś		Rate/Applica		ý ×	Target		Spray 🎗	/olume
				Formu		Active	aredia				°~~	
Plot		Test	No. of	Produc	ct.(fp)		(// h ·	<u> </u>	пети		<b>GPA</b>	LPHA
ID <sup>a</sup>	Year	Subs.	Apps.	fl oz fp/A	mi fp/ha	Name of ai	lb ai/A	g⁄ai/ha	(days)	(days)	min.	min.
UTC	1, 2	NAc	NA	NA 🖇		AMA (	» NA 1	* NA 🏷	NA 🤅	NA	NA	NA
TRTD	1, 2	Admire Pro	2 ~	7,0	512	Imidacloprid	0,25	280.5	8-10	A A	50	468
L					ŵ. (		LO″	Lý,	Ļ	۵ř		

Plot ID: UTC = untreated control plot. TRTD = Treated plot with two pre-bloom foliar spray applications of ADMRIE PRO with an appropriate additive.

PBI = Pre-bloom interval. It necessary, samples may be collected after the second application when b the blooms tart to pen. s L

с NA = Not applicable. Ľ

Plot TRAD received two folia applications of ADXIRE 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 (0.25)4 to 0.2599 b imidacloprid/A/application) in year 1 and from 0.2841 to 0.2908 kg imidacloprid/ha/application, 0.2539 to 0,2594 lb/midacloprid/A/application) in year 2. Total seasonal application rates ranged from 0.56 to 0.577 kg midacloprid/ha (0.504 to 0.515 lb imidacloprid A) in year 1 and from 0.569 to 0.580 kg midacloprid/ha (0.507 to 0.517 lb imidacloprid/A) in year 2. All applications were made between BBCIP 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 Cand between BBCH growth stages 31 and 61 (BBCH 31: beginning of shoot growth, axes of developing shoots visible; BBCA 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 t@70 garA. Arapplications 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 NF004-10ZA were sprayed with Provado, an insecticide containing imidacloprid, in both 2010 and 2011 Additionall Prey 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 imidactoprid added to the plots prior to the study, the residue values are notably higher in this trial.



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 triat NT004/12ZA, when leaves were collected at BBCHs of 55, 60, 61, 65, 67, and 83 (BBCH 55: flowers visible still dused (green hud), borne on single or multiflowered leafy or leafless inforescences; BBCH 89: fruit fue 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 a 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 (initial coord, 5 avdrox) imidacloprid, and initial oprid 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 ordantification (LOQ) for the total residue is the highest LOQ value for an individual analyte in a particular matrix

The limits of detection (LOOs) are shown below

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	K K		~0	
	Summary of LOOS and LO	Ďs S Š Ž .	OY	
44	Matrix 4		✓LOQ <sup>a</sup> (ppm)	LOD <sup>a</sup> (ppm)
	Citri & floworro		0.010	0.0009
	Citrus flowers	5-Hydroxy imidacloprid	0.010	0.0011
	Citiga nowers	Initiacloprid olefin	0.010	0.0033
		Total Imidas loprid	0.010	0.0033
~				
4	Mačijx 🔊	Analyte	LOQ <sup>a</sup> (ppm)	LOD <sup>a</sup> (ppm)
A A A		, midacleorid	0.010	0.0027
	Citizus leaves	5, tydroxy midacloprid	0.010	0.0030
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		Minidactoprid olefin	0.010	0.0030
. Ku		🖉 🏾 🏹 🖉 🖉 🖉 🖉	0.010	0.0030
$\sim$		Q Invidacloprid	0.001	0.0003
	Nectar (	5-Hydroxy imidacloprid	0.001	0.0007
		Anidacloprid olefin	0.001	0.0006
		🏾 👻 🛛 Total Imidacloprid	0.001	0.0007
		Imidacloprid	0.001	0.0004
1 and 1 and	Bollen 5	≪Q <sup>¯</sup> 5-Hydroxy imidacloprid	0.001	0.0005
a,×		Imidacloprid olefin	0.001	0.0003
~(?)		Total Imidacloprid	0.001	0.0005
K, K	Bollen S	Imidacloprid	0.005	0.0012
N a	Soil	5-Hydroxy imidacloprid	0.005	0.0015
	<u>"0" įš</u> y	Imidacloprid olefin	0.005	0.0024
	a Soil LODs and LOQs are reported.	orted in individual analyte equivaler		
°0,	is calculated. All other matrix	analyte LODs and LOQs are repo	rted in parent equi	ivalents.

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



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 (SP G.3.). A statistical evaluation of the total imidacloprid residues in the bee-relevant matrices of pollen and pectar 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 & given in Section 3. & 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 indidacloprid in the surface soil n of the second (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 vear (2 × 0.25 b) ai/ac. The first foliar spray was approximately 18 days see-bloom and the second follar spray was approximately 10 days prebloom. First samples were collected at early boom and there were more more more apple collection prior to petal fall. Due to differences in triads and weather over the 2 years, the first samples were collected as early as 4 days after the final application (D&A) and as late as 30 DAA

- Nectar residues declined over the bloom interval. Oothe six trial-years, for had data sets appropriate to analyze decline. One of the five exhibite on decline in nectar residues; the remaining four exhibited a significant decline, with half-lives ranging from 4 to 7 days. Therefore, nectar acute and chonic 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 form all of the trial-years are presented in the summary table below,
- Pollen residues among the six tral-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 trialyears that exhibited significant decline and based on all sampled residues for those trial-years that did not@xhibiCa significant decline. The highest resulting exposure estimates from all of the trialyears are presented in the summary table below,
- Resodues in both pollen and negar were lower overall in trial-years with a longer interval between the last foliar application and first bloom. Onis suggests that a longer pre-bloom interval for foliar <sup>2</sup> applications way result in reduced overal total imidacloprid residues in pollinator food items.

Citrus (2 x 0,25 lb avac)	Maximum Total Residue <sup>a</sup>	Trial
Pollen of S C S	4100 <sup>°</sup> ppb	NT006-12ZA
Nectars a rev	430 ppb	NT006-12ZA
	Median Total Residue <sup>a</sup>	
Pollen &	2900 ppb	NT005-12ZA
Néctar	290 ppb	NT006-12ZA

#### Ø1 Calculated Acute and Chronic Exposure Values

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.

>>M-503101-01-2@S-602311-01-1

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<b>Report:</b> Title:	Determination of the resid imidacloprid and imidaclo	4; <u>M-505618-01-2</u> lues of imidaclopri oprid olefin in bee	id and its metabo relevant matrices	lites 5-hydroxy
Depart No.	following foliar application EBNTY008	on of imidacloprid	over two success	ve years
Report No.:				N Q
Document No.:	<u>M-505618-01-2</u>			
Guideline(s):	US EPA OPPTS/OCSPP	850.SUPP, Ecolog	ical Effects	
Guideline deviation(s):	none	Ĉa	Å,	
GLP/GEP:	yes	- T		
< <m-505618-01-2@s-602383-01-1< td=""><td></td><td>L</td><td>,Ó¥</td><td>L S S O</td></m-505618-01-2@s-602383-01-1<>		L	,Ó¥	L S S O

#### **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 soll from cherry trees following five foliar applications per year of ADMIRP PRO Systemic Projectant, ADMIRE PRO is a suspension concentration containing 550 g/L imidacloprid. ADMIRP PRO was applied to cherry trees at target rates and timings as shown below.

#### Target Application Summary

					02							
				Formi	lated	Rate/Applica			Target	Jarget	Spray V	
Plot		Test	No. of	Rrodu	ctt∯fp) _	U	$\sim$	$\mathcal{O}_{\mathcal{N}}^{\mathcal{N}}$	Interval	<b>DAA</b>	GPA	LPHA
ID <sup>a</sup>	Year	Subs.	Apps.	fl oz fp/A	ml fp/ha	Name of ai	lb ai/A	🔓 ai/ha	∕(days⊅∂	(days) <sup>b</sup>	min.	min.
UTC	1, 2	NAc		\$, NA O°	NA	NA .	NA 🔬	NAS	NA	NA N	NA	NA
TRTD	1, 2	Admire Pro	5		ي205 ر	midacloprid	$\sim$	\$12 \$		6)5-7	50	468
		ITOAO					A		via a cal			

Plot ID: UTC12/UPC13 = Untreated cordinal plot/UTC printiated 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 = Day Safter application; the last application was targeted to occur 5 to 7 days prior to harvest.
- · NA = Not applicable.

Plot TRTD received five folia 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 (F097 to 0.1149 kg imidacloprid/ha per application (0.0978 to 0.4025 lbumidacloprid/A per application). Total seasonal application rates ranged from 0.560 to 0.569 kg imidacloprid/ha (0.500 to 0.507 lbumidacloprid/A). The first applications were made after cherry havest, at BBCth growth stage 1 (BBCH 91: shoot growth completed; foliage still fully green), and the interval between applications was & to 10 Gays. The spray volumes for plot TRTD ranged from 50 to 10 gal/A.

In 2013, individual application rates ranged from 0.1115 to 0.1145 kg imidacloprid/ha per application (0.0994 to 0.022 lk inidacloprid/Å per application). Total seasonal application rates ranged from 0.560 to 0.564 kg imida loprid/ha (0.499 to 0.503 lb imidacloprid/Å). The first applications were made prior to cherry havest, between BBCH growth stages 73 and 75 (BBCH 73: second fruit fall; BBCH 75: fruit about half find 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

Æ,



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 of 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 5 (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 18 days after the last application (QAA) of years 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 bayes were conjected when the trees were argrow the stages of BBCH 65 and 69 (BBCH 69: end of flowering; all perals fellen), corresponding to 2094 232 DAA in year 1 and 279 to 312 DAA in year  $20^{\circ}$ 

Nine soil samples were collected twice driving the first wear of the study, prio to the first a. . application in 2012 and after the last sampling per plot in 2013, with the exception of trials NC007-12ZA and NT008-12ZA, in which the last leaf collection look place after the year 1, 2013 soll 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 grial NT2017-12ZA, in which the 2004 samples were not collected due to the tree removal.  $\bigcirc$ 

The residues of ADMIRE PRO (imidacloprid, 5 bydroxy imidacloprid and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution gnass spectrometry (JC/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 mantification (LOQ) for the total residue is the highest LOQ value for an individual analyte in a particular matrix

The LOQs and LOOS are summari	zed in the table below.	e an	
Summary of OQs and LO		~ Ø	
Summary of COQS and LO	Qs A A	Ň	
	A Analyte	🖌 LOQª (ppm)	LOD <sup>a</sup> (ppm)
		0.0100	0.0010
	5-Hydroxy Whidacloprid	0.0100	0.0016
	Mimidadopřid olefity ()	0.0100	0.0013
		0.0100	0.0016
Matrix S	Analyte	LOQ <sup>a</sup> (ppm)	LOD <sup>a</sup> (ppm)
Matrix 7	midacloprid	0.0100	0.0020
	5-Hydroxy imidacloprid	0.0100	0.0031
	Imidaclopric ofefin	0.0100	0.0020
	🖉 🖉 Total Imidacloprid	0.0100	0.0031
	midacleprid	0.0010	0.0003
Charu port	5-Hydroxy imidacloprid	0.0010	0.0007
	Imi@cloprid olefin	0.0010	0.0006
	Total Imidacloprid	0.0010	0.0007
	Imidacloprid	0.0010	0.0004
	5-Hydroxy imidacloprid	0.0010	0.0005
	Imidacloprid olefin	0.0010	0.0003
the state of the s	Total Imidacloprid	0.0010	0.0005
	Imidacloprid	0.005	0.0013

5-Hydroxy imidacloprid

Imidacloprid olefin

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.

0 0 0 5

0.005

0.0015

0.0018

Soil



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 pror to extraction.

The imidacloprid residues in soil and cherry leaves, flowers, pollen, and prectar are given in Table 8 (SP C.3.). A statistical evaluation of the total imidacloprid residues in flowers, leaves, and the begrelevant matrices of pollen and nectar is described in Section 3.6.

 $\bigcirc$ A discussion of the imidacloprid residues in soil is even 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, of 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 ALSMIRE PROP Systemic Protectant on both 2012 and 2013 (target was 5 x 0.1 lb imidacloprid/A with & 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 of (BBCH 91, shoot growth completed; for age still fully green). In 2013, applications were made prior to cherry harvest, between BBCH growth stages 79 and 75 (BBCH 73: second fruit fall; BBCH 75: frait 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).  $\bigcirc$ 

In both years, nectae and pollen samples were collected at growth stages of BBCH 61 and 65 (BBCH 61: beginning of flowering, about 10% of flowers open; BBCH 55: full/flowering, at least 50% of flowers open and first petals falling) For the year, lesamples, these growth stages occurred between 205 and 218 days after the fast application (DAA). For the year 2 samples, these growth stages occurred between 274 and 303 DAA. The longer period between application and sompling in year 2 than in year 1 was largely due to the difference in application tiging 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 midactopric residues in negative and pollen were generally similar between BBCH 61 and 65. Additionally, for both nectar and poller, total imidacloprid residues were lower and less variable in year I than in year I. 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.

Cherry (5 x Q Ib a ac)	90 <sup>th</sup> Percentile Total Residue (ppb) <sup>a</sup>	Trial, Year
- Poten , O	660	NT008-12ZA, Year 1
	7.7	NT007-12ZA, Year 1
	Median Total Residue (ppb) <sup>a</sup>	
Pollen	400	NT008-12ZA, Year 1
C Nectar	4.6	NT007-12ZA, Year 1

# ič Exøosure Values

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; 2014; <u>M-506016-01-2</u>
Title:	Determination of the residues of imidacloprid and its metabolites 5-hydroxy
	imidacloprid and imidacloprid olefin in bee relevant matrices collected from bueberries
	following soil application of imidacloprid over two successive years - Admit pro
	systemic protectant (550 g/L) (imidacloprid SC 550 G)
Report No.:	EBNTY006
Document No.:	<u>M-506016-01-2</u>
Guideline(s):	US EPA OPPTS/OCSPP 850.SUPP_Ecological Effects
Guideline deviation(s):	
GLP/GEP:	yes
< <m-506016-01-2@s-602385-01-1< td=""><td></td></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 complex 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.

Targe	et Ap	plicati	on Su	mmary	T O				) D		Ŵ	<i>.</i>
				Q)		Rate/Appl			8			v Volume
				Formu	ulasted ct (fp)冷♀	Active	Ingredie	nt (ai)	Jarget	Parget	Š∕	
Plot		Test	No. of	Produ	င္ရင္ (၂၃) လူန		<u></u>		🗸 » App 🖉			
ID <sup>a</sup>	V											
	rear	Subs.	Apps.	∄)oz fp@A	ml fp/ha	Name of	ai lb ai/	A g ai/ha	Interval	(days)	GPA	LPHA
UTC	1, 2	NA <sup>c</sup>	Apps. NA	ØFJozfp@A N_AA	ml fo)na NA	Name of	ai lb ai/4 /	<b>g</b> ai/ha NA	Interval WA	(days) ≲NA	GPA NA	LPHA NA
	1, 2		NA 🄊	NA C	ŇA	Imidaciopi		NĂ		<b>NA</b>	NA	

 Plot ID: UTC = intreated control plot, size sufficient hold, wo 150-270 ftx 15-20 ft bee tents. TRTD = Treated plot with one post-harvest soil application of ADMIRE PRO, size sufficient to hold five 100-270 ft x 15-20 ft begtents.

- PHI = Pre Prarves Finterval. Single applications to occur 3 dags post garvest on target date of Oct. 1 each year.
- NA = Not applicable

Plot TRTD received one past-harvest soiDapplication 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 kgomidaetoprid/ta/application (0.498 to 0.504 lb imidacloprid/A/application) in year 1 and from 0.599 to 0.564 kg/midaeloprid/ha/application (0.499 to 0.503 lb imidacloprid/A/application) in year 2. The total seasonal application cates 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 range from 19.2 to 20.0 gal/A (180 to 187 L/ha) in year 1 and from 16.9 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



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: flowers adding 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). Blueberre leaves were to be collected at six target sampling periods, when the blueberry plants were between BBCHS 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 )

All samples were collected from within the erected bee tunnels on both plots exception tria T002/12Z when leaf samples taken prior to and after pollen and sectar sampling were collected from the boshes & 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 PRTD and two samples from untreated control plot UTC at each sampling period, corresponding to the sample per erected bee tunnel (also referred to as a subplot). In all trias, not mough plant pratrix materia was present at every sampling interval to allow for the full number of target samples of sampling intervals to be collected (see Appendix 1).

Nine soil samples were collected prior 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 onlosoil samples collected were after sampling in year 2 (no year 1 soil samples).

The residues of imidacloprid, 5-hydroxy inidacloprid and imidacloprid olefin were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (JC/MS/MS) and LC/high resolution mass spectrometry (FC/HRMS) using stable isoppically 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 LOOP 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 LO	Ds & S J	» <sup>*</sup>	
Matrix 7	Ds Analyte Ana	LOQª (ppm)	LODª (ppm)
	midaçîpprid 🖉 🔘	0.0100	0.0036
Plucharry flaw	5-Hydroxy imidacloprid	0.0100	0.0035
Blueberry flowers	Inidaclophid olefin	0.0100	0.0036
	🗸 🖉 Total Imutacloprid	0.0100	0.0036



### Imidacloprid Bee Studies Compilation of Study Summaries

Issue date 2017-11-22

Matrix	Analyte	LOQª (ppm)	LODª (ppm)
	Imidacloprid	0.0100	0.0055 🖉
Plucharry lagyas	5-Hydroxy imidacloprid	0.0100	0.0016
Blueberry leaves	Imidacloprid olefin	0.0100	0.0023
	Total Imidacloprid	0.0100	0.0055
	Imidacloprid	0.0010 🔗	0.0003
Nectar	5-Hydroxy imidacloprid	0.0010	0007 ° 0
Nectar	Imidacloprid olefin	0.0010	
	Total Imidacioprid	020010	<u>0.0087</u> 0 0
	Imidacloprid	<b>6</b> 8.0010	
Pollen	5-Hydroxy imidaclopr	0.0010 🖉	<b>0.0005</b> 0 y
Folieli	Imidacloprid olefin	🔨 0. <b>0</b> 0ຳ0 🦾	J 0.0003 J
	Total Imidacloprid	<u>0</u> .0010	0,0005
	Imidacloprid 🍇 💩 🔊	∞0.0050	©° õ₅õ005 ≪°
Soil	5-Hydroxy imelaclop	~ 0. <b>60</b> 50 ~	
	Imidacloprid olefin	Q.0050	0.0 <b>092</b>

Soil LODs and LOQs are reported in individual analyte equivalents, and no Otal invidual oprior residue is calculated. All other matrix analyte LODs and LOQs are reported in parent equivalents.

Storage stability studies and transit spikes indicate that the imidacloprod residues would have been stable during frozen storage for at least 1080 days (36 months) in blockerry 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 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 nector are given in Table 8 (SP C.3.). A statistical evaluation of the total midacloprid residues in the bee-relevant matrices of pollen and nector is described in Section 3.6.

A discussion of the midactoprid 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 midacloprid concentrations in nectar or pollen in the second year.

# Executive Summary, Part B

For blueberry pectar, oral initiacloped resolues 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.

A

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.

Matrix 😽	Exposure Estimate Type	Selected Summary Statistic (Source)	Total Residue (ppb)
Blueberry		Median (Trial NT003-12ZA, Year 1)	15
Pollen	Or Acute	90th Percentile (Trial NT003-12ZA, Year 2)	23
Blueberry	Chronic	Median (Trial NT003-12ZA, Year 1)	7
Nectar	Acute	90 <sup>th</sup> Percentile (Trial NT003-12ZA, Year 1)	12

# Blueberry Study Summary, Pollen and Nectar (1 x 0.5 lb ai/ac)

>>M-50601 0-2@S-602385-01-1



<b>Report:</b> Title:	02.02.01/46; 2016;	M-544990-01-2 es of imidacloprices collected from	d, 5-hydroxy imion stone fruit trees	dacloprid, and imitacloprid following application of
	imidacloprid over two succe	essive years	2	
Report No.:	EBNTN013		Ş	
Document No.:	<u>M-544990-01-2</u>		104	
Guideline(s):	US EPA OPPTS/OCSPP 85	50.SUPP (Ecolog	ical Effects)	
Guideline deviation(s):	none	Ĉa	\$."	
GLP/GEP:	yes		Ů	
< <n 01="" 1<="" 2@5="" 544000="" 602007="" td=""><td>-</td><td>Υ C</td><td>R</td><td></td></n>	-	Υ C	R	

#### -544990-01-2@S-602997-01-1

A total of nine field trials were conducted to measure the magnitude of imidacloped residues in 8n cherry, plum, apricot and peach (stone fruit) nectar and pollen and in/og stone built leaves following one soil and two foliar applications of Admire Pro® Systemic Protoctant in each of two successive years. Admire Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L imidacloprid. Admire Pro® Systemic Protectant is a suspensive container transferrates and timings as shown oblow.

## Target Application Summary

Julger	Applicatio	- Callin	, <i>"</i>	9. <i>i</i> .		$\sim$	~	C I		/	
			C	Rate	Application	( <b>≵</b> 5%)	ŝ N	ð S		Spray	Volume
			Formu	lated		2	r a	Target			
		Type/	Produ	ct (fp)	Active Ing	redien	t (a 🛵	App.	Target	1	
	Test	Number	≶fl oz`∕	ml	l O	«Ĵb		Interval	©"PHI⊳ <sup>®</sup> >	ł	
Plot ID <sup>a</sup>	Substance	of App.	fp/A	fp/A	Name of a.i.	a.i./A	a.i./ha	(Days)	(Days)	GPA	LPHA
UTC	NAc	NØ	NDA	NA	NA .	NA	NAS	NA	NA	NA	NA
	Admire Pro	Soil / 1	<u>105</u>	769	midacloprid	638	N N	NA (		13,500-	126,358-
TRTD	Systemic	Soil / 1	· / *	¥ '	P _0" 1	N N	<b>426</b>			28,000	262,076
	protectant	Foliar / 🙎	1,7	12	Imidacloprid	0.06	67 0	<sup>∞</sup> 8-16√	7	50-100	468-936

UTC = Untreated control plot.

TRTD = Treated plot Pictor soil and two foilar applications (first foliar 3-5 days after soil application second toliar 13-15 days after soil application).

b PHI = Preprarvest interval. Days listed apply to 2014 normal commercial fruit harvest; in 2013, all applications were made after format commercial fort harvest.

 $\bigcirc$ 

С NA = Not applicable

Applications were made in 2013 and 2019, post bloom Across both years, individual soil application rates were 0.38 lb midacloprid (0.42 to 0.43 kg/ha). The onterval between soil and first foliar applications was 3 to 7 days. For all coliar applications, individual rates ranged from 0.058 to 0.064 lb imidacloprid (0.065 to 0.671 kg/ha). The interval between first and second foliar applications was 7 to 11 days. Application volumes ranged from 13,000 to 26,600 gal/A (GPA) for the soil applications and from 53 to 90 GPA for the foliar appleation. 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 95 shoot growth completed, foliage still fully green; BBCH 99: harvested product). In 2004, sold 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 ope for consumption, fruit have typical taste and firmness).

All applications were made using ground-based equipment. The adjuvant Dyne-Amic was used in all folder applications at a rate of 0.25% v/v, except in trial NT027-13ZA, when a rate of 0.025% v/v was used.  $p_{a}^{O}$ 

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 handprocessed 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 conposite samples (one from each subplot) of cherry, plum, apricot, or peach leaves were collected after booms. once the leaves had expanded, at BBCH 69 to 75 (BBCH 69: end of flowering, all petals fallen; BECH 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 a 155 to 188 DAA. In 2015, flower samples were collected at 211 12 309 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 conjected from the treated plot

After collection, stone fruit flowers were hand processed at a facility near the field site to obtain the beerelevant matrices nectar and pollen. The processed flowers were discarded.

The residues of Admire Pro Systemic Protectant (imj Cacloprid, 5-horroxy, Phidacloprid, and imt Cacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadropole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The individual analyteresidues were summed to give a total imidacloprid residue. ñ

The limits of quantitation (LOQs) and limits of detection (LODs) are shown b Ø

DDs Q Qnalyte		
	LO@(ppm)	LOD (ppm)
Imidacloprid	s 0.005 <i>0</i>	0.0005
5-Hydroxy Indacloprid	r0.00\$	0.0004
Imida clopric olefin 🖉 📎	0.005	0.0016
Total Imidacioprid	0,0005	0.0016
	0.001	0.0003
5-Hydroxy imidaclogrid	0.001	0.0007
Imidacloprid olefin	مَرْ 0.001	0.0006
	0.001	0.0007
Whidactoprid K	y 0.001	0.0004
5-Hydroxy invidacloprid	0.001	0.0005
Imine a clopula olefin	0.001	0.0003
Tota Imidacloprie	0.001	0.0005
	5-Hydroxy incracloprid Imidacloprid Total Imidacloprid Inidacloprid 5-Hydroxy imidacloprid Imidacloprid olefin	5-Hydroxy interactorial       0.005         Imidactorial       0.005         Total Imidactorial       0.001         5-Hydroxy imidactorial       0.001         5-Hydroxy imidactorial       0.001         Midactorial       0.001         5-Hydroxy imidactorial       0.001         Midactorial       0.001         Foral Imidactorial       0.001         Midactorial       0.001

Storage Stability studies indicate that the inordacloprid residues would have been stable during frozen storage for at least 1080 days (36 months) in store fruit leaves prior to analysis. Transit spikes showed that imidacloprid residues were stable in polled 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.

Õ ×1 A summary of the total midactorial residues grouped by year and for the overall study is shown in the table below.



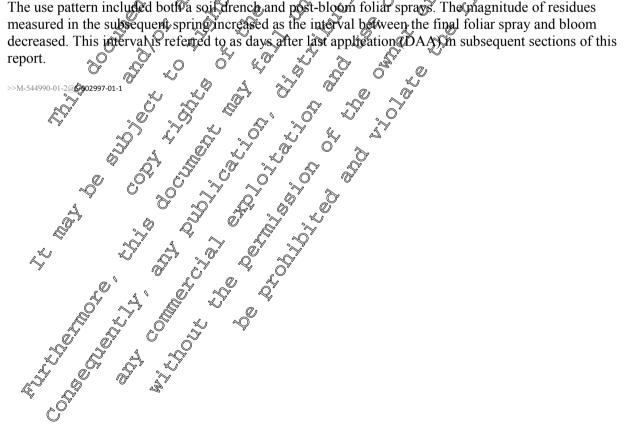
Summ	Summary of Residue Data for Imidacloprid in/on Stone Fruit, All Trials												
>			)a	c		Total Imidacloprid Residue Levels (ppm)							
Commodity	Plot Name	Sampling Year	DAA (days) <sup>a</sup>	Seasonal Application Rates (Ib a.i./A)	n°	Min	Мах	90 <sup>th</sup> Pars entile	Median	Mean	Standard Deviation	8	
		2014	133–160	0.50-0.51	18	<lod< td=""><td>0.034</td><td>016</td><td>0.0030</td><td>0.00</td><td>0.010</td><td></td></lod<>	0.034	016	0.0030	0.00	0.010		
Nectar	TRTD	2015	211-291	0.50-0.51	16	_ CODD	0.011	0.006	0.001	0.002	0 903	lø	
		2014, 2015	133-291	0.50-0.51	34	<lod< td=""><td>0.03</td><td>0.009</td><td><b>@002</b></td><td><b>9.005</b></td><td></td><td></td></lod<>	0.03	0.009	<b>@002</b>	<b>9.005</b>			
		2014	133–160	0.50-0.51	1	0.013	\$ 84	<sub>0</sub> .13	0.034	0.069	0.086		
Pollen	TRTD	2015	211-309	0.50-0.51	<b>G</b> é	0.002 🎮	1 0.	0.09%	Q.009	0,033	0,054		
		2014, 2015	133-309	0.50-0¢51	<b>30</b>		0.34	J. 0, 13	@.027°^	0.050	0.072		
		2014	155–188	0.50-0.51	AS	0.002	0.28	0.21	0.026	0.000	0,085		
Leaves	TRTD	2015	230-323	0.50-0.51	/18	9.006	0.20	0 ලි	0.021	<b>0</b> .058			
		2014, 2015	155-323	@.50 <b>-0</b> :51	<b>36</b> C	0.002	028	v 0.19 🖉	0.023	0.053	0.078		

a DAA = Days after the last application.

R For the purpose of calculating the total midacio prid residues any mividue analyse value reported as b <LOD was summed into the total at a default value equal 1/2 the LOD.

С n = Number of individual treated samples analyzed. The ear of alues epresent the target number of samples to be collected; Where the n value is lower, samples Were composited or vere of insufficient ~ ~ size to analyze. Ŵ n  $\bigcap$ 

Differences in pollen and nector residue levels do the 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





Report:	02.02.01/47; (2016; M-544778-01-2)	0
Title:	Determination of the residues of imidacloprid and its metabolites 5-hydroxy	
	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 ye	
Report No.:	EBNTN014	
Document No.:	<u>M-544778-01-2</u>	
Guideline(s):	US EPA OPPTS/OCSPP 850.SUPP, Ecological Effects	29° . 19
Guideline deviation(s):	none	Y Q
GLP/GEP:	yes a a a a a a a a a a a a a a a a a a a	<u> </u>

#### <<M-544778-01-2@S-602888-01-1

A total of nine field trials were conducted to measure the magnitude of imidacloped residues in apple nectar and pollen and in/on apple leaves following one soil and two foliar apploations of Admire Pro® Systemic Protectant in each of two successive years.

Admire Pro Systemic Protectant is a suspension contrate 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 Summarv

	repricatio			a			(C) .			$\sim$	
			d	<b>Rate</b>	Application Active lag	(±5%)	Č	õ.		Spray	Volume
			Form	lated		5° ~		Target		Ŵ	
		Type/	Produ	ct (fp)	Active lag	redien	t (a.i.)	App.	Target		
	Test	Number	fl oz	ml	W S	l hay		Interval	<b>∖©HI</b> ⊳ (		
Plot ID <sup>a</sup>	Substance	of App 🕄	, fp/A`^	∕fp/A	Name of a.i.	a i./A	a∕i.∕ha	(Days)	<b>₽Days</b> }	GPA	LPHA
UTC	NAc	NA 🖑	N/A,	NAC	ANA @	, NA ·	NA 🌣	🕺 NA 🖏	NA	NA	NA
	Admire Pro	Soil 71	10.5	<b>\$68</b>	Invacloprid	0,38	426		401	13,000-	121,678-
TRTD	Systemic		1	\$00				₩°	2 <sup>1</sup>	27,000	252,716
	protectant	Køliar / 2	<sup>*</sup> "1.7	<sup>•</sup> 124 <sup>·</sup>	midacoprid	0.06	67	🔬 10 🖕		50-100	468-936
		· · · · · · · · · · · · · · · · · · ·		@.	· ~ ~ ~	0		* <u> </u>			

Plot ID: UTC = Critreated control plot a

TRTD Treated plot K,

PHI = Pre-harvest interval the period between application and commercial apple harvest. For application in 2000 only if application could not be made goor to commercial apple harvest, it was acceptable to apply after apple harvest using the same application thereas.

NA = Not applicable,

Applications were made in the fall of 2013 and 2094, 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 "Jo are reported from this trial. Ő

Across both gars, 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 0,039 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 (GRA) for the sol applications and from 55 to 75 GPA for the foliar applications. Total seasonal application rates ganged from (950 to 0.52 lb imidacloprid/A (0.56 to 0.58 kg/ha). In 2013, trials NT031-122A and NT036-13ZA made applications prior to apple harvest, while the other trials made all applications post-karvest. Soil applications were made at BBCH growth stages 79 to 99 (BBCH 79: fruit about 90%/final Dize; BBCH 99 harvested product), and the two foliar applications were made at BBCH growth stoges 8 40 99 BBCH 81: beginning of ripening, first appearance of cultivar-specific color) and 85 (599 (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 I 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 pring of 2014 following the fall 2013 applications, and once in the spring of 2015, following the fall 2614 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 handprocessed to obtain apple nectar and pollen) and apple leaves were confected 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 (BBCH0/1: 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 DAO. In 2015, apple flower samples were collected at 131 to 287 DAA, and apple leaf samples were collected at 147 to 293 DQA.

Single composite samples of apple flowers and leave 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 btain the bees relevant matrices of apple nectar and posten. The processed towers were docarded. ×

The residues of Admire Pro Systemic Projectant amidacloprid, 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 (LQQs) and limits of detection (LODs are shown below.

N

Analyte O	🗸 🖓 Q (ppm)	LOD (ppm)
Imitraclop@d 🏑 🦄	الم <sup>(</sup> 0.001	0.0003
	≫ 0.001	0.0007
imida@oprid.olefin 🖉	م <sup>م</sup> 0.001	0.0006
Jotal Invdacloprid	0 <sup>°</sup> 0.001	0.0007
Imidacloprid & O	0.001	0.0004
S-Hydroxy imidacloprid	0.001	0.0005
Îmidaciopridolefin 🥎	0.001	0.0003
Total Imidacloprid	0.001	0.0005
	0.005	0.0009
9-Hydroxy imidacloprid	0.005	0.0005
Imidaciopric oletin	0.005	0.0008
Votal Imidacloprid	0.005	0.0009
	Imitaclop0d Hydroxy imidacloprid Total Imidacloprid Hydroxy imidacloprid Hydroxy imidacloprid Imidacloprid Total Imidacloprid Total Imidacloprid Midacloprid Hydroxy imidacloprid Hydroxy imidacloprid	Imidaclopud     0.001       Hydroxy imidacloprid     0.001       Imidacloprid     0.001       Total Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.001       Imidacloprid     0.005       Imidacloprid     0.005       Imidacloprid     0.005       Imidacloprid     0.005       Imidacloprid     0.005

Storage stability studies indicate that the imidacloprid residues would have been stable during frozen storage for at the storage for a imidaelopris residues were stable in pollen and nectar for the duration of the study. The maximum storage period of Pozen samples in this study for Admire Pro Systemic Protectant was 413 days for apple leaves and 208 days for apple nectar and pollen.

A summary of the residues grouped by year and for overall study is shown in the table below.



Summ	Summary of Residue Data for Imidacloprid in/on Apple, All Trials										
<b>`</b>			a	_		Total In	nidaclop	rid Resid	ue Leve	els (ppm	
Commodity	Plot Name	Sampling Years	DAA (days)	Seasonal Application Rates (Ib a.i./A)	n°	Min	Max	90 <sup>th</sup> performatile	Median	Měže Měže	Standard Deviation
		2014	138-193	0.50-0.52	17	0.001	0.036	003	0.0010	I 0. ≤	0.008
Nectar	TRTD	2015	131-287	0.50-0.52	16	<b>0</b> 001	0.004	0.003	0 001	0.002	<b>Ø</b> 001 @
		2014, 2015	131-287	0.50-0.52	33(	<lod< td=""><td>0.036</td><td>0.003</td><td>Ø.001</td><td>0.003</td><td>0.000</td></lod<>	0.036	0.003	Ø.001	0.003	0.000
		2014	138-193	0.50-0.52	Å8	0.001	Q047	0.030	0.015	0.096	0.012
Pollen	TRTD	2015	131-287	0.50-0.52		0.002	0.10	0,089	015	0.033	0.035
		2014, 2015	131-287	0.50-0.52	Ø	0.601	Ø10	0.057	0.015	0.024	0.027
Leaves TF		2014	151-214	0.50 0.52	718,	0.001	90.060	0.031	0.009	<b>Ø</b> Ø14	Ø <u>.</u> 017
	TRTD	2015	147-293	\$50-0.52	16	0.002	36		0.015	0.45	1.1
		2014, 2015	e e	0.50+0.52	34	<b>00001</b>	~3.6	0.11	0.002	0,22	0.81

DAA = Days after the last application.  $^{\prime O}$ а

For the purpose of calculating the total imidaclopric residues, any individual analyte value reported as <LOD was summed into the total at a default value equal to 1/20 E LOD. b

С

n = Number of individual use ated samples analyzed. The next highest residue from 2015 leaf samples in that NT029-132A was 0.004 ppm. d

Differences in pollen and nector residue levels do jor appear to be related to the differences in soil types. The use pattern included both a soit drenct and post-bloom foliar sprass. 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 (D

$$\begin{array}{c} D(\mathbf{A},\mathbf{A}) \\ \text{M-544778-01-2} \left( \mathbf{b}, \mathbf{C}, $



Report:	02.02.01/48; ; 2016	; M-525733-02-2		
	02.02.01/48,, 2010	$, \frac{101-323733-02-2}{102-2}$		
Title:	Determination of the residu	ues of imidaclopri	d, 5-hydroxy imi	idacloprid, and imidacloprid
	olefin in bee relevant matr	ices collected from	n cotton during t	wo successive years -
	Admire Pro Systemic Prot	ectant (550 g/L) (i	midacloprid SC?	\$50 G)
Report No.:	EBNTN011-01		Ş	
Document No.:	<u>M-525733-02-2</u>		10-	
Guideline(s):	US EPA OPPTS/OCSPP 8	350.SUPP (Ecolog	ical Effects)	. Ö <sup>r</sup> . S <sup>r</sup> . 1 <sup>Q</sup>
Guideline deviation(s):	none	Ĉa	de la companya de la companya	
GLP/GEP:	yes		Ű	
		e	×	

#### <<M-525733-02-2@S-602387-01-1

A total of nine field trials were conducted to measure the magnitude of imidacloped residues in Beerelevant cotton pollen and nectar samples and in/on cotton leaves following for applications of Admire Pro Systemic Protectant in each of two successive years, Admine Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L initidaclopfed. Admire Pro Systemic Protectant was applied to cotton at target rates and timings as shown below.

#### Target Application Summary

	Application		· · · · · <b>J</b>	0.1		ala	$\bigcirc$		1 V	N.	450
				Rate	Application (±	Target	Ũ,	🖓 Sp	Ray		
		Type/	c	O¥ (			ð "	App \$	Target	, Ô	
Plot	Test	Number	fl oz	r ml "	Name of active	lb 🤇	g a.iØ	Interval	DAA	≪∫ĩ	
ID <sup>a</sup>	Substance	of Apps.	fp/Ã∕∕	fp/ba	ingrødient (a.i.)	a∱∖A	ha	(Days)	(Days)	GPA	LPHA
UTC	NAc	NA	A A	₅_NA	NAS .	<b>W</b> Å	A A	WA 🏾	NA §	<b>⊘NA</b>	NA
TRTD	Admire Pro Systemic Protectant	Soil / 1	9.20	673 673		0.329	370			10 - 20	94 - 188
	Admire Pro Systemic Protectant	Fotiar / 3	9.59×	<b>9</b> 116	imidaçioprid	0.057	64 % 0		14	10 - 20	94 - 188

Plot ID: UTC = Intreated control plot

TRTO = Treated plot receiving an in-furrow spray application at planting followed by three foliar spray applications with an application interval of \$ 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.

- DAA = Days after application, the number of days between the most recent application and sample b collection.
- NA = Not approable

Only the first year of trial TO could be completed and reported because the plot location was no longer avaffable.

Plot TRTD received one soft (in-farrow) opray application of Admire Pro at planting (BBCH 00: dry seed) followed by 3 equivalent Admire Bro for a 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 follow application rates langed from 0.063 to 0.067 kg imidacloprid/ha/application (0.056 to 0.060 16A). All folia applications were made between BBCH growth stages 61 and 72 (BBCH 61: beginning of flowering; BBCP 72: about 20% of bolls have attained their final size). The interval between folia 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.



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 potten, floral nectar, and extrafloral nectar) and cotton leaves were collected from the treated ots 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 begrelevant samples of cotton pollen, floral nectar, and extrafloral rectar. The processed flowers were discarded.

The residues of Admire Pro Systemic Protectant and indecloprid, 5-hydraxy imidacloprid, and imida doprid olefin) were quantitated by high performance liquid chromatography triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LO/HRMS) using stable isotopically labeled internal standards. The individual analyte residues were summed @ give a total imidacloprid residue.

The limits of quantitation (LOQs) and fimits of detection (CODs) are shown bet

Summary of LOQs and L	DDŝ <sup>w</sup> 🧔 🧟		×° Ŭ
Matrix	Analyte 🔗	⊆LOQ((ppm) =	⊖~ Loop (ppm)
	imidacloprid 🛇	(9.005, Q	9h 0012
Cotton leaves 🐧 🖗	5-Hydroxy inidacloprid	′ °0.005 ×	_ <i>©</i> 0.0007
	Imidaclopid olefin (		0.0008
	Total Imidacioprid	$\bigcirc$ $\emptyset.005$	<b>0.0008</b>
, contraction of the second second second second second second second second second second second second second	Imidacioprid	Y ( <b>0</b> .001 / )	≫ 0.0003
Cotton extraflor prectat	5-Hydroxy midacloprid	0.001	0.0007
	linudaclorshid oletin	<u>ک</u> 0.001	0.0006
			0.0007
Cotton floral nectar	Imid@lopri& 2	y .001	0.0003
	5-Hydroxy imidacloprid	0.001	0.0007
	knidaciopho olettin ~	$\alpha_{\mu} = 0 \alpha \delta^{\mu}$	0.0006
	🖓 Total Imidacloprid 🛛	\$ <u>6</u> 001	0.0007
	1migacioprige 🔊 🔊	ຼ້ 🔊 0.001	0.0004
Cotton notion		A 0.001	0.0005
Cotton potten	Lefidacleoprid olefin	ک 0.001	0.0003
	Total Imidacloprid	§ 0.001	0.0005

Storage stability studies indicate that the midac opride sidues 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 poller and notar 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 poller, and 211 days for cotton floral and extrafloral nectar.

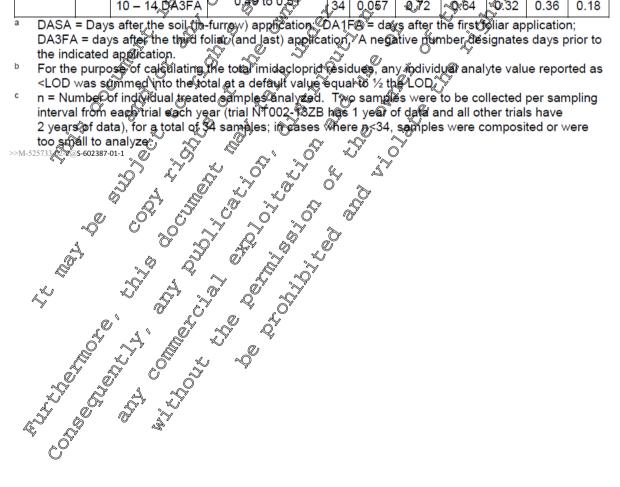
A summary of the residues is shown in the table below.



Issue date 2017-11-22

Summary of Residue Data for Imidacloprid in/on Cotton											
		<u>_</u>	_		Imida	cloprid	Residue	Level	s (ppm)	) <sup>b</sup> 🥠	
Commodity	Plot Name	Days after the Application <sup>a</sup>	Application Rates (Ib a.i./A)	n°	Min	Max	90 <sup>th</sup> Pargentile	Median	Mean	standard Deviation	
Floral		70 to 95 DASA (-4 to -5 DA1FA)	In-furrow application: 0.32 to 0.34	32	0.001	0.13	050	0.007	1 1	0.027.	
Nectar	TRTD	4 – 5 DA3FA	Total seasonal rate:	38	∲0.012	0.17	0.13	0.000	0.075	0.043	
		10 – 14 DA3FA	0.49 to 0.51	\$3	0.010	<b>£</b> 92	0.069	6035	<b>0</b> 040	<b>S</b> 027	ĸ°
Extroflorol		70 to 95 DASA (-4 to -5 DA1FA)	In-furrow application: 0.32 to 0.34	28	0.001	0.036	0.01			0.008	
Extrafloral Nectar	TRTD	4 – 5 DA3FA	Total seasonal rate:	33	0.047	,2,8	<i></i> 9.5	028	° <b>,0,5</b> 6	0,62	
		10 – 14 DA3FA	0.49 to 0.51	33	0.008	ð.14 ð	0.090	0.027	0.040	0.033	ρ
		70 to 95 DASA (-4 to -5 DA1FA)	In-furrow application: 0.32 to 0.34	34	0.081	0.043	0.014	0,001	0.006	0,010	
Pollen	TRTD	4 – 5 DA3FA	Total seasonal rate	34	0,004	ن 2.9 ر <u>ک</u>	0.38	0.046	0.26	00.58	
		10 – 14 DA3FA	0.49 0.51	34∕	0.003	0.18	0,19	0.05	0.050	0.058	
		70 to 95 DASA (-4 to -5 DA1F	In-furr@v appl@ation:	934 234	0,001	5.36	0.11	C,		0.068	
Leaves	TRTD	4 – 5 DA3 <b>FA</b>	Total seasonal rote:	34	0.48	3.2	23	1.5	1.6	0.68	
		10 – 14 DA3FA	0 0,49 to 0,54	34	0.057	Ø.72	~0.64	<b>0.32</b>	0.36	0.18	

DASA = Days after the soil the furrew) application DA1F® = days after the first value application; DA3FA = days after the third foliar (and last) application A negative number designates days prior to the indicated application





<b>Report:</b> Title:	02.02.01/49; 2016; M-525735-02-2 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 - Amire Pro Systemic Protectant (550 g/L) (imidacloprid SC 550 G)
Report No.:	EBNTN012
Document No.:	
Guideline(s):	US EPA OPPTS/OCSPP 850.SUPP (Ecological Effects)
Guideline deviation(s):	none v u u u
GLP/GEP:	yes

#### <<M-525735-02-2@S-602394-01-1

A total of nine field trials were conducted to measure the magnitude of indidacloprid 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. Admir Pro Stemic Protectant was applied to tomato at target rates and timings as shown below? tomato at target rates and timings as shown below?

Target	Application	on Sum	mary	Q	× ×		Ŵ		<u> </u>	Ŝ	0
		Type/		Rate/Application (±5%)				Target	S L	Spray	/olume
		Number	Form			, j	Ŝ,	App.	Target	Z°	
Plot	Test	of	Prodů		Active Ingre	dient	(a.i.)	Interval	<b>RAA</b> <sup>b</sup>	<sub>⊘</sub> GṔA	LPHA
ID <sup>a</sup>	Substance	Apps.	ft oz/A	ĥn∦ľha	Name of a.i.	<b>Ib</b> (Å	g/ha	(Days)	(Days)	min.	min.
UTC	NAc	NA <sup>°</sup>	<sup>⊘</sup> NA⊛	NA	NA ,	NA	Ň	^∧NA (	NA 🔊	NA	NA
	Admire Pro Systemic Protectant	Soil	0° 140.5	768 Ø		0	<sup>\$</sup> 422 <sup>\$</sup>	NAC	See belowd	NA	NA
	Admire Pro Systemic Protectation	Foliar (2	1.75 1.75	ж.	Imidaclopric	0.06 0.06	0 268		y ∀ See below <sup>d</sup>	50	468

Plot ID: UTC Untreated control slot.

TOT D = 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.

DAA Bays after application withe number of days between the application and sample collection. b NA Not applicable С

The PFI permanently marked the plots before planting. The soil application occurred 5-7 days after the tomato seedings were transplanted (June July) The first foliar application was made at target 1 day after the second sampling of collen and leaves (approx. 40-60 days after soil application). The d second foliar application was mad 4-5 days after the first (approx. 45-65 days after soil application). Ô

Across all trials and years plot TRTD received one soft (in-furrow) drip/drench application of Admire Pro 5 to Zdays after tomato transplantation followed by 2 equivalent Admire Pro foliar spray applications per planting season. Ordividual 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/hay. 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 00 101 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).

Alf 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.



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 TROD tunnel was erected. Bumble bee (Bombus impatiens) colonies (1 to 3 per tunne) were placed in each tunnel for the collection of pollen. One sample was collected per bee tunnel, were placed in each and one UTC sample at each sampling interval, except in trials NT013-13ZA, NT040-13ZA, and NT040-13ZA, and NT040-13ZA, 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 trom the flowers in the field due to a bee shortage.

Tomato leaf and pollen samples were collected at four sampling intervals each year. Wo samples vere 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 torage 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 brough 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 ten were composited together the 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 sample collected from the treated plots.

The residues of Admire Pro Systemic Protectant (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance inquid 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 (DOQs) and limits of detection (LODs) are shown in the following table.

Matrix ~	Analyte 🛇	LOQ (ppm)	LOD (ppm)
Ky Ly By	Ingidiaclophid	0.005	0.0022
Tomoto Idouvos	B-Hydroxy imidaeloprid	0.005	0.0007
Tomato leaves	midacioprid ofefin	0.005	0.0010
Polizo	<b>₹</b> øtal Imidacloprid	0.005	0.0022
	Imidaclophd	0.001	0.0004
Pollen	S Hydroxy imidacloprid	0.001	0.0005
	Imidaalaprid alafin	0.001	0.0003
	Total Imidacloprid	0.001	0.0005

Summary of LOQs and LODs 🖉 🔊

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

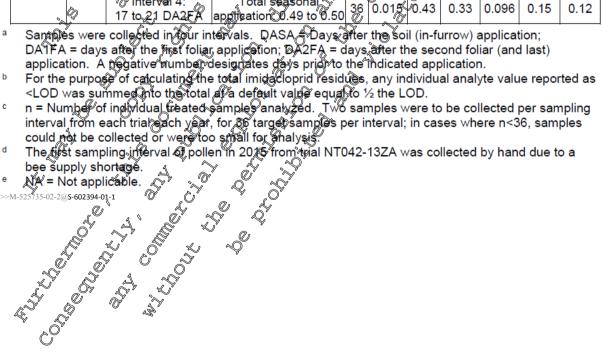


maximum storage period of frozen samples in this study for Admire Pro Systemic Protectant was 561 days for tomato leaves and 560 days for tomato pollen. 

A summary of the residues is shown in the table below

A	summary	of the	residues is shown	in the table below.				~		(	5	O,
	Summa	m. of	Paaidua Data fa	r Imidaalaarid in/aa	Tai	moto		, G	2	Ś		)
1	Summa	i y oi i		r Imidacloprid in/on			loprid	Pocid		els (pp		Ro
			u <sup>a</sup>	Ę		innuac		Resid		0	27	S
		Ĕ	ng I, atic	A) atic	6			tile	Ł		์ ย 🄊	, Qı
	rix	Ň	ilqr s a lica	ses ses			Q,	cen	À		iate	Å
	Matrix	Plot Name	Sampling Interval , Days after the Application	Application Rates (Ib a.i./A)	'n	Min	Max	ି ୦ 90 <sup>th</sup> ଜିତ୍ର percentile	Media	Mea	Standard	\$ <u>,</u>
			Interval 1:	In-furrow application:			ġ,	Ő.	Š	¢.	0.097	I A A A A A A A A A A A A A A A A A A A
			31 to 68 DASA	0.37 to 0.38	30	0,007	029	0.18	0.041	0.0724	0.077	
			(-20 to -10 DA1FA)	<u> </u>	Ŵ			- 4		°~~	K,	
	Pollen		Interval 2: 45 to 77 DASA	In-furrow application:	27	0.002	0.0	0.096	0.030	0.040	0.036	o
		TRTD	(-3 to -1 DA1FA)	0_37 to 0@8 _ C	, <b>~</b> '		0. pr	\$	0.00		Ū	
	collected)		Interval 3:	Total seasonal	26			$\approx$		0.59	<b>3</b> .40	
			2 to 8 DA2FA	application: 0 49 to 0.50	State of the second sec	0.25	(~ n	×1.0		, ,	J <sup>⊎.40</sup>	
			Interval 4:	,O <sup>™</sup> Total seasonal	32	0,017	() () () () () () () () () () () () () (	<b>C</b> S	0.066	0,0	0.064	
			14 to 22 DA2FA	application: 0.49 to 0.50		6 <sup>7</sup> (			No.	~~~~		
	Pollend	тото	Interval I:	NT042-137A In-ferrow	¢ G	R (? )	0.68	NA		/ NA	NA	
	(hand- collected)	TRTD	58 DASA (-7 DA1EA)	2015 application. 0.38	γ <b>z</b>	0.53	0.08	INA O		/ INA	INA	
	conected)		Interval 1:						Ô			
			31 to 63 DASA	In-therew application:	36	0.002	0.65	0.44	0.13	0.18	0.18	
			(-20 to -7 DA4KA)	0.37 0.38	Ô	<b>%</b> ,	~	. 6	<sup>v</sup>			
			Interval 2:	In-furrow application	1	$\bigcirc^{\nu}$	) Z	s.				
	Leaves	TRTD	45 to 77 DASA	~0.37 to 0.38	36	0.005	<b>9</b> .50	0.35	0.10	0.15	0.14	
		Å	Sto DA1FA		Ş							
		8	4 9 8 DA2FA	ू Totalseasonal application: 0,≰9 to 0.50	36	0,045	6.2	3.3	0.73	1.2	1.3	
		ĨÕ	Vinterstal 1:	Total angenet	(							
	٩,	Ô	17 to 21 DA2EA	application 0.49 to 0.50	36	0.015	0.43	0.33	0.096	0.15	0.12	
_ '		1			Cy .							

- b
- С
- d





Report:	02.02.01/50; 2016; <u>M-559999-01-2</u>
Title:	Amended final report - Field collection study to evaluate total imidacloprid residue
	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.:	<u>M-559999-01-2</u>
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

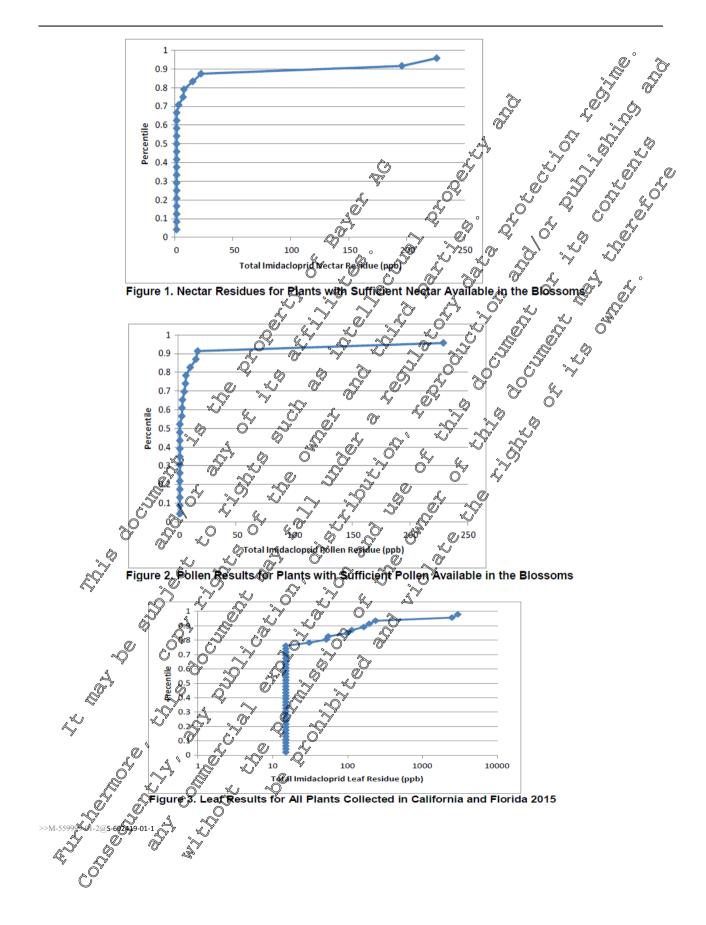
The purpose of this study was to determine the amount, if any, of imida popridor the follen, nectar, and leaves of annual or perennial flowering bedding plants in the live goods retail section of The Home Depot (Atlanta GA, 30339 USA) stores. There was no active reatment of the bedding plants with imidacloprid products included in this study; the purpose of the study was to impartially selectblooking 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 invidacloprid olefin and 5-hydroxy imidacloprid.

The plants for sampling in this study were collected in two State Florida and Salifordia. Five stores were visited for each trial with at least 4 different plant species purchased pecstore

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 Fotal indacloprid results of LOQ were given a value of 1/2 the LOQ for the purposes of determining these supernary statistics?  $\sim$ \_@

	یے	S d	^^^ ^^ ^ ^ ^ ^ ^	o <sup>∞</sup> ∕Max	<u> </u>			
Matrix		Results	(nnk)		90 <sup>th</sup> percentile	Median	Mean	Standard deviation
				Y OF T		<i>(</i> )		
	¢ 23		£002	(ppb) ( 226)	0127	, ¿LOQ	21.8	60.1
Pollen	22					<loq< td=""><td>14.5</td><td>48.1</td></loq<>	14.5	48.1
Folien	<u> </u>			Ox722	<u> </u>		14.5	40.1
Leaf	45	35 20 20 20	<loq ×LOQ</loq 	2947		<loq< td=""><td>153</td><td>561</td></loq<>	153	561
	*			2947 2947				







Report:	02.02.01/51; ; 2016; <u>M-559994-01-2</u>
Title:	Amended final report - Field collection study to evaluate total imidacloprid residue $\sim$
I IIIC.	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
Culueline(0):	blooming bedding plants from retail garden centers and in field planted blooming bedding plants US0593 <u>M-559994-01-2</u> Based on EPA et al. Guidance for Assessing Risk to Bees 2014 OCSPP 850.SUPP (Ecological Effects) none yes idy was to determine the amount, if any of imidacloprid in the pollen nector and rennial flowering bedding plants in the live goods retail section of The Home Depot per plants after being plants in the live goods retail section of The Home Depot
Guideline deviation(s):	none
GLP/GEP:	yes and the second seco
< <m-559994-01-2@s-602409-01-1< td=""><td>idy was to determine the amount, if any of imidacloprid in the pollen nector and</td></m-559994-01-2@s-602409-01-1<>	idy was to determine the amount, if any of imidacloprid in the pollen nector and
The purpose of this stu	and was to determine the amount, if any of immacrophic in the police, nectar, and
leaves of annual or per	ennial flowering bedding plants in the five goods retail section of The Home Depot
stores, and in those sar	ne plants alter being planted and wed to be blog in under neite conductors. There
was no active treatmen	nt of the bedding plants with mida doprid products included in this study; the
	as to impartially select blooming plants on the tetail garden centers and analyze for
potential residues. The	imidacloprid tofal residue method includes the parent imidacloprod and the bee-
relevant analytes imida	acloprid olefin@nd 5_hydrox&imida@foprid
The plants for use in th	nis study were collected in two regions greater Raleign area in North Carolina and
greater Kansas City ar	ea in both Kansas and Missour. Five Home Depot stores were visited for each trial
with at least 4 differen	t plant species purchased per store during April and May 2015.
with at ioust i uniteren	
Composite samples of	pollen and/or nectar and leaf matrix were collected from a least 20 individual
nlants per species. Les	wes and blossoms were collected from the canops of the plants. Pollen was
plains per species. Lea	soms using a vacuum pump and a filter pipette up. Nextar was collected from
	soms using a vacuum pullop and a mile pipelle up. Neotar was conected nom
biossoms using a revol	r 26 aL capillary tube. The quarkity 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
	The plants were then fransplanted and allowed to re-bloom under field conditions.
	ants were transplanted to sarden plots in Clayton, NC at a Bayer site; and the
	plants were transplanted to garden plots in Stifwell, KS at the SynTech site.
Sampling of pollen, no	ztar, and leaves was then repeated approximately 4 weeks later.
· /	
Results from the stordy	show total leaf imidacloprid residues in North Carolina retail garden centers
ranging from <loo -<="" td=""><td>2.3 pb for pre-planted flowers and <loq 85.7="" after="" flowers="" ppb="" td="" were<="" –=""></loq></td></loo>	2.3 pb for pre-planted flowers and <loq 85.7="" after="" flowers="" ppb="" td="" were<="" –=""></loq>
transplanted. Mectar re	sidues for pre-planted flowers ranged from <loq td="" –<=""></loq>
353 3 and all total imit	dactoprid residues for negar in post-planted flowers were <loq. pollen="" residues<="" td=""></loq.>
	35.3 ppb th pre-planted flowers and <loq 6.3="" ppb<="" td="" –=""></loq>
after flowers were not	

after flowers were planted.

Results for retail garden onters in the greater Gansas 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 sumpting of the residues are shown in the table below. The pre-planting results for each matrix (nectar, poller or leat), 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 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

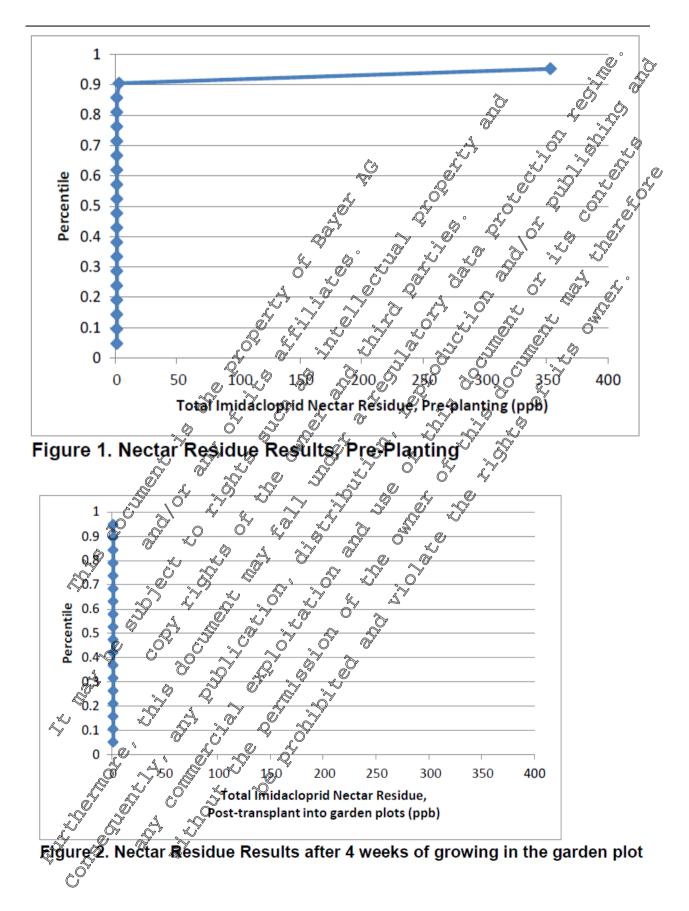
Please click on the hyperlink to order a Study Report.



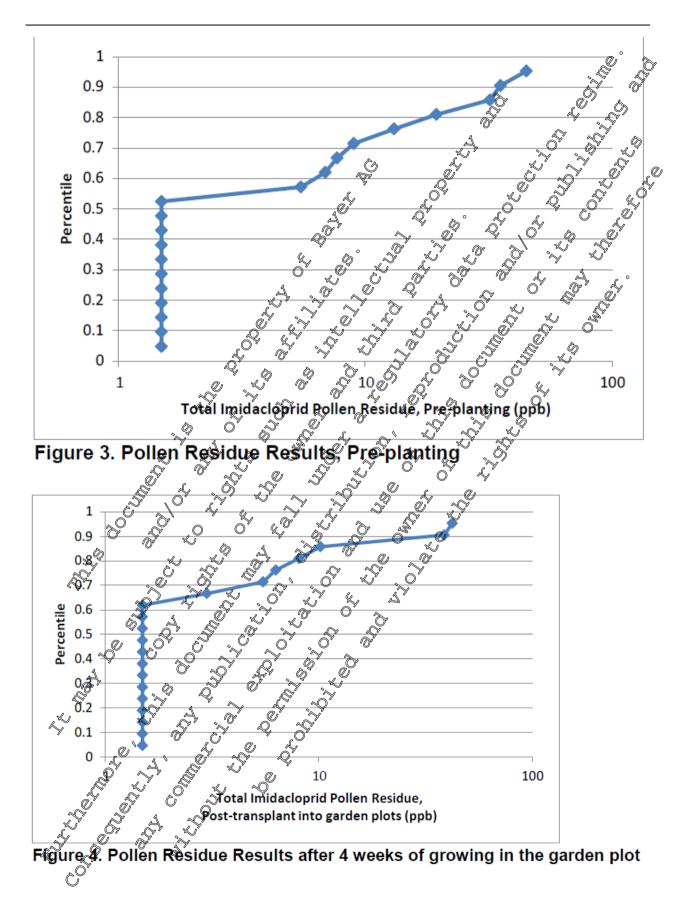
when the plants are transplanted by the consumer. The nectar and pollen taken from the plants that rebloomed in the garden plots indicated a decrease in residues and the mean results were <LOQ and 6.7 ppb, respectively. For those few plants that initially contained imidacloprid residues, the potential metary exposure was even lower 4 weeks after purchase.

						Madian	, , ,	
Matrix	Ν	# samples <loq< td=""><td>Min (ppb)</td><td>Max (ppb)</td><td>90<sup>™</sup> percentile (ഊb)</td><td>Median (ppb)</td><td>Mean (ppb)</td><td>Standard</td></loq<>	Min (ppb)	Max (ppb)	90 <sup>™</sup> percentile (ഊb)	Median (ppb)	Mean (ppb)	Standard
Residue	s in pl	ants purc	hased fr	om retail	garden cen	iters in Apr	il or Mag	20 <u>15</u> 4
Nectar	20	18	<loq< td=""><td>3530</td><td>3</td><td></td><td>19.2</td><td>2015 4 7896</td></loq<>	3530	3		19.2	2015 4 7896
Pollen	20	11	<loq< td=""><td>49.9 3812 at were tr</td><td><sup>*</sup> 3 ~ <sup>©</sup> 35 ~ <sup>©</sup> LOO ansplanted</td><td><u>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</u></td><td>9.5</td><td><u>↓</u>13.<u>1</u> ∘</td></loq<>	49.9 3812 at were tr	<sup>*</sup> 3 ~ <sup>©</sup> 35 ~ <sup>©</sup> LOO ansplanted	<u>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</u>	9.5	<u>↓</u> 13. <u>1</u> ∘
Leaf	40	37	<loq.< td=""><td>3812</td><td>LOQ</td><td>_≪LOQ</td><td>_≴¶12√</td><td>£00</td></loq.<>	3812	LOQ	_≪LOQ	_≴¶12√	£00
Residue to re-blo	es in th om pr	e same p ior to sec	olants the ond sam	iphing (ar		ks∧after niù	rchase)	d allowed
			× a	Ô	$\sim$			0
Pollen	20	130		42	35.9		<b>₹</b> 8.7	11.8
Leaf	40	_ <b>⇔</b> 39 ∂	<loq< td=""><td>85.7</td><td></td><td></td><td><loq< td=""><td>11</td></loq<></td></loq<>	85.7			<loq< td=""><td>11</td></loq<>	11

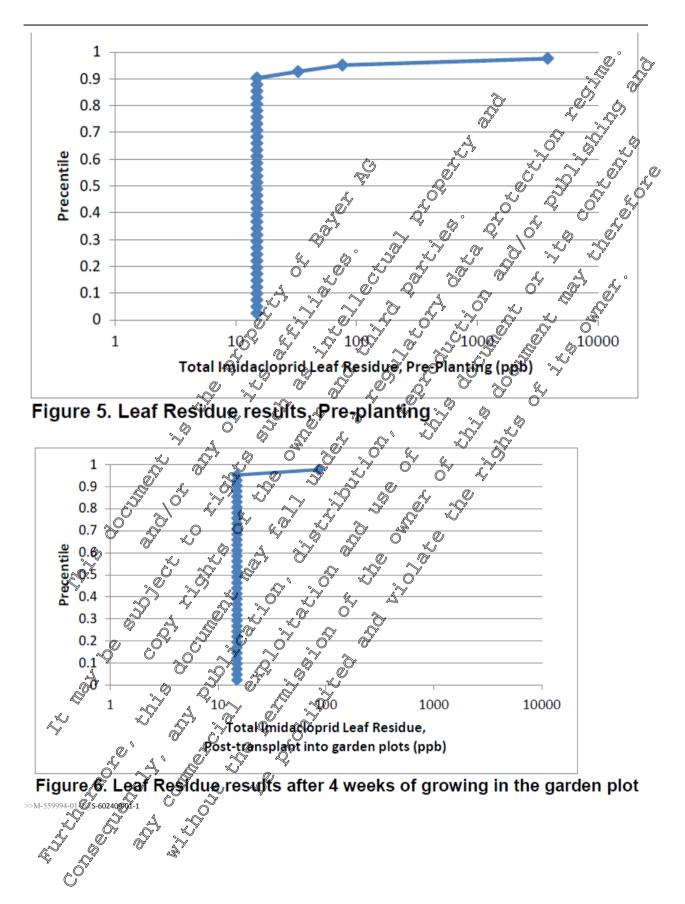














Report:	02.02.01/52; ; 2017	; <u>M-581858-01-2</u>		° .
Title:	Final report: Survey of imi	daclonrid residue	s in nectar and polle	en collected by honey
	bees in urban and suburbar	n environments ac	cross different region	ns of the United States
Report No.:	ESNTN015		Š	
Document No.:	<u>M-581858-01-2</u>		- S	
Guideline(s):	OCSPP 850.SUPP (Specia	l Design)	10-	
Guideline deviation(s):	none		A	
GLP/GEP:	no	Ĉŋ	4	
< <m-581858-01-2@s-602901-01-1< td=""><td></td><td>- A</td><td></td><td></td></m-581858-01-2@s-602901-01-1<>		- A		

A two-year, multi-state study was initiated in July 2014 with the goa $\mathbb{O}$  assessing exposure to perficide 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), DroZachary Huang (Michigan State University), Dr. Juliana Rangel and Mr. Pierre Lau (Fexas 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 Cexas (DX). The percentage of developed area in the primary foraging area (1-mile radius) of the study bives 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 potten samples were submitted periodically to the USDA-NSL for multi-residue analysis, which included imidadoprid parent, imidacloprid parent, imidacloprid olefin and imidacloprid -hydroxy at levels of detection oct, 10 and 25 ppb, respectively. A portion of the pollen samples collegied between July 2014 and June 2015 served for identification of floral resources in these environments through palynological analysis. Exception the sampling schedule included winter months for some regions and perfods of ollen and/or nectar dearth. A total of 1,628 samples were analyzed, of those 76 Divere sectar 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 the ctar samples with detectable imigacloprid residues in FL, MI, CA and TX was 0.8% (12263), 1.5% (1=1940, 11.2% (n=223)), and 0% (n=87), respectively. Likewise, the percentage of pollon samples with detectable invidacioprid residues in FL, MI, CA and TX was 5.5% (n=272), 4.7% (p=190), 20.7% (n=295), 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 hopey be colonies in these environments during the study was minimal. In addition, trees were identified a important pollen sources in urban and suburban areas. The results from this study will be published in one or more peer-reviewed journakarticles and have been presented at national and regional professional meetings and at beekeepers associations meetings.

Report: <u>A</u> 02.02.0053; <u>M-@2796-03-2</u>
Title: <i>Pollingtor</i> full field study evaluating chronic effects of a post seeding application of
imidačloprid in pumpkins (Qurcubita pepo pepo) - Final report
Report No.: 27 12798.4145 0 7
Document No.: $m_{2}^{2}-542p_{2}^{2}6-03-2$ $\sim 0^{\circ}$
Guideline(s): US ERA OC SOP 850 SUPP
Guideline deviation(s):
GLP/GEP:
<<

A field study was conducted to evaluate the potential long-term effects of imidacloprid exposure to honey bee and bumple 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 moved into the fields once sufficient blooming of the pumpkins had occurred. The hives remained in the pumpkin fields for weeks. Thereafter, hives were relocated to a post- exposure apiary near Durand, Will

Samples for residue analysis were collected from field soils pre-treatment and indicated very low background levels (0-19 ppb) of imidacloprid, clothianidin, and thianethoxam. Nectar and poller 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 nectian 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 residues (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 proved into pumpkin fields with a few hives having detections for imidacloudd. 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 imidacloudd residues theither the reference of treated fields. Imidacloprid residues, however, were more consistently detected in bee bread samples in the freated fields and demonstrate the largest difference in residues between reference and treated fields. After overwintering, no imidacloprid residues were detected in capped honey samples confected 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 overvantering 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 a Phives

Three surveys of non-*Apis* bees were conducted during the pumpkin bloom period using bee bowl traps containing soapy water tharge numbers of bees were collected across both reference and imidaclopridtreatment sites and no regnificant 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 boing outside of their normal range. Performance of the bumble bee colonies was not afficient to compare between reference and treated fields.

Overall, no adverse effects were observed in horey 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 tells, nor bee bread cells which previously were observed to be sensitive endpoints for chronic imidacloprid exposure.

Treatic.



Report:	02.02.01/54; ; 2011; <u>M-408424-01-3</u>		
Title:	Determination of exposure levels of honey bees previously treated with imidacloprid	foraging on flowe	
Report No.:	EBNTL056-7		
Document No.:	<u>M-408424-01-3</u>	~	
Guideline(s):	US EPA OCSPP 850.SUPP	19 T	
Guideline deviation(s):		°O''	
GLP/GEP:	no	A	

#### <<M-408424-01-3@S-605221-01-1

A series of field investigations were undertaken to determine to what effent honey bees for any officitrus blossoms may be exposed to imidacloprid when citrus trees are treated with systemic applications (soi treatments) of this insecticide.

#### **Tunnel Cage Study (Section 2)**

- The objective of this component of the study was to amine citrus groves that were treated with a soil application of imidacloprid systemic insective, to inderstand the levels of imidacloprid that occurred in (a) nectar extracted by hand from citrus flowers, (b) pectar collected by forager honey bees and transported back to the live, and (c) nectar of "uncapped honey" deposited by bees in cells of the brood comb Ľ
- Concentrations of imidacloped, 5-hydroxy, imidacloprid and initiacloprid olerin in nectar collected by hand from cittles flowers were similar to those in stomachs of bees for aging on the Ĩ same trees confined within tunnels. R
- The highest residue levels from the nectar sources were measured in the nectar deposited within the new comb (stored nector). 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 3fold higher while the levels of imidaetoprid defin were 5-fold higher. The higher measurements in the stored nectar may be because combonectar has lower water content and higher sugar content compared with upprocessed 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 incident of systemic precipite, 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, (cynectar 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 noctar 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 Store mectal samples were somewhat greater than in flower nectar. This may be because comb nectar has ower water content and higher sugar content compared with unprocessed negar, although Qur results are not conclusive based on refractometry measurements.
- The imidaclopric concentrations measured in the limited pollen available for analysis were equal those in the stored octar 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)

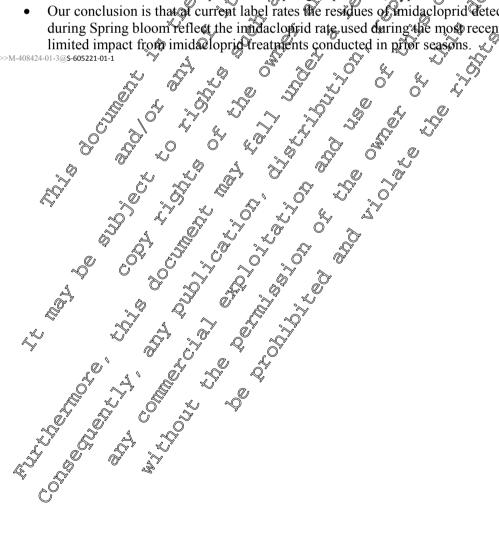
The objective of this component of the study was to determine to what extent increasing the imidacloprid application rate would impact residues in the nectar



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 5)

- The objective of this component of the study was to determine to what extent imidac loprid residues might persist and/or accumulate in citrus trees from year-to- year following multi-year applications.
- Based on experiments at the Hemet site, imidaclopfid residues a 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 condusion is based on a period of 1 year between applications, which would be the normal use under the current label recommendation for citius in California. There was a suggestion of some carryover between years with the 2X label rate treatments, although the result was not statistically significant. Ľ
- Nectar samples were obtained from 19 sites feitrus blocks in the gemec da region and at Lindcove Research and Extension Center where the 1X Soil application rate of imideclopped had been made in two successive years (2008, 2009) priordo sampling. in April 2010, Residue levels at these 11 sites averaged 8 ppl and ranged from 1 to 18 pp. ×1
- The application timing (fall S. 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-Qune) applications.
- Our conclusion is that of current label rates the residues of midacloprid detected in the nectar • during Spring bloom reflect the innitiaclostid rate used during the most recent application, with





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Issue date 2017-11-22

# 02.02.02 - Succeeding crops

Report:	02.02.02/01; ; 2002;	<u>M-061850-01-3</u>		N N N	
Title:	Imidacloprid (Admire) resid	lue levels follow	ving in-furrow a	prication in potato fields in	
	Prince Edward Island and N	ew Brunswick			
Report No.:	<u>M-061850-01-3</u>		-0		
Document No.:	<u>M-061850-01-3</u>				
Guideline(s):	US EPA OPPTS 850.3040	Ĉs	Š		
Guideline deviation(s):	not specified	- T	<u> </u>		1
GLP/GEP:	no	Å.	Ő		
- 34 0(1050 01 200 (02(75 01 1		,∭`	×		

Imidacloprid (ADMIRE® 240F), is a synthetic systemic chloronicotinyl insecticide, produced for the control of Colorado potato beetles, aphids, flea beetles, and leathoppers on potato crops (Elbert et al., 1991; 1999). Imidacloprid is an agonist at nicotinic acetylcholine receptors that demonstrates selective toxicity for insects over vertebrates, and has the fastest prowing 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 initial cloprid, and in April 1995, the PMRA granted tempotary 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 of over 55 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. 1999). The molecular ability of imidacioprid makes it an ideal candidate for the use on potatoes and numerous other crops (apples, lettuce, tomatoes, mustard, canola, cucumber, com, etc.). Due tools long/term action this chloronic tinoid 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 \$30 ml to 1.3 D/ ha. Due to its residual activity, imidacloprid has become the most popular control agent for Colorado potato beetle.

Ľ Despite worldwide recognition, the use of Admire® has been in question following reports by French bee keepers of disoriented honey bees that had been for aging 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 doe 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 hecta and posten of flowering crops at concentrations harmful to honey bees has been the focus of an extensive research program, A study conducted by Schmidt and (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 ppp (part 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, do danage 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.



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:

 son, clover leaves, and clover flowers, and wild flowers
 pollen, and nectar collected from honey bees foraging in previously treated clover fields
 uncapped honey collected from the hives placed in previously treated clover fields
 following report is a review of the protocol and result of the project. The following report is a review of the protocol and results of the project >M-061850-01-3@S-602677-01-1

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Report:	02.02.02/02; 2011; <u>11:406075-01-3</u>
Title:	Determination of residue levels of imidacloprid, infidacloprid-monohydroxy and
	imidacloprid-olefine in bee relevant matrices of winter type in accreat succeeding crop
	scenario at Bayer CropScience AG experimental farme Germany
Report No.:	E 319 3388-5
Document No.:	
Guideline(s):	US EPA OCSPP Guideline Number: 859.SUPP
Guideline deviation(s):	ves
GLP/GEP:	yes of the the the second seco
< <m-406075-01-3@s-602303-01-1< th=""><th>none yes</th></m-406075-01-3@s-602303-01-1<>	none yes

Experimental starting/completion date: October 17, 2007 August 25, 2009

#### Material and methods:

The imidacloprid containing test item (maxture of imidacloprid and fungicides), used for the purpose of this study, was fuberidazol + imidaclopric + triadimental FS 145.2 (72+8+70+60) G, TOX-No. of test item: 08068-00 analysed content of imidadoprid; 72.3 g@.s./L; density, 1.081 g/mL). In addition: imidacloprid-treated winter wheat seeds of the variety Dekard, dressed with the above mentioned test item (TOX-No. of reate Oseeds. 08079-00; analysed content of imidacloperd: 70.75 g a.s. /100 kg seeds; imidacloprid-free dressed winter wheat seeds of the variety "Dekan" as well as imidacloprid-free dressed Ľ, winter oil-see Prape (OSR) seeds of the variety "adriana". Õ

In autume 2007 (18 October 2007), the imidaclopric containing test item was applied and incorporated down to 20 cm soil depth on Hallow test plot (=treatment test plot) at a rate corresponding to nominally 126 g a.s. imidaclosoid/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 bomeans of a power-karrow on the same day, immediately after the establishment of the Cong-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 lest plot, inidacloprid-free Dessed wheat seeds (=control winter wheat seeds) of the same variety as the treatment seed? were sown at the same day (18 October 2007). These imidaclopridfree control winter wheat seeds deceived the same nominal loading of active fungicidal substances as the treatment seeds. The control seeds were sover on the control test plot also at a nominal sowing rate of 180 kg seeds/ha. On the control sest plot, no plateau concentration has been established, and as such, no spray application was performed.

In late symme 2008 (19 Apprist 2008), after harvesting of the winter wheat at 30 July 2008, winter OSR seeds with a vimida loprid-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 matment 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 m<sup>2</sup> 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 3009 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, nectao 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 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  $\mathcal{D}\mu g$  as kg dry soil.

After a period of nearly 10 months, directly before sowing winter OSR seed with an imidacloprid free seed coating, the mean imidacloprid concentration on the treatment set plot decreased to 48.8 up a.s./kg dry soil. The corresponding parallel sol residue analysis on the control test plot showed no residues of imidacloprid.

Analytical results for imidaclopfed, imitaclopfed-modohydrexy and imidaclopric olefine in bee relevant matrices of winter OSR:

		<u></u>	S R C			,9 ,5
Sample	Sample ≾	Sample Material	Treatmen®7		Residue	
Number	Sample Name		Control Test	<sup>V</sup> Imidacloprid	Imedaclopi/d- monohydroxy	Imidacloprid- olefine
001	Pollen C1	v 4	🦕 C 🕎	, <sup>7</sup> < LOD √ < 100D - 30	€ <sub>€</sub> ÇÕD	< LOD
003	Pollen Co	v,	O' a	,∜ < 100°D _3°	< LOD	< LOD
005	Pollen 🛛					< LOD
002 🔊	Pollen T2		ু কি ত	0.002	< LOD	< LOD
004	Pollen Ţ4		Т <u></u>	0.002	∫ < LOD	< LOD
006	Pollen 76	Pollen		, ∞,002 ∆′	< LOD	< LOD
001	Nectar C1	1 0		QLOD,	< LOD	< LOD
003	Nectar C3		p c x	< LQQ	< LOD	< LOD
005	Nectar 🔗		× ¢	k ≤ LƠD	< LOD	< LOD
002	Nectar T2	Nectar ~		LOD	< LOD	< LOD
004			O'T N	, ≪ < LOD	< LOD	< LOD
006	Nectar T&Y	4		~O <sup>Y</sup> < LOD	< LOD	< LOD

Limit of quantitation (LQQ) for initial aclop(d, imid eloprid monohydroxy and imidacloprid-olefine = 0.001 mg/kg, Limit of detection (LOD) for imidaclopsid, imidacloprid-monohydroxy and imidacloprid-olefine = 0.0003 mg/kg

#### Conclusion:

Under still uprealistic 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 voteat, followed by winter OSR as a succeeding crop), residues of imidacloprid in OSR plectar collected on the imidacloprid treatment test plot were always below the limit of detection (LOD). The inidacloprid concentration in the three pollen samples from the imidacloprid treatment test plot was determined to be 0.002 mg a.s./kg, respectively.

The impacloprid-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** 



<b>Report:</b> Title:	Determination of residue l imidacloprid-olefine in be	e relevant matrices	s of winter rape in	n a cereal succeeding crop
	scenario at Bayer CropSci	ence AG experime	ental farm Hoef	en, Germany
Report No.:	E 319 3387-4		Å.	
Document No.:	<u>M-406083-01-3</u>		10%	
Guideline(s):	US EPA OCSPP Guidelin	e Number: 850.SU	JPP A	
Guideline deviation(s):	none	Ĉa	£.''	
GLP/GEP:	yes	T.	Ň	
< <m-406083-01-3@s-602306-01-1< td=""><td></td><td>L</td><td>,Õ<sup>¥</sup></td><td></td></m-406083-01-3@s-602306-01-1<>		L	,Õ <sup>¥</sup>	

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 + triadimentor FS 165.2 (7.2+8+70+60) & TOX-No. of test item: 08068-00; analysed content of imidacloprid. 72.3 g as:/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 initiacloprid: 7075 g as: /100@ g seeds; imidacloprid-free dressed winter wheat seeds of the variety. Dekan, as well as 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 imidaciopric 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/hato conservatively establish a long-term softplateat concentration of imidacloprid, simulating the consecutive use of imidacloprid on the same weld plot over several years. Incorporation was achieved by means of a power-harrow. On the same day, inspediately after the establishment of the fong-term soil platea & conceptration of imidacloped, imitacloped, imitacloped winter wheat seeds, dressed with test item (=treatment winter wheat seeds) (were gown on the treatment test plot at a nominal sowing rate of 186/kg seeds/ha, sorresponding to normally 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 featment seeds were sown at the same day (19 October 2007). These imidaclopridfree 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." Ì

In late summer 2008 (2) August 2009), after harvesting of the winter wheat at 01 August 2008, winter OSR seeds with an initial oprid-free seed coating (insecticidal seed coating: Elado<sup>®</sup> (= 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 loneybes were exposed to the flowering winter OSR crop under confined conditions, one gauze tunner (approximately 50 m<sup>2</sup> 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 meltifera larnica*) 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.



#### **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@ /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 \$3.2 µg a.s./kg dry soil. The corresponding parallel soil residue analysis on the control test plot showed to residues imidacloprid.

Analytical results for imidacloprid, imidacloprid-monohydroxy and matrices of winter OSR: Ô

					N. N.	
Sample Number	Sample Name	Sample Material	Treatment Control Test Plot [FAC]	Amidacoprid	Residue [mg/kg] imidacloprid- menohygroxy	midaç@prid-
002	Pollen C2		Ĩ, Î	©Lop√y		olefine
004	Pollen C4			~ LQD		✓ LOD ✓ < LOD ✓ < LOD ✓ <lod< p=""></lod<>
006	Pollen C6		5 CO		S LOD	ି < LOD ଦି < LOD
008	Pollen C8	Bollena		OLOD 6		≷LOD
001	Pollen T1	Pollen	T O	-00.000	S < LOD S	Sy < LOD
003	Pollen T3			_<、 < LOĎ √、 ♀0.0003 、	∿ × LOD⊘	<pre>_ &lt; LOD</pre>
005	Pollen T5			0.0003	<	Contraction
007	Pollen T7			LOB K	< 100 0	< LOD
002	Nectar C2		√ <sup>2</sup> C	L~~~~~~~ O		< LOD
004	Nectar 🥨		S &	S ≲trod	O< LOD	< LOD
006	Nectar C6	ó >	1″ ××C ~~	, S LOD	S < SD	< LOD
008	Nector C8>			_^~ < LOD″ _Q	v <b>≪</b> ľod	< LOD
001	Nectar T	Nestar		୍	<lod< td=""><td>&lt; LOD</td></lod<>	< LOD
003	Nectar T3			έlod,	<lod< td=""><td>&lt; LOD</td></lod<>	< LOD
005	Vectar T5		T,	< LOO	y ≺ LOD	< LOD
007	Nectar T		T.Q	S LOD	< LOD	< LOD

Limit of quantitation (LOR) for initiaclopid, initiacloprid inonohydroxy and imidacloprid-olefine = 0.001 mg/kg, Limit of detection (LOD) for imidaclopre, imidacloprid menohydroxy and imidacloprid-olefine = 0.0003 mg/kg

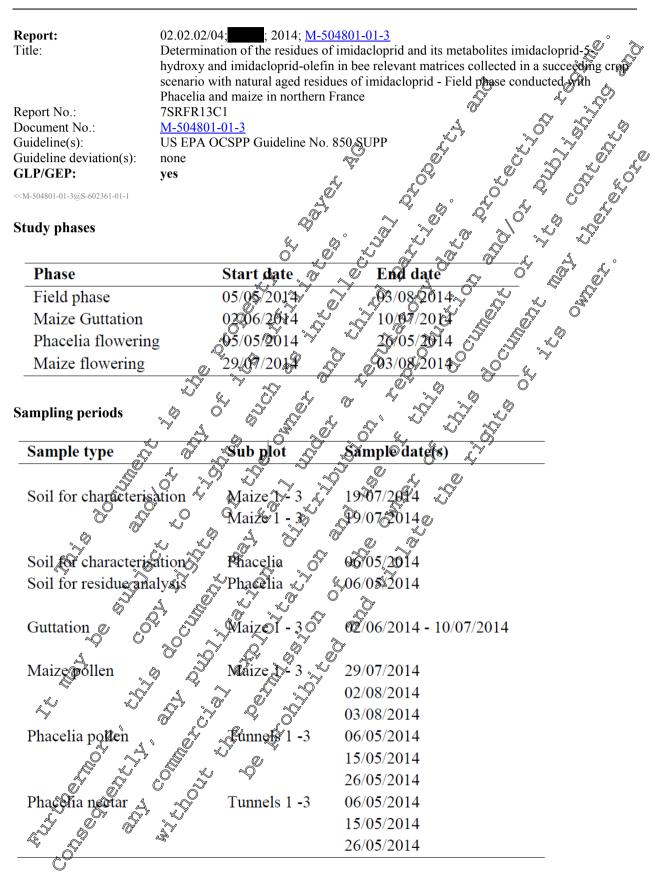
#### Conclusion:

Under still unrealistic worst case conditions (long-term imidacloprid plateau concentration conservatively simulated by fresh apple ation and incorporation of imidacloprid into the soil at the day of sowing imidaçloprid-dressed winter wheat, followed by winter OSR as a succeeding crop), residues of imidacloprid in OSR-poller and QSR-nectar confected on the imidacloprid treatment test plot were always below the limit of quantitation (LOQ)

The imidacloprid concentration in two poller 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 x as < LOD, respectively. The imidacloprid-monohydroxy and imidaclogrid-oldine concentration of all pollen samples from the treatment test plot was < LOD. The imidactoprid, midactoprid, bonohydroxy and imidacloprid-olefine concentration of all nectar samples from the treatment pest plot was < LOD. le,

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** 





Executive summary:



#### Objective:

The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee refevan 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 a Imidacloprid use and such with a likelihood of natural aged soil residers of this active substance An approximately one hectare plot located within the field was marked out, and divided into two evonly sized sub-plots. One sub-plot was sown with maize (Zea mays) the other sub-plot was sown with Phaceliar (Phacelia tanacetifolia).

#### Material and Methods:

Crops were sown according to Good Agricultural Practice (GAP). Maize and Phacelia without neonicoting d seed treatment were sown in 2014, using calibrated equipment (tractor and seed drill). The target so ving 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 onallers ub 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 negtar and pollen Ô

Soil sampling: From each of the maize sub plots and from the phace ha sowing area, two different types of soil sample were collected These Samples were used for. L L Õ

Soil characterisation of the upper 10 cm soil lager. 1.

Determination of the residues of parent imidacloprid and its metabolites in the upper 15 cm soil 2.

layer. Soil cores used for characterisation and residue analysis were collected from each of the three maize sub plots during the guitation sampling phase of the trial and from inside of the Phacelia sowing area prior to placement of the boneybee colonies into the tunnels.

Sampling of Sectar and Polen from Phacelia Coops:

Nectar and pollen sampling was conducted at aree 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 cropc Honeybees were exposed to the flowering Phacelia under confined conditions and were exclusively used as a sampling devoce 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 intrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Potten and nectar samples during bloom were analysed for residues of imidacloprid

Sampling of Guttation fluid and Pollen from Maize:

Guitation Fuid 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).



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.

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 BBCK scale 63) until male flowering had completed (BBCH scale 67). Ĩ Ò

At each time point  $\geq$  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 overlight. 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 of inseet debries remaining in the pollen sample by hand using forceps or a fine paint brush,

#### Residue analysis:

All samples (soil samples, pollen, nectar and guttation third) were analysed to their content of imidacloprid and and its metabolites 5-by droxy and offin by using fligh Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection. Analysis of the soil samples followed the provisions of method 90790 y1001 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 01435.

The Limit of Quantitation (LQQ) of imidac loprid, defined as the lowest validated fortification level, was 5.0 µg/kg for soil. The corresponding Limit of Detection (LOD) was & µg/kg

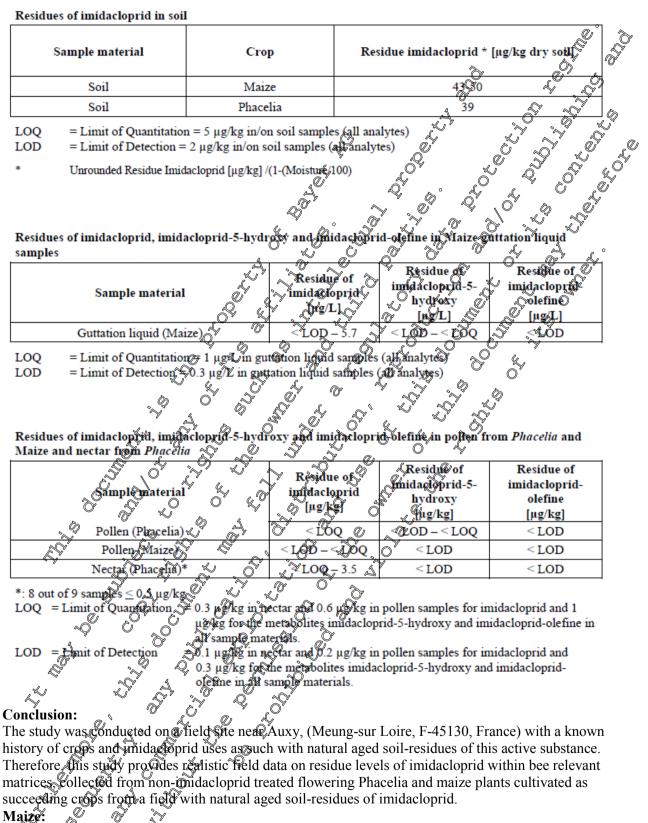
The LOQ levels for imiday loprid were 66 µg/kg for pollen, 0.3 µg/kg for nectar and 1.0 µg/L for guttation liquid while the LOQ Tevel of the metabolites way constant 1.0 µg/kg for all sample materials. The corresponding Limit of Detections (LOD) were 0.2 ug/kg for pollen, 0.1 ug/kg for nectar and 0.3  $\mu g/L$  (0.0003 mg/L) for guttation and  $\mu g/L$  for guttation and  $\mu g/L$  for the metabolites imidacloprid-5-hydroxy and insidacloprid-oletine for all sample materials

All results of the method validations were in accordance with the general requirements for residue

All results of the method validations were in accordance with the general requirements for residue analytical methods, the employed methods were validated, sccessfully. The average recoveries were within the acceptable range of 60 = 120% RSD values are below 20%. A summary of the analytical results acordance with the following tables of soil, gut tation begins of soil gut tation begins of soil gut tation begins of the method values are below 20%. A summary of the analytical results acordance with the following tables of soil, gut tation begins of soil gut tation begins of the method values are below 20%. A summary of the analytical results acordance with the following tables of soil, gut tation begins of soil, gut tation begins of soil, gut tation begins of soil accordance with the following tables of tables of tables of the following tables of tables of tables of tables of tables of tables of tables of tables of tables of tables of tables of tables of tables of tables of tables of tables of tables



Issue date 2017-11-22



One set of soil samples were taken from the maize sub plots during the trial. The residue levels of imidactoprid 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

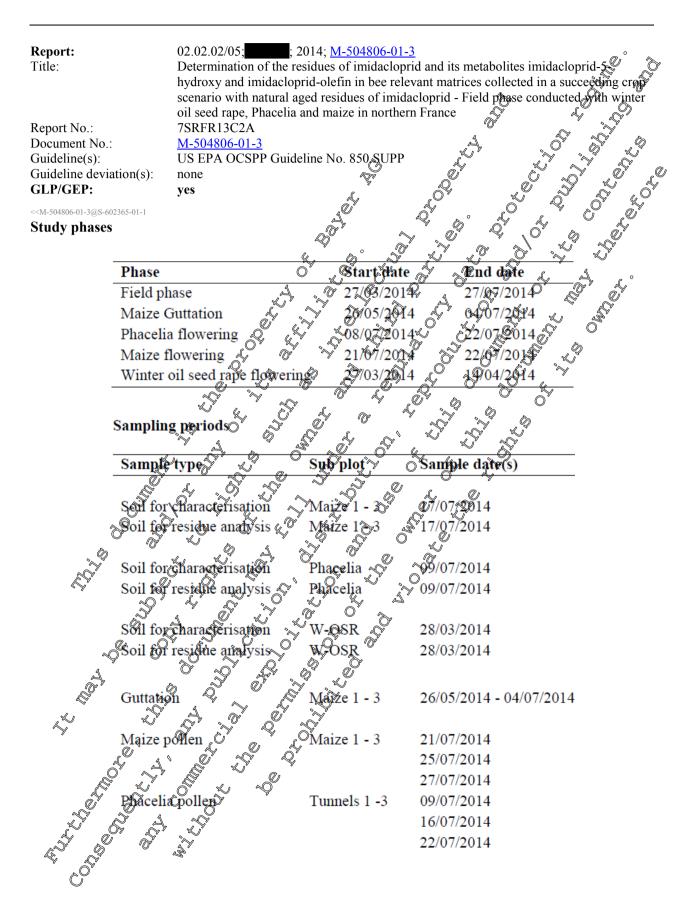


ug a.s./L and are thus several orders of magnitude below neonicotinoid values measured in droplets from seed treated maize plants.

The residue levels of imidacloprid in pollen, as sampled at three time points during bloom of the poince plants ranged from below the LOD ( $<0.2 \mu g a.s./kg$ ) to below the LOQ ( $<0.6 \mu g a.s.$ ). **Phacelia:** 

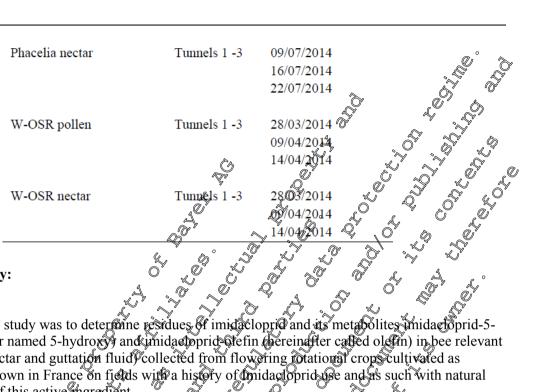
A contract of the probability of Soil cores used for residue analysis were taken from the entire field prior to placement of the honeybee colonies into the tunnels. The residue level of imidacloprid in the phacelia plot was 39 µg as./kg dry







Issue date 2017-11-22



# **Executive summary:**

#### **Objective**:

The objective of the study was to determine residues of imidacloprid and its metabolites imidacoprid-5hydroxy (hereinafter named 5-hydroxy) and imidaetoprid Stefin (pereinafter cated 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 midactoprid se and as such with natural aged soil-residues of this active ingredient.  $\cap$ 

#### **Study Site:**

The study was conducted on a field site near Groux (p-36159), France) known hypory of Imidacloprid use and such with a likelihood of matural aged son residies of this active substance. On this land, non imidacloprid treated Winter oil seco (Brassica napus) has been cultivated in 2013. During bloom in 2014, in total, three tunness were setup for Winter oil seed with one bee hive per annel. 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 midacloprid 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 provious from was removed from the and.

A

### 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 subplot 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  $\sigma$  m wide x 3 m high) were placed onto the phacelia plot after successful gertoination. A single hopeybee colony was placed into each tunnel at the start of Phacelia flowering

# Soil sampling:

From each of the market subplots 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. Soll 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 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 guitation 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 at the giffered grop. 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 forage? bees. Therefore, the hive entrange was blocked during bee flight activity for a short period of time and the eturning for ager bees were collected at the hive entrance. Pollen was collected from foragers returning to the Wony using apollen trap attached to each colony. Pollen and nectao 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 colomes were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the Dowering Phacelia under continued conditions and were exclusively used as a sampling device for both pectar and poten.

Nectar was sampled by extracting the hone stomachs from forager bees Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning for ager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Rollen and nector samples during bloom were analysed for residues of imidacloprid

Sampling of Guttation fluid and Pollen from Maize;

C. C Guttation fluid and poten sampling was conducted in the maize prop. Samples were collected directly from the crop by hand.

Sampling of guttation fluid was carried out on regular basis over a 40-day period. Guttation sampling started directly after energence of the maize crop (BBCH scale H-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 10ml 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  $\geq$  50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damplasses were ar 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 imidaeloprid. n Š

Residue analysis:

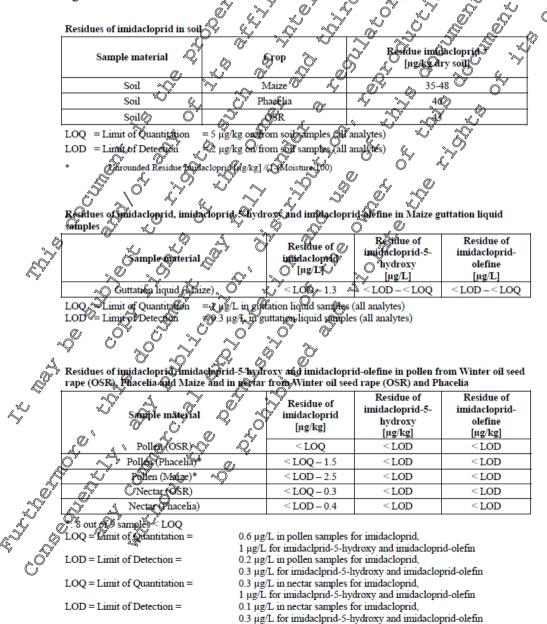
All samples (soil samples, pollen, nectar and guttation fluid) were analysed for their content of imidacloprid and and its metabolites 5-hydroxy and olefin by using High Performance Liquid

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  $\mu$ g/kg for soil. The corresponding Limit of Detection (LOD) was 2  $\mu$ g/.

The LOQ levels for imidacloprid was 0.6  $\mu$ g/kg for pollen, 0.3  $\mu$ g/kg for rectar and 1.0  $\mu$ g/L for guttation liquid while the LOQ level of the metabolites were constant 1.0  $\mu$ g/kg for all sample materials. The corresponding Limit of Detections (LOD) were 0.2  $\mu$ g/kg, 0.1  $\mu$ g/kg and 0.3  $\mu$ g/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-blefine for all sample materials.

All results of the method validations were in accordance with the general requirements for residue  $\sqrt[6]{}$ 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, guitation aquid pollen and next samples are provided in the following tables:





#### **Conclusion:**

The study was conducted on a field site near Auxy, (Meung-sur Loire, F-45130 France) with 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 imidaeloprid within the relevant matrices, collected from non-imidacloprid treated flowering winter oilsectrape, Phacelia and maize x Ś plants cultivated as succeeding crops from a field with natural aged soil residues of initial aged.

#### 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 image cloprid in the field was 43 up a.s. Rg drysoil. Residue analysis of pollen and nectar, collected at three time points during blooming of winter offseed rape, revealed generally low residue levels. <0.6 µg a.s. (bg)

The residue levels of imidacloprid in nectar ranged from below the L ( $<0.3 \ \mu g a.s./kg$ ).  $\sqrt{00}$  µg a.s./kg) to the  $\sqrt{00}$ 

#### Maize:

Maize: One set of soil samples were taken from the maize subgroots for ing the trial. The residue levels of imidacloprid in soils ranged from 35 ug a.s./kg to 48 ug a.s. kg drosoil during gottation Residues analysis of guttation fluid, collected directly after emergence until early bloom of the maize plants, revealed generally low residue levels. Ñ

The residue levels of imitacloprid in gultation 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. a.  $\bigcirc$ 

The residue levels of imidaelopri on poten, 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 pg a.s. (kg). 8 of 9 samples contained residues < LOQ.

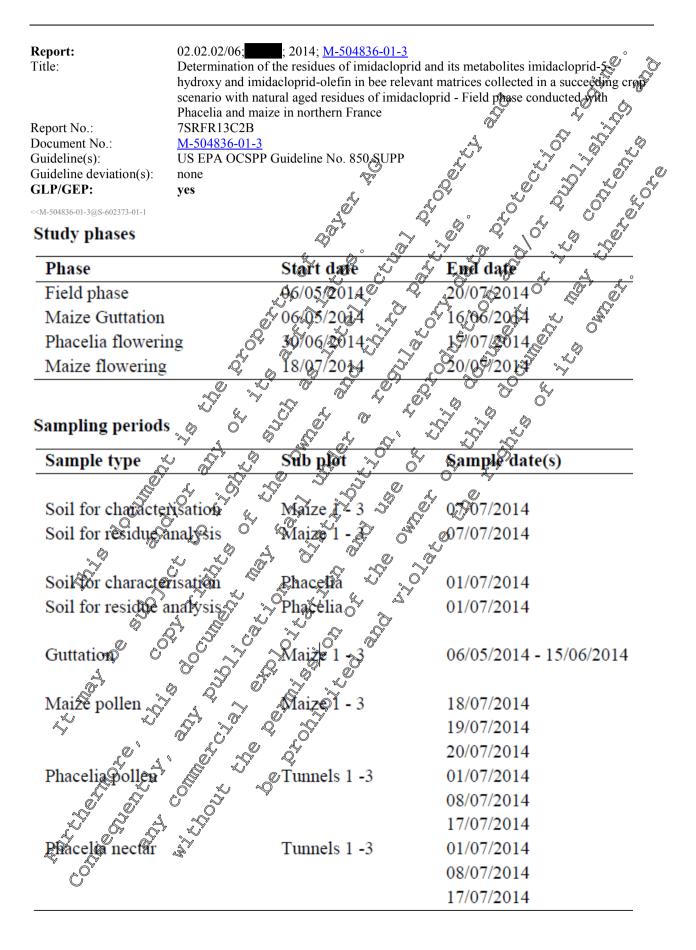
#### Phacelia:

Soil cores used for residue analysis were taken from the entire field prior to placement of the honeybee colonies into the tunines. The residue level of inite acloprid in the phacelia plot was 46 µg a.s./kg dry soil. Residue analysis of pollen and nestar, collected at three time points during blooming of Phacelia, revealed generally low residue levels.

The residue levels of midacloprid in pollen ranged from below the LOQ (<0.6 µg a.s./kg) to 1.5 a.s./kg. 8

The residue levels of initiacloprid in pollen ranged from below the LOQ (<0.6 µg a.s./kg) to 1.5 a.s./kg. of 9 samples contained residues \$200, 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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#### **Executive summary:**

#### **Objective:**

The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid 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 matural aged soil-residues of this active ingredient.

#### **Study Site:**

The study was conducted on a field site near Auxy (F#5340, 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 and was marked off, and divided into two evenly sized sub-plots. One sub-plot was sown with maize (Zea norys) the other sub-plot was sown with Phacelia (Phacelia tanacetifolia).

Crops were sown according to Good Agricultural Practice GAP The maize and phacelia plots were sown using calibrate sowing rates were 10 kg seeds/ ha for Di The sub plot of Material and Methods: Crops were sown according to Good Agricultural Practice (GAP) The maize and phacelia plots were sown using calibrated equipment (pactor and seed drill). The target

sowing rates were 10 kg seeds/ ha for Phacelia and 100000 kernel/ ha for maize.

The sub plot sown with maize was divided into three smaller sub posts, each similar in size that were large enough to have a sufficient numbers of plants available for both gutation fluid and for maize pollen sampling. Ì

sampling. Three bee proof tunnels (40 m long x 5 m wide x 3 m high) were placed on the phacelia plot after successful germination. A single honeybee colony was placed into each tunnel at the start of Phacelia flowering flowering

# Soil sampling:

From each of the main sub plots and from the phaselia sowing area, two different types of soil sample were collected. These samples were used for;

Soil characterisation of the upper 10 cor soil layer. @ 1.

Determination of the residues of parent implacion and its metabolites in the upper 15 cm soil 2. layer.

Soil cores used for haracterisation and residue analysis were collected from each of the three segregated maize sub plots, during the gutation sampling phase of the frial and from inside of the Phacelia sowing area prior to placement of the honeybee comies into the annels.

# 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 Phace into the phace into mesh covered tunnels erected over the crop. Howevbees 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 hone stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for 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. Dollen and nectar samples during bloom were analysed for residues of imidaç løprid 💦

### 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).



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). Ĩ Ñ

At each time point  $\geq$  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 overlight. 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 of inseet debries remaining in the pollen sample by hand using forceps or a fine paint brush, P Pollen samples during bloom as well as colleged gutation fluid were analysed for residues of imidacloprid.

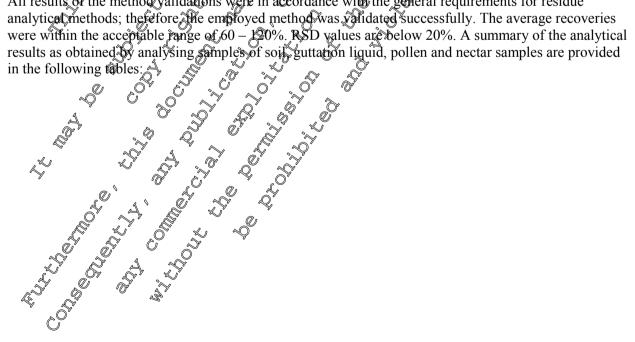
#### **Residue analysis:**

All samples (soil samples, pollen, nechar 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 spectrometer (MS/MS) detection. Analysis of the soil samples followed the provisions of method 0000/M001. Apprysis of nectar and pollen followed the provisions of method 91433 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 imitacloperd, defined as the lowest validated fortification level, was 5.0 µg/kg for soil. The corresponding/Limit of Detection (QOD) was 2 µg/kg.

The LOQ levels for insidacloprid were 0.6 µg/kg (0.0006 mg/kg) for pollen, 0.3 µg/kg for nectar and 1.0 µg/kg for guttation brouid while the LOQ level of the metabolites were constant 1.0 µg/kg for all sample materials. The corresponding Lumit of Detections (LOD) were 0.2 (ug/kg for pollen, 0.1 µg/kg for nectar and 0.3 µg/kg for guttation liquid, respectively for imidae loprid and 0.3 µg/kg for the metabolites imidacloprid-5-hydroxy and imidacloprid-olefing 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





Residues of imidacloprid in soil			0				
Sample material	Сгор	Residue imidacloprid					
Soil	Maize	45-55					
Soil	Phacelia	52 ي	\$ \$ \$				
$LOQ = Limit of Quantitation = 5 \mu g$	/kg on/from soil samples	(all analytes)					
$LOD = Limit of Detection = 2 \mu g$	kg on/from soil samp	(all analytes)					
* Unrounded Residue Imidacloprid	[μg/kg] /(1-(Moisture/100)		2 A A A A A A A A A A A A A A A A A A A				
	Q0						
Residues of imidacloprid, imidacloprid samples	d-5-hydroxy and imidad	loprid-olefine in Maize g	uttation liquid				
samples							
Sample material	Residuêdor finidaelopri	Residue of d d bydrozy (1994)	Residue of imidaclopric plefin (µg/L)				
Guttation liquid (Maize)	0 <10D-4.	1 \(\sec{LOD}{- <lod}) -="" <\sec{lod}{-<soq}<="" td=""><td><math>\sqrt{2}</math> <math>&lt; 200Q</math></td></lod})>	$\sqrt{2}$ $< 200Q$				
LOD = Limit of Detection $0.3 \mu$	$LOQ = Limit of Quantitation = 1 \mu g/L \mu guttation liquid sample (all adalytes)$						
Maize and nectar from PhaceDa	65-hydroxy and imidad	roprid glefine in poller h	com <i>Phacelia</i> and				
Sumple material 4	Residue of imitaclopa	Residue of	Residue of imidacloprid- olefine [µg/kg]				
🖉 Pollen (Phacelia)* 🖉	A. X-LOO-1.	2 <sub>2</sub> ,	< LOD				
Pollen (@aize)	0.00-0.91	S <lod< td=""><td>&lt; LOD</td></lod<>	< LOD				
Nectar Phaceha	$\langle \nabla \rangle \sim \log - 0$	4 X <lod< td=""><td>&lt; LOD</td></lod<>	< LOD				
*: 8 out 9 samples DOQ	<u>``\</u> ``````````````````````````````````	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~					
LOQ = Limit of Quantitation LOD = Limit of Detection LOD = Limit of Detection Conclusion: LOD = Limit of Detection Conclusion: LOD = Limit of Detection LOD = Limit of Detection Conclusion: LOD = Limit of Detection LOD = Limit of Detection L							
The study was conducted on a field s midacloprid uses as such with natura provides realistic field data oppresidu non-infidacloopid traffed flowering P with natural gred sol-residues of imi	ite war Auxy, (F-4534 aged soil-residues of e levels of imidaclopri hacelia and maize plan	40, France) with a know f this active substance. T id within bee relevant m	Therefore, this study atrices, collected from				

with patural aged soil-residues of imidacloprid.

Maize O 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 ( $<0.3 \ \mu g a.s.$ ) to  $4.1 \ \mu g$  a.s./L and are thus several orders of magnitude below values measured in droplets from seed treated maize plants.

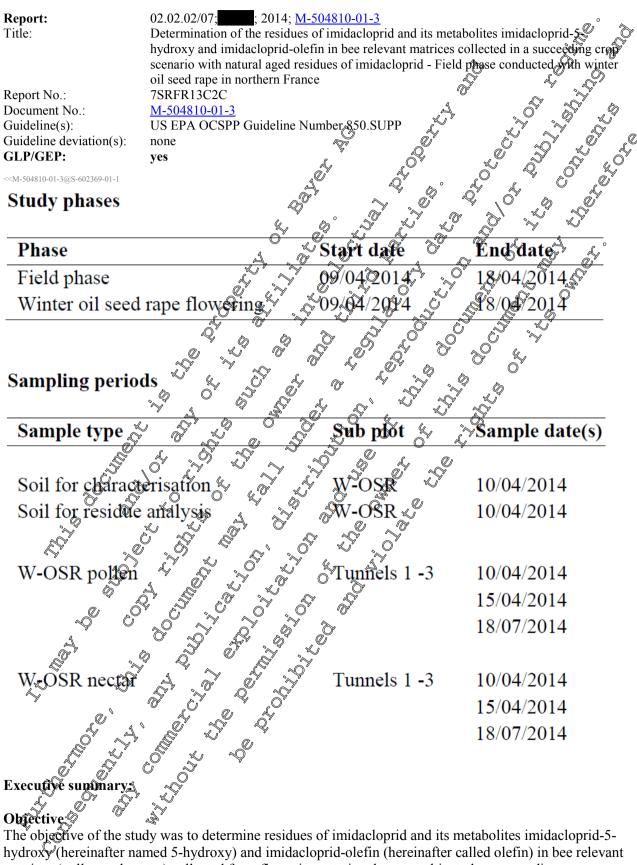
The residue levels of imidacloprid in pollen, as sampled at three time points during bloom of the maze plants ranged from 0.64 µg a.s./kg to 0.91 µg a.s./kg.

### Phacelia:

Soil cores used for residue analysis were taken from the entire field pror to placement of the honeybee colonies into the tunnels. The residue level of imidacloprid in the placelia plot was  $52 \mu ga.s./kg/dry sou.$ Residue analysis of pollen and nectar, collected at three time points during bloopring of Phacelia, revealed generally low residue levels.

The residue levels of imidacloprid in pollen ranged from below the kOQ (\$0% µg @s./kg) to 1.24µg





The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5hydroxy (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



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 the active substance. On this land, non imidacloprid treated Winter oil seed (Brassica napus) has been cultivated in 2018. 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 Grager 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 minels (10 m long x 5 m wide x 3 m high) were placed onto the winter oil seed rape plot poor to bloom. A single honorbee colony was placed into each tunnel at the start of winter of seed rape flowering

# Soil sampling:

From the winter oil seed rape, two different types of soil sample were collected. These samples were used for:

Soil characterisation of the upper 10 cm soil by er. 1.

Determination of the residues of parent initiacloprid and its metabolites in the upper 15 cm soil 2. laver. Ø1  $\bigcirc$ 

Soil cores used for characterisation and residue analysis were collected from inside of the winter oil seed sowing area prior to placement of the honey be colonies into the hunnels. O

# Sampling of Nectae and Rollen from Winter Oilseed Rape

Nectar and poller sampling was conducted at three different time points wiring 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 Rowering winter oilseed under confined conditions and were exclusively used as a sampling device for both nectar and pollen.

Nectar was sampled by extracting the noney stomach's 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. Poten was collected from foragers returning to the colony using a pollen trap attached to each colony. Porten and nector samples during bloom were analysed for residues of imidacloprid \_@

# **Residue** analysis:

All samples (soil samples, police and nectary were analysed for their content of imidacloprid and and its metabolites 5-hydroxy and elefin 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/MQ01. Analysis of nectar and pollen followed the provisions of method 01433

The Limit of Quantification (LOQ) of intedacloprid, 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 imidaclopfid were 0.6 µg/kg for pollen and 0.3 µg/kg for nectar. The LOQ levels of the metabolites were constant 1.0  $\mu$  kg for nectar and pollen. The corresponding Limits of Detections (LOD) were 0.2  $\mu$ g/kg for pollen and 0.1 (kg for nectar, respectively for imidacloprid and 0.3 µg/kg for the metabolites imitaclopfid-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:



Residues of imidacloprid in soil			
Sample material	Сгор	Residue Imidacloprid * [µg/kg dry soil)	<i>}</i>
Soil	Winter oil seed rape	245 × 5	
	= 5 μg/kg for imidacloprid in/on soil = 2 μg/kg for imidacloprid in/on soil		

Residue imidacloprid [µg/kg] /(1-(Moisture/100); For the calculation of midacloprid residue's related to dry soil, unrounded values were used. Therefore minor deviations may occur when rounded values shown with Qis table are used \*\* The given residue values and corresponding residue values related to dry soil are mean values of two introducing values are the given residue values of two interval and corresponding residue values related to the given residue values of two interval and corresponding residue values related to the given residue values of two intervals are related to the given residue values of two intervals are related to the given residue values of two intervals are related to the given residue values of two intervals are related to the given residue values of two intervals are related to the given related to the given residue values of two intervals are related to the given related to samples to assure maximal homogeneity.

Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid refine to oil seed rape fectar and pollen complex  $\mathcal{O}$ 

sampres				Ĉĩ M	· <u>~</u> (	
San	nple Material		Residue A		Residue midacloprid⊻5- \$droxy [ng/kg]	O'Restoue Imidacloprid oletine [µg4g]
Necta	r (oil seed rape)	Q	<			
Polle	n (oil seed rape)			-44.3	A DO N	
						- <u>~</u>

🖤 = 0 φμg/kg φaidaclowid in nestar samples an Ø.6 μg/kg in pollen samples, LOO = Limit of Quantitation 1  $\mu$ g/kg for imidaclprid-5-hydroxy and imidated oprid define in neutral samples of  $0.2 \mu$ g/kg/m/on pollen

LOD = Limit of Detection  $= 0.1 \mu g/kg$  for imidacloprid in neutral samples samples, 0.3  $\mu g/kg$  for imidacloprid-5-hydroxy and imidacloprid obsine in hettar samples

### **Conclusion:**

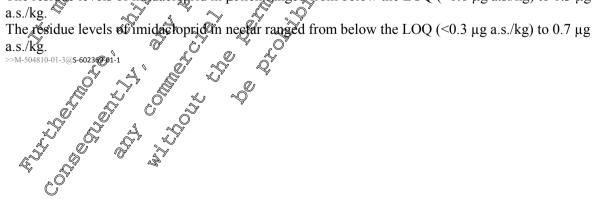
The study was condicted on a field site war Ribeaucourt (F-55290, France) with a known history of crops and imidacloprid uses as such with natural aged soil-residues of this active substance. Therefore, this study provides realistic field data on residue levels of imidad oprid within bee relevant matrices, collected from non-invidual oprid treated flowering winter oilseed rapegultivated as succeeding crops from a field with natural aged softeresidies of pridacloprid.

# Winter Øilseed Rape.

Soil cores used for esidue analysis were aken from the entire field prior to placement of the honeybee colonies into the winnels The residue level of midacloprid the Winter Oilseed Rape plot was 45 µg Ô a.s./kg dry soil

a.s./kg dry soil Residue analysis of pollen and nectar, collected at three time points during blooming of winter oilseed Ő

The resider levels of invidacloprid in collen, anged from below the LOQ (<0.6 µg a.s./kg) to 1.3 µg a.s./kg.





Report:	02.02.02/08; 2014; <u>M</u>	-504842-01-3	3	e °
Title:	Determination of the residues	of imidaclop	rid in bee relevar	t matrices collected from
	succeeding crops following aj	oplication of	imidacloprid FS (	500E G via soil incorporation
	to plateau concentration and s	owing of imi	dacloprid-treated	winter barley seeds. Field
	phase conducted in southern I	France		
Report No.:	7SRFR13C3		0	
Document No.:	M-504842-01-3		A	
Guideline(s):	U.S. EPA OCSPP 850.SUPP	(PA	"Ś"	
Guideline deviation(s):	none		Ű	
GLP/GEP:	yes	¢ V	R	
	•	and a start	L.	

### <<M-504842-01-3@S-602377-01-1

Aim: determination of the amount of residues which may be taken up and transported 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 imidad opridand its metabolites imidad oprid-5hydroxy (hereinafter named 5-hydroxy and imidacloprid-olofin (hereinafter called olefor) in ber relevant matrices (pollen, nectar and guttation of fuid) collected from succeeding crops for owing application of IMIDACLOPRID FS 600E G via soil incorporation to plateau conceptration and sowing of imidaclopridtreated winter barley seeds.

### **Study Site:**

The study was conducted on a field site pear Ninnes (E-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: phacelie (Phacelia tanacetifolia), invistard (Sinapis arvensis) and maize (Zea may@(each in an area of approx)0.2 hav.

### Material and Methods

# Test item and application:

The test item imidacloprid was applied in autumn 2013 with two different calculated plateau concentrations directly to bar 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	Sowing of treated winter barley
$\sim$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$	seeds * (10.10.2013)
Low plateau concentration + low 97.3 g 97.ha seed dressing rate (variant blue) 97.3 g 97.ha	85.8 g a.s./ha
seed dressing rate (variant blue) Q 44 L product/ha	184.5 kg seeds/ha
	118.5 g a.s./ha
High plateau concentration + high seed dressing rate (variant green)     154.0% g a.s./ha       *Actual concentrations     0.254.1 product/ha	189.5 kg seeds/ha
*Actival concentrations	·

In 2014, Winter barley crops were removed and untreated succeeding crops (Mustard, Phacelia and Maize) were fown on the areas with previous imidacloprid applications.

Three bee proof turnels (00 m long x 5 m wide x 3 m high) were placed onto the phacelia and the mustard bot after successful germination. A single honeybee colony was placed into each tunnel at the start of Phaceka, respectively mustard flowering

The spo plowown 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



### 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;

Soil characterisation of the upper 10 cm soil layer. 1.

Determination of the residues of parent imidacloprid and its metabolite on the upper of cm soil 2. laver.

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 Rhacelia or

Sampling of Nectar and Pollen from Phacelia or Mostard 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 colonic were placed Otcorresponding crop. Once the crop started to blown, Honeybee colonie were placed into mesh covered 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 for agers returning to the colony using a pollen trap attached to each colony. Pollen and negrar samples during bloom were analysed for residues of imidacloprid m

Sampling of Guttation fluid and Pollen from Maize: Ŵ from the crop by hand. Ì  $\bigcirc$ Sampling of guitation fluid was carried out on Fregular basis over a XYZ day period. Guttation sampling

started directly after emergence of the maize grop (BBCH seale 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 Š

Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub Ś plot 3.  $\bigcirc$ 

When guttation was present it was collected from >10 plant@throughout each of the sub plots. The target volume for each sample was find of suttation fluid S  $\bigcirc$ 

Pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering has completed (BBCH cale 67).

At each time point  $\geq$  50 howering tassets were collected from throughout each of the three sub plots and placed into paper bags Dams tassels were on dried, in the dark at room temperature overnight.

Next day, the pollen was slaken 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 inidadoprid in soil comples and samples of guttation liquid, nectar and pollen was performed by using High Performance Equid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection. Analysis of the soil samples followed the provisions of method 00796 M001 Analysis of guttation liquid mainly followed the provisions of method

00533 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  $\mu$ g/kg for pollen, 0.3  $\mu$ g/kg for nectar and 1.0  $\mu$ g/L for guttation liquid while the



Õ

LOO level of the metabolites were constant 1.0 µg/kg for all sample materials. The corresponding Limit of Detections (LOD) were 0.2  $\mu$ g/kg for pollen, 0.1  $\mu$ g/kg for nectar and 0.3  $\mu$ g/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% Å 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 a	
Residues of imidacionrid in soli (green g	and nine mors) $(// \hbar)$
itesitates of innoactoping in son (green i	into orac proco,

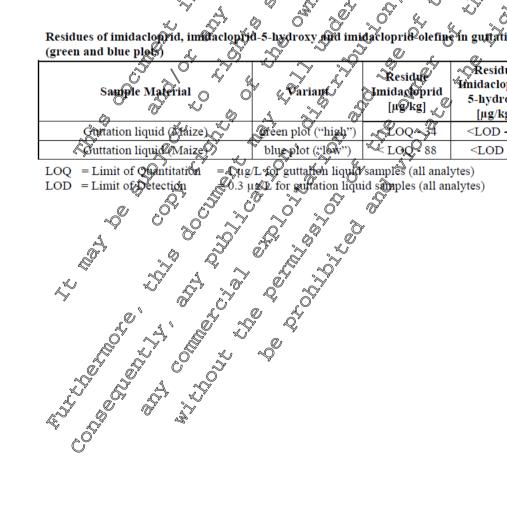
	-	0	· / ( ·	· ()	
Sam	ple material	Variant	Residue Intégacioprid & [µg/kg]** @uring@ioom_%		bloom
	Soil	green plot ("high")	26-72	1000 - 17.33	Ø - 93 Ø Ø
	Soil	blue plot ("low")	×12 - 62	0.53 - 1 .55	O <sup>×</sup> 2 34 - 82 <sup>×</sup> C <sup>×</sup>

 $LOQ = Limit of Quantitation = 5 \mu g/kg for invitacloperatin/on soil samples$ = 2  $\mu$   $\sigma$  g for maidacle prid in  $\sigma$  soil somples LOD = Limit of Detection

Residue imidacloprid [µg/kgD(1-(Moisture/100); For the calculation of imidacloprid residues related to dry soil, \* unrounded values were used. Therefore minor deviations thay occus when ounded values shown within this table are used. \*\* The given residue values and corresponding residue values related to set soil are mean onus of two individually extracted samples to assure maximal homogeneity.

Residues of imidaclopiid, imidaclopiid-5-hydroxy and imidacloprid olefine in guitation liquid samples

	Saupple Material		& Variant	Řesidu Jmidacoprid [µgkg]	Residue Unidacloprid- 5-hydroxy [µg/kg]	Residue Imidacloprid- olefine [µg/kg]
	Guytation liquid (Maize		een plot ("high")	~ LOQ~34	<lod -="" 12<="" td=""><td><loq -="" 2<="" td=""></loq></td></lod>	<loq -="" 2<="" td=""></loq>
4	Guttation liquid Maize	D bl	lueplot (:"low")	LOQ <sup>Q</sup> 88	<lod -="" 9<="" td=""><td><lod -="" 2<="" td=""></lod></td></lod>	<lod -="" 2<="" td=""></lod>





# 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 Imidacleprid- ole@ne [ng/kg] &
	Nectar (Mustard)	arean plat ("high")	<loq -="" 0.5<="" td=""><td><lod< td=""><td>~LOD ~</td></lod<></td></loq>	<lod< td=""><td>~LOD ~</td></lod<>	~LOD ~
	Nectar (Phacelia)	green plot ("high")	0.8 - 1.0	LOD	
	Nectar (Mustard)	blue plot ("low")	A.7 - 3.9	∮LOQ 5	LOR-XLOQO
	Nectar (Phacelia)	once plot ( low )	¶©OD – <loq< td=""><td><pre>LOD C</pre></td><td></td></loq<>	<pre>LOD C</pre>	
LOQ	= Limit of Quantitation = $($	0.3 μg/kg imidaclopa	fal in nectar sand	ples, 1 µg/kg/fo	r imidaelpra-5-

 $LOQ = Limit of Quantitation = 0.3 \ \mu g/kg imidaciopeta in nectar samples, 1 \ \mu g/kg for imidaciopeta-5$ hydroxy and imidaciopeta-olefine imprectar samples

LOD = Limit of Detection =  $0.1 \ \mu g/kg$  for initecloprid in nectar samples,  $0.3 \ Qg/kg$  for imideclopridhydroxy and imidacloprid-olefine in nectar samples

Residues of imidacloprid, imidaclop	orid-54	ydroxy an	d imit	laclopri	d-olefine	in poll	en samj	oles (gr	een and
blue plots)		, C	KŰ		«»	, Si	Ĩ	Š	$\bigcirc$

						0.*
Sample material		Varia		Qug/kg	μg/kg	Résidue Imidacloprid- olefine (µg/kg)
Pollen (Mustard)		, S	L	1.6 - 4.7	LOD& <loq< td=""><td><loq -="" 1.2<="" td=""></loq></td></loq<>	<loq -="" 1.2<="" td=""></loq>
Pollen (Maize) 🖉	) O'	green plot	Ønigh")	COQ - 0.93	ALOD X	<lod -="" <loq<="" td=""></lod>
Pollen (Phacelia)	Â			<b>4</b> ♥ − 2.0	K/KLOBC	<lod< td=""></lod<>
Pollen (Musturd)	S ×	ĝ O	ð	°∼1.8 - 5¥	CLOD S LOQ	<loq -="" 1.2<="" td=""></loq>
Pollen (Marze)		blu@plot (	Yow")	COQ - 1.2	Q <lod <="" <loq<="" td=""><td><lod< td=""></lod<></td></lod>	<lod< td=""></lod<>
Pollen (Rhacelia)			~ ^Ô	<1690-0.€	<b>%</b> LOD	<lod< td=""></lod<>
Loo Linita on Linita	tion of the					. toold. A standard a

 $LOQ = Limit \textcircled{of} Quantitation \neq 0.6 \ \mu g/kg \ madacloprid in/or \ polled \ samples, 1 \ \mu g/kg \ for \ imidaclprid-5- \ of \ droxy, \ and \ imple \ cloprid-olefing \ in/on \ pollen \ samples$ 

LOD = Limit of Detection =  $0.2 \ \mu g/kg$  for mutaclound in/on pollen samples,  $0.3 \ \mu g/kg$  for imidaclouid 5-hydroxy and imidaclouid-olefine in/on pollen samples Q

# **Conclusion:**

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-relevan matrices (nectar and polled) and guttation droplets of succeeding crops. In a model approach, two levels of bridacloprid 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 polletrand nectar, as collected at one time during blooming of Phacelia, in three tunnels per test rate revealed in ow 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



ranged from below the LOQ (< 0.3  $\mu$ g a.s./kg) to 3.9  $\mu$ g a.s./kg. Residue levels of imidacloprid in pollen ranged from 1.6  $\mu$ g a.s./kg to 5.1  $\mu$ g 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 imidacioprid in guttation fluid ranged from below the LOQ (< 1  $\mu$ g a.s./L) to 88  $\mu$ g 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  $\mu$ g a.s./kg) to 1.2  $\mu$ g a.s./kg.

Overall, transfer of Imidacloprid soil residues into bee-relevant matrices and guitation 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 Initiacloprid metabolities were only beasured in single guttation or pollen samples

Report:	02.02.02/09; (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)
Title:	Residues of instacloprid in nectar and pollop of flowering relation decrops in Western
Report No.:	
Document No .:	
Guideline(s):	Regulation (EC) Notisi 07/2007
Guideline deviation(s):	
GLP/GEP:	
< AL 504954 01 2 GE (00070 01 1	
< <m-504854-01-3@s-602379-01-1< th=""><th></th></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.

to determine residues of imicacloprid and its metabolites 5-hydroxy and olefine in nectar and pollen of the verine 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 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 hareach, 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:

Please click on the hyperlink to order a Study Report.



	Imidacloprid	Imidacloprid
	Application of the Plateau	Sowing of treated winter barley seeds*
	Concentration*	seeds*
	26.09.2013	0,9,10.2013
Low plateau concentration	95.4 g a.s./ha	69.2 g a.s./ha
+ low seed dressing rate	0.157 L product/ha	136 kg seeds/ha
(Variant blue)	S.157 Epiodadi, ng	(with 46.5 ga.s./db)
High plateau concentration	173.4 g a.s./ha	126.3 sa.s./bo
+ high seed dressing rate	0.286 L product/ha	Q <sup>°</sup> 2020kg seeds/ha
(Variant green)	× ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	َنْ (with 62.5) a.s. (طَرْ)
*Actual concentrations	O <sup>V</sup> Q <sup>V</sup> Z	A & A & A

In spring 2014, untreated phacelia, mustard and maize were sown on the study plots which contained soil residues from the previous Imidaclopric 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 howering. The following ranges of binidac oprid residues were determined:

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Nectar & Pollen sampling: Horeybee colonies were placed into mesh covered tunnels crected 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.

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\$ 1

Nectar was collected by honey bub extraction from for ager bees in mustard and phacelia crop. For each nectar sample about 800-P000 returning for ager bees were collected with a modified vacuum sampler, deep-frozen and transported to the laboratory for nectar extraction. Targeted nectar amount per sample was  $\geq 500 \text{ mg}$ .

Pollen of phacelia and pustart was conflected from foraget 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 hree times during flowering of maize plants (BBCH 63-65). The pollen, targeted were 1.5 g per sample, collected from at lease 30 plants, was shaken out of the flowers into paper bags and cleaned by siesing (mesh size 2 mm and 1 mm).

Maize guttation fluid, farget 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 (poller), nector and suttation fluid) were analysed for their content of imidacloprid and its metabolites 5-hodroxy and olorine via HPLC-MS/MS. Residues are reported in terms of µg active substance/kg for poller), nector and soil respectively µg/L for guttation fluid.

Results and Discussion

Imidacloprid residues - Measured range for soil and bee relevant components



# **Imidacloprid Bee Studies Compilation of Study Summaries**

Issue date 2017-11-22

		Imida	cloprid	
Matrix	Сгор	Variant blue (after application 95.4 g a.s./ha plateau + 63.2 g a.s./ha treated seeds)	Variant green (after application 173.4 g a.s./ha platedu + 126.3 g a@/ha treated seed	
	2013 PEC plateau	71	140 × × × ×	
Soil*	2014 Phacelia	9-13 💍		
[µg/kg]	2014 Mustard	12-18	CH-13 & 0	
	2014 Maize	9913 °		
	Phacelia		< 200 − 6x62 →	
Pollen [µg/kg]	Mustard			
	Maize	20 8 6 < 60 2 2 2		
Nectar	Phacelia 🦉		\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	
[µg/kg]	Musţa <b>¢a</b>	2 < LOD - 0.57	29	
Guttation [µg/L] calculated	Maize &		≪	

soib 0.6 µg as./kg for polleg 0.3 µg as./kg for nectar and 1 µg a.s./L for LOQ = Limit of Qontification = 5 µg a.s./ guttation liquid ample for imidacloprid /kg/for

LOD = Limit of Detection = 2 µg a.s./kg for soft 0.2 µg a.s./kg for polle@ 0.1 µ@.s./kg for nectar and 0.3 µg a.s./L for guttation for include amples for include apprile.

Imidacloprid metaboli	tes residues	s - Measured	range for bee	relevant components
•	. W & V			

~		Imidacloprid-olefine			
Matrix	Crop 🖒	Variant blue	Variato green	Variant blue	Variant green
Pallan	Phacelia	<sub>Δ</sub> <ἶΟ <u>Σ</u> →< LOΩ	<_ <b>00</b> - < LOQ	< LOD	< LOD
. <mark>Pollen</mark> [μ̃g/kg]	Mustard 🖓	Ĩ <sub>Š</sub> ≪ <mark>LOD</mark> Q	∽\$ < LOD	< LOD - < LOQ	< LOD
[µg/ ∿g]	<sub>@</sub> ,Maize	ر < ۲ <u>۰</u> ۵	Ky < LOD	< LOD	< LOD
Nectar	Phacelia		🖗 < LOD	< LOD	< LOD
[µg/kg]			< LOD	< LOD	< LOD
Guttation	Maize	C LOD – 2	< LOD – 11	< LOD - < LOQ	< LOD – 2

LOQ Limit @ Quantification = For the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine 1  $\mu$ g a.s./kg for all matrices.

LOD = Umit of Detection = For the metabolites imidacloprid-5-hydroxy and imidacloprid-olefine 0.3 µg a.s./kg for all matrices.

# Conclusion



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 barkey seeds (again with two different seed dressing rates) were sown (see overview below)

## Phacelia:

<u>Residues analysis of pollen and nectar</u>, as collected at three time points during blooping of phace that, 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  $\mu$ g a.s./kg) to 0.49  $\mu$ g a.s./kg. Residue levels of imidacloprid in pollen ranged between from below LOD (< 0.2  $\mu$ g a.s./kg) to 0.62  $\mu$ g a.s./kg.

# Mustard:

<u>Residues analysis of pollen and nectar</u>, 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  $\mu$ g a.s./b) to 0.63  $\mu$ g cs./L. Residue levels of imidacloprid in pollen ranged between from below LOQ of 0.6  $\mu$ g a.s./kg to 1  $\mu$ g a.s./kg.

# Maize:

<u>Residues analysis of guttation fluid</u>, as collected from directly after emergence that early bloom of the maize plants, revealed in generally low residues. The residue levels of initiacloprid in guttation fluid ranged from below the LOD (< 1 mg a.s./b) to 26  $\mu$ g a.s./L and are the 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 <u>pollen</u>, as sampled at three time points during bloom on three subplots was always below the LOD  $(< 0.2 \ \mu g \ ao \ kg)$ ,  $\mathcal{Q}$ 

Overall, transfer of Imidacloprid soil residues into bee-relevant matrices and guttation droplets of succeeding cross 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.

Plateau concentrations in soft 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 are Science and Nimes.



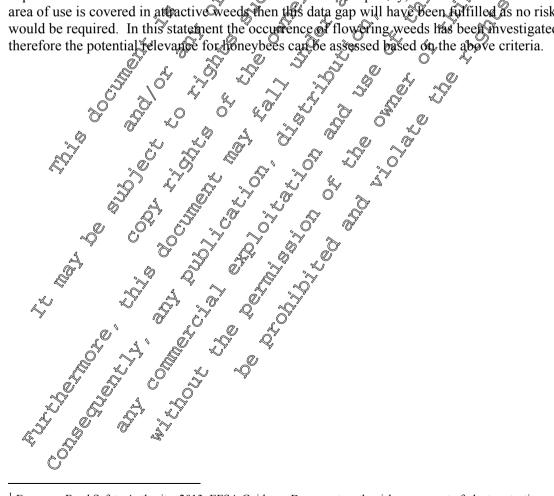
Issue date 2017-11-22

# 02.02.03 - Weeds

Report:	02.02.03/01; ; ; 2014; <u>M-505126-01-</u> ;	3	
Title:	Statement - Evaluation of the occurrence of	flowering weed on agricultur	alærops: 🔊
	Cereals, sugar beet and potatoes		4
Report No.:	<u>M-505126-01-3</u>		
Document No.:	M-505126-01-3		
Guideline(s):	US EPA OCSPP Guideline No. 850 SUPP	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Guideline deviation(s):	~~		
GLP/GEP:	no		
			Q, Q'

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 sundardized methods are available.

The European Food Safety Authority (GFSA) bee risk assessment scheme requires a first tier assessment through various exposure scenarios (DFSA 20131). To date this document has not been adopted as the official European guidance and remains the guidance of EFSA. One exposure route sited 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 File 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



<sup>&</sup>lt;sup>1</sup> 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



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Issue date 2017-11-22

# 02.02.04 - Honey dew

<b>Report:</b> 02.02.04/01; 2013; <u>M-453965-01-3</u>	
Title: Statement - Information on the occurrence or possible occurrence	e of the development of
resistance of the plant protection product Janus Forte (for spomis	sion in Europe) 🖉
Report No.: <u>M-453965-01-3</u>	
Document No.: <u>M-453965-01-3</u>	
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 submissio	ns on plant protection
products and their active substances $\sqrt[n]{0}$	4
Guideline deviation(s):	
	L A o

Resistance in arthropod pest species comprises a change of the genetic composition of a population in response to selection by pesticides such that control in the field may be impared repeatedly at recommended application rates. The report includes resistance management information regarding key invertebrate pests targeted in sugar beet in countries such as Belgium, Crech Republic, France, Germany, Poland, Romania, Slovakia and Serbia by seed treatments with Janus Porte® (JFS 280) containing the insecticidal ingredients clothianidin, imigaclopfed and Peta-colluthrin.

Ľ 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 invelved in any cross-resistance issue globally.

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 $\sim$ 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-cyfluthring are classified by IRAC (Insecticide Resistance Action Committee) in prode of action classo A, sodium channel modulators.

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Resistance of pyrethroid insectivities has been described for the free of the pests and the major mechanisms of resistance were identified as either metabolic (estorases and monooxygenases) or knockdown-resistance (kdf) due to a mutation in the US6 domain of the voltage-gated sodium channel. All of the pest insects interded to be targeted by Betaccyflut@rin in Janus Forte® as a seed treatment are not listed as high risk pests within PPO/ 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 ape 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 larvar (Agriotes ssp., wirgworms), weeves (Berhynoderes), flea beetles (Chaetocnema ssp.) and Atomaria linearis. Other important pests target in subar beet include aphid pests such as Aphis fabae and Myzus persicae, thrips (Thrips tabacy), dipterans (Pegomyia), millipedes (e.g. Blaniulus guttulatus) and myriapodes (e.g. Scutiger ha immaculate). Neonicotinoid insecticides such as clothianidin and imidaclopfed are Gassified by ISAC 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 B-subunit. No reports are known from any secondary host species yet, including sugar beet and vegetables.



In sugar beet no resistance to clothianidin, imidacloprid and beta-cyfluthrin seed treatments is vet described for any of the pests or pest groups mentioned above, including aphid species such as Aphis A. J. fabae and Myzus persicae (particularly targeted by systemically acting clothianidin and imidaclopsed).

mi. des as pr pred as ner pr fabee and Myzus persicae (particularly targeted by systemically acting clothianidin and imidaclongd). General resistance management guidelines for neonicotinoid and pyrethroid inserticides as published IRAC are usually followed with products such as Janus Forte® and regionally clapted as necessary in the products such as Janus Forte® and regionally clapted as necessary in the products such as Janus Forte® and regionally clapted as necessary in the products such as Janus Forte® and regionally clapted as necessary in the products such as Janus Forte® and regionally clapted as necessary in the products such as Janus Forte® and regionally clapted as necessary in the products of the product and the second state of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner of the owner ow General resistance management guidelines for neonicotinoid and pyrethroid insecticides as published by

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Issue date 2017-11-22

# 02.02.05 - Guttation

Report:	02.02.05/01; 2014; <u>M-498939-01-3</u>	) v
Title:	Field study to monitor potential effects on honey bees from posure to guttation fluid of	f
	Field study to monitor potential effects on honey bees from posure to guttation fluid of winter wheat (W-WHT), seed-treated either with an imida poprid or a clothanidit	
	combi-product	
Report No.:	R09247-4 $(x^{2})^{2}$ $(x^{2})^{2}$ $(x^{2})^{2}$	
Document No.:	<u>M-498939-01-3</u>	<i>a</i> .
Guideline(s):	U.S. EPA OCSPP 850.SUPP	Ç,
Guideline deviation(s):	not applicable	)"
GLP/GEP:	no $\sqrt{Q^{\prime}}$ $\sqrt{\gamma}$ $\sqrt{Q^{\prime}}$ $\sqrt{Q^{\prime}}$ $\sqrt{Q^{\prime}}$	
< <m-498939-01-3@s-602266-01-1< th=""><th></th><th></th></m-498939-01-3@s-602266-01-1<>		
Aim of the Study		

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 affacent to fields sour 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, Bader Württemberg) for Sputhern Germany and the Institute of Apiculture in Celle (Dr, you der Ohe, Dower 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 pertest location. One of the two treatment groups per test location was imidaeloprid-treated (=imidaeloprid-treatment group) and comprised one individual study field, on which imidaclopride reate OW-WAT seeds were sown, the other treatment group per test location was chathianidin-treaded (=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 sowrk(seed-treated with a routine fungicide (EfA®)). O

Õ Ò Moreover, all seeds were additionally seed-treated with commercial INTECO<sup>®</sup>, in order to minimize dust abrasion.  $\bigcirc$ 21

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As such, treatment is defined by the presence and the potential exposure of honey bees to either the systemic neonicotinoid insecticide imidaclopfie or to the systemic neonicotinoid insecticide clothianidin. *P* 

All W-WHT seeds were seed treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim, Germany. In total, two different WWHT 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.

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Key study of evelopment and the overwintering performance of posed 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 exuctation of guttation fluid of W-WHT and flight activity of honey bees occurred singultaneously.

In case 10 ght 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 thirtyfive 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 F& + 70 + 7.2 + 8) or clothianidin-treated (Clothianidin & Beth-Cyfluthrin 58 375 + 80) despectively.

### 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) ast 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 heated at the Joinger Hof experimental field station for plant cultivation and protection of the University Hohenheim fin the following called Ihinger Hofo

On each of the two test locations one study told was assigned as phidacloprid-treated fild (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 assoned as control field (on which non-insecticide treated ( wontrof) W-WHT seeds were sown, respectively As there were in total two test locations, the study comprised in total two imidaclopride freated fields, two clothianidin-treated fields and two control fields, giving overall six study fields under investigation. , V

# Set-up of honey bee hives

At each of the six stuffy fields under investigation five koney 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.

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# 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 m-crop) covered the immediate area in front of the bee hives and Zone 1 % 2 m broad hand, shaped like an inverted 'U', with a vertical distance of the band to the field margin of 7 m prside the crop). The see hives were placed into the Off-Crop Zone, directly adjacent to the W-WHT crop (width: 10 relengther along the field margin, 1 m depth into the offcrop). In addition, two 1 moassessment plots were established to record the proportion of W-WHT displaying guttation and/or dew

# Honey bee mortality

Each five was equipped with a dead be grap. 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 guitation 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



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 ô occurrence of guttation fluid and/or dew. If guttation was still present at the start of honey begactivity. the numbers of honey bees resting or walking on the ground or on the W-WHA 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 a (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 ono complete observation cycle of the assessment area and its associated two segregated plots of A m<sup>2</sup>, at which guttation- and honey bee assessments wore conducted during the presence of guttation flyid on the W-WHT crop. Ý

# Honey bee colony strength and hearth assessment

<u>Honey bee colony strength and hearth assessment</u> At both test locations (i.e. Ihinger Hot 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) cology 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 Whow (Salix caprea) until beginning of winter oil-seed flowering in the respective region. Maintaining of the bee hives as we has all boney 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. Rosenkanz, Baden-Württemberg) in Southern Germany, respectively.

Imidacloprid and clothanidin esidues in the various samples were analysed by an analytical laboratory of Bayer CropScience AG. in the

Results <u>Frequency of guttation</u> During the assessments in the morping, 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 WWHT and becactivity in the evening in autumn 2009 and spring 2010 was observed.

# Duration of guttation

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Whenever guttation was observed of a respective day, it was already present in the early morning. Depending on the advalation with the 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, guitation lasted for several hours.

# Honey bee activity on 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.



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 hone bees observed per monitoring on plants in the respective assessment areas in both, meatments and control, was mostly higher in spring 2010 than in autumn 2009. With the exception of honey bees on soft surface: in autumn 2009 the observed relative proportion was three to four times higher in Zone 0 than in spring to 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 homey bees were recorded taking up guttation fluid within the assessment zones (which includes the Off-Crop Zone). Most of the bees faking op dew or guttation fluid were observed in Zone 0, i.e. directly in front of adjacent to the hixes. Accounting for all honey bees directly observed during the individual monitoring sessions within the assessment area to 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 gattation fluid were observed during springtime (341 of 343 bees), which gives a relative propertion of bees taking up guttation fluid in autumn of 2 bees / 404 bees = 0.5 % and of 341 bees / 2,872 bees = 1 % doping 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 ZNG and TZMU (clothianidin treatment/group) or their content of the imida oprise metabolites in idacloprid-5-hydroxy and imidacloprid-olefin (imidacloprid treament group). Chromatography and detection by MS/MS was performed according to method 00554/MO01 (clothiandin, T2NG and TZMU) or method 00537/M002 (imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin).

The Limit of Quantingtion (LOQ), of each analyte in guitation faid was 0.01 mg/L and the Limit of Detection (POD) of each analyte was (COO1mg)L, respectively.

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The residue levels of Sothian din 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 guttator water were within the range of LOD to 6.9mg/L. The residue levels of imidacloprid-9-hydroxy in Outtation water were within the range of <LOD to 0.61mg/L. The residue levels of inidacloprid-olefin in suttation water were within the range of <LOD to 0.12 mg/L.

# Honey bee mortality

At both study sites, honey bee mortality in automn 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 fifluenced by the experimental setup.

During spring time, the modality found in the traps was generally low, but still variable from colony to colony and with higher portality 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.



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 from of the hivest. The relative proportion of boney 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 dev 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; 400 bees during autumn and 2,872 bees during springtime) overall amoderate 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 / 2,802 bees = 11.9% during springtime.

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. Baring 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 metabolities TZNG and TZMF in guttation water ranged between <LOQ to 0.49mg/L and between LOD to 0.32mg/L, respectively.

Also for imidadoprid 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 clothranidin, 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 0.64mg/L or between <LOD to 0.12 mg/L, respectively. No treatment related differences in kiney be 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 the atment 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 neomicoticoids, these not have unacceptable effects on honey bee colonies under typical commercial use conditions.



<b>Report:</b> Title:	02.02.05/02; 2012; M Field study to monitor potent winter barley (W-BAR), seed	<u>-498922-01-3</u> ial effects on h l-treated either	oney bees from ex with an imidaclo	xposure to guttation fluid of
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### Aim of the Study

The study was conducted on two separated test locations (study sites) from the and 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 begeolonies were set up directly adjacent to fields sown with winter barley (W-BAR) seeds, in order to investigate the potential offects from exposure to guttating W-BAR, starting from seedling emergence in autumn 2009 until beginning of wither 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- Wurttemberg) for Southern Gemany and the Institute of Apiculture in Celle (ID. von der Ohe, Lower Saxony) for Northern Germany gespectively. 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: Que of the two treatment groups per test location was insidacloprid-treated (=inidacloprid meatment group) and comprised one individual study field, on which invidacloped treated W BAR seeds were sown; the other treatment group per test location was clothfanidin-freaded (=clothfanidin-treatment group) and comprised also one individual study field on which clothianidin-treated W-BAR seeds were sown. For test location there was in addition one control group, comprising one individual study fold on which non-insecticide treated (=control) W-BAR geeds were sown (seed-treated with a routine fungicide

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 imidation in the systemic neonicotinoid insecticide initiation.

All W-BAR seeds were seed-treated of 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 jest 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 pypica Commercial use conditions.

Key study objectives were to evaluate and to compare the colony development and the hibernation performance of exposed hones 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 explation of guttation fluid of W-BAR and flight activity of honey bees occurred simultaneously. In case thight 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-fiveminute 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.



# Material and Methods

Test item

W-BAR seeds, either imidacloprid-treated (Triadimenol & Imidacloprid & Fuberidazol & Imazalit 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 Ibinger Hof).

On each of the two test locations one study field was assigned as imidaclopred-treated field (on which imidacloprid-treated W-BAR seeds were sowo), one Study field as plothianidintreated field (on which clothianidin-treated W-BAR seeds were sown) and one stady 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 of total two in adacloprid-treated fields, two clothanidin freated 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 alones were placed along a line one to eight days before sowing, either directly adjacent or within a maximum distance of 9.5 m to the W-BAR crop, depending on the actual local field signation.

Assessment area A specified area (assessment area) in front of the boney 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 pr to each side of the hives, 2 m depth into the in@rop) covered the immediate area in front of the beedives and Zone 1 (a m broad band, shaped like an inverted 'U', with a vertical distance of the band to the field margin of 7 m inside the grop). The beenives were placed into the Off-Crop Zone, directly adjacent to the W<sub>2</sub>BAR crop (width: 10 m length along the field margin, 1 m depth into the offcrop). In addition, two Om2 assessment plots were established to record the proportion of W-BAR displaying guttation and/or dew. A

# Honey bee mortaby

Each hive was equipped with a dead bee trop. The praps were emptied daily during the monitoring period to record the number of dead honey bees After of October 2010, also dead bees found on the soil surface in front of each colony, respectively, were recorded

### j, Guttation fluid sampling

In case guttation was observed in the maning are respective field, up to three samples of guttation fluid, each with a volume of approximately , mL were collected from various plants of W-BAR. The samples were thereafter deep frozen (20°C) for later analysis.

# Monitoring

The monoring activities stared 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 moniforing 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,



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 n (autumn and spring), one observer was continuously responsible for two study posts. At the story 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 mL 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 of both est locations (i.e. Ihinger Hoff and celle), the colony strength and the colony development were estimated according to the Liebefeld method (Indorf et al. 1987). The first assessment was performed in mediately after coony set up; further assessments were performed every 21 days until end of October 2009. In spring 2010, colour development was assessed in the same manner from the beginning of inflorescence of the Goar Willow (Satix capica) until beginning of winter oil-seed flowering in the respective region. Maintaining of the bechives 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 Fermany, respectively.

0 Ő Residue analysis Imidacloprid and cloppianidifresidues in the various samples were analysed by an analytical laboratory of Bayer CropScience AG

Results

Frequency of Suttation

Frequency of guttation with the morning guttation fluid was observed on W-BAR at 84.2 % of all observation days in automn 2009 and at 80.7 % of the observation days in spring 2010. No remarkable coincidence of guttainin of W-BAR and bee activity in the evening in autumn 2009 was observed. A coincidence during his period of time occurred, with few exceptions only, just on those days where guttation anyhow prevailed for the whole day due to damp of rainy weather. In spring 2010, no coincidence between presence of guttation in the gening and bee activity was observed at all.

# Duration of guttation

Whenever guttation was observed on a respective way, 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 afternoop and on some occasions even until evening. On most observations days, guttation lasted for several hours.

Honey bee a wivity in the assessment area

During the entire field monitoring periods in autumn 2009 and spring 2010 (comprising a total of 264 individual moniforing session 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 \$11 in the In-Orop Zones and 319 in the Off-Crop Zone; 1,918 honey bees were resting on plants, with 1,380 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.



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 entre 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 boney bees directly observed during the individual monitoring sessions within the assessment areas mosth, treatments and control, a moderate proportion of bees was observed taking up guttation fluid, i  $\bigcirc 334$  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  $\bigcirc$  autumn of 33 bees / 1,267 bees = 2.6 % and of 301 bees / 1,887 bees = 16 % during springtime.

# Residue analysis of guttation fluid

All samples of guttation fluid collected from the treatment fields were analysed either for residuer of imidacloprid or clothianidin, respectively. Selected samples of guttation fluid collected from the treatment fields were additionally analysed for their content of the clothianidin metabolities. TZNG and TZMU (clothianidin treatment group) or their content of the imidacloprid metabolities imidacloprid 3-hydroxy and imidacloprid-olefin. Chromatography and detection by NS/MS was performed according to method 00554/M001 (clothianidin, TZNG and TZMU) or method 00537/M002 (imidacloprid and its metabolites imidacloprid-5- hydroxy and inidacloprid Stefin). The Limit of Quantitation (LOQ) of each analyte in guttation fluid was 0.01 mg/L and the Limit of

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 Flothiandin in guttation water were within the range of  $\leq$  LOD to 2.3 mg/L. The residue levels of TZNG in guttation water were within the range of  $\leq$  LOD to 0.05 mg/L. The residue levels of TZMU in guttation water were within the range of  $\leq$  LOD to 0.05 mg/L. The residue levels of imidacloprid in guttation water were within the range of  $\leq$  LOQ to 15 mg/L. The residue levels of imidacloprid in guttation water were within the range of  $\leq$  LOQ to 0.64 mg/L. The residue levels of imidacloprid of the range of  $\leq$  LOQ to 0.64 mg/L. The residue levels of imidacloprid-5-hydroxy in guttation water were within the range of  $\leq$  LOD to 0.64 mg/L. The residue levels of initial cloprid-olefin in guttation water were within the range of  $\leq$  LOD to 0.05 mg/L.

Synoptic assessment of honey bee mortality and colony performance Effects during the autumn expositive performance

*Effects during the autumn expositive period* During the approximately 5 week's continuous autumn exposure period, none of the treatment colonies revealed adverse effects in terms of mortality rate 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 apringtime, 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 transfates into an overwintering success (total success) rate of 80 (80)% in the control group and 89 (801% in the imidacloprid treatment group, indicating that guttating W-BAR seedlings, carrying wigh 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 autumper post of (0 colonies in control, 1 jothe 2 imidacloprid treatment group and 2 in the clothianidin treatment group), a clear corelation 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 overgeinter and/or to survive overwintering (see below).

# Methodological deficiencies resulting in experimental biages, particularly for the clothanidin treatment group

The autumn- and overwintering conditions for the clothranidin treatment group were substantially less favourable as compared to the control and/or to the innidacleprid treatment group due to three key factors:

- Higher number of weak colonies at study initiation Colonies which have a below average colony strength in automn 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  $\leq 8,000$  bees with 2/2 and 3 of such colonies being assigned to the control (colonies 7/2 and 7/4), the imidaeloprid-meatment 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 ( $\sqrt[6]{4}$ ) 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 hiberinate successfully.

In the imidacloprid reatment group one of the two weak colonies (8/1) was removed before overwintering as front 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 overwritered successfully, the restoring of this colony during springtime would have required favourable sincumstances.

In the clothianidin-freatment group, twoof 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 autumer but remained too weak too inally survive the winter.

When comparing the colony performance of all initially weak colonies, they all showed a similar pattern across experimental groups, i i no restore of colony strength except forv control colony (7/2) due to better brood ross. 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 overwintenng. 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



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 on Celle) during the study), it is a matter of fact well known in apiculture that the anti-*Varroa* treatment score yield 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 nate drop ( $\Box$ : 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 coronies 15/3 and 15/5 (both: study site Celle) in the clothianidin treatment group suffered from a mgh *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 Cetre) exhibited during both, the preoratic 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 colonie (15/3) and 15/5 However, the mite drop in the colony 13/1 decreased more significantly after treatment as compared to the colonies (5/3) and (15/5) (period 05 - 11 NOV versus period 29 OCP – 05 NOV) which indicated a more effective *Varroa* control as compared to the colonies 15/3 and 15/5. The poor overwine ring performance of the colonies 9/2, 15/3and 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 blased colony shality of the obthian din treatment groups, these colonies also suffered from more unfavourable ambient conditions prevailing at the assigned study plots in comparison to the control and the midacloprid study sites.

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At the Ihinger Hof study site, the honey bee colories 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 meroclimate.

Also on the study location Celle, environmental factors differed on the individual study locations. Particularly the clothianidit study plot was affected, as the honey bee colonies were placed in a slight landscape depression. The soil abound 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 duc 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, seedtreated 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



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 peonicotinoid reatment groups were exposed differently over time. Both nitro-substituted neonicotinoid compounds share an identical intrinsic honey bee toxicity (imidacloprid – lowest LD<sub>50</sub> value: 3.7 ng/bee; clothianidin – lowest LD<sub>50</sub> value: 2.5 ng/bee; source: Bayer CropScience).

- Recorded symptoms during exposure to guttation exudates were comparable between impactoprid and victorianidin colonies

The number of bees with behavioural abnormalities did not differ between the clothiantdin (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 guitation fluid from seedtreated W-BAR plants, neither during the automn period nor during springtime (control group – autumn/spring/total: 7/53/60 bees; imidacloprid treatment group autumn/spring/totab 12/117/123 bees; clothianidin treatment group – autumn/spring/totat: 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 implactored 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, the the allocation of a higher number of weaker colonies (colony strength and brood), higher initial *Varroa* intestation levels as well as a flower 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 dreas 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 ap guttation fluid and dew in both, treatment and control, was mostly higher in all assessment zones in spring 2010 as compared to an autumn 2009.

Accounting for all boney 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 poney 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 mp a.s./L. Residues of inidacloprid 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



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 imidacloprist treatment group. The same conclusion could be drawn for the clothianidin treatment group if propriate corrections are made for experimental biases concerning colony vitality at study initiation.

reatment group. The same conclusion could be drawn for the clothandin treating group if appropriate corrections are made for experimental biases concerning colony vitality at study initiation. without the particular of the opening of the openin





Report:	02.02.05/03; 2014; <u>M-501261-01-4</u>
Title:	Field study to monitor potential effects on honey bees from exposure to guttation fluid
The.	
	clothianidin + imidacloprid FS 100 + 175 G in Germany in $2011/2012$
Report No.:	winter barley (W-BAR), seed-treated with the insecticidal seed-treatment product clothianidin + imidacloprid FS 100 + 175 G in Germany in 2011/2012 R11130 <u>M-501261-01-4</u> U.S. EPA OCSPP 850.3040 not specified yes
Document No.:	<u>M-501261-01-4</u>
Guideline(s):	U.S. EPA OCSPP 850.3040
Guideline deviation(s):	not specified
GLP/GEP:	yes a way way way way way way way way way w
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Aim	
The field study was co	nducted in winter barley (WBAR), grown from seeds treated with the cereals
seed-treatment product	Clothianidin + Imidacloprid FS 100 + 475 G, in order to investigate the potential
	to guttating W-BAR, stating from sectling energence in aprumn 2011 until
	-seed rape (W-OSR) flowering in spring 2002. The Assessment Phase and Bee
Health Phase lasted fro	om middle of September 2011 until beginning of April 2012. The study fields were
located in Hesse, Germ	
Honey bee colonies we	ere set up at the fudy herds either directly adjacent to the grop or a distance of
approximately 4.5 m to	the crop margin. The study comprised one treatment group and one control group:
The treatment group	omprising four study fields with altogether five study plots on which W-BAR
and a good troated with	h Clothianidin + Inidae Sprid IS 100 175 G and Study piots ar which w-DAR
seeus, seeu-ireateu with	roun and main thind a supplicities 100 - 1/3 stand of unglitude (baytane) were
W DAD goods good tr	roup, eschiprising also four study fields with altogether five study plots on which
w-DAK seeds, seed-uk	eated with a fungicide (Baytan®, defined as control) were grown. Moreover, all
	ent were additionally seed-treated with commercial NTECO® in order to reduce
dust abrasion.	the presence and the potential exposure of honey bees to the systemic
I reatment is defined p	s the presence and the potential exposure of noney bees to the systemic
neonicotinoid insection	des clothiandin and imidaeloprid
All treatment seeds we	reseed-treated at the Seed Treatment application Centre of Bayer CropScience
AG in Monheim Gern	any. The seed variety was "Campanile".
	y typical pneumatic cereal sowing machines under typical commercial use
conditions.	
Key study objectives w	vere to assess acute honey bee mortality and to evaluate and to compare the long-
term colony developm	ent along with the overwint ring performance of exposed honey bee colonies in the
two study groups (i )	treatment and contol). Furthermore, the guttation behaviour of W-BAR was
surveyed and it was ex	amined whether studation of guttation fluid of W-BAR and flight activity of
honey bees occurred si	Qu'Itané Busly v v S

In case bee flight activity and guttation councided, 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 ostalled at the entrance of the bee hives to record the number of dead@ees.

# Material and Methods

Test item  $\sqrt[6]{}$   $\sqrt[6]{}$   $\sqrt[6]{}$   $\sqrt[6]{}$  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



(Baytan<sup>®</sup>)) 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

Ś At each of the ten study plots (i.e. five treatment and five control plots, respectively), five honey been 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 structions. total, the treatment and the control group comprised each 25 honey bee colonies. Ò

## Assessment area

A specified area (assessment area) in front of the honey be colonies was intensivel monitored. The whole assessment area was divided into two In-Crop Zones (Zone Q and Zone 1) and an Off-Crop Zones 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-Cro Zon width 10 m ength along the field margin, 1 m depth into the off-crop) or in a distance of approximately \$5 m to the W-BAR crop (Off-Crop Zone) width: 10 m length along the field margin 5 m depth into the off-croph Eacloassessemtn 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 WeBAR displaying guttation and/or dew.

# Honey bee mortality

Each hive was equipped with a dead bee trap. The traps were empted daily to resord 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 10 x 0.5 m<sup>2</sup> in front of each dead trap were recorded 

# Guttation fluid sampling

In case guttation was beerved in the morning at the spectrive treatment plot, up to three samples of guttation fluid, each with a volume of approximately binL were collected from various plants of W-BAR. The samples were thereafter stored deep frozen ( $\leq -\frac{1}{2}$  °C) for later residue analysis.

### 0 × \* 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 automn 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 studo fields were located

During morning hours, the respective assessment area on the study plots under investigation was systematically checked for the accurrence of gattation 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 optake 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 starts 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" laged 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,



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 condens, 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 altumn 2011 and at 87.6% of the observation days in spring 2012. Guttation in the berbaceous of 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 cleased, on average at about 12 p.m. both in automn 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 asted for the whole day, due to rainy or damp weather (24.1% on W-BAR and 9.3% 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).

Altogether 734 monitoring sessions (355 in auturn 2014) 379 in spring 2012) were carried out, which lasted 388 hours (197 h / 191 h). In the morning, bee fight activity and guttation coincided on approximately 70% of all observation days ( $\overline{33.1\%}$ ) 69.7%) in the event of only on 11.0% of all observation days ( $\overline{33.1\%}$ ) 69.7%) in the event of only on 11.0% of all observation days ( $\overline{33.1\%}$ ) 69.7%) in the event of only on 11.0% of all observation days ( $\overline{33.1\%}$ ) 69.7% of  $\overline{33.1\%}$  ( $\overline{33.1\%}$ ) 69.7% of  $\overline{33.1\%}$  ( $\overline{33.1\%}$ ) 69.7% of  $\overline{33.1\%}$  ( $\overline{33.1\%}$ ) for the event of only on 11.0% of all observation days ( $\overline{33.1\%}$ ) 69.7% of  $\overline{33.1\%}$  ( $\overline{33.1\%}$ ) for the event of  $\overline$ 

If there was an overlap between the presence of guttation and bee flight activity during morning hours, the mean overlap time for autumn 2018 was 2 h 35 min and 2 h in pring 2012. In the evening, the mean temporal overlap during autumn 2011 lasted 34 monutes. 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. directle in front of the hives followed by the Off-Crop Zone and Zone 1. In spring 2012 most hones 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 2 times observed) or dew (nine times observed) was a rare phenomenon, while it was more compon in pring 2012, were 502 honey bees were 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.



## 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 cetts. 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  $2M^2$ , at the final colors 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 antra-group variability in two out of the five control plots, which ranked behind the other control coloures concerning number of adult bees at the 3rd and 4th colony assessment. Statistical analysis of colony strength withouth considering the data of these AP colonies (C1-1 to C1-5 and C5-1 to C5-5), eliminated every difference between control and treatment groups. Ũ Ŵ

Development of brood cells was on an empirically normal level and comparable between control and treatment colonies in autumn 2011 and at the 1st colory assessment March 2010. There were no distinct, biologically relevant differences between treatment and control Grrespective Wether 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 assessmen ((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, folony strength had decreased in both groups when compared to the before-winterevaluation, which is a typical apiological phenomenon. That equates to an average overwintering index of  $57.8 \pm 21.1\%$  in control colonies and to an average overwintering index of  $67.0 \pm 14.1\%$  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 stody, 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-Varoa 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 hove yield, six control colonies and one treatment colony were not too promising (one control and one treatment colony when excluding the C1 (CM to C1-5) and the C5 (C5-1 to C5-5) group).

### Ŵ Varroa destructor \

In autumn 2014, the mean daily Varioa mite fall was on a moderate level. The maximum mean was detected on the last assessment at the end of October 2011 with  $16.6 \pm 28.2$  mites per day in the control group, and  $5.1 \pm 5.2$  mites per day in the treatment group. There were no distinct, biologically relevant differences between treatmen and control, irrespective whether the colonies were set-up directly adjacent to the field margins of 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 fiving 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  $0.4 \pm 0.4$  mites per day in the control group and  $1.7 \pm 3.1$  mites per day in the treatment group end of April 2012 and with  $0.2 \pm 0.4$  mites per day in the control group and  $0.9 \pm 2.5$  mites per day in the treatment group beginning of May 2012. Again, there were no distinct, biologically relevant differences



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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 should 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 imidaclopric was 6.65 mg/L (01 October 201) 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 14,78 mg/L (11 October 2011)

### Conclusions

°~~/ Ľ Guttation of W-BAR plants was a regular occorring phenomenon during the autopin and spring growth period of the investigated W-BAR crop. Time overlap between presence of guttation build and bee flight activity was a common phenomenmon during morning hours, but rarely obsorved in the evening of at all, only on a few days in autumn). Honey bees were observed visiting the study plots frequency in spring, but rared in autumn. The relative

proportion of honey bees observed for monotoring on plants in the respective assessment areas in both, treatment and control, was higher in spring 2012 than a autumn 2019. Moreover, as 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 on spring 2012 as compared to autumn 2019, 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. 2

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 op guitation fluid.

Residue analysis of guttation fluid, as collected throughout the duration of the study on the treatment plots, revealed that clothianidin and midactoprid tesidues generally peaked shortly after emergence of the dressed W-BAR prop. Residues of clothianian and midac oprid 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 midacipprid was about 6.65 mg a.s./L, the maximum residue level of clothianidin was 8,51 mg as./L; the overall maximum observed combined residue level of imidacloprid and clothianidin was 11/78 mg total ao //L (all maximum values first half of October). Regarding honey bee montality brood and colony development, colony strength and varroa infestation levels during antumn and spring, there were no diginet, bologically relevant differences between treatment and control (irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 25 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 gudy S

Overall, it can be concluded that suttation fluid, excreted by winter barley, seed-treated with Clothianidia 4 Imidacloped FS 100 + \$75 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 Grength and -development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality, colony health, or on overwintering performance. M-50157 -01-4@s 622298-01



Report:	02.02.05/04; ; 2014; <u>M-500724-01-3</u>
Title:	A long-term field study to monitor potential effects on the honeybee (Apis mellitera 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.:	<u>M-500724-01-3</u>
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:	ves
< <m-500724-01-3@s-602286-01-1< th=""><th></th></m-500724-01-3@s-602286-01-1<>	
Material and methods:	

Test item:

Name: Sugar Beet Pills, prepared with clothanidiff, imid cloprid and beta-cythathrin; TOX number TOX10065-00; Batch: ZR02931; content of a r. (nominal): 0 Omg/pill clothanidiff = 0.3 mg/pills imidacloprid + 0.08 mg/pill beta-cyfluthrin

The potential effects of exposure of honeybees (*Apis mellifera*).) to gattation liquid from sugar beet plants, grown from sugar beet pills, confinercially prepared with the insected es epothianidin, imidacloprid and beta-cyfluthen at a meatment rate corresponding to nominally 9.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrit/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 clothighidin + imide toprid + 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 \$ 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 a well as the number of honeybees visiting sugar beet plants and the occurrence and propertion of guttation on sugar beet plants was assessed daily after set-up of the bee colonies at the field sizes from 1DAG 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 intestation level was evaluated and samples of honeybees for bee disease and bee virus analysis as well as nectar for ACB analysis was collected to monitor colony health. Samples of guttation liquid from sugar bee plants fiest item treatment group T only) were collected for residue analysis

The influence of the set item was valuated by comparing the results in the test item treatment to the corresponding control under consideration of the results of:

- Mean number of dead bees of the linen sheets and in the dead bee traps;
- Flight intensity in the field (mean number of forager bees / 5 x 2 m<sup>2</sup> / min);
- Observation of herebybees 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 colonicand assessment date);
- Bee health (bee disease and bee virus analysis);
- Coverwintering performance

Dates of work: 15 May 2013 to 26 May 2014



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Issue date 2017-11-22

# Findings

	Treatment group	Control (C)	Test item         Image: Construction of the second se
Daily mean mortality	5DBE to 1DBE (Pre-exposure)	21.5 ± 26.2	
(dead bees/colony) ± STD	1DAE to 42DAE (Exposure)	12.9 ± 4.7	516.6 ± 5.4 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 bee /colony/day for the control group C and reatment group T, respectively).

Throughout the entire field exposure period of the columnes, no constituous 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 (2.9 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 (portality within the copp area), throughout the entire exposure period, a freen of 0.3 and 0.2 field bees/day as found in C and F, 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 boneybees was observed in the observation areas in the control group, whereas a total of 4 boneybees was observed in the test item treatment group. In the control group, 4 hone bees were flying over the cop and 4 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 gritation flying were observed in both, the control and the test item treatment group during the entre 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 sest 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 of 42 days in th

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 of 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 bec/8 colonies and assessment date), and also on 1 out of 42 days in the control to bee/8 colonies and assessment date).

Small numbers of inactive honeybees were observed on 2 out of 42 days in the test dem treatment group (range: 24–31 bees/8 colonies and assessment date). No inactive honeybees were corded 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 honeybess on all assessment dates in both, of the test item treatment group and in the control group. Thus, no test-item related adverse effects on honeybe@behaviour were observed.

Occurrence of Guttation and Percertage 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 Colonfes

Strength of the Colonies The mean number of bees per colony assessed during the first colony assessment on 11 Jun 2013 (2DBE) shortly before state of exposure revealed a mean colony strength of 16981 bees/colony in the control C (range: 11115 to 2060) and 7152 bees/colony in the test item treatment group T (range: 17355 to 20800).

At the second colony assessment on 03 rul 2013 (2017AE), during exposure, the mean colony strength had increased in C (21060 bec) colony, range. 16345 to 31525) as well as in T (20914 bees/colony, range: 13910 to 31785). The increase of corony spength was approx. equal in the control and in the test item treated group.

At the third colony assessment on 25 Jul 2693 (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: 13916 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: 0515 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,



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 I, 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 VI Jun 2013 (2DBE) op 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 Te and Tf on 20 Aug 2013 (68DAE); see below) contained all brood stages fluring the brood assessments.

In colony Ca, no larvae were present on 250 ul 2013. This was most probably due to closs 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 170 ul 2013 as well as a hatched queen cell. Since Ga contained cells with eggs on 25 Jul 2013, a new queen had been gaised by the colony.

In colony Th, no eggs were present on 03 Jul 2003 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 cogs and larva 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 07 Sep 2013, all brood stage overe 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, G-Cg, Tc, Te, Tg) or only a relatively small number of cells with brood (Cb, Ch, Ta, Tb, Ta, 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, notest 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 poller and nectar storage cells throughout the entire observation period. Thus, no test form-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.



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 Warrow infestation level in the (future) test item treatment group (on 11 Jun 2013) was sughtly 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, Varioa destructor and Paenibacillus karvae. O In the bee samples taken from the control colonies before start of exposure, Northan spt, spores were found in colonies Cb, Cc and Ch (medium infestation level). Control co free of analysable spores. No bee sample was available from control colony Qf. ° In the bee samples taken from control colonies at encore for exposure the control colony Ca had a low infestation level and the control colony Cg had a medium nfestation level with Noseroa 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 Rosema sp. spores were found in any sample taken from control colonies.

In the control bee samples taken arend of overwintering Nosena sp poresovere analysed only in control colony Ce (low infestation levely. All other control colonies were tree of analysis a spores. The highest infestation rate with Varroa miles was 2.5 % in the bee sample taken from the control colony Ca at end of exposure. In all other bee samples gramined the Varroa offestation rates was between 0.0 % and 2.1 %. 

In the bee samples taken from the test item to atment colornes before start of exposure, Nosema sp. spores were on a medium level in test item treatment colonies Ra, Tc and Tf. dest 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), sivest item treatment colonies were free of analysis are spores (Ta, Tb, Tc, Td, Te and Tg). In the samples taken at start of overwintering, test item treatment colories Ta and Th had a medium infestation level. No Nosema spersovere bund in any of the other test item treatment colonies (Tb, Tc, Td, Tc, Tf and Tg) 2  $\bigcirc$ 

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. pores was analysed

The highest investation rate with Warroa mites in samples taken from the test item treatment colonies was found in colony Te with 10.8 % followed by colony of 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 controk 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 treament group could be obser@d. Ċ

#### Bee Viruses

The objective of the bee virus analysis was to determine the following bee viruses in bee samples collected at alfferent time points of the year: DWV (deformed wing virus), SBV (sacbrood virus), ABPV (active begeparalysis 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



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 (To, Tg) but not in the control group at the time point 'start of overwintering' on 15 Oct 2003. At the time point after & overwintering' in spring 2014, all colonies of the control group and the jest item treatment group wore 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 ful 2017 in three outoff eight colories of the test item treatment group, but not in the control. But the start and at the ond of overwintering, the colonies of both treatment groups were free of ABRV. Ø Q

The fact that increased infestation levels of DWVYSBX 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 beg health status on terms of virus infestation between the colonies of the control group and the test@tem\_treatment group could be observed.

#### **Residue Analysis**

The determined clothianidin residues in guttation liquid, as malysed in the samples collected on each day where guttation droplets were actually present on the sugar beet plants in the tegratem treatment group T, were within the range of 153 327, 25-57 and 36-55 µg/kg for parent clothianidin and its metabolites TZNG and TZMU respectively. The corresesponding midac loprid residues were within the range of 18-61, 6.9-16 and 1, 4.0 ug/kg for parent imidac loprid and its metabolites midacloprid-5-hydroxy and imidacloprid-olofine, respectively. Residue of beta-cyflathrin in all guttation liquid samples were virtually inexistent.

#### Conclusion

 $\bigcirc$ The objective of this study was to determine the potential effects of exposure of honeybees (Apis mellifera L.) to guitation liquid from sugar beet plants grown from pills, commercially prepared with the insecticides clothanidin imidacioprid and beta-cyfluthrin and rate corresponding to nominally 0.6 mg clothianidin/pill + 0.3 mg imgtacloped/pill 0.08 mg be@ cyfluthrin/pill during the first 6 weeks after emergence under field conditions

Guttation in the test fields was abserved on 1 but of AP 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 noneybees observed in the crop was therefore on the same level in both the control and the lest item treatment group. Overall, guttation occurred only infrequently in sugar beets, and if, the overal abundance of guttation droplets is rather low, particularly when compared to adjacent off-crop meas

No test itero-related adverse effects were observed on mortality and behaviour of the honeybees.

No test item-rotated 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 automn, 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 cologies Te and This howed normal Varion infestition rates at the end the overwintering period in early spring of the following gear. Overall, it can be concluded that the exposure of the neybee colonies to Guttation liquid from Sugar Beet plants, grown from pills, commercially prepared with the insecticides clothianidin midacloprid and betacyfluthrin at a rate corresponding to nominall  $0.6 \text{ mg/clothanidin/pill} \pm 0.3 \text{ mg/midacloprid/pill} \pm 0.08$ mg beta-cylluthrinyhill during the first 6 weeks after emerce.c. dol neither cause actic, shoerd long-term adverse effects on mortality, howeybe behaviour, colony strength Colony, health and brood- and food development and overgentering performance in the exposed colonies. mg beta-cyfluthrin/pill during the first 6 weeks after emergence, did neither cause acute, shotterm hor long-term adverse effects on mortality, howeybee behaviour, colony strength colony, health and vitality,



Report:	02.02.05/05; 2014; <u>M-500734-01-3</u>
Title:	A long-term field study to monitor potential effects on the honeybee (Apis mellitera 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.:	<u>M-500734-01-3</u>
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< th=""><th></th></m-500734-01-3@s-602289-01-1<>	
<11-1	

#### 1.1 Material and methods:

Test item:

Sugar Beet Pills, prepared with clothianidin, inidacloprid and beta eyfluthrin; TOX number: TOX10065-00; Batch: ZR02931; content of al. (nominal) 0.6 morphill containin + 0.3 mg/pill imidacloprid + 0.08 mg/pill beta-cyfluthrin

The potential effects of exposure of boneybers (Apis mellifera L ) to gutation build from sugar beet plants, grown from sugar beet pOIs, commercially prepared with the insecticides cothianidin, imidacloprid and beta-cyfluthrin at a treatment rate corresponding to dominally 0.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrin/pith, 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.

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 + bera-cyfluthrin) and the control group C (noninsecticide-treated sugar beet pills). Commercial bee colonies were plated at the field sites shortly after emergence of the plants (T: BBCH 72, C BBCH 2-14). The exposure plase 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 40DAF. 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 and regularly thereafter after of the of overwintering. The Varioa infestation level was evaluated and samples of honeybees for bee disease and bee virus analysis ad well as nectar for AFB analysis was collected to monitor colony heath. 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 finen sheets and in the dead bee traps;
- Flight intensity in the field (mean number of forager bees / 5 x 2 m<sup>2</sup> / min);
- Observation of honeybees visiting sugar beet plants displaying guttation;
- Occurrance and propertion 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 colory and assessment date);
- Bee health (bee disease and bee virus analysis);

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Dates of vork: 15 May 2013 to 26 May 2014
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#### 1.2 Findings

	Treatment group	Control (C)	Test item
Daily mean mortality	15DBE to 11DBE (Pre-exposure)	22.4 ± 5.7	
(dead bees/colony) ± STD	1DAE to 40DAE (Exposure)	₹13.1 ± 2.9 €	¢4.1 ± 30 x y y

DAE: days after start of exposure; DBE: days before shart of exposure; STD; Randard deviation

#### 1.2.1 Mortality

During the pre-exposure period at the monitoring site (15) BE to (1DBE), the mean daily mortality, assessed by using dead bee traps, was on the same level in the control group and in the test them 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 10 AE to 40 DAE), the mean daily mortality, assessed by dead bee traps was 13.1 and 14.1 dead beet 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 Intensition the Field and Observation of Honeybers Visiting Sugar Beet Plants The assessments of thight intensity in the field and the observation of Koneybees 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 1DAF 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, 55 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 term treatment group during the entire observation period. Overall, the number of boneybees observed in the tive 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 around group.

#### 1.2.3 Behaviour of the Be

During the assessment period from IDAE 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 (fibering 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).

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 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 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 (onge: 4 bees/8 colonies and assessment date). Small numbers of inactive honeybees were observed on 29 out of 40 days in the test, them treatmenderoup (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 tobe 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 of 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 homeybeedbehaviour were observed. Ŵ  $\mathcal{O}$ Occurrence of Guttation and Percentage of Plants Displaying Guttation 1.2.4 In the control group, guttation of sugar best plants in the assessment meas was observed on 3 out of 40 assessment days. In the concurrently assessed off-cropy area guttation occurred on 25 out of 40 assessment days. In the test item treatment goup, gottation of sugar beet plants of 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 perassessment area varied from 2.9% to 57 %. In the test item treatment group, the percentage of plants xhibiting guttation per assessment area varied from 3.0 % to 82.1 %, when guttation was detected. Overall grittation 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. ð Condition of the Colonies 1.2.5 ô  $\hat{\circ}$ shortly before start of exposure revealed a mean colory strength of 15933 bees/colony in the control C (range: & 90 to 24635) and 15240 bees/colory 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 begs colony, range; 10599 to 23400) as well as in T (24651 bees/colony, range: 17355 to 29250). The increase of colony strength was more pronounced in the test item treatment group. At the third cotony assessment on 24 Jul 2023 (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: 16545 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 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: 10070 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,



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 tem 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, latval and pupal stage, occurred during the observation period. From 12 Jun 2003 (2DBE) up to an Omcluding 13 Xug 2013 (60DAE), all colonies in the control (except colony Ce on 04 Jul 2003 (2DBE), 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 queer was added to colony Ce on 26 Jun 2013. In early autumn, when the natural period of breeding activity of the colonies orded, 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, Te and *C*d).

The overwintering period lasted from 14 October 2015 until 0 Mar 2014. After overwintering, all colonies of the test item treatment group and he control were viable and all were found to have resumed breeding activity (except colony Color 2017).

Thus, no test item-related adverse offects were observed on colony vitality and brood development, including queen privital and overwintering performance.

1.2.5.3 Food Storage In the colorises 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 A Evaluation of Varroa Intestation in the Colonies

Varroa pute 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 2040 (Vartisa 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 pathogene. Nosema sp., Malpighamoeba mellificae, Varroa destructor and Paenibacillus larvae. In the be 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.



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 O 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 colory Ch at start of overwintering. In all other bee samples examined, the Varioa infestation rate was between 0.0 % and 1.1 %.

In the bee samples taken from the test item treatment solonies before the start of sposure Nosona 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 , v 4 spores. K)

spores. In the bee samples taken at the end of exposuo, one wist item treatment coordinate 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). ð

In the samples taken at the start of oversy intering, the test items treatment colonies Te and Th had a medium infestation level. No Nosema sp. spores were found in any of the other test item treatment

In the bee samples taken at the end of overwintering, no Nosetia sprease vere found in any sample taken from test item treatment detonication of the same set item treatment detonication of the same taken from test item treatment Monies. ñ

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 care of all other test item treatment colonies varied between 0.0% and 0.5%.

No Malpighamoeba mellificar and no spore of Pachibacillas 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 bechealth status between the colonies of the control group and the test item treatment group could be observed

# Beenviruses

1.2.5.4.3 Beoviruses The objective of the beevirus analysis was to be termine 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), SBPV (chronic bee paralysis virus), KBV (Kashmir bee virus), IAPV (Israeli acute paralysis virus, BQCV (black queen cell virus)

In this study the Gruses BPV DWV KBV and IAPV were not detected in any of the samples taken between 'before exposite' in 2013 and 'end of overwintening' in 2014.

BQCV was detected in one amples 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, BOOV 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 stightly higher in the test item treatment group. At the time point 'end of exposure on 249ul 2013, SBO was detected in five different colonies of the test item treatment group (Tc, Te, Tf, Te, Th), but not in the control group.

ABPV was setected 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



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 catonies. 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 colleged on each day where guttation droplets were actually present on the sugar beet plants in the test item treatmen groups were within the range of 17-64, 2.9-12 and 3.1-11 µg/kg for parent clothanidin and its metabolites #ZNG 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-54 droxy and % imidacloprid-olefine, respectively. Residues of beta cyfluthrin in a guttation liquid samples were virtually inexistent.

#### 1.3

1.3 Conclusion The objective of this study was to determine the potential offects of exposure of hone bees (appis 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 to mg clothianidin/pill + 0.3 mg imidaclopric pill # 0.08 mg bete evflut prin/pill during the first approximately 6 weeks after emergence under field conditions.

Guttation in the test fields was observed on 5 or of 40 days is 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 ather fow, particularly when compared to adjacent off-crop areas.

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 colory health, colory 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 fronitoring period until the end of overwintering in spring 2014. overwintering in spring 2014. A Woreover, the overwintering performance of the coordination of the test item treatment group was not

adversely affected when compared to the performance of the control group.

Overall, it can be soncluded that the sposure of honeybee colonies to guttation liquid from sugar beet plants, grown from pills, commercially prepared with the insecticides clothianidin, imidacloprid and betacyfluthrin at a rate corresponding to nominally 0.6 mg olothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cylluthrin/pill during the first approximately weeks after emergence, did neither cause acute, short-terror nor long-terrin adverse effects on prortativy, honeybee behaviour, colony strength, colony health

short-teres nor long-term adverse effects on priortality, honeybee behaviour, colony strength, colony and vitality, brood and food development and overwintering performance in the exposed colonies.



Report:	02.02.05/06; 2015; <u>M-503349-03-2</u>
Title:	A long-term field study to monitor potential effects on the honeybee (Apis mellitera L.)
	from exposure to guttation fluid of potato plants, grown from seed tubers treated with
Damant Maria	Monceren G in southern Germany in 2014 and 2015 - Final port
Report No.: Document No.:	S14-01385
Guideline(s):	$\frac{M-303349-03-2}{OFPP/EPPO Guideline No. 170(4) (2010)}$
Guideline(3).	<ul> <li>nom exposure to guitation hard of potato plans, grown nom seed tabers dealed with a Monceren G in southern Germany in 2014 and 2015 - Final eport S14-01385</li> <li><u>M-503349-03-2</u></li> <li>OEPP/EPPO Guideline No. 170(4) (2010)</li> <li>US EPA OCSPP Guideline # 850.3040 Field Testing for Pollinators none yes</li> <li>nethods:</li> <li><u>MD+PCC FS 370 (120*250) Go Spect No. 102000008024</u> FOX number: 2014-001766-01; content of a i. (nominal) f20 gH imidaeloprid + 250 g/L of a i. analysed: 120.5 g/L infidaeloprid + 254.2 g/L pencycuron</li> </ul>
Guideline deviation(s):	none
GLP/GEP:	yes yes
< <m-503349-03-2@s-602314-01-1< td=""><td></td></m-503349-03-2@s-602314-01-1<>	
	nethods:
Test item:	
Monceren G, I	nethods: IMD+PCC FS 370 (120*250) (2) Spec: No. 102000008024, FOX number. 2014-001766-01; content of a 1. (noninal) 20 gC imidacloprid + 250 g/L f a.i. analysed: 120.5 g/L initiacloprid + 254.2 g/L pencycuron
TOX10501-00; Batch:	2014-001766-01; content of a.i. (nominal) 20 ge imidadoprid + 250 g/L
pencycuron; content of	f a.i. analysed: 120.5 // init dacloprid + 251.2 g/L peneocuron
The potential effects of	f exposure of hopeybees (Apis mellifera L.) to guttation flaid from potatoplants,
grown from seed tubers	s, treated with Monceven G (active ingredients: invidacloprid + pencycyron) during
	sponding to pominally 1.5 L product/ha were investigated under field conditions in
	rst 59 days after emergence by following the QEPP/EPPO Guideline No. 170(4),
2010.	
The field study	y consisted of two treatment groups: The test item treatment group T (seed tubers
reated with Monceren	G) and the Control group C (test item untreated seed (ubers). Commercial bee
colonies (8 per treatme	ent) were placed at the field sizes shortly after emergence of the plants (BBCH 10).
The mortality of the ho	meybers was assessed over a period of 5 mays shortly before start of exposure and
	é colonies agine field sites from DAE to 58DAE. Flight intensity and behaviour as
	honeybees visiting potato plants and the occurrence and proportion of guttation on
potato plants was asses	sed daily after set-up of the bee colonies at the field sites from 0DAE to 58DAE.
The condition of the condition	Sonie Swas assessed once Before for up of the colonies at the field sites and
regularly increased and	er until end of overwintering. The Varroa infestation level was evaluated and for bee disease and bee virus analysis as well as nectar for American foulbrood
(AER) analysis were a	of guttation fluid from potato plants
(AFD) analysis well w	bup <b>T</b> only) and deal worker bees from dead bee traps were collected for residue
The influence of	the test item was evaluated by comparing the results in the test item treatment to
the cor	responding control under consideration of the results of:
	number of dead bees on the line sheets and in the dead bee traps;
	intensity in the field (mean number of honeybees per m2 and minute);
	vation of honeybees visiting potato plants displaying guttation;
« Oceurr	rence and proportion of guttation;
🦘 🔹 Behavi	ion of the bees in the coop and around the hive;
• ©ondit	tion of the colomes (pumber of bees (colony strength), total values of the different
	stagesper colony and assessment date);
	ealth (bee disease and bee virus analysis);
	viotering performance;
	ue analysis.
$\sim$ $\sim$ $\sim$	
Dates of work: 02 Apr	r 2014 until 23 Jul 2015
Dates of work: 02 Apr	r 2014 until 23 Jul 2015
Dates of work: 02 Apr 1.2 Findings	r 2014 until 23 Jul 2015



#### Mortality of Honeybees

	Treatment group	Control (C)	Test item	
Daily mean mortality	7DBE to 3DBE (Pre-exposure)	10.6 ± 5.4	10.5 ± 5.1	
(dead bees/colony) ± STD	1DAE to 58DAE (Exposure)	16.0 2.8	0 13.8 ± 4.9 0	

DAE: days after start of exposure; DBE: days before start of exposure; STD: standard deviation, O

#### Mortality 1.2.1

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 so treatment group T (10.6 and 10.5 dead bees/colony/day for the treatment group's 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 itern 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/cotony/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 them treatment group concerning mortality during the exposure peood. ° Ox  $\bigcirc$ 

 $\bigcirc$ Flight Intersity in the Field and Observation of Hone bees Visiting Potato Plants 1.2.2 The assessments of flight intensity in the field and the observation of honeybees visiting potato plants were conducted early the morning when the occurrence of guitation droplets was expected. The concomitant fight activity of the colonies at the lave entrance. Owas plonitored 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 \$8DAP 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, al 1124 honeybees were flying over the crop, whereas no hone were located of potate plants or were observed on the soil during the entire observation period. In the test item treatment group, 3023 Joneybees 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 hopeybees taking up gutation 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 homeybees that were only accidentally passing through the observation areas due to their close vicinity to the hives. However, witually no honeybees were observed in direct contact with potato plants or soil in both reatment 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 untrated) did notoccur during all assessments.

# 1.2.35 Bebaviour 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: 1–465 bees/8 colonies and assessment date). It should be noted that the assessments were conducted early in the again and the numbers of inactive honeybees may as well include cold-topaired bees/2 and 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 diabnormal behavior was observed in the test item treatment group compared to the control.

## 1.2.4 Occurrence of Guttation and Percentage of Plants Displaying Guttation

In the control group, guttation of potate plants in the assessment areas was observed on 18 out of 59 assessment days. In the concurrently assessed off-trop area, guttation of potate plants in the assessment days. In the test item treatment group, guttation of potate 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.

on 33 out of 59 assessment days. When guttation occurred in the in-grop 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: 942% 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 strength 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 upfil the start of overwritering on 13 Oct 2014 and the end of overwritering in spring of the following year (17 Mar 2005).

At the start of overwintering in antumn 2014, the mean colony strength was 13731 bees/colony in C (range: 10505 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 Q (range: 4160 to 1295).

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 adderse 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 fuctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage (carefed 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.

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 water present again.

In colony Th, for undetermined reasons, no queen, and therefore no eggs and lawae, 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 natural period of cells with brood had declined in both the control and the test item treatment group on the day of the cology assessment on 15 Sep 2014 (135DAE). On the last colony assessment before start of overwintering on 12 Oct 2014 (163DAE), the breeding activity of the colonies of the study had almost ended. Wirtugby no eggs and larvae, but still residual amounts of pupae were observed in the control and in the test items treatment group, respectively.

The overwintering period lasted from 13 Oct 2014 until 17 Mar 2015 After Overwortering, 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 colong Cc, which showed an interdiption of egg. laying activity for unknown reasons).

Thus, no test item-related adverse effects were observed on colony sitality 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, Pespectively the natural and typical changes and fluctuations in the relative amount of nectar and posten storage cells occurred during the observation period. The treatment groups C and T showed approximately equal mean numbers of pollen and nectar storage vells 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

Evaluation of Warroa Infestation in the Colonies 2 1.2.5.4.1

Varroa mite occurrence in the colonies was assessed via a, Varroa board beneath the hives. The infestation level of a colong was monitored by counting dead rates of the board.

During the assessments from 06 Aug 2014 to Of Sep 2014, only relatively small mean numbers of mites were detected. Moderafely elevated mean numbers of mites were observed on 01 Oct 2014 in both treatment groups. This was due to a previous Varioa treatment of the colonies with formic acid on 16 Sep 2014.

The Varroa infestation levels of the test item weatment colories were approximately on the same level as or even lower than those of the control colonies doing the course of the study and at the end of the honeybee season. Notest i Om-related adverse effects were detected.

#### Bee Diseases 1.2.5.4.2

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 leved with Nosema sp. spores. All other control colonies were free of analysable spores. 67 Ľ

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.

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 T that 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 That 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 To had a high  $\frac{1}{\sqrt{2}}$  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 of 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 Viruse

The viruses CBPV, KBV, and APV 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 (Co), and in the sample of one colony of test them 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 colory of the test tem group (Tc) at the time point 'start of overwintering' an 2016

In 2014, BQCV was detected in the samples of seven colonies of the control group (Cb-Ch), and in the samples of xix 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 bechealth status in terms of virus infection between the colonies of the control group and the test item group were observed.

#### 1.2.6 Resider Analysis

Analysis of residues of initial and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine in gattation 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  $\mu$ g/L for imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, respectively. The Limit of Detection (LOD) was 0.3  $\mu$ g/L for imidacloprid,



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 ug a.i./L to 583 ug a.i./L and from below the LOD (0.3 ug a.i./L to 583 ug a.i./L respectively. They were thus several orders of magnitude below the values measured for the parents 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

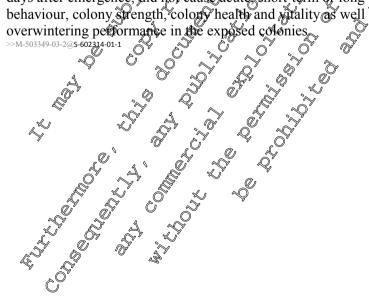
The objective of this study was to determine the potential effects of exposure of honeybees (Apis mellifera L.) to guttation fluid from potato plants grown from seed tublets, treated with Market to nominally 1.5 L product (active ingredients: 120 g imidacloprid/L + 250 g peneveuron/L) during planting and rate corresponding to nominally 1.5 L product/ha, during the first 9 da s after emergence under field conditions, Guttation in the test fields was observed on 17 out of 59 days in the test fiem treatment group and on 18 out of 59 days in the control. During the entire assessment period of 59 days of 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 stal of 2 honeybees to cated on potato plants was observed in the test item treatment group. The number of koneybers 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 Goneybees from potato planes (treated and untreated) did not occur during

all assessments. No test item-related adverse effects were observed on mortality and the having of the honeybees.

No test item-related adverse effects were observed on colong health status, colong development (including colony strength, brood devolopment 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 spling 2005.

Moreover, the overwintering performance of the colonies in the test item freatment 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 Moncered 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 sprength, colony health and vitality as well as brood and food development and





Report:	02.02.05/07; 2015; <u>M-503344-03-2</u>
Title:	
	from exposure to guttation fluid of potato plants, grown from seed tubers treated with
	Monceren G in Southern Germany in 2014 and 2015 - Final port
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 a v v v v
< <m-503344-03-2@s-602313-01-1< td=""><td></td></m-503344-03-2@s-602313-01-1<>	
1.1 Material and m	nethods:
Test item: Monceren G	$G_{\rm r}$ , IMD+PCC FS 370 (120+250) $G_{\rm r}$ ; Spec. No. 102000008024. TOX number.
TOX10501-00: Batch	2014-001766-01; content of a.i. (nominal) 20g/Simida@oprid+250g/L
nencycuron: content of	A long-term field study to monitor potential effects on the honeybee (Apis mellitera L.) of from exposure to guttation fluid of potato plants, grown from seed tubers treated with Monceren G in Southern Germany in 2014 and 2015 - Final peport S14-01392 <u>M-503344-03-2</u> Regulation 1107/2009 (Europe) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3040 none <b>yes</b> hethods: G, IMD+PCC FS 370 (120+250) G; Spec.No, 10200008024, TOX number. 2014-001766-01; content of a.1. (nominal) 20g/ Amidactoprid + 2500/L a.i. analysed: 120.59/L imidactoprid + 251.2g/L pencycuron
peneyeuron, content of	
The notential e	effects of exposure of honeybees (Apis mellifera L to gunation third from potato
nlants grown from see	d tubers, treated with Moncesen G active ingredients: initiacles in + pencycuron)
during planting at a rat	e corresponding to nominally 1.5 L product/ha were investigated under field
during planning at a fat	a corresponding to nominary 1,5 L product/ha swere novesnighted under netu
conditions in Germany $N_{\rm e} = 170(4) - 2010$	during the first 58 days after emergence by following the OEPP/EPPO Guideline
No. 170(4), 2010.	
The field study	v consisted of two treatment groups: The fost item treatment group T (seed tubers
treated with Monceren	G) and the Control group C (test item untreated seed (ubers), Commercial bee
colonies (8 per treatme	nt) were placed at the field sites shortly after emergence of the plants (BBCH 10).
	meybers was assessed over operiod of 5 days shortly before start of exposure and
	e colonies agene field sites from MAE to 57DAP. Flight intensity and behaviour as
well as the number of l	non-ybees visiting potato plants and the occurrence and proportion of guttation on
potato plants was asses	sed daily after set-up of the bee colories at the field sites from 0DAE to 57DAE.
The condition of the co	nonie was a set once before set-up of the colonies at the field sites and
regularly thereafter after	er 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 of	flected to monitor colony health. Sample of guttation fluid from potato plants
(test item treatment and	oup T only and dead worker bees from dead bee traps were collected for residue
analysis.	
The influence of the te	A tam the automated by comparing the results in the test item treatment to the

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 fold (mean number of honeybees per m2 and minute);
- Observation of honeybees visiting potato plants displaying guttation;
- Cocurrence and proportion of gutation
- Behaviour of the bees in the crop and bound the hive;
- Condition of the colorues (number of bees (colony strength), total values of the different brood stages per colory and assessment date);
- Bee bealth (bee disease and bee frus analysis);
- Overwintering performance;
- Residue analysis.

Dates of work: 02 Apr 2014 until 12 Aug 2015

Findings 1.2



#### Mortality of Honeybees

	0000			° .
	Treatment group	Control (C)	Test item (T)	
Daily mean mortality	5DBE to 1DBE (Pre-exposure)	45.9 ± 42.0	35.7 ± 20.6	A Q
(dead bees/colony) ± STD	1DAE to 57DAE (Exposure)	20.7 ± 6.1	18.3 ± 5 3	
DAE: days after start of ex	posure; DBE: days before s	tart of exposure; STD. stan	idard deviate	
1.2.1 Mortality	sure period at the mo	nitoring site (5DBE	to 1DBFX the mean	

#### 1.2.1 Mortality

Ŷ During the pre-exposure period at the monitoring site (5DBE (71DBE), 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 preatment group T, respectively).

Throughout the entire field exposure period of the colonies, no conspicuous differences regarding the mortality levels were observed on a doly basis between the test item treatment group and the control group. During the entire exposure period at the field sites (assessed from 1DAB to 57DAE), the mean daily mortality, assessed by dead bee traps, was 20.7 and 18.2 dead bees/colony/day for the control group C and test item treatment group , respectivel . 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 been day was found in the control group C and in the test item treatment group T, respectively.

0 treatment group during the efficience period  $\bigcirc$ 

Flight Internsity in the Field and Observation of Honeybeer Visiting Potato Plants 1.2.2 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 gottation droplets was expected. The concomitant/flight activity of the colonies at the hive entrances was monitored at about the same time. The flight assessment ateas were all located close to the colonies with a distance of 10–15 m to the hives. During the entire assessment period from DAE 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 locate on portato plants and I honeybee was observed on the soil during the entire observation period. If the test item freatment group, 1788 honeybees were flying over the crop, whereas only 3 honeybees were located populate 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 vi@nity to the higes. However, virtually no honeybees were observed in direct contact with potato planes or soft 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.

#### Ô Benaviour of the Bees 1.23

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.

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 to 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 freatment group (range: 1–20 bees/8 colonies and assessment date), and on 13 out of 58 days in the control (range: 1–44 bees/8 colonies and assessment date). It should be noted that the assessments were conducted early are the Q 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 of d frequency of the decurrence of abnornal behavior was observed in the test item treatment group compared to the control.

Consequently, no test-item related adverse effects on an environment of the province of the second device of the s

1.2.4 Occurrence of Guttation and Percentage of Plants Displaying Guttation In the control group, guttation of potator lants in the sesentient areas was observed on 39 out #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 concorrently assessed off-crop area, guttation occurred Ø on 21 out of 58 assessment day Ľ

on 21 out of 58 assessment day 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 Source 1 to the control group group.

#### Condition of the Colonies 1.2.5

1.2.5.1 Strength of the Colonies

The mean number of bees per colony assessed during the first colory assessment on 16 May 2014 (3DBE) shortly Defore start of exposure revealed a mean colony strength of 17184 bees/colony in the control C (range: 9685 to 23140) and 17704 bees colorg in the lest item treatment group T (range: 9750 to 31135) 🔊

At the second colony assessment on B Jun 2014 (2SDAE) during exposure, the mean colony strength had decreased in C (14365 bees/colony) as well as in F (14121 bees/colony). However, the decrease of colony strength was equally pronounced in both, the test item freatment 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 them traiment group T, followed the natural course of colony strength development, with an inexcasing 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 overworkering in automn 2014, the mean colony strength was 15633 bees/colony in C (range: 11700 to 19500) and 15836 been colored 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 77.1 bees/colon@in T (fange: 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

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 betweeper 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 pupe were observed in the control and with the test item treatment group, respectively.

The overwintering period lasted from 14 Oct 2014 witil 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 are 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 result of queen 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 not 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 itality 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 arount conectar 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 05 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 Varioa Intestation 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 cooling that mites on the board.

During the assessments from 05 Aug 20 J/ 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 Varroe treatment of the colonies with formic acid on 16 Sep 2014.

The varroa infestation levels of the testment 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 honeyber samples take from the control colonies before exposure, Nosema sp. spores were found in control colonies Ce (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 koneybee samples taken at end of exposure, no Nosema sp. spores were found in any sample taken from control colonies.



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 of 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 of from control colonies examined, the Varroa infestation rate varied between 0.0 % and 4.6 % taken we have a 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 set 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.

were free of analysable spores. In the honeybee samples taken at end of exposure of the start of overwintering and at ond of overwintering, no infestation with Nosema's p. spores could be analysed in an of of the test item treatment colonies.

The highest infestation rates with Varioa mites in samples taken from the test item treatment colonies were found in colony Th with 6.6 % and To with 6.2 % before start of every intering. 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 Malpighanoeba mellificae was found in the troneybee samples taken in 2014 and 2015, neither in samples taken from the control for from the test item treatment columes.

No spores of Paenibacillits larvae were found for 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 (6, Ce and Cf as well as from test item treatment colony Tabelore exposure).

treatment coloro Ta before exposure a status compared between the honeybee colonies of the control group and the test item treatment group were observed.

1.2.5.4.3 Bee Fruses

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 Oerwintering in 2014, DWV was detected in the samples of two colonies of the control group (Ce, Cg) and if 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 (Cg-Te) at the time point 'after overwintering' in 2015.

At the time point 'before exposure' 2010, 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 are 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 betected 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-blefing 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 ()  $\mu$ g at L) to 4749  $\mu$ g a.i./L.

The residue levels of the metabolites imidacloprid-5-bydroxy and initial cloprid-olefine in cuttation fluid ranged from the LOQ (1  $\mu$ g a.i./L) to 1042  $\mu$ g a.i./L and from below the LOD (63  $\mu$ g a.i./L) to 19  $\mu$ g 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 spectmen collected on 2DAE, 3DAE, 23DAE, 25DAE and 29DAE, which most likely have caused the high residue values to 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 pencycurorf ) 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 97 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 sufface was observed in the assessment areas in the control group, whereas a total of 0 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 first item treatment group.

Uptake of guttation droplets by honeybees from potato plants (treated and antreated) did not occur during all assessments

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 colong health status, colony development (including colony strength, brod 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 2005.

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 hone bee colonies to guttation fluid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: 120 g imidacloprid/L + 250 g pencycuroh/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 form 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 calonies.

benaviour, colony strength colony health and stality overwintering performance in the exposed colonies.



*a*.

Issue date 2017-11-22

#### 02.02.06 - Dust

Report:	02.02.06/01; 2010; <u>M-366273-01-3</u>
Title:	Monitoring of dust drift deposits during and after sowing of winter barley (WBAR)
	treated with Triadimenol & Imidacloprid & Fuberidazol & Smazalil 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.:	R09247-1
Document No.:	M-366273-01-3 US EPA OCSPP Guideline No 850 SUPP
Guideline(s):	US EPA OCSPP Guideline No. \$50.SUPP
	91/414/EEC of July 15, 1991, 0 <sup>1</sup>
	SAINUU/3029/99 Kev. 4. 2090/07-11 👘 🖉 🔗 🔗 👘
Guideline deviation(s):	not specified
GLP/GEP:	no & 6° 5° 5′ 4″ 6° 5° 5′
< <m-366273-01-3@s-602225-01-1< th=""><th></th></m-366273-01-3@s-602225-01-1<>	
Matarial and Mathada	

#### **Material and Methods**

#### Test item

Two different W-BAR varieties (i.e. Lomerit and Highlight) were purchased untrated and commercially cleaned-up from a commercial seed ostributor (Gut Peterhof, D-50127 Bergheim, Getrany) and were thereafter seed-treated at Bayer CropScience's Seed Treatment Application Centre in D-40989 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: TOX08780400 (vanety Lomerit) TOX08779-00 (vanety Highlight)

and

Ő, Smaragd® for FS 453 (TO \$0874 900) treated winter barley seeds, dressed with 133mL product/100 kg seeds (= nominall \$50 g. Jothian din/100 kg seeds); identification of treated seeds: TO\$0877500 (variety komerit); TO\$08774600 (variety Highlight)

After seed-dressing, the seeds were subject to chemical analysis for the determination of the actual seed loading. Finally, the weed bags were unequivocally labeled and shipped via road transport to the respective study sites in Germany.

A

#### Study sites and sowing

Ô The multiple site study was condicted at two different regions in Germany: one in Southern Germany in the federal state of Baden Wüttemberg in Renningen, southwest of Stuttgart at the experimental station Ihinger Hof of the Uppersite Hohenheim on the Ollowing called Ihinger Hof) and the second in Northern Germany in the rederal state of Lower Saxon 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® flus-treated W-BAR seeds at Ihinger Hof and Celle were 4.8 ha and 8.0 ha, respectively. The fields drilled with Sharagda forterreated W-BAR seeds at Ihinger Hof and Celle were 3.9 ha and 7.0 ha, respectively. The variety of W-BXR sown at famiger Hof was 'Highlight' and the variety drilled at Celle was 'Lomerit'. More detailed to formation 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 Smarged® 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 cm<sup>2</sup>) 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 Petrizush was then individually transferred to a separate polyethylene flask. To ensure that all possible deposits of impactorial or respectively clothianidin from the inside of the Petri-dish were transferred to the corresponding polyed vlen flask each Petri-dish and its corresponding funnel was additionally rinsed with fresh tap water ( $\approx 20$  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 fightly closed, To avoid crosscontaminations the Petri-dishes were always oproached from the downword 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 model 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 clothianidil residues in the samples were subsequently determined by Bayer CropScience AG by High Performance Fiquid Chromotography, coupled with Tandem Mass Spectrometry. Until shipment, the samples were stored at room temperature.

#### Results

A total number of 279 samples were contected from fields didled with Manta® Plus or Smaragd® forte treated seeds. One Petricdish was inadvertently left closed. Of these 279 samples, 208 samples (74.5 %) were found to contain no quantifiable residues of inidacloprid or clothianidin, respectively (LOQ1); this included 194 samples (69.5% of Al 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 & OQ1035 of these samples were taken at the time of sowing, the remaining 8 were taken 24h after driffing 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 BOD 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 borde, 0.029 g a.s. a 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 was below the LOQ. The 90<sup>th</sup>%ile residue values during the sowing operation were 0.037 & a.s./ho 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 90<sup>th</sup>%ile residue value was below the DOD (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.



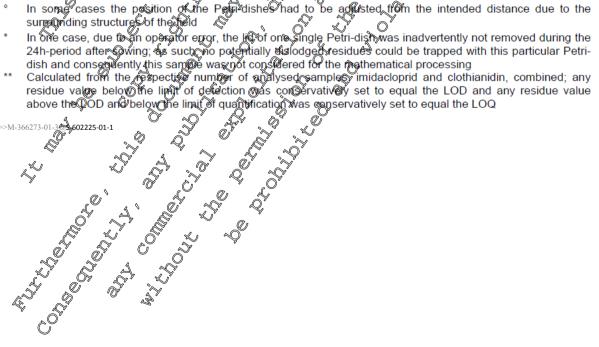
The results of the imidacloprid and clothianidin residue analysis of the dust drift samples are summarised in the table below and are detailed in the Analytical Phase Report ( , 2009; Report-No.: MR 09/153).

#### Table S1: Summary of residues at respective distances to the sowing borders (imidacloped and clothianidin, combined)

				4	
		During Sowing	j O	A4h-sampling	Total
Nominal distance	1 m	3 m	<sup>™</sup> 5m	Ç 1 m Ø	
(actual distance)°	(1 m)	(3 m)	(4.5 - 5 m)	(0.8 - 1 m)	
No. of samples analysed	40	40	40		279 279 279 279 279 279 279 279
No. of samples not recovered in the field *	0			sidues lexels [n]	<i>″</i>
Residue level	~	Number of s	amples with re	sidues levels [n] 🔿	
< LOQ	22		, <sup>42</sup> , 0		() 2163 <sup>°</sup>
0.014-0.050 g a.s./ha		4 16 S	J 17 0		59 59
0.051-0.100 g a.s./ha					°≫ 0
>0.100 g a.s./ha		~ 3 0	~ 1 0 <sup>°</sup>		4
		y Ø Res	due levels [g	a.s./hat y	
Average **	0.01%	0.029	<u>,</u> 00.020€		
90 <sup>th</sup> %ile **	0,000	0.001	y 0,027	© ́≹od	n.a.
Maximum *	£0.045	~ 0.283 ×	-30.272 O	٥.026 O.026	

LOD = 0.004 Ca.s./ha@midacOprid, cOthianitin); LOG = 0.00 g a Sha (imidacloprid, clothianidin); n.a. = not applicable L.

In some cases the position of the Positidishes had to be adjusted from the intended distance due to the





#### Imidacloprid Bee Studies Compilation of Study Summaries

Issue date 2017-11-22

Report:	02.02.06/02; <u>M-366277-01-3</u>
Title:	Monitoring of dust drift deposits during and after sowing of winter wheat (W-WHT)
	treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (6) 70 +
	7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields m
	Germany
Report No.:	R09247-2
Document No.:	<u>M-366277-01-3</u>
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 v s v v v
	Germany R09247-2 <u>M-366277-01-3</u> 91/414/EEC of July 15, 1991, SANCO/3029/99 Rev. 4, 2000-07-11 US EPA OCSPP Guideline Number, 850.SUPP not specified <b>no</b> Is C varieties (i.e. Hermann and Manager) were purchased untreated and commercially imercial seed distributor (Cut Peterhof, D-5012) Bergheim, Germany) and were
< <m-366277-01-3@s-602228-01-1< td=""><td></td></m-366277-01-3@s-602228-01-1<>	
Material and Method	S A A A A A A A A A A A A A A A A A A A
Test item	
Two different W-WHT	varieties (i.e. Hermann and Manager) were purchased untreated and commercially
cleaned-up from a com	mercial seed distributor (Gut Peterhof, D-50127) Bergbeim, Germany) and were
thereafter seed-treated	at Bayer CropScience's Seed Freatment Application Centre in D 40789 Nonheim
am Rhein, Germany (n	in Difference in the second second second second second second second second second second second second second
ani feleni, Germany (ii	FS 145.2 (TOX08744-007 treated winter wheat seeds dressed with 1000mL
• Monto® Diva I	25.145.2 (TOV 09/244 00/2 tracto Wining to when Quanda Olymono Winith MOOmI
• Manta® Plus r	15 145.2 (TOAU8444-009Areated winter wheat seeds dressed with 1000mL
	g seeds (Chominally 70 g imigacloprid/100 kg seeds); identification of treated
seeds: TOX08	781-00 (variety Manager); TOX08782-00 (variet) Hermann)
and	
Smaragd® for	te FS 455 (TOX08741-90) treated wigter wheat seeds, dressed with 133mL
product/100 ks	seeds ( nonmally 50 g clonianidn/100 kg seeds); identification of treated
seeds: TOX0	776-00 (variery Magager) FOX0\$777-00 (variery Hermann)
Secus. 10Mg	
After good dragging th	exeeds were subject to chemical analysis for the determination of the actual seed
After seed-dressing, th	e seeus were subject to chemical analysis for the determination of the actual seed

After seed-dressing, the seeds were subject to chemical analysis for the determination of the actual seed loading. Finally, the seed bags were inequivocally labeled 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-Würtenberg in Renningen, Southwest of Stuttgart at the experimental station Ihinger Hof of the University Hohenhaim (in the following called Ihinger Hof) and the second in Northern Germany in the federal state of Lower Saxony fear 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® Phys-treated W-WHT seeds at Thinger Hof and Celle were 6.0 ha and 16.21 ha, respectively. The fields drifted 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 imidacloprida.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 seving

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



(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 ( $\emptyset$  13.7 cm, 147.41 cm<sup>2</sup>) 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 Petrizush 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 polye@vleneflask. each Petri-dish and its corresponding funnel was additionally rinsed with fresh tap water ( $\approx 20$  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 lightly closed. To avoid crosscontaminations the Petri-dishes were always opproached from the downword direction. Each polyethylene flask was labelled with the sampling date and an individual sample dentification 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 model of each field side at a distance of 1 m to the field borders to give a total of 40 Petri-chishes 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 continuitin restrictes in the samples over subsequently determined by Bayer CropScience AG by High Performance Liquid Chromatography, coupled with Tandem Mass Spectrometry, Ontil supprent, the samples were stored at room temperature.

#### Results »

A total number of 280 samples were collected from Fields Filled with Manta® Plus or Smaragd® forte treated seeds.

Of these 280 samples, 272 samples (97,4%) were found to contain no quantifiable residues of imidacloprid or clothiandin, respectively (LOQ<sup>1</sup>); this included 228 samples (81.4% of all 280 samples) with no detectable residues ( $\mathcal{LOD}^1$ ). A totable f 8 somples ( $\mathcal{L}$ .8%) were found to contain residues of imidacloprid or clothianidid above the light of quantification (LOQ<sup>1</sup>). 5 of these samples were taken at the time of sowing, the remaining 3 were take 24h after drilling was completed. The maximum observed residue level was 0.258 g a.s. Da (see Table SI).

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 some roatively 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 < 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.



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 ( 2009; Report-No.: MR-09/159).

<sup>1</sup> LOD = Limit of Detection = 0.004 g a.s./ha, LOQ = Limit of Quantification = 0.014 g a.s./ha imidacloprid and clothianidin, respectively)

### Table S1: Summary of residues at respective distances to the sowing borders (imidaclopsi and clothianidin, combined)

	-1	L.	Ć	Ň K	<u> S</u>
		During Sowing	dd 2	24h-sampling	Total
Nominal distance (actual distance)°	1 m (1 - 2 m)	3.07 (34 m)	5 mr ° (5 √8 m) √	(,100 or 100)	
No. of samples analysed	40	0°40 20°	č 40 ov	S. 160 4	
No. of samples not recovered in the field	0				
Residue level	, Oq	(Number of s	amples with re	sidues levels [n]	, Q
< LOQ		87 °			a 272
0.014-0.050 g a.s./ha		3 '0'		6 <u>6</u> 6	7
0.051-0.100 g a.s./ha 🚬 🖗	ð "	r _w	Ø O Å		0
>0.100 g a.s./ha	A O S	8°0 80			1
			sidue levels [g	Ds./ha]	-
Average	↓↓LOQ		SLOQU	S < LOQ	
90 <sup>th</sup> orile *	< LOQ 5			ک < LOD	n.a.
Maximum *	\$0.034 g	9.030	Ø.258	0.027	

LOD = \$ 004 g a.s./ha (midacloprid, clothianidio), LOQ = 0.014 g a.s./ha (imidacloprid, clothianidin); n.a. = not **%** applicable A O \$ 1

In some cases the position of the Petridisher that to be adjusted from the intended distance due to the surrounding structures of the ford.

During sowing: The close proximitly of a doinage often to the downwind border of study field 18 prevented samplers from being deproved autside the study field as required for sampling. In order to circumvent this problem, the farmer sovied a 60 strip parallel is the field's downwind margin. Samplers were then placed in the source 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 head a distance of Thm inside the study field of study field 11 required the samplers along side C to be placed at a distance of Thm 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) C Calculated from the respective number of analysed samples, imidacloprid and clothianidin, combined; any residue value below the smit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the light of quantification was conservatively set to equal the LOQ.



Report:	02.02.06/03; ; 2014	; <u>M-502885-03-3</u>		orid treated winter arley of in autumn 201
Title:	Investigation of dust drift of	deposits of clothi	anidin & imidaclop	orid treated winter warley
	seeds with pneumatic sowi	ing machinery on	fields in Germany	in autumn 201
Report No.:	R11129		8	
Document No.:	<u>M-502885-03-3</u>		Ą	
Guideline(s):	BBA Drift Guideline Part	VII, 2-1.1	10%	
	US EPA OCSPP Guideline	e Number: 850.8	SUPP A	
Guideline deviation(s):	none	Ĉa	\$.	
GLP/GEP:	yes		<u> </u>	
< <m-502885-03-3@s-605130-01-1< td=""><td></td><td>Å</td><td>ĴŐ<sup>¥</sup></td><td></td></m-502885-03-3@s-605130-01-1<>		Å	ĴŐ <sup>¥</sup>	

Aim

This study was conducted in order to determine occurring aerial and ground dust drift deposits doing 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 (Clothanidin + Imidacloprid FS 100 + 175 G at a nominal seed treatment rate of 200 mL product/100 kg, corresponding to nonnally 20 g clothianidin and 55 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 noninally 20 g clothianidm and 35 g imidacloprid/100 kg). Each pneumatic solving machine was filled on the farm site. Solving of the dressed seeds was exclusively performed by typical commercial pneumatic solving machine, 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 aerial field boundary (corphology): Petri-dishes, horizontally arranged at a height of approximately 2 cm above the soft surface and vertically erected gauze-netting-samplers (effective sampling area:  $2m \times 3^{-7}m$ ). 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  $\leq \pm 30^{\circ}$ . The border of the downwind study field side was described as "zeto" line".

Samples of dressed seeds were taken at the time of bagging and from the used seed bags shortly before filling that the time for Meubach analysis by the Seed Growth Center of Bayer CropScience AG (non-GLP)

Two lines of 3 × 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 the 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-



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 ug, 0.1 ug, 10 ug 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 gothered 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 word direction was 280° (± 19°). The mean wind speed was  $33^{\circ}$  m/s ( $\neq 0.9$  m/s). For study field the target wind direction was 120°. The measured mean wind direction was 120° ( $\pm 33\%$ ). The mean wind speed was 2.4 m/s ( $\pm 0.9$ m/s). The target wind direction for streey field 3 was 140°. The measured mean wind direction was 128° (± 14°). The mean wind speed was  $\frac{1}{2}$  8 m/s(± 0.9 m/s).

Residue analysis Residues of clothianidin and inidacloprid in all Petri-dishes and gauze fletting samples as well as all field fortification samples, filters used in the Heubachabrasion tests obtained from the seed samples taken shortly before drilling and in soil samples were analysed by taboratory of the Analytical Test Site (BCS-R, Report # MR+12/006). Chromatography and D-HS-RA, Bayer CropScience AG) detection by MS/MS in Heubach filters, gauze netrings and Petri-dish colutions was done according to method MR-338/00 (clothanidin and MR-06/144 (inodaclopfid). Analysig in soil samples was done according to method MR-106/02 (clothianidia) and MR-106/03 (ignidacloprid). The Limits of Quantitation (LOQ) for clothianidin and infidacloss of the ganze 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 inidacoprid were 0.007 g a. Tha, respectively, the corresponding LODs were 0.02 g a.s. ha. For the soft samples the OQs were 50g 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.

2 The Heubach value determined shortly after seed it atment 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 Heuback 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 stody field 3, respectively (non-GLP).

The filter from the neubach test that were conducted after drilling were also analysed for their content of clothianidity 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 cospectively. 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.



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 imidate lopride 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 clothian din and imidacloprid was below the LOD and none of the 45 gauge samples from study field 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 6.02 g.a.s./ha in Petri-thish samples and 0.01 g a.s./ha in gauze netting samples, residue values below or equal the LOO were set conservatively 0.07 g a.s./ha in Petri-dish samples and 0.04 g s./ha in gauze netting samples. If all residue values of one sample type of one study field were < OD of < LOO 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 200D at study field 2 and 3. At a distance of 3 m o the zero line the average residue level of clothianidin in the Petri-dishes was 0.65 g a.s. ha at study field 1 and 2LOD at study field 2 and 3. For inidad oprid 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 2LOD at study field 2 and 3. At a distance of 3 m to the zero line the average residue level of inidad oprid 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 2LOD at study field 2 and 3. At a distance of 3 m to the zero line the average residue level of inidacloprid in the Petridishes was 0.07 g a.s./ha at study field 1 and 4

The mean residue level of clothianidia and inidaclogrid in the gauge netting was 0.040 g a.s./ha for all three study fields, as values 200 and  $\leq 1000$  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 Phace Report (Attachment 1).

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~ ¥	Residue jevels or ciotnian (g a.s./na)									
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	Q		্ৰ জাৰ্ম	netting	<u> </u>	) <sup>v</sup> 3m 🛷	netting	1m	3m	netting
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× 1	OD Potri	dich $= 0.0$	2 d a c /bc		tri-dish = 0.0	Zaac/ba:				

Table S1: Summary of clothiandin Amidactoprid residues in Petri-dishes and gauze nettings

SLOD 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



#### **Imidacloprid Bee Studies Compilation of Study Summaries**

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Issue date 2017-11-22

>>M-502885-03-3@\$-605130-01-1

Report:	02.02.06/04; 2015; <u>M-504522-02-2</u>	
Title:	Assessment of potential impacts on honeybee colony development, their hilfernation	
	performance and concurrent monitoring of aerial dust drift during the sowing operation	
	of imidacloprid FS 350A G - Treated winter barley with typical commeterial vacuum-	
	pneumatic sowing technology, directly adjacent to full flowering Phacelia tanacetifolia	
Report No.:	R1440009	
Document No.:	<u>M-504522-02-2</u>	
Guideline(s):	in United Kingdom R1440009 <u>M-504522-02-2</u> US EPA OCSPP Guideline 850 SUPP none yes	
Guideline deviation(s):	none	
GLP/GEP:	yes a way of the second s	
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Aim		
	(1, 1)	
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 Placelia tanacetifolia. The employed winter barley seeds were		

commercially treated with Imidacloprid FS 350A G (nominal cate: 70 g imidacloprid/100 kg seeds). Moreover, dust drift deposits during the sowing operation of the treated where badey seeds were L, concurrently monitored. 0ì The study comprised in total four study fields, two treatment fields and two control fields, both of similar

size. The Imidacloprid FS \$50A O-treated winter barley seeds were sown on treatment fields, while untreated winter barley seeds dressed with the standard fungricide Prothioconazore FS 100 G were sown **K** on the control fields.  $\mathbb{R}^{\times}$ on the control fields. All fields were sowned with typical commercial available pneumatic sowing machines. Possible impacts on

the colony development and their hiberhation performance were investigated. All assessments made on bee colonies placed at the two treatment fields were compared to concurtent and equal assessments made 0° Ŵ on the two coord fields.  $\bigcirc$ Ô Ő

Furthermore, concurrent dust driftomeasurements of the active substance of Imidacloprid FS 350A G (a.s. imidacloped) were performed by placing vertical gauze-neurong-covered construction fences directly adjacent to the sowing area of the two treatment folds.

### Material and Methods

Test item Conventional winter barley seeds the sseed with Inudaclopfid FS 350A G (nominal treatment rate of 70.0 g imidacloprid/100 kg seed .

The test item was bagged at the Seed Freatment Application Centre of Bayer CropScience AG in D-40789 Monheim am Rhein, Germany (non SLP) by employing typical seed-treatment and bagging practices. 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 taracetifold, a highly becattractive crop. The dimension of the winter barley-sown area inside the Phacelia macetifolia 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 hove was straightened in the direction to the Phacelia to correspond to the appeultural practice. After the prosure period the honeybees were relocated to a monitoring site on 10 July 2014 in the region of Nork without intensive agricultural activities in the near vicinity.

#### Honeybee mortality and behaviour

The mortality of honeybees (e.g. workers, pupae, grones) 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 abnormatives of the honeybees at the entrance hole were recorded during the mortality assessments. Ľ.

Population development and health assessment of cells filled with ages, larvae of capped brood) as well as food stores (i.e. pollen and neotar) 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 trame 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 oquivalents per hive. The quotient between honeybee numbers after and before hibernation was calculated as a value for the Š

hibernation success of honeybee colonies.

During the Field Phase and the Bee Fealth Phase, bee colonies were kept according to Good Apicultural Practice and all typical apicultural measures were respected.  $\bigcirc$ 

#### Dust drift sampling

S 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 effected gauze-netting-samplers were set up on each assessment plot of the treatment fields. The solving 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-samples (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 conjent 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 jug@before@he starf 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 withered and immediately transferred into separate polyethylene wide mouth bottles. Ôĭ

### Residue analysis

Imidactoprid 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 appost al colonies. Here on most days, in both groups a mean of  $\leq$  one dead large or pupa percolony was found in the dead bee traps. Therefore it can be assumed, that there was no test item related effect, also recarding to the worker bee brood mortality.

#### Honeybee colony development

At the pre-sowing assessment, the number of orker dees was very similar in the control and treatment group. At both groups the colony strength increased in a similar way towards the first olony assessment after sowing, which resulted in still very sumilar numbers of adult worker bees. Also, during the following assessments in 2014 and at the assessment after hibertration 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 colory 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 begon r for the total brood amount. Also the lebernation index indicates that there is no effect of the test iters, as the colorbes from the test itero group hibernated even slightly better than those of the control group (hipernation index of 0, \$46 in test item group and 0.443 in control group). Altogether, it can be concluded that the test them did not affect the honey bee colonies in any manner. J.

During the field Phase and the Bee Health Phase, the queens of three colonies were replaced by another sister queen according of Good Apicentural Practice due to different reasons. As the replacements had to be done also in the control colonies, there is no hint for a test item related effect on the health of the queens.

# Varroa destructor infestation

Natural daily white fall was recorded during all of ony assessments. Though it was on a generally very low level, the Varroa infestation was lightly 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 hones bee colonies in any manner.

#### i, Residues

No residues were found in the control gauze samples. In the field spike samples, the mean recovery at study field T 2% study field T2 101 % ± 2.5 %. The Limit of Quantification (LOQ) referring to the determination of midacloprid from gauze netting samples was 1 µg imidaclopfid/L gauze extract @quivalent to 0.04 g a.s./ha. The corresponding Limit of Detection (LOD) was 0 Mug inoraclopind/L gauze extract, equivalent to 0.004 g a.s./ha.

Due to changing word 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.



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  $(0.18 - 0.32 \text{ g a.s./ha})_{a}$ compared to those determined on the upwind assessment plots, which were below the LOQ (<0.04) 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 approximately on the level of the LOO.

#### Conclusion

To assess the potential effects of Imidacloprid FS 350A G on the colony development of hone wees (Apis mellifera L.), Imidacloprid FS 350A G - treated winter barley seeds (nominal treatment rate \$0.0 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 imrdacloprid/100 kg scods) indicate that seedtreatment dust, abraded and released during the sowing operation with type al, commercial available pneumatic sowing equipment, resulted in a measurable of field exposure, which was distingly higher at the downwind borders of the winter barlewsowing areas as compared to the corresponding upwind borders. The maximum vertical dust deposition, as measured by vertically erected gauge retting units, directly adjacent to the winter barley swing areas, corresponded to a maximum drift rate of 0.32 g a.s./ha. m

The application of Imidacloprid F\$350A G did not cause any effects on the survival of adult bees and bee pupae, foraging activity, belavior, also not on coon development, hibernation performance and colony strength as well as on the bee brood

colony strength as well as on the bee brood Thus this study demonstrated that midactoprid to 350AG – treated winter barley seeds (nominal

colony strength as well as on the bee brood? Thus this study demonstrated that midacioprid US 350AG – treated winter barley seeds (nominal treatment rate 70.0 g imidacioprid/100 kg seeds), so you during bee hight, and not adversely affect honeybee colonies.



Report:	02.02.06/05; 2014; <u>M-504065-01-3</u>	
Title:	Assessment of potential impacts on honeybee colony development, their hibernation	
	performance and concurrent monitoring of aerial dust drift during the sowing operation	
	of Donoho Dato Dive. Tracted grant host fully with trained of momental years of the	
	of Poncho Beta Plus - Treated sugar beet pills with typical commercial vacuura-	
	pneumatic sowing technology, directly adjacent to full-flowering Phacelia tanacetite ia	
	in Germany	
Report No.:	R12261A	
Document No.:	<u>M-504065-01-3</u>	
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According to the Regulation (EC) 110/2009 (2009) the possible adverse effects of crop protection products on honeybees have to be assessed. Therefore this study a med 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 Phacetia tanagetifolta. The employed sugar beet pills were commercially treated with Poncho Beta Plus (nominal rate; 0.60 ng clothanidin a.s./pill, 0.08 mg a.s. beta-cyfluthrin/pill and 0.30 ng a.s. imidactoprid/pill). Moreover, dust thift deposits during the sowing operation of the treated sugar beet pills were concurrently monitored.

The study comprised in total three study fields, one freatment field and two control fields, all of similar size. The Poncho Beto Plus-treated sugar beet pills were drilled on the reatment field only, while maize seeds dressed with the standard fungicide Thiram SC 700 were drilled on the control fields.

Maize seeds at the cortrol fields were sown with a typical deflected vacuum-pneumatic sowing machine, while the Poncho Bora 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 lieta-cyfluthring were performed by placing vertical gauze covered construction fences directly adjacent to the sowing area on the reatment 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 cyfludrin 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 wed-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 Fymerazol WP 70.

The coated pills were bagged into 1 Unit (=100,000 pills) cardboxes, and were labelled with a unique label and the OX-Number.

The praize 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 so wing 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./ka, 10.4 g beta cyfluthin a.s./ha and 39.0 g imidacloprid a.s./ha. In order to keep driving distances with fulled 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-preumatic 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 violity. 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 thoneybee hives from each study field were relocated randomly to the monitoring sites cone third of the hives of each study field be 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 prectice to the Phace a 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 tabelled 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 (humber 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, Buchmann 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 actual 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 ganze 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



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/betacyfluthrin/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 ansport 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 al field fortification samples were analysed by Bayer CropScience AG ( R. & 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-cyfluthrif). The Limit of Quantitation (LOQ) of the gauze samples (0.25 m2) was 0.04 g a.s./b) for all analytes. The Limit of Detection (LOD) was 0.004 g a.s./hator bolk clothanidin and invidaclop id and 0.012 g a.s./ha for beta-cyfluthrin. Results Honeybee mortality In control and treatment group, worker beemortality was on the same generality low fevel, mostly around five to ten dead bees per day in mean. Agatatistical significant difference between control and treatment worker bee mortality could be seen on some days before the application, so that 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 confol 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 worket 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:  $0.52 \pm 1.90$ ; mean treatment group:  $0.28 \pm$ 0.67). On most days, no brood was found in the dead bee traps. Honeybee colopy development  $\bigcirc$ 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 colonie con the study fields. Due to the excellen 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 breading 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 pro-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 if 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.



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 Muller 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 the clothianidin mida opricand bet 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 LQD (0.0 10 g a.s. ha forbetacvfluthrin and 0.004 g a.s./ha for clothianidin@nd im@acloprid) were deteoted in my of the gauze samples obtained from the study field during sowing of the test item.

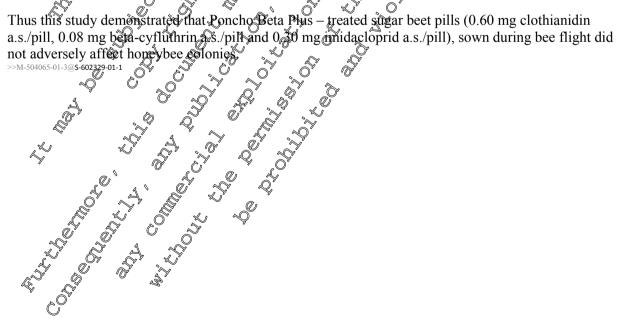
#### Conclusion

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 clothanidin a.s./pfl, 0.08 mg betacyfluthrin a.s./pill and 0.30 mg imitacloprid a.s./pill) were sown (139,500, og ar 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 grop.

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 °, Ô and the hibernation sugcess.

The dust drift measurements made during the sowing operation of Ponoho 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 soming equipment and dismounted chassis of the discharged air system did notresult in a measurable off-field exposure as all analysed samples were below their respective LOD (0.012g a.s. tha for Deta-cylluthrin and 0.004 g a.s./ha for clothianidim and imidacloprid).

Ś Thus this study demonstrated that Poncho Beta Plus – treated sugar beet pills (0.60 mg clothianidin





preumatic drilling of imidacloprid reated cotton seeds in Greece during springime 204 enument No.: M424386-01-2 Working document 1607/VU97 rev. 1 with the partial integration of the BBA DPH Guideline Part VII, 2-1.1 (1992) and 2010/21/EU uideline deviations): more EP/GEP: yes without a star sum: Cotton seeds treated with Pinidacloprid FS 350, Active Amerediant: midacloprid Analyzed content of active ingredients 484 of <i>g</i> 100 Kg seeds Batch: EAA88 11 10 23 arises site Name: Cotton seeds treated with Pinidacloprid FS 350, Active Amerediant: midacloprid Analyzed content of active ingredients 484 of <i>g</i> 100 Kg seeds Batch: EAA88 11 10 274 the field study was conducted in Greece Conc final in flet vicipity of Vefla (tral S11, 2028)-011 and a cond trial in the vicinity of Larissa (fral S12, 2028, 202). Cotton seeds, pre-treated with Imidacloprid S30 (provided by Bayer CropScieße CA)9, were Sown in Giadmina game Peblic (RL 2028)-011 and a cound trial in the vicinity of Larissa (fral S11, 2028, 3-02) and 13 May 2014 and 14 Maxy 3011. The purpose of the study was to determine the deposition of dust from the seed freatment emitted from a neuro-pneumatic drilling miching during Sowijke of Lajdacloprid FS 350 prated cptton seed. Dust mechanical abrasion of the 9 treated seed from prefersed during seeding of ection seed. Dust mechanical abrasion of the firther at a Larise (S11-02083-02) and 13 May 2014 and 14 Maxy 3011. The purpose of the study was to determine the deposition of dust from the seed freatment emitted from a neuro-pneumatic drilling miching during Sowijke of Lajdacloprid FS 350 prate degiton seed. Dust mechanical abrasion of the 9 treat at Larise (dist colpected in Petri fishes). The plots where dust mission was measured lad a total size of 10490.4 mg for the firth of the drilling rate for the dust fail and 1070 m x 52m for the trag at Lajdas (dist colpected in Petri fishes). The plots where dust mission was measured lad a total size of 10490.4 mg for the firth be to 10.776.8 m <sup>-1</sup> for trial at arissa (dust c	arissa site Name: Cotton seeds treated with Invide Ignid ES 350 Active meredient: Invide Ionrid Analyzed content of active ingredient: 555.07 g/100 kg seeds Batan: EA385 11 40 274 The field study was conducted in Greece one that in the vicinity of Pella (trail S11 502083-01) and a econd trial in the vicinity of Larissa (hfal S14 202083-02). Cotton seeds, pre-treated with Imidaeloprid S 350 (provided by Bayer CropScience AG), were sown in Gjatinitsa mar Pella (S14 502083-01) on 12 May 2011 and in Glafki near Larissa (S11-02083-02) on 13 May 2014 and 14 May 2011. The purpose of the study was to determine the deposition of dust from the seed freatment emitted from a acuum-pneumatic drilling machine, during sowing of Imidaeloprid F9 550 treated cotton seed. Dust mechanical abrasion of the treated seed frem) refeased during seeding of eorion seeds was collected using Petri dishes and cellulose air filters attached in the air fan of the driller (see FIGERE 10 and FIGURE 2). The size of the field plot where dust deposition was measured was 200 x 52.08 m for the trial at Pella and 176 m x 52 m for the trial size of 10490.4 m for the trial at Pella 3rd 10776.8 m <sup>2</sup> for trial at arissia (dust collected in air filters which were fitted to a filter box connected to the fan exhaust outlet via ubing). Before the filter trials stated 20 m were drilled to prime the tube. The actual drilling rate for the Pella site S11-02083-01) was 88.84 g air./ha, equivalent to 081,126 seeds fila. A total area of 2.0906 ha was drilled. For the Larissa site (trial \$11-02083-02) the Perif dishes trial the actual applied drilling rate for the dust rial at 202,241 seeds.ha was drilled for the Perif dishes trial and 1.0777 ha for the air filter trial. For the Larissa site (trial \$11-02083-01), the average wind speed during drilling was 2.33 ± 0.89 m/s (0.39 m/s o 4.79 m/s) and the average deviation to the intended wind direction was 19.91° ± 23.85° (range -54.95° o 193.22°).	Report:	02.02.06/06; ; 2012; <u>M-4</u>	24386-01-2	
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arissa site Name: Cotton seeds treated with Ifridacloprid ES 350 Active ingredient: Imidacloprid nalyzed content of active ingredient: 555.07 g/100kg seeds Bath: EA 385 1140 27. he field study was conducted in Greece one thal in file vicinity of Pella (trial S1/202082-01) and a cond trial in the vicinity of Larissa (trial S1/202083-02) oftoto seeds pie-treated with Imidacloprid S 350 (provided by Bayer CropScieace AG), were sown in Gjathitsa near Pella (S1& 02083-01) on 12 lay 2011 and in Glafki near Larissa (S11-02083-02) on 13 May 2014 and 14 May 2011. he purpose of the study was to determine the deposition of dustform the seed freatment emitted from a neuro-meumatic drilling michine during sowing of Taudacloprid FS 350 prated cotton seed. Sus collected using etri dishes and cellulose air filters attached to the artian of the driller (see FIGURE 10 and FIGURE 2). The size of the trigd plots where dust deposition for our better and the dust of the trial at ella and 176 m x 35m for the trigd at LaGssa (doit collected in Petri dishes). The plots where dust mission was measured thad a total size of 10400.4 m for the trial at Pella 3fd 10776.8 m² for thrial at arissa (dust collected) air filters which were fitted to a filter box connected to the fan exhaust outlet via bing). effore the filter trials started 20m were drilled to prime the stude. The actual drilling rate for the Pella site S11-02083-01) was 88.84 g to /ha, equivalent to R81.126 seeds tha. A total area of 2.0906 ha was drilled. or the Larissa site (trial S11-02083-02) the gerif dishes trial, the actual applied drilling rate for the pella at at 202,241 seeds/ha was guivalent to ma application fare of 109.18 g a.i./ha. For the filter trial the trial applied filling frate at 95 905 seeds ha was equivalent to an application rate of 105.76 g a.i./h. A tal area of 1.0032 ha was drilled for the Petri dishes yrial and 1.0777 ha for the air filter trial the trial applied filling frate at 95 905 seeds ha was equivalent to an application rate of 105.76 g a.i./h	arissa site Name: Cotton seeds treated with Ibidactopid ES 350 Active ingredient. Imidactopid Analyzed content of active ingredient: 555.07 $\frac{1}{2}$ /100 kg seads Bator. EA 385 11 90 274 The field study was conducted in Greece, one that in the vicipity of Falla (tch S11 202082-01) and a coond trial in the vicinity of Larissa (tohal S12 202082-02). Gotton Seeds, pre-treated with Imidactoprid S 350 (provided by Bayer CropScieace AG), were sown in Giatfinitsa near Pota (S14 202082-01) on 12 Aay 2011 and in Glafki near Larissi (S11 202032-02) and 13 May 2014 and 14 May 2011. The purpose of the study was to determine the deposition of dustoriom the seed freatment emitted from a acuum-pneumatic drilling machine during sowing of Inuidactoprid F3 550 treated gotton seed. Dust mechanical abrasion of the treated seeds term) preased during seeding of epticon seeds was collected using ever i dishes and cellulose air filters attached by the air fan of the driller (see FIGERE 10 and FIGURE 2). The size of the field plot where dust deposition for the trial at file and 176 m x 52m for the trial at Lafssa (tist collected in Petri dishes). The plots where dust mission was measured had a total size of 10400 4 m for the trial at Petle and 1076.8 m <sup>2</sup> for trial at arissa (dust collected air in filters which were drifted to a filter box connected to the fan exhaust outlet via ubing). Petror the first trials started 20m were drifted to prime the tube. The actual 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 rial at 202,241 seeds/ha was enuivalent to 81,126 seeds/fa. A total area of 2.0906 ha was drilled total applied filling fate a f195 908 seeds ha were equivalent to an application rate of 105.76 g a i./ha. A total area of 10032 ha was drilled for the Petri dishes trial, the actual applied drilling rate of the dust rial at 202,241 seeds/ha was enuivalent to the intended wind direction was $9.91^\circ \pm 23.85^\circ$ (range -54.95° o 193.22°).	Report No.:	S11-02083		tion seeds in Greece during springsme 270
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/s to 6.77 for/s) and the average deviation to the intended wind direction was $9.60^{\circ} \pm 39.07^{\circ}$ (range - 9.11° to 73.31). O Petri dishes, filled with grycerol/water (1/1, v/v) were placed at 1, 3, 5, 10, 20, 30 and 50 m distance out the zero line of sowing (first driller pass). The Petri dishes were placed horizontally on the ground. oil samples from the upper 10 cm were taken before drilling for soil characterization and for analysis of otential residues of imidacloprid in the soil that might have originated from previous treatments. Soil umples from the upper 5 cm were taken for the moisture content determination.	h/s to 6.77 m/s) and the average deviation to the intended wind direction was $9.60^{\circ} \pm 39.07^{\circ}$ (range - 9.11° to 73.31). 0 Petri dishes, filled with plycerol/water (1/1, v/v) were placed at 1, 3, 5, 10, 20, 30 and 50 m distance room the zero line of sowing (first driller pass). The Petri dishes were placed horizontally on the ground. oil samples from the upper 10 cm were taken before drilling for soil characterization and for analysis of otential residues of imidacloprid in the soil that might have originated from previous treatments. Soil amples from the upper 5 cm were taken for the moisture content determination.	or Larissa site (trial-\$	11-02083-02) the average win	d speed durin	ng drilling was $2.44 \pm 0.89$ m/s (0.79
9.11° to 73.315. O Petri dishes, filled with prycerol/water (1/1, v/v) were placed at 1, 3, 5, 10, 20, 30 and 50 m distance out the zero line of sowing (first driller pass). The Petri dishes were placed horizontally on the ground. oil samples from the upper 10 cm were taken before drilling for soil characterization and for analysis of otential residues of imidacloprid in the soil that might have originated from previous treatments. Soil imples from the upper 5 cm were taken for the moisture content determination.	9.11° to 73.319. O Petri dishes, filled with prycerol/water (1/1, v/v) were placed at 1, 3, 5, 10, 20, 30 and 50 m distance com the zero line of sowing (first driller pass). The Petri dishes were placed horizontally on the ground. oil samples from the upper 10 cm were taken before drilling for soil characterization and for analysis of otential residues of imidacloprid in the soil that might have originated from previous treatments. Soil amples from the upper 5 cm were taken for the moisture content determination.	n/s to 6.77 m s) and th	e average deviation to the inte	nded wind di	rection was $9.60^\circ \pm 39.07^\circ$ (range -
out the zero line of sowing (first driller pass). The Petri dishes were placed horizontally on the ground. oil samples from the upper 10 cm were taken before drilling for soil characterization and for analysis of otential residues of imidacloprid in the soil that might have originated from previous treatments. Soil amples from the upper 5 cm were taken for the moisture content determination.	out the zero line of sowing (first driller pass). The Petri dishes were placed horizontally on the ground. oil samples from the upper 10 cm were taken before drilling for soil characterization and for analysis of otential residues of imidacloprid in the soil that might have originated from previous treatments. Soil amples from the upper 5 cm were taken for the moisture content determination.	9.11° to 73.31%.			
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	ease click on the hyperlink to order a Study Report.	<b></b>			



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 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 indacloprid 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, 39.857 mg a.i./ha, equivalent to 0.040 % of the field rate for 1 m distance, 39.857 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 ari/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 p distance.

For trial at the Larissa site (S11-02083-02) the average amount of imitaclopric was 257.390 mg a.i./ha in a distance of 1 m, 151.810 mg a.i./ha in a distance of 30 n, 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, 2497/56 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 3 m distance, 249.546 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 3 m distance, 249.546 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 3 m distance, 249.546 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 3 m distance, 249.546 mg a.i./ha, equivalent to 0.229% of the field rate for 3 m distance, 136.510 mg a.i./ha, equivalent to 0.425 % of the field rate for 10 m distance, 22.218 mg a.i./ha, equivalent to 0.084 % of the field rate for 26 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 4/ha, equivalent to 0.041 % of the field rate for 50 m distance. The air filters attached to the far exhaust including the tube trapped a total of 1101.5 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).

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Le la la la la la la la la la la la la la	Distance from Zero Line [m]	Imidacloprid Dieno Deposition [mg a Mha]	percentile (mg a ¢)ha]	% of Mield rate (90 <sup>th</sup> percentile)	Imidacloprid mean Deposition [mg a.i./ha]	% of applied a.i./ha
D.Y	Ô	~~~ 0	″∕∕S11-02	803-01		
	1 2	33.115	35.7690 <sup>7</sup>	0.040		
, K		27.316	@/31.85⁄7√	0.036		
$\sim$			♀ 25,849	0.029		
	<u>∿10</u>	18,444	23,823	0.027	1151.5	1.3
*	× 20ٍ ∕	05.090 S 8.733	2.526	0.031		
	<b>3</b> 9/ <sup>10</sup>	8.733	@ 10.200	0.011		
Š		0° 6. <b>9</b> 96 ^	♀ <mark>6.986</mark>	0.008		
e Circle	Ű,			803-02		
J A		~237.390	370.616	0.339		
Á .Ű		151.810	249.756	0.229		
	5	195.543	249.546	0.229		
Contraction of the second seco	10	96.549	136.510	0.125	4415.8	4.2
U	20	65.530	92.218	0.084		
	30	36.887	51.278	0.047		
	50	26.827	44.921	0.041		



>>M-424386-01-2@ <b>S-603073-01-1</b>				
				a s.
Report:	02.02.06/07; ; 2005; <u>M-2</u>	<u>57837-01-2</u>		
Title:	Summary of particle size measured	urements of dust	generated by six seed	drillingmacine®
Report No.:	MEF-05/429		<sup>o</sup>	SU D
Document No.:	<u>M-257837-01-2</u>		1 Contraction of the second se	
Guideline(s):			A	64 54 Q
GLP/CFP·	 po	(Pa		
	02.02.06/07; 2005; M-2 Summary of particle size measu MEF-05/429 M-257837-01-2  no nes were tested at the Arvalis al characteristics with respect sons included observations of		Û Û	
< <m-257837-01-2@s-605935-01-1< th=""><th>as more tested at the Armalia</th><th>antituta (Daian</th><th></th><th></th></m-257837-01-2@s-605935-01-1<>	as more tested at the Armalia	antituta (Daian		
Six seed drilling machin	es were tested at the Arvans	to the series	eville, 91/20 Franc	e, in order to
compare then operations	sons included observations of	to the generation	on on clust monthline	seed defining
manufactoria and a second and a second and a second a sec	r outlet dimensions, maximum	offentation of v	wit of the formation	l maguranta
of the particle size distri	bution of the dust emitted by	the blower The	alatter maastreman	ts are
summarised here with r	espect to their possible effect	s on the drift of	due from seed deit	ling
summarised here, with r				ing operations.
The principal objective	of the measurements was to d	etermine if the	machinery type had	wlarge affect on
the quality of the dust ge	enerated during the drilling p	ocess Althonyol	h the quantity of dri	st generated by
different machinery doe	s differ depending on the med	harrism for atta	ching the seed to the	e distribution
wheel the age of the equ	uipment, ere., it is more likely	that the mality	On the dust is most	strongly affected
by formulation and form	ulation@dditives rath@ than	Souinment diffe	erence &	strongly uncetted
>>M-257837-01-2@S-605935-01-1		squipment are	erences.	1
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	al characteristics with respect sons included observations of r outlet dimensions, maximum bution of the dust emitted by respect to their possible efficient of the measurements was to denerated during the drilling of s differ depending on the measurement, etc., it is more likely nulation additives rather than the dependence of the drilling of the measurement of the measurement of the drilling of the measurement s differ depending on the measurement upment, etc., it is more likely nulation additives rather than the drilling of the measurement of the measurement of the drilling of the measurement of the drives rather than the drives rather than the drilling of the measurement of the drives rather than the drilling of the drives rather than the drives rather than the drilling of the drives rather than the drives rather the drives rather than the drives rather than the drives rather the drives rather than the drives rather than the drives rather the drives rather than the drives rather than the drives rather than the drives rather than the drives rather than the drives rather than the drives rather than the drives rather than the drives rather than the drin the drives rather than the drives rather than the drives rather			
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### 03 - Bumble bees

# **03.01 – Effects**

# 03.01.01 - Lab Studies

03.01 – Effects	
03.01.01 - Lab S	
	03.01.01/01; 1999; M-0167&001-3 Bumblebee (Bombus terrestris L.) oral toxicity study in the laboratory with imidaelopric techn. AH99.4.22.2 M-016786-01-3
Report:	03.01.01/01; 1999; M-0167& 01-3
Title:	Bumblebee (Bombus terrestris L.) oral toxicity study in the laboratory with imidae loprid
	techn.
Report No.:	AH99.4.22.2 <u>M-016786-01-3</u>
Document No.:	
Guideline(s):	<u></u>
Guideline deviation(s):	y y y y y y y y y y y y y y y y y y
GLP/GEP:	yes
	An199.4.22.2 <u>M-016786-01-3</u>  yes
< <m-016786-01-3@s-602475-01-1< td=""><td></td></m-016786-01-3@s-602475-01-1<>	
I ne purpose of the tox	icity study was to examine the effects of imidae loprid techn on bumblebees when
applied in the laborator	y. Que the the the the the the the the the th

Per concentration 30 bumblebees were fed individually with 10-ul sucrose solution 50%, containing a range of concentrations of imidacloprid techn Ż

A range finding test preceded the definitive test. Kappeared that 1.1 up imida doprid techn, per bumblebee killed 97% of the bumblebees, within 24 hours. The oral intake of 0.1 us imidacloprid techn. per bumblebee, affected 90% of the bumblebees within 24 bours Mortality was 2% after 48 hours. No effects on behaviour of survival were observed for closes of 0.010 g or less imidacloprid techn. Based on these data, 0.96 µg, @72 µg, 0.53 µg, 0.35 µg and 0.11 µg imidacloprid techn. per 10 µl was offered to the bumblebees. S  $\bigcirc$ 

All concentrations feelin the definitive test resulted in effect of the bymblebees. 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 farsus. Beside that, spasms and paralysis were observed as well. These effects lasted at deast during the observation period of 72 hours. Most of the affected bumblebees which had taken in amounts of initiacloped techn. of \$33 µg bumblebee or more died within 24 hours.

Ľ Amounts of imidaclop techo. higher than 9.11 us per bumblebee, cause effect and mortality of the bumblebees.

the bumblebees. The LD<sub>50</sub> of imidacloprid echn, based  $\rho q$  the linear regression is:

- $0.33 \text{ µg} \text{ imid} \text{ for ide chn} \text{ (}r^2 = 0.73 \text{)}$ LD<sub>50</sub> (24 bours):
- $\sqrt[7]{0.22}$  µg imidacloprod techn. (r<sup>2</sup> = 0.53) LD<sub>50</sub> (48 hours):
- $LD_{50}$  (72 hours): 0.22 µg inductoring techn. (r<sup>2</sup> = 0.53)

The effect of imidaclopric techn, in the concentrations higher than 0.1  $\mu$ g / bumblebee is obvious. The  $ED_{50}$  is between 0.1 and 0.01 g/ burgblebes. The data provide no basis for an accurate  $ED_{50}$  calculation.

and 0.01 rg / burbleb



Demont	02.01.01/02
<b>Report:</b> Title:	03.01.01/02; 1999; M-017116-01-4 Bumblebee (Bombus terrestris L.) contact toxicity study in the laboratory with imidacloprid techn. AH99.4.22.1 M-017116-01-4 US EPA OCSPP Guideline no 850.SUPP none yes ity study was to examine the effects of imidacloprid techns on bumblebees when
Title.	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 A O A Q
GLP/GEP:	yes
The nurness of the toxic	ity study was to examine the affects of imid Monrid techn on humblehand when
applied in the laboratory	
applied in the laboratory	ity study was to examine the effects of imidal oprid techns on bumblebess when $\frac{1}{2}$
Der concentration 20 hur	nblebees were exposed individually to midgeloprid technoly way of
Per concentration 50 but	notebees were exposed individually toorindactophic company of a second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second s
administration on the ver	ntral part of the thoras with bal acetone, containing a range of concentrations of
imidacloprid techn.	
A (* 1' / /	
A range finding test prec	ceded the definitive test. In order to determine a toxic concentration, based on the
data, obtained in the rang	ge finding test and inconsultation with the sponsor, six concentrations 901 µg,
	and 0.1 µg midac oprid techn. por 1 µl acetone were administered to the
bumblebees.	
All concentrations tested	I in the definitive test, resulted in effect on the bumblebees. The most significant
effect was the "frozen be	ehaviour" at which the by habebees are motion tess except for a little trembling of
	antennae or tarsus. Besides that, spasms and paratysis were observed. These
effects lasted at least dur	ring the observation period @ 72 hours.
There was no correlation	h between the amount of invidacloprid techn. and the pumber of dead and affected
bumblebees, whether the	e bumblebees were treated with $\mu$ g imidacloprid techn. or with 101 $\mu$ g
imidacloprid techts With	10 72 hours these treatments resulted in 90% to 100% dead or affected
bumblebees. Imidaclope	d techn, administered to bumblebees in the amount of 0.1 µg resulted in 47%
mortality and 60% effect	t. Concentrations inidactoprid techn. of 0.05 mg / 1 µl or less, administered per
bumblebee. did not caus	e effect and mortality (result range finding fest). Mortality continued during the
observation period. Bup	plebees that were affected may have died of starvation.
The data provide notasi	is for an accurate $\mathbb{Q}D_{50}$ and ED by but it is obvious that the exposure of 0.1 µg
imidacloprid techa, or m	pre per Bumblebees des seriously affect bumblebees
>>M-017116-01-4@S-602493-01-1	
	A N O Y
s" "	pore per Sumblebees des seriously affect bumblebees
a, <sup>\</sup>	
S A'	
The data provide nopasi imidacloprid techa? or m >>M-017116-01-4@s-602493-01-1	is tor an accurate 2.D <sub>5</sub> , and ED <sub>5</sub> out les obvious that the exposure of 0.1 µg wre per bumblebees does seriously affect bumblebees
Ô	
The data provide not pass imidacloprid techo or m >>M-017116-01-4@5-602493-01-1	



Report:	03.01.01/03; 2014; <u>M-494283-01-3</u>
Title:	Clothianidin + imidacloprid FS 275 (100+175 g/L): Acute contact toxicity to the bumble
Report No.:	bee, Bombus terrestris L. under laboratory conditions
Document No.:	M-494283-01-3
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 179(4)
(b).	(2010) and OECD Guideline 214 (1998), and on the review article of (an 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< td=""><td>S13-05151 <u>M-494283-01-3</u> No specific guidelines are available. The test design is based on OEPP/EPPO 179 (4) (2010) and OECD Guideline 214 (1998), and on the review article of on der Steen (2001) US EPA OCSPP Guideline No. 850 SUPP not applicable yes s: anidin + Imidacloprid &amp; 2750 100+175 g/L 00025006-01 L clothianidin (analysed) 176 7 g/L ippdacloprid (analysed)</td></m-494283-01-3@s-602260-01-1<>	S13-05151 <u>M-494283-01-3</u> No specific guidelines are available. The test design is based on OEPP/EPPO 179 (4) (2010) and OECD Guideline 214 (1998), and on the review article of on der Steen (2001) US EPA OCSPP Guideline No. 850 SUPP not applicable yes s: anidin + Imidacloprid & 2750 100+175 g/L 00025006-01 L clothianidin (analysed) 176 7 g/L ippdacloprid (analysed)
<b>Materials and Method</b>	s: Q a a a a a a
Test item: Name: Clothi	s: anidin + Imidacloprid ES 2750100+135 g/L 00025006-01 L clothianidin (analysed) 176.7 g/L ipidacloprid (analysed)
TOX No.: 10068-00	
Specification No.: 1020	
Content of a.s.: 100.3 g/	L clothianidin (analysed) 176. se/L ipodacloprid (analysed)
The contact toxicity of (	Clothianidin Frindacioprie FS 275 (100 775 g.S) to the bumble bee (Bombus
<i>terrestris</i> L.) was detern	nined in a gose-response test according to OERP/EPHO 1/0 (3) (2010), the OECD
Guideline No. 214 (199	8) and the review article of VAN DEPSTEEN (2009).
In the laboratory, the bu	mble bees were exposed to 1.23, 3.70, 14.11, 33.33 and 100 µg total a.s./bumble
bee by topical application	on. Mortal Dy and Sub-let bal effects were assessed 24, 48 and 72 hours after
	roup was exposed for the same period of time under identical exposure conditions
to tap water.	
Ű	
Dates of work: 27 Nove	ember 2003 – 02 February 2019 6 4 2
Findings' ("	
In the control group, the	ated with tap water, no mortality was observed during the 72 hour test period.
In the test item treatmen	t group, a mortality of 63.33 % was observed at the highest dose level
corresponding to 100 µg	total a y/bumble bee at the final assessment after 72 hours.
In the reference item gr	Sup, $m_{s}$ restriction of the test. Thus, the test was considered to
be valid.	
Table 1: LD50 values in	the bumble free contact toxicity test with Clothianidin + Imidacloprid FS 275
(100+175 g/L) (100+175 g/L)	
Clothianidin + Im	jdacloprid 🖉 💭 💭 Contact toxicity test
PS 275 (100+1	75 g/Ľ) ~ (μg total a.s./bumble bee]
LD <sub>50</sub> (24)	$h_{\mu}$
$L_{50}^{6}$ (48)	h) 2 2 79.2
LD50 (72)	h) 54.9
In the test iter Treatmen	t groap, moribund, affected and apathetic bumble bees were observed at all tested
	and 72 hour assessments.
	corresponding to 3.70 µg total a.s./bumble bee was determined to be the NOED
(No Observed Effect Do	
Conclusion:	/ · · · · ·

The 72 hour contact LD50 value for Clothianidin + Imidacloprid FS 275 (100+175 g/L) was determined to be 54.9  $\mu$ g total a.s./bumble bee.



>>M-494283-01-3@S-602260-01-1

Report:	03.01.01/04; (2014; M-494307-01-3)
Title:	Imidacloprid FS 350 (350 g/L) - Acute contact toxicity to the bumble bee, Box out
	terrestris L. under laboratory conditions
Report No.:	S13-05153
Document No .:	<u>M-494307-01-3</u>
Guideline(s):	M-494307-01-3 No specific guidelines are available. The test design is based on OEPPEPPO 70 (4)
	(2010) and OECD Guideline 214 (1098), and on the veview article of van der Steer
	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	not applicable
GLP/GEP:	yes $\mathcal{O}^{\mathcal{V}}$ $\mathcal{V}$ $\mathcal{O}^{\mathcal{V}}$ $\mathcal{O}^{\mathcal{V}}$ $\mathcal{O}^{\mathcal{V}}$ $\mathcal{O}^{\mathcal{V}}$
< <m-494307-01-3@s-602261-01-1< td=""><td>No specific guidelines are available. The test design is based on OEPPEPPO970 (4) (2010) and OECD Guideline 214 (1098), and on the view article of van der Steers (2001) U.S. EPA OCSPP 850.SUPP not applicable yes</td></m-494307-01-3@s-602261-01-1<>	No specific guidelines are available. The test design is based on OEPPEPPO970 (4) (2010) and OECD Guideline 214 (1098), and on the view article of van der Steers (2001) U.S. EPA OCSPP 850.SUPP not applicable yes
Materials and Method	
Test item: Name: Imida	acloprid FS 350 (350 g/L) 2 0 0 0 0 0 0 0 0
TOX No.: 10231-00	
Specification No.: 1020	
Content of a.s.: 355.2 g	No specific guidelines are available. The test design is based on OEPP EPPO 70 (4) (2010) and OECD Guideline 214 (1098), and on the view article of van der Steers (2001) U.S. EPA OCSPP 850.SUPP not applicable yes ds: acloprid FS 350 (350 g/L) (200007262 (L imidacloprid FS 350 (350 g/L) to the bumble bee (Boanbus terrestris L.) was esponse test according to OEPP EPPO 770 (4) (2010), the OECD Guideline No.
The contact toxicity of	Imidacloprof FS 350 (350 g/L) to the bomble bee (Bombus terrestris L.) was
	iew article of VAN DER STEEN (2001).
In the laboratory, the bi	umble bees were exposed to 1.23, 3.70, 14.11, 33.33 and 100 µg imidacloprid
	cal application. Mortality and sub-lethal effects were assessed 24, 48, 72 and 96
nours after treatment. I	he control group was exposed for the same period of time under identical
exposure conditions to	rap wayer. L
Datas of works 17000	and an 2012 and the house 2000 to the
Dates of work. 1 Dec	tap water. ember 2013 – 08 February 2014 52 54 55
Findings:	
In the control group tr	eated with ap water, no portality was observed during the 96 hour test period.
In the test war treatme	it group a motality of 46.7% was observed at the highest dose level
approximate 100 @	g imidacloprid a.s./bumble@ee at the final assessment after 96 hours.
At the dose level correct	spording to \$3.33 Qg imitaclopful a.s./bumble bee, a mortality of 53.3% was
observed after 96 hours	sponging to 55.55 the initial topped a.s. outlittle bee, a mortainty of 55.570 was
In the reference item of	with the test was the end of the test. Thus, the test was considered to
be valid.	$\phi$ $\mu$ , $\mu$ $\phi$ $\mu$ $\mu$ $\phi$ $\mu$ $\phi$ $\mu$ $\phi$ $\mu$
Table 1: 10050 values i	the burnele bee contact toxicity test with Imidacloprid FS 350 (350 g/L)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
	dacloprid FS <b>359</b> Contact toxicity test (340 g/L), O U (µg imidacloprid a.s./bumble bee]
	100 so (24 kb) >100
	$\Delta LD_{50}$ (Appl) $\Delta V$ >100
	$LD_{2}$ ( $Z_{2}$ ) ( $Z_{2$
	LO50 (96 lb) 85.3*
Moribund affected and	a week do response, no meaningful confidence limits can be derived I apathetic bumble bees were observed at all tested dose levels during the entire
test period of 96 hours.	
The NOED (No Observ	$\sqrt{20}$ Effect Dose) was determined to be < 1.23 µg imidacloprid a.s./bumble bee.
Conclusion:	
	D50 value for Imidacloprid FS 350 (350 g/L) was determined to be 85.3 $\mu$ g
imidacloprid a.s./bumb	
>>M-494307-01-3@S-602261-01-1	

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Report:	03.01.01/05; (2014; <u>M-494321-01-3</u> )
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 $\frac{1}{2}$ $\frac$
Guideline(b):	(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:	No specific guidelines are available. The test design is based on OEPP/EPPO 179(4) (2010) and OECD Guideline 214 (1998), and on the review article of on der steen (2001) US EPA OCSPP Guideline No. 850 SUPP not applicable yes ds: acloprid + Pencycuron KS 370 (20+250 g/L)
< <m-494321-01-3@s-602263-01-1< td=""><td></td></m-494321-01-3@s-602263-01-1<>	
<b>Materials and Metho</b>	ds: $Q$ $Q$ $Q$ $Q$ $Q$ $Q$ $Q$ $Q$
Test item: Name: Imid	ds: acloprid + Pencycuron KS 370 (320+250 g/L) 000008024-02
TOX No.: 09865-00	
Specification No.: 102	)00008024-02 A TO A A TO A A TO A A A A A A A A A A
Content of a.s.: 119.8 g	z/L imidacloprid (analysed) 252 s/L perforycuran (analysed) 🖉
	000008024-02 z/L imidacloprid (analysed) 252 g/L perceycuron
terrestris L.) was deter	mined in a dose-response test according to OERP/EPRO 170 (4) (2010), the OECD
Guideline No. 214 (19)	98) and the review article of VAN DEROSTEEN (2001).
In the laboratory, the b	umble bees were exposed to \$23, 3,0, 11,9, 33,33 and 000 µg imidacloprid
a.s./bumble bee by top	ical 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 a star water water and a star water
5	The set of the set of
Dates of work: 03 Dec	ember 2014 08 February 2014 O
Š.	
Findings <sup>.</sup> C	
In the control group, the	eated with tap water, no mortality was observed during the 96 h test period.
In the test item treatme	nt group, a mortality of 89.0 % was observed at the highest dose level
corresponding to 100 µ	gunidactoprid as /bumble bee at the final assessment after 96 hours.
	Sup, nortality was $\geq 50$ % of the end of the test. Thus, the test was considered to
be valid.	
	The burnble free contact toxicity test with Imidacloprid + Pencycuron FS 370
(120+250 g/L)	
Imic	lacioprid + Bencycycon 🖉 🖉 Contact toxicity test
F F	\$ 370 (120) 250 gf 3 [μg a.s./bumble bee]
	<sup>2</sup> LD <sub>50</sub> 24 b >100
	200 (48 D) 200 >100
×	$\mathcal{O}_{LD_{50}} \mathcal{O}_{2} h) \mathcal{O}_{2} \mathcal$
L' A	LDG (96 b) 28.1
	nt roup moribuid, affected and apathetic bumble bees were observed at all tested
	entire 96 hour test period.
	I corresponding to 3.70 $\mu$ g imidacloprid a.s./bumble bee was determined to be the
NUE A (INO Observed	Effect Dose) for mortality.
Constant S	
Conclusion:	

### 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.



### 03.01.02 - Field

**Report:** 

03.01.02/01; ; 2001; M-081939-01-3

Title:

Report No.: Document No .: Guideline(s): Guideline deviation(s): **GLP/GEP:** 

Evalution of the effects of a soil treatment of ornamental plants with imidacloged on nectar and pollen sampling bumblebees (Bombus terrestris) in the semified

BT001 M-081939-01-3 U.S. EPA OCSPP 850.SUPP none ves

<<M-081939-01-3@S-603226-01-1 Material and methods: Ornamental plants, *Kobelig Grinus* received soil breatmost at a rate of 15 mg a.i./l soil substrate before flowering and/or at full blossone with Ioidacloprid WG 5 (NTN 33893: article No. 0004897447, formulation No. 03584/0344(0285), a.r. content 4.95%, T@X No. 05672-00. Control plants received no treatment. 

The following 5 treatments with two splicates 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-bowering apploation, 90% treated and 50% untreated plants in the tent;

B: 15 mg a.i./l soil substrate, me flowering application plus application at full bossom, 50% treated and 50% untreated plants in the tent;

C: 15 mg a.i./l soft substrate, pre-flowering application plus application a full blossom, 10% treated and K. 90% untreated plants in the tent;

D: 15 mg a 2/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 burblebee colory (companing approx 50 bumblebees) was allocated.

The bumblebees were observed for the parameters mortality, foraging activity and colony strength and condition.

-96-19, te 2001, 97-10 Findings: Findings for the treatments are presented in table 1. Dates of biological work



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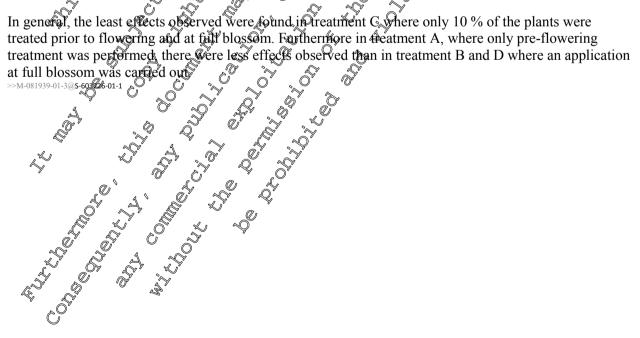
Issue date 2017-11-22

Treatment	к	Α	В	С	D
Average mortality pe treatment and day in front of the hive [n]		0.09	0.09	0.25	0.17
Average mortality pe treatment and day inside tents [n]	or 0.15	1.80	2.42	0.42	2.76
Average foraging activity per treatment and day [n]	t 137.09	51.75	17.75	66.00	ر پر ۲ 31.92
Weight decrease of the mini-hives during the study [%]	30.95	21.65	25.40	28.69	°22.90
Average number of bumblebees alive at study termination in the mini-hives [n]	50.00	22.00	0 <sup>4</sup> 20.60 č	33.56 <sup>7</sup>	2 2 14.00 2 14.00 2 1 4 2 14.00 2 14.00 2 14.00 2 14.00 2 14.00 2 14.00 2 14.00 2 14.00 2 14.00 2 14.00 2 14.00 14
Average number of bumblebees dead at study termination in the mini-hives [n]	0.00	Q50 %	7,00	0.00	Č 3.50
Food stores at study termination	yes @	¥ ¥es	ves/60	yes	
Non-capped brood a study termination	t yes	yes 🖓		ýěs	
Conclusion:					

#### **Conclusion:**

Increased mortality was observed in the bumblebee colonies of treatment A, B and D. Foraging activity was higher in the control & that 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 reatment C. Food was stored in all treatments except in treatment D and one replicate of greatment B. Capped brood was found in all treatments except B and D.

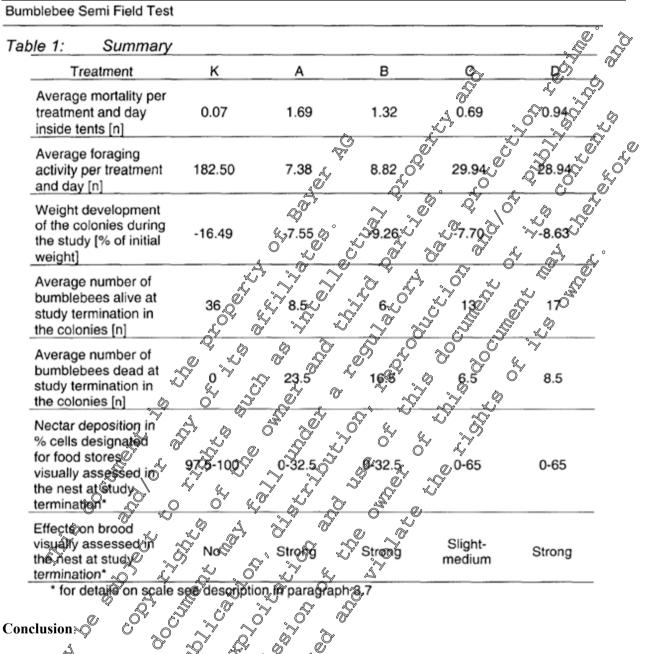
In general, the least effects observed were found in treatment C where only 10 % of the plants were





Report:	03.01.02/02; (M-060086-01-3)
Title:	Evaluation of the effects of a soil treatment of ornamental plants with Imidacloped WG
	5 on nectar and pollen sampling bumblebees (Bombus terrestris) in the semifield (test
	plants: Erica and Lobelia)
Report No.:	<u>M-060086-01-3</u>
Document No.:	<u>M-060086-01-3</u>
Guideline(s):	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s): GLP/GEP:	not specified
GLP/GEP:	5 on nectar and pollen sampling bumblebees (Bombus terrestris) in the semifield (test plants: Erica and Lobelia) <u>M-060086-01-3</u> U.S. EPA OCSPP 850.SUPP not specified yes : Ornamental plants, <i>Lobelia Frinus</i> and <i>Enica gracilis</i> , received soil treatment at a substrate at full blossom with Imidacloprid WG (NTN033895 article No.
< <m-060086-01-3@s-604656-01-1< td=""><td></td></m-060086-01-3@s-604656-01-1<>	
Material and methods	: Ornamental plants, Lobelia, Frinus and Erica gracilis, received soil treatment a
rate of 15 mg a.1./l soil	substrate at full blossom with imidacloprid WG (NTN 33895 article No.
0004897447, formulation	on No. $03584/0344(0285)$ , a.i. content 4,93%, 40X No. $05672-00$ . Controc plants
received no treatment.	on No. 03584/0344(0285), a.i. content 493%, 40X No. 05672-00. Control plants
5 treatments with two r	eplicates 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 pla	ants for the treatments A and B and a propertion of 10% of the ground overed
with treated and 90% of	f the ground overed with intreated plantofor the treatments C and Do When
taking into account the	different sizes of the two plant species used, the numbers of plants for the
treatments and the cont	rol were as follows: of a b b a b
K: control: no treatmen	t, 130 untreated Lobelia erunus and 90 untreated Erica gracilis in the tent
	strate, application at full blossom, 139 treated Lobelia erings and 90 untreated
A: 15 mg a.i./l soil subs	strate, application at full bloscom, 130 treated Lobelia erupas and 90 untreated
Erica gracilis in the ter	f a si a si a si a si a si a si a si a s
	trate, application at full blossom, 90 treated Erica gracilis and 130 untreated
B: 15 mg a.1./I soufsubs	trate, application at full blosson, 90 greated Erica gracilis and 130 untreated
Lobelia erinus in the te	
	estrate, application at full blosson, 22 treated Lobelia erinus and 160 untreated
C: 15 mg a 11 soil sub	strate, application at full blosson, 22 treated Lobelia erinus and 160 untreated
Erica gracius in the ten	
D. 15	ostrate, application at full Plosson, 22 treated Erica gracilis and 198 untreated
D: 15 mg a.1.11 sourgut	strate, application at the plossor, 22 meated Effica gracills and 198 untreated
Lobelia erinus in a tent	
The sultants and Quite and	inside tents (floor space 4 Sm x 4.5m) on the experimental farmland "Laacher
I ne plants were placed	Inside tents (1006 space 4 Sm x 4.5m) on the experimental farmland "Laacher
Hof . In each tent one t	burgolebee (Bombus terrestris) colony (containing approx. 50 burblebees) was
allocated	punplebee (Bomous terrestris) folony (containing approx. 50 bumblebees) was
	bset ved for the parameters mortality, foraging activity and colony strength.
Condition of the coloni	exbrood, food storage were assessed according to an internal assessment
scheme.	
scheme.	
Dates of biographics	$-\nu = 0.01.00.10 + 0.001.00.27$
	rk 2001-09-10-09-27
Finding	
Findings for the treatme	are presented in table 1.
i maines for the treath	sass are presented in more 1.
Ô	
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The effects of the treatment to pumble be 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 antifectant response caused by the treatments. This antifeedant response may act protectively to pollenating 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 life adult bumblebees at study termination.

Ľ, 6-00 S-604656-01-1



Report:	03.01.02/03; (2003; M-109444-01-3)
Title:	Assessment of the effects of a soil treatment of ornamentals with imidacloprid we 5 on
	nectar and pollen collecting bumblebees (Bombus terrestris) in the field (test provident:
	Lobelia erinus)
Report No.:	<u>M-109444-01-3</u>
Document No.:	<u>M-109444-01-3</u>
Guideline(s):	U.S. EPA OCSPP 850.SUPP
Guideline deviation(s):	not specified
GLP/GEP:	Lobelia erinus) M-109444-01-3 U.S. EPA OCSPP 850.SUPP not specified yes S: Ornamental plants of the species <i>Lobelia Finus</i> received a soil treatment at a il substrate at full blossom with Imidacloprid WG 3 (NTO 33889). article No 50-
< <m-109444-01-3@s-604664-01-1< td=""><td></td></m-109444-01-3@s-604664-01-1<>	
Material and methods	s: Ornamental plants of the species Lobelia Sinus received a soil treatment at a
rate of 0.015 g a i /I soi	il substrate at full blossom with Imidacloprid WG (NTO 3388)? article No 60-
05439280, formulation	No. 03584/0460(0460) and content 4 68% TAX No. 06066-00) Control mants
received no treatment.	No. 03584/0460(0460), a.P. content 4.68%, TOX No. 06066-00). Control plants
The study was carried o	out in 34 gardens in the surfoundings of Cologne/Düsseldorf and the Berginehes
I and" in Nordrhein-We	estfalen, Germany, In each garden, 50 <i>Dobelig erinus</i> and a bumblebee ( <i>Bombus</i>
terrestris) colony (cont	aining approx. 80-10(kbumblebees) were placed. In 17 gadens, the plants were
tracted and in the other	17, the plant were printeger. Endpoints of the study were moreality, dight
a stivity and fanasing a	17, the plants were understed. Enclosing of the space were independently, angle
activity and foraging ac	ctivity on Lobe/ia and on alternative plants growing in the gardens. As far as
	nts were conducted every day. Six weeks after setting up the colonies in the
gardens, a final monito	ring of the bumblebee nests was conducted to examine the brood cells and the
condition of the nests.	
Dates of biological wo	ring of the bumblebee nests was conducted to examine the brood cells and the prk? 2002 05-23 to 2002 07-26
ž L	
Findings:	
"Š	
Findings for the freatman	ent groups are presented in Table 1.
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### Imidacloprid Bee Studies Compilation of Study Summaries

Issue date 2017-11-22

Bumblebee Field Test	Imidacloprid WG 5		
Table 1: Summary results	of the bumblebee monitoring		
Treatment group		Control	Treament
Total mortality of all bumblebee	e species per treatment group [n]	<b>B</b> <sup>1</sup>	49*
Average mortality of all bumble	bee species per garden [n]	<b>0.29</b>	0 <sup>°</sup> 2.86 <sup>°</sup> 2 <sup>°</sup>
Average mortality of all bumble	bee species per garden and day [n]	a 0.01	33° x x
Total mortality of B. terrestris p	er treatment group [n]	5 5 2	→ 33* ↔ → 33* ↔ 1.64* ↔
Average mortality of individuals	of B. terrestris per garden [n]	° 0.29€ ∠	1.94
Average foraging activity on Lo	belia per garden and assessment [n]	© 0.39	Q.17
Average foraging activity on alto gardens per garden and assess	of <i>B. terrestris</i> per garden [n]	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Average flight activity per garde	en and assessment [n]	A 4382 V	A.25
Nest structure** [n] - +- + ++ ++	en and assessment [n]		₹4.25
Average number of bumblebee assessment [n]	s found alive in the nest at the final	× 16935	16.24
Average number of bumblebee assessment [n]	s found dead in the next at the final	2.1205	2.18
Average increase weight duri	ng the study [%] S	0 12.65	14.94
Average nest size at the final as		~67.29	73.65

\* Statistically significarly different from control (Mann-Whitney U Dest, one-sided, p<0.01) \*\* Nest structure as a figure of quality of the brood cells: it was voually assessed and classified from "-" i.e. the nest was in a poor condition, to "+++" which means that the calls were very well developped

**Observations:** 9 nests of the control and 10 nest of the treatment were parasitized by the bee moth *Aphomia sociella* (Cepidoptera: Poralidae) during the study. The larvae of A. *sociella* live on the wax cells and on the bamblebee'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 toraging 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 Imidacloprid WG 5 at a rate of 15 mg a.i./I soil poses only a nearigible risk to foraging bumblebees. Mortality was slightly increased in the treatment, but it remained on a rather the 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** 

D (		02.01.02/04 00.14 14 50/174.01 2
<b>Report:</b> Title:		03.01.02/04;; 2014; M-504174-01-3 A field study to subjust a effects of Managerer C on the humble has (Dombus torrestric L)
I itle:		A field study to evaluate effects of Monceren G on the bumble bee (Bombus terrestris L;
Report No.:		sin opticia, Apidae) in polato in southern Germany in 2014
Document No.:		M-504174-01-3
Guideline(s):		No specific quidelines are available. The test design is has $0$
Guideline(3).		SETAC/ESCORT recommendations (BARRETT et al. 1994)
		OEPP/EPPO Guideline No. 170 (4), 2010
		US EPA OCSPP Guideline No. 850,3040
Guideline devia	tion(s):	none
GLP/GEP:		yes V Q V J V V
< <m-504174-01-3@s-602< td=""><td>2337-01-1</td><td></td></m-504174-01-3@s-602<>	2337-01-1	
1.1 Mater	ial and M	lethods
Test item:	Monce	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 S14-03554 <u>M-504174-01-3</u> No specific guidelines are available. The test design is basedoon: SETAC/ESCORT recommendations (BARRETT et al. 1994) OEPP/EPPO Guideline No. 170 (4), 2010 US EPA OCSPP Guideline No. 850 3040 none yes lethods ren G; TOX number: TQX10501-00; Batch; 2014-001766-01; content of a.i. dacloprid + 250 g/L pencycuron is terrestris L. (Hymenoptera, Apidae) Id study was carried out on agricultural fields in southers Germany (Heilbronn) ng the SET AC/ESCORT recommendations and the OEPP/EPPO Guideline No. , 2010. The field grop was potato; Solarum upperosum L.
(nominal): 120	) g/L imic	dacloprid + 250 g/L pencycuron & a a a
	0	
Test species:	Bombu	is terrestris L. (Hymenoptera, Apidae) 🖉 🦨 🖉 🗸
1		
Test design:	The fie	ld study was carried but on agricultural fields in Southern Germany (Heilbronn)
8	followi	ng the SETAC/ESCORT recommendations anothe OBPP/EPPO Guideline No.
	170 (4)	, 2010. The field grop was potato; Solanum tuberosum L.
	The stu	dy included 2 treatment groups (C $\neq$ control / T = test-item) with six replicates (6
	replicat	te bumble bee colonies) pet treatment group for biologigal assessments.
	Bumble	e bees wer cassessed for freir flight activity within the crop, flight activity at the
	entranc	es of the hives. The weight of the hives and the sugar consumption were assessed.
	Moreo	er, the mortality of adult bees and larvae was observed at every assessment date
	during	the field phase and at the pronitoring site. Additionally, three samplings of pollen
	forvesi	due 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
ŝ	residue	sampling. Before set-up and aftenthe field phase, brood assessments were done to
	doctime	ent all stages of development and the vitality of the colonies.
Endpoints	Flight a	wivity in the crop, flight activity at the entrance of the hives, mortality of adults
, ja	and lar	vae, weight of hive and sugar consumption, initial and final brood assessment
* *	includi	ng the production of young queens and drones.
Application:	The app	plication was done at a separatostudy S14-01392. The insecticide Monceren G bled as n-furrow application at planting at a rate corresponding to nominally 1.5
	was ap	offed as m-furrow application at planting at a rate corresponding to nominally 1.5
	©L pro€	uct/hat equivalent to 180 g midac loprid/ha and 375 g pencycuron/ha) under field
	conditio	ons en potaro (Setanum taberosom L.).
Test condition	s: Exposy	ure of the bumple bee colonies started at the beginning of potato flowering. After
	end of	flowering, the colonies were transferred to a monitoring site were the assessments
×.,		blowed until the colonies reached their peak of colony development and switched
N.	over to	the reproduction has ve. young queen and male (drone) production.
	a	
Dates of work	🐓 01 Jai	201496 09 Oct 2014
la la la la la la la la la la la la la l		
1.2 Findin	ıgs∜	
The montality	of adult b	2014 to 09 Oct 2014
the hives, flight	it activity	in the crop, the sugar consumption and the weight of the hives were assessed.
		and differences were observed between treatment groups for the total mean
		ble bees and larvae (Table 1).
õ		



Table 1: Mean numbers of dead bumble bees (adults and larvae)							
Mean number of dead bumb	e bees (adult	and larvae)	oer day a	nd per treatn	nent	A CO	
			Treatmen	nt group		5	
Date	DAE	Cont	rol	Treatm	ent 🕎		
		Mean	STR	Mean	SPD	L.	
02 Jul 2014	0	.0	00	0.5	<del>~0.8</del> @		
03 Jul 2014	1	ر 0.3	<u>, 07.8</u>	<b>Q</b> 2	0.4	<u>k</u> O <sup>Y</sup>	
04 Jul 2014	2	0.8	Q 1.6 °	مري <mark>0.0 م</mark> ر	90.8 0.4 9.0	,O <sup>v</sup>	
07 Jul 2014	5 🕎	1.8 💭	~ <u>2</u> 3	2.5 ×	2.8	/	
10 Jul 2014	8 🖑	1.8 ×	2.3	Q2.5 ×	2.9		
13 Jul 2014	<u> 11</u> ő	<b>63</b> Q	2.3 2.2 2.2	1.30	A.2	° 1	
16 Jul 2014	×14 ~	~4.7 ~	x3.6 x	p 🕺 🕺 🕺	3.4		
18 Jul 2014	Q 185	U 1.5 1	( <sup>0</sup> 1.4 <sup>2</sup> )	Q 2.2 S	20		
21 Jul 2014	ç, <b>6</b> 3 , ≫	<b>'3</b> .3 🕎	<b>B</b>	2 3 A	\$ 1.1		
24 Jul 2014	222	6.30	x 8.5 ~	2 3 5 ×	3.7		
28 Jul 2014 🏾 🍼	`∼> 26,		5.65	<b>7.80</b>	8.9		
31 Jul 2014 👸 🕺	<sup>*</sup> <sup>2</sup> 26, <sup>7</sup> 529 0 <sup>7</sup> <sup>9</sup> 33, <sup>4</sup>	34.3	~ <del>@3</del> .8 `	y 1405	8.7		
04 Aug 2014 🦂		0°78° u	5.0	<b>\$</b> 5.7	3.4		
07 Aug 2014		<b>12.3</b> O	18/2	<sup>*</sup> 16.5	8.9		
11 Aug 2014 🖇 , 🔿	¥0	~ <sup>3</sup> 4.5 <sup>©</sup>	2.1	6.3	3.8		
14 Aûg 2014 🔨	& 43V A	19°N a	2.1 0 2.1 0 2.1 0	11.8	6.7		
18 Aug 29 14 🖉	50 °	20.0 5	<u>_</u> @-	11.0	7.1		
Aug 2014	50 0	<sup>07</sup> 17.0	<u>o</u> -	9.0	-		
Mean exposure poase			7	1.5			
Total sum of means exposur	e phase x	×11.8		10.2			
Mean post-exposure phase		12,9		8.3			
Total sum of means post-exe	sosure phase	133.2		91.6			
Total mean over all phases		0 8.1		5.7			
Total sum of means over all	phases	145.1		101.8			

. ..

DAE = days after exposure (goey indicates dates on monitoring site) STIX = standard deviation

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STID standard deviation and all available replicates, mean values calculated with unrounded values - = data not available as hives whe already deep frozen a) mean values of 3 hives, <sup>b)</sup> mean values of 4 hives, <sup>c)</sup> mean values of 4 hives, <sup>d)</sup> value for 1 hive

A slight ficrease 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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Mean numbers of foraging bumble bees in the crop (4 m <sup>2</sup> areas)								
			Treatme	ent group				
Date	DAE		С	A	T			
		Mean	STD	Mean				
02 Jul 2014	0	0.7	0.6	2.7				
03 Jul 2014	1	1.0	\$10	ر <sup>0°</sup> 2.3	<b>Q</b> 6 5 <sup>3</sup> 40			
04 Jul 2014	2	0.3	-0.6	<sup>Q</sup> b <sub>3</sub> 3 Å				
07 Jul 2014	5	0.7	Q 1.2	∕ <sub>∿</sub> ¶.0 _				
10 Jul 2014	8	2.0	\$ <u>\$</u> ?0 ~	لي <sup>2</sup> 1.7℃	A 7.2			
13 Jul 2014	11	1.3	× 0.6 Č	<u> 21</u>	0 5 1.2 x x			
16 Jul 2014	14	0.0	20 AV 20	<b>2.7</b> * Ô <sup>%</sup>	× 0.8			
Mean flight act	ivity	<b>∧</b> ``		0 <sup>°</sup> 2.0 <sup>°</sup>				
DAE = days after ex STD = standard devi	otion		aced with Onrouneed					
Mean = mean values	s of all replica	ates, means calcul	acod with Onrounded	values . O	<u> </u>			

\* = statistically significant differenc@to control (t-tes $\theta(p \le 0.05)$ )

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 meatment groups.

# Table 3: Mean numbers of bumble bees entering the colonies

	<u> </u>			~ 0*				
🖉 Mean	Mean numbers of burnble bees entering the colonies / 15 minutes							
× ÿ			🔊 (Treatm	ent group				
Date	DAE	Mean y		-	Г			
		🕺 Mean 📏	SSTE	Mean	STD			
02 Jul 2094	0,0		6 87	0.9	1.2			
03 Jul 2014	1	S 23 .	√0.6	1.8	1.5			
04 10 2014	×2 ^		1.8	4.3	1.9			
07 Jul 2014	8	∖_‴ <b>3.8</b> ° ∝	3.1	5.4	1.7			
10 Jul 2014	80	° 2,8 ,	1.9	8.5*	2.7			
13 Jul 2014	A 11 &	3.7 4	1.1	3.6	1.2			
16 Jul 2014	A 11 0 V 148	10.8	3.5	8.8	2.8			
Mean föght a	tivity	<sup>©</sup> 3.8		4.8				

DAE = days after exposure

STD stand deviation

Magn = magn values of all epicates, calculated with unrounded values

\* = statistically significant difference to control (t-test (p ≤ 0.05))



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.

#### Mean consumption of sugar solution Table 4:

<b></b>						
Mea	an consumpt	ion of the sug	ar solution	<b>1</b>		2
		<u> </u>	Treatme	groups 💍	j 🤊	S a.
Date	DAE	Cont	N	groups & Treatn	nent S	
		Mean SA.7	S78	ം° Medan	nent S	
03 Jul 2014	1	\$1.7	7.5	20.0	J. 3 ~~	r"
04 Jul 2014	2		7.5	23,3	<sup>*</sup> 16 <sub>3</sub> 3	
07 Jul 2014	5		9.8 7.5 29,8 29,8	°° <b>96.7</b> °	10.6	ç°
10 Jul 2014		106.7 \$ 51.7	A42.2	×~86.7×~	STD 3.3 16,3 16,3 10.6 3 41.3 5 83.4 5 83.4 5 83.4 5 83.4 5 83.4	
13 Jul 2014	112	× 154,7 ~	× 48%	ن 95	83.4	
16 Jul 2014	Á4 (	181.7	<b>3</b> 4.2 0	<u>(</u> 71.7 5	<b>₹</b> ¥30.3	
18 Jul 2014	0 16 2	<b>☆173 \$</b>	0 <sup>7</sup> 43,5 <sup>7</sup>	°∂175,0 (	48.1	
21 Jul 2014	0 16 2 0 19 2 0 22 5	¥ 141.7	49.8 ·	2 141.7 <sup>C</sup>	51.5	
24 Jul 2014 🧳	022	W65 0	<b>48.9</b>	A40.0	67.5	
28 Jul 2014	26	2967 (	52.0	2783	77.8	
		193.3 📈	126.4 0	<b>\$</b> 3.3	126.8	
04 Aug 20 4		193.3 V ~440.0	\$ 226 \$	<b>468.3</b>	83.0	
07 Aug 014	36	430.0ª	<u>8</u> 4.1	✓ 425.0	97.1	
	640 1	<b>\$96.4</b> *	967.0 C	571.7	99.9	
14 Aug 2014		670-0° ~		412.5 <sup>b)</sup>	95.4	
18 Aug 2014	2 AT 5	54∕0.0°	67.02 0 0 0 0 0 0 0 0 0 0 0 0 0	260.0 <sup>°)</sup>	169.7	
21 Aug 2614	40 43 43 47 50 50	670.0 <sup>4</sup> 540.0 <sup>1</sup>		359.0₫		
Consumption during ex	pusule	> 545.0 🔗	>	493.3		
Consumption during pe	st.exposure	\$876.4		3384.8		
Total consumption		~ 4424 4		3878.2		ļ

DAE = dos after exposing (grey indicates dates of monitoring site)

STD = standard deviation Ø Ø

Mean = mean values of all eplicates, calculated with unrounded values

- = data not available as hives were alread deep for an

a) mean values at 3 hives

<sup>b)</sup> mean values of 4 hives Ô

 <sup>a)</sup> mean values of 4 threes
 <sup>a)</sup> value for hive
 <sup>a)</sup> total sum of mean consumption values

c) mean values of 4 hives

Table 5: Mean we	ights of bu	mble bee hive	es		٩)°	
	Mean wei	ghts of bumble	bee hives	(g)		Ê.
				ent groups		
Date	DAE	Contro		Treatn		Řo
		Mean	STD	Mean	N OTR K	
02 Jul 2014	0	554.8 🗇	16.3	<b>552.0</b>	91.8 J	
03 Jul 2014	1	559.3	15.3	549.5	Q 11 6 16.0 4 201 0 201 0	
04 Jul 2014	2	560 7	17.4	\$ 56102	16.0	)
07 Jul 2014	5	<b>590.8</b>	<b></b>	5667.8	· 24.0 9	
10 Jul 2014	8	0601,2 <sup>0</sup>	39.6	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	293.3	, ,
13 Jul 2014	11	🔿 634.3 🕎	41.4 ~58.6 0 7.2.0	6 <b>≰§</b> .0	\$0.8	
16 Jul 2014	14 🖉	2°650.8	<u>,</u> 58.6 Ô	<b>3</b> 35.3	× 895	
18 Jul 2014	16.0 <sup>9</sup>	€50.8 Ø √687.3 €	73.0	<b>చ్ 664</b> 00 ్ల	1033.8	
21 Jul 2014	18	743.3	<b>8</b> .0	<b>695.5</b>	~116.5	
24 Jul 2014	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	765.0	© 55.90	713.8	V 123.9	
28 Jul 2014	<sup>2</sup> 26	O 859.0 V	40./8	y 779.2	135.4	
31 Jul 2014 🛛 😽	26 29 0	\$97.5 -{->	~ <del>8</del> 9.1 <sup>©</sup>	819.2	135.5	
04 Aug 2014 😓	29 33 33 29 29 29 20 20 20 20 20 20 20 20 20 20	9055 4	93.3	<u>لار 913,5</u>	162.4	
07 Aug 201	. 36 ~	✓ 1051.6³ 次	53.4	955.8	172.9	
11 Aug 2014 🔨	م <sup>4</sup> 0 م	<b>√1139.5</b> ¥	288.20V	<b>2</b> 91064.3	209.9	
14 Au@2014	40 0 430 67 5	≤ 125 .5 °	L SAN	⊘ 1097.5 ి	235.6	
18 Agug 2014 🔬	97 5 37 5 37 50 5	1254.5 °C	0 - 0	934.5°) 1)	344.4	
2 Aug 2014	397 A 65 50 C	1227 5 1 2	×0×	773.0 <sup>d) f)</sup>	-	
		SSN2.1 √	0 0 - 0 5 -0 5 5 5 5 5 5 5 5 5 5 5 5 5	585.1		
Mean weight exposure Weight increase expo Mean weig@t postexp	sure v	<u>,</u> ≪96.0	Q°	83.3		
		0 9938	¢	855.1		
Weight increase post	exposure ?	35.3		455.8		
Total mean weight		837.8		750.1 <sup>1</sup>		
Total weight increase		567.8		567.8		

DAEx days after exposure prey indicates dates on monitoring site); STD = standard deviation

Mean = mean values of all replicates, calorated with unrounded values

- = data not available as thives vere already deep frozen <sup>a)</sup> mean values of 3 nives, <sup>b)</sup> bean values of thives, <sup>c)</sup> mean values of 4 hives, <sup>d)</sup> value for 1 hive, <sup>e)</sup> calculated as mean values of single periods values of lower values due to the fact that remaining hives during these assessments were lower in weigh compared to the ones that were already deep-frozen

The results of the foral brood evaluation showed a statistically significant difference in one out of all parameter 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.



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 pumble bees
Sampling date	
5 DAE	47.4
12 DAE	
16 DAE	$40.0  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  $

Residue analysis was carried out on pollen samples collected from forager bumple bees at 5,92 and 16 days after exposure (DAE). No resolues of imidacloprid and its metabolites (midacloprid )-hydroxy and imidacloprid olefine) were detected in pollen from the control field Residue level of imidacloprid in samples from the treated field were below the limit of quantification at sampling date DDAE and below the limit of detection at 16 DAE. The maximum residue bevel of 0.71 ug/kg was found at the sampling date 12 DAE (Table 7). Acall sampling dates, the residue levels of indactoprid-Shydroxy and imidacloprid olefin were below LOD

#### dues of indactoprid and is metabolites in potato pollen Table 7:

Treatment			Residues [µg/kg]	
group	Sampling date	almidaclopdid	Imidaclop#id-	lmidacloprid olefine
<u> </u>			N SPOD	< LOD
C		S XLOD	LOD	< LOD
ģ		or log of	S < LOD	< LOD
~~~	C5 DAE	^> <∿ດດ i∾	< LOD	< LOD
T	12 DAE	\$0.71,\$ <sup>2</sup>	< LOD	< LOD
<u> </u>	16 DAE		< LOD	< LOD

DAE - days after exposure

LOQ = limit of quantification = 0.6 m/kg for imidaç oprid, 1.0 µg/kg for imidacloprid metabolites = 0.2 µg/kg for imidaclogid, 0.3 µg/kg for imidacloprid metabolites

-U.2 UD Kg for imi -U.2 UD Kg for imi -U.2 UD Kg for imidack >>M-504174-



Report:	03.01.02/05; 2014; <u>M-503597-01-3</u>
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 S14-03553 M-503597-01-3 No specific guidelines are available. The test design is based on: SETAC/ESCORT recommendations (BARRETT et al 4994) OEPP/EPPO Guideline No. 170 (4), 2010 US EPA OCSPP Guideline No. 850-3040 none yes lethods ren G; TOX number: TOX 10501-00; Batch, 2014-001766 91; content of a.i. dacloprid + 250 g/L pencycuron is terrestris L. (Hymenoptera, Apidae) eld study was carried out on agricultural fields in southern formany (Kathsruhe) ESCORT recommendations and the OEPP/EPPO Guideline No. 170 (4). The field um tuberosum L.
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 A994)
	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 M	lethods
Test item: Monce	ren G; TOX number: TQX 10501-00; Batch 2014-001766 91; content of a.i.
(nominal): 120 g/L imi	dacloprid + 250 g/L percycurph & & & & & & & & & & & & & & & & & & &
Test species: Bombu	is terrestris L. (Hyménoptera, Apidae)
1	
Fest design: The fie	Iethods ren G; TOX number: TOX 10501-00, Batch 2014-001766-01; content of a.i. dacloprid + 250 g/L pericycuron is terrestris L. (Hymenoptera, Apicae)
following the SETAC/I	ESCORT recommendations and the OEPP/EPPO Guides in Southern Sermany (Kabstulle) aum tuberosum L. eatment groups $C = \text{control} \partial I = \text{test-item}$ with six replicates (b replicate bumble nent group for biological assessments.
crop was potato: Solani	um tuberosum L.
r r r r r r r r r r r r r r r r r r r	
The study included 2 tr	eatment groups $C = control \partial T = test-item with six renlicates (by renlicate humble)$
pee colonies) per treatn	nent group for biological assessments
bee colonies) per treatil	
pives. The weight of the	ssed for their flight activity within the crop, flight activity at the entrances of the whive and the sugar consumption were assessed. Moreover, the mortality of adult
nives. The weight of the	served at every assessment date during the field phase and at the monitoring site.
Additionally throad	served at every assessment date quining the neldphase and at the monitoring site.
Additionally, the same	plings of pollen for residue analysis and palenological analysis at different dates
were carried out by tak	ing the pollen loads from forager bumble bees of additional colonies only used for
residue samprag. Bere	re setop and after the field phase brood assessments were done to document all
stages of development a	and the vitality of the cohonies of $\sqrt{2}$
Endpoints. Flight	activito in the crop, flight activity at the entrance of the hives, mortality of adults
and larvae, weight of a	ive and sugar consomption, initial and final brood assessment including the leens and arones.
production of young qu	eens and drones. <sup>y</sup>
Application:	The application was done at a separate study (S14-01385). The insecticide
	ed as in-furrow application at plating at a rate corresponding to nominally 1.5 L
	to 180 gomidaeloprid/ba and 375 g pencycuron/ha) under field conditions on
ootato (Solanum tubero	Bum L.Y.
	in of the pumble bee of onies started at the beginning of potato flowering. After
end of flowering, the co	plonies were transferred to a monitoring site were the assessments were followed
until the colordes reach	ed their peak of colory development and switched over to the reproduction phase
i.e. young queen and m	alectorie) production.
Ĩ, S	
Dates of work: 11 Jun	2014 08 Oct 2014
.2 Findings	$\overrightarrow{G}$ was evaluated by assessing the mortality of adult bumble bees and bumble bee
ringanings -	
The effect of Monceren	flight activity at the entrance of the hives, foraging activity in the crop, the sugar
The effect of Monceren arvae within the hives,	flight activity at the entrance of the hives, foraging activity in the crop, the sugar eight of the hives.
The effect of Monceren	



ð

Issue date 2017-11-22

No statistically significant differences were observed between treatment groups for mortality of adult bumble bees and larvae within the hives (Table 1). Ø

							A O
Table 1: Mean number of	dead bumble	e bees (a	adul	ts and	larvae)		<i>®</i>
					<u>Ĝ</u>	<del>\</del>	) 
Mean numbe	er of dead bum	ble bees (	adu	It and la	rvae)		Ô
		<u> </u>		reatmen	it groups		
Date	DAE	Č,	C		<u> </u>		Å
		Mean		<u>®ŤD</u>	Mean 👌	STO	K <sup>O</sup>
12 Jun 2014	0	4.7	- <sup>2</sup> C	∲ <u>3.8</u> °	<u>مَرْ 2.3 المَرْ الْمَرْ /u>	9.4	Ø
13 Jun 2014	1 🕅	1.0	2	×4,5	× 0,2 ×	9.4	
14 Jun 2014	2	1.0 0.2	,	ç <u>0.4</u>		<sub>4</sub> 0.8	
17 Jun 2014	54 0	2 2 2	Ŕ	1.0	1.30	0°1.5 ×	0
20 Jun 2014		∕́	>	x7.5 ·~	2 2 ,	0,8	
23 Jun 2014	Q 11 4	× 1,2	×	0.8	3.8	9.1	
26 Jun 2014	5 19 ×	5.0	$\gg$	ð¥	<sup>2</sup> 6.3	\$ 4.6	
30 Jun 2014 🧷	× _ 918 @	21.5 <sup>0</sup>	)	~11.9 <del>~</del>	<sup>^</sup>	4.1	
03 Jul 2014 🏾 🍼	· * 01	21%	Ó	1205	<b>22.3</b> 0	7.1	
07 Jul 2014		¶4.3	×¥	~ <del>3</del> .8	y 1∲23	5.2	
10 Jul 2014 📎 🙏	28 28	∅ 25 🔊	, ,	16.3	24.2	11.2	
14 Jul 2014	* * &		Ő	13:0	18.7	12.5	
17 Jul 🎘 14 🔬 ,	) 2 <sup>85</sup>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		L 5.6 O	7.3 <sup>b)</sup>	4.8	
21 Jui 2014	& <b>39</b>			<u>, 5.0</u> -2	9.0 <sup>c)</sup>	3.7	
Mean exposure phase	re phase O	2.1 (	Š.	<sup>©</sup>	2.5		
Total sum of means exposure	re poase 📎	14.6		, Q	17.2		
Mean post-exposure phase			°~0″		15.7		
Total sum of means post-eq	posure phase	106.2	1		110.2		
Total mean over all phases		⊳ <b>9</b> ,3ĭ			9.1		
Total sum of means over all	phases N	120 7			127.3		
DAE - dave after exposure (even indice	tos dattes on manit	oringeito					

DAE = days after exposure (be) indicates dags on regnitoring site)

DAE = days after exposure (mey introduces upges of atomics) and, STD = startbard deviation Mean = prean values of all replicates, mean values calculated with unrounded values - = data not available actives were all eady deep-frozen a) mean values of 4 hives

- mean values of 4 hives
- c) value for 1 hive <sup>()</sup>

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 of DAE and 14 DAE) the flight activity in the crop was statistically significant lower coorpared to the control (Table 2).



#### Mean numbers of foraging bumble bees in the crop (4 m<sup>2</sup> areas) Table 2: Mean numbers of foraging bumble bees in the crop (4 m<sup>2</sup> areas / 10 min) Treatment group Date DAE С т STD Mean Mean **0.6** 12 Jun 2014 3.3 0.3 0 0.7\* 13 Jun 2014 1 4.0 1.7 14 Jun 2014 2 1.7 0.6 07 17 Jun 2014 5 5.0 0 1. 20 Jun 2014 8 3.7 6 23 Jun 2014 4.0 2. 11 26 Jun 2014 14 5 Mean flight activity DAE = days after exposure Mean = mean values of all replicates mean values of control $(p \le 0.05)$ \* = statistically significant difference to control $(p \le 0.05)$ ¢,

Flight activity at the entrance of the hives was statistically ognificant lower compared to the control at

Flight activity at the entrance of the hives was statistically significant lower compared to the control at two assessment dates (2, 14 bAE) (Lable 3). For the other assessment days no significant differences were observed. The Gverall mean dight aenvity was slightly lower for the test item but no statistically significant difference was found:

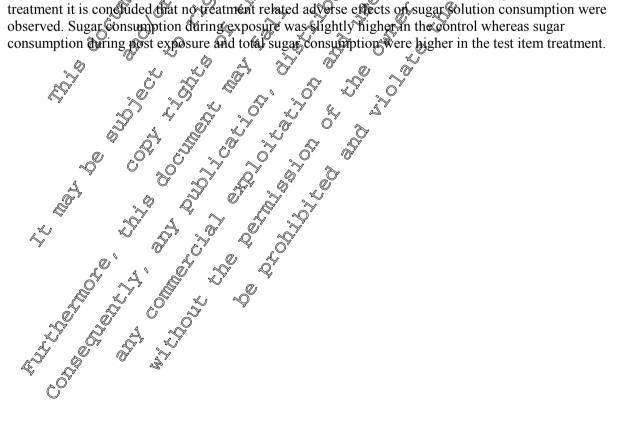


			e entening a	
Mean numbe	rs of bum	ble bees ente	ring the color	nies / 15 minutes
			Treatme	nt group
Date	DAE	(	C	T S
		Mean	STD	Mean STO U
12 Jun 2014	0	4.2	Q <sup>2</sup> 2.7	0 3.5 0 00 0 3.3 0 0 1.7 0 40
13 Jun 2014	1	4.8	2.1	<b>3.3</b> 2 Q 1.7 9 4
14 Jun 2014	2	5.6	1.9 🖓	© 2.5 € 4 2.0 K
17 Jun 2014	5	10.6	· 307 >	1.1.4 , 5.3 ,
20 Jun 2014	8	10.8	2.2 5	9.6 3.2
23 Jun 2014	11	A15.10	<u>€ 6.4</u>	11,9
26 Jun 2014	14	22,8	× \$4 64	<b>9</b> /8* <sup>a)</sup>
Mean flight activity		∢10.5 ↔	19 W	
DAE = days after exposure STD = standard deviation	Â,	<u>~~~~</u> & & (		

#### Table 3: Mean numbers of bumble bees entering the colonies

Mean = mean values of all replicate, mean values ealculated with enrounced values \* = statistically significant difference to control a) = t-test ( $p \le 0.05$ )

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 of sugar colution consumption were observed. Sugar consumption during exposure was slightly higher in the control whereas sugar





### **Imidacloprid Bee Studies Compilation of Study Summaries**

Issue date 2017-11-22

	Mean con	sumption of su	ugar solution	n (g)				
			Treatment group					
Date	DAE	С		ŤΤ				
		Mean	STD	<b>Mean</b>	STOF J			
13 Jun 2014	1 DAE	23.3	Q 12.1	<b>23.3</b>	52 5			
14 Jun 2014	2 DAE	28.3 🦼	, 14.7 <u>(</u>	∽ 35.0 <sub>×</sub>	240 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			
17 Jun 2014	5 DAE	115.0 🔬	<b>4</b> 0.9 Q	. ₀103,2 <sup>O</sup>	243			
20 Jun 2014	8 DAE	193.2	38.8	<u>163.5</u>	<b>\$0.7</b>			
23 Jun 2014	11 DAE	19(1.7 0		438.3	°∕√36.6″√			
26 Jun 2014	14 DAE	288.3	0153.50°	ð 158.3 <i>/</i>	4 4 × × ×			
30 Jun 2014	18 DAE	288.3 ×		4	6.2			
03 Jul 2014	21 DAE	200.0	» <b>2</b> 3.0°	208:3** 326.7 2 3233	× 1810			
07 Jul 2014	25 DAE 🦉	476.7	£ 241 P	2 323 3	148.0			
10 Jul 2014	28 DAE	6 <b>1200 </b>	580	092.7	°∕772.1			
14 Jul 2014		331.7	L 99.1 P	240.0	87.9			
17 Jul 2014				290 <sup>9</sup> 0 <sup>10</sup> 6	141.1			
21 Jul 2014	32 DAE % 35 DAE % 39 DAE			370.0				
Total consumption	on exposure "	<b>8</b> 40.0		در <mark>62</mark> 4				
Total consumption	pri post-	840.0 1607.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 2447.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5 247.5						
Total consumption	on 4 &	2447.5		< <b>√2672.7</b>				

#### Table 4: Mean consumption of sugar solution

DAE = days aft@exposure (gre)Ondicates dates on monitoring see Õ Ì L

STD = standard deviation

Mean = mean values of all replicates Ø O

Mean = mean values of all replicates \* = statistically significant difference to control, total values calculated with unrounded values = = data hot available as fives were already decorrozen mean values of 4 hives mean values of 3 hives value for 1 hive total sum of mean consumption values \* = t-test (p < 0.05) \* = t-test (p < 0.05) \* = Mann Whitney Exact (p < 0.05) \* = Mann Weight development of the hives showed no statistically significant treatment related adverse effects (Table 5). Mean weights adviring exposure phase, total mean weights and total weight increase of the

The weight development of the hives showed no statistically significant treatment related adverse eff (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.



	Mean weigh	ts of bumble be	ee hives (g)				
			Treatmen	t group			
Date	DAE	С	C				
		Mean	STD 🖑	Mean 🍾	SPD X		
12 Jun 2014	0	637.2	15.60	647.70	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
13 Jun 2014	1	635,0	2390	648.8	38,2		
14 Jun 2014	2	568.7	₩1.4 °	647.5 L	39.5 🖉		
17 Jun 2014	5	@604.0	~ 42.2	669.3	48.6		
20 Jun 2014	8	68 f.2 🗸	48.0	669.3 ( 739.0	7,7.0		
23 Jun 2014	11 🦽	<b>46.8</b>	Q80.4	790.30	2003.5 ×		
26 Jun 2014	14	760.7	Ø 97¢8 ∘	° 829.5	120		
30 Jun 2014		¥ <b>8</b> \$48.5 ×	<b>404.7</b>	<b>900.8</b>	140.2		
03 Jul 2014	21 4 0 25 4 25 4 27 28 7 4	857.3	√1058	_∕ <sup>_</sup> 918,7€	× 945.2		
07 Jul 2014	25 2	9493	) 113.3 ×	1089.0	≫ 153.0		
10 Jul 2014	<sup>م</sup> 28 کې	🤉 🦻 952.2 🔨	022.60	1016.50	136.1		
14 Jul 2014		<b>942.</b>	1023	≫ 9820	142.5		
17 Jul 2014		906 3 <sup>a) e)</sup> 🖓	59.7 <i>√</i> _	983,0 <sup>b)e)</sup>	157.6		
21 Jul 2014	¥ 8 <sup>3</sup> 39× <sup>9</sup>	1 ~~ <u>`</u> 7	0 <sup>7</sup> - <sup>4</sup>	791.0 <sup>c) e)</sup>	-		
Mean weight du	ring(exposure 🚿	6659		710.3			
Weight increase		123.5 ~	Jan Ja	181.8			
Mean weight po		0 907		943.0			
Weigh@ncrease	post-exposure <sup>d)</sup>	°C 93.0 ₪	. 7	61.7			
Total mean weig	H. 3 & .	∫ 075.3√	. 0 . 5 . 5	826.6			
Fotal weight inc	rease <sup>d)</sup>	× 29 <b>4</b> ,3	\$°	314.8			

DAE= days after axposure on monitoring site

(grey adicates

Mean= mean values of all available replicates mean values calculated with unrounded values \* = statistically significant ofference to control

- = staustically significant (over the second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second

The results of the kinal brood evaluation did not show any statistically significant differences between the control and the test iten 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 itemOreatment.

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



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 th	e forager bumble bee polle	n analysis			
% of potato pollen in pollen samples of forager bumble bees					
Sampling date	С	T 🖏			
5 DAE	0 🖉	24.8	E A D		
12 DAE	0	<u> </u>			
15 DAE	0 4	Q 6° \$4.8	L C L		

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-by droxy, and imidacloprid olefine) were detected in pollen from the control field. Residue levels in samples from the treated field were below the limit of quadrification at the sampling fates 5 DAE and 12 DAE. The maximum residue level of imidacloprid of  $1/4/\mu g/kg$  was found at the sampling fates 15 DAE (Table 7).

		$\sim$	A- Ro	° A	s and the second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second	()	õ	Ĉı	·~~
Table 7.	Desidues	fimiðlau	alanrid an	d it is n	notaho		in Vata	to be	ممالم
Table 7:	Residues o	I MIIIO	1000100-211	U AS I	IIBECIDU	Junes			Jien
		0//				0			V.

N

	Residues [µg/kg]	
Treatment group	Sampling	midacloprid
group	Sampling date Imidacloprid 5-hydroxy	olefine
	S DAE S AND S ALOD	< LOD
		< LOD
		< LOD
		< LOD
E. T	12 DAE & LOQ & ST STOD	< LOD
ν.Ψ.	To DALE NO DA LOQ	< LOD

DAE = days after prosure 2

LOQ = limit of quantification = 0.2 yg/kg for imidactoprid, 50 µg/kg for imidacloprid metabolites LOD = limit of detection = 0.2 µg/kg for imidactoprid, 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 nortality 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 as a seven as the 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.

